Universitat Autònoma de Barcelona

Process development for the synthesis at industrial scale of active pharmaceutical ingredients

Javier Santos Ramos

Universitat Autònoma de Barcelona
Facultat de Ciències
Departament de Química

Process development for the synthesis at industrial scale of active pharmaceutical ingredients

Ph.D. Thesis

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Javier Santos Ramos

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Supervisors:

Dr. Ramon Alibés Arqués

Dr. Xavier Pujol Ollé

Universitat Autònoma de Barcelona
Facultat de Ciències
Departament de Química

Thesis presented by Javier Santos Ramos
to apply to the degree of Doctor.

Javier Santos Ramos

With the approval of,

RAMON ALIBES I ARQUES
Firmado digitalmente por RAMON ALIBES I ARQUES
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Dr. Ramon Alibés Arqués **Dr. Xavier Pujol Ollé**

Bellaterra, 22 October 2020

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Sincerely,

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Table of abbreviations

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Abbreviation:	Meaning:
2-MeTHF	2-Methyl tetrahydrofuran
ACN	Acetonitrile
AEMPS	Agencia Española de Medicamentos y Productos Sanitarios
ALARP	As Low As Reasonably Practicable
AML	Acute Myeloid Leukaemia
ANDA	Abbreviated New Drug Application
API	Active Pharmaceutical Ingredient
APPI	Atmospheric Pressure Photoionization Ionization
ASTM	American Society Testing and Materials
b.p	Boiling point
BSA	Benzene Sulfonic Acid
CAPA	Corrective Action/Preventive Action
CDMO	Contract Development and Manufacturing Organization
CIDT	Crystallization-Induced Diastereoisomer Transformation
CMA	Critical Material Attribute
CMO	Contract Manufacturing Organization
CPME	Cyclopentyl Methyl Ether
CPP	Critical Process Parameter
CPPA	Critical Process Parameters Assessment
CQA	Critical Quality Attribute
CRO	Contract Research Organization
D9·HCl	**D9** hydrochloride form (**DH0517** process intermediate)
DBN	1,5-Diazabicyclo[4.3.0]non-5-ene
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
DET	Detectability
DFT	Density Functional Theory
DIPEA	*N,N*-Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	It can refer to Drug Master File or to *N,N*-Dimethylformamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic Acid
DoE	Design of Experiments
DR	Deviation Report
DSC	Differential Scanning Calorimetry
DSTR	Discontinuous Stirred-Tank Reactors
EDQM	European Directorate for the Quality of Medicines and Healthcare
EMA	European Medicines Agency

Table of abbreviations

Abbreviation:	Meaning:
FDA	Food and Drug Administration
FMEA	Failure Mode and Effects Analysis
FMECA	Failure Mode, Effects and Criticality Analysis
GC	Gas Chromatography
GMP	Good Manufacturing Practices
HAZOP	Hazard and Operability study
HMDS	Hexamethyldisilazane
HPAPI	Highly Potent Active Pharmaceutical Ingredient
HPLC	High Performance Liquid Chromatography
HT	Head Tank
ICH	The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IND	Investigational New Drug
INDA	Investigational New Drug Application
IP	Intellectual Property
IPA	Isopropyl Alcohol
IPC	In-Process Control
IR	Infrared Spectrometry
KF	Karl Fischer
KIE	Kinetic Isotopic Effect
KPP	Key Process Parameter
LC	Liquid Chromatography
LC-MS	Liquid Chromatography coupled to Mass Spectrometry
LOD	It can refer to Loss On Drying or to Limit Of Detection
MA	Material Attribute
MBR	Master Batch Record
MDS	Myelodysplastic Syndromes
MIT	Minimum Ignition Temperature
MoA	Method of Analysis
MPLC	Medium Pressure Liquid Chromatography
MPRA	Manufacturing Process Risk Assessment
MS	Mass Spectrometry
MSDS	Material Safety Datasheet
MTBE	Methyl Tert-butyl Ether
N/A	Not Applies
NA	Not Available
NASA	National Aeronautics and Space Administration
NCE	New Chemical Entity
ND	Not Detected
NDA	New Drug Application
NIST	National Institute of Standards and Technology
NKPP	Non-Key Process Parameter
NME	New Molecular Entity

Table of abbreviations

Abbreviation:	Meaning:
NMR	Nuclear Magnetic Resonance
NOR	Normal Operating Range
OCC	Occurrence
OECD	Organisation for Economic Co-operation and Development
OEL	Occupational Exposure Limit
OOS	Out Of Specification
OSHA	Occupational Safety and Health Administration
OTC	Over The Counter
OVAT	One Variable At a Time
p	Parts per weigh of limiting reagent (see footnote on page 150)
PAR	Proven Acceptable Range
Pd/C	Palladium on Activated Charcoal
PDE	Permitted Daily Exposure
PEL (Spanish acronym)	Standard Laboratory Procedure (Proceso Estándar de Laboratorio)
PMDA	Pharmaceuticals and Medical Devices Agency
PMP	Pentamethylpiperidine
PP	Process Parameter
PPE	Personal Protective Equipment
PQRA	Process Quality Risk Assessment
PSCI	Pharmaceutical Supply Chain Initiative
PSD	Particle Size Distribution
PSI	Process Safety Information
PTC	Phase-Transfer Catalyst
PTFE	Polytetrafluoroethylene
PTSA	p-Toluene Sulfonic Acid
PVP	Process Validation Protocol
QbD	Quality by Design
QSAR	Quantitative Structure–Activity Relationship
QTPP	Quality Target Product Profile
R&D	Research and Development
RLD	Reference Listed Drug
RLT	Range Limit Test
RNA	Ribonucleic Acid
ROI	Residue On Ignition
RoS	Route Of Synthesis
RPN	Risk Priority Number
RRF	Relative Response Factor
RT	Retention Time
SEV	Severity
SOP	Standard Operational Procedure
TBAI	Tetrabutyl Ammonium Iodine
TBD	1,5,7-Triazabicyclo[4.4.0]dec-5-ene
TED	1,4-Diazabicyclo[2.2.2]octane

Table of abbreviations

Abbreviation:	Meaning:
THF	Tetrahydrofuran
TMG	Tetramethylguanidine
TMP	Tetramethylpiperidine
TMSOTf	Trimethylsilyl Trifluoromethanesulfonate
TÜV SÜD	Safety Swiss Institute
US	United States
w.up	Work Up
XRPD	X-Ray Powder Diffraction
λ	Wavelength

CHAPTER 1

INTRODUCTION

INDUSTRIAL PhD THESIS

PROCESS DEVELOPMENT FOR THE SYNTHESIS AT INDUSTRIAL SCALE OF ACTIVE PHARMACEUTICAL INGREDIENTS

Chapter 1

1. Company presentation

This thesis, has been developed thanks to a collaboration between the Universitat Autònoma de Barcelona (UAB) and Farmhispania S.A. It is an industrial doctorate project enclosed in the Industrial Doctorates Plan of the Catalan government (Generalitat de Catalunya). The main feature of an industrial PhD is that the research project is developed at a company or institution. The doctoral students must develop their research and training in collaboration with a university or research center. Therefore, the industrial doctorates act as a bridge for knowledge transfer between industry, universities and research centers.[1]

Farmhispania S.A. is company founded in 1970 that belongs to the Farmhispania Group. The Farmhispania Group is formed by two companies, Farmhispania S.A. and Rolabo S.L. both located in Spain. The Farmhispania S.A. facilities (Figure 2) are located in Montmeló (Barcelona) while, the Rolabo S.L. plant is placed in Zaragoza (Figure 1).[2]

Figure 1. Images of the Rolabo S.L. plant situated in Zaragoza.

The Farmhispania Group is mainly dedicated to the manufacturing under GMP (good manufacturing practices) of API's (active pharmaceutical ingredients) and HPAPI's (highly potent active pharmaceutical ingredients) and to the development of the processes required for the large scale production of these substances. Historically, the Farmhispania Group has strongly invested in R&D and still nowadays dedicates an important part of its resources to investigation and development activities. It has five R&D laboratories and counts with more than 65 scientists dedicated to process and analytical research and development.[2]

The company is a worldwide leader CDMO (contract development and manufacturing organization) that collaborates with several top pharmaceutical companies. Farmhispania has been successfully audited by the PSCI (pharmaceutical supply chain initiative), the Food and Drug Administration (FDA), the *Agencia Española de Medicamentos y Productos Sanitarios* (AEMPS), the European Directorate for the Quality of Medicines and Healthcare (EDQM) and many national health authorities. It is a key supplier of multiple companies and manufacturers and many of its active pharmaceutical ingredients can be found in either oral or injectable

commercially available drugs. It has more than 45 different products in its portfolio and is considered one of the major manufacturers of high-grade Metformin Hydrochloride API, a widespread Type II antidiabetic drug. For more than 30 years the Farmhispania Group has manufactured yearly thousands of tons of Metformin Hydrochloride and this substance can be considered as one of its flagship products. Chlorambucil, Tipiracil, Gemcitabine, Methotrexate and other substances appearing in this thesis can be also found in the company's portfolio.[2]

In its state-of-the-art multipurpose facilities, the Farmhispania Group produces several API's and HPAPI's and also develops new synthetic routes to obtain complex molecules, advanced intermediates and building blocks. These facilities allow to perform large scale synthetic and fermentation processes and to cover a wide range of operating conditions making possible to work from milliliter-scale to 8000 L, at temperatures from -70 °C to 200 °C and in a wide range of pressures.

Thanks to its advanced and varied equipment, which includes API/HPAPI monodedicated fermentation development laboratories as well as a kilo-HPAPI area and, preparative high-performance liquid chromatography/medium pressure liquid chromatography (HPLC/MPLC) the Farmhispania Group has capabilities to manufacture under GMP from few grams to multi kilograms of synthetic or fermentative highly potent API, including cytotoxics with an occupational exposure limit (OEL) below 30 ng/m^3. The above-mentioned equipment allows the company to perform processes requiring challenging techniques such as hydrogenation reactions, cryogenic conditions, wet milling and freeze drying treatments.[2]

Figure 2. Farmhispania S.A. facilities located in Montmeló.

The company was recognized by the Spanish government as a Preferential Industry of National Interest, it has passed successfully 12 FDA inspections since 1993 and with a sales turnover over 80 million of euros is considered a world leader in the pharmaceutical ingredients manufacturing field.[2]

The main part of the work done during this industrial doctorate was carried out on the Research and Development Department of Farmhispania S.A. (Figure 2). This Department is formed by more than 40 scientists that are focused on:

- The development synthetic routes for the obtention of different compounds (API's, impurities, and synthetic intermediates).
- The development and validation of the analytical methods required to control the different studied processes.
- The development of robust, safe and efficient manufacturing processes through the scale-up of some of the designed synthetic routes from grams to kilograms.

2. Introduction

The modern pharmaceutical industry, started in the second half of the XIX century when, Thomas Beechman in 1859 founded in the United Kingdom the first world's factory producing only medicines. The origin of the pharmaceutical industry lies back in apothecaries and pharmacies that offered traditional remedies. Some of these small businesses have become important pharmaceutical companies such as Merck, Hoffman-La Roche or Upjohn which is nowadays part of Pfizer.[3]

The pharmaceutical companies are between the companies that spend more resources in R&D (Figure 3). In average they spend the 17.7 % of its benefits in R&D activities. The main reason is the huge effort that is required in terms of investment to develop a new drug. Not only to find a chemical compound with high activity, good selectivity and low side effects but also to demonstrate that the drug is safe and effective in humans and to get the approval of the authorities. In average the development of a new drug lasts for 13 years and costs around 2600 millions of dollars.[4]

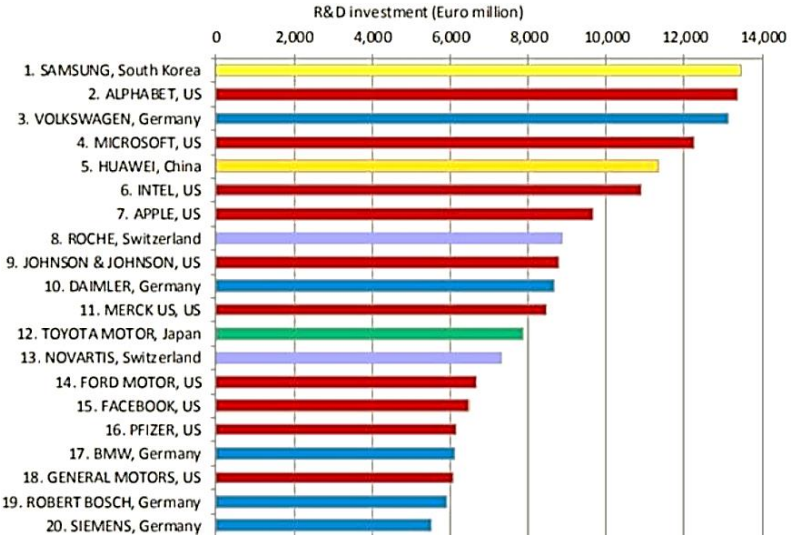


Figure 3. Total R&D spending (in billions of dollars) for the 20 top R&D investors during 2018. Reproduced from Ref [5] with permission of the copyright holder.

The discovery of new active pharmaceutical ingredients (API) has allowed to develop new drugs with enhanced effects (analgesic, antibiotic or antipyretic) compared to previously existing medicines. It has allowed to fight against illnesses without an efficient known treatment and, to treat the diseases producing to the patient fewer side effects. As a consequence of all these innovations, an improvement of the life quality of the patients has been achieved and treatments against many diseases which were mortal in the ancient times have been developed

contributing to the increment of the life expectancy (see Figure 4). Derived from the mentioned advances in the pharmacological field, other social benefits have been generated. The reduction of hospitalization costs by reducing or eliminating the inpatient stays, the reduction of sick leave or the increase of the patients productivity when they are at work are examples of the generated social benefits.[6]

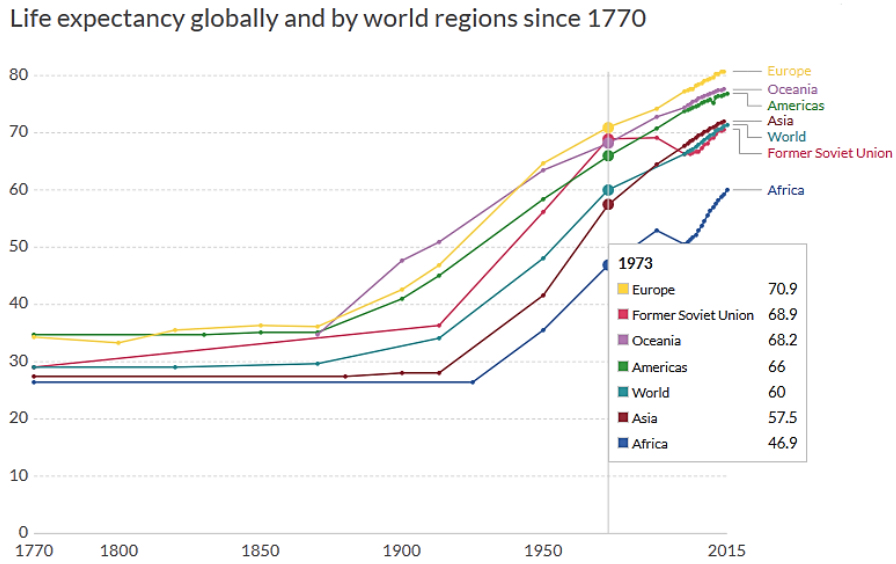


Figure 4. Progress of the life expectancy during the last 245 years. Reproduced from Ref [7] with permission of the copyright holder.

One of the keys for the achievement of the above-mentioned social wealthiness derived from the drug use has been the industrialization of the drug manufacturing processes. The development of robust, reliable and cost-effective methods for the large scale drug obtention has allowed to restrain the increase of its prices while increasing its quality and efficacy. The large scale manufacturing of APIs has made the medical treatments available for a significant part of the world population and, has given access to millions of people to the benefits derived from the drug usage.

2.1. The drug approval process

In order to get the approval of regulatory and health authorities such as the FDA (Food and Drug Administration) in United States, the PMDA (Pharmaceuticals and Medical Devices Agency) in Japan or EMA (European Medicines Agency) in Europe and to obtain the license to commercialize a drug in an specific territory, the pharmaceutical companies have to complete a complex, expensive and long process.

The number of steps, complexity, cost and duration of the drug approval process depends on the type of product that is going to be marketed. The approval process for new products either from synthetic or biological origin is, in general, more complex and expensive than the approval process for generics since, in this case, part of the required information has been already generated by the innovator. The requirements to get the commercialization authorization are the same for the drugs being subjected to doctor's prescription than for the over the counter (OTC) drugs (remedies that can be used by the general public without doctor's supervision). Although the approval process is regulated and reviewed by the corresponding regulatory

authority depending on the world region, the steps to be followed are generally quite similar in all the cases (see Figure 5).[8] There are different guidelines that the applicants should follow in order to complete the different phases during the process. These guidelines are published by the ICH (International Harmonization Conference) and describe the contents or the structure that these phases must follow and achieve.

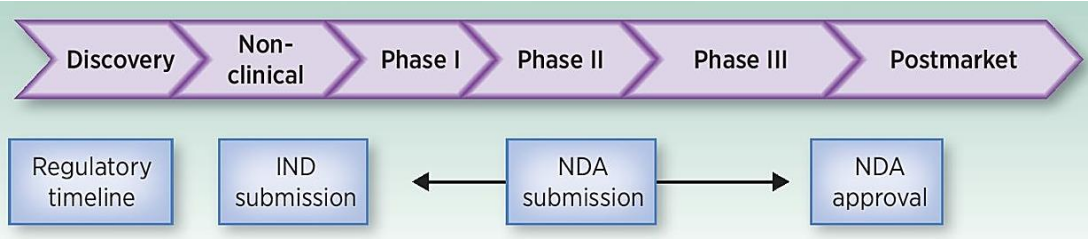


Figure 5. Different phases of the drug approval process. Being IND (investigational new drug) and NDA (new drug application). Reproduced from Ref [9] with permission of the copyright holder.

The complexity of the drug approval process requires a deep knowledge in many different areas, such as API manufacturing, process scale-up, clinical studies or drug formulation. Due to not all the pharmaceutical companies have the resources and the expertise required to cover all the knowledge areas involved in the approval process, in many cases partnerships are stablished. This kind of alliances involve companies which are specialized in specific areas of the drug approval process. The collaboration between different companies increases the possibilities of complying the FDA requirements and, reduces the time required to reach the market. This kind of partnerships, starts when the enterprise who wants to put a drug into the market outsources part of the work related to the drug approval process to other companies specialized in some of the different tasks to be performed. Typical examples of contract organizations are contract manufacturing organizations (CMO) also known as contract development and manufacturing organizations (CDMO) which, may offer stability study, API manufacturing, process scale-up or analytical method development services and, contract research organizations (CRO) which are mainly focused on the tasks related with the clinical trials.

2.1.1. Approval of new drugs

With an average development time of 13 years, the approval process for a new drug (from synthetic or biological origin) begins with the drug discovery phase where the identification of a new chemical entity (NCE) or a new molecular entity (NME) takes place (Figure 6). A NME is a drug with a chemical structure completely different from the structure of any other API already approved while, a NCE is a molecule that has not been previously registered but that is based on the same non-active moiety that other approved drugs. The objective in this phase is to find a compound capable to strongly interact with the therapeutic target molecule (enzyme or gene involved in the disease to treat).

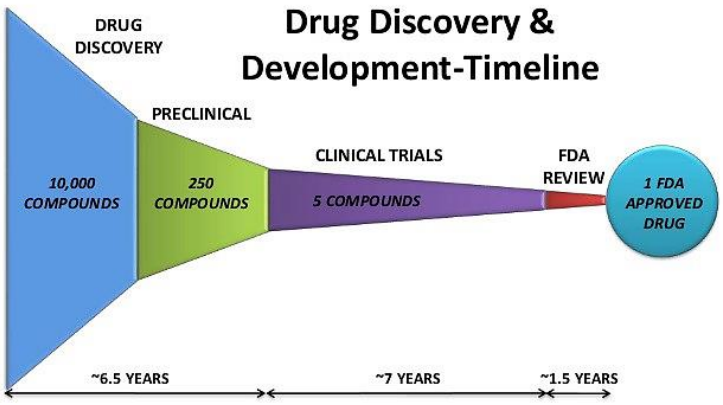


Figure 6. Summary of the different phases constituting the drug discovery process.[10]

There are many different methodologies for the NCE/NME discovery. Nowadays, the trend is to define the set of molecules to be tested using two *in-silico* techniques: combinatorial chemistry and virtual screening. The combinatorial chemistry technique is based on the creation of virtual libraries, containing thousands to millions of 'virtual' compounds. These virtual libraries are formed by all the possible structures that can be obtained from the reaction of a specific building block with all the available reactants. From the obtained *in-silico* compound library, the subset of compounds with higher potential to have the desired therapeutic effect is determined using virtual screening. The virtual screening software, estimates how likely is that a specific molecule binds to the therapeutic target based on computational chemistry calculations or quantitative structure-activity relationships (QSAR).

The selection process continues by synthesizing and testing the molecules selected in the previous *in-silico* studies. In this phase the properties of the candidates are evaluated in front of its pharmacological target. The substances with more promising activity and toxicity profiles are taken as starting point for the development. These candidates are systematically modified and tested looking for improvements in activity and/or reductions in toxicity. The actual synthesis and testing of the large sets of selected compounds is possible thanks to the advances in robotics that have allowed to automate and faster the process (Figure 7). The rate of success of the molecules entering in this phase is considerably low, in average of each 10000 evaluated substances only 1 leads to a drug approved by the regulatory authorities.[10]

Figure 7. Robotic arm working in a laboratory.[11]

The substances showing the best pharmacological properties are then evaluated in the pre-clinical phase which may take from 3 to 6 years to be completed. In this phase, *in-vitro* and *in-vivo* studies are performed to evaluate the toxicity, metabolism and excretion of the studied molecules. The dosage form, the safety initial dose or the formulation are also evaluated and determined for the most successful candidates. According to the statistics only 1 of each 250 compounds entering in the pre-clinical assays becomes approved for its use as a drug in humans (see Figure 6).[12,13]

Taking as example the FDA requirements for the candidates giving favorable results in the pre-clinical phase, the next step before to proceed with the clinical trials is to fill an investigational new drug application (INDA). The INDA must contain information about the mechanism of action, the clinical trial plan, the pre-clinical safety data or the manufacturing process. This document is reviewed by the FDA that will authorize the initiation of the clinical trials once has been confirmed that the compound to be tested and the proposed treatment are safe and potentially effective.

After the INDA approval the clinical trials of the new drug candidate can be started. This part of the drug development process where the potential new drug is tested in humans is divided in three phases (Figure 8):

- Phase I: this phase is mainly focused on the study of the safety of the potential new drug evaluated. In this phase the product is tested using limited doses given to small groups of volunteers (20 to 80).
- Phase II: it is initiated only in case that the toxicity levels observed at phase I are considered acceptable. The main objective of this phase that involves between 100 and 300 patients is to determine if the studied drug is effective for the treatment of the disease in humans. The tested drug is given to some subjects that are compared with other patients receiving inactive substances (placebo) or a different drug. The drug safety and side effects are also evaluated in this part of the clinical studies.
- Phase III: this final part of the clinical trials is started if in phase II is confirmed that the drug candidate does not present any safety of efficacy issue. It is mainly oriented to the obtainment of drug safety and efficacy additional information in different populations. Phase III studies involve between 1000 and 3000 patients that are treated with different dosages and different drug combinations. As in phase II, the performance of the tested drug is evaluated giving it to some subjects that are compared with other patients receiving inactive substances (placebo) or a different drug.

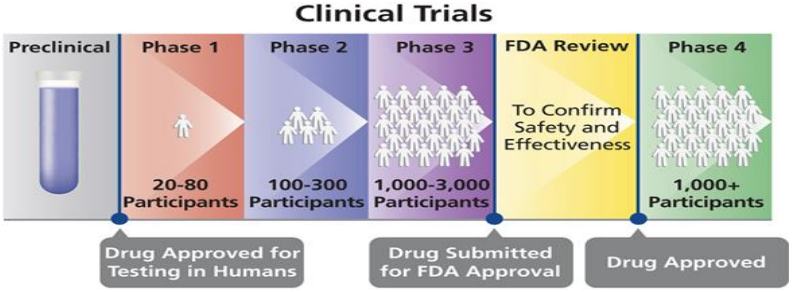


Figure 8. Illustrative summary of the different phases of the clinical trials. Reproduced from Ref [14] with permission of the copyright holder.

Approximately, 1 of each 8 compounds entering in clinical trials succeeds and reaches the following step in the process for being approved for its use in humans.[4,13] In case that the information obtained during the clinical studies indicates that the new drug candidate is safe and capable to offer a significant improvement over the already existing therapies the approval process may be continued. The next step is the preparation of an application for approval that must be reviewed by the corresponding regulatory organization. In the FDA case this document is called new drug application (NDA). The objective of an NDA is to demonstrate that the developed product is safe for its use in humans and that beneficial effects derived from its use are not achievable using any other existing drug. The NDAs are typically 100000 pages or more, they must provide data related with the clinical research, drug abuse effects, patent and manufacturing information or directions for use. Concerning the manufacturing of the drug it must be proved that the developed production method is robust, reproducible and well documented. The provided information must demonstrate that the manufacturer can obtain the desired drug within the stablished quality requirements, meeting good manufacturing practices (GMP) and using reliable/well controlled raw materials.

In order to ensure that the methods used to manufacture and control the quality of the drug candidate are effective, accurate, precise and reliable they must be normally validated. For the validation of the manufacturing methods the most extended procedure is to perform a series of validation batches (a minimum of three according to the Farmhispania S.A. approach). These lots are commercial scale batches that are manufactured consecutively according to a validation protocol that in advance describes the equipment to be used, the expected results or the manufacturing procedure.

The drug is finally approved for its commercialization if from the review of its NDA, the FDA scientists conclude that the new drug candidate is safe, effective and that it is manufactured according to the corresponding quality requirements. During the first three years after the approval of the drug, the pharmaceutical companies are required to perform a monitoring of the drug side effects. Within this period, a report has to be presented to the FDA every 3 months describing and studying any adverse reaction not observed in phase III due the relatively small population studied. This stage of the process is also known as the fourth phase of the clinical trials (see Figure 8).[8]

2.1.2. Approval of generics

A generic drug is a copy of an innovative synthetic drug for which, the enforcement period of its patent has expired. In the generics field, the drug being copied is defined as the reference listed drug (RLD). To be considered a generic drug, the manufactured product must have the same characteristics that the RLD (active ingredient(s), dosage form, strength, route of administration and conditions of use).

Compared with the new drugs, the approval process for generics is faster, inexpensive and simpler. It does not involve the target discovery and optimization phases and additionally, it is not required to perform again the expensive and time consuming pre-clinical and clinical studies. In this case, the safety and efficacy data previously generated for the RLD is accepted. To obtain the authorization for the commercialization of a generic drug, the regulatory organizations

request to the manufacturers to present a dossier. This document must contain the product manufacturing information, the data used to support the validation of the manufacturing/analytical methods and, the information required to demonstrate that the generic product is bioequivalent to the RLD. In the case of the FDA this dossier is called abbreviated new drug application (ANDA).

In some cases, the information related with the validation process and the manufacturing of the API is collected in a document known as drug master file (DMF). This document, is requested by the FDA to the API manufacturers during the process of obtention of the commercialization license in the United States. The FDA requests the DMF for each product and manufacturer unless the substance manufacturing information is already presented in a different format in the NDA or INDA being prepared. The DMFs are commonly used to support NDAs, INDAs and ANDAs especially, when the active pharmaceutical ingredient is being manufactured under contract by another company. In these cases, is common that the API manufacturer has already filled a DMF to get the FDA authorization to commercialize its product and, to be a more attractive supplier for the pharmaceutical companies.[15]

3. Project justification

Despite of the progresses made in the area of the active pharmaceutical ingredients manufacturing, over the last years the overall price of the commercialized drugs has increased gradually. Since 1990 the mean cost of drugs has grown yearly about 1 to 5 %. This trend could be related to the price variations of the protected brand drugs (products protected by patents) and of the generic drugs. According to the statistics, from 2014 to 2018 protected brand drugs have increased its price by over 60 % while generics cost has dropped down by 37 %.[16] The price of the protected brand drugs has increased over the last years due to:

- The manufacturing costs have increased because of the elevated complexity of the synthesized new chemical entities. In general, more advanced, time consuming and expensive techniques such as fermentative processes or preparative HPLC are required for the manufacturing of these substances. The increase in the complexity of the target molecules has also induced the use synthetic routes with a higher number of steps, lower overall yields and based on the use of expensive reagents such as deuterated substances or certain catalysts. In some cases, tailor-made manufacturing plants are required to produce the desired products due to the safety and/or technical requirements of the developed processes with the corresponding associated investment increase.
- The cost of the process required to develop a new drug and introduce it on the market has increased over the last decades. While in 2003 the cost of this process was estimated to be around 800 million of dollars nowadays it costs to the pharmaceutical companies near to 2600 millions of dollars. The cost increase has been associated with the higher failure rates observed for the drugs entering in the testing in humans phase. Only 12 % of the drugs entering clinical trials get the final approval. The increase in the failure rates, has been mainly attributed to the increased clinical trial complexity, the larger clinical trial sizes, the focus on targeting chronic/degenerative diseases and, the testing

on comparator drugs to accommodate payer demands for comparative effectiveness data.[4]

- The increase in the tightness of the quality standards at which the new developed drugs are subjected. These products, must fulfil strict specifications related to the levels of impurities, polymorphism or particle size distribution among others to guarantee its efficacy and safety. The implementation of this policy has led to a rise in the complexity of the manufacturing procedures and to a higher number of batch rejections and batch reprocessing/reworking operations increasing the drug manufacturing costs.
- The necessity of obtaining benefits during the relatively short period of enforcement of the patents. It is important to consider that due to the duration of the drug approval process the pharmaceutical companies only have, in average, 8 to 13 years of market exclusivity. This is the reason why in certain cases the authorities grant to the pharmaceutical companies with an additional 5 year patent enforcement period.[13]

As it has been mentioned in the previous section, generic products also need to pass through a marketing approval process from regulatory authorities. The cost, the complexity and the duration of this process is significantly lower compared with the process required to put a new drug into the market. This characteristic has contributed to the price decrease observed for the generic drugs. The price reduction can be also associated to the characteristics of the generics market. In this market, the pressure from the competitors forces the companies to reduce its margins of benefits and, to develop manufacturing procedures that allow the obtention of the desired product at lower costs.

The overall drug price increase combined with the changes in the social paradigm observed over the last few decades (rise in the occurrence of some chronical diseases such as diabetes or hypertension, population aging and, expansion of the population access to health care in emerging countries) have promoted a severe increase in the expense per capita in medical treatments. For example, the expense per capita in prescription drugs in the U.S. increased from 90$ in 1960 to 1.025$ in 2017 (data adjusted to inflation; Figure 9).[16]

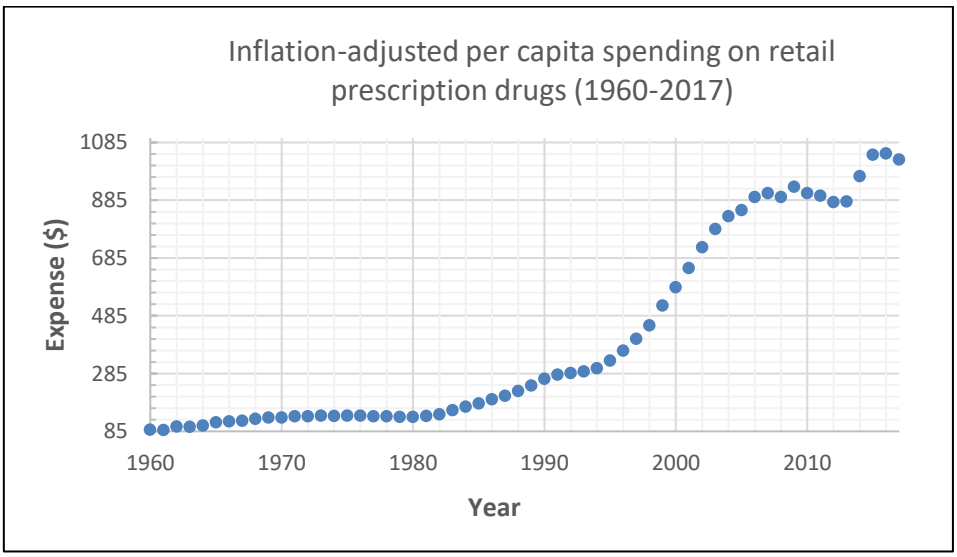


Figure 9. Prescription drug spending in the U.S. from 1960 to 2017.[16]

Currently, in the U.S already a 24 % of the patients have difficulties to afford its medical treatments and, the drug demand will continue to increase over the next years. Therefore, the decrease of the drugs price and the achievement of higher manufacturing capacities should be considered a priority.[16] The development of robust and well controlled drug manufacturing methods as well as its optimization is key for the price reduction and the manufacturing capacity increase of both generic and brand protected drugs. The suitability of this philosophy has been proved by the generics industry where it is successfully being implemented with the corresponding price decrease of its products.

To achieve the reduction of the manufacturing costs and the increase in production capacity, changes in the typically used process development and validation methodologies are being implemented. The traditional approach, based on extensive testing or the use of tight operating ranges within the process response is known is being replaced by, a new approach centered in the obtention of a more accurate process understanding and control. This new approach allows to reach the requested quality standards and to ensure the patients safety in a faster and more efficient way. It is based in the determination of those process parameters truly influencing the product quality/process performance and, in the use of high throughput experimental methodologies such as the design of experiments.

Further improvements in the manufacturing processes should contribute to the reduction of the production and poor-quality associated costs allowing to the companies to reduce the price of its products while maintaining the capability of obtaining a revenue from them. Additionally, the reduction of the production times and the increase of the manufacturing capacity achieved through the optimization of the API production processes should help to satisfy the increasing demand on this type of goods.

CHAPTER 2

OBJECTIVES

INDUSTRIAL PhD THESIS

PROCESS DEVELOPMENT FOR THE SYNTHESIS AT INDUSTRIAL SCALE OF ACTIVE PHARMACEUTICAL INGREDIENTS

Chapter 2

1. Objectives

This industrial doctorate is focused on the development and optimization of processes for the synthesis at industrial scale of active pharmaceutical ingredients. It is intended to contribute to the creation of safe, fast, robust and profitable methods for the obtention of this type of goods. The developed processes should help to the reduction of the manufacturing costs and to the increase of the manufacturing capacity. To achieve the mentioned goals, the process development will be based on the use of high throughput experimental techniques, *in-silico* studies and manufacturing process risk assessments among others. The adopted strategies will allow to reach the levels of process knowledge and control required for the obtention at industrial scale of products within the requested specifications.
The final goal of this industrial PhD is, to contribute to the amendment of the demand and price increase problems nowadays associated to many medical treatments (see Project justification section).

In the following chapters, it is described the work done for the development and validation of manufacturing processes for the obtention at industrial scale of two active pharmaceutical ingredients, **DH0517** and **FH0317** (Figure 10).

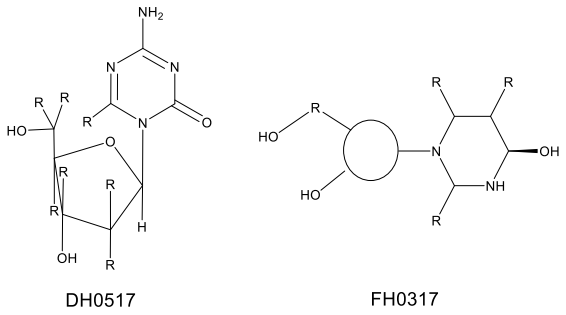


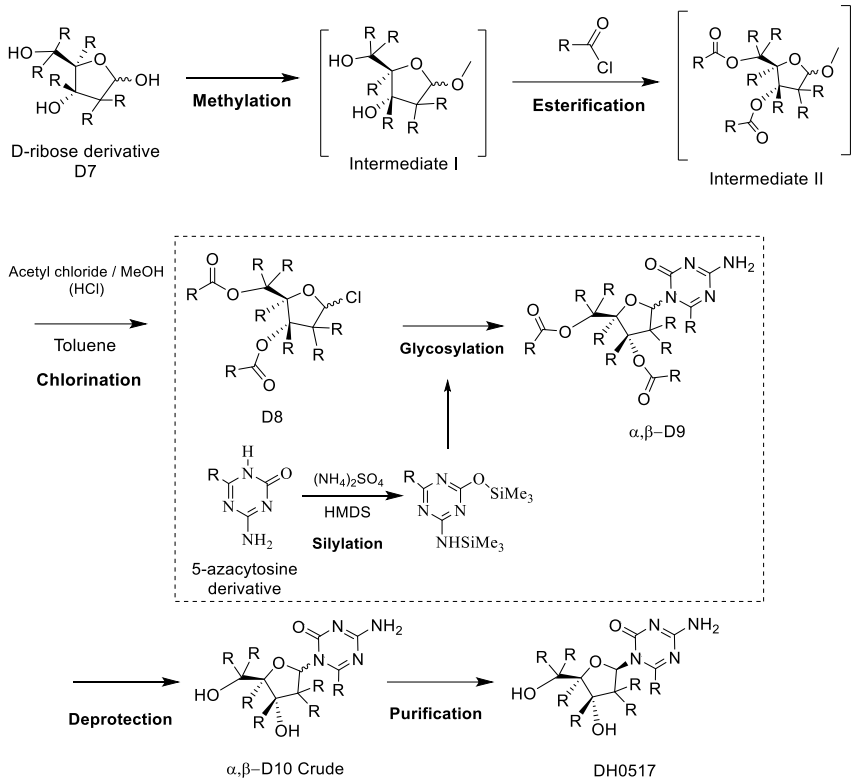
Figure 10. DH0517 (left) and FH0317 (right) chemical structures.

Disclaimer: the information generated during the **DH0517** and the **FH0317** projects is property of Farmhispania S.A. In order to protect the trade secret and the interests of the company the different figures, schemes, procedures and results presented in this thesis have been censored.

1.1. DH0517 project objectives

The compound **DH0517** is a highly potent active pharmaceutical ingredient (HPAPI) already approved by the food and drug administration (FDA) for the treatment of acute myeloid leukaemia (AML) and myelodysplastic syndromes (MDS). It is a parenteral drug, nowadays commercially available as a generic. The **DH0517** project was a contract manufacturing project that was initiated in Farmhispania S.A. under the request of an external company. The customer requested the development of an industrial process that allowed the obtention of 17 kg of **DH0517** with API quality per batch. In the company, there was no previous knowledge on the industrial preparation of **DH0517** and, therefore, the development of a new industrial process

was necessary. In this case, a synthetic route starting from a D-ribose derivative was adopted (Scheme 1).



Scheme 1. Route of synthesis selected in Farmhispania S.A. for the obtention of DH0517.

This thesis is focused on the industrialization of the synthetic steps involving the silylation and the Vorbrüggen glycosylation reactions (Scheme 1). Thus, in the presented study the main goal is the development and validation of an industrial process that allows the obtention at commercial scale of the intermediate **D9** using as starting material **D8**. The developed process had to comply with the robustness, reproducibility, safety and cost-effectiveness requirements necessary for its industrialization. During the **DH0517** project, all the process knowledge required to validate the developed manufacturing method had to be also generated.

To reach the above-mentioned goals, the **DH0517** project will be divided in different phases:

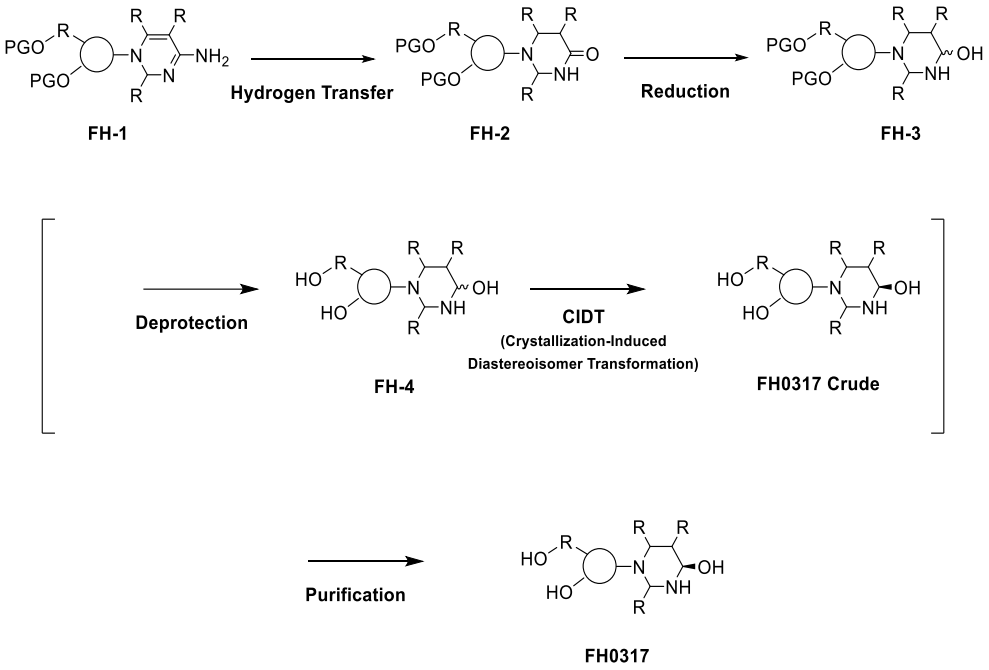
a) Screening of the glycosylation conditions: based on information from the literature, different experimental conditions will be tested to determine which are the optimal ones. During this phase it will be evaluated among others the use of different solvents, catalysts and reaction temperatures.
b) Process optimization: from the preliminary conditions defined in the screening phase, different studies will be performed to increase the yield, reduce the formation of impurities, enhance the reaction selectivity and to correct other weaknesses observed in the **DH0517** process.
c) Process scale-up: during this phase it will be evaluated and minimized the effect of variations in mixing, heat transfer, operation time and other factors which typically can not be kept constant during the scale-up. The performed studies will lead to the

obtention of a suitable process for the manufacturing of large quantities of **D9** and for its validation.

d) Study of process impurities: to guarantee the proper control of the process and of the quality of the obtained product, different process impurities will be isolated and characterized. The information and analytical standards generated, are fundamental for the process validation and, for the obtainment from the regulatory authorities of the authorization to commercialize the produced drug.

1.2. FH0317 project objectives

The **FH0317** research project involved the scaling-up from gram to kilogram scale of a synthetic process for the obtention the new chemical entity (NCE) named as **FH0317**. This NCE, is under clinical trials (at phase III). It is intended to be used for the treatment of certain types of cancer via oral dosage. In this case, a preliminary process description was received from the NCE innovator. The mentioned process consisted of a five-step synthetic route (Scheme 2) and, it was based on a lab procedure used to obtain small amounts of **FH0317**.



Scheme 2. FH0317 route of synthesis provided by the innovator of this NCE.

In order to conduct the clinical trials, the NCE innovator requested to Farmhispania S.A. to manufacture **FH0317** at kilograms scale. The main goals of this project are to develop and validate an industrial process for the manufacturing of **FH0317**. Moreover, throughout the project all the process knowledge required to support the NDA filling necessary for the drug approval must be generated. The developed process has to ensure that the batches of product are consistently produced and controlled according to the required quality standards.

The strategy adopted to reach the stablished goals will be based on:

- The extensive optimization of the **FH0317** synthetic process provided by the NCE innovator. Different studies will be performed to make this process suitable for the

industrial scale operation by reducing costs, solving robustness problems, increasing the quality of the obtained product and circumventing safety issues.

- The evaluation of the suitability of the developed process for its application at industrial scale through its gradual scale-up. The scale-up tests will allow to detect and solve problems related with the heat transfer, mixing, mass transfer variations associated to the use of large scale equipment.
- The development and implementation of a quality risk assessment methodology. The performed quality risk assessments will allow to define which process parameters are critical for the product quality/process performance and, those points of the process where corrective actions are required to reduce the risk of failure. As a result of these studies, the process robustness and reliability will be increased to the levels required for its validation.

CHAPTER 3

FUNDAMENTALS OF PROCESS SCALE-UP

INDUSTRIAL PhD THESIS

PROCESS DEVELOPMENT FOR THE SYNTHESIS AT INDUSTRIAL SCALE OF ACTIVE PHARMACEUTICAL INGREDIENTS

Chapter 3

1. Fundamentals of batch process scale-up

In order to successfully carry out a chemical process at industrial scale a wide variety of parameters must be studied and considered. Although cost plays often a leading role, the process can be constrained by lots of other factors such as time, safety, available equipment, waste generation or regulatory issues. All these parameters may have a big impact in the characteristics of the developed manufacturing method and often represent the driving force of the decisions taken during the optimization of the studied chemical process. The developed manufacturing method may be influenced even by the region where it is performed. Is important to keep in mind that, typically in Europe and in the United States the main contributors to the process cost are the manufacturing costs (waste disposal, energy, labour and equipment occupation). However, in China or India the situation is normally different. In these areas, the costs related to the raw materials usually constitute the main part of the required investment. These variations may lead to completely different manufacturing approaches depending on the location of the chemical plant.[17]

Although, the development of a manufacturing process is a complex activity in any industrial area, in the pharmaceutical industry it is especially complicated. Some of the particularities of the pharmaceutical business that make even more challenging the development of processes for the manufacturing of APIs are:

- The high robustness and quality standards requested by the customers and by the regulatory authorities. Normally, the manufactured products are subjected to very tight specifications and a high degree of repeatability and process knowledge is demanded (e.g. structure of the potential impurities or acceptable operating ranges).
- The adjusted timelines under the pharmaceutical companies normally operate. In the case of new drugs, the sooner the drug reaches the market the higher profit may be taken during the period of enforcement of the product patent. In the generics field is also important to reach as fast as possible the market in order to be in a position of advantage in front of the competitors.
- The high investment performed in the drug discovery process and in the development of the drug manufacturing method. As it has been mentioned in the previous chapter, the obtention of the desired compound at industrial scale is a key step in the process to put a new drug into the market. Due to the huge investment that the pharmaceutical companies make during the drug development and the drug discovery process, a failure or even a small delay in the commercial launch of a new product may have a severe impact in the price of the company's stock.
- The high investment carried out in every industrial batch of API. Usually, the manufacturing of an API involves large amounts of expensive raw materials and reagents. The failure of an API industrial batch may represent for the company an economical loss of hundreds of thousands of dollars.

To successfully develop an industrial method for the manufacturing of an API, the process must be thoroughly studied, optimized and controlled in order to ensure that it will behave equally at large scale than at laboratory scale. If the developed process is not robust enough, during the scale-up the use of different equipment or timings can originate deviations from the results obtained in the laboratory and lead to the batch failure. Due to the limited amount of time and resources available it is important to reduce unnecessary testing and to obtain the maximum information from the experimental work performed.

In the following sections there are described some characteristics and particularities of the industrial equipment, tools, methodologies and typical industrial procedures to be considered during the development of a chemical process. This information could be useful for the successful/fast development of profitable, safe and effective industrial manufacturing methods for the obtention of small organic molecules. In this thesis, special emphasis in the synthesis of active pharmaceutical ingredients and the techniques used to produce them at industrial scale is made. However, in most of the cases the described information could be applied to other industries manufacturing other chemical specialities such as flavours, cosmetics, surfactants, fragrances and agrochemicals.

1.1. The industrial plant equipment

Due to the high degree of complexity of the used facilities (Figure 11) and the large number of process parameters to be considered (vessel geometries, temperatures, stirring rates and stoichiometry among others) it may be difficult in certain cases to predict the process behaviour during the scale-up. To adapt the process to the plant capabilities will allow to increase the rate of success during the scale-up. For this reason, it is important to know the potential limitations and advantages offered by the industrial equipment to be used.

Figure 11. Industrial scale reactor set-up formed by the reaction vessel and multiple pipes and connections required for its operation.

In the following sections, there is a brief description of the most usual equipment used at manufacturing plants dedicated to the manufacturing of fine organic chemicals making special emphasis in the apparatus used for the APIs and HPAPIs manufacturing.

1.1.1. Reactors

The reactor is the centre of the chemical plants, a wide variety of sizes, shapes and configurations depending on its purpose can be found. In the pharmaceutical industry, the reactors are typically used for more than one process. The most common reaction vessels used are the batch stirred reactors also called discontinuous stirred-tank reactors (DSTR). They are widely used because they require the lowest investment among all the reactor types, allow fast scale-up and provide high flexibility (many different operations and manufacturing processes can be carried out in one of this vessels). Due to the above presented advantages, the batch operation mode is more extended in the pharmaceutical industry than the continuous methods. However, in the last years continuous methods are attracting the attention of pharmaceutical companies since they are less labour intensive and faster than the discontinuous ones.

Discontinuous stirred-tank reactors are typically cylindrical with a centred mechanical stirrer (see Figure 12) and, in some cases with internal coils or baffles. Internal coils and bafflers are incorporated in the reactor inner cavity with different purposes. Both allow to improve the reactor mixing capacity because they promote the turbulent flow of the mixture acting as an obstacle for the undesired laminar flow. In the case of baffles, they can also act as gas injection or sample collection pipes or they can house different types of in-line sensors (e.g. temperature, pH, oxygen). The use of internal coils promotes the increment the heat transfer capacity of the reactor and allows to achieve faster cooling/heating rates.

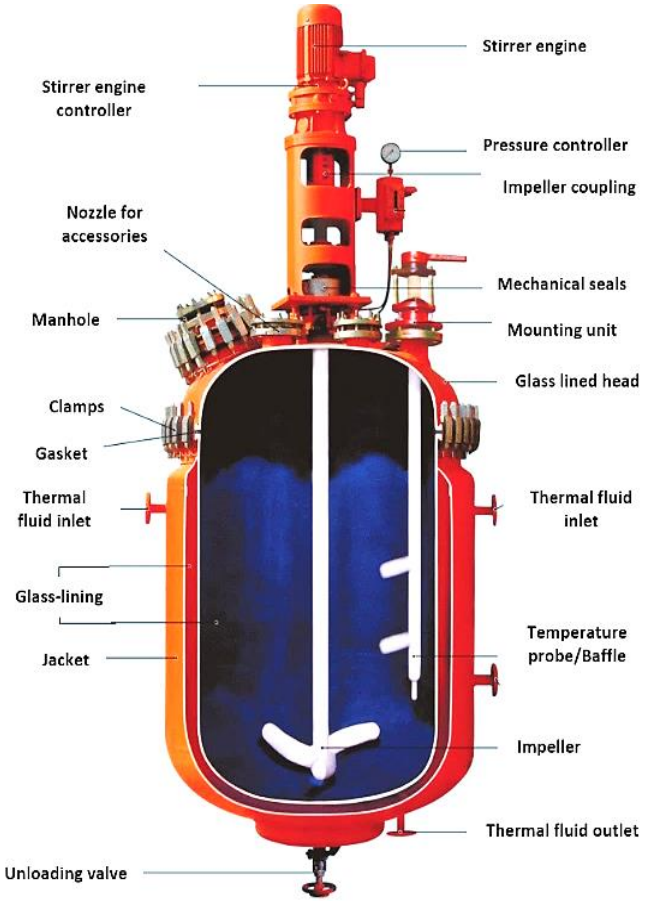


Figure 12. Cross section drawing of a typical batch stirred reactor.[18]

Batch stirred reactors are often manufactured in two parts that are joined by a gasket that ensures the device hermeticity. The biggest part is composed of the bottom and body of the reactor and is covered with a second smaller piece constituting the reactor head (Figure 12). The reactor bottom and body are surrounded by a jacket which is filled with a thermal fluid (propylene glycol-water mixture, hot water or steam are examples of thermal fluids). At the reactor bottom, a valve is placed to unload the reactor content when required (see Figure 13).

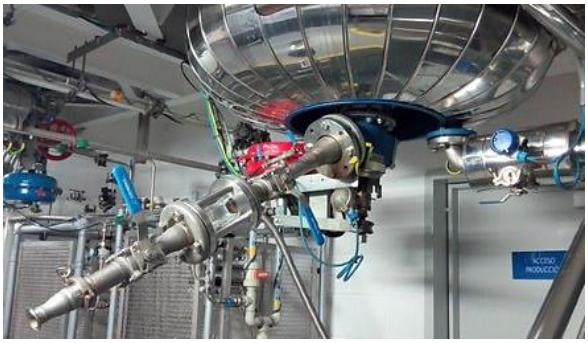


Figure 13. Detail of an industrial reactor unloading valve.

The reactor head contains a series of auxiliary connections that are used to install all the devices required for the vessel operation. It may contain connections to feed vessels, to reflux condensers, to nitrogen or vacuum pipes and to scrubbers (Figure 14). Normally, in the head of the reactor is also placed the reactor manway which is a circular opening of around 50 cm of diameter that communicates the reactor interior with the outside (see Figure 14). The reactor manways are occasionally used when solids have to be loaded in the reactor or to take samples. The manways are normally equipped with a glass window which often offers a limited picture of what happens inside the reactor.

Figure 14. Reactor head picture showing the reactor manway and the auxiliary connections used for the vessel feeding, for the gas venting and for the reflux condenser connection among others.

Glass is the most popular material of construction for this kind of industrial equipment. In most of the cases the industrial systems are based on a steel structure coated by an internal glass

layer of 1-2 mm thickness. Glass is commonly employed because it can be easily cleaned in most of the cases and because of its excellent chemical resistance and great mechanical strength. However, this material presents some limitations, and for certain applications the use of other materials may be required. Stainless steel, polytetrafluoroethylene (PTFE) also known as Teflon or Hastelloy which is a nickel-based alloy are examples of materials used to replace glass.

As it is detailed above, glass is the most typically used material in the industrial equipment used at the pharmaceutical companies. The main limitations of glass are that it degrades under extremely basic conditions and that it cannot stand large temperature differentials or extremely high or low temperatures. In order to prevent the crack of the glass, the maximum temperature differential recommended between the internal content and the fresh added materials is 50 °C in the case of steel glass-coated equipment and, 100 °C in the case of fully glass-based vessels.

1.1.2. Filters

The processes where no intermediates are isolated (so called telescopic processes) have a lower cost because generally lead to higher yields, require less analytical work and, avoid filtration or drying operations. However, in the active pharmaceutical ingredient manufacturing area is recommended not only to isolate the final product but also some of different process intermediates. The isolation of different process intermediates allows to detect yield or reactivity problems and, to precisely determine the equivalents of reagents required in each step enhancing the control over the synthesis and making the process more robust. The introduction of isolation operations also facilitates the purge of some of the process impurities in the filtration mother liquors leading to the obtention of more pure products.

From a regulatory perspective, the isolation and characterization of as much process intermediates as possible is also attractive since it leads to more robust and well controlled processes.

The systematic implementation of filtration operations in the pharmaceutical industry makes the filters a key piece of equipment in any API manufacturing plant. There is a wide variety of filter types and within each filter type many sizes, shapes and configurations may be found. The filter and the type of filter cloth to be used is normally selected depending on many factors such as the slurry characteristics, solid properties or operating scale. The filter cloths typically used at industrial scale are meshes formed by fibres of stainless steel or polymers, which may have many different porosities. The material and porosity of the filter cloths must be selected carefully considering, among others, the particle size of the isolated product, the chemical compatibility and the thermal compatibility (additional details on the filter cloth selection may be found in section 1.4.3). The most popular filter types in the pharmaceutical industry are:

1. Nutsche filters: they are normally cylindrical vessels with a perforated bottom where the filtration media is placed. Usually, they have a sight glass on the top and a pressure gauge to control the operation (see Figure 15).

Figure 15. 292 L capacity Nutsche filter.

The filtration driving force in this kind of devices may be gas pressure applied over the filter cake and/or vacuum applied at the filter draining pipeline. They may be jacketed to accommodate hot or cold filtrations and, normally, their material of construction is stainless steel. In this case, it is also important to check the chemical compatibility of the filter alloy with the components of the mixture to be filtered.

The use of this type of filters is not recommended for large scale production because the unloading operation may be hazardous, labour intensive and slow when large amounts of solid are involved.

2. Agitated Nutsche filters: they are similar to the above described Nutsche filters, but they include mechanical stirring arms (Figure 16).

Figure 16. Detail of the stirring arms of an 80 L capacity agitated Nutsche filter property of Farmhispania S.A.

This kind of devices are very useful when an efficient rinsing of the solid cake is required since, they allow to perform slurries of the filtered solid with the rinsing solution in the own filter. Their capability to break the solid cake also allows to perform the solid drying in the filter itself reducing solid handling operations, product exposure and product contamination risk. The agitated Nutsche filters may incorporate automatic discharge screws for totally enclosed operation required when dealing with highly hygroscopic/air sensitive substances or highly toxic compounds. The main problems derived from the use of this filters are caused by the friction induced during the solid mixing. In some

cases, this friction may favour the crystal breakage and the generation of heat or static charges.

3. Centrifuges: they are based on perforated baskets covered with a permeable material that are filled with the slurry that is going to be filtered. The solid is separated from the mother liquors spinning at high-speed the centrifuge vessel (Figure 17). The filtration driving force is in this case the centrifugal force induced by the spinning movement. The main advantages of this kind of devices are that they allow to remove much more efficiently traces of residual solvents from the isolated solids which can reduce drying times. Additionally, they may be easily equipped with mechanical unloading systems allowing much higher production rates than Nutsche filters and reducing product exposure and contamination risk.

   However, the centrifuges present some drawbacks since they require a relatively high maintenance, the efficiency of the rinsings is not completely reproducible within loads due to the lack of uniformity of the obtained solid cakes and, they are complex to clean between batches.

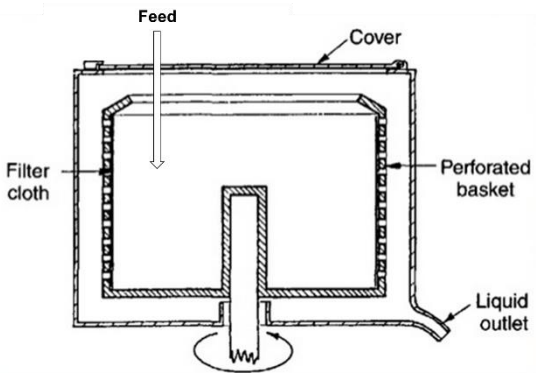


Figure 17. Scheme of a in industrial centrifuge.[19]

Due to the low similarity of the filters typically used in the laboratory (Buchner funnels) with the industrial scale ones, the filtrations are considered one of the most challenging operations to be studied. The laboratory experiments can be useful to determine the most suitable type of filter cloth, to predict the filtration behavior during the scale-up and, to anticipate possible residual solvent problems derived from the inefficient solid cake draining among others. When it is required, laboratory scale centrifuges or agitated Nutsche filters can be used to obtain the information required for the filtration study and scale-up (Figure 18). The utilization of these devices allows to minimize the lack of similarity within laboratory and industrial filtration devices and, to obtain more representative results. Additional details on the filtration study are given in section 1.4.3.

Figure 18. Laboratory scale centrifuge (360 g capacity) from Comteifa used at Farmhispania S.A. to perform filtration studies.

1.1.3. Dryers

Drying is an important operation that can influence not only the residual solvent content of the isolated product but also some other important properties such as particle size distribution, purity, bulk density and polymorphism. Therefore, the drying process applied may have a relevant impact in the bioavailability, solubility or handling of the produced substance. Depending on its working principles, dryers may be divided in two categories:

- Convective dryers: based on the use of preheated air or other gases that are passed through the solid to gradually remove the solvent. The main advantages of kind of dryers are that they tent to maintain the product relatively cool and that they can be easily scaled-up.
- Conductive or contact dryers: in this kind of devices the product is in direct contact with a heated surface. They may operate using a gas flow (not preheated in this case) that is used to remove the evaporated solvent or under vacuum. In the vacuum operated dryers, a smooth passage of gas is normally applied to increase the solvent removal capability.

The conductive dryers operated with vacuum are the type of dryers most commonly used in the API manufacturing plants. This kind of devices are particularly suitable for temperature or air sensitive products and are recommended for the removal of toxic or flammable volatiles. Many different types of conductive vacuum dryers may be found in the market. However, the most typical ones are the tray dryers (Figure 19) and the tumble dryers (Figure 20).

Figure 19. Laboratory scale vacuum tray dryer.

Tray dryers are the most common apparatus for laboratory and moderate size scale. They are very reliable due to its lack of moving parts and the availability of this type of devices at the laboratories allows an easy scale-up. Additionally, tray dryers prevent the particle attrition during the drying which, may induce undesired variations in the particle size distribution of the isolated solid. However, the use of this type of devices presents some drawbacks:

- They normally require larger drying times than agitated dryers due to the internal part of the wet cake is not completely exposed.
- The dried product often requires an additional milling or blending treatment to eliminate agglomerates and ensure the batch homogeneity.
- They are less suitable for large size batches due to their space and efficiency limitations.

The use of agitated driers such as the tumble dryers (also known as rotary cone vacuum dryers) could allow to circumvent some of the tray dryers drawbacks. However, these devices present have their own limitations (they may induce particle attrition or product agglomeration). In this case, the drying procedure has to be carefully studied to ensure the obtention of a product within the desired residual solvent content, particle size distribution or polymorph specifications.

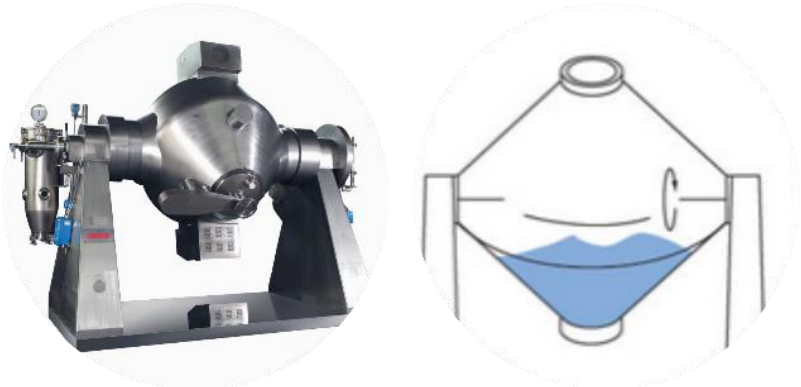


Figure 20. Industrial tumble dryer (left) and scheme of this type of device (right). Reproduced from Refs [20,21] with permission of the copyright holders.

Certain processes may require from the use of more complex drying techniques such as spray drying or lyophilization (also known as freeze drying).

- In lyophilization, a solution containing the product is frozen and the solvent is removed through its sublimation using vacuum.
- Spray drying is based on the nebulization of a product solution against a steam of a dry hot gas (normally air or nitrogen). The solvent is removed from the solution droplets by the gas steam leading to the obtention of dry solid product. Due to the applied temperatures this technique is normally not suitable for the isolation temperature-sensitive substances.

The use of spray drying or lyophilization allows to obtain dry solid product directly from a solution containing it. They are useful to avoid problems typically observed when the traditional crystallization/filtration/drying approach is used (formation of dense slurries, slow filtrations and obtention of undesired polymorphic forms). The main disadvantages of the lyophilization and spray drying compared to the traditional drying methods are the limited manufacturing rates that can be achieved and, its higher cost/complexity in terms of equipment and operation.

1.1.4. Scrubbers

The scrubbers are pieces of equipment designed to avoid the emission to the atmosphere of gases and dust originated during the plant operation. They are designed to promote an intimate contact between the gas stream coming from the industrial system and a liquid solution that is used to absorb the pollutants. In many cases, the scrubbers are filled with a porous matrix. The gas flow is injected at the bottom of the device and the rinsing solution is sprayed from the top in order to favour the contact between the liquid and gas phases (Figure 21).

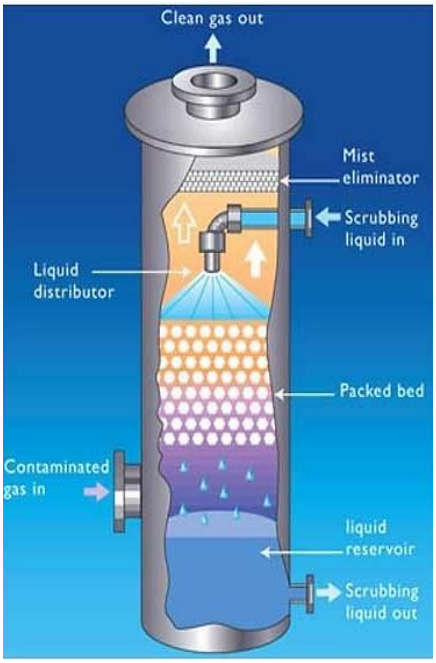


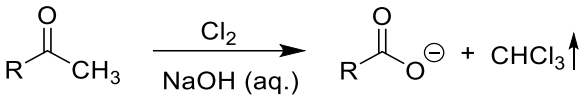
Figure 21. Scheme of a typical scrubber working principle.[22]

The composition of the liquid solution used in the scrubbers is adapted according to the contaminants that are expected to be generated in the system. For example, it is common to use alkaline solutions to absorb acidic vapours such as HCl or to use solutions containing bleach

or other oxidising agents to destroy some toxic and/or odorous organic contaminants for example, mercapto derivatives.

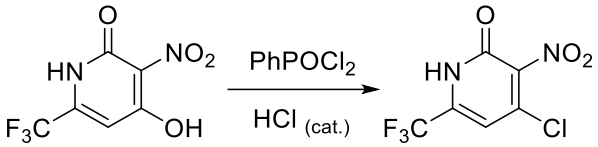
Although the scrubbers are not directly involved in the synthetic processes, the use of this devices may have a relevant impact in the process performance. The use of scrubbers implies the application of a gas flow and smooth vacuum over the industrial system (the applied gas is normally nitrogen). It is known that, at industrial scale, the efficiency of the nitrogen blanketing is considerably higher compared with the one observed during the standard laboratory operation. The higher inertization efficiency may induce an involuntary removal of part of the volatile substances involved in the process. This phenomenon is sometimes not considered in the laboratory studies and, it may cause variations in the process behaviour during the scale-up. See some examples below:

1. In the presented example of an haloform reaction (Scheme 3) the use of inert gas flow could allow to remove the chloroform formed as a by-product shifting the equilibria towards the product side and increasing the process performance at industrial scale.



Scheme 3. Example of an haloform reaction favored by chloroform removal.

2. The reaction presented in Scheme 4 did not reach the completion when it was scaled-up. It was found that the higher efficiency of the nitrogen flow applied was detrimental for the reaction since, it promoted the elimination of the hydrogen chloride that was acting as a catalyst. [23]



Scheme 4. Chlorination reaction inhibited by high nitrogen flow rates.[23]

In the same way than in the volatile stripping case, the higher efficiency of the inertization may lead to variations into the results achieved during the scale-up process. These variations may be related to phenomena such as the more efficient oxygen removal. As in the above presented examples, depending on the process features the oxygen absence may positively or negatively affect the reactivity or impurity profile obtained.

1.2. Safety at industrial scale

In order to evaluate process safety at industrial scale, lots of different parameters concerning capabilities of the used equipment, characteristics of the chemical reactions and toxicity of the used substances must be considered. The development of a safe process is a multidisciplinary task that involves different departments of the company since it requires a deep knowledge about the process and the facilities. To favour the collaboration between the different experts of the company (chemists, chemical engineers and plant personnel) involved in this process, the most usual procedure is to hold a series of meetings known as hazard and operability study (HAZOP). According to the occupational safety and health administration (OSHA) regulations, the process safety information (PSI) must be reported for all processes involving hazardous chemicals. This means that the safe operating limits, the equipment information and the material safety datasheets (MSDS) must be obtained.[24]

To perform a reaction at industrial scale or even at a scale of few kilograms often involves the use of high amounts of solvents, reagents or catalysts. Additionally, most of the industrial processes are designed to be performed at high concentration conditions in order to increase the manufacturing capacity per volume unit. The employment of high concentrations and large amounts of materials makes difficult to keep the safety at similar levels to the ones achieved at laboratory scale. The use of large quantities of chemical substances means that, inherently there are inside of the reactor important amounts of energy that can be released in case of an uncontrolled reaction. The criticality of this scenario is increased by the high concentration conditions often applied.

In cases where toxic substances are used, it should be assured that the plant operators are not exposed to them. The use of closed atmosphere systems (Figure 22) and of specific personal protective equipment (PPE) (Figure 23) are the most common solutions.

Figure 22. Closed atmosphere system used in Farmhispania S.A. for the manipulation of hazardous chemicals at industrial scale.

Figure 23. Farmhispania S.A. plant operators wearing a Tychem suite during the manipulation of a hazardous chemical.

1.2.1. Solid manipulation

The addition of solids into the reaction mixture is a usual operation at laboratory scale. However, at industrial scale this means the manipulation of several kilograms of material and may result in one of the most challenging and dangerous process operations.

During the solid load, the operators may be exposed not only to dust generated by the solid being dosed but also to vapours released by the volatile substances contained in the reactor. Additionally, depending on the operation mode, during the solid loading the system may be exposed to the atmosphere increasing the risk of having a contamination caused by the entrance in the reactor of dust, moisture, foreign matters or chemicals being used in the surroundings. When the risk of having a contamination and/or the hazards derived from the exposure to the chemicals involved in the process are unacceptable, closed solid addition systems such as the ones presented in Figure 24 must be used.

Figure 24. Closed solid addition systems used in Farmhispania S.A. Left: isolator system. Right: scheme of a device from Ezidock Systems. [25]

Throughout the solid loading dust explosions can be easily originated by a discharge of static electricity. As it is shown in Figure 25, this kind of accidents can have dramatic consequences for the facilities and the personnel involved.

Figure 25. Consequences of a dust explosion in a sugar refinery.[26]

To reduce the dust explosion probability, the use of antistatic clothes and shoes is specially recommended. In these cases, it is also convenient to ground properly the reaction vessel and the powder container, to maintain an inert atmosphere inside the reactor and, to use antistatic or paper bags for the solids to be loaded.

Similar precautions are recommended for the drying and the manipulation of the solid products isolated at plant. Generally, it is recommended to dry the collected solids below two thirds of its minimum ignition temperature (MIT) to reduce explosion risk. The MIT is the minimum surface temperature at which flames are observed when a cloud of the solid powder contacts the mentioned surface.[27–29]

1.2.2. Thermal hazards

The industrial equipment heating and chilling capacities are several times lower than the ones observed for the laboratory glassware because of the differences in the surface/volume ratios. As it is illustrated in Figure 26, the industrial equipment typically presents lower surface/volume ratios than the laboratory scale material.

- Assuming spherical shape. For a 100 mL round bottom flask filled to the 50 % of its capacity (50 mL):

Radius: 2.87941 cm Sphere Area: 104.18 cm^2 Contact Surface (Sphere Area/2): 52.09 cm^2

Surface/volume ratio: 1.04 cm^{-1}

- Assuming cylindrical shape and 50 cm of radius. For a 1000 L (10^6 cm^3) reactor filled to the 50 % of its capacity (500 L; $5x10^5$ cm^3):

Height: 127.3 cm Contact Surface (Base surface + Surface of the cylinder/2): 27850 cm^2

Surface/volume ratio: 0.056 cm^{-1}

Figure 26. Surface/volume ratio comparison between a round bottom flask and an industrial reactor.

Due to the lower surface/volume ratio, it may happen that during a certain process operation the release of energy could be higher than the one that the reactor is able to evacuate. In this case, the excess of energy not absorbed by the reactor ends up in the reaction mixture and increases the temperature of the reactor contents. Since the reaction rate and consequently the heat release rate are temperature dependent this phenomenon is self-catalysed and may lead to an exponential increase of the temperature. In extreme cases, if the process is not controlled and there are present large amounts of reactants, the temperature may raise leading to the violent boiling and massive evaporation of the solvent. To avoid the pressurization of the reactor, safety systems such as rupture disks connected to blow down tanks[1] are used. These systems are designed to evacuate and storage the effluents generated during an uncontrolled violent boiling event (Figure 27).

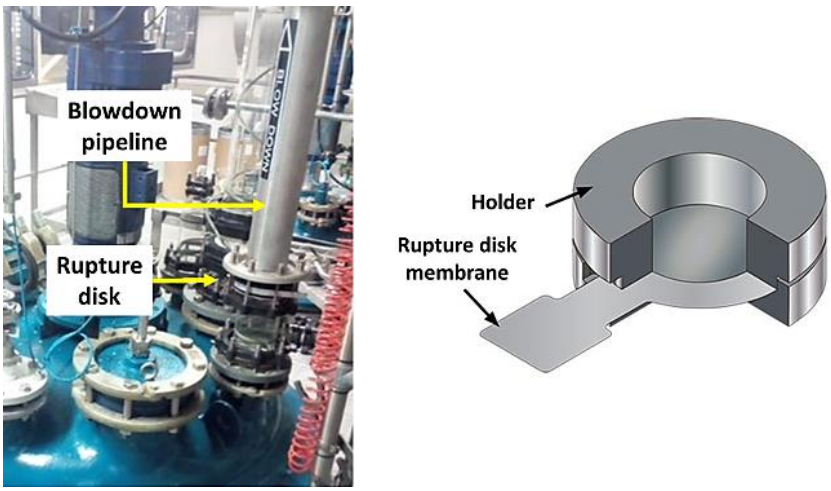


Figure 27. Safety system installed in the head of a Farmhispania S.A. reactor (left) and scheme of a rupture disk (right). Reproduced from Ref [30] with permission of the copyright holder.

[1] Rupture disk: safety device formed by a membrane that only brakes when the internal pressure of the reactor overcomes a determined value. When the membrane breaks the reactor becomes connected to an auxiliary vessel (the blow down tank) which has the purpose of receive the content emitted by the main reactor.

In the worst cases, when the solvent evaporation takes place in such a fast way that the safety devices are not able to avoid the reactor pressurization the reaction vessel may explode. This phenomenon is known as runaway and may have dramatic consequences (Figure 28).

Figure 28. Effect of a runaway explosion in a pesticide plant.[31]

According to the Farmhispania S.A. approach, before the execution at industrial scale of any synthetic step its safety assessment must be completed. The study must guarantee that there is no risk associated to the reactions being performed and, that the process is safe enough to be carried out at industrial scale. To evaluate a given synthetic step in terms of safety, the enthalpy value of the reaction being studied is used. The reaction enthalpy value can be estimated from the literature, from calorimetric studies or from theoretical calculations carried out using software tools such as the Chetah software (see section 1.3.2). To perform the safety evaluation is also required some calorimetric data of the reaction crude and/or of the different substances involved in the process (starting materials, products and reagents). The most typical technique used to obtain the mentioned calorimetric data is the differential scanning calorimetry (DSC). Using this technique, it is possible to determine the temperature at which takes place the decomposition reaction of the different chemical species involved in the process. From the DSC data it is also possible to obtain the enthalpy of the decomposition reaction of the different samples being studied (see in Figure 29 a typical DCS curve).

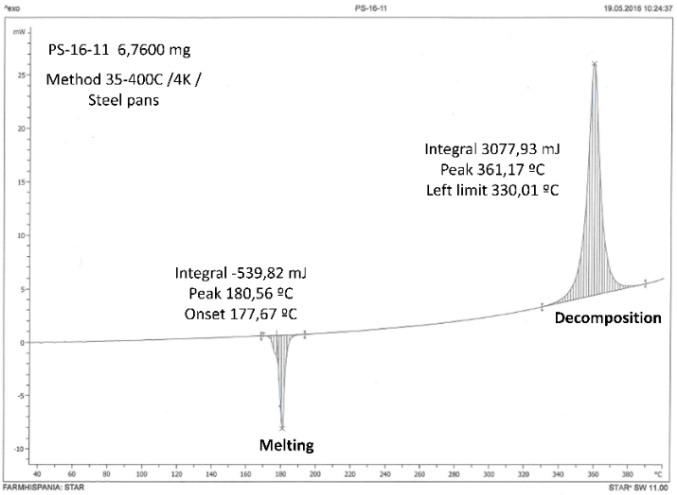


Figure 29. Example of a DSC curve obtained in Farmhispania S.A. from the analysis of a process intermediate.

The safety assessment studies give an indication of the process hazardousness and are commonly carried out assuming the worst-case scenario (adiabatic conditions and the highest values of reaction enthalpy found). At Farmhispania S.A., an automated Excel spreadsheet created by the safety experts of the company is used to conduct the mentioned safety evaluations (Figure 30). The developed tool, requests information related to the DSC analyses, reaction enthalpy, reaction crude mass or reaction crude heat capacity. Based on the expected temperature increase derived from the heat release of the studied reaction and, in the reaction mixture boiling point among others, this tool determines the hazardousness of the studied process.

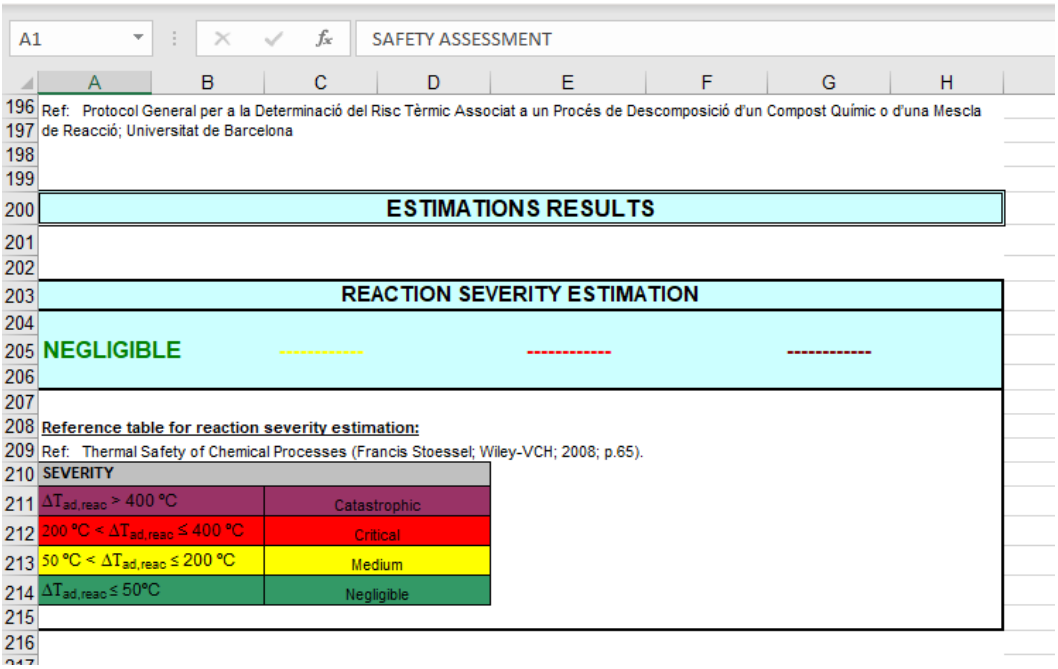


Figure 30. Fragment of the tool developed at Farmhispania S.A. to evaluate the process safety.

If from a safety assessment study it is concluded that the preformed reaction is safe, no further actions must be taken. However, if the study gives a safety alert, additional tests are required in order to determine in a precise way the process behaviour under stress situations and, to define accurately the process risk. In cases where additional studies are necessary, isothermal calorimetry or adiabatic calorimetry experiments are normally carried out (see Figure 31).

Figure 31. Adiabatic calorimeter from Thermal Hazard Technology (left) and isothermal calorimeter RC-1 from Mettler Toledo (right). Reproduced from Ref [32] with permission of the copyright holder.[33]

In isothermal calorimetry it is measured the energy required to maintain the reaction crude at a constant predefined temperature while the reaction evolves. From the isothermal calorimetry experiments the reaction enthalpy may be precisely determined. The obtained results are used to replace in the safety study the initial value of reaction enthalpy, which is normally estimated from the literature or *in-silico* calculations assuming the worst case. The experimental reaction enthalpy value may serve to accurately determine the expected adiabatic temperature increase of the vessel contents for the studied reaction. In case that the predicted adiabatic temperature increase leads to a final temperature above the boiling point of the mixture or, higher than the decomposition temperature of any of the species present in the studied mixture, adiabatic calorimetry experiments are recommended.

In adiabatic calorimetry, the studied reaction is carried out in a pressurized/thermally isolated vessel and the internal temperature/pressure increase are monitored. These experiments allow to determine the consequences in case of loss the control of the studied process at industrial scale and, to stablish the actual process hazardousness.[33]

In case that the results of the safety study indicate that the studied process is not completely safe, corrective actions must be applied. The most usual techniques applied to increase the process safety are:

- Ensure the proper mixing of the reaction crude, especially during reagent additions. If the reaction mixture is not well agitated the accumulation of some of the reagents can take place. This substance can react violently once the mixture is homogenised due to an increase of the stirring speed or temperature.
- Increase the dilution of the mixture when it is possible. Reduce the concentration of the reactants in the reaction crude allows to decrease the reaction rate and to increase the overall mass of the reactor. When the total weight of the reactor content is enlarged, the same amount of energy leads to a lower temperature increment. See Figure 32.

$$\Delta t = \frac{\Delta H_r \times n}{C_p \times m}$$

ΔH_r: *reaction enthalphy in* $^{KJ}/_{mol}$ n: *n° of unreacted mols of limiting reagent*

C_p: *reaction crude specific head capacity in* $^{KJ}/_{K \times Kg}$ m: *total reaction crude mass in* Kg

Figure 32. Formula used to calculate the temperature variation under adiabatic conditions caused by the energy release or absorption of a reaction.

- Control the reagent addition rates. Adjust the addition rate of determined reagents permits to control the amount of starting material available for the reaction. Therefore, the dosage of these substances allows to modulate the reaction rate as well as the heat release and to adjust them to the heat dissipation capabilities of the used reactor. For highly exothermic reactions the use of this technique may lead to additions of hours or even days at large scale. Therefore, to prevent degradation problems during the scale-up it is recommended to evaluate the stability of the reaction crude when this strategy is adopted.

- Work at high temperature. The increase of the internal reactor temperature leads to an increase of the reaction rate. To accelerate the reaction can be useful in cases wherein reagents must be added over the reaction mixture. High reaction rates avoid accumulation of unreacted materials and decrease the thermal runaway likelihood since, under these conditions the reaction proceeds gradually during all the reagent addition.
- Use taller and thinner reactors and/or internal coils (Figure 33). For a fixed vessel capacity, the reactors with higher height to diameter ratios and internal coils present greater heat transfer areas and therefore they have a higher cooling capacity. The use of this kind of technical solution is less usual because it requires large investments and, because this type of vessels typically present worse mixing capabilities.

Figure 33. Inside view of a reactor equipped with an internal coil.[34]

1.3. *In-silico* predictive tools

As it has been mentioned in previous sections, a failure or even a delay in the development of a process for the obtention of a drug at industrial scale may cause important economical loses to the pharmaceutical companies. For this reason, increasing as much as possible the ratio of success during the process development and scale-up is considered a priority in this sector. The traditional approach used for the development of manufacturing methods was based on the expertise of the company personnel (R&D team, plant operators and chemical engineers) and, in the obtention of the required information from large sets of experiments and complex calculations.

The use of *in-silico* tools is being adopted by the pharmaceutical industry in order to accelerate the development of the new processes and to increase the robustness of the created manufacturing methods. The final goals are to reduce the testing and time needed to reach the industrial scale, to reach the levels of process knowledge required to obtain the desired quality/yield and also, to reduce as much as possible the probability of failure during the scale-up.

The above-mentioned software solutions have been designed to simplify complex calculations and to increase the amount of information extracted from the available experimental data. Based on iterative methodologies, statistical treatments and user-friendly interfaces with complex mathematical backgrounds, this type of software creates mathematical models based on the available experimental data and, in chemical/physical constants. Using the obtained mathematical models, thousands of simulations about the process response in front of variations in the experimental conditions can be carried out in minutes or seconds. The levels of process knowledge and, the solutions found using these tools are in most of the cases impossible to achieve using a traditional experimental approach since it will require thousands of experiments. However, the mentioned programs may present in certain cases some drawbacks. They may require a specific training to be operated, may need from data complex to be obtained experimentally or, may require the validation of the obtained mathematical models through additional confirmatory experiments.

There is a wide variety of different software solutions designed to facilitate the process development. During the development of this thesis, the Dynochem and the Chetah software were mainly used. However, other software packages available at Farmhispania S.A. such as the VEGA and the QSAR toolboxes were also seen and applied in collaboration with company experts for punctual studies (see Table 1).

Software name	Developer	Application field
MODDE	Sartorius Stedim Biotech	Design of Experiments (DoE) studies
QSAR toolbox	Organisation for Economic Co-operation and Development (OECD)	Genotoxicity studies
VEGA QSAR	Istituto di Ricerche Farmacologiche Mario Negri (IRCCS)	Genotoxicity studies

Table 1. Summary of the different programs used during the development of this thesis.

1.3.1. The Dynochem software

Property of Scale-up Systems, this software has been created as a Microsoft Excel add-in (see Figure 34). The Dynochem software is considered the world's leading drug substance and process development software for scientists and engineers. This program is used by chemists and chemical engineers at hundreds of leading pharmaceutical, agrochemical and CDMO companies of Asia, Europe and North America. The first version of the Dynochem software was created in 1994 as a piece of software developed by scientists of Zeneca (now Syngenta) to be used in the scale-up of agrochemical processes. Since software development is not a core activity for Syngenta, the commercialization of this tool was assumed by Scale-up Systems.

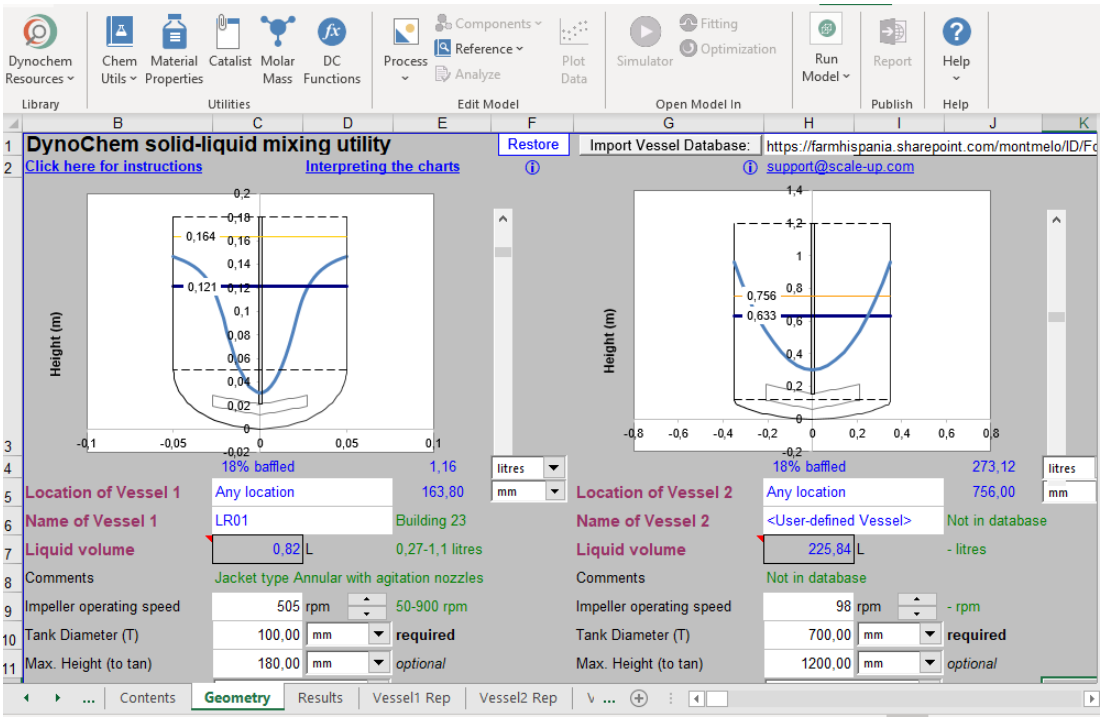


Figure 34. Example of the Dynochem software interface taken from its mixing utility.

The Dynochem software allows to obtain models capable to predict the behaviour of the studied system from the introduced experimental information. Based on the obtained models, the optimization and simulation of any rate-based unit operation can be performed. The use of the Dynochem software may help to study the reaction mechanisms and the work up/isolation operations. The performed simulations can be used to predict and to avoid formation of impurities, incompatibilities with the industrial equipment and yield loses during the process development and scale-up. This software is divided in different tools that have been created to study specific unit operations. See in Table 2 a summary of the most popular Dynochem tools.[35–37]

Tool name	Features	Main applications
Fit UA from solvent tests	Calculates heat transfer capacity of the studied reactors.	1. Predict cooling/heating times during scale-up. 2. Determine stability during the cooling/heating time required at plant 3. Safety studies.
Quick scale-up of dosing-controlled reactions without kinetics	Predicts temperature profile during the scale-up for exothermic/endothermic additions.	1. Estimate addition times/internal temperatures during the scale-up. 2. Determine stability during the addition time required at plant. 3. Safety studies.
Solid liquid mixing utility	Scales-up preserving mixing efficiency	Study of unit operations sensitive to mixing (crystallizations or extractions).
Liquid mixing times utility	Calculates meso, macro and micromixing times. Estimates feed pipe dimensions to avoid back mixing.	Study feed processes in solution (crystallizations or reaction quench).
Reaction kinetics model	Determines kinetics from experimental data.	1. Optimize reaction conditions 2. Study process response in front of variations in the process conditions. 3. Predict scale-up results based on the selected process parameters.
Filter cake formation model	Predicts filtration times for pressure driven filtrations (requires this type of set-up at lab scale).	1. Predict filtration time. (filter size *vs.* filtration time). 2. Characterize filter cake and filtration medium to determine the optimal filtration. technique; centrifuge or Nutsche filter.
Vapour-Liquid and Liquid-Liquid equilibrium prediction utility	Predicts phase composition and boiling points for binary/ternary solvent mixtures. Predicts phase compositions in series of partially miscible solvent extractions.	Solvent swaps: 1. Study of distillations (azeotropes and b.p). 2. Study solvent removal through extractions.
Solvent swap distillation model	Predicts duration/residual solvent content during solvent exchange operations.	1. Predict distillation time. 2. Optimize distillations. 3. Calculate solvent consumption.
Crystallization model	Help to design crystallizations based on antisolvent addition and cooling ramp with seeding (may include breakage, agglomeration and secondary nucleation).	Design crystallizations to obtain the desired yield and PSD (optimal seed PSD, cooling and addition rates).
Solubility	Predicts solubility curves at different temperatures and %wt of antisolvent.	1. Process development (crystallization, reactions and w.up). 2. Calculation of theoretical yield lost. 3. Development of cleaning methods.
Early phase solvent selection: solubility prediction utility	Predicts solubility in >160 solvents and mixtures of 2 – 3 solvents (not valid for ionic compounds).	Automatic screening of solvents for crystallization and cleaning.

Table 2. Summary of the most relevant Dynochem software tools and its potential applications.

1.3.2. The Chetah software

In general, during the initial phases of the development of a process the potential hazards associated to the chemicals involved on it are not known. In these situations, the experimental thermochemical data that should be considered for the process design and to predict the process hazardousness is often not be available. The Chetah software allows users to predict thermochemical properties for compounds and reactions easing the process design and the hazard evaluation.

The Chetah software from the American society for testing and materials (ASTM) is an *in-silico* tool created for predicting the thermochemical properties and the hazards associated with pure chemicals, mixtures of chemicals and chemical reactions. This software contains also extensive databases of thermodynamic properties such as heat capacities, entropies or formation enthalpies. The information provided by the Chetah software is useful for classifying materials according to their ability to decompose with violence, for estimating heats of reaction or combustion, for predicting lower flammable limits and, for the obtention of some other safety related parameters such as the amount of gas released in decompositions. During the development of this thesis the Chetah software was mainly used to obtain heat capacities and to estimate reaction enthalpies. The obtained results were used to complement the available experimental data during the process safety assessments performed and, during the *in-silico* studies carried out using the Dynochem software.[38]

1.4. Development of industrial chemical processes

Due to the characteristics of the industrial equipment and the large amounts of chemical substances manipulated some considerations have to be taken into account when a process is scaled-up. Some procedures commonly used in the laboratory such as distillation to dryness or heating the reaction mixture once all the reagents have been loaded in the reactor may result challenging to be carried out at industrial scale due to technical limitations and/or safety issues. Other common synthetic operations such as phase separations, filtrations, distillations and crystallizations must be thoroughly studied and optimized. A smooth interphase or a slightly slow filtration at laboratory scale may lead to an important problem or even to the batch failure at the manufacturing plant where, because of the scale increase the observed phenomena may be magnified.

During the industrialization of a process is common to have a more accurate inertization of the reactors, more effective rinsing of the solid cakes, lower mechanical loses and larger operation times. These variations may have a relevant impact in the behaviour of the process compared with the one observed during the laboratory tests. To keep into account the particularities of the industrial scale equipment/operation during the process development is important to avoid deviations from the expected results and, to reduce the risk of batch failure during the process scale-up. It may lead to the obtention of more robust, safe and effective industrial processes in shorter periods of time.

In the following sections the main drawbacks and advantages of the industrial scale operation and how to overcome or take profit of them during the development of an industrial chemical process are described.

1.4.1. General considerations

During the process development, the use of ice baths, preheated baths or quenching pouring the reaction crude in ice should be avoided because, due to the relatively small heat-transfer capacity of the industrial equipment, the maximum achievable heating/cooling rates are limited. In cases where fast cooling is required, the addition of pre-cooled solutions or the use of vacuum to promote solvent evaporation could be useful to accelerate the chilling process. In order to achieve higher heating rates, the addition of pre-heated solutions can be also considered.

The use of hot filtrations, hot washings, hot polish filtrations[2] and the manipulation of highly concentrated solutions out of the reaction vessel should be avoided. At industrial scale, during these operations there is a high risk of having an uncontrolled crystallization which may lead to the blockage of the used pipes or filters.

The visibility of the plant reactors interior is very limited since normally only the reactor manway can be used for this purpose (see Figure 35).

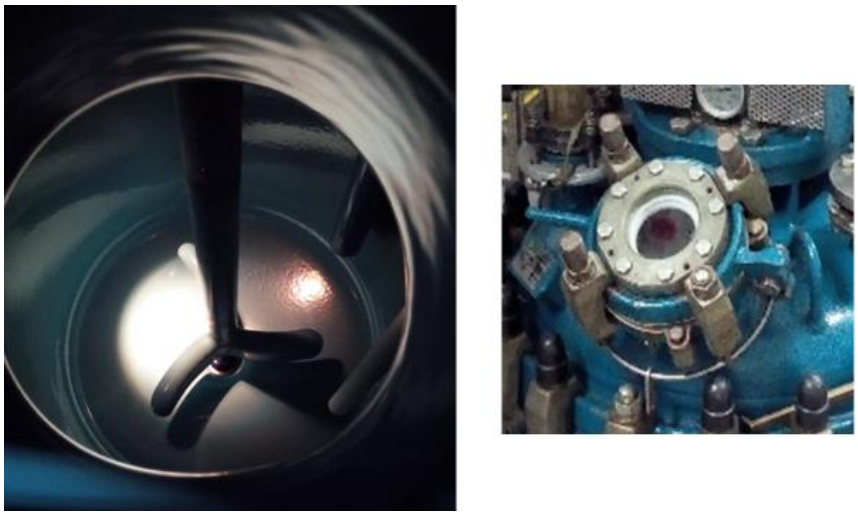


Figure 35. Manway of a reactor located in the Farmhispania S.A. plant (right) and view of a reactor inside from a manway (left).

If during the process a sticky solid, an agglomerate or an interphase is formed, the problem probably will not be detected by the operators leading to an important issue or even to the batch failure. In order to study these phenomena is convenient to perform tests at scale of several litres using transparent glass reactors with similar geometry, occupation and stirrer type that the ones that are going to be used at industrial scale (see Figure 36).

[2] Polish filtration: filtration of a solution carried out through a small porosity filter (typically 0.45 microns). It is performed to remove traces of solids that may interfere into the process causing foreign matter contaminations, acting as undesired nucleation centers or increasing the impurity content of the product.

Figure 36. 1 L glass reactor used at Farmhispania S.A. to study a phase separation.

In some occasions the solids formed during the process tend to aggregate around the pH probe, temperature sound or other devices used to control the process conditions applied. The fouling of the used sensors may conduct to misleading lectures and deviations from the optimal process conditions. In the case of agglomerate/sticky solid formation, special attention has to be paid to this phenomenon during the process development since probably it will be not detected at plant. If the blockage of the reactor sensors is detected alternatives such as the use of at-line discontinuous measurements should be evaluated. In the case of at-line methods, the used sensor can be rinsed between measurements avoiding the mentioned fouling problems.

The addition of solids into the reaction mixture is a usual operation at laboratory scale. However, as it is mentioned in section 1.2.1 at industrial scale this means the manipulation of several kilograms of solid and, may result one of the most challenging and dangerous process operations.

To avoid the direct addition of solids into the reaction vessel, the addition of the reagents in solution is recommended. In case that the addition of solids can not be avoided it should be carefully evaluated. Differences in the particle size distribution or the polymorphic form may induce changes in the dissolution rates, surface areas or other physical properties causing variations in the process behaviour.

The completion of a synthetic step at large scale may take several days or even weeks in some cases. Due to plant requirements (plant personnel schedule and equipment availability) it may happen that the process must be stopped for some hours or days at some point during the synthesis. For this reason, it is important to evaluate and stablish safe holding points where, the process can be stopped without causing an impact into the product quality or yield. It is also interesting to determine the stability of the isolated intermediates and the storage conditions required to preserve them while the synthesis cannot be continued.

The lack of robustness and process knowledge is another common source of failure and/or deviations during the scale-up. Normally, in the laboratory experiments the process parameters are maintained within narrow ranges around the target values. However, large fluctuations may be observed at plant due to the technical limitations of the used equipment and the variability

associated to the large scale operation. To evaluate process behaviour within the expected plant operation ranges is advisable to increase the rate of success during the process industrialization. These studies should serve to detect those parameters having a relevant impact in the quality or yield.[39] Based on the gained knowledge, control strategies may be implemented to reduce the variability associated to those parameters influencing the process behavior or, process modifications may be done to reduce the process sensitivity in front of variations.

1.4.2. Distillations

Distil to dryness is a technique commonly used at laboratory scale. However, it is rarely used in industry because to unload large amounts of a dry solid from the interior of an industrial reactor may become extremely challenging in terms of safety and operation. During the industrialization of a process, the distillation to dryness operations are commonly replaced by alternative techniques such as solvent swaps/solvent exchanges, anti-solvent crystallizations, crystallizations of solvates or salts of the product or the use of telescoped processes.[3]

Distillations that at laboratory take several minutes or few hours may require several hours or even days at industrial scale because they imply the removal of important volumes of solvent. It is important to study the product stability during the distillations and to perform laboratory tests trying to mimic the large scale distillation rates, stirring conditions and bath temperatures. Below are listed some considerations to be taken for the suitable development of industrial processes including distillations.

- Study the stability of the product during the distillations. As it is mentioned above distillations at industrial scale may require several hours or days, for this reason is convenient to evaluate the product stability during this operation. In some cases, degradation takes place only during the final part of the process when the mixture is highly concentrated. For this reason, it is common to apply higher bath temperatures at the beginning of the operation and to use softer conditions for the final part of the distillation. Another effective technique to solve this kind of problem is to perform continuous distillations. In this operation mode, the reactor volume is kept constant during all the process since fresh solvent is continuously added.
- Establish vacuum and bath temperature limits for the distillations. During the laboratory experiments is important to determine practical limits for bath temperature and vacuum levels. The distillation conditions must be adapted to the limitations of the industrial equipment and should allow as fast as possible distillation rates without causing product degradation. The use of a nomographs (see Figure 37) can be very useful to determine suitable vacuum and temperature operating conditions.

[3] In telescoped processes some of the intermediates obtained are not isolated as a solid. In this type of processes, solutions containing the intermediate dissolved are obtained at the end of each step. These solutions are used as starting material at the following synthetic step.

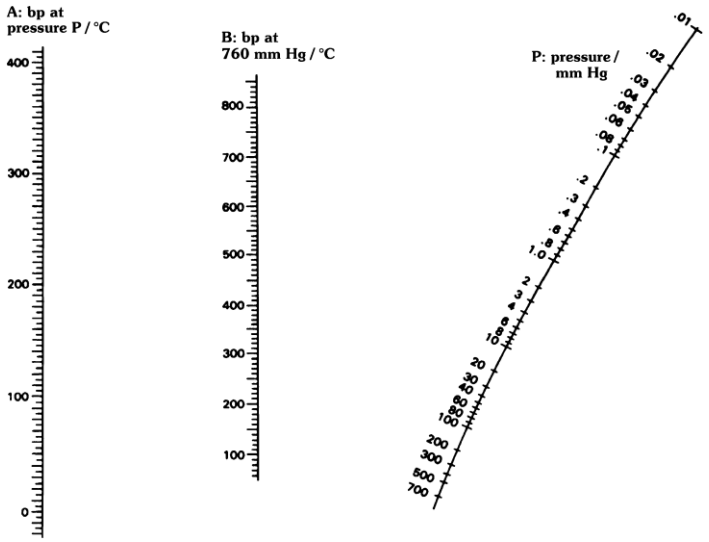


Figure 37. Standard nomograph used to calculate boiling point of a solvent (A) under an specific pressure (P) from its boiling point at atmospheric pressure (B).[40]

- Monitor internal temperature and set an internal temperature limit. To specify internal temperature limits could help to avoid product degradation or changes in process behaviour during evaporative crystallizations among others.
- For evaporative crystallizations, it is useful to determine if the solid formed gets stuck in the reactor walls during the solvent stripping and, to quantify the mechanical loses caused by this phenomenon also known as crusting (see Figure 38).

Figure 38. Crusting observed in a 2 L reactor during an evaporative crystallization.

In some cases, the solids stuck in the vessel walls may constitute the main source of product loss during the industrial batches. In order to determine if at industrial scale the stuck solid could be recovered from the reactor walls it may be useful to test at small scale some solutions typically used at plant. Increase the stirring speed punctually during the distillation to splash the reactor walls with the slurry, load the solid cake rinsings

into the reactor to unload the remaining product or induce controlled foaming at some points of the distillation are examples of this type of techniques.

- Determine the stability and quality of the solids stuck in the vessel walls. The solids adhered to the reactor walls during the distillations are directly heated to jacket temperature while the suspended solids remain at the boiling point of the mixture, which may be several degrees lower.

  Due to the differences in its crystallization conditions, the solid recovered from the reactor walls may present a completely different quality compared to the one observed in the product isolated initially from the slurry. The recovered solid may be enriched in some impurities, present a different polymorphism or a different residual solvent content. In these occasions, to recover the product from the vessel walls can bring the entire batch out of specifications.

  The observed quality differences between the stuck and the suspended solids may be caused by many different reasons such as the degradation of the product due to excessive heating or the accumulation in the agglomerated solid of some impurities or immiscible solvents that tend to oil out of the slurry during the distillation.
- Fix end points for the performed distillations. In some cases, the end point can be settled as a certain amount of solvent that has to be removed from the reactor. When this approach is used, measure the residual volume inside of the reactor using internal graduation or radar measurements (Figure 39) is considered more precise than the monitoring of the volume of collected distillate. The measurement of the collected distillate volume leads to more inaccurate results because not all the solvent removed from the reactor is condensed during the distillation.

Figure 39. Radar based on radiofrequency reflection used for the measurement of the liquid level in industrial reactors.[41]

Another approach used in order to set up the distillation end points is the measurement of the amount of undesired residual solvent present in the reactor mixture or in the collected distillate.

Normally Gas Chromatography (GC) is used because it allows to avoid matrix interferences caused by the product of the synthesis or other substances present in the studied samples. However, in many cases the same information can be obtained from refractive index measurements. Refractometry is a simple, fast and sensitive technique that can avoid the development of new chromatographic methods. In Figure 40 is presented a refractometer apparatus. Due its low size it allows fast at line measurements leading to a more dynamic process operation.

Figure 40. Refractometer from Atago used in Farmhispania S.A.

1.4.3. Filtrations/Crystallizations

Filtrations and crystallizations are considered one of the most critical operations at industrial scale since they may have an important impact in the residual solvent content, the yield, the required drying times, residue on ignition (ROI) contents or purity. Due to the differences between the industrial and the laboratory equipment these operations are complex to be studied and optimized at small scale. For example, a filtration that at laboratory requires 15 min. can take several hours or even days at plant.

The use of process conditions (stirring, temperature or pH among others) that guarantee the stability of the slurry obtained is recommended in order to prevent possible problems derived from slow filtrations. During these studies is convenient to evaluate the particle size distribution (PSD), polymorph, residual solvent content, impurity profile and yield obtained after several hours of slurry aging. Another factor to consider is the induction time required by the studied product to fully crystallize. This information will help to avoid loss of yield due to premature filtration. It is also important to consider that, the product crystallization may take longer at large scale than at the laboratory. To prevent low unexpected yields during the industrial batches it is recommended to take samples from the obtained slurry and to determine product loss in mother liquors before to start the whole batch filtration.

In order to determine the capacity of the filter that will be required for the industrial filtration it may be useful to measure the volume of the wet solid cake obtained in the filtrations performed during the laboratory tests. From the obtained value, the volume of the industrial wet solid cake can be easily estimated applying a simple calculation (see Figure 41).

$$Industrial\ U.Op\ (Kg) \times \frac{Volume\ of\ wet\ cake\ (dm^3)}{U.Op\ at\ lab.test\ (Kg)} = Volume\ of\ wet\ industrial\ cake\ (dm^3)$$

$$U.Op = Operational\ unit\ (Amount\ of\ limiting\ reagent)$$

Figure 41. Formula to estimate the volume of wet solid cake that will be obtained in an industrial filtration. Any units can be used if they are used consistently.

The behaviour of an industrial filtration will depend mainly of the selected filter type, the particle size distribution of the isolated product, the solid load of the filtered slurry, the filter cloth, the

filter area/slurry volume ratio and the applied filtration conditions (pressure level applied or rpm selected in case of use a centrifuge).

The type of filter cloth used may have a relevant impact in the filtration behaviour. To select the most suitable filtration media, filtration tests using filters (sintered glass or paper filter) with different porosities can be carried out at laboratory scale. In Table 3 are reported the pore sizes of the more typically used sintered glass Buchner funnels. Once the most suitable porosity has been determined at laboratory scale, the selection of the industrial scale filtration media can be carried out based on the advice provided by the industrial filter cloth suppliers.
According to the Farmhispania S.A. criterion, which has been stablished based on the accumulated experience, a sintered glass Buchner funnel grade n°3 behaves normally as an industrial filter cloth with 12 L air/min*dm^2 permeability, 510 g/m^2 weight, 0.8 mm thickness, warp of 25 threads and a weave of 10 threads (filter cloth typically used in the filtrations performed at the Farmhispania S.A. plant).

Sintered glass Buchner funnel grade n°	Pore size
0	160-250 µm
1	100-160 µm
2	40-100 µm
3	16-40 µm
4	10-16 µm
5	4-10 µm

Table 3. Equivalences between the porosity of the laboratory sintered glass Buchner funnels and its grade number.[42]

To have an accurate prediction of the filtration behaviour during the scale-up, the filter used must be carefully selected to mimic the industrial filtration conditions. It is important to keep constant the filter area/slurry volume ratio, the filter diameter/filter height ratio, the filter type and the filter loading/solid cake rinsing times. To perform a filtration test using a piece of the filter cloth that is going to be used at plant could give very useful information. In cases where the selection of the filter cloth remains unclear, to know more about the product particle size distribution and crystal shape can ease the selection. The determination of some parameters described in the following paragraphs such as the slurry settling time and the solid cake compressibility can help also to take the decision and to stablish the ideal filter type to be used.

Anticipate the behaviour of a filtration at industrial scale is feasible through some specific laboratory tests and the support of *in-silico* tools. These tests can give an idea of the characteristics of the studied filtration and about which corrective actions can be implemented in order to improve the process performance.

- Settling tests: they are based on the determination of the settling time required to have a clear solution with the entire solid at the reactor bottom. This test must be carried out with around one litre of slurry. If after half an hour the solid has not completely settled, the filtration will be probably difficult at industrial scale. Long settling times are normally

related with the presence of small particles or fines[4]. The small sized particles contribute to the blockage of the filter cloth and of the solid cake pores.

In case that the studied product presents a certain thermal stability, an annealing step can be introduced into the process in order to reduce fines and increase particle size. The annealing technique consists in heating the obtained slurry in order to favour the dissolution of the fine particles of solid. Once the small particles have been dissolved, a cooling ramp is applied. During the cooling ramp the remaining undissolved solid crystals act as a nucleation center inducing the formation of larger particles.

- Cake permeability tests: these studies are carried out using a product cake of around 5.08 cm (2") of thickness and measuring the rate at which the already filtered mother liquors pass through it. In cake permeability tests vacuum must be used as a driving force of the filtration and the formation of cracks in the solid cake has to be avoided. The results obtained from the experiment must be expressed in litres of mother liquor collected per minute and surface square meter of filter (L/min.*m^2). If values greater than 40 are obtained the filtration probably will perform well at large scale. However, results below 20 may indicate that the filtration will not perform properly at large scale. Low values are normally correlated with the presence of important amounts of fines or with highly compressible solid cakes[5].

  In case of poor results, the same approaches described for settling tests can be used to enhance the filtration performance and successfully scale-up the process.
- Pressure-filter tests: these experiments are based on carry out the filtration applying a constant pressure or vacuum during all the process. It is recommended to use similar pressure or vacuum levels to the ones that are going to be used at plant. From pressure-filter tests, useful information can be obtained if the plot derived from Figure 42 (Strauss equation) is represented.

$$\frac{P \times t}{V/A} = m\frac{V}{A} + b$$

Figure 42. Strauss equation used to evaluate filtration performance. Being: P=pressure (psi); A=area of the filter (in^2); V=weight of filtrate (lbs); t=filtration time (min.)[43]

The straight line obtained by regression of the collected data will allow to calculate the value of *m*, which corresponds to the slope of the obtained regression. *m* values of around one thousand will indicate fast filtrations while, poor filtrations are expected for slopes near four hundred thousand. In case that the obtained test result indicates that the filtration will not be fast the same corrective actions indicated in cake permeability tests and settling tests can be applied.

[4] The term "fines" is commonly used when a fraction of the product particles has a smaller size than the rest.

[5] Compressible solid cakes reduce its volume closing the gaps between the solid particles and reducing its permeability during the filtrations. This kind of solid cakes can lead to extremely slow filtration rates since, to more pressure or vacuum is applied more compression/solid cake blockage is promoted.[198]

If the pressure-filter tests are performed using pressure/vacuum levels like the ones that will be applied at plant, predictions about the time that will be required to carry out the large scale filtration can be performed introducing in the obtained equation the industrial values of A and V (see Figure 42). This type of predictions may be also performed using software tools such as the Dynochem software (see section 1.3.1).[43]

In order to improve the filtration performance, the use of filter aids can be also considered. Filter aids are inorganic solids (commonly celite or silica) that can modify the properties of the filtration. Filter aids can act as crystallization nucleus if they are added just before the solid precipitation, can help to modify the properties of the obtained solid cake if they are mixed with the slurry once crystallization has been completed and, can help to avoid the loss of fines if are used to pre-coat the filter cloth. In some cases, the materials used as filter aid can present affinity towards some compounds present in the treated mixture. This phenomenon must be studied in each case since it may represent an advantage if the trapped substance is an impurity or a drawback if the substance being captured is the target product.

CHAPTER 4

DH0517 PROJECT

INDUSTRIAL PhD THESIS

PROCESS DEVELOPMENT FOR THE SYNTHESIS AT INDUSTRIAL SCALE OF ACTIVE PHARMACEUTICAL INGREDIENTS

Chapter 4

1. DH0517 project

Disclaimer: the information generated during the **DH0517** project is property of Farmhispania S.A. In order to protect the trade secret and the interests of the company the different figures, schemes, procedures and results presented in this chapter have been censored.

1.1. Introduction

DH0517 is a highly potent active pharmaceutical ingredient already approved by the FDA for the treatment of acute myeloid leukaemia (AML) and myelodysplastic syndromes (MDS). It is a parenteral drug nowadays commercially available as a generic drug. This substance is a monosaccharide derivative in its β-furanose form (see Figure 43). It belongs to a family of nucleoside analogues used as anticancer drugs that contain a cytosine or a chemically equivalent moiety. Apart from **DH0517** other members of this family of compounds are described in Figure 43.

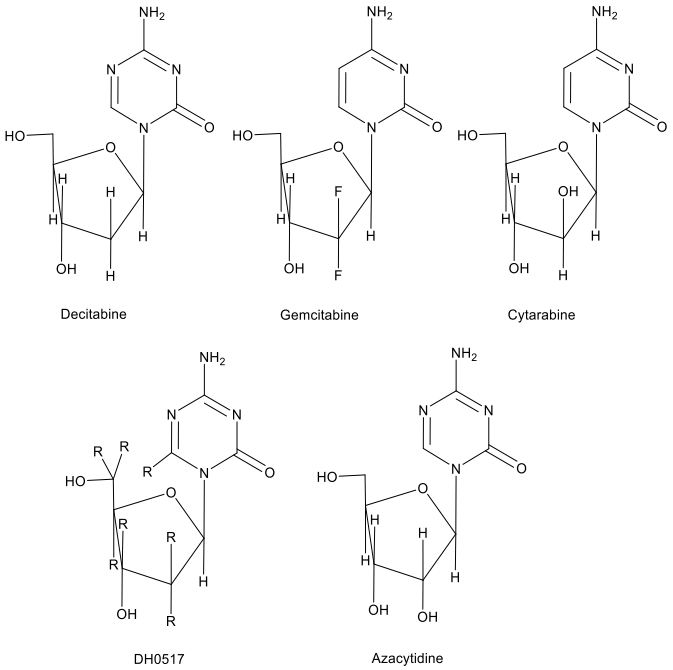


Figure 43. Chemical structure of the different nucleoside analogues belonging to the DH0517 family.

The nucleosides are preeminent in the normal cell functioning. They are combined with one or more phosphate groups in order to form nucleotides.[44] The nucleotides are the molecular building blocks of the nucleic acids (DNA and RNA). They are involved in some enzymatic processes where they play an important role acting as co-enzymes and they participate in the cell metabolism (e.g. cytidine triphosphate (CTP) similar to adenosine triphosphate (ATP) may carry out energy storage and supply functions in the cell).[45]

MDS are a type of cancers caused by alterations in the maturation process of the blood cells that takes place in the bone marrow. As a consequence of these alterations abnormal immature

blood cells are produced. These cells do not become functional heathy blood cells; they accumulate and die in the bone marrow or they go into the bloodstream instead. In patients with MDS the production of healthy blood cells is decreased, as a consequence there is a high risk of developing infections, anaemia or suffer easy bleeding. In cases where important amounts of immature blood cells accumulate in the bone marrow there is also a high risk of developing acute myeloid leukaemia.[46]

AML is an aggressive (fast-evolving) type of cancer that starts in the bone marrow. It is caused by a fast and uncontrolled grown of abnormal immature blood cells. The immature cells affected by AML do not become mature healthy blood cells, in most cases they quickly move into the blood and sometimes they spread to other parts of the body. The symptoms of AML are very similar to the ones described for MDS, they are also in this case related with the decrease of the production of healthy blood cells.[47]

MDS and AML are uncommon in people younger than 50, in the majority of the cases the patients are around 70 or 80. The treatment for MDS and AML normally includes supportive care that is used to relieve the symptoms caused by the disease and chemotherapy/radiation therapy that are used to slow the progression of the illness and to delay the development of AML. In certain cases, the patient may be cured through the use of aggressive chemotherapy/radiation therapy combined with a steam cell transplant using steam cells from a donor.[46,47]

It is known that AML and MDS are related with the silencing of multiple genes induced by its hypermetilation. Gene methylation increases the likelihood of suffering cancer since it may induce the mutation or deletion of genes related with tumour suppression or with other critical cell protection mechanisms. The aberrant methylation of genes may be the cause of the disease but also may take place during the disease progression leading to resistance to therapy and tumour progression.[48]

DH0517 is a hypomethylating agent whose mechanism of action is based on the inhibition of the DNA methylation process. Once in the cells, it is phosphorylated and incorporated into the DNA where it is able to covalently bind the enzyme responsible of the DNA methylation (DNA methyltransferase). It has a dual dose dependant mechanism of action; at high doses it has a cytotoxic activity caused by its capability to suppress the DNA synthesis while administrated at lover doses, **DH0517** acts inhibiting DNA hypermethylation inducing the reactivation of tumour suppressor genes and reducing tumour growth. The optimal **DH0517** dosage (number, duration, periodicity and size of the doses) is still being evaluated since due to its dual concentration dependant activity it remains unclear how to extract the maximum potential from the drug.[48]

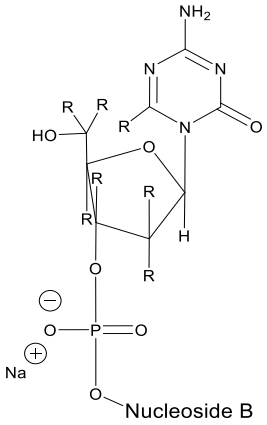
DH0517 degrades in aqueous media, it is supplied in form of a dry powder that is mixed with water and other typical additives for injectable drugs such as sodium chloride and dextrose solutions at the moment of its administration.[49–53]

The **DH0517** DNA incorporation pathway is more direct compared to other cytidine analogues such as azacytidine which makes it a more potent drug. For **DH0517**, significant activity towards MDS has been reported in low dosage treatments. This was considered an interesting feature since, MDS and AML are mainly suffered by patients of advanced age which cannot stand the

current treatments based on high intensity chemotherapy combined with stem cells transplant.[48]

Although the current **DH0517** worldwide consumption is low (approximately 14 kg per year) the high price of each **DH0517** dose (around 1700 Eur) makes it commercially attractive. Additionally, in the next years an increase of the demand is expected since there are two new drugs based on **DH0517** at advanced stages of the clinical trials. The two potential new applications of **DH0517** include:

- A new combo drug which is in phase 3 of the clinical trials. The mentioned combo would allow developing an oral and more effective antineoplastic treatment.
- Its use as an intermediate in the synthesis of the dinucleotide called **PDH0517** (see Figure 44). **PDH0517** is not degraded by cytidine deaminase; it releases **DH0517** gradually in the cell through the breakage of its phosphodiester bond carried out by phosphodiesterase enzyme. This optimized delivery pathway allows to increase the exposure time to **DH0517** and should allow to increase treatment efficiency. **PDH0517** is nowadays at the third phase of clinical studies for treatment of MDS and AML and at phase 2 for the treatment of solid tumours.



PDH0517

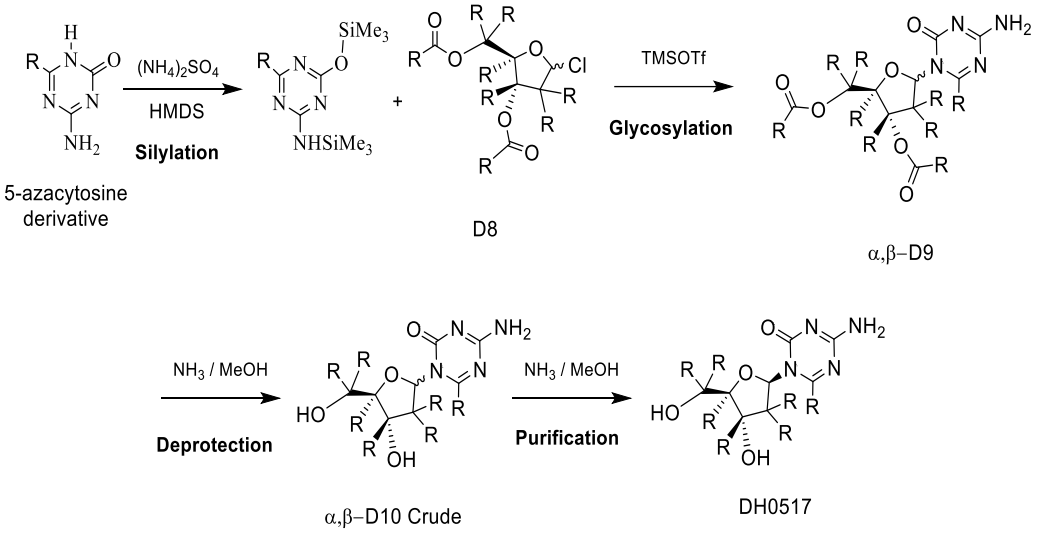
Figure 44. PDH0517 chemical structure.

If eventually these two new drugs receive the approval of the regulatory authorities, an increase of the **DH0517** demand to around 200 kg per year is expected. These potential further applications make **DH0517** even a more commercially attractive product.

1.2. Project features

The **DH0517** project was a contract manufacturing project that was initiated in Farmhispania S.A. under the request of an external client. Farmhispania S.A. was selected in front of other competitors not only by its GMP/HPAPI capabilities and its expertise in process development but also because it already had in its portfolio other nucleoside analogues with similar structure such as Gemcitabine (see Figure 43).

The customer initially requested the development of an industrial process for the manufacturing of **DH0517** using the synthetic pathway described in Scheme 5. In this case, the customer did not provide the details of the process. The suitable synthetic conditions and w.up procedures must be determined by Farmhispania S.A. through the corresponding experimental studies.



Scheme 5. Synthetic route selected for the obtention of DH0517 using as starting material D8.

The process developed by Farmhispania S.A. must allow to obtain 5 kg of **DH0517** with API quality[6] per batch. In this case, **D8** which was commercially available was used as starting material. The selected synthetic route started with the silylation of the commercially available 5-azacytosine derivative. The obtained silylated intermediate was not isolated, it was directly coupled to **D8** through a Vorbrüggen glycosylation reaction to afford the intermediate **D9**, which was isolated as a mixture of anomers enriched in the β form (Figure 45). Using ammonia in MeOH, the **D9** intermediate was deprotected to afford **D10 crude**, which was also isolated as a mixture of anomers enriched in the β form. Finally, a purification step was carried out to eliminate the undesired anomer and to deliver pure **DH0517**.

[6] The quality requirements for **DH0517** API were: purity≥98.0%, unknown impurities ≤ 0.10 % (each), known impurities (including **α-DH0517**, 5-azacytosine derivative and **monodeprotected DH0517**) ≤ 0.15 %, total impurity content ≤ 2.0 %, residue on ignition (ROI) ≤ 0.1 %wt, particle size distribution (PSD) [d(0.9)=13 µm/d(0.5)=5 µm].

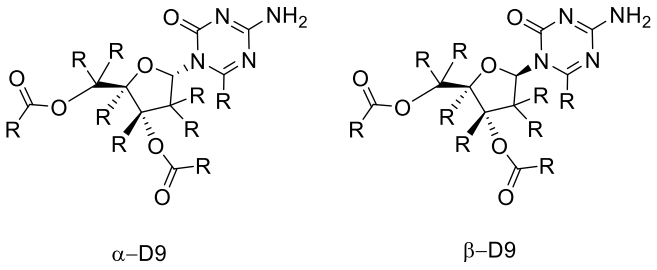


Figure 45. Chemical structure of the two D9 anomers differing in the configuration of the glycosidic bond. α-D9 (left) and β-D9 (right).

Initially, the synthetic conditions already applied in Farmhispania S.A. for the preparation of similar nucleoside analogues were considered. This approach was abandoned because of the differences observed in terms of temperature, solvent, stoichiometry and catalyst compared to the ones described in the literature for the **DH0517** synthesis. The synthetic conditions to be used for the **DH0517** industrial process were eventually stablished according to the expertise accumulated by the R&D Farmhispania S.A. team, the experimental studies performed and, the information extracted from the literature.

This chapter of the thesis is focused on the industrialization of the synthetic step involving the silylation and the Vorbrüggen glycosylation reactions. Thus, in this study the main goal was the development of an industrial process to obtain at commercial scale the intermediate **D9** using as starting material **D8** (see Scheme 5).

The obtention of the intermediate named as **D9** was considered the key step of the **DH0517** synthesis. It was the most challenging step of the synthesis because:

- It has to be directed towards the formation of the **β-D9** anomer since only the β form is suitable for the obtention of **DH0517** (Scheme 5).
- The **D9** intermediate presented low solubility in many of the solvents typically used at industrial scale (acetonitrile, toluene or EtOAc)
- The **D9** intermediate was prone to crystallize as a sticky solid complex to manipulate.

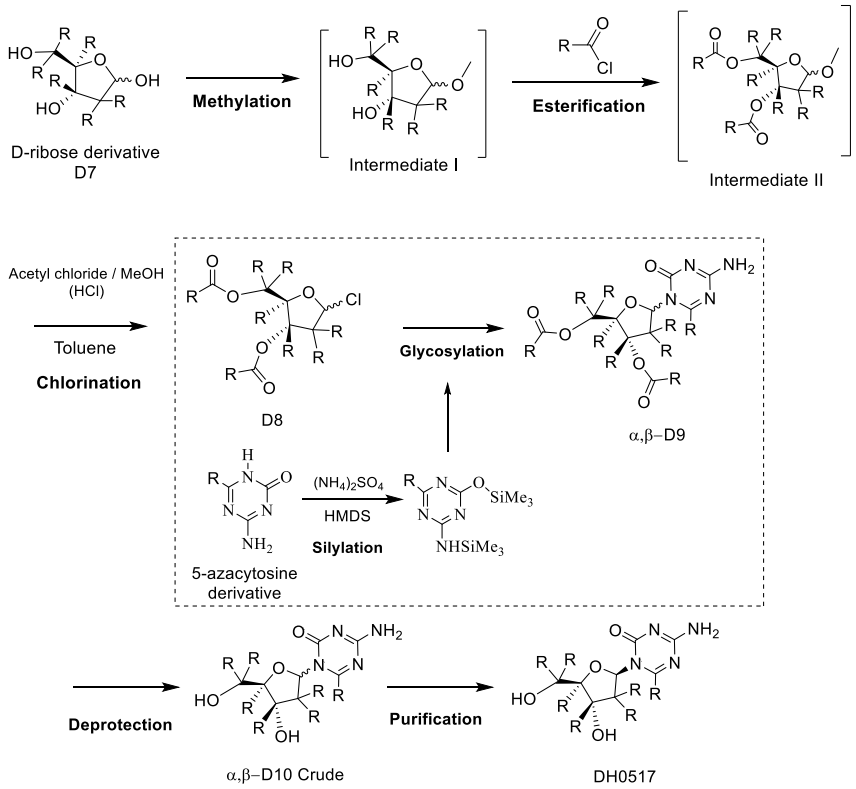
In the last two steps of the synthesis (deprotection and purification), the **α-D9** anomer and its derivatives are purged and consequently removed (see Scheme 5). Favouring the formation of **β-D9** would allow to increase the overall yield of the synthesis and the manufacturing capacity while reducing both, the manufacturing cost and the content of impurities associated to the α-anomer forms in **DH0517**.

The work up (w.up) procedures described in the literature for the obtention of **DH0517**, were not suitable for the large scale manufacturing. They were performed at highly diluted conditions, using distillation to dryness or flash chromatography.[54,55,64,65,56–63] The development of a procedure that allowed the isolation of pure **DH0517** working at high concentration conditions and using the reactors available at the company was also considered essential for the project.

Although in the initial stages of the project the objective was to develop a **DH0517** manufacturing process that started from the intermediate **D8** (Scheme 5), a more extended synthetic route based on the use of a D-ribose derivative as raw material was adopted

afterwards (Scheme 6). The number of steps of the synthetic route was increased for many reasons:

- The price of the D-ribose derivative was considerably lower than the **D8** price. Consequently, the use of this starting material allowed to reduce the manufacturing costs.
- The extended process was more solid under a regulatory and industrial perspective. The number of FDA accepted industrial suppliers for the D-ribose derivative was much higher than for **D8**. Therefore, the use of the D-ribose derivative allowed to reduce the dependence from specific external suppliers.
- The route presented in Scheme 6 included also more isolation steps between the starting material and the final product, this represents an advantage from a regulatory point of view because, it allows through the analysis of the different process intermediates a higher control of the quality and leads to the obtention of a more robust process.



Scheme 6. Synthetic route developed at Farmhispania S.A. for DH0517 preparation.

Additionally, during the course of the project the customer requested to increase the manufacturing scale. As a consequence, an industrial process capable to yield around 17 kg of **DH0517** per batch starting from 70 kg of D-ribose derivative was developed.

The use of the D-ribose derivative as starting material increased the complexity of the project since, it is known that it may exist in four different forms which are: the five-membered ring (furanose); the six-membered ring (pyranose), the open chain and the open chain hydrated form (see Figure 46). In solution, all the mentioned forms are in equilibrium (open chain hydrated form only occurs in aqueous medium). Therefore, the absence of these pyranose and open chain

derivatives in the different intermediates and, in the final product obtained must be guaranteed through the corresponding studies.

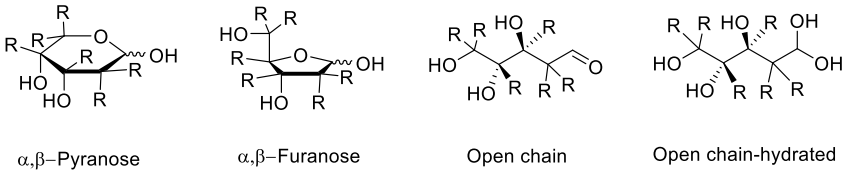
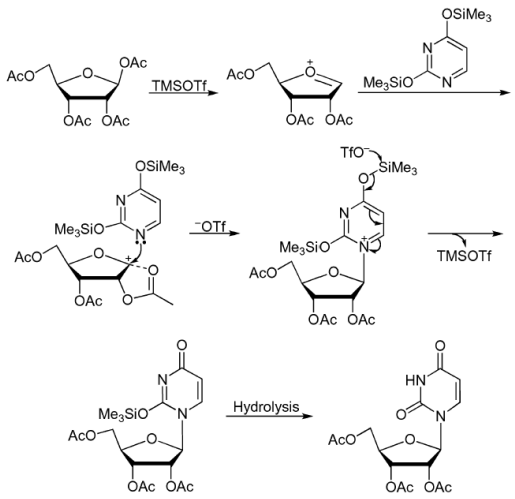


Figure 46. Four different forms of the D-ribose derivative.

1.3. Precedents

The reaction performed to obtain the **D9** intermediate from **D8** (Scheme 6) is based on the Vorbrüggen glycosylation which is a modification of the Hilbert-Johnson reaction. Both methods allow to prepare *N*-glycosides from the reaction of carbohydrate derivatives and heterocyclic bases. The first example of the use of the Vorbrüggen glycosylation was published by Niedballa and Vorbrüggen and dates from 1970 while in the case of the Hilbert-Johnson reaction, it was reported by first time by Hilbert and Johnson in 1929.[66,67]

The Hilbert-Johnson reaction is used to obtain pyrimidine or purine nucleosides as well as other nucleoside analogues from the reaction of the corresponding protected monosaccharide halide and a pyrimidine or a purine derivative.[68–71] The Vorbrüggen glycosylation allows the preparation of nucleosides and nucleoside analogues from peracetylated or perbenzoylated monosaccharides and silylated heterocyclic bases. In Scheme 7 is presented the proposed mechanism for the Vorbrüggen coupling.



Scheme 7. Proposed Vorbrüggen glycosylation mechanism. Reproduced from Ref [72] with permission of the copyright holder.

The Vorbrüggen glycosylations are typically performed using dichloromethane (DCM) or acetonitrile (ACN) as solvent and in presence of a Lewis acid, which normally is $SnCl_4$ or trimethylsilyl trifluoromethanesulfonate (TMSOTf). It has been found that peracetylated sugars react better than the corresponding perbenzoylated analogues. The anomeric configuration of the products obtained from this reaction is strongly dependent of the substituents situated at the C2 group of the reacting sugar. While in certain cases a (1:1) anomer mixture is obtained, in

others almost, pure β or α-anomers are formed. According to the literature, the use of temperatures above 25 °C is generally beneficial for the reaction and leads to higher yields. The regioselectivity of this synthetic method is remarkably affected by the nature of the silylated heterocycle used; for pyrimidine derivatives it often leads to N1 couplings, nevertheless, mixtures of regioisomers are commonly obtained when purines are used.[73–78]

This reaction has been broadly applied in the preparation of nucleosides and nucleotides analogues such as Gemcitabine or Cytarabine (Figure 43).

1.3.1. State of the art

Before to start the development of the **DH0517** process, a bibliographic search was carried out to stablish preliminary reaction conditions and w.up procedures for the glycosylation and the silylation reactions (Scheme 5). Since the objective was to industrialize the **DH0517** synthesis, the bibliographic search was focused on patents describing manufacturing methods for the obtention of the **D9** intermediate. Although some articles and enforced patents were consulted, special attention was paid on expired and abandoned patents which would allow the free use of the procedures described on it. [54,55,64,65,78,56–63]

In Table 4 are summarized the most relevant results obtained from the bibliographic review performed. The review was focused on the Vorbrüggen glycosylation because, the procedures and results reported for the silylation reaction were acceptable and similar in all the examined publications.

Entry	Patent code	Yield	(α:β) Anomer ratio	Yield considering β-anomer	Expiry date
1	EP2048151	71 %	1:2.2	49 %	Abandoned
2	EP2201020B1	99 %	1:1.7	62 %	10/10/2028
3	CN101311184B	51 %	1:1	26 %	25/05/2027
4	EP2341772B1 (Example 1)	46 %	1:2.4	33 %	02/10/2029
5	EP2341772B1 (Example 5)	78 %	1:1.3	44 %	02/10/2029
6	US2010249394 (Example 4)	88 %	1:1.45	52 %	Abandoned
7	US2010249394 (Example 6)	83 %	1:2.5	59 %	Abandoned
8	CN103232512B	44 %	1:11	40 %	13/04/2033
9	CN101497639B	27 %	Pure β-anomer	27 %	13/03/2029
10	CN101307084A	28 %	Pure β-anomer	28 %	Abandoned

Table 4. Summary of the results reported in the literature for the glycosylation reaction performed to obtain D9 intermediate. [54,55,64,65,56–63]

The initial conditions from which the development was started are described in section 1.5.1. These conditions were selected based on the raw materials cost, toxicity of the reagents used, maximum volume of synthesis, anomer ratio obtained, yield or operational costs. The main goal was to initiate the development from a process with a high potential to be scaled-up successfully to commercial scale (safe, profitable and robust).

1.4. Objectives

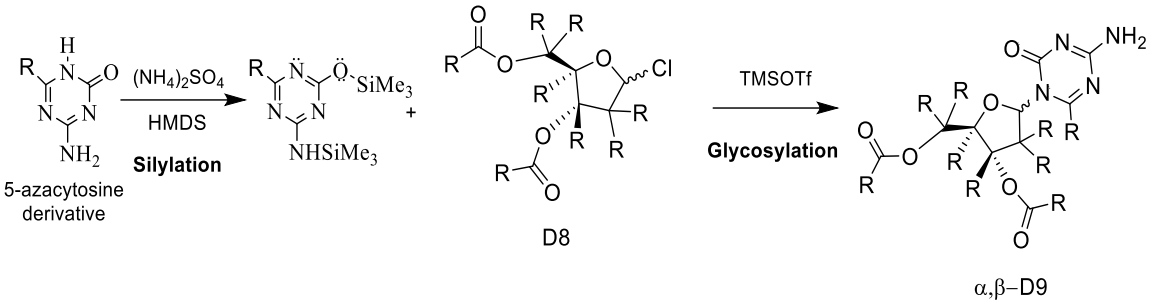
The main goals of the **DH0517** project were:

- The optimization of the Vorbrüggen glycosylation reaction conditions in order to favour the formation of the **D9** β-furanose form which was the anomer required for the obtention of **DH0517** (Scheme 6).
- The optimization of the reaction parameters in order to achieve a high conversion of the used raw material and to reduce the formation of impurities. The performed optimizations should allow to reach an acceptable quality and yield.
- The development of a w.up based on procedures suitable for the large scale operation. The w.up should also allow to operate at the high concentration conditions required for the manufacturing at large scale of the mentioned intermediate.
- The optimization of the w.up to achieve the obtention of **D9** with the highest possible purity and yield.
- The development of a robust, reproducible, safe and cost-effective process that could be executed and validated using the industrial equipment available at Farmhispania S.A.
- The characterization of the different intermediates and impurities involved in the **DH0517** synthesis.
- Scale-up the developed processes within the timelines agreed with the customer. In the case of the batch starting from **D8** a 30-week timeline from the beginning of the project was set. For the scale-up of the first batch using as starting material the D-ribose derivative a development period of 32 weeks was defined.
- Support the scale-up of the process. Perform investigations to determine the root causes and find solutions for all the problems arising during the pilot and industrial batches.
- The investigation of the formation of pyranose impurities during the whole **DH0517** synthesis. It must be guaranteed the absence of these pyranose derivatives in the different intermediates and in the final product obtained.

In the next sections are described the different studies carried out during the development of this thesis in order to reach the above indicated objectives.

1.5. Process development

As it has been mentioned in section 1.2, the most challenging step of the **DH0517** synthesis was the obtention of **D9** from **D8** (Scheme 8). This step was considered the key step of the process since the process yield and the quality of the obtained **DH0517** were highly dependent on its performance and reproducibility.



Scheme 8. Synthetic route developed for the obtention of D9 using as starting material D8.

In the following sections are collected the different studies performed in order to optimize this synthetic step in terms of performance, robustness and safety. The activities derived from the process scale-up are also included in this part of the thesis.

1.5.1. Initial screening

The development of the process was started using as a reference the procedures described in a paper from Vujjini SK focused in the synthesis of 5-azacytidine and, in the examples 4 and 6 of the US20100249394 patent (Table 4).[54,61] In order to evaluate the feasibility of the reference synthetic methods, no modifications were applied over the reaction conditions and w.up procedures described. Since the objective at this stage was to perform a fast-preliminary screening, a set of small-scale experiments (≈3 g **D8**) was performed using round bottom flasks and commercially available reagents/solvents.

The silylation reaction was successfully performed using commercially available raw materials and according to the literature conditions. The 5-azacytosine derivative was heated up to 90-130 °C using hexamethyldisilazane (HMDS) as solvent in presence of catalytic amount of ammonium sulphate. Once the reaction was completed, the HMDS excess was distilled and the obtained residue was used for the glycosylation step. The silylation reaction progress was controlled visually since it was known from other Farmhispania Group projects that the reaction is complete once total dissolution of the initially loaded 5-azacytosine derivative is observed.

The performed Vorbrüggen glycosylation tests were monitored using HPLC. In these first trials, anhydrous solvents (ACN, EtOAc and chloroform) were used to eliminate possible interferences caused by water. The reactions were carried out using TMSOTf (catalytic) or triflic acid (1.2 eq.) and temperatures between 25 °C and 45 °C.

Anomer ratios of **D9** close to 1:1, were observed in the reaction crudes. For the w.up of these tests, the reaction mixtures were diluted with more reaction solvent and were treated with a sodium bicarbonate solution ($NaHCO_{3\,(aq)}$) and brine. After the aqueous rinsings, the organic phases were dried with magnesium sulphate and **D9** was crystallized in a MeOH-hexane mixture or in toluene. During the crystallization a sticky solid appeared in all the cases leading to important mechanical loses and to extremely slow filtrations.

The w.up was carried out at highly diluted conditions. The use of 40 to 80 vol of organic solvent was required and, even at these conditions, interphase formation problems were observed. In the performed experiments, yields around 45 % were obtained. The low yields observed were

attributed to the above-mentioned interphase formation problems and to the mechanical loses promoted by the formation of sticky solids during the final crystallization. Table 5 summarizes the results achieved in the initial experimental screening.

	Patent example 4	Patent example 6		Vujjini SK paper[61]
Glycosylation solvent	ACN	Chloroform		EtOAc
Glycosylation reagent	TMSOTf	TMSOTf		Triflic acid
Glycosylation temperature	25 °C	25 °C		45 °C
Anomer ratio	Almost no **D9** formation observed	≈1:1		No **D9** formation observed
Crystallization solvent	Not evaluated	Option 1: MeOH:hexane	Option 2: toluene	Not evaluated
Yield	Not evaluated	≈45 %		Not evaluated
Comments	Route abandoned	Product loses observed in the w.up		Route abandoned

Table 5. Summary of the experimental results achieved during the first glycosylation trials. The patent examples were taken from the US20100249394 patent.[54]

From the results obtained in the first round of experiments it was decided to focus the research in the optimization of the procedure described in the example 6 of the patent US20100249394.[54] The mentioned procedure presented some limitations that made it non-suitable for its scale-up to industrial scale. Below are listed the main drawbacks observed for the mentioned process:

- It included a distillation to dryness operation after the silylation reaction. As it is mentioned in previous chapters this type of operations is normally avoided at industrial scale since they may originate a series of safety and operation problems.
- Chloroform was used as solvent. The use of this substance is not recommended due to its toxicity.[79]
- The amount of **β-D9** anomer (target product) formed in the reaction was low. Because of the low selectivity of the reaction, about half of the used starting material (**D8**) was lost in form of undesired **D9** α-anomer.
- During the w.up, interphase formation, slow filtration and crystallization problems were observed. The mentioned issues made the process unsuitable for its application at large scale and caused relevant product loses.
- A drying operation using magnesium sulphate was applied during the w.up. This kind of operation is normally avoided at industrial scale since it requires to perform an additional filtration and is time consuming.
- It required highly diluted w.up conditions. The maximum volume reached during the synthesis was above 50 volumes. The use of large volumes implies the use of large reaction vessels for the obtention of small amounts of product. This limits the manufacturing capacity per unit volume and makes the process unsuitable for its application at industrial scale.
- Because of the mechanical loses observed in the w.up and in the crystallization the obtained yield was low (≈45 %). Considering that only the β-anomer of **D9** is useful for the obtention of **DH0517** (Scheme 5) and that the obtained product was a 1:1 mixture of β and α-anomers the corrected yield was even lower (≈23 %).

After the evaluation of the obtained results and despite the observed problems it was concluded that the process had potential to be industrialized. Therefore, the process development was continued and the optimization phase was started.

1.5.2. Process optimization

The objective of this stage was to solve the reactivity and w.up problems identified in order to develop an effective industrial process for the manufacturing of **D9** (Scheme 5).

During this phase, the influence on the process performance of the different process parameters (volumes of solvents, process temperatures, stoichiometries or stirring rates) was determined. Afterwards, a series of experiments focused on the optimization of the process was carried out. The ranges evaluated in these experiments were adjusted to the plant equipment limitations i.e. extremely high or low temperatures were avoided and reasonable stirring rates were maintained. In this case, our efforts were focused on the improvement of the anomer ratio and yield.

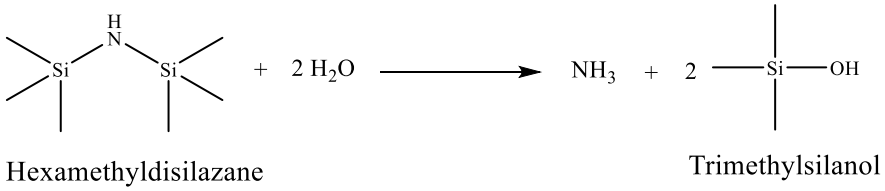
1.5.2.1. Reactivity

All the reactivity tests were carried out at 8 g scale of **D8** using 250 mL glass jacketed reactors (see Figure 47). The use of these reactors allowed to mimic the plant equipment features from the beginning of the development and to achieve a good level of similarity in terms of heat exchange, mixing or geometry. Performing the experiments directly in this type of vessel permitted to detect/correct issues related to the characteristics of the jacketed reactors and to facilitate the scale-up.

Figure 47. 250 mL reactor used during the reactivity optimization experiments.

From the beginning of the optimization process, a post-silylation solvent swap operation was implemented to avoid the distillation to dryness performed initially. A study was carried out to determine the influence of HMDS traces remaining after the solvent exchange. It was found that HMDS traces did not affect the reactivity. However, they could cause several phase separation problems during the w.up. The observed phase separation problems, were attributed to the residual trimethylsilanol formed from the HMDS hydrolysis occurring during the aqueous w.up

(see Scheme 9). To avoid further problems, the solvent exchange was monitored through gas chromatography (GC). After some experiments, a 2 %wt limit of residual HMDS in the silylated 5-azacytosine derivative (Figure 48) slurry obtained at the end of the solvent exchange was stablished.[80,81]



Scheme 9. Reaction of HMDS hydrolysis that leaded to the formation of trimethylsilanol.[80,81]

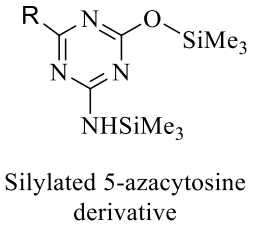
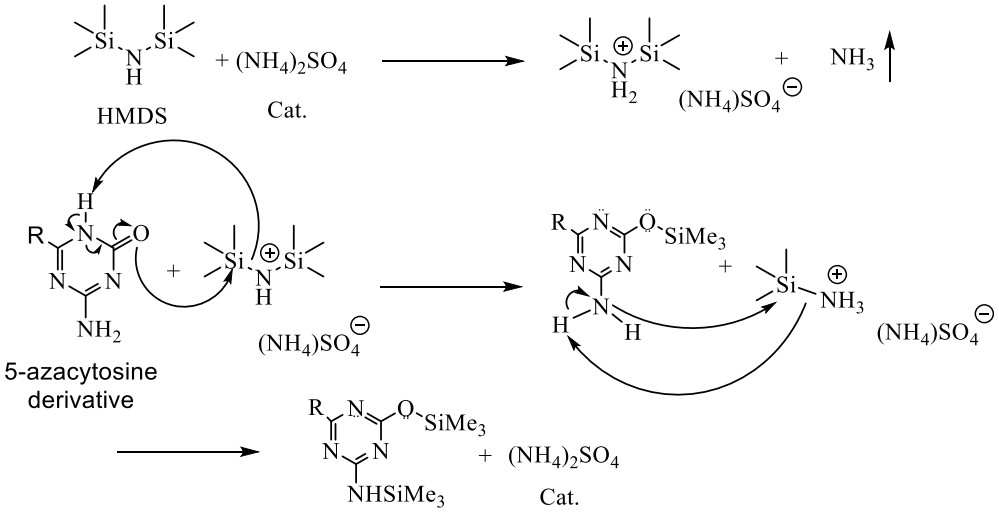


Figure 48. Molecular structure of the silylation reaction product (silylated 5-azacytosine derivative).

Ammonia formation was detected during the silylation experiments. The reaction mechanism presented in Scheme 10 was suggested to justify the release of ammonia during the reaction.[82,83] To quench the generated ammonia, in the industrial scale batches the reactor was connected to a scrubber containing an acid solution during this step (see the Scrubbers section).



Scheme 10. Proposed mechanism for the silylation reaction.

The use DCM instead of chloroform was evaluated in the glycosylation reaction (Scheme 5) since, according to the literature both solvents present similar properties.[84] DCM was finally adopted as reaction solvent because under the same conditions it allowed to reach higher β-anomer levels and to reduce the formation of impurities (see entries 1 and 2 on Table 6). During the glycosylation study, the addition order of the different reagents was also studied and modified. In the initial process, a **D8** solution was prepared in the reactor and then the silylated 5-azacytosine derivative and the catalytic TMSOTf were loaded over it. According to the literature, the slurry obtained at the end of the silylation reaction is moisture sensitive.[61]

Therefore, it was decided to maintain this mixture in the reactor and add the rest of the reagents over it. The implementation of this strategy allowed to prevent silylated 5-azacyotsine derivative loses and to avoid its degradation.

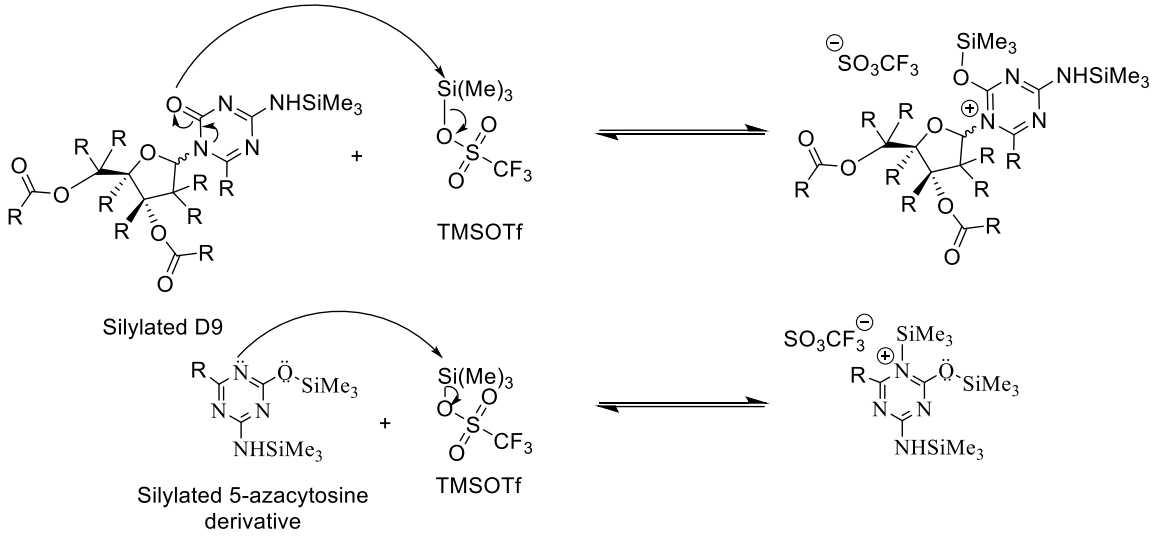
The procedure eventually stablished was based on the loading of TMSOTf over the silylated 5-azacytosine derivative slurry, followed by the **D8** loading. During these trials, it was observed that the TMSOTf was able to degrade the **D8** if they contacted out of the reaction medium. Therefore, before the **D8** loading a DCM rinsing of the reactor walls was implemented.

Entry	Test	Reaction conditions	Purity	Yield	α:β ratio	Main impurity	Comments
1	Reference conditions	$CHCl_3$, 1 °C	89.3 %	41 %	1.05:1	7.5 %	Conditions from patent ex.6.[54]
2	Reference conditions in DCM	DCM, 1 °C	98.7 %	69 %	1:2.04	1.0 %	Diluted w.up. Phase separation problems, product lost in the aq. phases
3	DCM/no TMSOTf	DCM, 1 °C	No product isolated. Reaction evolved slowly and mainly forming α-anomer.				
4	DCM/high temp.	DCM, 25 °C	Small test a r.t no product isolated. Worse α:β ratio than at low temp.				
5	DCM/low temp.	DCM, low temp.	97.3 %	78 %	1:2.55	1.1 %	α:β ratio: after o.n 1:3.3 (15 % **D8**) 10' at 25 °C 1:2.8 (12 % **D8**) 1 h at 25 °C 1:2.5 (0.88 % **D8**)

Table 6. Summary of the results achieved during the glycosylation reactivity trials. The patent examples were taken from the US20100249394 patent.[54]

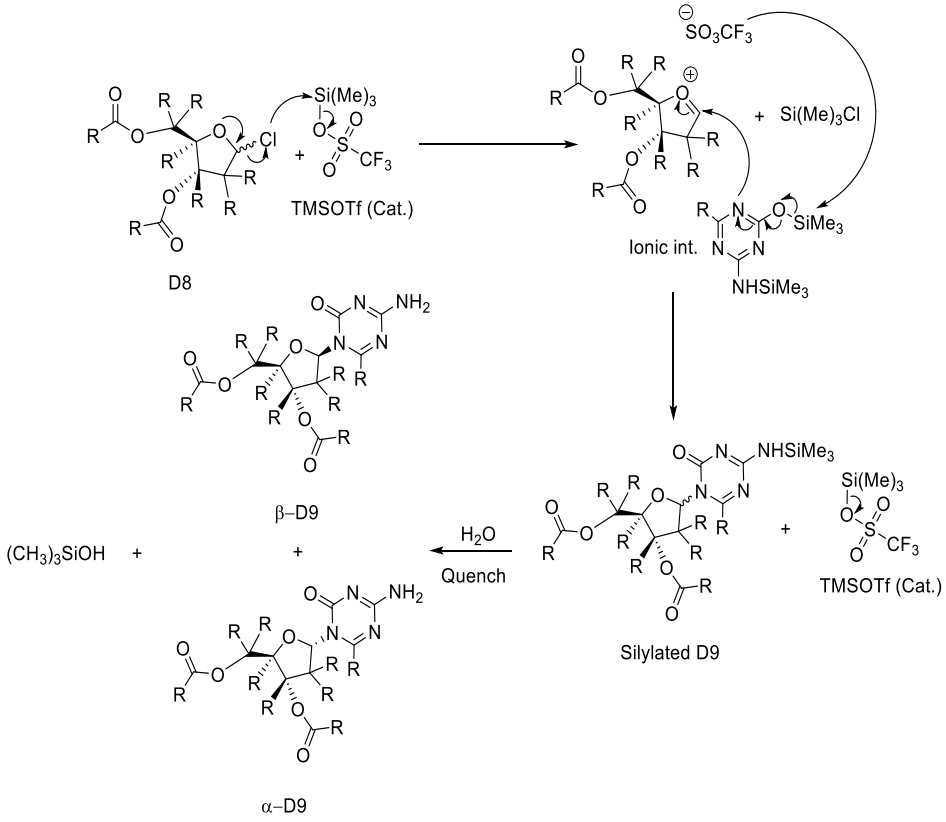
During the reactivity study, the influence of the reaction temperature and the TMSOTf amount on the stereoselectivity was also studied. As it can be seen in entries 3, 4 and 5 (Table 6) high temperatures and absence of TMSOTf favoured the formation of **D9** α-anomer. The anomer ratio during the course of the reaction at low temperature was also studied (entry 5). It was observed that the reaction stopped after overnight. When higher temperatures were applied to induce the reaction completion, the α:β-anomer ratio decreased. From the obtained results, it was suggested that the TMSOTf catalyst lifetime is limited. Once the catalyst loses its activity the reaction stops and if temperature is applied the undesired **α-D9** anomer is formed.

The decrease of the TMSOTf catalytic activity was related to the high reactivity of this substance which has been already reported by other authors.[85] The decrease in the TMSOTf activity was also associated to the different equilibria generated in presence of the silylated 5-azacytosine derivative and the silylated **D9** formed during the glycosylation reaction. These equilibria have already been described in the literature (see Scheme 11).[86–89]



Scheme 11. Equilibria between the silylated D9 and the TMSOTf (up) and equilibria between the silylated 5-azacytosine and the TMSOTf (down).[86–89]

According to the literature and the information obtained from the study of the reaction, the reaction mechanism shown in Scheme 12 was proposed.[69,72,86]



Scheme 12. Proposed reaction mechanism for the glycosylation reaction lading to the formation of D9.

Next, a set of experiments designed to favor the formation of the **β-D9** anomer were carried out. Eventually, it was found that the use of more TMSOTf, the addition of **D8** as a solid and working at lower reaction temperatures allowed to promote the formation of β-anomer. Based on the obtained results and, in the information extracted from the literature it was proposed that the **β-D9** anomer was the kinetically favored one. According to the available information,

the **α-D9** anomer was the most stable one (thermodynamically favored), these differences in stability were attributed to the anomeric effect contribution.[78,90–93]

Adding **D8** as solid, allowed to perform fast addition without remarkable temperature increase or formation of reaction impurities. This addition mode favoured the formation of higher amounts of **β-D9** anomer specially at the beginning of the reaction (Figure 49). During the initial phases of the glycosylation, the presence of larger amounts of unreacted silylated 5-azacytosine promoted the formation of the kinetically favoured product (**β-D9**). Based on these observations, it was suggested that the obtained anomer ratio could be increased using a larger excess of 5-azacytosine derivative. The implementation of this optimization was finally dismissed because the 5-azacytosine derivative was the most expensive raw material of the synthesis and to reduce its consumption was considered essential to maintain the process profitability.

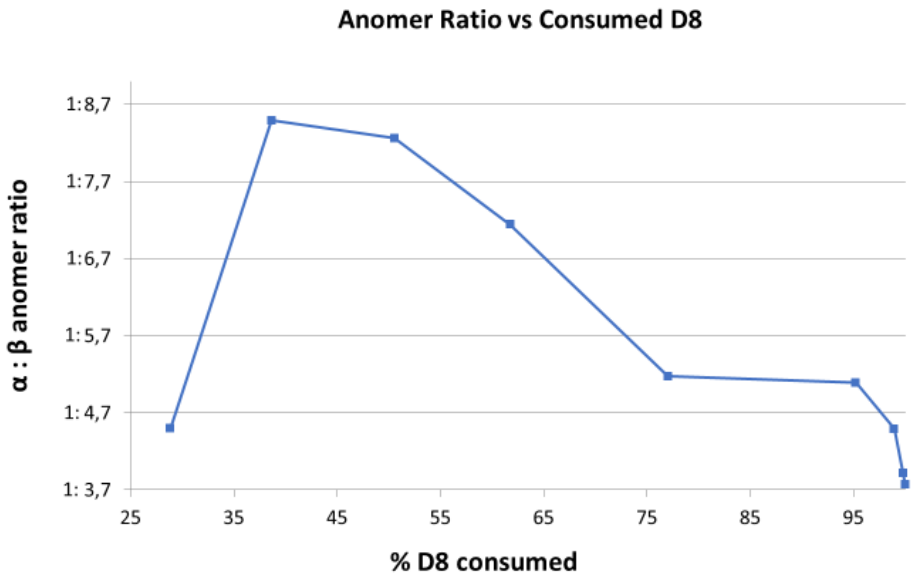


Figure 49. Study of the anomer ratio evolution during a glycosylation reaction performed adding D8 as a solid.

The use of larger amounts of TMSOTf favoured the formation of the **β-D9** anomer. It allowed to increase the reactivity and to complete the reaction maintaining low temperatures. The addition of higher amounts of TMSOTf avoided the α-anomer increase observed during the first trials. In these experiments, the formation of the thermodynamically favored product (**α-D9**) was promoted because the temperature had to be increased to reach the reaction completion (see entry 5, Table 6).
In the experiments performed at lower temperatures, larger amounts of **β-D9** were formed. The observed results were also in agreement with the hypothesis that the **β-D9** anomer was the kinetically favored one.

The optimal reaction conditions found were considered potentially hazardous because all the **D8** was added at once over a mixture containing large amounts of unreacted silylated 5-azacytosine derivative and catalyst. In this scenario it is not possible to stop the reaction in case of failure of the reactor cooling bath or any other unexpected event. After the corresponding safety study, it was determined that the process was safe. Considering the enthalpy of the performed reaction (-250 kJ/mol) and, the enthalpy of the **D9** decomposition reaction (-36

kJ/mol)[7] it was concluded that there was no risk of having a runaway even in the worst-case scenarios (see the Thermal hazards section).

In the final phase of the reactivity optimization, some experiments were performed to evaluate the process robustness and to stablish some safe holding points required for the large scale operation (see the Fundamentals of batch process scale-up chapter). It was determined that the silylated 5-azacytosine derivative slurry obtained after the solvent swap from HMDS to DCM was stable during 16 h at low temperature stored under inert gas. Additionally, it was found that once completed the glycosylation, the reaction crude obtained could be stored at 25 °C for three days without causing affectation into the process.

The optimal conditions found during the reactivity study were successfully scaled-up in the laboratory to 200 g using a 5 L reactor (Figure 50). Using the optimized conditions, the reaction reached the completion after overnight. An α:β ratio of 1:4 and a remarkably low impurity content was observed in the HPLC analysis of the reaction crude.

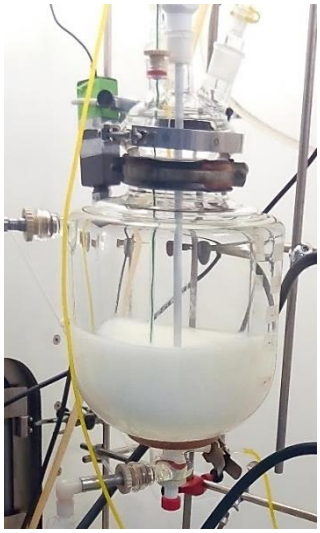


Figure 50. 5 L reactor used in the DH0517 project for the scale-up of the glycosylation step.

During the industrialization of the process, a method for the manipulation of large amounts of TMSOTf was developed. The use of this smoky, flammable, corrosive and very hygroscopic reagent required from a set up similar to the one presented in Figure 22 in section 1.2.[94] The use of the presented set up allowed to reduce the exposition of the operators to the TMSOTf increasing the process safety. The developed addition system allowed also to reduce the contact of this reagent with the atmosphere, decreasing the risk of degradation and minimizing possible process affectations.

1.5.2.2. Work up

During the w.up optimization the conditions described in several articles and patents were evaluated.[54,61] The development was carried out in 250 mL reactors as in the case of the

[7] The **D9** decomposition enthalpy was calculated from the results of the DSC analysis of a **D9** sample. The reaction enthalpy value given was extracted from the reference tables created by the Safety Swiss Institute (TÜV SÜD) available in Farmhispania S.A.

reactivity tests in order to reproduce the plant equipment heat exchange ratios, stirring and geometry.

Initially, it was found that the 5-azacytosine derivative excess could be efficiently removed by rinsing the reaction crude with an aqueous HCl solution. During this treatment, no undesired **D9** crystallization was observed even working at concentrated conditions. However, sticky solid formation and phase separation issues were found during the subsequent aqueous and basic rinsings even with alternative solvents such as 2-methyltetrahydrofuran (2-Me-THF).

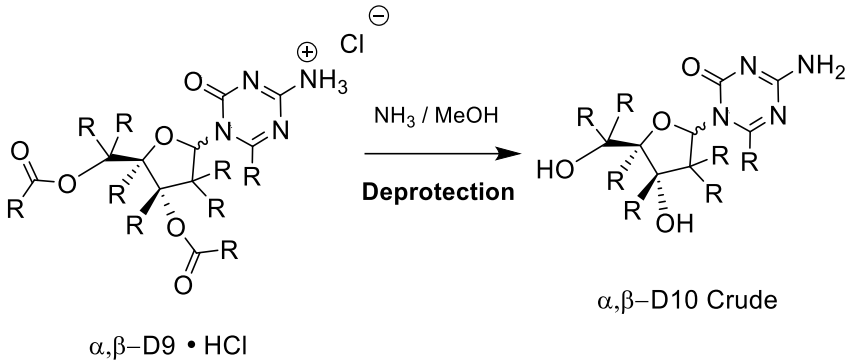
Despite salts are typically more soluble in aqueous media, the **D9** hydrochloride (**D9**·HCl) formed during the hydrochloric acid rinsing had a higher solubility in the organic phase than the **D9** free base. During the basic and aqueous rinsings, the formation of the free base form was favoured promoting the observed crystallization and phase separation problems. In order to maintain highly concentrated conditions during the w.up, basic and aqueous rinsings were avoided and a crystallization procedure for the obtention of **D9**·HCl was developed.

In the **D9**·HCl crystallization tests sticky solid formation problems were found. Up to nine solvent/antisolvent pairs were evaluated at small scale using direct and reverse additions (Table 7). Eventually, it was found that using a precise ratio of DCM and methyl tert-butyl ether (MTBE) a suitable process for the crystallization of **D9**·HCl was developed and successfully scaled-up to 8 g scale in a 250 mL reactor (Table 7; entry 12).

Entry	Solvent	Anti-solvent	Addition type	Solid morphology	Scale-up
1	MeOH	n-Heptane	Direct	Not acceptable	-
2	MeOH	Toluene	Direct	Not acceptable	-
3	MeOH	Acetone	Direct	Not acceptable	-
4	MeOH	Water	Direct	Acceptable	Fail
5	MeOH	Water	Reverse	Not acceptable	-
6	MeOH	EtOAc	Direct	Not acceptable	-
7	MeOH	MTBE	Direct	Not acceptable	-
8	DCM	n-Heptane	Direct	Not acceptable	-
9	DCM	n-Heptane	Reverse	Not acceptable	-
10	DCM	Toluene	Direct	Not acceptable	-
11	DCM	MTBE	Direct	Not acceptable	-
12	DCM	MTBE	Reverse	Acceptable	Succeed

Table 7. Summary of the crystallization experiments carried out to isolate D9 hydrochloride.

The deprotection reactions (Scheme 13) on the isolated **D9**·HCl evolved slowly showing relevant degradation and low yields. This behaviour was not observed when **D9** free base was used as raw material. Based on the observed degradation and yield problems it was concluded that **D9**·HCl could not be used for the obtention of **DH0517** and that it was necessary to isolate **D9** in its free base form.



Scheme 13. Deprotection reaction performed to obtain D10 crude from D9·HCl.

The study of an alternative w.up that allowed the controlled crystallization of **D9** in its free base form was started using conditions found in the literature.[64] The adopted approach was based on a solvent swap from DCM to EtOAc after the glycosylation. The solution obtained was then added at low temperature over a mixture of ACN, cyclohexane, EtOAc and $NaHCO_{3\ (aq.)}$ to perform a three-phase crystallization involving a solid and two immiscible liquid phases. The described procedure allowed to isolate **D9** in its free base form in 97.0 % purity; 85 % yield. However, it required a deep optimization process in order to make it suitable for the industrial scale operation. The main drawbacks observed for this crystallization process and the implemented optimizations are summarized in Table 8.

Entry	Drawback	Action
1	Low temp. applied (ice formation risk)	Temperature increased; no impact on yield.
2	Complex mixture of solvents used	Cyclohexane and ACN substituted by MTBE.
3	Cyclohexane toxicity	
4	5-azacytosine derivative traces in **D9**	HCl rinsing implemented before the crystallization.
5	Initial EtOAc phase moisture sensitive	
6	Reverse addition required	Addition of MTBE and $NaHCO_3$ over the EtOAc phase.
7	Thick slurry in DCM to EtOAc solvent swap	Residual volumes and number of co-distillations increased.
8	Thick final slurry formed	Amount of MTBE increased; no impact on yield/Dynochem software mixing study.
9	Slow filtration	Over-sized industrial filter allotted.

Table 8. Summary of the optimizations implemented over the D9 free base form crystallization process.

As it is shown in entry 5 of Table 8, the EtOAc solution obtained after the solvent swap from DCM was very moisture sensitive. It formed dense jelly mixtures that were difficult to manipulate if it was minimally exposed to air or any moisture source. The observed problems were related to the residual products formed from the hydrolysis of the traces of HMDS, silylated **D9** and/or silylated azacytosine present in the reaction crude (Scheme 9 and Scheme 12). This hypothesis was reinforced by the observed stability increase after the implementation of an HCl rinsing before the solvent exchange. This acid washing allowed to eliminate HMDS and silylated intermediates, incrementing the stability of the EtOAc phase and reducing the residual azacytosine content in the final product. In order to avoid product loses the residual acid phase obtained was rinsed with DCM before to continue with the process.[80,81]

In entry 8 it is reported that the slurry formed during the **D9** crystallization was thick. In the laboratory tests, vigorous stirring was required to ensure a proper mixing (e.g. during a set of 45 g scale experiments carried out in a 2 L reactor, the crystallization mixture had to be stirred at 350 rpm to ensure the homogeneity). In order to guarantee that the stirring applied at plant

would lead to an adequate mixing of the slurry an *in-silico* scale-up study was performed using the Dynochem software (see The Dynochem software section).

The *in-silico* scale-up studies are based on simulations where it is evaluated the result of applying the laboratory scale conditions at industrial scale (reactor occupation, temperatures, mixing or addition rates). For these simulations, the amounts of raw materials, reactor geometries and the reactor heat capacities that will be used during the large scale batches are considered. Performing *in-silico* scale-up tests allows to determine which will be the behavior of an industrial process under a specific set of operating conditions. These studies are useful to evaluate the suitability of the process modifications before its implementation at large scale, to prevent failures and deviations during the scale-up, to study parameters complex to be evaluated through laboratory scale experiments (addition rates or mixing) and, to detect possible safety issues among others.

In the performed *in-silico* study, the industrial scale stirring rate equivalent to the agitator speed that was applied in the laboratory experiments was calculated through the Dynochem software (see Table 9). The calculations were based on the geometries and the contents of the laboratory and the plant reactors. In order to calculate the equivalent stirring rate for the industrial reactor, the Dynochem software calculates a series of parameters typically used to characterize the mixing efficiency (e.g. power per unit mass, tip speed or liquid velocity). The software determines the stirring rate to be applied at industrial scale in order to keep constant the selected mixing parameter.

Since the impeller geometries included in the Dynochem software were not exactly equal to the actual geometry of the industrial reactor impeller, two types of simulations including the more similar available impellers which were the retreat curve impeller (RCI) and the curved blade turbine (CBT) were performed (see Figure 51). The industrial reactor had a baffler but as in the case of the impeller, in the software was not available a baffler with the same geometry. To evaluate the influence of the baffler and its geometry some simulations using different types of bafflers were carried out. Additionally, simulations based on different mixing parameters were carried out. Special attention was paid to the results obtained maintaining constant power per unit mass (criterion more typically applied) but other mixing parameters were explored. The main goal of the performed simulations was to determine if, in the worst-case scenario stirring rates higher than 100 rpm were required at industrial scale. The 100 rpm threshold was set based on the specifications of the industrial scale mixing system.

Simulation	Type of impeller	Mixing parameter	Baffler	Result (rpm)
1	RCI	Constant power per unit mass	Dip pipe	81
2	RCI	Constant vessel averaged turbulent shear rate	Dip pipe	81
3	RCI	Constant power per unit mass	Beavertail baffler	69
4	RCI	Constant tip speed	Dip pipe	33
5	RCI	Constant bulk liquid velocity	Dip pipe	34
6	CBT	Constant power per unit mass	Dip pipe	56
7	CBT	Constant vessel averaged turbulent shear rate	Dip pipe	55
8	CBT	Constant power per unit mass	Beavertail baffler	44
9	CBT	Constant tip speed	Dip pipe	33 (Solids not suspended)[1]
10	CBT	Constant bulk liquid velocity	Dip pipe	21 (Solids not suspended)[1]

Table 9. Simulations at different conditions carried out using the Dynochem software to predict the stirring rate at plant equivalent to a 350 rpm stirring rate in laboratory. Note 1: the solid not suspended warning given in entries 9 and 10 indicates that, according to the Dynochem software, if the described conditions are applied the solids will remain in the reactor bottom as a consequence of an inefficient mixing.

Figure 51. Impeller geometries used for the Dynochem software simulations. Left: retreat curve impeller (RCI). Right: curved blade turbine (CBT)

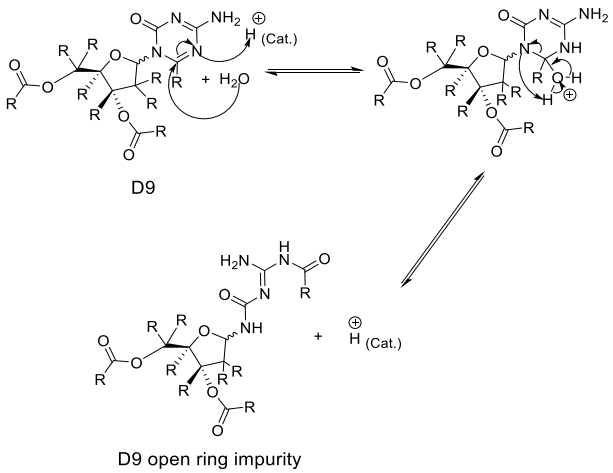
As it is shown in Table 9, even at the worst-case scenarios (simulations 1 and 2) stirring rates below 100 rpm were obtained. According to the simulations, the mixing would be more efficient at plant than at the laboratory considering stirring rates of 100 and 350 rpm respectively. Since increasing mixing efficiency was considered positive for the crystallization, no mixing issues were expected during the scale-up. The simulation results were confirmed during the industrial batches performed afterwards.

According to entry 9 of Table 8, slow filtration rates were observed during the **D9** isolation. As it mentioned in the Development of industrial chemical processes section, an slow filtration at laboratory scale may lead to a filtration of days or even weeks at the manufacturing plant where, because of the scale increase the observed phenomena may be magnified. The corrective action applied to solve the slow filtration problems observed was to employ an oversized filter for the industrial scale batches. The use of a larger filter would allow to increase the filtration area and, therefore the filtration rate. This approach was successfully applied at laboratory scale where, through the use of oversized filters, fast filtration rates were achieved (Figure 52). In order to stablish the industrial filter size, a laboratory experiment was performed starting from 200 g of **D8**. In this case a wet solid cake of **D9** with a volume of 1040 cm^3 was obtained. This information was used to calculate the volume of the wet solid cake to be obtained at plant, considering the industrial operational unit. The size of the filter required for the industrial batches was stablished based on these calculations. As it is reported in section 1.5.3, slow filtration rates were observed in the first industrial scale batch. Since the observed behaviour was not in agreement with the laboratory scale results, additional experiments and process optimizations were carried out. The corrective actions taken after the first industrial batch to solve this issue are described in the following section.

Figure 52. Filtration set-up used during the filtration tests formed by a 5 L Kitasato and a Ø=26 cm Büchner funnel.

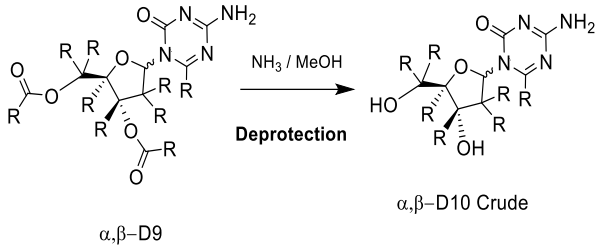
Once defined the final w.up procedure, some additional studies were carried out to evaluate the process robustness.

- The product loss in the mother liquors and the aqueous phases generated during the w.up was monitored through HPLC. The analyses indicated that almost no **D9** either in α or β form was lost during the w.up. Based on the high conversion and selectivity towards the **D9** formation (both anomers) observed during the glycosylation reaction it was suggested that the yield loses observed in this step were related to the **D9** degradation and to the mechanical loses.
  It was found that the ring opening impurity presented in Scheme 14 was formed during the acid washing because of the azacytosine ring hydrolysis. This degradation reaction was reversible and, although around a 13 % of this impurity was observed during the acid rinsing most of the formed impurity was converted again into **D9** during the subsequent DCM distillations. It is suggested that the removal of the water traces during the mentioned solvent swap shifted the equilibria towards the **D9** formation. Several examples were found in the literature where this degradation pathway was studied and described for compounds similar to **D9** (see **D9 impurity A** study in section 1.6).[49–52,95]



Scheme 14. Suggested mechanism for the formation of the D9 ring opening impurity.

- It was observed that minimum amounts of moisture in **D9** caused severe degradation problems in the subsequent deprotection reaction (see Scheme 15).



Scheme 15. Synthetic route used for the obtention of DH0517 using as starting material D8.

Since water traces in **D9** may lead to batch failure it was considered mandatory to determine the maximum amount of water tolerated by the process and to establish specifications for the residual moisture content of the **D9** intermediate. From the deprotection study, suitable limits for the **D9** residual water content were obtained. It was found that this parameter must be controlled through the use of a Karl-Fischer (KF) oven apparatus since analytical interferences were observed when a volumetric KF was used (the mentioned devices are presented in Figure 53).

Figure 53. KF oven (left) and volumetric KF (right) both from Metrohm used during the DH0517 process development.

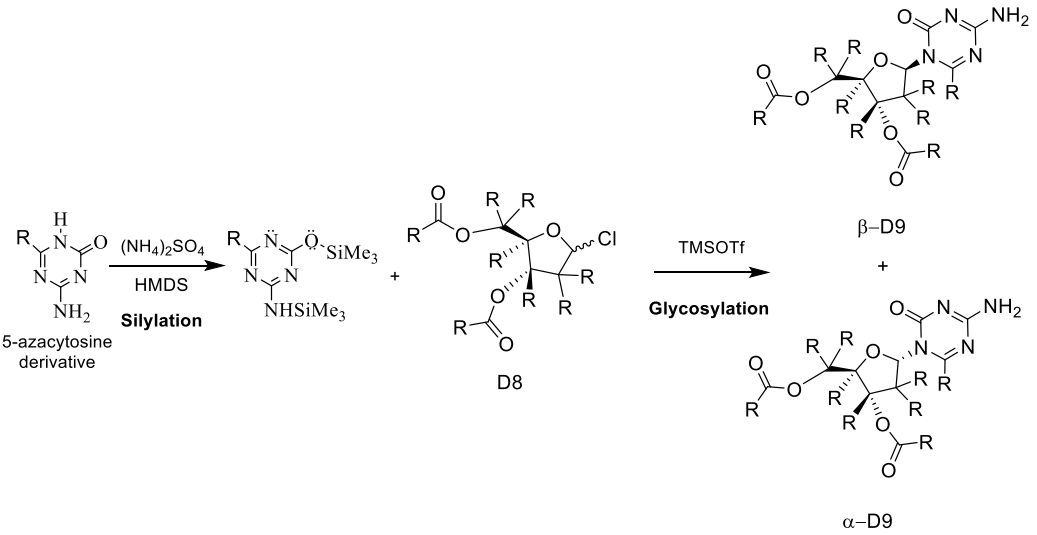
- During this final phase of the w.up study, some **D9** drying tests were carried out. The goal was to find suitable drying conditions that allowed to reach the low residual water content specification stablished in an acceptable amount of time and, without causing the intermediate degradation. Eventually, appropriate drying conditions were found from the study of the **D9** stability at different drying temperatures.

The optimal conditions found during the w.up study were successfully scaled-up in the laboratory to a 200 g scale using a 5 L reactor (see Figure 50). Appling the optimized conditions, a 86 % yield was obtained (68 % yield considering only β-anomer), the isolated **D9** presented a 98.2 % purity and a α:β-anomer ratio of 1:3.7. The obtained yield considering only the **β-D9** anomer was above the highest yield reported in the literature (62 %; entry 2 of Table 4 in section 1.3.1). The high reported **β-D9** anomer yield was achieved as a result of the reduction of the product loses during the w.up/crystallization and of the selectivity increase achieved for the glycosylation reaction.

The developed procedure was applied at the first industrial batch, which was performed at scale of 45 kg of **D8** in a 1600 L reactor. Although the synthesis was successfully completed and the requested amount of **DH0517** was obtained some problems related to slow filtration and long operation times were found during the execution of this batch. In section 1.5.3 are described in detail the studies and optimizations carried out after the first industrial batch in order to solve the above-mentioned process weaknesses.

1.5.2.3. Process optimization conclusions

- A suitable method for the obtention at industrial scale of the **D9** intermediate based on a silylation and a glycosylation reaction was developed (Scheme 16). The isolated **D9** presented a 98.2 % purity and a α:β-anomer ratio of 1:3.7. The obtained yield was 86 % (68 % considering **β-D9**).



Scheme 16. Synthetic route used for the obtention of D9 from D8.

- The silylation reaction conditions taken from the literature leaded to acceptable results. However, the procedure described in the literature for the glycosylation required from an extensive optimization in order to solve problems related to yield, volumes of synthesis, **D9** anomer ratio, toxicity of the used solvents or phase separation/crystallization problems.

- The solvent of the glycosylation was exchanged from chloroform to DCM. The use of DCM allowed to reduce the concerns related to the solvent toxicity.

- During the reactivity optimization, the main parameters affecting the conversion and the selectivity of the glycosylation reaction were investigated. It was determined that the finally obtained anomer ratio was highly dependent on the reaction temperature, the amount of TMSOTf and the procedure used for the **D8** loading.

- The performed investigations revealed that the **β-D9** anomer formation was favoured by low reaction temperatures, the use of larger amounts of TMSOTf and by the fast addition of **D8** in solid form.

- The w.up of the silylation and the glycosylation reactions were modified in order to work at higher concentration conditions, avoid interphase formation and solve crystallization issues. In the glycosylation w.up an acid rinsing of the reaction crude and a crystallization based on the use of $NaHCO_{3\,(aq)}$, MTBE and EtOAc were implemented.

- As part of the w.up development, studies were performed to avoid possible mixing and filtration issues during the scale-up.

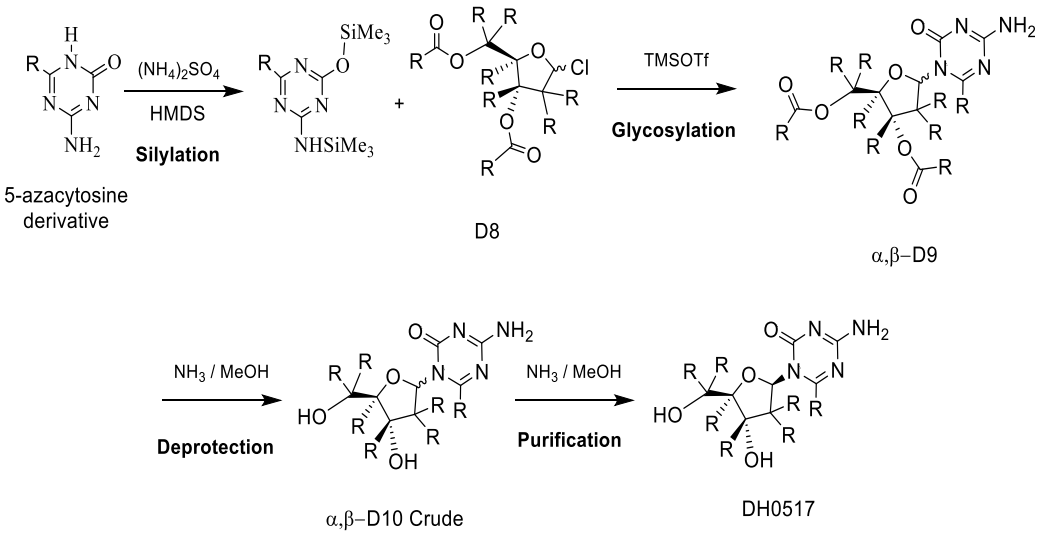
- A suitable drying process was defined to reach the desired levels of residual moisture in the isolated **D9**. The defined drying conditions allowed to avoid reactivity problems during the subsequent deprotection reaction (Scheme 18).

1.5.3. Process scale-up

In order to synthesize the large amounts of intermediate **D9** required for the obtention of **DH0517** at commercial scale, the manufacturing process obtained after the completion of the initial development and optimization phases was transferred from the laboratory (200 g of **D8**; 5 L reactor) to the industrial scale (45 kg of **D8**; 1600 L reactor). As it is mentioned in the Fundamentals of batch process scale-up chapter, the differences between the laboratory and the plant operation may affect the process performance during the scale-up. In order to obtain a process suitable for a large scale operation that could be validated, a series of pilot batches of **D9** were executed. As consequence of the characteristics of the used industrial equipment and of the scale increase, during the large scale batches of **D9** different minor issues were found. For each problem, an investigation was carried out and the corresponding corrective action/preventive action (CAPA) was implemented. In the following sections are listed the problems found during the **D9** process scale-up and the different modifications implemented to solve them.

1.5.3.1. Residue on ignition (ROI) out of specifications

The main problem found during the first **D9** industrial batch was the high residue on ignition (ROI) observed in the isolated product, which was related to an elevated inorganic salt content. The observed values were above the **DH0517** specification (≤0.1 %). This was considered a problem since typically the ROI values found in **D9** were maintained in **DH0517**. Due to the use of MeOH as solvent, the inorganic salts were not eliminated during the deprotection and the purification steps (Scheme 17). Normally, the use of aqueous treatments is required for the elimination of this type of impurities due to its low solubility in organic solvents. Aqueous treatments can not be applied over **DH0517** because it degrades when it is dissolved in water. For this reason, it was concluded that the implementation of an additional water slurry to reduce the ROI content of **D9** was required.[49–52]



Scheme 17. Synthetic route employed for the obtention of DH0517 using as starting material D8.

During the initial trials performed to develop the mentioned **D9** treatment it was found that, this intermediate presented good stability in aqueous media and, that it was highly hydrophobic. Therefore, it could not be properly suspended and purified using only water. The hydrophobicity problem was solved adding a certain amount of MeOH to the mixture in order to increase the homogeneity. Once the amount of MeOH and the total volumes of solvent were adjusted to achieve the formation of a homogeneous slurry with capability to eliminate residual inorganic salts, this additional treatment was implemented into the **D9** manufacturing process. In this case, MeOH was selected as a cosolvent because it was the solvent of the following step, **D9** presented a low solubility on it and also it allowed to displace water traces efficiently during the solid cake rinsings easing the subsequent product drying.

After the corresponding laboratory scale suitability tests, the use of an agitated Nutsche filter was adopted (Figure 54) (see Filtrations/Crystallizations section). The use of this type of filter allowed to reduce operation time and simplified the process avoiding reactor discharge and filter unloading operations. In the industrial batches the initial **D9** filtration, the water/MeOH purifications and the primary drying were carried out in the filter itself. Additionally, to ensure that the obtained solid met the ROI specification before to discharge the filter an in-process control (IPC)[8] was implemented.

[8] In-process control (IPC): analysis performed during the course of a batch that must meet a certain specification in order to ensure that the quality of the isolated product and/or the process performance (safety, yield or timing) will be maintained within acceptable terms.

Figure 54. Outside view (right) and inside view (left) of the laboratory scale Nutsche agitated filter used during the laboratory scale tests performed. The filter was provided by Pope Scientific INC.

1.5.3.2. Slow filtration problems

In the first industrial batches performed, the **D9** filtrations were considerably slow. In average, near to 3 days were required only to complete the filtration of the first slurry. The magnitude of this problem increased due to the implementation of the water slurries used to reduce the ROI content and its corresponding additional filtrations. Therefore, the optimization of the **D9** crystallization in order to increase the filtration rate and reduce the operation time was considered a priority. During the study of the crystallization the use of annealing (see Filtrations/Crystallizations section), alternative organic bases and different solvents was evaluated. Eventually, a crystallization procedure based on the use of ACN and $NaHCO_{3\,(aq.)}$ was developed. The use of this mixture of solvents allowed to reduce the filtration time around a 70 % and simplified the crystallization that initially involved three-phases (the solid and two immiscible liquid phases). The product isolated using this new crystallization process had lower purity. This issue was solved introducing MTBE purification slurries after and before the aqueous slurries used to reduce the ROI. To simplify the process, as in the case of the MeOH/water purifications the MTBE treatments were carried out in the agitated Nutsche filter employed in the initial filtration.

As part of the studies related with the filtration optimization, the stability of the obtained slurries was evaluated. The objective of the stability studies was to determine if degradation could arise in case of slow filtration. The slurries were found to be stable, confirming that the product quality would not be affected in case of a delay in the filtration.

1.5.3.3. Drying problems/solid homogeneity problems

One of the first **DH0517** industrial batches failed at the deprotection step (see the **DH0517** synthesis described in Scheme 17) due to product degradation issues. The impurities formed were typically observed when **DH0517** degrades in presence of water. After an investigation, it was found that the source of the moisture that caused the batch failure was the **D9** intermediate.

During the routine operation, an IPC analysis (see foot note on page 100) of a **D9** sample taken from the Nutsche agitated filter was performed before to conclude the drying. This analysis was carried out to ensure that its residual water content was below the acceptable limits. From the investigation it was concluded that the stirring arms of the used Nutsche agitated filter did not efficiently mix the bottom of the formed solid cake. Therefore, the filter content was not homogeneous and the IPC result obtained in the failed batch was not representative of the overall moisture content of the isolated **D9**. In the failed batch, the amount of water accumulated in the not stirred solid was higher than in previous cases and caused the formation of critical amounts of impurities during the deprotection reaction.

To circumvent drying problems in further **DH0517** batches, the process was revised and modified. On one hand, taking profit of the **D9** stability at the applied drying conditions the water removal capacity was increased through the application of extended drying times at the Nutsche agitated filter.

On the other hand, a sieving operation was introduced after the primary drying conducted at the filter (see in Figure 55 the detail of the used sieve). The sieving was introduced to eliminate the solid aggregates formed during the primary drying because they could occlude moisture. The implementation of a sieving operation allowed also to increase the water removal rate because of the increment of the contact surface promoted by the solid disaggregation. After the sieving, an additional drying carried out in a rotary cone vacuum dryer was applied to remove completely water traces while ensuring the batch homogeneity (see Dryers section). Eventually, the endpoint of the drying process was determined based on an IPC Karl-Fischer analysis.

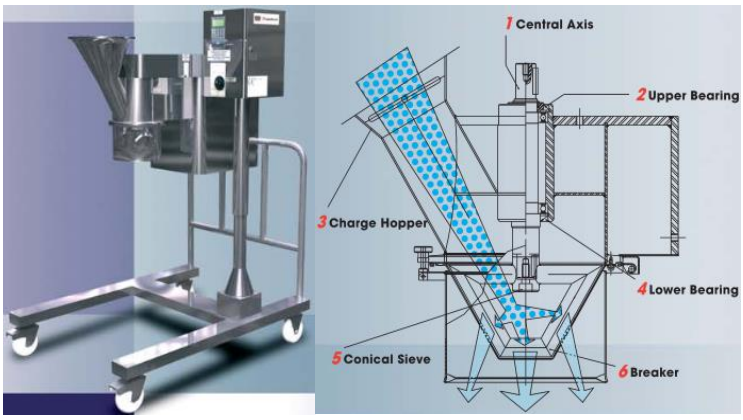


Figure 55. Sieve Glatt Labortecnic model TGC-220 used in the D9 industrial batches (left) and scheme of its working principle (right).[96]

1.5.3.4. Excessive operation time

In front of the lack of process knowledge during the w.up of the first **D9** industrial batch a more conservative but time-consuming approach was adopted to try to avoid degradation and yield loss problems. This w.up included a rinsing of the residual acid aq. phase obtained after the HCl treatment and, the use of low temperatures during the DCM to EtOAc solvent swap performed before the crystallization. As it is mentioned in the Fundamentals of batch process scale-up chapter, time is one of the main cost contributors in manufacturing processes carried out Europe and in the US. For this reason, reducing the manufacturing time was considered a priority. In order to reduce the time required to complete each batch of **D9** three different areas of the process were studied and optimized:

a) At industrial scale, the HMDS stripping took around 11 h because in addition to the three standard co-distillations, an extra co-distillation close to the minimum level stirrable by the reactor (1.3 residual volumes) was required to reach the desired residual level of HMDS (below 2 %wt). The study of this operation revealed that the HMDS remained constant within the DCM distillations and that the desired level was only reached when the mixture was distilled at up to 1.3 residual volumes. It was suggested that HMDS and DCM does not form azeotrope (no information was found in the literature) and that because of the large difference in boiling point (b.p) (126 °C *vs.* 39.6 °C) in presence of DCM only this solvent was being distilled. Considering this data, a more robust and faster solvent swap procedure based on distillations to lower residual volumes was implemented. The optimized procedure allowed to increase the distillation range where HMDS was being removed and leaded to the desired residual level of HMDS in a single HMDS/DCM co-distillation. This optimization allowed to reduce in an 86 % the time required to complete this operation and reduced the DCM consumption during the solvent exchange in a 50 %.
b) In the first industrial scale batch, the acid aq. phase obtained after the HCl treatment applied over the reaction crude was rinsed with DCM in order to recover product. Although from the HPLC analyses performed it was known that some **D9** was recovered, the precise amount of product recovered was not quantified during the initial phase of the development. From the study of the amount of product lost in the residual aq. phase was concluded that less than a 1 % of yield was lost in this phase. It was also found that the recovered product was not as pure as the one finally isolated. Based on the obtained results it was decided to eliminate the DCM rinsing of the aqueous phase.
   The elimination of this DCM rinsing allowed to reduce the DCM consumption and to reduce the manufacturing time since all the operations derived from the DCM rinsing (solvent transfers, phase mixing or phase separation) were avoided. Additionally, the elimination of this rinsing reduced the time required for the subsequent solvent swap to EtOAc since the amount of DCM to be stripped was reduced.
c) In the initial **D9** manufacturing process, the internal temperature was limited to 10 °C during the distillations performed in the solvent exchange from DCM

to EtOAc. This temperature limit was established because the **D9** thermal stability was unknown. The limit was based on the maximum distillation temperatures observed during the laboratory experiments, where, no product degradation was detected. The mentioned 10 °C limit leaded to slow distillation rates and caused that this operation required from 26 h to be completed at plant. After the study of the distillation temperature, it was found that the quality of the isolated product was not affected when the distillation was performed at 15 °C. This change was implemented into the manufacturing method leading to an increase in the distillation rate and to reduction of the solvent swap duration. The increase of the distillation temperature in combination with the decrease of the total volume of the DCM solution to be distilled leaded to a reduction of a 63 % of the time required to perform this solvent exchange operation. Afterwards it was determined that this new temperature limit was also suitable for the DCM to ACN solvent exchange implemented during the filtration problems study (see above in this section).

1.5.3.5. Microfiltration implementation

As part of the Farmhispania S.A. quality policy, a microfiltration of the solution containing the product should be included, if possible, before the final crystallization in all the processes. The objective of this microfiltration was to reduce the risk of having foreign matter contaminations. After the completion of the first industrial **D9** batch some extra development was conducted in order to incorporate a microfiltration in the synthesis. In this case, the microfiltration was performed over the organic phase obtained after the HCl treatment. The microfiltration was implemented at this point because it was the last operation of the synthesis at which total dissolution was observed. The stability of the organic phase obtained after the HCl rinsing was evaluated in order to anticipate possible degradation problems that could arise due to unexpected delays in the microfiltration. Eventually, it was confirmed that the mentioned organic phase was stable and that the process robustness was not affected by the implementation of a microfiltration operation. See in Figure 56 the detail of the used industrial microfilters.

Figure 56. Cuno filters (right) and Cuno filter housing (left) used to perform the microfiltration at industrial scale.[97]

1.5.3.6. Anomer ratio decrease

The manufacturing method finally obtained after the optimization derived from the industrial scale observations was scaled-up to a scale of 118 kg. During the scale-up process a decrease in the **D9** anomer ratio was observed (see Table 10). Although the anomer ratios obtained during the first industrial batch performed at 45 kg of **D8** scale were even higher than the ones observed in the laboratory, during the final phase of the scale-up a decrease on the amount of β-anomer being formed was observed. The mentioned results were related to the decrease on the selectivity of the glycosylation reaction observed in the IPC analysis of the reaction crude. Since the reaction conditions and the operating procedure were maintained during the scale-up, the observed trend was related to variations derived from the increase of the scale (longer addition times, changes in mixing efficiency or reactor geometry) and/or to variations in the morphology of the used starting material. As it is mentioned in section 1.5.2.1, the **β-D9** anomer formation was favoured by the fast addition of the **D8** as a solid. This method of addition promoted the formation of a slurry that turned into a solution upon mixing and time.

Entry	Batch size	Reactor size	Stirring speed	Power per unit mass	(α:β) Anomer ratio
1	50 g (lab. scale)	1 L	350 rpm	0.03 W/kg	1:4.2
2	200 g (lab. scale)	10 L	180 rpm	0.08 W/kg	1:4.0
3	45 kg	1600 L	90 rpm	0.37 W/kg	1:4.4
4	118 kg	4000 L	90 rpm	0.21 W/kg	1:3.0

Table 10. Mixing efficiency and anomer ratio obtained at the end of the glycosylation at four different scales. The mixing study was carried out using the Dynochem software.

The **D8** used in the 118 kg batches was produced in-house using the synthetic route described in Scheme 6 (section 1.2) while for the 45 kg batch a commercial raw material was used. It was suggested that variations in the particle size, polymorph or residual solvent content of the used **D8** could have promoted a slower dissolution of the **D8** leading to the observed results. In order to confirm this hypothesis a series of laboratory experiments were performed using as raw material **D8** prepared in-house. The anomer ratios observed where comparable to the ones obtained using the commercially available material. The obtained results allowed to discard the hypothesis of the solid morphology and to focus the investigation in the features of the large scale operation.

It is suggested that the extended amount of time required to load the **D8** in the reactor at 118 kg scale and the less efficient mixing provided by the larger industrial reactor used promoted the slow dissolution of **D8** influencing the obtained anomer ratio. To confirm the stirring hypothesis, the mixing efficiency at laboratory and at the two used industrial reactors was evaluated through the Dynochem software simulations (Table 10).
As it is shown in entries 3 and 4, the power per unit mass applied in the larger industrial reactor was a 43 % lower compared to the one applied during the 45 kg synthesis. The anomer ratio results achieved in the laboratory compared well with the results observed in the 45 kg batch (entries 1, 2 and 3). However, in the laboratory experiments the power per unit mass applied was even lower that at 118 kg scale. The performed simulations indicated that the mixing efficiency can not be considered as the main cause of the anomer ratio decrease. From the obtained results a direct correlation between the anomer ratio and the mixing intensity can not be stablished. Therefore, the variations in the reaction behaviour observed were attributed to

the extended **D8** loading time required in the 118 kg batches. This hypothesis was in accordance with the trends observed during the reactivity optimization (see section 1.5.2.1).

To increase the selectivity reducing the **D8** loading time was not feasible due to the complexity of the solid manipulation at industrial scale (see1.2.1 section 1.2.1 in chapter 3). However, despite the decrease on selectivity observed, the yield considering only **β-D9** anomer was 63 %. This result was equivalent to the highest yield found during the initial bibliographic search (62 %; entry 2 of Table 4). The results achieved considering yield, purity and anomer ratio were encouraging and triggered the process validation. The validation was eventually performed at a scale of 118 kg of **D8** using 4000 L reactors.

During the validation, three consecutive **D9** batches were carried out without remarkable incidences. The average yield, purity and α:β-anomer ratio obtained were 86 %, 98.0 % and 1:2.8 respectively. The success of this campaign demonstrated that the developed method has the robustness and reliability required for the obtention of this intermediate at large scale. Around 115 kg of **D9** were obtained per batch, this material after the subsequent deprotection and purification steps allowed to obtain three lots of pure **DH0517** with an average size of 16.7 kg.

1.5.3.7. Process scale-up conclusions

- An industrial manufacturing process for the obtention of **D9** was successfully developed and validated. The mentioned process allowed to obtain 115 kg of **D9** per batch with a yield, purity and α:β-anomer ratio of 86 %, 98.0 % and 1:2.8 respectively.
- During the scale-up from 45 kg to 118 kg of **D8** a decrease in the amount of **β-D9** formed during the glycosylation reaction was observed. The decrease on selectivity was attributed to the extended **D8** loading time required in the 118 kg batches.
- In the different pilot batches of **D9** executed a series of minor issues were found. The corresponding studies and process modifications were performed to increase the process robustness at the levels required for the large scale operation and for the process validation.
    - The drying method was modified to adapt it to the large scale operation and ensure the efficient moisture removal form the isolated **D9**.
    - A series of MeOH/water treatments were included after the initial filtration in order to decrease the residue on ignition of the isolated **D9** intermediate.
    - A microfiltration was included into the process. The objective of this microfiltration was to reduce the risk of having foreign matter contaminations.
    - A series of studies were performed in order to reduce the operation time. The mentioned studies allowed to reduce the amount of time required to complete the solvent swaps performed, avoided unnecessary rinsings of the residual aqueous phase and reduced the solvent consumption.

- A new crystallization process based on the use of ACN and $NaHCO_{3\ (aq)}$ was developed to solve slow filtration issues. The developed process included a series of MTBE slurries which were performed to maintain the purity level.

1.6. Study of process impurities

1.6.1. Introduction: Regulatory context, types of impurities and permitted limits

1.6.1.1. Regulatory context

The impurities that are present in a drug substance, are one of the fundamental subjects covered in the reports required to obtain the authorization from the regulatory authorities to test and/or commercialize a drug (see The drug approval process section). The impurities are normally classified in organic impurities (by-products, degradation products or reagents), inorganic impurities (catalysts, heavy metals or inorganic salts) and residual solvents. However, more complex classifications can be found in the literature. These classifications include categories such as enantiomeric impurities, polymorphic forms and exogenous contaminants that get into the drug from sources that are external to the process.[98–100]
The report required to obtain the drug testing or commercialization authorization must contain information about the actual and the potential impurities that can arise during the drug synthesis or storage. For the preparation of this report, it should be considered that the used raw materials, reagents and solvents may have associated non-desired substances and also, that the own solvents or reagents used during the synthesis may constitute an impurity into the final product. For the identification of those impurities that can arise as degradation products of the API during its storage, information obtained from stress tests must be included. Moreover, all the derivatives that could be formed from the transformations that the evaluated impurities may suffer in the different steps of the synthesis must be considered as potential impurities. The study where all the transformations that can suffer the process impurities during the different steps of the synthesis are considered is called the carry over study of impurities. The applicant should also describe into the presented report all the laboratory studies that have been done in order to detect impurities in the final product.

1.6.1.2. Types of impurities and permitted limits

As it is mentioned above, the impurities are normally classified in organic impurities, inorganic impurities and residual solvents. In this section are described the main features of each group of impurities and the limits stablished for each of them.

- Inorganic impurities: in the case of the inorganic impurities they are normally detected and quantified using procedures described by the international council for harmonisation of technical requirements for pharmaceuticals for human use (ICH) guidelines. The maximum amount allowed of each of them in a drug is stablished base on its PDE (permitted daily exposure) value. The PDE values define the maximum daily intake permitted for a determined substance. These limits are based on the ICH standards that rely on known safety data.

- Residual solvents: this type of impurities is typically determined by chromatographic techniques such as gas chromatography, the method used for the residual solvent determination must be described by the ICH guidelines as in the case of inorganic impurities. However, if there is not an appropriate method available, the applicant can select the most suitable validated analytical method for the required application. The residual solvents are classified in four classes depending on its hazardousness:

    a) Class 1 solvents (solvents to be avoided) they have an unacceptable toxicity and/or may generate severe environmental hazards. Benzene, tetrachloromethane and 1,1-dichloroethylene are examples of class 1 solvents.

    b) Class 2 solvents (solvents to be limited) are those suspected to have significant but reversible toxicities. In this category solvents such as ACN, DCM, toluene or *N,N*-dimethylformamide are included.

    c) Class 3 solvents (solvents with low toxic potential) they have low short-term toxic potential to the human beings. Nevertheless, the long-term toxicity for many of them is not well known. For this kind of solvents, amounts in the final drug which represent a daily intake of 50 mg or less are allowed without justification. Additionally, higher limits are accepted in some cases if is demonstrated that the existing manufacturing capabilities does not allow to achieve lower levels in the final product. EtOH, acetic acid and pentane are examples of class 3 solvents.

    d) Class 4 solvents, they are substances for which no adequate toxicological data exists. Trifluoroacetic acid or 2,2-dimethoxypropane are considered class 4 solvents. The presence of these substances in the drug or API must justified by the candidate in front of the regulatory authorities.

  Similar to the inorganic impurities, the limit at which each residual solvent that can be present into a drug is defined according to its PDE value. It must be noted that there are some class 1 solvents such as 1,1,1-trichloroethane that have higher PDE values that other solvents belonging to the class 2. This is because 1,1,1-trichloroethane is classified as a class 1 since it represents an environmental hazard and not because of its toxicity for the human beings. Therefore, the maximum daily intake allowed for this substance is higher than from some class 2 solvents.[98,101]

- Organic impurities: for the organic impurities, as it can be seen in Table 11, the ICH guidelines define three different thresholds (reporting, identification and qualification respectively). The impurities below the reporting threshold should not be described. All those impurities present at levels between the reporting and the identification limits had to be summed and reported as total unknown impurity content. The contaminants found between the identification and the qualification thresholds must be reported

individually identifying its structure. Finally, those impurities above the qualification limit must be qualified.

If the structure of an impurity that must be identified cannot be determined or, if the impurity must be qualified additional studies are required. The performed studies must ensure that this compound does not represent a hazard to the patients at the levels at which is present in the final drug. These studies are quite expensive, for this reason normally they are performed only when it is not possible to reduce the amount of impurity below the established limits.

Maximum daily dosage of the drug	Reporting threshold	Identification threshold	Qualification threshold
≤2 g/day	0.05 %	0.10 % or 1 mg per day intake (whichever is lower)	0.15 % or 1 mg per day intake (whichever is lower)
>2 g/day	0.03 %	0.05 %	0.05 %

Table 11. Thresholds for impurities in APIs proposed by ICH and expressed in percentage of weight.[98]

The compounds with high toxicity constitute an exception to the above presented rules; this kind of impurities must be identified and quantified at levels lower than the identification threshold. The impurities with high toxicity that must be sought in the produced drug or API are determined through the study of the toxicity of all the compounds described in the carry over study of impurities. The toxicity of this kind of compounds is determined by consulting known safety data or is predicted based on quantitative structure–activity relationship (QSAR) models using software tools such as QSAR toolbox or VEGA QSAR. These *in-silico* studies allow to estimate the toxicity of a compound through the comparison of its structure with the structure of other molecules that have known toxicities.

In the case of generic drugs an impurity profile of the product has been already defined by the company who initially patented it. The impurity report prepared by the drug innovator to obtain the approval of the regulatory authorities defines which impurities must be identified and which ones may be present at levels higher than the qualification threshold. To avoid complex qualification or characterization studies, when a company starts the manufacturing of a generic drug, the impurity profile of its product must be as similar as possible to the one already described by the drug innovator. Only in case of finding a new impurity or an impurity above the limits of the innovator's specifications the previously detailed criteria must be applied.

The organic impurity levels must be determined by using validated analytical methods. This type of impurities is identified and quantified by comparing the analytical response obtained from the product analysis with the response observed from a standard of the impurity or of the product itself if the relative response factor (RRF) is known. Due to the necessity of standards of the impurities, the synthesis and characterization of this kind of compounds is a usual practice in the pharmaceutical industry. The synthesized impurities make possible to identify/quantify them in the different batches of API produced, allowing to comply with one of the main requirements needed for the API testing or commercialization. It must be seen that in the case of API's, the limits established for each impurity are based on the customer requirements. In this case, the customers are companies that are dedicated to formulating drugs containing the

mentioned API's and request for specifications that normally are equal or even lower to the ones described by the ICH guidelines.

1.6.2. Impurities in DH0517

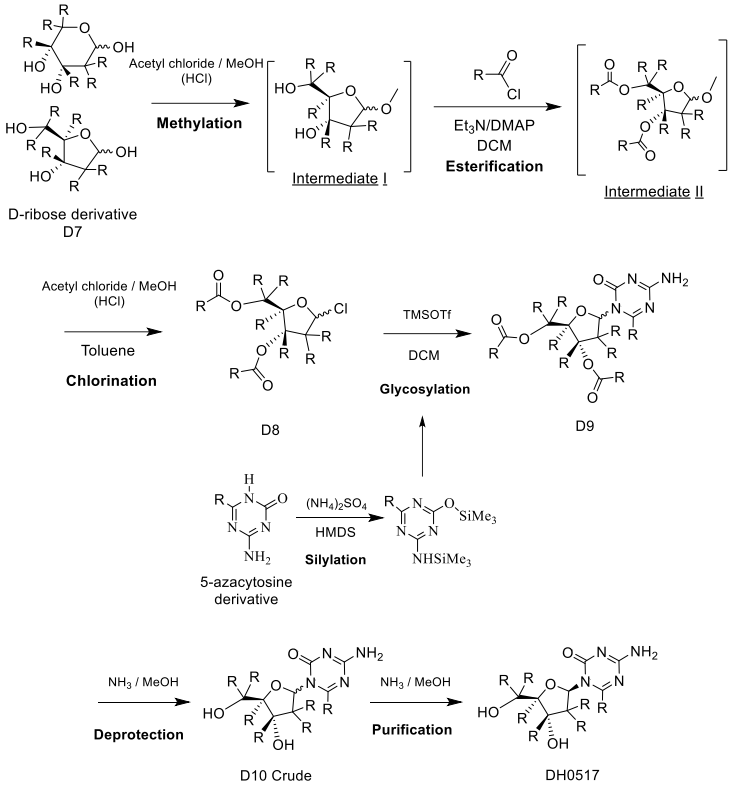
For **DH0517**, which is a generic drug, the study of some of the process impurities was undertaken since they were not reported in the already accepted innovator's impurity profile and were present in the HPAPI obtained at Farmhispania S.A. at levels above or near the identification threshold. For the non-commercially available substances the studies were performed to obtain standards of these compounds in-house. These studies included the development of suitable methods for the obtention of a pure sample of the impurity and the full characterization of the obtained compound (structural elucidation, calculation of RRF *vs.* **DH0517** and HPLC purity determination). The mentioned studies were also used to confirm that, using the available analytical methods there was no possibility of having impurities in the obtained product not detected or underestimated because of its RRF.

The investigations related with the process impurities presented in the following sections of this thesis allowed to demonstrate to the customers and to the regulatory authorities that the quality of the HPAPI being produced at Farmhispania S.A. is well controlled since all the impurities are being properly identified and quantified.

1.6.3. Furanose/pyranose forms and anomeric configuration study

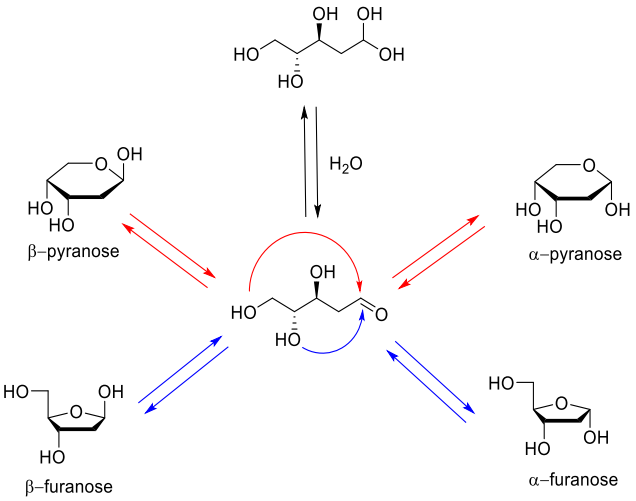
1.6.3.1. General concepts

As it has been mentioned in the previous sections, the synthetic route developed by Farmhispania S.A. to prepare **DH0517** uses a D-ribose derivative as starting material (Scheme 18). This substance may exist in different forms and this characteristic behavior may lead to the formation of different impurities.



Scheme 18. Synthetic route developed at Farmhispania S.A. for the DH0517 preparation.

The D-ribose and its derivatives may exist as a five-membered ring (furanose) a six-membered ring (pyranose) and also, in open chain form. There is an additional form that must be considered in aqueous medium which is the open chain hydrated form. In solution, all the mentioned forms are in equilibrium. The position of this equilibrium depends on the solvent, the temperature and the nature/number of substituents of the studied carbohydrate. Moreover, cyclic forms may appear as α and β-anomers depending on the configuration of the anomeric center. In solution, the α and β-anomers are also in equilibrium through the open ring form (Scheme 19).[102–104]



Scheme 19. Equilibria between 2-deoxy-D-ribose forms. These equilibria may be also observed for the studied D-ribose derivative.

It is known that for the cyclic structures, the acetal formation allows to lock the ring opening. This phenomenon has been studied through density functional theory (DFT) pathways by other authors. The mentioned studies indicated that the transition states involved in the ring opening mechanism of methylated sugars were considerably more energetic.[105] It can be concluded from the study that the ring opening is avoided if the C1 hydroxyl group is derivatized unless a functional group capable to donate hydrogen is introduced (-SH, -NHR or -NH_2 among others).[105]

The results reported in the literature indicate that in solid state, the D-ribose derivative is in its β-pyranose form.[106–108] However, in solution there is an equilibrium between all the previously mentioned forms. The time required to reach this equilibrium depends on the solvent. For example, in water the equilibrium is reached in 15 min., in MeOH/EtOH it takes around 4 h to reach the steady state and in pyridine near to 30 h are required.[109]

In different published nuclear magnetic resonance (NMR) studies performed for D-ribose derivative in water solutions at different temperatures it was demonstrated that the pyranose forms are more abundant than the furanose forms and that the open chain form is so scarce that it is not detected (Table 12).[106,110,111]

Temperature	β-Pyranose	α-Pyranose	β-Furanose	α-Furanose	Aldehyde
90 °C	30	30	22	18	--
30 °C	35	40	12	13	--
0 °C	42	43	5	10	--

Table 12. Ratio of D-ribose derivative forms in water solutions in equilibrium at different temperatures.[106,110,111]

From the collected data it can be concluded that the amount of furanose forms increases at higher temperatures.

The prevalence of the pyranose forms can be justified taking in to account that six membered saturated rings present lower structural tensions and, therefore, higher stability than other cyclic structures. Moreover, the substituents in the six membered rings are more staggered in comparison with its situation in other cyclic arrangements allowing to reduce steric hindrance.[111] When functional groups are removed from the basic structure the amount of furanose forms increases due to the decrease of the steric hindrance between substituents. This effect is especially important when the removed functional groups are bulky, although when the removed functional groups are small (e.g. hydroxyl groups) some furanose stabilization is observed. [103,111]

It has also been reported by other authors that the amount of the furanose forms increases in organic solvents and if the carbohydrate hydroxyl groups are derivatized. These experimental observations were related with the fact that pyranoses are more efficiently solvated by water through hydrogen bonding than furanoses. When the number of hydrogen bonds formed is reduced, the difference in stability between pyranoses and furanoses decreases and, therefore, the amount of five membered ring forms increases.[103,111,112]

It is important to consider that the pyranose:furanose ratio can be also modified by the ionic content of the studied solution. The presence of some cations may favour the formation of the forms that strongly interact with the charged species. Some Fe (III)/2-deoxy-D-ribose complexes where the monosaccharide acts as bidentate ligand in its pyranose and furanose forms have

been described by other authors (Figure 57). In this case the presence of Fe (III) ions favoured the formation of furanose forms. It is also known that the anomeric ratio is strongly influenced by the C2 substituent of the studied carbohydrate.[111,113]

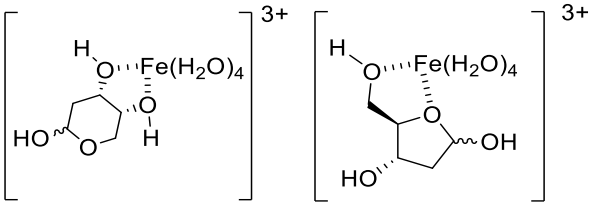
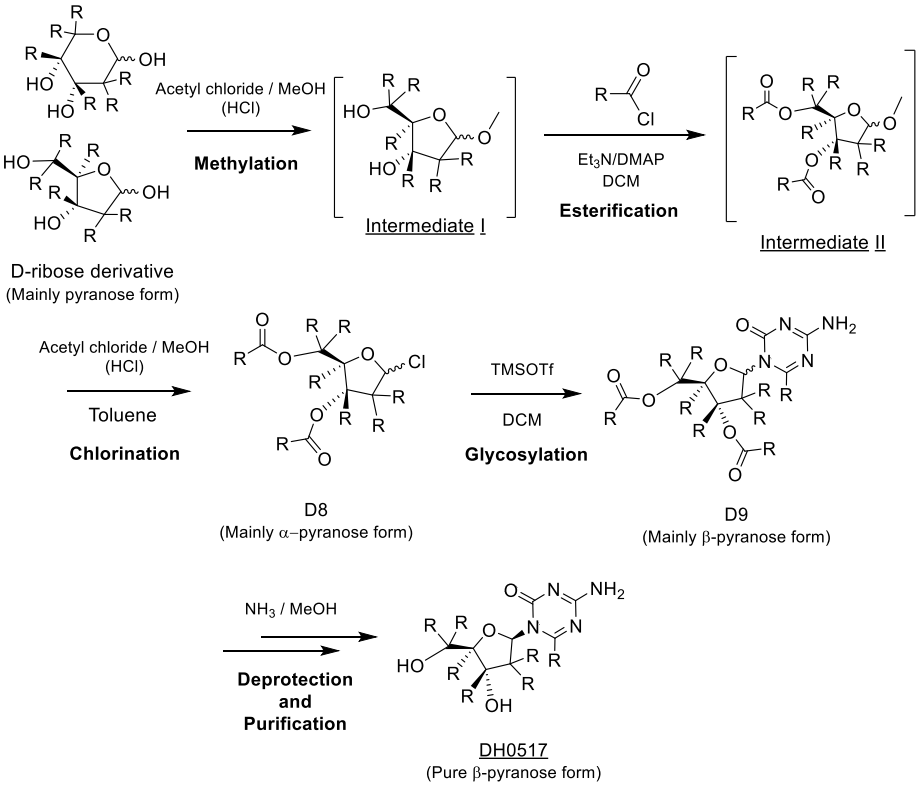


Figure 57. Fe (III)/2-deoxy-D-ribose complexes.

1.6.3.2. Objective

The study presented here aims to demonstrate that the **DH0517** obtained using the manufacturing method developed at Farmhispania S.A. is pure β-furanoside and does not contain impurities derived from the different structures that carbohydrates may adopt. In the **DH0517** synthetic route adopted by Farmhispania S.A. (Scheme 20), the first solid product isolated is compound **D8** because methylation, esterification and chlorination are carried out without isolation of the corresponding intermediates.



Scheme 20. Synthetic route developed at Farmhispania S.A. for the DH0517 preparation.

In the **DH0517** synthetic route presented in Scheme 20 the equilibrium between the different forms is locked from the first step and the formation of additional amounts of pyranose/ring opening impurities is not expected after methylation reaction. For this reason, this study is focused on the characterization of the starting material, the first isolated intermediate in the synthesis (**D8**) and the final product (**DH0517**).

1.6.3.3. D-ribose derivative study

The D-ribose derivative provided by Carbosynth (batch: 1572.60662) which was the industrial supplier of Farmhispania S.A. was analysed through NMR. Its ^{13}C, ^{1}H, COSY, HSQC, DEPT and HMBC-NMR spectra were collected at 25 °C using deuterated MeOH as solvent. See the obtained results in the **DH0517** project experimental section.

MeOH was selected because it is the solvent used in the methylation reaction performed in the first step of the **DH0517** synthesis. The use of MeOH allowed studying the amounts of pyranose/furanose forms present in equilibrium just before starting the reaction.

Signals from the α and β-anomers of the pyranose and furanose forms were observed in the collected D-ribose derivative spectra. The signals were assigned using 1D and 2D NMR experiments. The site of attachment leading either to a pyranose or to a furanose ring was corroborated by HMBC experiments. The two lower intensity set of signals showed interactions between C4 and H1 confirming the furanose structure while the two higher intensity set of signals displayed interactions between C5 (CH_2) and H1 corroborating the pyranose structure (Figure 58).

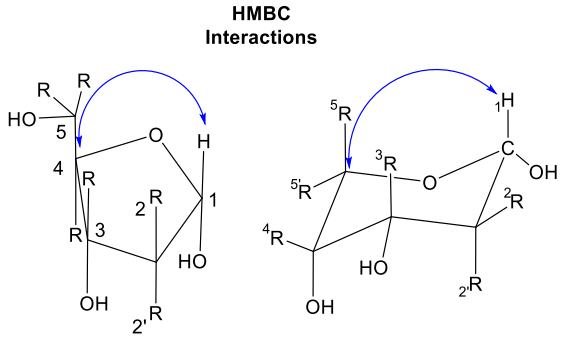


Figure 58. Diagnostic HMBC interactions between C4 (CH) and H1 observed in furanose; interaction between C5 (CH_2) and H1 observed in pyranose.

From the intensities of the four sets of signals observed in the ^{1}H NMR spectrum of the studied sample it was concluded that, 82 % of the population was in the pyranose form (63 % β-anomer and 19 % α-anomer) and that the remaining 18 % was in the furanose form (1:1 ratio of α and β-anomers). The differences observed in the population distribution compared with the one adopted when water is used as solvent (see Table 12) were related to the ability of the studied solvents to solvate and stabilize the different forms involved in the equilibrium.[106,111]
The collected spectra, compared well with the results reported by other authors and indicated that the main form in the studied conditions was the pyranose form.[106,108] Additionally, the D-ribose derivative was characterized using alternative analytical techniques such as specific optical rotation, HPLC and IR spectroscopy. The obtained results confirmed the identity of the studied compound since they were in agreement with the supplier specifications and data obtained from the literature (see the **DH0517** project experimental section).[109,114–116]

1.6.3.4. D8 intermediate study

In order to study the structure of the **D8** intermediate the ^{13}C and ^{1}H NMR spectra of three different samples obtained at industrial scale were collected (see the **DH0517** project

experimental section). The experiments were carried out at 25 °C using $CDCl_3$ as solvent. (batches used: 407.170001.01.01; 407.170002.01.01 and 407.170003.01.01)

The collected spectra compared well with already published data.[117,118] The spectra were almost identical for the three analysed batches confirming that there were no relevant differences between products and that the developed process yielded **D8** with the same structure and impurity profile in a reproducible way. The structure of the **D8** intermediate was also studied through different 2D NMR experiments. In this case COSY, HSQC, HMBC and NOESY experiments were performed (see the **DH0517** project experimental section).

The **D8** connectivity was determined through an HMBC experiment. In the analysed sample interaction between the carbon C4 and the proton H1 was observed confirming the furanose structure (Figure 59).

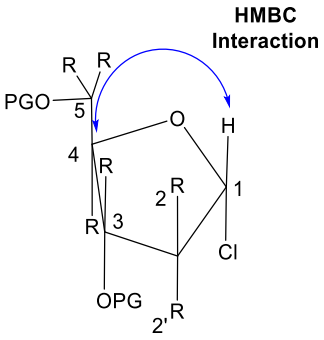


Figure 59. Interaction between C4 and H1 observed in the HMBC experiment. Protective groups abbreviated as -OPG.

No other signals corresponding to the pyranose or the open chain forms could be detected in the ^{1}H and ^{13}C NMR spectra. Considering the limit of detection of the used spectrometer it can be concluded that the **D8** synthesised at Farmhispania S.A. does not contain pyranose/ring opening impurities above the 1.0 %.

The configuration of the anomeric centre of the main form present in the **D8** was established by means of a NOESY experiment. In our case, interaction between proton H1 and proton H3 (described as R- in all the presented figures) was observed indicating that both protons are on the same face of the furanose ring (Figure 60). Therefore, it was concluded that the main anomer in the studied samples was the α one.

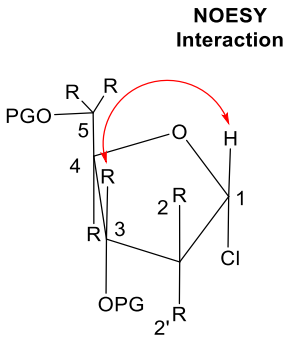
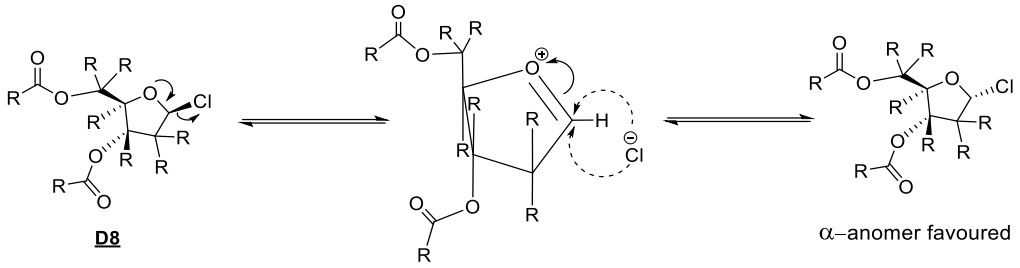


Figure 60. Interaction between H3 and H1 observed for the α-anomer of D8 in the NOESY experiment. Protective groups abbreviated as -OPG.

The exact anomer ratio could not be determined since different ratios were observed when the same **D8** sample was analysed at different times. For freshly prepared samples, α:β ratios close to 5:1 were observed but the amount of α-anomer increased with time. Since degradation by-products were not observed in the collected spectra the anomer ratio variations were related with the proposed interconversion reaction (Scheme 21). It is suggested that the nucleophilic attack of the chlorine atom over the cationic intermediate that leads to the formation of the mentioned α-anomer is favoured.



Scheme 21. Proposed interconversion reaction between α and β D8 anomers.

Although **DH0517** presents β configuration, the observed anomer ratio was not considered a problem since it was known that the anomer ratio could be modified in the Vorbrüggen glycosylation reaction and, that the residual α-anomer could be purged in the final steps of the synthesis.

Considering the good yield of the first three steps of the synthesis (73 %, Scheme 20), it can be concluded that in the methylation reaction, the furanose forms of the intermediate I are mainly formed. Some examples can be found in the literature where it is reported that that D-ribose derivative methylation reaction using MeOH as solvent can be directed towards the furanoside or pyranoside depending on the used synthetic conditions (Table 13).[114,119]

Test	MeOH volumes	Temperature	HCl concentration	Product obtained
Example 1	18.3	25 °C	1 %	Pyranoside
Example 2	16.8	15 °C	0.1 %	Furanoside
Farmhispania S.A. conditions	>17	>15 °C	0.05-0.5 %	Furanoside

Table 13. Summary of the results obtained from the methylation reaction depending on the synthetic conditions applied.[114,119]

1.6.3.5. DH0517 study

The structure of a **DH0517** sample taken from an industrial scale batch was determined using different NMR experiments (^{1}H, ^{13}C, COSY, DEPT, HMBC, NOESY and HSQC). The analyses were performed at 25 °C using deuterated dimethyl sulfoxide (DMSO-d_6) as solvent. From the study of the collected spectra it was concluded that the analysed sample was the β-furanose form. As it can be seen in the **DH0517** project experimental section, the presented NMR results were in accordance with the proposed structure and with data from the literature.[120,121] In the HMBC experiment, interactions between C4'/H1' and C1'/H4' (described as R- in all the presented figures) were observed confirming the furanose structure (Figure 61). Taking in to account the limit of detection of the used spectrometer it can be assured that, the **DH0517** synthesised at Farmhispania S.A. does not contain pyranose/ring opening impurities above the 1.0 % as signals or interactions attributable to this kind of compounds were not observed in the NMR spectra.

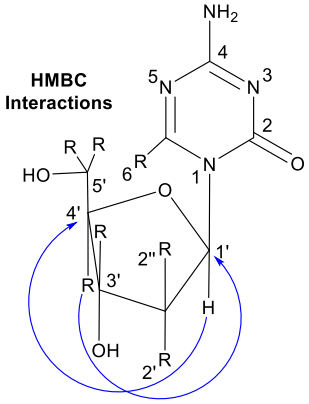


Figure 61. Interactions between C4'/H1' and C1'/H4' (described as R- in all the presented figures) observed in the HMBC experiment.

The NOESY experiment showed interaction between proton H6 and protons H3'; H5'; H5''; OH5' and H2'' (Figure 62) (most of the mentioned protons are described as R- in all the presented figures). The observed NOESY interactions are all in accordance with the β-anomer orientation previously suggested. In the studied sample, signals that could be assigned to the α-anomer were not observed. Therefore, it can be assured that the **DH0517** obtained using the synthetic procedure developed by Farmhispania S.A. does not contain the α-anomer above the 1.0 % (limit of detection of the used spectrometer).

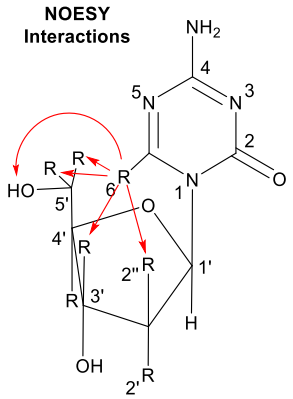


Figure 62. Interactions of proton H6 with H3'; H5'; H5''; OH5' and H2'' protons observed in the NOESY experiment. Most of the mentioned protons were described as R- in all the presented figures.

Additionally, an exhaustive characterization of the synthesised **DH0517** was carried out using a wide range of different analytical techniques such as infrared spectrometry (IR), X-ray powder diffraction (XRPD), mass spectrometry (MS) and HPLC (see the **DH0517** project experimental section). The obtained results compared well to the results obtained from standards and with data from the literature. Special mention requires the analysis performed using single-crystal x-ray diffraction that allowed unequivocal structural characterization of the molecule and confirmed that the product was not a solvate or a salt. All the performed analyses were in agreement with the NMR elucidation and confirmed that the compound obtained from the synthetic route developed at Farmhispania S.A. was pure **DH0517** (β-furanose form).[49–52,122–125]

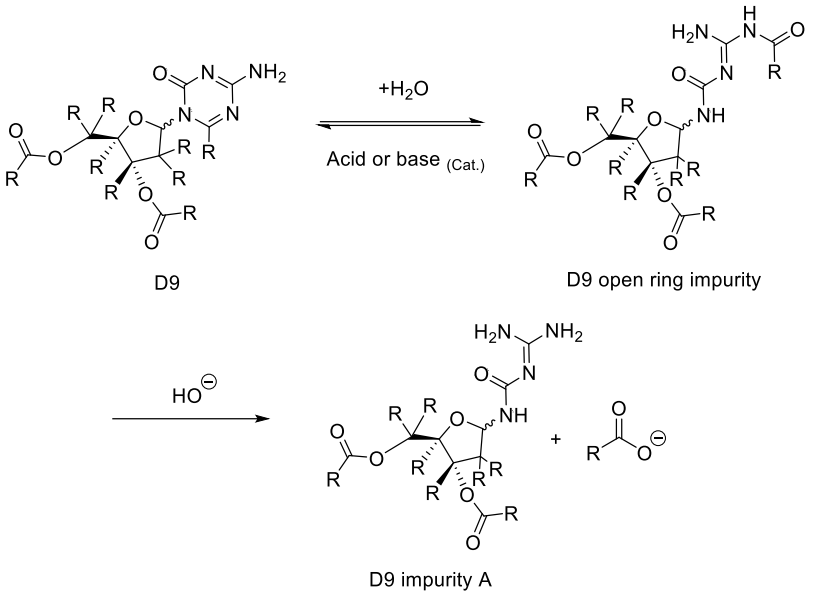
1.6.3.6. Conclusions from the furanose/pyranose forms and anomeric configuration study

- D-ribose derivative mainly adopts pyranose structure when it is dissolved in MeOH.
- The equilibrium between pyranose and furanose forms is locked from the methylation reaction.
- The **D8** intermediate obtained using the synthetic route developed at Farmhispania S.A. has furanose structure. The α:β-anomer ratio of the synthesised intermediate is around 5:1 but in chloroform solutions it varies over the time.
- The observed anomer ratio in the **D8** samples is not a problem since the anomer ratio is modified in the subsequent Vorbrüggen glycosylation reaction. Eventually, the residual α-anomer is purged in the final steps of the synthesis.
- It was determined by NMR spectroscopy that the pyranose and open chain impurities are below the limit of detection (1.0 %) in the isolated **D8** intermediate.
- Pyranose or open chain form impurities were not detected in the final product (**DH0517**) in any of the analytical techniques used to study it.
- It was confirmed that the **DH0517** obtained using the synthetic route developed at Farmhispania S.A. is the desired β-furanose.

1.6.4. D9 impurity A study

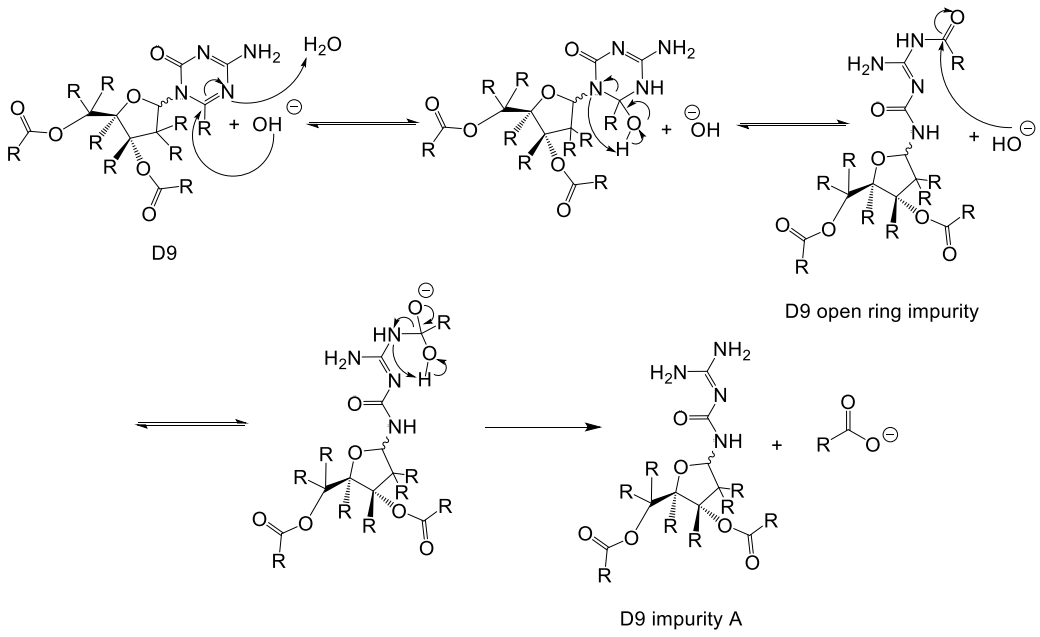
1.6.4.1. General concepts

An impurity was observed at levels around 0.7 % in the LC analyses of the **D9** samples isolated during the study of the glycosylation reaction. This substance was identified as **D9 impurity A** which was generated due to the hydrolysis of the **D9** open ring impurity in presence of base (see Scheme 22). Several examples were found in the literature where this degradation pathway was studied in compounds similar to **D9**. [49–52,95]



Scheme 22. D9 degradation pathway that leads to the formation of the D9 impurity A. In the presented scheme base is abbreviated as B.

As it can be seen in Scheme 22, the first step of the **D9** intermediate decomposition is the reversible nucleophilic attack of a hydroxyl molecule over the C-6 atom of the 5-azacytosine derivative ring. The attack at this position is coherent with the results obtained from quantum chemical calculations found in the literature.[95] These calculations indicated that this position is the more susceptible to a nucleophilic attack since the electron density of this carbon atom is lower. Eventually, the irreversible hydrolysis of the **D9** ring opening impurity formed furnishes **D9 impurity A**. The suggested mechanism for the **D9 impurity A** formation is presented in Scheme 23.[49]



Scheme 23. Suggested mechanism for the formation of the D9 impurity A.

The existence of an equilibrium between the **D9** and the **D9** ring opening impurity was confirmed during the experiments performed to isolate this intermediate. In these experiments, an

aqueous solution enriched in **D9** ring opening impurity was obtained using medium pressure liquid chromatography (MPLC). During the distillation of the solution enriched in **D9** ring opening impurity obtained from the MPLC it was observed that the HPLC impurity peak decreased and, that **D9** was formed. This behavior was attributed to the shift towards the **D9** side of the equilibria as a consequence of the water removal during the solvent stripping process (see Scheme 23).
According to the literature, for Decitabine the complete hydrolysis takes place in basic aqueous solutions while, at neutral or acid pH the ring opening impurity is formed but no Decitabine **impurity A** analogue is observed (other unknown impurities are formed instead). For 5-azacytidine a similar behavior is observed.[49,52,95]
Similarly to Decitabine, the **D9 impurity A** was not formed in acid or neutral solutions of this substance even heating to 40 °C. The impurity was only obtained in relevant amounts when basic solutions of **D9** were heated to 40 °C during extended periods of time.

1.6.4.2. Objectives

The process impurity was detected at significant levels in the **D9** intermediate manufactured according to the procedure developed at Farmhispania S.A. The elucidation of the structure of this substance was required in order to determine the potential impurities that may arise in the final product derived from its carry over (see section 1.6.1). The carry over study of this impurity was necessary to justify that no highly toxic substances that must be controlled at lower levels that the ones described by the ICH guidelines (Table 11) are being formed during the process. Additionally, the obtention of an analytical standard of this substance was needed to properly identify it in the LC analyses of further **D9** batches. This standard would be used to confirm that the observed LC peak is related to the mentioned compound.

This study describes all the experimental and analytical work done in order to isolate and characterize the **D9 impurity A**.

1.6.4.3. D9 impurity A obtention

In order to characterize the **D9 impurity A**, a pure sample of this substance was required. In this sense, 5 g of a **D9** sample with a 75.9 % purity of β-anomer were used as starting material. The experiment was monitored through HPLC, using the analytical method developed and validated by the Analytical Development Department of Farmhispania S.A. for the analysis of **D9**.

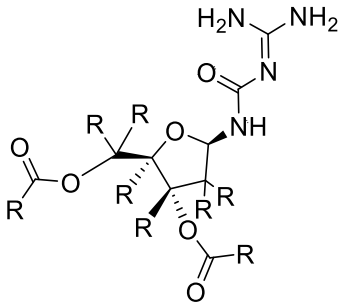
The **D9** intermediate was suspended in 10 volumes of 0.24 M $NaHCO_{3\,(aq)}$ (1.1 equivalents). The slurry was heated to 44 °C for 4 days, the solid obtained from the filtration of the mixture was enriched in **D9 impurity A**. Its HPLC analysis showed a 73.0 % purity. The obtained sample was purified through MPLC (Figure 63) and, finally, a solid with an HPLC purity of 96.0 % was obtained.

Figure 63. MPLC apparatus Puriflash 450 from Interchim used in Farmhispania S.A.

The selected experimental conditions were based on the results obtained from the **D9** stability studies described above. Sodium bicarbonate was chosen as base for the obtention of **D9 impurity A** because stronger bases could promote the hydrolysis of the protective groups of the molecule leading to undesired degradation by-products. This base was also preferred because it was already used in the synthesis of **D9**, where, small amounts of this impurity are formed.[126]

1.6.4.4. D9 impurity A characterization

The structure of the obtained **D9 impurity A** sample was determined using different NMR experiments (^{1}H, ^{13}C, COSY, DEPT, HMBC, NOESY and HSQC). The analyses were performed at 25 °C using CD_3OD as solvent. From the study of the collected spectra it was concluded that the analysed sample had the structure presented in Figure 64; it was the β-anomer with furanose form of the **D9 impurity A**.



D9 impurity A

Figure 64. D9 impurity A molecular structure.

As it can be seen in the **DH0517** project experimental section, the presented NMR results were in accordance with the proposed structure and with the literature.[51,52] The absence of signals from the amide group present in the **D9** ring opening impurity presented in Scheme 23 confirmed the suggested structure.

In the HMBC experiment, carbon C4'/proton H1' and proton H4'/carbon C1' interactions were observed. These interactions were in accordance with the proposed furanose structure (Figure 65). An interaction proton H1'/carbon C2 was also observed confirming the atom connectivity

proposed in Figure 65. Note that most of the mentioned protons were described as R- in all the presented figures.

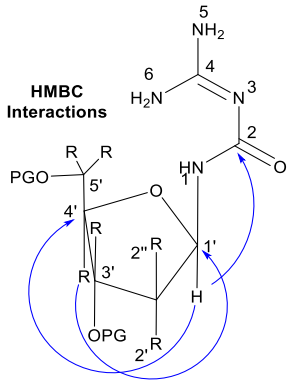


Figure 65. Interactions between C2/H1'; C4'/H1' and C1'/H4' observed in the HMBC experiment (most of the mentioned protons are described as R- in all the presented figures).

The NOESY experiment showed interaction between proton H1' and proton H4' (Figure 66). The observed NOESY interaction was in accordance with the β-anomer orientation previously suggested (most of the mentioned protons are described as R- in all the presented figures).

No other signals corresponding to the pyranose or the α-anomer forms could be detected in the ^{1}H and ^{13}C NMR spectra. Considering the limit of detection of the used spectrometer it can be concluded that the **D9 impurity A** isolated at Farmhispania S.A. does not contain pyranose or α-anomer forms above the 1.0 %.

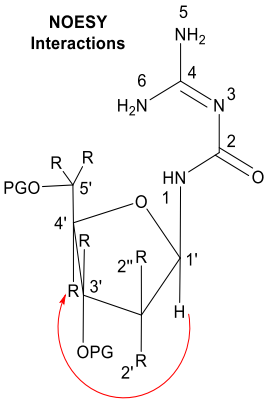


Figure 66. Interaction of proton H1' with H4' proton observed in the NOESY experiment (most of the mentioned protons are described as R- in all the presented figures).

Additionally, the isolated **D9 impurity A** was analysed through MS (see **DH0517** project experimental section). The obtained results were in agreement with the NMR elucidation and confirmed that the compound obtained from the synthetic route developed at Farmhispania S.A. was **D9 impurity A**.

1.6.4.5. Conclusions from the D9 impurity A study

- **D9** intermediate shows the same behaviour that other nucleoside analogues. It tends to degrade to its hydrolysed derivative in basic aqueous solutions.

- **D9** enriched in its β-anomer was aged in basic aqueous media to afford a solid enriched in **D9 impurity A** impurity. The obtained solid was purified through the use of MPLC to obtain a pure sample of **β-D9 impurity A** in its furanose form.

- The NMR analyses of the obtained **D9 impurity A** sample confirmed that the isolated solid was the β-anomer of the **D9 impurity A** in its furanose form.

- No signals corresponding to the pyranose or the α-anomer forms of the impurity were detected in the performed NMR analyses. Considering the limit of detection of the used spectrometer it can be concluded that the **D9 impurity A** isolated at Farmhispania S.A. does not contain pyranose or α-anomer forms above the 1.0 %.

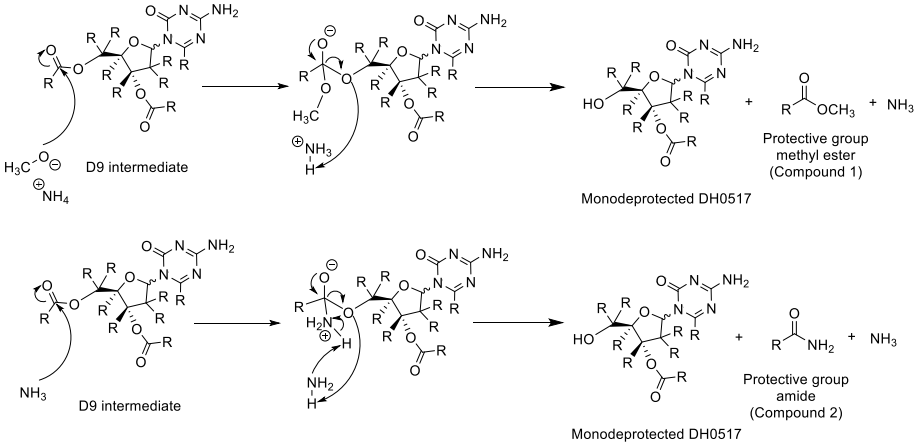
- The obtained MS results agreed with the NMR elucidation and confirmed that the compound obtained at Farmhispania S.A. was pure **D9 impurity A**.

- The obtained sample was used to generate the analytical standard required for the **D9 impurity A** analysis.

1.6.5. Monodeprotected DH0517 impurity study

1.6.5.1. General concepts

During the **D9** deprotection step presented in Scheme 24, the two protective groups of the **D9** intermediate were removed using an ammonia solution in MeOH. In the liquid chromatography (LC) analyses of the reaction crude the methyl ester (Compound 1; Scheme 24) and the amide (Compound 2; Scheme 24) of the protective group were detected. These substances were generated as by-products of the deprotection reaction and its formation is in accordance with the proposed deprotection mechanisms[9] (see Scheme 24).[127–129]

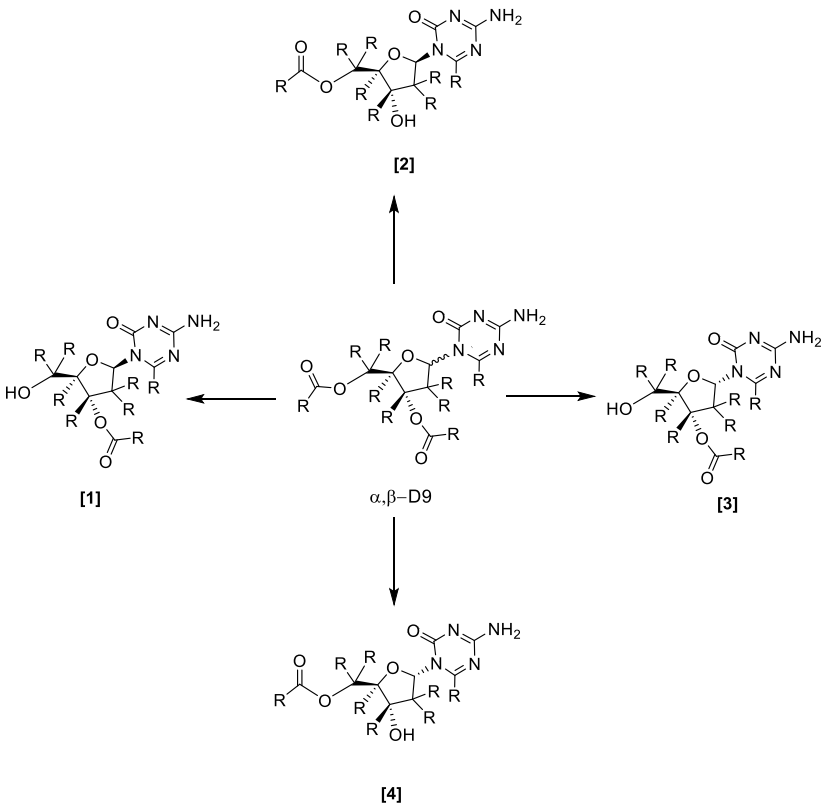


Scheme 24. Proposed mechanisms for the D9 deprotection reaction leading to the formation of the methyl ester of the protective group (compound 1; up) and to the formation of the amide of the protective group (compound 2; down).

[9] In the presented reaction mechanisms, the removal of one of the two **D9** protective groups is described. It is suggested that the removal of the second protective group follows the same reaction mechanism.

During the study of the **D9** deprotection it was observed that, in the reaction mixture the amount of the Compound 1 was several times higher than the amount of the Compound 2 (data obtained through HPLC analyses). These substances were identified and quantified using commercially available pure standards. The obtained results indicated that, under the applied reaction conditions the main occurring deprotection pathway was the one leading to the Compound 1 formation (upper part of the Scheme 24). The study of the reaction crude revealed that, at the used reaction conditions part of the generated Compound 1 was slowly converted in the Compound 2. This side reaction difficulted the precise determination of the extent of each of the presented mechanisms. However, the Compound 2 was observed from the beginning of the reaction at amounts not attributable to the mentioned slow interconversion reaction. The obtained results indicated that the mechanism described in the lower part of the Scheme 24 also takes place during the deprotection.

In the LC analyses of the reaction crude performed during the study and the development of the deprotection step, the formation and the consumption of an intermediate was observed. The mentioned intermediate, had a RT lower than **D9** but higher than **DH0517**. It was suggested that this intermediate was one of the **DH0517 monodeprotected** isomers (see Scheme 25) that was formed at early stages of the reaction from the partial deprotection of **D9** (Scheme 24). The **D9** synthesized at Farmhispania S.A. is enriched in its β-anomer but it still contains certain amounts of the undesired α-anomer. Therefore, from the deprotection reaction the formation of four different monoprotected intermediates could be expected depending on the configuration of the anomeric center and the protective group removed. The structures proposed for the mentioned intermediates are presented in Scheme 25.



Scheme 25. Suggested structure for the four possible monodeprotected intermediates that could be formed from D9.

Several examples were found in the literature where the formation of this kind of intermediates has been studied and described in deprotection and protection reactions carried out over **DH0517** analogues with ester protective groups.[130–135]

1.6.5.2. Objectives

The above mentioned deprotection intermediate was a potential impurity of **DH0517**. This compound was included in the **DH0517** specifications with a limit of 0.15 %. The specification was below the qualification threshold but above the identification threshold. According to the ICH criterion (see Table 11), this substance must be properly quantified and identified in the final product by means of an analytical standard.

The obtention and characterization of a pure sample of this substance was required in order to prepare the mentioned analytical standard. This analytical standard was necessary to confirm the identity of the peak observed in the HPLC analyses and to obtain its RRF *vs.* **DH0517** which is necessary for the quantification of the impurity in the manufactured HPAPI.

This study describes all the experimental and analytical work done in order to isolate and characterize the **D9** deprotection intermediate detected in the LC analyses of the deprotection rection crude.

1.6.5.3. Monodeprotected DH0517 obtention

In order to obtain a **monodeprotected DH0517** sample, a **D9** deprotection reaction was conducted using 92 g of **D9** intermediate. The starting material used had a 95.8 % purity and contained a 75.1 % of β-anomer. The HPLC method used to control the synthesis was developed and validated in-house by Analytical Development Department of Farmhispania S.A. to analyse the produced **DH0517**.

During the study of the deprotection reaction it was observed that the formation of the monodeprotected intermediate is faster than the complete **D9** deprotection. The **monodeprotected DH0517** synthesis was carried out using the typical process conditions employed in the **DH0517** synthesis, based on the use on an ammonia solution in MeOH. However, in this case, DMSO was added to the reaction crude. During the development of the process it was observed that **DH0517** presents a much higher solubility in DMSO than the monodeprotected intermediate. It was also known that the deprotection reaction tends to stop leading to the formation of important amounts of monodeprotected intermediate when it is performed using MeOH solutions with low ammonia concentrations. The addition of DMSO allowed to decrease the ammonia concentration in the reaction crude and to dissolve the **DH0517** formed from the complete **D9** deprotection. This favoured the formation of higher amounts of monodeprotected intermediate and facilitated its isolation from the direct filtration of the reaction crude.

The reaction was monitored by LC, after 45 h the reaction crude analysis showed a 30 % of the intermediate peak attributed to **monodeprotected DH0517**. At this point the obtained slurry

was cooled to 25 °C and filtered. The isolated solid was washed with MeOH and was dried under vacuum to afford 11 g of **monodeprotected DH0517** (Yield: 17 %).

Due to the aromatic ring of its protective group, the **monodeprotected DH0517** has a much higher absorption coefficient than **DH0517**. Because of its higher RRF a low amount of this substance in the reaction crude leaded to a 30 % peak in the LC analysis performed just before the slurry filtration. Therefore, the low observed yield was related with the high RRF of **monodeprotected DH0517**.

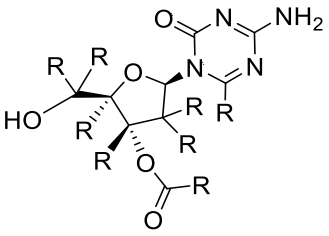
From the LC analysis of the isolated solid it was found that it was the target deprotection intermediate with a 98.3 % purity. In the analysis of the isolated sample, **DH0517** was observed at a 1.4 %.[10]

1.6.5.4. Monodeprotected DH0517 characterization

The structure of the obtained **monodeprotected DH0517** sample was determined using different NMR experiments (^{1}H, ^{13}C, COSY, NOE and HSQC). The analyses were performed at 25 °C using DMSO-d6 as solvent.

Due to the low solubility of the **monodeprotected DH0517** it was not completely dissolved under the test conditions (30 mg in 0.8 ml DMSO-d6). In comparison, **DH0517** shows a much higher solubility in DMSO than the monoprotected intermediate and, therefore, the analysed solution was enriched in this substance. Consequently, signals attributable to **DH0517** could be observed in the collected NMR spectra of the prepared sample.

From the study of the collected NMR spectra (see **DH0517** project experimental section) it was concluded that the analysed sample had the structure presented in Figure 67 (it was β-anomer with furanose form and, deprotected at position 5').



Monodeprotected DH0517

Figure 67. Proposed structure for monodeprotected DH0517.

NOE experiments selectively irradiating at protons H1', H3', H5' and H6 were performed. The interactions observed in the performed experiments were all in accordance with the proposed structure. For example, interactions between proton H6 and protons H2''; H5'; H5''; OH5' and H1' were observed (Figure 68). As it is mentioned above in all the cases the observed NOEs were

[10] Due to the mentioned differences in the absorption coefficient of **monodeprotected DH0517** and **DH0517** the %w/w of **DH0517** in the isolated sample is expected to be higher.

the expected ones for the β-anomer of the **monodeprotected DH0517** (deprotected at position 5'). Note that most of the mentioned protons were described as R- in all the presented figures.

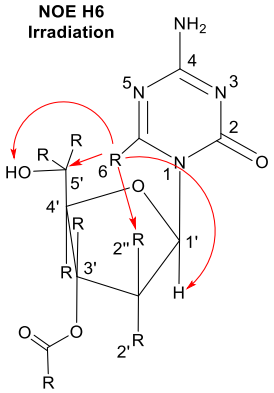


Figure 68. Interactions of proton H6 with H1'; H5'; H5''; OH5' and H2'' protons observed after irradiation of proton H6 (most of the mentioned protons were described as R- in all the presented figures).

In the performed NMR experiments, signals that could be assigned to the α-anomer of the molecule were not observed. Therefore, it can be concluded that the **monodeprotected DH0517** obtained does not contain relevant amounts of the mentioned α-anomer.

Additionally, an exhaustive characterization of the obtained **monodeprotected DH0517** sample was carried out using IR, MS and HPLC (see **DH0517** project experimental section). The performed analyses agreed with the NMR elucidation and confirmed that the compound obtained was pure **monodeprotected DH0517** (β-furanose form).

1.6.5.5. Conclusions from monodeprotected DH0517 study

- In the IPCs of the **D9** deprotection reaction of the **DH0517** synthetic process developed at Farmhispania S.A. the formation of an intermediate was observed. A pure sample of this intermediate was obtained and characterized through several analytical techniques.
- It was confirmed that the sample obtained is pure **monodeprotected DH0517** with β-furanose form and with its secondary hydroxy group already protected (Figure 67).
- Two deprotection reaction mechanisms were proposed. The proposed mechanisms agreed with the literature and with the experimental data. They allowed to justify the formation of the amide and methyl ester by-products observed in the reaction crude.
- The obtained sample was used as the analytical standard required for the **monodeprotected DH0517** analysis.

1.7. DH0517 project conclusions

- The main goal of this project has been achieved. A suitable method for the preparation at industrial scale of **D9** intermediate has been developed and validated within the requested timelines. The obtained **D9** had the quality required for its use in the manufacturing of **DH0517** at commercial scale.

- The **D9** synthetic method initially tested and based on the literature reference conditions was not suitable for the industrial scale operation. This method was systematically optimized to solve all the initially observed weaknesses and eventually a process for the obtention of the desired intermediate was obtained. Through laboratory scale experiments, the reaction conditions were studied and optimized in order to obtain the desired anomer ratio, conversion and purity. The w.up was also modified to adapt it to the limitations of the large scale equipment and reduce product loses. The optimized process allowed to reach at laboratory scale higher anomer ratio and yield (regarding the **β-D9** anomer) than the ones reported in the examined literature.

- During the development of the process a safety assessment was performed in order to guarantee that it was safe and that it could be scaled-up without risk of having a runaway event.

- The obtained process was successfully scaled-up to 118 kg. During the industrialization of the process different problems related with the limitations of the industrial equipment used and the scale increase were found (high ROI contents, drying problems or extended operation times). In front of the mentioned difficulties, series of laboratory scale experiments were performed. The objective of these tests was to determine the root causes of the mentioned problems and to find which modifications had to be applied over the initial process to avoid them.

- A decrease on the reaction selectivity was observed at the large scale batches. This result was related to the variations associated to the scale increase (slow solid loading). Despite the selectivity decrease, the yield obtained in relation to the **β-D9** anomer was equivalent to the highest yield found during the initial bibliographic search (62 %; entry 2 of Table 4).

- The study of the structure of the different process intermediates allowed to confirm that pyranose or open chain form impurities were not present in the **DH0517** obtained using the synthetic route developed at Farmhispania S.A. It was also confirmed that the product being manufactured is the desired β-furanose conformation.

- Two process impurities present in the **D9** intermediate and in the **DH0517** respectively were successfully isolated and characterized. The obtained samples were used to create the corresponding analytical standards required to guarantee an accurate control of the synthesis.

CHAPTER 5

FH0317 PROJECT

INDUSTRIAL PhD THESIS

PROCESS DEVELOPMENT FOR THE SYNTHESIS AT INDUSTRIAL SCALE OF ACTIVE PHARMACEUTICAL INGREDIENTS

Chapter 5

1. FH0317 project

1.2. Introduction

1.2.1. The cancer disease

Cancer is defined as a group of genetic diseases caused by the uncontrolled and unstopped division of some of the body cells. A particularity of this type of diseases is that, they may spread to other tissues causing metastasis. Cancer can start almost anywhere in the human body. Normally, the cells grow and divide to form new cells according to the body necessities. During the normal function of the organism, the new generated cells assume a specific function once they mature (structural, energy storage or reproductive). When they became old or are damaged, they die and are replaced by new cells. The cancer disease starts when the normal cell cycle is altered and therefore, new cells are produced when they are not needed. The produced cells, in contrast to normal cells, show lack of functionality. They are capable to survive and generate other cancer cells indefinitely even when they became old. The accumulation of unfunctional cells (solid tumours), the consumption of the body resources to produce these usefulness tissues and the induced displacement of the functional cells by cancer cells are responsible of most of the cancer symptoms which may include fatigue, infections, uncontrolled bleeding or anaemia.

Many cancers form solid tumours (Figure 69), which are masses of tissue consequence of the abnormal cell growth. However, the blood cancers such as leukaemia, generally do not form these solid mases of tissue. Cancer is produced by mutations in the genes that control the functioning of the cell, its growth and its division. The genetic changes leading to the cancer appearance may be inhered from parents, caused by errors occurring during the cell division process, triggered by certain viruses or may be induced by the exposition to certain chemicals or radiations capable to damage the cell's genetic material which is comprised by the deoxyribonucleic acid (DNA) and the ribonucleic acid (RNA).

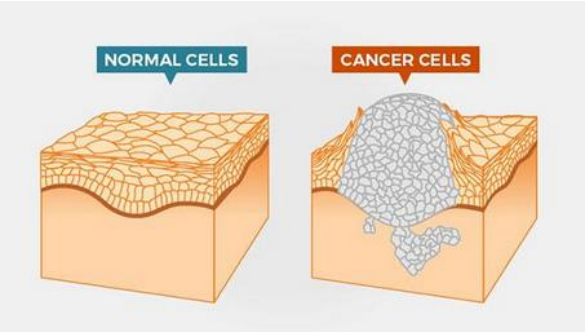


Figure 69. Representation of the abnormal cell growth promoted by cancer. Reproduced from Ref [136] with permission of the copyright holder.

The human body has several protective mechanisms against cancer. The three main protective systems are the tumour suppression genes which are involved in the control of the cells growth, the DNA repair genes which are capable to repair the damaged DNA strains and, the immune

system which can detect and attack the cancer cells. The disease normally progresses in cases where the accumulation of several genetic changes leads to the inhibition of the mentioned protective mechanisms and at the same time to the alteration of the cell division/growth pattern. Each patient's cancer is the result of the combination of a unique set of genetic modifications and consequently, the response in front of the same treatment may be completely different in each case. The appearance of cancer requires from the accumulation of several genetic changes. The risk of cancer occurrence increases during the life course because, the cancer-causing changes naturally accumulate during the persons ageing.

More than 100 types of cancer have been described, they are named as the organ/tissue where they have been originated or as the type of cells from which they come from. In general, the cancer disease causes a severe affectation of the body normal function and in certain cases it may lead to patient's death. Some of the most widespread types of cancer are:

- Carcinomas: they occur in the epithelial cells, which are the cells that form the tissues covering the internal and external body surfaces. The carcinomas are the most common type of cancer. Although they may be induced by many other factors, they are more related to external agents such as radiation or chemicals than other cancers since the epithelial cells are typically more exposed. Most of the colon and prostate cancers are carcinomas.
- Leukaemias: cancers that begin in the bone marrow and do not form solid tumours. In patients suffering this disease, large amounts of abnormal white blood cells (theoretically forming part of the immune system) are accumulated in blood and in the bone marrow. The non-functional cancer cells displace the normal blood cells affecting the body capacity to transport oxygen to its tissues, to control bleeding or fight against infections.
- Lymphomas: diseases affecting the functionality and production of lymphocytes which are white blood cells generated in the lymph nodes, lymph vessels and other body organs. Since lymphomas affect the immune system, they cause difficulties to fight against infections.
- Myelomas: they affect plasma cells which are a type of cell forming part of the immune system. The normal plasma cells release antibodies that bind to the harmful foreign agents to neutralize and destroy them. The abnormal plasma cells are produced in the bone marrow and may form tumours in any bone of the body.[136–139]

1.2.2. History of the cancer treatments

The historical records confirm that, already in the ancient times, cancer was treated using natural remedies prepared from plants and other sources. From the end of the 16th century until the mid-part of the 20th century, arsenicals remained as the most popular remedy to treat cancers such as leukaemia and Hodgkin lymphomas. Arsenic trioxide was the main active ingredient of most of these arsenic based remedies. With the development of modern chemotherapy and because of the toxicity associated with this first generation remedies the use of arsenicals was almost completely abandoned. This type of treatments caused frequently cardiac, skin, neurological and gastrointestinal side-effects among others (Figure 70).

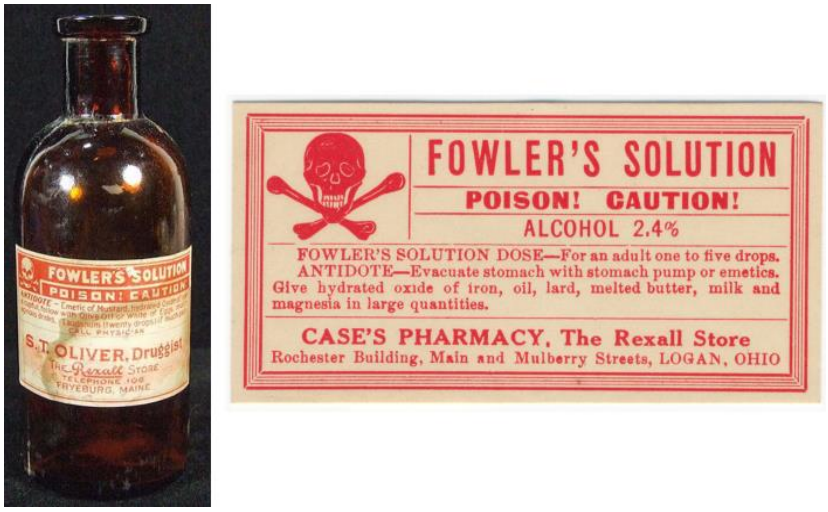


Figure 70. Can (left) and labelling (right) of one of the most popular arsenic based remedies (Fowler's solution).[140,141]

Some modern studies indicate that the effectivity of the arsenic trioxide treatments towards cancer was based on three lines of action:

1. Induction of the cancer cells differentiation through the inactivation of the malignant proteins causing the absence of cell maturation. Through this mechanism the formation of cells with a complete lack of functionality typically observed in cancer is avoided relieving one of the main effects of the disease.

2. Generation of reactive oxygen species that induce the cell death. It was found that the cancer cells, present a lower capability to buffer reactive oxygen species. Consequently, they are more sensitive to the presence of this kind of compounds than healthy cells and are preferentially eliminated during the treatment.

3. Accumulation of arsenic in the unhealthy cells. It was determined that cancer cells tend to accumulate more arsenic due to the alterations in the cell functioning caused by the disease. This phenomenon increases the effect of the two previously described mechanisms and makes unhealthy cells more sensitive to the treatment.

A new generation of drugs based on arsenical compounds is currently being evaluated for the treatment of various types of leukaemia and myelomas. Some of these drugs have shown high effectivities, nevertheless, the real key for its success is the reduction of its toxicity through the study of the optimal dosage and the use of less harmful/more selective arsenic derivatives.[142–144]

It was not until the half of the 20th century that the first effective treatments against cancer were developed. The discovery of these treatments, and the subsequent advances made in the anticancer drugs field are mainly attributable to the discovery of the transplantable animal cancers. This finding has made possible to obtain mice populations suffering from the same tumour allowing to test systematically series of potential anticancer drugs, to better understand the disease and, to develop more efficient treatment strategies.[142,145,146]

From the mid part of the 20th century the arsenical drugs were replaced by a second generation of anticancer remedies based on small molecules. This new drug generation caused fewer side effects and presented a higher effectivity than its predecessors. The first second-generation drug that reached the market was Chlormethine (Figure 71). This nitrogen mustard was approved by the FDA in 1949, it is still nowadays used to treat lymphomas and commercially available under the tradename of Mechlorethamine. Its discovery was derived from the serendipitous observation of the bone marrow and lymph node depletion capabilities shown by the sulphur mustard gas used as chemical weapon during the first world war. It was found that the anti-cancer effect shown by the chlormethine was consequence of its cytotoxicity, which is associated to a process called DNA alkylation. This substance is capable to alkylate some of the amino groups of the cell DNA strains damaging them. As a result of the induced DNA damage the cells become unable to divide and eventually die. Since cancer cells divide faster than healthy cells, they are more sensitive to the DNA alkylation. Though this mechanism, the growth of cancer cells in the patient's body can be slowed down or even stopped.[142,146–149]

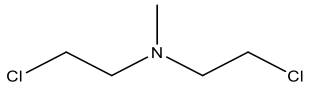


Figure 71. Chlormethine molecular structure.

The following decades brought to a massive development of new drugs based on small molecules that affected the integrity of the cell's genetic material (cytotoxic treatments). The developed second-generation drugs were based on the DNA alkylation (as it is the case of Chlorambucil; Figure 72) as well as other alternative DNA damaging mechanisms, for example:

- The interruption of the DNA biosynthesis caused by antimetabolites such as antifolates, base analogues (altered nucleobases) or nucleoside analogues. Methotrexate (based on an antifolate; see Figure 72), Gemcitabine (based on a nucleoside analogue; see **DH0517** project chapter), Thioguanine and 5-Fluorouracil (Figure 72) both based on altered nucleobases are examples of drugs based on this type of mechanism.
- The DNA platination caused by platinum complexes such as *cis*-platin (Figure 72). These complexes lead to platinum species capable to covalently bond to the cell's DNA strands inducing the damage of its genetic material and eventually the cell death. *Cis*-platin was approved by the FDA in 1978. Because of its high effectivity towards many cancer types and despite its toxicity, *cis*-platin is nowadays one of the most widely used anticancer drugs.[149,150]
- The alteration of the correct DNA distribution between cells during the mitosis that leads to the cell death. The vinca alkaloids and the taxanes are the main representatives of the active ingredients acting through this mechanism. They are currently used in anticancer drugs such as Vinblastine (Figure 72) based on a vinca alkaloid and in Paclitaxel where a taxane (Figure 72) acts as the active ingredient.[142,146,149,151]

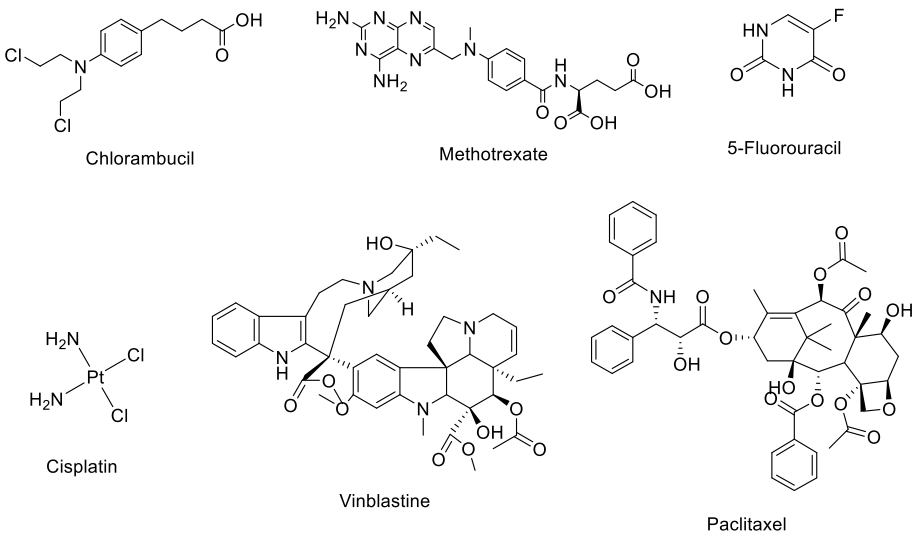


Figure 72. API of six popular anticancer drugs based on the DNA damage principle.

Regardless of the improvements in effectivity achieved with the second-generation of anticancer drugs, the necessity of more effective treatments triggered the development of a third generation of antineoplastics. The anticancer drugs of third generation are targeted to certain cell receptors, this allows to increase the selectivity of the treatments leading to higher rates of success while reducing the side-effects. Generally, the drugs belonging to this generation are chemically more complex than the second-generation ones (see Figure 73 and Figure 74). Consequently, they require from more advanced manufacturing methods such as fermentation or preparative chromatography. For this reason, they have normally higher marketing prices (see Table 14). The hormone analogues and the cytokines are remarkable examples of third-generation drugs.

The hormone analogues are used to treat some cancers which are known to be sensitive towards this type of substances. The cells of this class of cancers contain hormone receptors which induce the cell growth once they bind to the target hormone. The hormone analogue therapies are mainly based on the inhibition of the production of the hormones responsible of the cancer cell growth or in the blockage of the hormone receptors present in the cancer cells. The suitability of this kind of treatments must be individually evaluated for each patient, since it must be determined case per case if the abnormal cancer cells contain the mentioned hormone receptors or not. The most common type of cancer treated using hormone therapies is breast cancer since, in many of the cases it is hormone sensitive. Zoladex, Faslodex and Suprelin approved in 1997, 2002 and 2007 respectively are examples of this kind of drugs (Figure 73).[152]

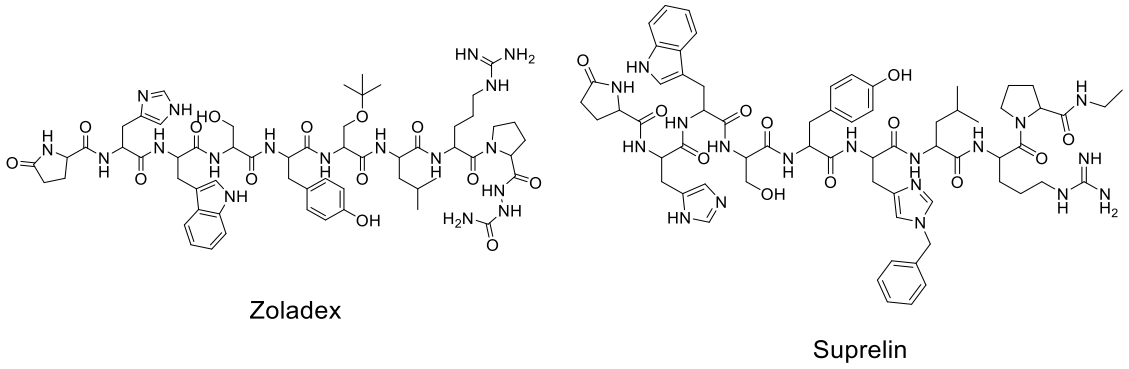
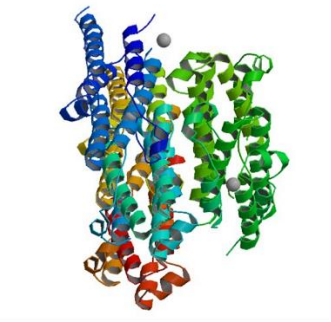


Figure 73. Molecular structures of the APIs of Zoladex and Suprelin.

Cytokines constitute a family of proteins including Interferons and Interleukins see Figure 74. These high molecular weight substances are targeted to the cells that supply the tumour cells from the nutrients and resources that they require for its development. Cytokines induce the activation of the immune system and the attack to the cells constituting the vital support of the tumour, causing tumour necrosis. Therefore, this type of therapies allows to fight against the illness without targeting the cancer cells.[153]



Interferon alfa-2b

Figure 74. Interferon alfa-2b structure. Reproduced from Ref [154] with permission of the copyright holder.

With the aim of increasing even more the efficacy of the anticancer treatments, in the last years the development of a fourth generation of antineoplastic drugs has been initiated. The drugs belonging to this class of therapies are based on the use of monoclonal antibodies. The antibodies, also known as immunoglobulins, constitute a large family of proteins secreted by a specific class of cells forming part of the immune system (Figure 75). Each type of antibody binds selectively to a specific molecule or molecular fragment present in a pathogen. Once they bind to its target, immunoglobulins may induce the elimination of the pathogen through many different mechanisms. The high selectivity towards a specific target shown by the immunoglobulins leaded to the development of antineoplastic therapies based on monoclonal antibodies. This kind of antibodies are isolated in the laboratory from cloned cells though complex and advanced techniques. The fourth generation of anticancer drugs use monoclonal antibodies capable to bind selectively to the cancer cells promoting its elimination. Due to its high selectivity they present a higher efficacy and a lower toxicity. The fourth-generation anticancer drugs may be based on wide variety of therapeutic approaches such as:

- Attachment of chemotherapeutic or radioactive drugs to the immunoglobulins to use them as delivery vehicles. Ibritumomab (containing a radioactive drug) and Mylotarg (containing an antibody conjugated to a molecule with anticancer properties) are examples of this type of drugs.
- Flag the cancer cells to induce the attack of other parts of the patient immune system. This is the case of Alemtuzumab.
- Inhibition of the cancer cells growth such is the case of Trastuzumab.
- Induction of the cancer cells death. Rituximab is an example of this type of therapy.

As in the case of the third-generation, the fourth-generation anticancer drugs present as a main drawback its elevated manufacturing cost, that leads to elevated marketing prices (see Table 14).[155–157]

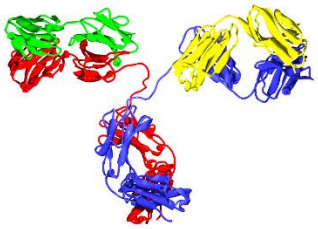


Figure 75. Antibody IgG2 structure.[158]

Entry	Drug name	Approx. Dose price (Eur)[11]	Drug generation	Chemical structure
1	Trisenox	636	1st	Small molecule
2	Gemcitabine	14	2nd	Small molecule
3	Chlorambucil	23	2nd	Small molecule
4	Methotrexate	7	2nd	Small molecule
5	Vinblastine	41	2nd	Small molecule
6	Interferon-alfa 2b	791	3rd	Protein
7	Faslodex	1857	3rd	Hormone
8	Zoladex	1738	3rd	Hormone
10	Mylotarg	8060	4th	Monoclonal antibody
11	Brentuximab	7596	4th	Monoclonal antibody
12	Alemtuzumab	22156	4th	Monoclonal antibody

Table 14. Dose Price, drug generation and molecule type of some popular anticancer treatments.[159]

Despite the achieved improvements in efficacy and in the reduction of side effects, the commercial success of the anticancer drugs of third and fourth generation is being limited. The commercialization problems observed for this type of treatments are mainly attributable to its high sell prices which are directly associated to the complexity of their manufacturing.

The demand and the overall price of the drugs is currently increasing. The necessity of inexpensive and more effective treatments towards cancer has led to a new opportunity for the second-generation anticancer drugs. The objective is to offer at lower prices, treatments with efficacies comparable to the ones shown by the dugs belonging to the third and the fourth generation.

The performed investigations have revealed that depending on its mechanism of action, the effectivity of the above-mentioned second-generation drugs is maximal at a specific phase of the cellular cycle. Therefore, the effectivity of these treatments could be increased dosing the drug at the phase of the cell cycle where it is more active (spacing the treatment) and, combining APIs that interfere on different phases of the cancer cells cycle. This approach is currently being explored and is leading to the development of optimized dosing schedules and, to treatments where different second-generation antineoplastic drugs are combined to promote synergistic effects (see entries 1 and 3 on Table 15). The better understanding of the drug metabolism has also triggered the development of some combo drugs where, an antineoplastic drug is combined with a second API that is used to avoid the degradation of the substance with anticancer properties. The degradation may be avoided through the use of inhibitors of the enzymes

[11] Related to Table 14: when it was required, the amount of drug per dose and therefore the dose price was deduced assuming 1.9 m^2 as the body surface of the patient (1.9 m^2 is the average body surface area for an adult men)[199]. For those drugs valid to treat more than one type of cancer and, presenting a different of amount of drug per dose depending on the treated condition an average dose price was estimated from all the values found.

responsible of the drug metabolization (see entry 2 on Table 15) or encapsulating the drug in liposomes to protect it from the environment (see entry 3 on Table 15).

Entry	Drug composition	Application	Remarks
1	Letrozole and Ribociclib	Breast cancer treatment	Letrozole and Ribociclib present a synergistic effect when they are combined.
2	Tipiracil and Trifluridine	Colorectal cancer treatment	Tipiracil inhibits the enzyme responsible of the Trifluridine degradation.
3	Cytarabine and Daunorubicin liposomal	Leukemia treatment	Cytarabine and Daunorubicin present a synergistic effect when they are combined. Daunorubicin is encapsulated in liposomes to avoid its degradation.

Table 15. Examples of different anticancer combo drugs currently available and its application/working principle.[160]

The better understanding of the drug metabolism and mechanism of action is also leading to the development of optimized second-generation drugs that contain fluorine and deuterium atoms in its structure.

On one hand, the utilization of fluorine in drug synthesis has increased during the last years. This element can be already found in many blockbuster drugs, in 2018 around the 45 % of the APIs approved by the FDA contained at least one fluorine atom. Fluticasone, Lipitor and Trifluridine are examples of drugs containing fluorine (see Figure 76). Fluticasone is the 16th most prescribed drug in the US while Lipitor is the 3rd one.

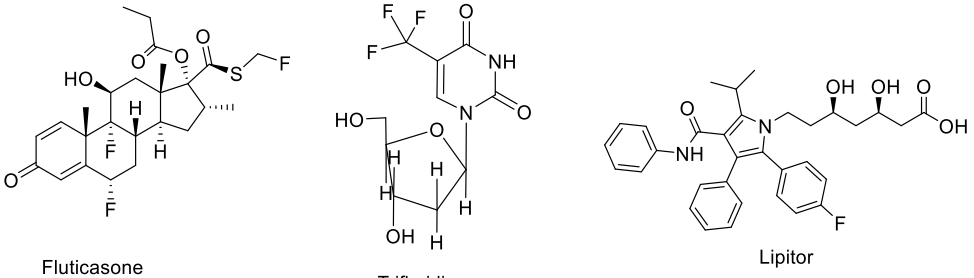


Figure 76. Example of the molecular structure of three APIs containing fluorine. Fluticasone (right), Trifluridine (center) and Lipitor (left).

The inclusion of fluorine in the structure of an API may have different effects. It may influence the drug potency, the drug safety (side effects) or the lifetime of the API in the organism. The changes in the drug properties derived from the introduction of fluorine in an API may be, among others, attributed to:

- The high strength of the C-F bond. The carbon fluorine bond is very strong and is not easily metabolized. This provides the fluorinated molecules with an extra stability that may positively impact in the drug lifetime once it enters the organism and, that may reduce the drug side effects by minimizing the formation of toxic metabolites derived from the drug decomposition.
- The high polarization of the C-F bond. As a result of the high electronegativity of the fluorine atom, new electrostatic interactions between the API and its target molecule may be induced. The fluorine presence may vary the π electron density in aromatic systems or lead to the formation of fluorine-hydrogen bonds. These new

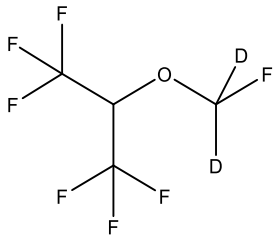
interactions could lead to an enhancement of the drug capacity to bind to its target and cause an increase in the drug potency.

- The variations in the most stable conformation of the molecule induced by the fluorine presence. These variations may affect the drug-target interaction and consequently the drug potency. The mentioned conformation variations can be related with the polarization of the C-F bond that can affect to the overall dipole moment of the molecule.
- The increase in lipophilicity and the modifications in the drug basicity caused by inductive effects. The alteration of these properties through the introduction of fluorine may allow to modify the tissue distribution, molecular recognition, stability or bioavailability of the drug.

On the other hand, it has been demonstrated that the deuterium-carbon bond is more stable than hydrogen-carbon bond. The higher stability of the carbon-deuterium bond may be used to tune the properties of the studied API and to increase the treatment efficacy or to reduce the dosage frequency. The deuteration of specific positions of the molecule could allow to:

- Reduce the formation of toxic/reactive drug degradation products. The reduction on the formation these spices allows to increase drug tolerability and to reduce its side effects.
- Enhance the stability of the active drug metabolites. To favor the formation/stability of the desired metabolite leads to a higher bioavailability and efficacy.
- Increase the stability of the API itself. The API stability increase may enhance the treatment efficacy in case that the API molecule is directly the active towards the studied disease.

The stability enhancements achieved through the introduction of deuterium in an API may cause a relevant impact in the drug effectivity. Deuterated Sevoflurane is an example of the benefits that can be achieved using deuterium (Figure 77). According to *in-vitro* studies, this substance degrades 17 times slower than its hydrogenated homologous.



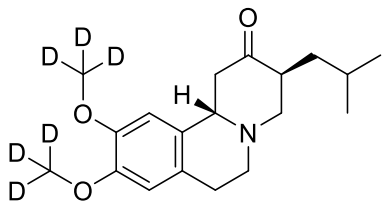
Sevoflurane-D_2

Figure 77. Deuterated Sevoflurane molecular structure.

Despite the use of fluorine and deuterium is attracting the attention of the pharmaceutical industry it presents some challenges. The synthesis of fluorinated compounds is often difficult. The fluorination methods can be functional group intolerant and, moreover, they may require the use of hazardous reagents.

The manufacturing costs of the deuterated drugs are often very high because the deuterated reagents are tens to hundreds of times more expensive than its non-deuterated equivalents. To save costs, it is important to reduce the product loses during and after the deuteration step. To

minimize the product loses, the deuteration step is situated as close as possible to the final product and the final steps of the synthesis are extensively optimized to reach high yields. Despite the optimization of the synthetic routes, deuterated drugs are considerably more expensive than its hydrogen-based analogues. For example, Tetrabenazine dose price is around 16 Eur while the dose price of its deuterated analogue (Deutetrabenazine; Figure 78) is close to 90 Eur.[159,161,162]



Deutetrabenazine

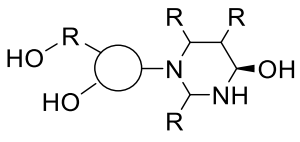
Figure 78. Deutetrabenazine molecular structure.

The progresses made in the development of anticancer drugs and in the treatment of the disease combining, chemotherapy with surgery and radiotherapy have led to the high rates of success currently observed. The use of modern treatments allows to achieve remission in around the 85 % of the cancer diagnostics. However, the limits of the anticancer therapies have not been reached and, research will continue in order to enhance the effectivity of the anticancer drugs while reducing its toxicity and manufacturing cost.[145]

1.3. Project features

Disclaimer: the information generated during the **FH0317** project is property of Farmhispania S.A. In order to protect the trade secret and the interests of the company the different figures, schemes, procedures and results presented in this chapter have been censored.

FH0317 is an active pharmaceutical ingredient (API) used to treat certain types of cancer via oral dosage. This substance is a new chemical entity (NCE) that is currently at the final stages of the drug approval process. On June of 2019 it passed successfully the phase III of the clinical trials and its NDA was filled by the end of the same year (see Approval of new drugs section). **FH0317** is a pyrimidine derivative containing some fluorine atoms in its structure (Figure 79). As reported in section 1.2.2, the use of fluorine could allow to enhance the properties of the presented drug (stability, bioavailability and selectivity).



FH0317

Figure 79. FH0317 chemical structure.

According to the classification presented in section 1.2.2, **FH0317** belongs to the second generation of anticancer drugs, which is formed by molecules of low molecular weight but with enhanced properties compared with the previous generation of antineoplastic remedies. This substance can be categorized as one of the second-generation drugs that are currently being

developed in order to solve the cost challenges associated to third and fourth-generation anticancer drugs.

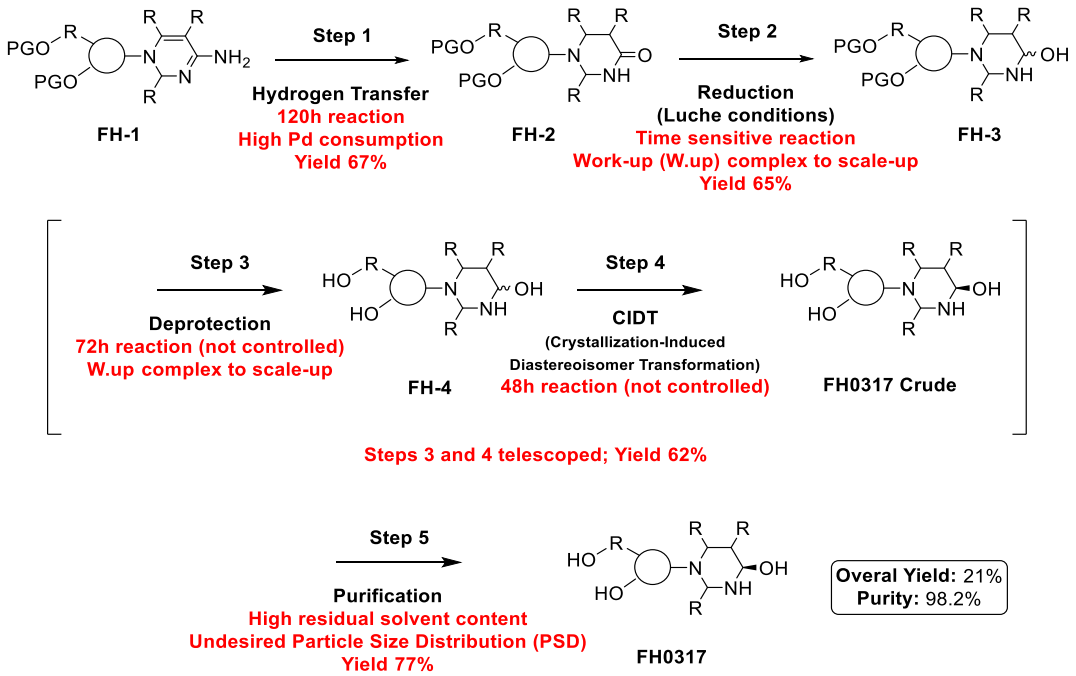
The **FH0317** project was a contract manufacturing project that was initiated in Farmhispania S.A. under the request of the drug innovator. The main targets of this project were the development and the validation of a manufacturing method that will allow to obtain within the desired specifications (residual solvents, purity or particle size) 5 kg of **FH0317** per batch. In this case, it was especially important to complete the different development and validation tasks within the requested timelines.

Large amounts of material were required to support the **FH0317** clinical trials. The fast obtention of industrial batches of this product executed in accordance to the GMP guidelines, was critical for continuing with the clinical trials and with the drug approval process. The compliance of the marked timelines was crucial because, as it is mentioned in Fundamentals of batch process scale-up chapter, a failure or even a small delay in the commercial launch of a new product may have a severe impact in the stock price of the pharmaceutical companies involved.

This project was started using a five-step manufacturing process provided by the innovator itself (Scheme 26). It was considered a tech transfer project since, a preliminary manufacturing method from which the development was started was given to Farmhispania S.A. before the project initiation.

1.4. Precedents

The different steps of the process and its more important drawbacks are summarized in Scheme 26 and described in detail in the following sections.



Scheme 26. Initial synthetic route of FH0317 including the main drawbacks of each step (ester protecting groups abbreviated as -OPG).

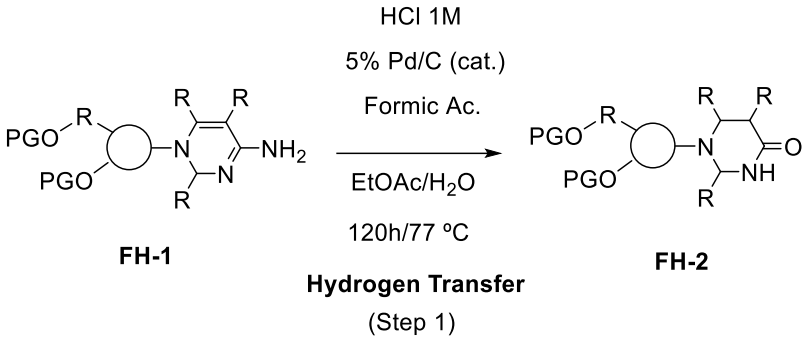
By the time the project was started, the manufacturing process above described had been tested twice by an external company at a scale of 1 kg of **FH-1**. One of these batches, failed due

formation of impurities at unacceptable levels in step 2 while the other yielded a product out of specifications in terms of residual solvents. In the batch where **FH0317** could be isolated the overall yield was 21 % and, the purity of the obtained product was 98.2 % (low purity considering the standards for an API). The initial trials performed using the provided manufacturing method revealed that all the steps presented several drawbacks (see the following sections). The overall process required from an extensive optimization in order to make it suitable for the industrial scale operation and for the process validation. The mentioned process needed from modifications to reduce costs, solve robustness problems, increase the quality of the obtained product, and circumvent safety problems.

1.4.1. Step 1: Hydrogen transfer reaction

The provided manufacturing method involved a hydrogen transfer reaction that was performed in a by-phasic mixture of EtOAc and water. The main problems of this step were found in the reaction although the w.up also presented some drawbacks.

In the non-optimized process, the reaction was carried out using a 5 %wt palladium on activated charcoal catalyst (Pd/C) and formic acid as hydrogen donor. A small amount of hydrochloric acid 1 M was also added in the reaction media (see Scheme 27).



Scheme 27. Detail of the step 1 (hydrogen transfer) of the FH0317 synthesis (non-optimized conditions), (ester protecting groups abbreviated as -OPG).

This reaction was monitored through HPLC and showed low reaction rates. The hydrogen transfer reaction was performed in a heterogeneous media with 3 different phases involved (water, solid catalyst and EtOAc) therefore, the reaction rate was dependent on the scale and the mixing efficiency. During the 1 kg batch trials mentioned above, 120 h and several Pd/C and formic acid refeedings were required to reach the reaction completion.

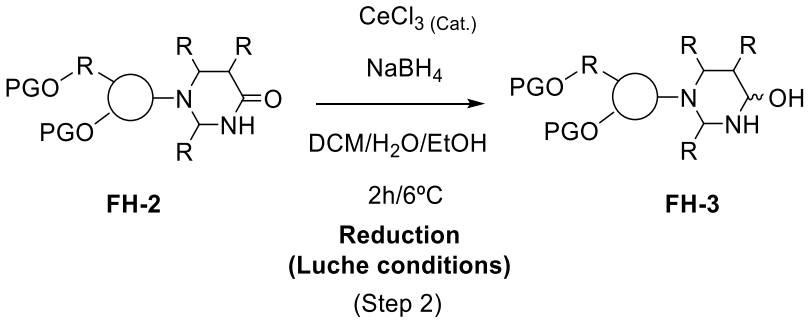
Despite a good conversion was observed in the in-process controls performed, the yield obtained for this step was low (67 %), indicating that some product was lost during the w.up. The w.up described in the initial process, was based on the filtration of the Pd/C catalyst and in a series of aqueous rinsings performed using brine and concentrated $NaHCO_{3\,(aq)}$. A solvent exchange from EtOAc to methyl tert-butyl ether was finally carried out to crystallize the product, which was isolated by filtration. The purity of the **FH-2** intermediate obtained using the initial process was around 93 % and it was considered acceptable.

The above described process was not feasible for the industrial scale manufacturing due to the high catalyst consumption and the extended reaction time. The use of these synthetic conditions

would lead to a poor manufacturing capacity and would cause a severe increase in the **FH0317** manufacturing costs.

1.4.2. Step 2: Reduction reaction under Luche conditions

The second step of the **FH0317** synthesis was a reduction performed under Luche conditions. This reaction was carried out at low temperature in a by-phasic mixture of DCM, EtOH and water. Catalytic amount of $CeCl_3$ was added and $NaBH_4$ was used as a reducing agent (Scheme 28). The product isolated at the end of this step was an epimer mixture formed by **FH-3** having *(R)* or *(S)* configuration.



Scheme 28. Detail of the step 2 (reduction) of the FH0317 synthesis (non-optimized conditions), (ester protecting groups abbreviated as -OPG).

Before its optimization, this step was the most critical of the **FH0317** synthesis, either the reaction or the w.up presented low robustness. The reaction was extremely time sensitive; it was left to evolve during approximately 2 h and was monitored through HPLC. This reaction must be stopped immediately once it reached the completion. If it was not quenched after a high conversion has been reached, the formed **FH-3** intermediate degraded rapidly leading to the batch failure.

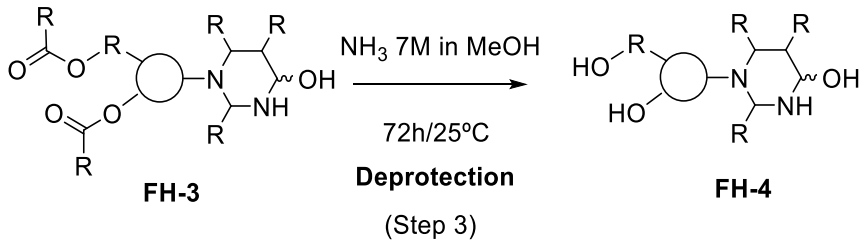
During the w.up there were also high probabilities of batch failure due to the formation of impurities. The reaction quench was exothermic and was performed using acetone. It was found that during this operation, **FH-3** degradation also took place. The acetone addition must be performed as fast as possible while maintaining a low temperature and, even in these conditions the formation of impurities may lead to batch failure. Nevertheless, the batch failure risk was not fully mitigated after the acetone quench, the obtained **FH-3** intermediate was sensitive to the extremely basic pH of the obtained mixture. Therefore, a pH adjustment using hydrochloric acid must be performed without delay. This pH adjustment was critical since **FH-3** was also sensitive to acid pH and, deviations from the target pH could lead to the formation of impurities. Additionally, the addition rate of the HCl solution must be carefully controlled in this operation. Product degradation was promoted in case of fast acid addition due to the formation of low pH hot spots. After the pH adjustment, a series of aqueous rinsings were performed. In some of these rinsings formation of interphases was observed. This phenomenon, was identified as a potential source of problems during the scale-up since the formation of interphases may lead to serious issues at industrial scale (product loss or increase of inorganic salt content in the final product).

The **FH-3** crystallization was identified as another area of concern. In the provided process, the organic phase was dried using sodium sulphate. Over the dried phase, a solvent exchange from DCM to MTBE was performed in order to crystallize the product that was eventually isolated by filtration. The use of salts for the organic phase drying is typically avoided at industrial scale since it increases the cost, reduces the process robustness and impacts in the operation time required to complete each batch. Perform an organic phase drying using a salt increases the risk of having inorganic salts in the isolated product (high ROI values), requires from an additional filtration, from reactor/filter cleaning and, generates additional residues. Additionally, during the solvent swap from DCM to MTBE a thick slurry was formed. This behaviour, could cause yield, mixing, unloading and filtration problems at industrial scale.

In the cases where this step was completed successfully, the obtained yield was around 65 % and the purity of the isolated intermediate was 90 %. Due to the reactivity, w.up and crystallization drawbacks above presented, the described process was considered not suitable for the industrial scale manufacturing. An exhaustive process optimization was required before the industrialization of this step.

1.4.3. Step 3: Deprotection reaction

The third step of the **FH0317** synthesis was a deprotection reaction using an ammonia solution 7 M in MeOH. This reaction was left to evolve for 72 h at 25 °C. The product formed (**FH-4**), as in the case of **FH-3** was an epimer mixture.



Scheme 29. Detail of the step 3 (deprotection) of the FH0317 synthesis (non-optimized conditions).

In the non-optimized process, the progress of this reaction was not monitored, it was left to evolve for 72 h before to initiate the w.up. The lack of control of the performed reaction was one of the main problems detected in this step. The lack of reaction control increased the risk of initiating the w.up before the reaction completion and the risk of unnecessarily extent the reaction time. The use of a fixed reaction time approach could lead to product degradation caused by an extended exposition to the reaction conditions, may contribute to unnecessarily extent the time required for the obtention of each **FH0317** batch and could reduce the yield and/or increase the impurity content of the isolated product due to a premature w.up initiation.

Once the reaction has been completed a solvent swap from MeOH to water was performed. The obtained aqueous phase was rinsed several times with EtOAc to remove reaction by-products. In the provided process, the **FH-4** intermediate was not isolated; steps 3 and 4 were telescoped. In order to proceed with step 4, the aqueous phase obtained at the end of the step 3 was distilled almost to dryness (until a mixture of **FH-4** in 0.4 volumes of water was obtained). As it is mentioned in Development of industrial chemical processes section, distillation to dryness

operations are normally avoided at industrial scale. The use of a telescoped process eliminated the opportunity to purge some impurities in the mother liquors of a hypothetic **FH-4** isolation. Moreover, the telescoped approach reduced process robustness since, the accuracy in the calculation of the amount of **FH-4** obtained at the end of the step 3 decreased and, as a consequence, the magnitude of the error associated to the equivalents of reagents and volumes of solvent used in step 4 increased.

To enhance the process robustness at the levels required for the manufacturing of **FH0317**, the step 3 required at least the implementation of a reaction control method and the elimination of the final distillation to 0.4 residual volumes of water.

1.4.4. Step 4: Crystallization-induced diastereoisomer transformation (CIDT)

The **FH-4** intermediate obtained from the deprotection reaction was an epimer mixture formed by **FH0317** with *(R)* configuration and by its epimer having *(S)* configuration at the alcohol group (Figure 80). The ratio of epimers in **FH-4** was approximately 50:50. However, through the Crystallization-induced diastereoisomer transformation (CIDT) performed in step 4 the main part of the **FH0317** undesired epimer was transformed into the desired product allowing to increase the yield of the process.

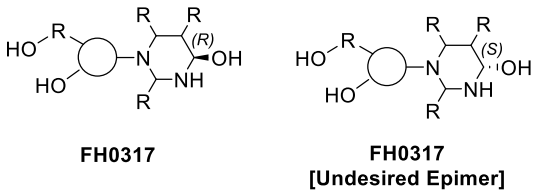
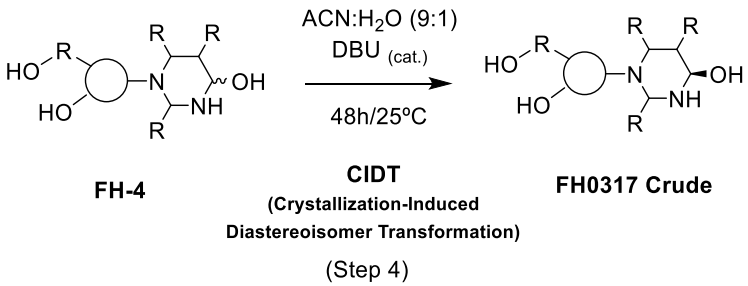


Figure 80. Molecular structures of FH0317 with *(R)* configuration (left) and FH0317 undesired epimer with *(S)* configuration (right).

In the manufacturing method provided by the innovator, this step was performed suspending **FH-4** at 25 °C in a mixture of ACN and water containing catalytic amount of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). In the mentioned conditions, the undesired epimer of **FH0317** was more soluble. It gradually epimerized in solution crystallizing as **FH0317**. As in the previous step, the progress of this reaction was not monitored, it was left to evolve for 48 h before to initiate the product isolation.



Scheme 30. Detail of the step 4 (CIDT) of the FH0317 synthesis (non-optimized conditions).

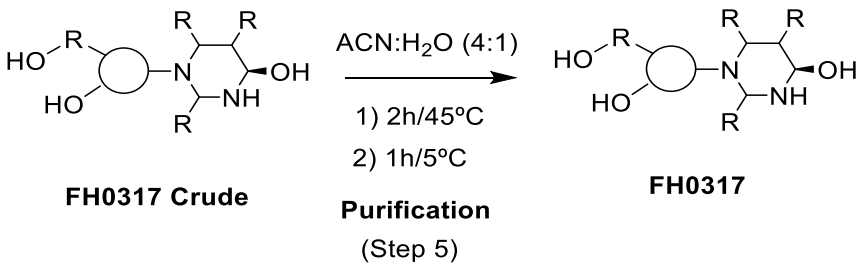
In this case no w.up was performed, the slurry obtained at the end of the reaction was cooled and after 2 h at low temperature, the product (**FH0317 crude**) was isolated by filtration. The lack of control of the performed reaction could lead to the same problems described for step 3. The

yield of the telescoped steps 3 and 4 was around the 62 % and the purity of the obtained product was around the 93 %. Although through the subsequent purification the impurity content was reduced, the relatively low purity observed in the **FH0317** was related with the impurities formed during the steps 3 and 4. Since step 4 was almost the last step of the **FH0317** synthesis (only a purification was done after step 4) telescope steps 3 and 4 could be considered as a high-risk approach.

The implementation of a control method for the performed reaction was essential to make this step suitable for the industrial scale. The enhancement of the yield of the telescoped steps 3 and 4 and of the purity of the obtained **FH0317 crude** were also considered key points for the successful industrialization of these two steps.

1.4.5. Step 5: Purification

In order to purify the obtained **FH0317 crude**, the solid was suspended in a mixture of ACN:water (4:1). The obtained mixture was heated to 45 °C for 2 h and cooled to 5 °C. Pure **FH0317** was isolated by filtration of the slurry after mixing for 1 h at low temperature (see Scheme 31).



Scheme 31. Detail of the step 5 (purification) of the FH0317 synthesis (non-optimized conditions).

Using the above presented procedure, a product with a 98.2 % of HPLC purity was obtained in a 77 % yield. The isolated **FH0317** contained some unknown impurities above the identification threshold and the qualification threshold (see Study of process impurities section in **DH0517** chapter).
The optimization of the purification conditions and the purity of the **FH0317 crude** used as raw material in this step was considered one of the main goals of the project. The objective was to meet the standard ICH criteria for impurities present in the APIs (see Study of process impurities section in **DH0517** chapter; Table 11) and, to eliminate the necessity of toxicologically qualifying the mentioned impurities.

The obtained **FH0317** was above the ICH limits in terms of ACN content. It contained 9000 ppm of this solvent (ACN is a class 2 solvent and considering the estimated **FH0317** dose, its content should be maintained below 3000 ppm; ICH Q3C option 2 criterion). The ACN levels could not be reduced through extended drying at high temperatures. It was found that the **FH0317** crystals formed during the purification occluded ACN. In order to meet the ACN specification, it was required to mill the whole batch and to perform three EtOAc slurries of the milled batch. The implementation of the EtOAc slurries and the product milling was not considered feasible in terms of timing, yield loses and risk of impurity formation. Additionally, it was found that the product obtained from the described purification process presented a particle size distribution

that caused manipulation problems during its tableting (low flowability and static charge accumulation).
An optimization in the purification conditions was required in order to eliminate the necessity of additional milling and reslurry treatments. The obtention of a product with a lower ACN content and within the desired particle size ranges was considered essential for the project success.

1.5. Objectives

The main goals of the **FH0317** project were:

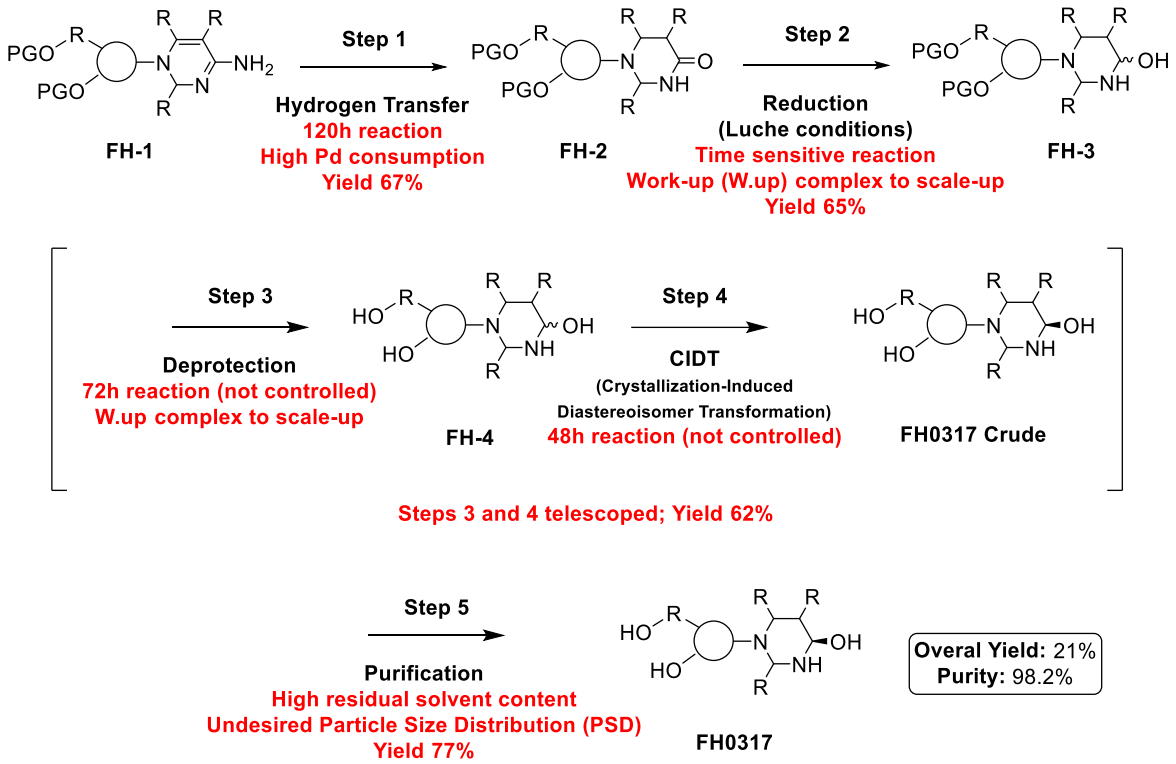
- Scale-up the process to 5 kg of **FH0317** per batch and generate the large amounts of material required to support the **FH0317** clinical trials.
- Optimize the initially provided manufacturing method in order to obtain a robust, profitable and safe industrial process capable to provide **FH0317** within the desired specifications (residual solvents, purity and particle size). Below are summarized step by step the main objectives to be accomplished:
    - Step 1: increase both, the yield and the reaction rate while reducing the consumption of palladium catalyst.
    - Step 2: modify the reaction parameters and w.up conditions to avoid the product degradation. Optimize the process to avoid the formation of thick slurries and, eliminate the organic phase drying with sodium sulphate.
    - Step 3: develop an analytical method to follow the reaction progress. Eliminate the final distillation to 0.4 residual volumes and, if possible, implement a product isolation operation before the step 4.
    - Step 4: develop an analytical method to follow the reaction progress. Increase both the yield initially obtained when steps 3 and 4 were telescoped and, the purity of the obtained **FH0317 crude**.
    - Step 5: obtain a more pure product and eliminate the necessity of additional milling or reslurry treatments. Increase the purification capacity and meet the requested specifications (residual solvent, particle size distribution and purity).
- Support the scale-up process investigating the different issues found during the industrial manufacturing and, find solutions for all the problems arising during the pilot and industrial batches.
- Collaborate in the validation of the developed process performing the process quality risk assessment and participating in the preparation of the required master batch records (MBR) and validation protocols.
- Generate the process knowledge necessary to support the NDA (New Drug Application) filling required for the drug approval.

In the next sections are described the different studies carried out during the development of this thesis in order to reach the mentioned objectives.

1.6. Process development

1.6.1. Process optimization

As it has been described in section 1.4, the process given to Farmhispania S.A. for the obtention of **FH0317** required optimization in all its synthetic steps (see Scheme 32).



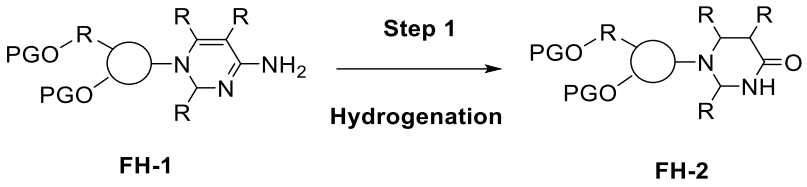
Scheme 32. Initial synthetic route of FH0317 including the main drawbacks of each step (ester protecting groups abbreviated as -OPG).

In the following sections are collected the experiments performed in order to enhance the process in terms of robustness, performance and safety. The customer requested to maintain the synthetic route as similar as possible to the initial one. Therefore, the main part of the development was focused on the optimization of the process already described in Scheme 32.

All the activities and studies performed in order to solve the different issues found during the scale-up of the synthesis are also included in this section.

1.6.1.1. Step 1: Hydrogenation

1.6.1.1.1. Reaction optimization



FH-1 FH-2

Scheme 33. Step 1 of the FH0317 synthesis (ester protecting groups abbreviated as -OPG).

During the optimization of step 1 (Scheme 33), two hydrogenation approaches were investigated (hydrogen transfer and gas hydrogenation). In this phase of the development a series of experiments were performed: to evaluate the feasibility of each hydrogenation approach, increase the reaction rate and reduce the formation of impurities or the catalyst consumption. In the following section are collected the different studies performed.

1.6.1.1.1.1. Hydrogen transfer optimization

A series of screening experiments were carried out in order to determine which were the parameters influencing the time required to reach the hydrogenation completion and to increase the reaction rate. In the mentioned study the amount of catalyst, the volume of HCl solution and the number of equivalents of formic acid were evaluated (Table 16).

Since the objective was to perform a fast screening in order to find the more suitable reaction conditions, the experiments were performed at 1 g scale using round bottom flasks. During all the study mechanical agitation was used. The utilization of magnetic stirring was avoided because this reaction involves mass transfer processes which are typically mixing sensitive. The objective was to mimic the stirring conditions that will be found in an industrial reactor in order to extract useful information for the scale-up. All the reactions were carried out at the reflux temperature (77 °C), using the same amounts of EtOAc and water.

From an early phase of the process development, the catalyst used was changed from 5 %wt palladium on activated charcoal to 10 %wt Pd/C. The use of 10 %wt Pd/C catalyst was preferred because:

- It had a shorter delivery time than the 5 % Pd/C originally used.
- Allowed to reduce the total weight of loaded catalyst, making the process simplest (easiest loadings, less solid to filter at the end of the reaction and lower amount of residue to manipulate).
- It had a lower cost (per Pd unit) compared to the 5 %wt Pd/C.

As it can be seen in Table 16, the reaction rates obtained using 5 %wt catalyst (entries 1 to 4) are comparable to the ones obtained using 10 %wt catalyst (entries 5 to 8).

Entry	Description			Reaction time	Comments
	Catalyst (p)[12]	HCl (1 M)	Formic Ac. (Eq.)		
1	0.1+0.3	0.08 V	9.9+5	Not completed	Evolved mainly after cat. refeeding
2	0.6+0.05	0.08 V	9.9+2.5	45 h	20 h: almost completed. cat. refeeding ineffective. 40 h: started to evolve after formic ac. addition
3	0.1+0.3	0.8 V	9.9+5	Not completed	Not evolved after cat./formic ac. addition
4	0.6+0.3	0.8 V	9.9+5	Not completed	Not evolved after cat./formic ac. addition
5	$0.1_{(0.2)}$	0.08 V	9.9+2.5	44 h	After formic ac. refeeding reached the completion
6	$0.1_{(0.2)}$	0.08 V	14.9+2.5	Not completed	44 h: even after formic ac. refeeding evolved slowly
7	$0.15_{(0.3)}$	0.08 V	9.9+2.5	27.5 h	At 20 h it stopped. Fast evolution after formic ac. refeeding
8	$0.1_{(0.2)}$	0 V	9.9+2.5	Not completed	Slow reaction rate

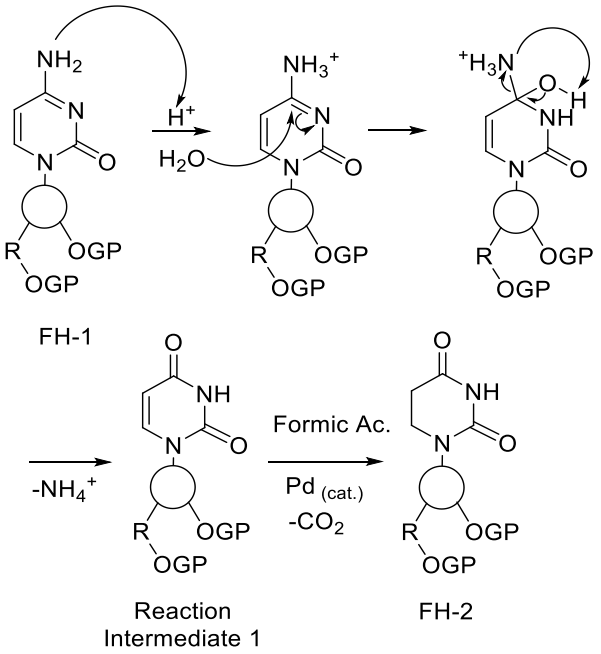
Table 16. Summary of the results obtained during the hydrogenation reactivity trials. For entries 5 to 8 the equivalent amount of 5 %wt Pd/C catalyst in terms of Pd loading is included in brackets.

From the results presented in Table 16 it was concluded that, small amounts of hydrochloric acid were necessary for the reaction (entries 7 and 8). Nevertheless, high amounts of HCl inhibit the hydrogenation process (entries 4 and 5). The results obtained in entries 5 and 6 seems to indicate that adding large amounts of formic acid at the beginning of the reaction could be detrimental for the reaction. Starting with lower amounts and refeed once the reaction stops seemed the best approach (entries 1 and 7). Regardless the used conditions, in all the experiments large amounts of formic acid must be added. Despite the formic acid boiling point (101 °C) was higher than the reaction temperature (77 °C), it was suggested that part of this reagent was lost due its relatively high volatility and the low efficiency of the small-scale reflux condensers used.

Concerning the amount of catalyst, it was found that using 0.1 parts per weigh of limiting reagent (p)[12] the reaction was very slow and refeeding was required (entry 1). Using 0.6 p of catalyst the reaction evolved faster (around 20 h of reaction were required; entry 2), however, as it can be seen in entry 7, similar results were obtained using only 0.3 p. When amounts below 0.3 p were tested, the reaction rate was considerably lower (see entry 5). Therefore, to add 0.3 p (0.15 p of 10 %wt Pd/C) seemed the better choice as it gave the best catalyst consumption/reaction rate ratio.

The results obtained during the reaction conditions screening were in accordance with the information found in the literature and in the LC-MS analyses of the reaction crude. In these analyses, a peak with an m/z ratio that could be associated with the intermediate 1 presented in Scheme 34 was detected.[163,164]

[12] (p) refers to parts per weigh of limiting reagent, this concept is widely used in industry. For example: 0.1 p equals to 0.1 g of catalyst per each gram of **FH-1** used.

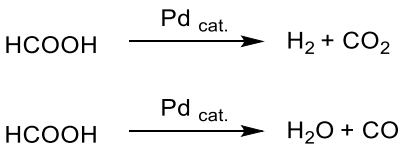


Scheme 34. Suggested mechanism for the hydrogen transfer reaction performed in the step 1 of the FH0317 synthesis (ester protecting groups abbreviated as -OPG).[163,164]

1.6.1.1.1.2. Hydrogen transfer fine tuning and scale-up

The best reaction conditions found during the screening phase (Table 16; entry 7) were successfully scaled-up from 1 g to 400 g (10 L reactor). In the large scale tests, the reaction behaviour was different that at the small-scale trials. At large scale, the formic acid refeedings were not effective. The reaction only reached the completion after the addition of fresh catalyst. Despite the observed differences, the obtained results were positive. The reaction finished after only one Pd/C refeeding and 32 h (120 h and several refeedings with catalyst and formic acid were required when using the non-optimized conditions).

The decrease in the amount of formic acid required to reach the reaction completion, reinforced the hypothesis of the formic acid volatilization occurring at small scale because of the low efficiency of the reflux condensers. Despite no formic acid refeeding was required, a large excess of this substance had to be initially loaded. According to the literature, the formic acid decomposition is catalysed in presence of Pd. The large amounts of formic acid that must be added even at large scale were related to these catalytic decomposition processes (Scheme 35). It was also found that the CO and CO_2 generated either during the hydrogenation reaction (Scheme 34) or during the formic acid decomposition (Scheme 35) were able to strongly adsorb into the catalyst active sites promoting its poisoning.[165,166]



Scheme 35. Formic acid decomposition reactions catalyzed in presence of palladium.[165,166]

It is suggested that, at 1 g scale the acid evaporation was the dominant process preventing its decomposition and the catalyst poisoning but making necessary formic acid refeedings. The

opposite behaviour was observed at large scale, the catalyst poisoning was favoured versus the formic acid evaporation, therefore, it was necessary to add fresh Pd/C catalyst but not additional formic acid.

The process based on the use of a hydrogen transfer reaction was successfully scaled-up to 50 kg using 1600 L reactors. After six industrial scale batches no relevant reactivity issues were found. As in the laboratory 400 g scale run, the industrial scale reactions reached the completion after 32 h and required catalyst refeedings leading to a total consumption of 0.22 p of Pd/C per batch.

In order to enhance the reactivity and reduce catalyst consumption a series of additional experiments were performed.

- Considering that the reaction crude was heterogeneous, and that the Pd/C catalyst showed a marked hydrophilic character use of a surfactant was evaluated. The objective was to enhance the phase mixing efficiency and to use tetrabutyl ammonium iodine (TBAI) as a phase-transfer catalyst (PTC). The use of TBAI should allow to enhance the mass transfer processes between the three different phases involved in the reaction (org. phase, aq. phase and solid catalyst) and, therefore, to increase the reaction rate while reducing the mixing sensitivity. However, the use of surfactants was rejected since no significant improvements were observed in the reactions performed adding TBAI.
- As it was mentioned above, it is known that Pd/C catalysts may become inactivated in presence of CO and CO_2. In order to prevent the catalyst poisoning, a hydrogenation experiment was performed bubbling nitrogen into the reaction crude. The objective was to avoid the accumulation of CO and CO_2 in order to prevent the catalyst poisoning. In the conducted experiment, no reaction rate increase was observed. From the obtained results it was concluded that, bubbling nitrogen into the reaction crude was not an effective way to avoid the catalyst poisoning. Therefore, this approach was also abandoned.
- It was found in the literature that hydrochloric acid may promote the Pd dissolution through the formation of soluble chloro-complexes.[167–169] In order to prevent the catalyst leaching, alternative acids with better compatibility towards the palladium catalyst were tested. During the performed studies, benzene sulfonic acid (BSA), *p*-toluene sulfonic acid (PTSA) and phosphoric acid were evaluated. All the experiments were performed at 1 g scale using the conditions described in Table 16; entry 7. In all cases no formic acid refeeding was performed, and 0.042 eq. of the corresponding acid were loaded. In all cases, the reaction rate increased compared with the standard HCl conditions (see Figure 81).

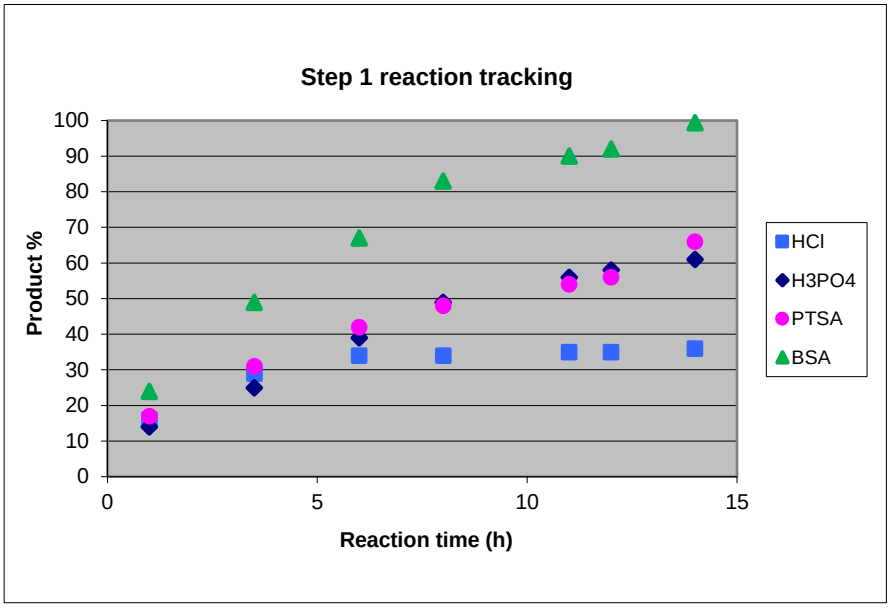


Figure 81. Step 1 amount of product formed *vs.* time data obtained using different types of acids.

For BSA, the reaction reached the completion without necessity of refeeding after less than 14 h. The obtained data seems to indicate that avoiding the catalyst leaching higher reaction rates could be achieved. Moreover, the impurity profile observed in the in-process control analysis of the BSA test was identical to the one obtained using HCl indicating that the use of this acid would not affect the quality of the obtained product. Despite the improvements observed, the use of HCl was maintained because sulfonates such as BSA or PTSA may react with alcohols to generate alkyl benzene sulfonates. These compounds are currently considered as genotoxic. Since MeOH and EtOH were used as solvents in the steps 2 and 3, and MeOH was also used for the reactor cleanings performed during the synthesis, there was a certain risk of generate traces of alkyl benzene sulfonates. Concerning the use of phosphoric acid, it was finally not adopted because by the time this study was concluded the customer requested to modify the step 1 reaction from a hydrogen transfer hydrogenation to a gas hydrogenation (see section 1.6.1.1.1.3). The use of alternative acids with similar properties to BSA but, without potential genotoxic carry-over impurities was left as an option to be studied for the improvement of the **FH0317** manufacturing process in case that the use of the hydrogen transfer approach will be reconsidered.

- The reutilization of the Pd/C catalyst for more than one batch was also studied. It was found that either using HCl or alternative acids the reactions evolved slowly when catalysts on its second cycle were used. For the alternative acids, the obtained results were related with the CO and CO_2 catalyst poisoning occurring during the step 1 reaction. In the case of HCl the decrease on the catalyst activity was attributed to the catalyst poisoning as well as to the catalyst leaching process.

1.6.1.1.1.3. Gas hydrogenation approach

Despite the customer requested to maintain the initially provided synthetic route, after the sixth industrial batch he requested to change the step 1 reaction from a transfer hydrogenation to a gas hydrogenation. The gas hydrogenation conditions from which the development was started

were provided also by the customer. The reaction was performed in a mixture of water, EtOAc and acetic acid 80 % at 70 °C, applying 3 bar of hydrogen gas. During the initial familiarization experiments it was found that using the gas hydrogenation approach the catalyst consumption, the reaction time and consequently, the manufacturing cost could be significantly reduced. In Table 17 are summarized the results obtained using gas hydrogenation (entry 2) and transfer hydrogenation (entry 1).

Entry	Reaction type	Scale (Reactor size)	Description				Reaction time
			Catalyst (p)	HCl (1 M)	Formic Ac.	Acetic Ac. 80 %	
1	Hydrogen transfer	400 g (10 L)	0.16+0.4+0.2	0.08V	9.9 eq	-	32 h
2	Gas hydrogenation	60 g (1.6 L)	0.025	-	-	16.4 eq	14 h

Table 17. Gas transfer hydrogenation and gas hydrogenation reactivity results obtained during the step 1 optimization.

A second round of gas hydrogenation tests was carried out in order to evaluate the suitability of the process for its use at industrial scale. In the performed tests the stability of the reaction crude, the suitability of a catalyst refeeding in case of incomplete conversion and the capability of the process to purge potential impurities of the used raw materials were studied.

The gas hydrogenation process was scaled-up in the laboratory to a 60 g scale using a 1.6 L hydrogenator (Figure 82). No relevant differences in terms of product purity, impurity profile or yield were observed within the **FH-2** intermediates obtained using the two different mentioned hydrogenation protocols.

Figure 82. 1.6 L hydrogenator used to perform laboratory gas hydrogenation tests.

1.6.1.1.2. Work up optimization

As it is mentioned in the precedents section, some adjustments were required to make feasible for the industrial scale operation the step 1 w.up. In this section are collected the different studies performed during optimization of the step 1 w.up.

1.6.1.1.2.1. Aqueous rinsings and catalyst cake wash

In the non-optimized process, despite a good conversion was observed in the IPC, the yield obtained for this step was low (67 %) suggesting that some product was lost during the w.up. In order to determine at which point of the w.up the product was lost an LC study was conducted. The amount of product lost in all the residual aqueous layers, in the Pd/C solid cake and in the filtration mother liquors was evaluated. Relevant amounts of product were observed in the catalyst cake and in the crystallization mother liquors.

It was found that the EtOAc rinsing applied in the original process over the Pd/C solid cake was not completely efficient. The study performed revealed that, the use of several rinsings of 2.3 vol was more efficient than the single wash of 5 vol initially performed. Through the quantification of the amount of product recovered in each solid cake wash, it was determined that three washes of 2.3 vol were required for an efficient product recovery from the catalyst cake.

During the step 1 w.up, a series of aqueous rinsings using a concentrated $NaHCO_{3\,(aq)}$ solution and brine were performed. Decreasing the concentration of the used solutions was evaluated. The use of concentrated solutions increased the risk of having a high content of inorganic impurities in the isolated **FH-2** intermediate and could led to operation problems derived from the uncontrolled crystallization of salts. Additionally, in the case of the $NaHCO_{3\,(aq)}$, it was a certain risk of having product degradation due to the high pH of this phase. The performed studies revealed that the concentration of the $NaHCO_3$ solution could be decreased from a 10 % to a 7 %wt without causing a relevant effect into the process. Related to the NaCl solution, it was found that decreasing its concentration reduced the capacity of this rinsing to remove impurities and the efficiency of the phase separation. However, the quality of the final product was not affected because the impurities not removed in the NaCl rinsing were purged during the product isolation. Finally, the use of a NaCl solution 10 %wt was selected because this was the minimum concentration that lead to a good phase separation.

1.6.1.1.2.2. Solvent swap

The step 1 crystallization was based on a solvent swap from EtOAc to MTBE. As it is mentioned in section 1.6.1.1.2.1, relevant product loses were detected in the crystallization mother liquors. In order to reduce the product loses, which were associated to the presence of residual EtOAc this operation was optimized. The initial procedure was based on the measurement of the weight of solvent collected during the distillations. This methodology may present some important problems since, typically, not all the solvent distilled is condensed. A variable fraction of solvent is normally lost through the vacuum pump and its scrubber system depending on the applied distillation conditions (vacuum, condenser temperature or distillation temperature). Additionally, the total mass of solvent to be distilled may vary due to the necessity of charge more EtOAc during the reaction (e.g. to perform refeedings) or during the w.up (due to phase separation issues, additional catalyst cake rinsings or rinsings of the unloading lines). In order to enhance the robustness of the solvent swap, a procedure based on the control of the residual volume of the reactor content was implemented. The procedure implemented was based on a distillation of EtOAc to two residual volumes and then in the loading of 6 volumes of MTBE. The

obtained slurry was cooled and then filtered to obtain **FH-2**. The use of this procedure combined with the additional product recovery achieved in the palladium catalyst filtration allowed to increase the yield from the 67 % initially reported to a 94 %.

1.6.1.1.2.3. Crystallization

In order to prevent safety issues derived from an abrupt heat release associated to a fast crystallization, a seeding operation was introduced during the step 1 solvent swap. The seeding was also introduced to try to control the **FH-2** polymorph. In the x-ray powder diffraction (XRPD) analyses of the **FH-2** samples obtained in Farmhispania S.A. it was observed that the polymorphic form varied and was not consistent from batch to batch. Two different polymorphic forms (Form I and Form II) and mixtures of these two crystalline phases were detected. A study was conducted in order to determine which were the process conditions that induced the formation of the different observed **FH-2** polymorphs and to determine if the **FH-2** crystallization could be controlled to favour the formation of the Form I. The Form I was set as the target polymorph because it was the crystal phase observed in most of the **FH-2** samples that had been used during the development of the subsequent steps of the process. Therefore, it had been already demonstrated that this polymorphic form was suitable for the obtention of **FH0317**. In this study different cooling ramps, distillation temperatures and seeded polymorph were evaluated. It was found that the crystallization process described in Table 18 allows to obtain **FH-2** in Form I consistently whether the seed polymorph is controlled.
In order to evaluate the potential impact for the process of the **FH-2** polymorphic form, different **FH-2** samples of Form I, Form II and of mixtures of both polymorphs were used in the subsequent step of the synthesis (reduction reaction). During the performed experiments, no noticeable differences in the process behavior were detected. Any incidence in terms of drying, filtration or residual solvent content issues was either observed during the isolation of the mentioned **FH-2** samples. Despite the observed **FH-2** polymorphs and its mixtures were all considered suitable for the obtention of **FH0317**, the process described in Table 18 was finally adopted. The implementation of the Table 18 procedure allowed to guarantee the obtention of the Form I in all the batches and, to increase the process robustness by reducing the probability of having different **FH-2** crystal structures.

Step	Operation
1	Distillation below 55 °C to 8 vol
2	Heat to 70 °C (total dissolution)
3	Cool to 50 °C
4	Seed with 0.001 p **FH-2** (Form I)
5	Maintain at 50 °C at least 1 h
6	Distillation below 55 °C to 2 vol
7	Load at 45 °C 6 vol MTBE
8	Cool to 5 °C in not less than 2 h
9	Maintain at 5 °C at least 4 h
10	Filter the obtained slurry

Table 18. Summary of the crystallization process used for the obtention of FH-2 in Form I.

1.6.1.1.2.4. Inorganic impurities

Once the development of the w.up was concluded, the amount of inorganic impurities present in the **FH-2** intermediate isolated in the laboratory was studied through ICP-MS and residue on ignition (ROI) analyses (see Table 19; sample 1). The obtained results demonstrated that the developed process yielded intermediate **FH-2** with very low levels of this type of impurities. These results were confirmed during the scale-up (see sample 2 in Table 19).

	Sample 1 (lab scale)	Sample 2 (industrial scale)
ICP-MS (Na)	791 ppm	Below LOD (550 ppm)
ICP-MS (K)	Below LOD (550 ppm)	Below LOD (550 ppm)
ICP-MS (B)	Below LOD (11 ppm)	Below LOD (11 ppm)
ICP-MS (Pd)	Below LOD (1.1 ppm)	Below LOD (1.1 ppm)
ROI	0.18 %	0.08 %

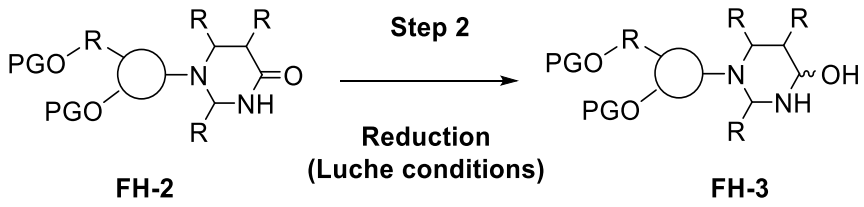
Table 19. Summarized results from ROI and ICP-MS analyses carried out for two different samples of FH-2 intermediate. Limit of detection abbreviated as LOD.

Using the optimized w.up and reaction conditions a 300 g scale laboratory test was performed. Compared with the non-optimized procedure the obtained yield was increased from a 67 % to a 94 % while purity was increased from a 93 % to a 99 %. The implemented modifications also allowed to increase the robustness, profitability and safety of the process making it suitable for the industrial scale operation. The synthesis of **FH-2** based on the optimized w.up and in the described hydrogenation protocols (gas hydrogenation/transfer hydrogenation) was eventually scaled-up to commercial scale (50 kg scale) without relevant issues.

Because of the advantages offered by the gas hydrogenation protocol (lower catalyst consumption and reaction time), the **FH0317** manufacturing process was eventually validated using this approach. On average, the yield and purity obtained during the industrial scale batches were 90 % and 98.9 % respectively.

1.6.1.2. Step 2: Reduction

1.6.1.2.1. Reaction optimization



FH-2 **FH-3**

Scheme 36. Step 2 of the FH0317 synthesis (ester protecting groups abbreviated as -OPG).

In order to reduce the time sensitivity of the reduction reaction (Scheme 36) some experiments were performed. The objective of these experiments was to reduce the product degradation observed when the process conditions included in the process provided by the customer were applied. The results obtained during the step 2 reactivity optimization are collected in this section.

1.6.1.2.1.1. Reaction temperature (from 6 °C to 3 °C)

Initially some experiments were performed decreasing the reaction temperature from 6 °C to 3 °C. The experiments were performed in 0.5 L and 1 L reactors at 10-30 g scale. The main goal was to decrease the reaction rate in order to minimize the product degradation observed once the reaction concluded. It was critical to reduce the impurity formation at the end of the reaction because, in the laboratory, the time required to start the w.up once the reaction concluded was only 30 min. (time required to carry out the LC analysis used to verify the reaction completion). However, to initiate the w.up at industrial scale will require from much larger times. It must be seen that, at large scale, before to continue the process the IPC sample had to be carried out to the Quality Control laboratory, had to be analysed and, the result of the analysis must be communicated to the plant operators.

The reactions that were run using the initially provided conditions but at lower temperature (3 °C) required around 2.5 h to reach the completion. In the performed LC analyses, it was observed that during the reaction progress, important amounts of impurities were formed. Around a 15 % of degradation by-products were already observed at the end of the reaction (Figure 83). Despite the use of a lower reaction temperature, the degradation problems observed once the reaction reached the completion persisted. One hour after the rection completion the amount of impurities increased to 40 %.

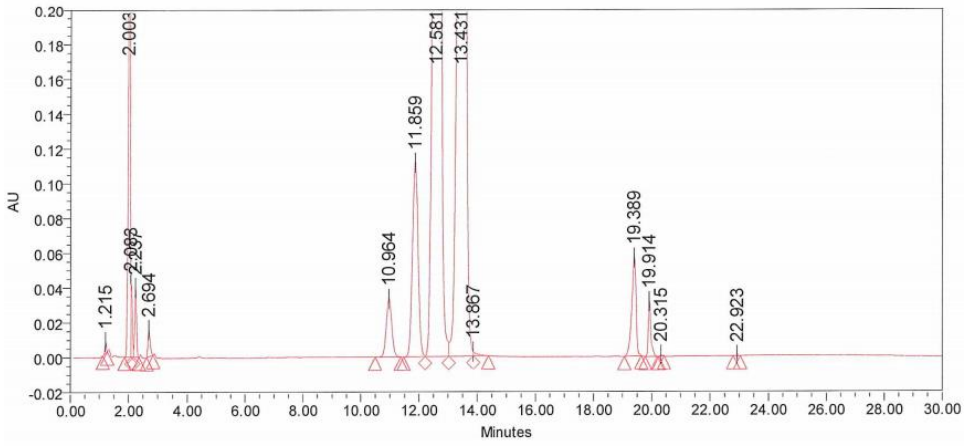
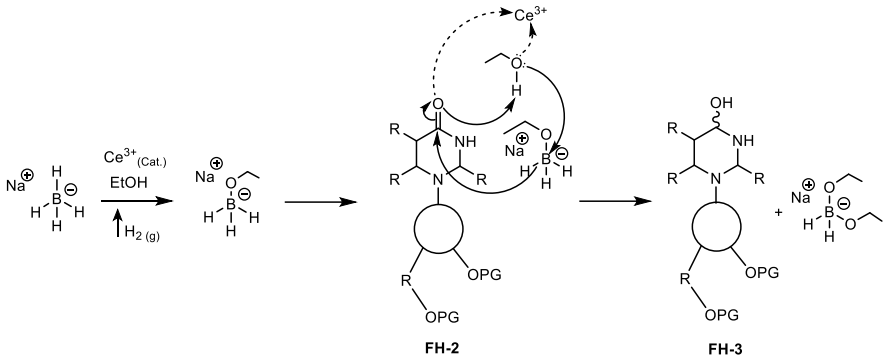


Figure 83. Typical LC profile observed during the step 2 tests once the reaction reached the completion (after 2.5 h of reaction). Retention times (RT) 12.58' and 13.43' corresponds to FH-3 (epimers 1 and 2; Figure 85). Peak appearing at RT 19.91' was identified as FH-1.

A bibliograrphic research was performed in order to reduce the reaction rate and the time sensitivity of this reaction. It was found that Luche reductions are typically used to achieve selective 1,2 reduction of conjugated ketones and take around 5 min. to reach the completion. This type of reaction normally does not affect carboxylic acids, halides, cyano groups, nitro groups, esters or amides. Since the step 2 reaction is not a 1,2 reduction of a conjugated ketone, the performed reaction is not properly a Luche reduction. In this case, the reduction of carbonyl group situated in the heterocyclic moiety of **FH-2** was carried out using the reaction conditions employed in the Luche reductions. Considering that **FH-2** is not a typical substrate for a Luche reduction, the observed reaction times of around 2.5 h were not challenged. The suggested reduction mechanism is based in the formation of alkoxy borohydrides catalyzed by Ce^{3+} (see Scheme 37). These compounds are more reactive than the borohydride ion and allow the

reduction of **FH-2**. The alkoxy borohydride formation was in accordance with the hydrogen release observed during the experiments performed in the laboratory.[170–172]

It is suggested that the formed alkoxy borohydrides reduced the **FH-2** intermediate through the mechanism described in Scheme 37. Although cerium (III) cations are capable to interact with the carbonyl groups of the substrate, according to the literature this interaction does not have a relevant effect in the reaction mechanism.[170–172]



Scheme 37. Alkoxy borohydride formation reaction catalyzed by cerium (III) and, suggested mechanism for the reduction reaction taking place in the second step of the FH0317 synthesis (ester protecting groups abbreviated as -OPG).[170–172]

1.6.1.2.1.2. Reaction stoichiometry

Since no clear improvement was achieved by decreasing the reaction temperature to 3 °C, a set of 1.5 g scale experiments were performed. These experiments were focused on the reduction of the impurity formation through the optimization of the reaction stoichiometry. The reaction stoichiometry was studied through two 2^2 DoE that were performed in a Radleys multi-reactor (see Figure 84) maintaining 3 °C as a reaction temperature. The reactions were carried out at 3 °C since a slight increase in the reaction time was observed compared with the initial process (performed at 6 °C). The mentioned DoEs were designed to study the influence of the amount of $NaBH_4$ and $CeCl_3$ in the formation of impurities. Initially, a screening DoE was performed in order to determine the influence in the reaction performance of the studied parameters and to define the tentative ranges to study. It was decided to explore the effect of decreasing the amounts of $NaBH_4$ and $CeCl_3$. The ranges studied for sodium borohydride and cerium trichloride were 0.15 to 0.10 p and 0.06 to 0.04 p, respectively.

The results obtained from this DoE indicated that, to decrease the amount of $NaBH_4$ to 0.10 p was detrimental for the reaction. The experiments where the low range of this reagent was used evolved slower. Initially they generated less impurities but during the extra time required to reach the reaction completion more impurities were formed. Related to the $CeCl_3$, a smooth degradation decrease was observed for the reactions performed at the high range for this reagent.

The information obtained in the first DoE was used as starting point for an optimization DoE. In this second set of experiments the ranges explored for $NaBH_4$ and $CeCl_3$ were 0.15 to 0.13 p and 0.06 to 0.08 p respectively. From the optimization DoE it was concluded that the use of 0.13 p of $NaBH_4$ and 0.08 p $CeCl_3$ allowed to decrease the reaction rate while maintaining the impurity levels observed in the initial 3 °C trials. Using the mentioned conditions, the reaction required 3.5 h to reach the completion and, a 16 % of degradation by-products were observed at the end

of the reaction. Since the optimal value for $CeCl_3$ was in the edge of the explored range, an additional experiment using 0.10 p of this reagent was performed. In this case, no clear improvement was observed. Therefore, it was decided to maintain the conditions found during the optimization DoE (0.13 p of $NaBH_4$ and 0.08 p $CeCl_3$).

Figure 84. Radleys multi-reactor used to perform the step 2 DoE studies (right). Detail of a Radleys 25 mL reactor provided of mechanical stirring (left).

1.6.1.2.1.3. Reaction temperature (from 3 °C to -8 °C)

Although the reaction time was increased from 2 h to 3.5 h reducing the time sensitivity, this step still had associated a high risk of batch failure because the reaction quench must be immediately performed to avoid product degradation. In order to solve this process weakness, the use of an even lower reaction temperature was evaluated. During the reaction temperature studies performed, it was assumed that there was no risk of ice formation because, the added water was mixed with EtOH forming a phase containing an 89.5 %wt of this alcohol. According to the literature the freezing point of this mixture is -95 °C. Therefore, it was concluded that the reaction temperature could be decreased far below 0 °C.[173]

Two experiments were performed using temperatures of 0 °C and -8 °C. Although no significant improvements were observed at 0 °C, the use of -8 °C allowed to reduce significantly the reaction rate and the rate of the product degradation reactions occurring once the reaction concluded. Using the optimized temperature and stoichiometry the reaction required around 13 h to reach the completion. At this point, the reaction crude contained around a 10 % of impurities; a 5 % less than the observed in the reaction crude obtained during the initial trials performed at 3 °C. The reaction crude obtained using the optimized conditions was stable for 7.5 h if it was kept at -8 °C. After 7.5 h, the impurity contents only increased in a 4 % which was considered a remarkable improvement compared with the 25 % increase observed in 1 h when using the non-optimized conditions.

As a result of the reactivity optimization the time sensitivity was decreased, and the process robustness was increased to acceptable levels for the industrialization of this reaction.
The optimized conditions were scaled-up in the laboratory to 400 g scale using a 10 L reactor without relevant issues. **FH-3** was obtained as a mixture 50:50 of the *(R)* and the *(S)* epimers (Figure 85).

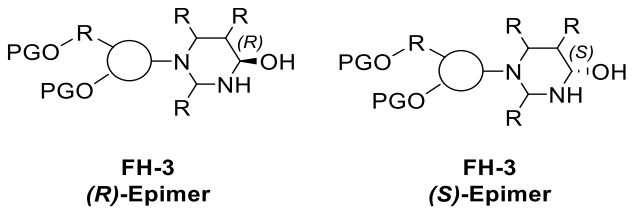


Figure 85. Molecular structures of the FH-3 epimers; *(R)* configuration (left) and *(S)* configuration (right), (ester protecting groups abbreviated as -OPG).

1.6.1.2.2. Work up optimization

The step 2 w.up required from optimization in order to reduce the product degradation, to increase the yield and to make it feasible for the industrial scale operation (eliminating the sodium sulphate drying and avoiding the formation of thick slurries). In this section they are collected the different studies performed during the development of the step 2 w.up.

1.6.1.2.2.1. Product degradation

During the treatment with acetone and the pH adjustment performed in the initial part of the step 2 w.up, relevant product degradation issues were found. After a LC-MS study it was determined that the main impurities being formed were the same detected during the reaction. They were identified as the over-reducted and the deprotected by-products (Figure 86).

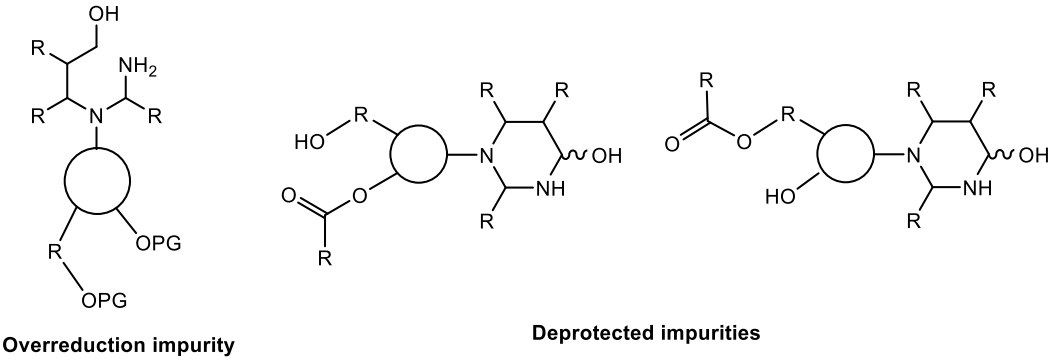


Figure 86. Molecular structures of the over-reducted impurity (left) and the deprotected impurities (right), (ester protecting groups abbreviated as -OPG).

In order to solve the mentioned degradation problems, a series of modifications were implemented over the original process. The w.up temperature was decreased to -8 °C to slow down the impurity formation. The amount of acetone used to quench the reaction was increased in order to minimize the over-reducted impurity formation since it was found that the quantity of acetone added in the initial process was not enough to completely neutralize the sodium borohydride excess. To reduce the formation of the deprotected impurities, the acid used during the quenching was changed from concentrated hydrochloric acid to diluted citric acid. The use of diluted citric acid allowed to avoid the formation of acid hot spots during the pH adjustment and reduced the exothermic character of the acid addition. As a result, the exposition of **FH-3** to basic or acid pH or to high temperatures was reduced decreasing the product degradation.

In the process provided by the innovator, a rinsing of the organic phase was performed using a 16 %wt $NaHCO_3$ solution after the pH adjustment. The concentration of this rinsing was studied and optimized. It was found that it could be reduced to a 4 %wt without affecting the process. This optimization allowed to reduce the risk of impurity formation by reducing the pH of the rinsing. Additionally, the optimization allowed to reduce the probability of having a high content of inorganic impurities in the isolated **FH-3** intermediate.

1.6.1.2.2.2. Sodium sulphate drying elimination

Continuing with the w.up optimization, the sodium sulphate drying performed before the product crystallization was eliminated without causing any impact into the process. The residual water content after and before the drying could not be determined using Karl-Fischer (KF) titration because **FH-3** interfered with the KF reagents. However, it was found in the literature that water forms azeotropes with DCM and MTBE; 97:3 %wt (DCM:Water) and 96:4 %wt (MTBE:Water) respectively. Considering that the water solubility in the DCM organic phase was rather low (1.76 g/L) and the large amounts of solvent distilled during the solvent swap, it was concluded that the water traces could be efficiently eliminated by the stablished process. Therefore, no sodium sulphate drying or control of the residual amount of water was required at the end of the distillations.[174,175]

1.6.1.2.2.3. Crystallization

The solvent swap from DCM to MTBE performed in order to crystallize the product was also studied and optimized. Initially, the possibility of telescope steps 2 and 3 was studied in order to avoid the **FH-3** isolation. A solvent exchange from DCM to MeOH (step 3 solvent) was developed and the step 3 was carried out successfully using the obtained **FH-3**/MeOH mixture. This approach allowed to solve the filtration/low yield problems observed at the end of the step 2. Nevertheless, the telescoped approach was finally not implemented because to isolate as much intermediates as possible during the process allows to purge impurities, to enhance the process control in terms of yield and residual solvents and, to assess in a more accurate way the quality of the different process intermediates.

After the rejection of the telescoped approach, an optimization of the DCM to MTBE solvent swap was performed. During the first trials low yields were obtained. It was suggested that the initial process was not allowing to effectively remove the DCM affecting the **FH-3** crystallization. The volume of solvent to be distilled and the number of co-distillations required to obtain a higher yield were evaluated experimentally and *in-silico* using the Dynochem software presented in The Dynochem software section.

As it is shown in the Figure 87 legend, using the solvent swap distillation model included in the Dynochem software many different parameters can be studied. The software requires information about the distillation conditions (vacuum, jacket temperature, minimum and maximum volumes reached during the distillation and initial contents of the reactor). Based in the values provided by the user and, in other parameters such as azeotropic data or densities which are extracted from data bases, the Dynochem software is able to calculate the volume of solvent that must be distilled to reach the desired residual level of the undesired solvent as well as may other distillation parameters (see Figure 87).

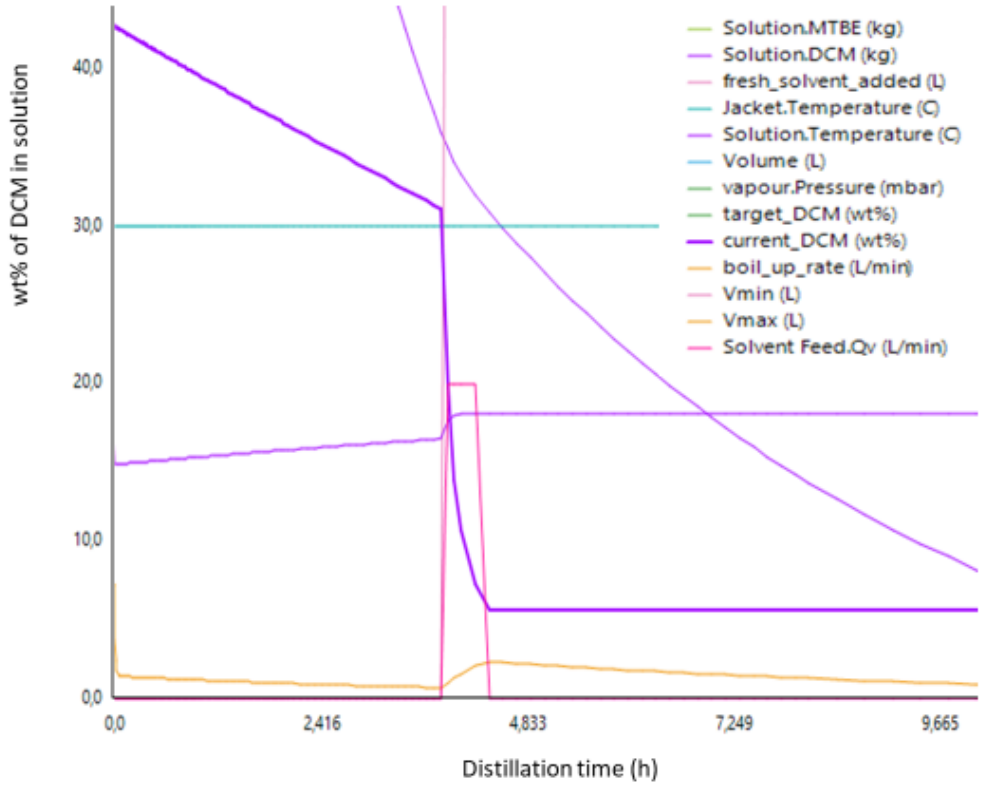


Figure 87. Plot of a series of distillation parameters *vs.* time obtained from the simulations performed using the Dynochem software. The plotted series are within the list of the main parameters that can be simulated and plotted during the distillation studies performed using the Dynochem software (see the attached legend).

The formation of thick slurries was observed during the experimental studies performed to increase the yield. Therefore, the crystallization procedure was adapted to solve issues related with the thickness of the slurry formed during the distillations. It was determined that to reduce the residual volume of the first DCM phase to 2 residual volumes and to maintain the mixture volume above 7 residual volumes during the subsequent DCM:MTBE co-distillations allowed to avoid slurry thickness issues. As it is mentioned above, the number of co-distillations required was evaluated using the Dynochem software. In Table 20 is presented the comparison between the optimized and the non-optimized solvent swap processes carried out using *in-silico* simulations.

Non-optimized solvent swap (thickness problems)						
Step	**Distil to 3 residual vol.**	**Add 6 vol MTBE**	**Distil to 3 residual vol.**	**Add 6 vol MTBE and cool**		
%wt DCM in solution	100 %	43.1 %	31.2 %	11.1 %		
Optimized solvent swap						
Step	**Distil to 2 residual vol.**	**Add 10 vol MTBE**	**Distil to 7.5 residual vol.**	**Add 5 vol MTBE**	**Distil to 7.5 residual vol.**	**Add 3.4 vol MTBE and cool**
%wt DCM in solution	100 %	23.4 %	21.0 %	12.8 %	12.0 %	7.9 %

Table 20. Residual %wt of DCM in solution in the different steps of the solvent swap process (optimized and non-optimized). Data obtained using the Dynochem software solvent swap distillation model.

Despite the optimized process, still remarkable amounts of DCM were present. Moreover, the final crystallization volume was higher and experimental yields around the 85 % with purities around the 92 % were obtained. Considering that the yield of the initial process was 65 % and

the obtained purities were around the 90 %, the above presented solvent swap procedure was considered suitable for the large scale manufacturing.

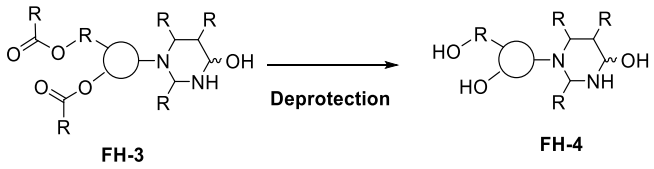
Further investigations revealed that, reducing the crystallization volumes or the residual content of DCM through additional distillations resulted in thick slurries and slow filtrations without remarkable yield increases. As it is presented in Table 20, the residual amount of DCM at the end of the distillations was similar in both procedures, demonstrating that the DCM content of the final slurry was not critical for the process yield. The yield and purity increase observed in the optimized process were related to the improvements achieved in terms of mitigation of the product degradation.

The optimized w.up conditions were scaled-up in the laboratory to a 400 g scale using a 10 L reactor and no relevant issues were observed. During the scale-up, the process robustness was evaluated studying the stability of the organic phase at different points of the w.up and the stability in front of temperature deviations and pH excursions. Additionally, it was confirmed that through the developed drying procedure it was possible to achieve the desired levels of residual solvents without causing the **FH-3** degradation.

The developed process was successfully scaled-up to industrial scale (42 kg scale; 1600 L reactor) obtaining similar results to the ones observed in the laboratory trials.

1.6.1.3. Step 3: Deprotection

1.6.1.3.1. Reaction optimization



FH-3 FH-4

Scheme 38. Step 3 of the FH0317 synthesis.

During the development of the process it was found that an important amount of the impurities detected in the final API were formed during the deprotection step. After several trials, it was determined that the decrease of the levels of impurities that could be achieved through the optimization of the CIDT (step 5) and of the purification (step 6) was limited. Therefore, an optimization of the deprotection step was required in order to decrease the formation of these impurities. In this section are collected the investigations performed in order to minimize the impurity formation during the deprotection reaction.

1.6.1.3.1.1. Reaction conditions

In order to study the step 3 reactivity, an HPLC method of analysis (MoA) that allowed the reaction monitoring was developed using the equipment available at Farmhispania S.A. The chromatographic method was developed using as a starting point the LC MoA provided by the drug innovator to control the steps 1 and 2 of the synthesis. During the initial phases of the development the wavelength (λ) used in the initial method (235nm) was maintained. In the performed trials it was found that, **FH-4** and all the associated impurities generated during the

deprotection reaction were not detected at this wavelength. It is suggested that the absence of the protective groups modified the UV absorption profile of all these substances causing the observed lack of absorption. The modification of the used λ to 195nm allowed the detection of the mentioned substances. However, the chromatographic resolution of the peaks corresponding to the different substances being studied was poor, complicating the analysis. The variations in the molecular structure induced by the removal of the protective groups caused important modifications in the chromatographic behaviour of the analysed substances making the used MoA unsuitable for the reaction control. The mentioned analytical problems were eventually solved using a completely different LC method more similar to the final product (**FH0317**) MoA than to the steps 1 and 2 MoA.

The monitoring of the impurity content of the reaction crude and of the different phases obtained during the w.up revealed that, the mentioned impurities were formed either during the deprotection reaction or during the solvent swaps performed in the step 3 w.up. In order to reduce the amounts of impurities generated during the reaction, alternative temperatures and bases such as NaOH, NH_4OH, Et_3N and NaOEt were evaluated. Additionally, the utilization of water, 2-methyl tetrahydrofuran (2-MeTHF) and EtOH as solvents was studied. The obtained results are summarized in Table 21.

Entry	Base	Solvent	Temperature	Results
1 (Ref.)	NH_3	MeOH	25 °C	**FH-4** with 97.7 % purity
2	NH_3	MeOH	40 °C	No improvement in reaction rate/impurity profile (lower NH_3 solubility)
3	NH_3	ACN/MeOH	25 °C	Deprotection not completed
4	NH_3	DCM/MeOH	25 °C	Deprotection not completed
5	NH_3	THF/MeOH	25 °C	Deprotection not completed
6	NH_3	MEK/MeOH	25 °C	Deprotection not completed
7	NH_3	EtOAc/MeOH	25 °C	Deprotection not completed
8	NH_3	2-MeTHF/MeOH	25 °C	Deprotection not completed
9	KOH	H_2O/2-MeTHF	25 °C	Good reactivity/High degradation
10	DBU	MeOH	40 °C	Reaction completed within 3 h **FH-4** with 97.1 % purity
11	NaOH	H_2O/2-MeTHF	25 °C	Good reactivity/High degradation
12	LiOH	MeOH	25 °C	Good reactivity/High degradation
13	NH_4OH	H_2O/2-MeTHF	25 °C	Deprotection not completed
14	NaOEt	EtOH	78 °C	No reaction
15	LiOEt	THF	25 °C	Good reactivity/High degradation
16	Et_3N	2-MeTHF	80 °C	No reaction

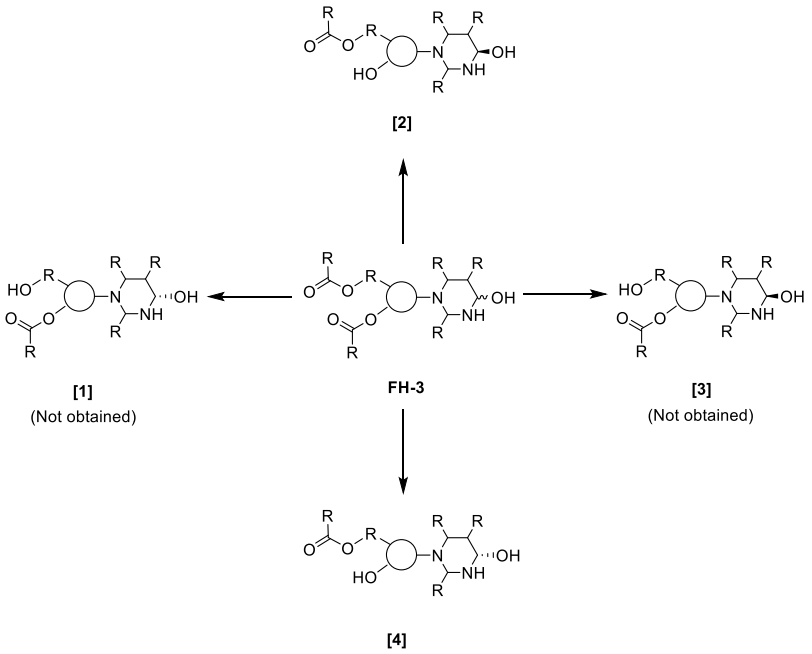
Table 21. Summary of the results obtained during the optimization of the deprotection reaction. Reaction time applied was 17 h in all the cases except for entry 9.

No clear improvement was achieved during the deprotection optimization trials. The reference conditions based on the ammonia/MeOH approach (entry 1) leaded to the lowest product degradation. Therefore, it was decided to maintain the ammonia/MeOH conditions and to focus our efforts in the optimization of the reference conditions and the w.up.

1.6.1.3.1.2. Reaction time

The LC-MS study performed using the developed chromatographic method revealed that the reaction reached the completion after 17 h. The study indicated that to extent the reaction time to 72 h as it was done in the non-optimized process caused an increase in the product impurity

content. During the reaction monitoring it was observed that **FH-3** was totally consumed during the first 2 h of reaction leading to the formation of two monodeprotected intermediates and the corresponding amide/methyl ester by-products. The reaction rate of the monodeprotected intermediates was considerably slower. They required around 17 h to be totally consumed. The suggested mechanism of formation of these intermediates is identical to the one presented for **DH0517** in **Monodeprotected DH0517** impurity study section. Similarly to **DH0517** from the **FH-3** deprotection, 4 possible isomers could be obtained (see Scheme 39). The monodeprotected intermediates were identified as the epimer pair [2] and [4] (Scheme 39) by an NMR study. In order to obtain a pure sample of these intermediates a step 3 reaction was left to evolve for 2 h and then distilled to dryness. The resulting oil from the distillation was purified through MPLC to obtain a solid containing the two monodeprotected intermediates with an overall LC purity close to the 100 %.



Scheme 39. Suggested structure for the four possible monodeprotected intermediates that could be formed from the FH-3 partial deprotection. Compounds [1] and [3] were actually not obtained.

The analysis of the reaction crude obtained at the end of the reaction revealed that **FH-4** was obtained as an epimer mixture (see Figure 88). The obtained results were in accordance to the stereochemistry of **FH-3** which already was a mixture of epimers.

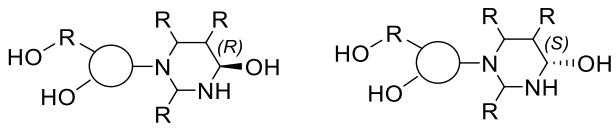


Figure 88. Molecular structures of FH-4 intermediates with *(R)* configuration (left) and with *(S)* configuration (right).

The deprotection reaction conditions based on the reduced reaction time were successfully scaled-up in the laboratory to a 240 g scale in a 10 L reactor.

1.6.1.3.2. Work up optimization

In the non-optimized process, a solvent swap from MeOH to water was performed once the deprotection reaction was completed. The obtained aqueous layer was rinsed several times with EtOAc to eliminate the amide and the methyl ester generated as deprotection by-products. After the rinsings, a second solvent swap from water to ACN was carried out and, the obtained slurry was directly used in the step 4 (steps 3 and 4 were telescoped).

As it is mentioned above, the reduction of the impurity formation during the step 3 was critical for the improvement of the **FH0317** purity. The LC method developed to monitor the deprotection reaction (see section 1.6.1.3.1) was also used to study the w.up. It was found that most of the impurities formed during the step 3 w.up were generated during the performed solvent swaps. Despite vacuum was applied, the high temperatures required to distill water promoted the product degradation. The impurity formation was particularly relevant during the distillations performed before the aqueous phase rinsings, which were carried out in presence of the reaction by-products. This section includes the different w.up studies performed in order to increase the purity of the obtained **FH-4** intermediate.

1.6.1.3.2.1. Alternative purification strategies

In order to increase the capability of the process to purge the generated impurities, different approaches were considered:

- The use of alternative solvents such as DCM, heptane, toluene, cyclohexane, cyclopentyl methyl ether (CPME) and 2-MeTHF for the aqueous phase rinsings as well as the increase in the number of washes performed was evaluated. During these studies it was found that in all the cases the rinsings were useful for the elimination of the amide and methyl ester by-products but not for the removal of other impurities. The EtOAc initially used as a solvent demonstrated to be the more efficient extractive agent allowing the maximum removal of amide and methyl ester with the minimum number of extractions.
- The introduction of a purification treatment for the aqueous phase obtained before the second solvent swap was evaluated. The utilization of silica, functionalized silica (C-18), basic alumina, neutral alumina, acid ion exchange resin, basic ion exchange resin and celite was studied. Only in the case of neutral alumina a slight reduction in the impurity content was achieved. However, in this case considerable amounts of product were lost causing a reduction of around the 20 % of yield compared with the non-optimized process.
- The isolation of the **FH-4** from the slurry obtained after the second solvent swap to ACN was considered. It was determined that in the filtration mother liquors a small part of the impurities were purged and, that the amount of product lost was low. The implementation of an isolation step was also attractive because, as it is mentioned in previous chapters, the isolation of the process intermediates allows to increase the process robustness and control. Despite some product was lost in the filtration, the overall yield of the steps 3 and 4 was slightly increased from a 62 % to a 66 %. The

observed yield increase was related to the reduction of the product loses achieved because of the more optimal adjustment of the volumes of solvent used in the step 4.

- The implementation of a purification based on a MeOH slurry applied over the isolated **FH-4** was considered. It was observed that the impurity levels were slightly reduced. However, considerable amounts of product were lost during the purification causing a decrease of around the 40 % of yield versus the telescoped process.

Despite no significant purification was achieved through the **FH-4** isolation, it was decided to incorporate it into the process because it allowed to increase the process robustness as well as slightly the yield. From the obtained results, it was concluded that removing the impurities is complex. Therefore, it was decided to modify the w.up in order to reduce the product degradation observed during the solvent swaps.

1.6.1.3.2.2. Solvent swap

The adopted approach was based on the elimination of the aqueous rinsings. Over the reaction crude a solvent swap from MeOH to ACN was performed to obtain a slurry from which **FH-4** was isolated by filtration. The reaction by-products (amide and methyl ester) were eliminated in the filtration mother liquors in this case. The elimination of the water distillations allowed to reduce the distillation time and the distillation temperatures leading to a lower product degradation. The main problem found during the implementation of this approach were the low yields caused by the product aggregation in the reactor walls (see Figure 89).

Figure 89. Product aggregation issues observed during the development of the step 3 w.up.

In order to avoid the observed product aggregation different procedures were evaluated:

- Perform the distillations maintaining high residual volume in the reactor to avoid the solid crusting.
- Perform several ACN distillations in order to eliminate the MeOH traces from the medium. It was suggested that the observed phenomenon was related to a higher affinity of **FH-4** towards MeOH. Therefore, in presence of small amounts of MeOH, **FH-4** agglomerated around this solvent. In this case, it was hypothesized that the complete removal of the MeOH from the system through several ACN distillations would allow to resuspend and disaggregate the product agglomerates.
- Seed at a certain point of the solvent swap to modify the crystallization behavior.

- Increase the scale to verify that the observed mechanical loses were not related the small scale of the experiments.
- Directly perform the step 4 of the **FH0317** synthesis (Scheme 32) using the slurry obtained after the solvent swap from MeOH to ACN. The aim was to determine if the solid agglomerates were resuspended during the step 4.

In all the above described trials, negative results were obtained. The obtained yields were low and product agglomeration persisted. Eventually, it was found that the agglomeration was promoted by the methyl ester generated as a by-product of the reaction. Despite this substance was completely miscible in ACN, in presence of **FH-4** it oiled out from the mixture agglomerating the **FH-4** crystals. The study of the behavior of the ACN/**FH-4**/methyl ester system in presence of different co-solvents allowed to determine that the agglomeration could be avoided by alcohols (see Table 22).

Entry	Co-solvent	Me-ester miscibility	Agglomeration in presence of FH-4
1	MeOH	Full	No
2	-	Full	Yes
3	THF	Full	Yes
4	Toluene	Full	Yes
5	EtOH	Full	No
6	MTBE	Full	Yes
7	IPA	Full	No

Table 22. Summary of the results obtained during the study of the FH-4 agglomeration. Experiments performed mixing 0.1 mL of ACN, 0.1 mL of the corresponding co-solvent and 0.1 mL of methyl ester to determine miscibility. Over the mixture used to determine miscibility, 25 mg (approx.) of FH-4 were added to evaluate agglomeration.

The agglomeration problems were solved through the addition of isopropyl alcohol (IPA) to the mixture before the first loading of ACN. IPA was selected as solvent because **FH-4** presented a lower solubility in this solvent than in MeOH or EtOH. Moreover, it was found in the literature that ACN forms an azeotrope with IPA; 37:63 %v/v (IPA:ACN) which allowed to maintain a certain amount of IPA during all the solvent swap preventing the agglomeration issues.[174]

The volume of IPA to be added and the number of co-distillations required was evaluated experimentally and *in-silico* using the Dynochem software solvent swap distillation model already introduced in section 1.6.1.2.2.3. The study of the solvent swap process carried out using the Dynochem software simulations is presented in Table 23.

Optimized solvent swap							
Step	**Distil to 3 residual vol.**	**Add 1.5 vol IPA + 5 vol ACN**	**Distil to 3 residual vol.**	**Add 7 vol ACN**	**Distil to 5 residual vol.**	**Add 2 vol ACN**	**Distil to 5 residual vol. and cool**
%wt MeOH in solution	100 %	27.9 %	20.9 %	6.3 %	0.6 %	0.4 %	0.2 %
%wt IPA in solution	0 %	16.6 %	29.7 %	9.0 %	4.2 %	3.0 %	2.3 %
%wt ACN in solution	0 %	55.5 %	49.4 %	84.7 %	95.2 %	96.6 %	97.5 %

Table 23. Residual %wt of IPA, ACN and MeOH in solution during the different steps of the optimized solvent swap process. Data obtained using the Dynochem software solvent swap distillation model.

The solvent exchange process was designed to minimize the amount of IPA and MeOH into the final crystallization mixture without causing agglomeration. The objective was to increase the process robustness and yield. By reducing the residual MeOH and IPA content in the isolated **FH-**

4 the possibility of having issues in the subsequent steps of the synthesis related to the presence of residual amounts of these solvents was minimized. Considering that the solubility of **FH-4** was higher in IPA and in MeOH than in ACN, the reduction of the total amount of these solvents in the slurry obtained before the filtration allowed to increase the yield of this step.

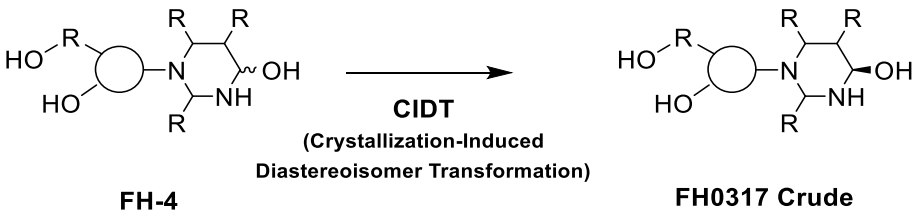
The impact on product quality related to the use of IPA was evaluated through GC analyses. It was found that after drying, **FH-4** contained small amounts of IPA (around 600 ppm). Furthermore, no IPA was found in the **FH0317 crude** and in the **FH0317** obtained after the subsequent CIDT and purification steps.

The optimized reaction conditions coupled to the w.up based on the **FH-4** isolation were successfully scaled-up in the laboratory to 240 g scale in a 10 L reactor. **FH-4** was obtained with a 90 % yield and a 99.4 % purity (considering both product epimers). These conditions were eventually industrialized to a 36 kg scale using a 1000 L reactor without any relevant issue. See in Figure 90 the industrial filter used for the **FH-4** isolation.

Figure 90. 292 L capacity Nutsche filter used at plant for the FH-4 isolation.

1.6.1.4. Step 4: CIDT

1.6.1.4.1. Reaction optimization



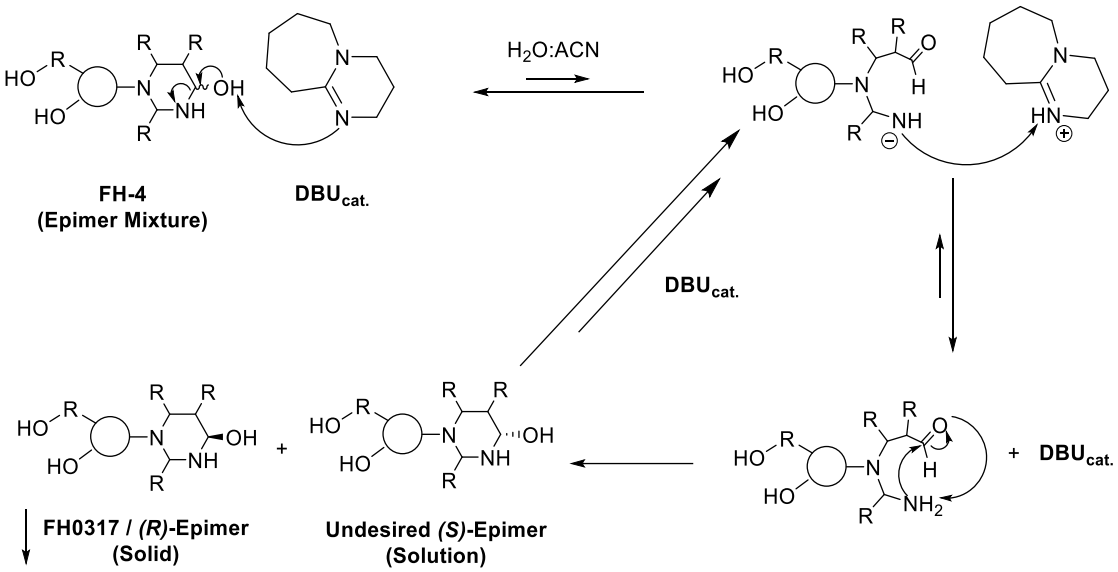
Scheme 40. Step 4 of the FH0317 synthesis.

It was found that the LC method developed to control the step 3 reaction was also suitable for the study of the CIDT. The analysis of different samples taken from the step 4 reaction crude revealed that impurities were formed during the CIDT. These results triggered the studies presented in this section which were mainly focused on the reduction of the impurity formation.

1.6.1.4.1.1. Reaction conditions

This step was performed suspending **FH-4** at 25 °C in a mixture of ACN and water containing catalytic amount of DBU. According to the information from the literature, this kind of dynamic resolutions is based on the interconversion between the two studied isomers in presence of a base or an acid. The reaction is driven by the difference in solubility of the two involved isomers.[176,177]

As it is commented in section 1.4.4, the desired *(R)*-epimer was less soluble than the *(S)* one. Scheme 41 shows the mechanism suggested for the studied CIDT.



Scheme 41. Suggested mechanism for the crystallization-induced diastereoisomer transformation (CIDT) performed in the fourth step of the FH0317 synthesis.

In order to reduce the formation of impurities and increase the purity of the obtained product, the use of alternative bases and the reaction stoichiometry were studied (see Table 24). All the experiments were performed at 1 g scale in 16 mL vials with magnetic stirring. In all the tests, a **FH-4** batch with a purity of 98.44 % and an epimer ratio (53:47) was used as starting material.

Entry	Base	Reaction time (h)	Yield	Crude FH0317 purity	Epimer ratio	Impurities
1	DBU 0.05 eq. [Reference]	66	81.3 %	95.36 %	97.1:2.9	1.80 %
2	DBU 0.028 eq.	111	81.1 %	96.59 %	97.9:2.1	1.33 %
3	DBU 0.014 eq.	111	92.2 %	96.68 %	98.0:2.0	1.05 %
4	DBU 0.007 eq.	111	92.2 %	96.69 %	97.9:2.1	1.28 %
5	DBU 0.001 eq.	66	67.2 %	94.40 %	95.7:4.3	1.39 %
6	Et_3N 0.05 eq.	111	85.6 %	97.23 %	98.2:1.8	1.02 %
7	Et_3N 0.001 eq.	66	55.1 %	93.90 %	95.2:4.8	1.38 %
8	Collidine 0.05 eq.	111	76.7 %	63.44 %	64:36	0.88 %
9	DIPEA 0.05 eq.	111	82.2 %	97.10 %	98.1:1.9	1.04 %
10	TBD 0.05 eq.	111	74.4 %	90.25 %	94.5:5.5	4.49 %
11	Bu_3N 0.05 eq.	111	81.1 %	96.67 %	97.7:2.3	1.07 %
12	Bu_3N 0.001 eq.	66	56.4 %	91.18 %	92.5:7.5	1.43 %
13	$BuNH_2$ 0.001 eq.	66	53.4 %	90.18 %	91.5:8.5	1.46 %
14	NH_3 0.001 eq.	66	52.9 %	94.13 %	95.4:4.6	1.29 %
15	DBN 0.05 eq.	66	82.8 %	95.89 %	97.4:2.6	1.57 %
16	TED 0.05 eq.	66	61.9 %	95.45 %	96.7:3.3	1.30 %
17	DMAP 0.05 eq.	66	61.8 %	93.25 %	94.5:5.5	1.33 %
18	K_2CO_3 0.05 eq.	66	83.1 %	95.71 %	97.4:2.6	1.77 %
19	LiOH 0.05 eq.	66	77.1 %	88.93 %	93.7:6.3	5.10 %
20	LiOEt in THF 0.05 eq.	66	79.5 %	87.91 %	93.0:7.0	6.61 %
21	PMP 0.05 eq.	66	86.0 %	96.89 %	97.9:2.1	1.00 %
22	TMP 0.05 eq.	66	85.5 %	96.80 %	97.8:2.2	1.07 %
23	Piperidine 0.05 eq.	66	83.5 %	96.99 %	97.9:2.1	0.97 %
24	Sodium trimethylsilanoate 0.05 eq.	66	78.5 %	90.25 %	94.4:5.6	4.43 %
25	^tBuOK 0.05 eq.	66	73.5 %	88.60 %	93.0:7.0	4.69 %
26	Lutidine 0.05 eq.	66	54.0 %	94.10 %	95.2:4.8	1.15 %
27	TMG 0.05 eq.	66	81.0 %	95.38 %	97.0:3.0	1.70 %

Table 24. Summary of the results obtained during the study of the step 4 reaction. See in the abbreviation index the meaning of the abbreviations used for some of the evaluated bases.

Compared with the original conditions, smooth improvements were observed for DBU, Et_3N, Bu_3N, DIPEA, PMP, TMP and piperidine (see entries 1 to 6, 9, 11 and 21 to 23 in Table 24). The most interesting results were obtained for DBU itself. The utilization of DBU, would allow a fast process optimization because, the impurity profiles of the products obtained were already familiar. Additionally, the analytical methods required to control this substance at ppm levels in the final API were already available. The experiments collected in entries 1 to 5 revealed that the amount of DBU could be reduced to 0.007 eq. leading to improvements in product quality and yield. During the scale-up of the reduced DBU conditions carried out in a laboratory reactor with mechanical stirring (see Figure 91), a reduction on reactivity was observed. The observed behaviour was related to the variation of the stirring mechanism. It was suggested that the magnetic stirrer used during the small-scale experiments promoted the milling of the **FH-4** crystals favouring the solubilization of the undesired epimer and the crystallization of **FH0317**. This hypothesis was confirmed taking an aliquot from the reactor and placing it in a round bottom flask with a magnetic stirrer. After some time under magnetic stirring, the reaction evolved and reached the completion. The reduced DBU conditions were considered not suitable for the industrial scale manufacturing because, at large scale, mechanical stirring is applied. For this reason this approach was abandoned.

Figure 91. Example of a mechanical stirrer used during the scale-up studies carried out in the laboratory.

The utilization of an alternative base was not further investigated because, after discussed it with the customer it was concluded that the smooth improvements observed at small scale experiments (Table 24) were not significant enough to justify additional work in this area (development of new analytical methods and scale-up of the new conditions).

In order to increase the yield of the synthesis, an additional experiment was performed using half of the ACN/water volumes and reducing the amount of DBU added to 0.025 eq. Since the concentration of DBU in solution was kept constant, during this experiment no reactivity issues were observed despite mechanical stirring was applied. The obtained results demonstrated that, to reduce the reaction volumes does not represent any advantage in terms of purity and yield compared to the standard conditions. Therefore, this approach was abandoned, and the reaction conditions described in entry 1 of Table 25 were maintained.

1.6.1.4.1.2. Reaction time

As it can be seen in Table 25 (entry 3), the impurities formed during the CIDT were mainly purged in the filtration mother liquors and had a limited impact in the quality of the obtained **FH0317 crude**. The observed trend was attributed to the fact that the main part of the **FH0317** present into the pre-resolution epimer mixture (**FH-4**), remains undissolved during the resolution influencing the purge of the formed impurities which remain dissolved. Additionally, it was found that the reaction time could be reduced since the steady state is reached relatively fast. The reduction in reaction time allowed to minimize the product degradation and the erosion of the formed crystals increasing slightly the yield and the product quality (see Table 25).

Entry	Reaction time	FH-4 purity	Epimer ratio	FH0317 crude purity	Impurities in reaction crude (before filtration)	Yield
1	2 h	98.06 %	96.9:3.1	98.29 %	3.06 %	74 %
2	20 h	98.06 %	97.0:3.0	98.18 %	1.88 %	68 %
3	9 days	99.96 %	99.1:0.9	99.23 %	13.65 %	35 %

Table 25. Summary of the results obtained during the study of the step 4 reaction time.

In entries 1 and 2 it can be observed that the amount of impurities in the reaction crude decreased slightly during the first 20 h. The obtained result was related to the low homogeneity of the samples taken from the reaction slurry. This hypothesis was reinforced by the results obtained after 9 days of reaction (entry 3) where, a clear increase of the impurity content was

observed. The obtained results confirmed that the product degraded if extended reaction times were applied. It is suggested that the erosion of the formed crystals promoted by the extended stirring times applied increased the amount of **FH0317** dissolved. The observed decrease of yield when the reaction times were increased was related to this phenomenon as well as to the product degradation that, leaded to the formation of more soluble impurities. Based in the obtained results it was decided to reduce the reaction time from 48 h to 2 h.

1.6.1.4.2. Work up optimization

In the w.up of this step, the reaction crude was cooled to 5 °C, after 2 h at this temperature the obtained slurry was filtered to isolate **FH0317 crude**. The studies performed during the step 4 w.up optimization were focused on increase the purity of the isolated **FH0317 crude** while reducing the amount of product lost during the filtration.

1.6.1.4.2.1. Crystallization conditions

In order to increase the yield, during the study of this step the utilization of larger crystallization times and lower temperatures was evaluated. Either in the case of the low temperature or the extended crystallization time no relevant yield improvements were achieved. Moreover, it was found that in both cases the crystallization of impurities was induced reducing the quality of the obtained product. Therefore, the initial process based on the filtration of the obtained slurry after 2 h at 5 °C was maintained.

1.6.1.4.2.2. FH0317 crude drying

The study of the step 4 w.up revealed that, the obtained **FH0317 crude** degraded during its drying. In Table 26 are presented some of the drying results obtained during the study of this operation.

Entry	Wet FH0317 crude purity	Dry FH0317 crude purity	Drying temp.	Drying time
1	98.47 %	98.31 %	55 °C	20 h
2	99.46 %	99.10 %	55 °C	20 h
3	98.29 %	96.60 %	50 °C	20 h
4	99.24 %	99.17 %	40 °C	20 h
5	98.17 %	98.04 %	40 °C	20 h
6	97.70 %	97.51 %	40 °C	20 h

Table 26. Purity results obtained after and before the drying of FH0317 crude at different temperatures.

Initially, the obtained **FH0317** was dried under vacuum at 55 °C for 16-20 h (Table 26; entries 1 and 2). In order to reduce the product degradation, the drying temperature was decreased from 55 °C to 40 °C. However, during the dying at 40 °C degradation was still observed (see entries 4 to 6). Eventually, it was found that, if the product was dried under vacuum at 25 °C no degradation took place. These drying conditions were adopted once it was confirmed that, the **FH0317 crudes** dried at 25 °C could be used in step 5 to obtain pure **FH0317** within the residual solvent specifications (Table 27). The use-tests of the **FH0317 crude** samples dried at 25 °C demonstrated that, the residual solvent levels in **crude FH0317** were acceptable (see Table 27).

The study of the **FH-4** and the pure **FH0317** thermal stability revealed that they must be also dried at 25 °C in order to avoid its degradation. Thus, once it was confirmed its suitability in

terms of residual solvent content, the drying conditions found for **FH0317 crude** were also applied in steps 3 and 5 (see Table 27).

Entry	Product	Acetone (ppm)	ACN (ppm)	MeOH (ppm)	Isopropanol (ppm)
1	**FH-4**	ND	2032	6270	10006
2	Crude **FH0317**	ND	1642	4193	115
3	Pure **FH0317**	2002	138	200	ND
FH0317 spec: acetone ≤ 5000 ppm; ACN ≤ 3000 ppm; MeOH ≤ 3000 ppm; isopropanol ≤ 5000 ppm					

Table 27. Typical residual solvent levels observed in FH-4, crude FH0317 and pure FH0317 dried at 25 °C. The FH0317 residual solvent specifications are included in the table for comparison. ND is abbreviation of not detected.

Eventually, the optimized CIDT process was successfully scaled-up in the laboratory to a 120 g scale using a 2 L reactor. Despite the introduction of an intermediate isolation step, the yield of the steps 3 and 4 was increased slightly from a 62 % to a 66 % after the optimization. The purity of the isolated **FH0317 crude** was also increased from a 93 % to a 95 %.

The described process was used for the industrial scale manufacturing of **FH0317** without remarkable issues. At industrial scale the process was performed at 17 kg scale in 250 L reactors.

1.6.1.5. Step 5: Purification

As it is mentioned in section 1.4.5, optimization of the purification conditions was required in order to increase the purity of the obtained **FH0317**, to reduce its residual ACN content and, to achieve a particle size distribution (PSD) that allowed an easy processing and tableting of this API. In this section are collected the different studies performed to optimize the purification and to achieve the obtention of **FH0317** with the desired quality.

1.6.1.5.1. The wet milling approach

During the first phase of the step 5 optimization, the utilization of rotor-stator wet milling was evaluated. This technique allows to reduce the size of the particles present in a slurry to sizes of 10 µm or even below through the application of large amounts of shearing energy. As it is shown in Figure 92 the slurry is passed through a rotor-stator system. The high rotor spinning rates applied (which can reach the 26000 rpm or more) induce the particle breakage and the mentioned reduction of the particle size. The wet mill apparatus typically include cooling systems in order to dissipate the large amounts of heat that are generated during its operation.[178]

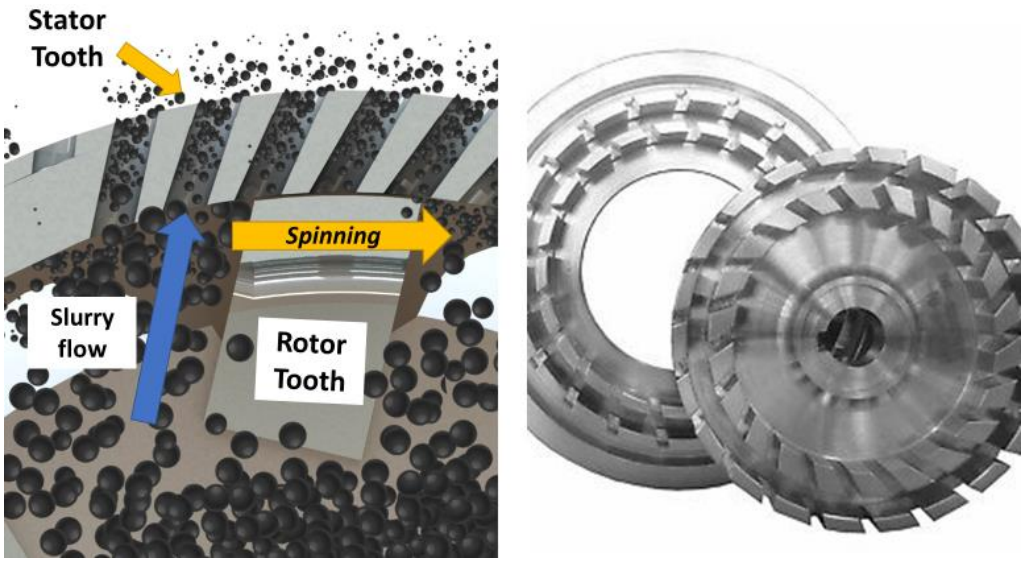


Figure 92. Wet milling working principle (left). In the figure situated at the right, an example of a wet mill rotor (front) and of a stator (bottom) are presented. Reproduced from Refs [179,180] with permission of the copyright holders.

The implementation of this technique into the **FH0317** process was considered because it could be easily scaled-up and it would allow to eliminate additional product drying and product manipulation steps after the purification. It was suggested that the crystal grinding achieved using a wet mill would allow to obtain the desired PSD and would solve the residual solvent problems caused by the occlusion of ACN into the **FH0317** crystals already described in section 1.4.5.

The wet milling trials were carried out using a Magic Lab wet mill from IKA. During the performed tests, it was observed that the use of a wet mill did not allow to reduce the ACN content of the **FH0317** obtained. In all the cases, even drying at high temperatures for extended periods of time, residual ACN values well above the product specification were obtained. Based in the obtained results, this approach was finally abandoned.

1.6.1.5.2. Purification conditions study

In a second phase of the purification optimization process, a set of experiments was performed using as a starting point the purification conditions already available. The experiments were focused on the optimization of the purification conditions in order to achieve the desired residual solvent levels and purity. During this phase of the purification development different water:ACN ratios, heating temperatures and heating times were evaluated.

1.6.1.5.2.1. Initial screening

Initially, the experiments were performed at small scale using magnetic stirring. It was found that the obtained results in terms of PSD and residual solvent content could not be reproduced when the same conditions were applied using mechanical stirring (Table 28 entries 1 and 2). It was suggested that, as it was observed during the step 4 optimization (section 1.6.1.4.1.2) the magnetic stirring promoted the product erosion. The mentioned erosion caused the low PSD and residual solvent contents observed in the entry 1 experiment. Considering that, at industrial scale the process will be performed using mechanical stirring, it was concluded that the

optimization experiments must be performed using mechanical stirring in order to have representative results.

Entry	Conditions	Purity Increase	Epimer ratio	Yield	Residual ACN content	PSD d(0.9) (µm)
1	40 °Cx2 h + 5 °Cx2 h, 20 % H_2O; magnetic bar	5.03 %	98.9:1.1	76.0 %	406 ppm	17.18
2	40 °Cx2 h + 5 °Cx2 h, 20 % H_2O	3.90 %	98.4:1.6	74.1 %	7366 ppm	166.14
3	20 °Cx2 h + 5 °Cx2 h, 20 % H_2O	2.78 %	97.2:2.8	74.4 %	3961 ppm	NA
4	40 °Cx2 h + 5 °Cx2 h, 20 % H_2O	4.11 %	98.2:1.8	69.0 %	5104 ppm	NA
5	40 °Cx2 h + 5 °Cx2 h, 10 % H_2O	2.53 %	97.0:3.0	71.6 %	3071 ppm	NA
6	45 °Cx10min + 5 °Cx2 h, 20 % H_2O	2.63 %	97.1:2.9	61.4 %	3014 ppm	NA
7	40 °Cx10min + 5 °Cx2 h, 10 % H_2O	0.39 %	95.7:4.3	85.6 %	2158 ppm	NA
8	40 °Cx10min + 5 °Cx10min. 10 % H_2O	0.37 %	95.7:4.3	80.8 %	2133 ppm	NA

Table 28. Summary of the results obtained using different ACN:water mixtures and heating times. In all cases except for entry 1 mechanical stirring was applied. NA is abbreviation of not available.

As it is reported in Table 28 (entries 7 and 8), only in the cases where the purity increase was low, acceptable residual ACN levels were achieved. From the results obtained, it was concluded that through the use of ACN:water mixtures was not possible to achieve the desired purity while maintaining the residual ACN levels below the product specification (3000 ppm). Therefore, it was decided to study the utilization of alternative solvents to substitute the ACN.

1.6.1.5.2.2. Solvent selection

A solvent screening was performed slurring **FH0317** at 25 °C in different solvents in order to determine which of them leaded to a more efficient impurity removal (see Table 29).

Entry	Solvent	Impurity Improvement	Purity Increase	ICH class
1	ACN	YES (low)	0.30 %	2
2	Acetone	YES (low)	0.53 %	3
3	Toluene	NO	0.16 %	2
4	THF	YES (low)	0.51 %	2
5	Diisopropyl ether	YES (low)	0.42 %	NA
6	IPA	YES (low)	0.35 %	3
7	n-Propanol	NO	0.15 %	3
8	Methyl ethyl ketone	YES (low)	0.31 %	3
9	MTBE	NO	0.25 %	3
10	MeOH	YES (low)	0.51 %	2
11	n-Heptane	NO	0.17 %	3
12	EtOAc	NO	0.28 %	3
13	EtOH	YES (low)	0.56 %	3
14	Dioxane	NO	0.28 %	2
15	Dimethyl sulfoxide	Total dissolution		3
16	DCM	YES (low)	0.46 %	2
17	Butyl acetate	YES (low)	0.39 %	3

Table 29. Results obtained during the solvent screening performed to find candidates to replace ACN in step 5. NA is used for these fields which are not available.[101]

Based in the purity increase results obtained for pure solvents and for ACN:water mixtures (Table 28/Table 29) and, considering the high **FH0317** solubility in water it was concluded that, the use of a water:organic solvent mixture was required to achieve an optimal purification capacity while maintaining a good yield.

IPA, EtOH and acetone were selected as candidates to replace ACN considering the purity increase promoted and its ICH class (see Table 29). Only ICH class 3 solvents were selected because the limits accepted for these compounds in APIs are higher. Thus, the utilization of ICH class 3 solvents increased the chances of success during the subsequent step 5 optimization.

Finally, acetone was selected to replace ACN because, the results obtained using mixtures of IPA, EtOH and acetone with water revealed that, using this solvent a higher product purity could be achieved causing only an smooth decrease on the yield (see Table 30).

Entry	Conditions	Purity Increase	Epimer ratio	Yield	Residual ACN content
1	45 °Cx2 h, 90:10 acetone:H_2O	12.17 %	99.3:0.7	67 %	100 ppm
2	45 °Cx2 h, 90:10 EtOH:H_2O	8.86 %	98.7:1.3	72 %	522 ppm
3	45 °Cx2 h, 90:10 IPA:H_2O	8.70 %	98.5:1.5	72 %	721 ppm

Table 30. Results obtained during the evaluation of different water:organic solvent mixtures in step 5.

1.6.1.5.2.3. Acetone:H_2O purification optimization

Once it was decided that an acetone:water mixture will be used as the step 5 solvent, a two-phase optimization was performed. During the first phase of the optimization, the main goal was to reach the desired purity and residual solvent levels while the second phase was focused on the obtention of the desired PSD. The optimization was divided in two phases to reduce the **FH0317 crude** consumption because, its availability at this stage of the process development was low.

1.6.1.5.2.3.1. Purity and residual solvent optimization

To reach the target during the first phase of the optimization, a set of 5 g scale experiments were carried out using mechanical stirring and 250 mL reactors. After the completion of the initial phase of the optimization it was concluded that slurring the **FH0317 crude** for 2 h at 25 °C in a mixture of acetone:water (50:50) an API within the requested purity and residual solvent specifications could be robustly obtained (see entry 1 in Table 31). However, the **FH0317** obtained using the mentioned purification conditions presented a bimodal PSD plot shape with approximately half of the solid particles presenting a size below 10 microns (see Figure 93). These PSD results indicated the presence of fines, which could cause manipulation problems during the manufacturing of the drug tablets.

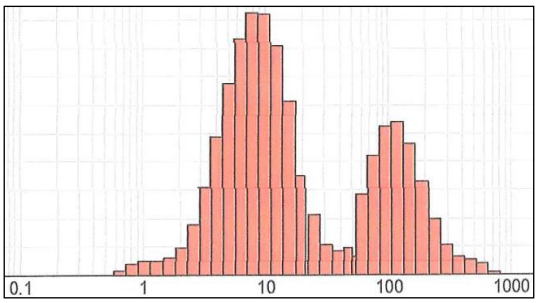


Figure 93. Bimodal PSD plot shape observed in the analysis of a FH0317 sample. The scale units presented in the plot are microns.

1.6.1.5.2.3.2. PSD optimization (the annealing approach)

In the second phase of the optimization, several experiments were carried out at 10 g scale using 250 mL reactors. The scale of the experiments was increased for these studies in order to mimic the reactor occupation of the industrial reactor. The objective was to achieve in the laboratory a mixing efficiency as similar as possible to the industrial one. Since PSD could be influenced by the mixing efficiency, at this stage of the development it was important to reproduce the industrial scale conditions. The approach used to solve the mentioned PSD problems was the implementation of an annealing step during the purification (see Filtrations/Crystallizations section).

In the annealing tests initially performed, low yields were obtained (see entries 2 to 4 in Table 31). Through the LC analysis of the obtained mixture, it was detected that during the slurry heating the **crude FH0317** initially loaded epimerized. The **FH0317** undesired epimer formed, presented a higher solubility and was lost in the filtration mother liquors leading to the observed low yields. It was suggested that the mentioned epimerization was catalyzed by the residual DBU present in the used **FH0317 crudes**. The temperatures applied during the annealing favored the dissolution of the desired *(R)*-epimer. Once in solution, it was converted in the *(S)*-epimer through the mechanism presented in section 1.6.1.4.1.1. This hypothesis was confirmed through the study of the residual DBU content of the **FH0317 crudes** used. The performed analyses revealed that, the starting materials used during the annealing tests contained 200-300 ppm of DBU. This behavior was not observed in previous experiments since, the batches of **FH0317 crude** used presented lower residual DBU contents and, because the *(R)*-epimer was not dissolved due to the lower temperatures applied.

In order to avoid the epimerization, a pH adjustment using diluted formic acid was implemented before the heating ramp. The adjustment was performed using formic acid because it is an organic substance and, therefore, it can not contribute to increase the ROI content of the final product. Additionally, the formic acid is a volatile compound which could be eliminated during the product drying avoiding problems related to the presence of traces of this substance in the **FH0317**.

To determine the optimal pH at which the annealing must be performed, several tests of step 5 were carried out (see entries 5 to 8 in Table 31). The best results were obtained when the pH of the mixture was adjusted between 7.25 and 6.10. Therefore, a range of 6.50±0.2 was adopted.

Entry	Conditions	Epimer ratio	Purity	Yield	Residual acetone content	Residual ACN content	PSD d(0.5) (µm)	PSD d(0.9) (µm)	PSD plot shape
1	2 h x 25 °C (slurry)	98.4:1.6	97.52 %	61.0 %	1180 ppm	184 ppm	18.3	169.4	BS
2	15' x 45 °C (annealing)	98.3:1.7	99.06 %	31.0 %	2064 ppm	ND	83.9	164.5	BS
3	15' x 35 °C (annealing)	98.3:1.7	99.12 %	45.0 %	2402 ppm	48 ppm	101.4	215.3	BS
4	5 h x 45 °C (annealing)	97.8:2.2	99.31 %	33.0 %	1314 ppm	ND	252.6	450.8	GS
5	pH adjusted to 7.25 1.5 h x 45 °C (annealing)	99.5:0.5	99.28 %	58.0 %	1308 ppm	53 ppm	145.6	235.1	GS
6	pH adjusted to 6.50 1 h x 45 °C (annealing)	99.6:0.4	99.40 %	64.5 %	1676 ppm	ND	127.7	211.6	GS
7	pH adjusted to 6.10 1 h x 45 °C (annealing)	98.4:1.6	99.46 %	57.0 %	NA	NA	160.0	298.2	GS
8	pH adjusted to 5.10 1 h x 45 °C (annealing)	96.3:3.7	99.18 %	45.5 %	NA	NA	149.7	276.3	GS

Table 31. Summary of the most relevant tests performed during the optimization of the step 5 using acetone:H_2O (50:50) as solvent. BS means bimodal shape and GS gaussian shape. The used starting material was an FH0317 crude batch with 95.0 % purity; 1853 ppm ACN; 214 ppm DBU; PSD d(0.5) 14.3 µm; PSD d(0.9) 180.8 µm; PSD plot shape BS.

The finally adopted purification conditions are described in entry 6. The developed purification allowed to obtain **FH0317** in acceptable yield and high purity. The optimized process was successfully scaled-up in the laboratory to a 75 g scale using a 2 L reactor without remarkable issues. In all the laboratory tests performed, the product was obtained within the residual solvent specifications and with a PSD plot shape and a particle size that allowed the easy manipulation of the solid during the tableting process (see Figure 94).

Compared with the non-optimized process the yield decreased from 77 % to 64 %. However, because of the yield improvements achieved in steps 1 to 4, the overall yield of the synthesis was increased from a 21 % to a 28 %. Additionally, it is important to highlight that, the non-optimized process yielded to a product out of specifications in terms of residual solvent content, with an undesired particle size and with a lower purity (98.2 % *vs.* 99.0 %).

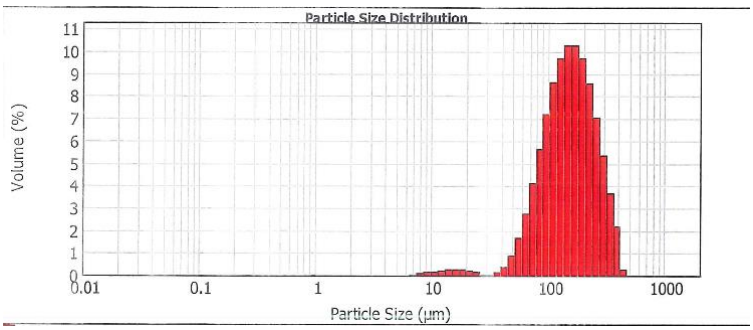


Figure 94. PSD plot with gaussian shape obtained from the PSD analysis of an FH0317 sample obtained using the optimized purification process.

The developed procedure was successfully applied at industrial scale (see Table 32) obtaining around 8.5 kg of **FH0317** per batch. Maintaining the scale, seven **FH0317** pilot batches followed

by three validation batches were performed. Although the industrial batches were successfully completed and the requested amounts of **FH0317** were obtained, some problems related with slow filtrations, interphase formation and foreign matters were found during the manufacturing of this product. In section 1.6.2, are described the studies and optimizations made to correct the observed process weaknesses and to end up with a robust manufacturing process that could be validated.

Entry	Step n°	Amount of starting material	Size of the reaction vessel
1	1 (Hydrogenation)	50 kg of **FH-1**	2300 L
2	2 (Reduction)	42 kg of **FH-2**	1600 L
3	3 (Deprotection)	36 kg of **FH-3**	1000 L
4	4 (CIDT)	17.4 kg of **FH-4**	250 L
5	5 (Purification)	11.5 kg of **FH0317 crude**	250 L
6	8.5 kg of **FH0317** obtained (28 % overall yield)		

Table 32. Summary of the amount of starting material and the reaction vessel used in each step of the FH0317 industrial batches.

1.6.1.6. Process optimization conclusions

- A suitable method for the obtention at industrial scale of **FH0317** based on the preliminary process provided by the customer was developed. The yield was increased from a 21 % to a 28 %. The purity of the obtained product was also increased from a 98.2 % to a 99.0 %.

- The corresponding studies were performed to optimize the different steps of the process. The performed optimizations allowed to reduce the formation of impurities, to increase the process robustness and, to reduce the time of synthesis/raw material consumption.

- The w.up of the different steps of the synthesis were also modified to reduce the product loses, avoid the formation of interphases and increase the purity of the isolated products. In order to make the **FH0317** process suitable for the industrial scale operation, the w.up were developed considering the limitations of the industrial equipment that was going to be used.

- The control of the process was improved through the implementation of the corresponding IPCs. The precise monitoring of the process allowed to reduce the time of synthesis, increase the yield and avoid the formation of impurities.

- The final purification step was completely redefined in order to achieve the requested particle size, residual solvent content and purity. Different sets of solvents, pH conditions and temperatures were evaluated in order to develop a process that yielded an **FH0317** with the requested quality while maintaining an acceptable yield.

1.6.2. Process scale-up

In order to support the **FH0317** clinical trials, large amounts of material were required. To generate the requested amounts of **FH0317** (at least 5 kg of **FH0317** per batch) the previously optimized process was transferred from the laboratory to the manufacturing plant. Seven pilot

batches of **FH0317** were successfully carried out and, eventually, the process was validated through the execution of three additional batches. As consequence of the characteristics of the industrial equipment used and of the scale increase, a series of minor problems were found during the execution of the mentioned pilot batches. For each problem an investigation was carried out and the corresponding corrective actions/preventive actions (CAPA) were implemented. The final goal was to increase the process robustness at the levels required to assure the success in the subsequent process validation. Below are listed the problems found during the performed pilot batches as well as the different process modifications implemented to solve them and to avoid its occurrence in further batches.

1.6.2.1. Foreign matter contamination in FH0317

During the visual inspection of the product obtained in the first pilot batch of **FH0317** some small dark particles were detected (see Figure 95). In an optical microscope study of the detected particles, two different faces were observed. One face with a fibrous aspect and another one with a smooth surface and a slightly blue color.

Figure 95. Optical microscope image of one of the particles detected in FH0317.

In order to determine the source of the contamination, a particle isolated from the product was analyzed using infrared (IR) spectroscopy and scanning electron microscopy (SEM). The performed analyses indicated that the blue side of the particle was formed by a polymeric resin and barium sulphate while the fibrous side contained Kaolin (a type of aluminosilicate) and non-identified organic compounds. In the analyses performed, traces of diatomaceous earth (SiO_2) and titanium were also detected.
On the basis of these results, the particles were tentatively identified as fragments of paper or cardboard.[181] The gaskets present in the manholes of the used train of equipment, were identified as most likely source of particles with this composition. These gaskets were composed of a PTFE envelope with a cardboard compressible filling. The review of the used reactors revealed that, the gasket of the reactor used in the second step of the synthesis was damaged. In the analyses of the damaged gasket filling, similar results to the ones observed for the particle isolated from **FH0317** were found. These results confirmed that this gasket was the source of the product contamination.

In order to reduce the risk of a foreign matter contamination in further batches, besides to the replacement of the damaged gasket a series of corrective actions were implemented:

- A more exhaustive revision of the reactor gaskets was included in the preventive maintenance program.
- A polish filtration (see footnote in page 64) using a 0.45 µm filter was included in the purification step of the **FH0317** process. Since total dissolution was

required to perform the polish filtration, a purification based on the recrystallization of the **FH0317 crude** was developed. The recrystallization process was used to replace the annealing based procedure described in section 1.6.1.5. In order to maintain the performance (yield, PSD and purity) reached with the annealing purification the volumes of acetone and water used, the cooling rate and the seeding performed were deeply studied and optimized. Eventually, a recrystallization process with a performance comparable to the annealing one was obtained and implemented (see **FH0317** project experimental section for more detail on the implemented procedure).

- After the implemented polishing filtration, the product was totally protected from the environment to prevent contamination. The loading of solids in the reactor was carried out through the reactor manhole using flexible isolators while the liquids were loaded through 0.45 µm filters.
- A sieving operation was included after the purification to reduce the risk of having foreign matters in the final product.

1.6.2.2. Step 2 phase separation problems

During w.up of the first step 2 industrial scale batch, some phase separation problems were detected. In order to avoid the formation of interphases in the $NaHCO_3$ rinsing and in the subsequent water rinsings additional amounts of DCM must be loaded and, extended settling times (around 5 h) must be applied. In the laboratory experiments the phase separation was slightly slow, however acceptable phase separations were observed after 30 min. of settling. Initially all these rinsings were carried out at low temperature. In order to solve the mentioned problems some scale down experiments were carried out. In these tests the phase separations were performed at 25 °C.

The scale down methodology is based on the simulation of the industrial scale conditions (reactor occupation, temperatures and addition/mixing rates) in an experiment performed at small scale in the laboratory or simulated *in-silico*. Performing scale down tests allows to determine which will be the behavior of an industrial process under a specific set of operating conditions. These experiments are useful to evaluate the suitability of process modifications before its implementation at large scale and, to determine the root causes of the unexpected results observed at industrial scale.

The scale down tests indicated that the **FH-3** stability at 25 °C was acceptable and, that the temperature can be increased during the extractions. The temperature increase allowed to eliminate the mentioned phase separation problems without causing product degradation.

1.6.2.3. Step 2 reactor unloading issues

During the execution of some of the step 2 pilot batches smooth difficulties to discharge the final **FH-3** slurry from the reactor were found. The unload could be completed in all the cases applying vacuum and nitrogen pressure cycles into the reactor. However, during the third validation batch of the step 2 severe unloading problems were encountered. A large amount of product (approx. 2/3 of the total) remained at the bottom of the reactor when the slurry was

unloaded. The recirculation of part of the collected mother liquors was required to complete the discharge. Despite the discharge was eventually completed and the process could be successfully validated, an optimization of the step 2 crystallization process was performed to avoid issues in further batches. In order to find the root cause of the mentioned problems a study of the crystallization was conducted. The conditions applied during the product crystallization at the different industrial batches and, the rheology of the slurry obtained in each case were evaluated. In Table 20 is summarized optimized the evaporative crystallization process that was used during the industrial batches. From the study it was concluded that the unloading problems were mainly related to differences in the conditions applied during final addition of MTBE and during the subsequent cooling ramp. The more relevant unloading issues were found in batches where:

- The stirring rate applied was lower. It was suggested that lower stirring rates favored the formation of solid agglomerates that difficulted the unloading.
- The temperature of the slurry before the final MTBE addition was lower due to the conditions applied during the previous distillation (vacuum level and jacket temperature). The addition of MTBE at a lower temperature could promote a fast product crystallization and the formation of a thinker slurry.
- The temperature of the slurry at the beginning of the cooling ramp was lower. In this process, a 2 h cooling ramp to an internal temperature of 5 °C was applied. However, the initial temperature of the ramp was not defined. It was found that in certain cases, the ramp was started from temperatures around 10-15 °C when, most of the product had already crystallized. This phenomenon was observed specially in winter because, the previously loaded MTBE was cool. The variability of the cooling ramp could allow to explain the unloading problems observed in certain batches.
- Lower jacket temperatures were applied. It was suggested that the utilization of jacket temperatures below 4 °C at certain points of the crystallization could promote the product agglomeration in the reactor walls. Because of the excessive cooling, the formation of solid agglomerates that difficulted the downloading operation could be promoted.

Unloading issues were found during the pilot batch execution despite that during the step 2 crystallization development the process was optimized to avoid the formation of thick slurries and discharge problems (see section 1.6.1.2.2.3). As it has been presented above, the source of the unloading problems observed were smooth differences in the batch to batch operation. This illustrates the complexity of the process scale-up and is a good example of how small details that typically are not considered during the laboratory experiments may have a relevant impact during the large scale operation.

In order to avoid further agglomeration problems, the implementation of the following changes was proposed: perform the MTBE addition maintaining the internal temperature at 25 °C, increment the stirring rate in a 20 %, start the cooling ramp from 25 °C, set as a minimum jacket temperature 4 °C and, perform an extra rinsing of the reactor/solid cake using 2 vol of cool MTBE. A 2 vol rinsing was proposed because this is the minimum stirrable volume of the

industrial reactor used in step 2. In case of unloading problems the use of two fresh volumes of MTBE should allow to resuspend the solids and to obtain a homogeneous mixture easy to discharge from the reactor. In order to determine the suitability of the proposed changes a scale down experiment was performed.

According to the scale down approach, a step 2 test at 42 g scale in a 1 L reactor was performed. In order to detect possible filtration problems, the plant filtration conditions were reproduced in the laboratory. The size of the filter used in the laboratory experiment was adapted to the size of the filter used at plant (see Table 33). The laboratory filtration procedure was also adapted to the procedure used at plant. I.e. the washings were leaved in contact with the solid cake for 10-15 min before applying vacuum and, the draining of the solid cake between each rinsing was carried out using gentle vacuum and as slowly as possible.

Parameter	Industrial scale	Lab. scale
Reactor capacity	1000 L	1000 mL
Scale	42 kg	42 g
Reactor height	1560 mm	300 mm
Clearance	60 mm	12 mm
Clearance/Height ratio	0.04	0.04
Filter capacity	234 L	250 mL
Filter diameter	600 mm	65 mm
Filter height	828 mm	90 mm
Filter diameter/Filter height ratio	0.72	0.72
Slurry volume	516 L	516 mL
Filter Area	2827.4 cm^2	3318.3 mm^2
Filter area/Slurry volume ratio	5.48	6.43

Table 33. Comparison of the laboratory and the industrial scale filters used to study the step 2 filtration. The criterion used to have filter similarity was to use filters with similar diameter/height ratio and area/slurry volume ratio. This criterion had been applied in other studies in Farmhispania S.A. with good results.

During the laboratory tests no unloading problems were observed, the obtained slurry was fluid enough to be uploaded into the filter without problems. The yield lost in the extra MTBE rinsing performed and the mechanical loses were also considered acceptable (see Figure 96 and Table 34). The obtained results confirmed the suitability of the proposed changes.

Figure 96. Detail of the mechanical loses observed during the step 2 test performed to solve the unloading problems.

Entry	Fraction	Weight (g)	Loss on yield
1	Mother liquors + 1st wash	6.39	15 %
2	2nd wash	0.65	2 %
3	3rd wash	0.56	1 %
4	Mechanical loses	2.34	6 %

Table 34. Summary of the yield loses observed during the experiment performed to solve the step 2 downloading problems.

In the performed test, the filtration was slower compared to the filtration rates observed normally in the laboratory (although it was still acceptable). This behavior was associated to the size of the used filter rather than to the introduced changes since, slow filtrations were already observed at plant using the standard conditions. As it was mentioned above, to mimic the plant filtration conditions, the filter used in this test was narrower than the filter that would have been used in a routine experiment (i.e. 85 mm diameter x 65 mm height or bigger). The slower filtration rate observed confirmed that the filter used at plant is slightly undersized considering the size of the step 2 batches. Although the filtration at industrial scale required from around 16 h, the filtration time was considered acceptable and the use of a larger filter was kept as a possible further improvement.

Based on the obtained results it was agreed with the customer to include in the step 2 a master batch record (MBR)[13] with the following minor changes:

- Increase from 90 to 110 rpm of the stirring rate after the completion of the final distillation.
- Include a target temperature of 25 °C during the final addition of MTBE.
- Include a minimum jacket temperature of 4 °C during the crystallization process.

In order to avoid the introduction of major changes in the already validated process it was agreed with the customer the no implementation of the extra MTBE wash. The suitability of the above-mentioned minor changes will be evaluated in further **FH0317** batches. The inclusion of the extra MTBE wash will be only considered in case that the unloading problems persist when using the modified process.

1.6.2.4. FH0317 process quality risk assessment

The methodology described in Annex 1 for the process quality risk assessment (PQRA) was applied before the validation of the **FH0317** process in order to guarantee its robustness and reliability. The information available from the process development and the pilot batches (see section 1.6) was used to feed the critical process parameters assessment (CPPA) tool. The CPPA tool allowed to determine, which were the process parameters that required additional studies and, to stablish the risk associated to each process parameter/material attribute (see Attachment 1).

[13] The master batch record (MBR) is the document containing all the instructions that the plant operators must follow to perform the synthesis of an API or a process intermediate at industrial scale. This document specifies among others the industrial equipment to be used, the amounts of raw materials and, the process temperatures/stirring rates to be applied. It also includes the detail about the sampling procedures or warnings about hazardous operations.

1.6.2.4.1. Attachment 1

Example of the CPPA tool; Process evaluation table:

Process parameter/Material attribute	Target value	Proposed range	Existing acceptable range	Process attributes affected[1]	Potential effect on quality or process performance[2]	Probability of minor deviation from existing acceptable data range	Initial risk Evaluation	Evaluation strategy/comments
Reaction								
Water loading temp.	25±5 °C	25±5 °C	25±5 °C	NONE	N/A	N/A	N/A	-
NaBH4 charge	1.38 Eq (0.110 p)	1.32-1.44 Eq (0.105-0.116 p)	1.38 Eq (0.11 p)	Purity Yield	HIGH	HIGH	HIGH	RLT: test using 0.099 p and 0.121 p. Combine with the corresponding positive of negative temperature, concentration conditions
Loading temp.	-8 °C	-8±3 °C	-10 °C to -5 °C	NONE	HIGH	HIGH	HIGH	See cell below.
Maintain reaction temperature	-8 °C	-8±3 °C	-10 °C to -5 °C	Purity Yield	HIGH	HIGH	HIGH	Based in process knowledge it is known that the use of higher reaction temperatures/reaction times may lead to formation of impurities. Confirm the impact on quality of using -3 °C and -12 °C
Reaction time	13 h	12-14 h	10-20.5 h	Purity	HIGH	LOW	MEDIUM	No need to study shorter reaction times (IPC). Confirm stability of the reaction crude at -5 °C (NOR limit)

[1]Note 1: Cells containing N/A correspond to parameters not considered critical in terms of quality, PAR not studied.

[2]Note2: Yield, safety and costing are not quality attributes, but are also considered as relevant responses for the study.

After the execution of the experimental work derived from the CPPA tool utilization, the proven acceptable ranges (PAR) and the normal operating ranges (NOR) ranges as well as the parameter criticalities were defined. In Attachment 2 is included an example of the process parameter evaluation performed for the 2nd step of the process. According to the performed evaluation, the step 2 and the step 3 reaction temperatures were defined as critical parameters. The performed studies revealed that, when temperatures above the explored PAR were applied, impurities where formed at levels close to the maximum tolerated by the process.

1.6.2.4.2. Attachment 2

Example of the CPPA tool; Process ranges and evaluation of critical parameters table

Process parameter/Material attribute	PAR[3]	NOR[3]	Target	Potential effect on quality or process performance[2]	Probability of minor deviation from PAR	Final risk evaluation	Definition	Comments
Reaction								
Water loading temp.	25±5 °C	25±5 °C	25±5 °C	N/A	N/A	N/A	NKPP	-
NaBH4 charge	1.24-1.52 Eq (0.099-0.121 p)	1.32-1.44 Eq (0.105-0.116 p)	1.38 Eq (0.110 p)	HIGH	LOW	MEDIUM	KPP	Ranges experimentally confirmed in CPP-PAR-NOR evaluation activities.
Loading temp.	-3 °C to -12 °C	-8±3 °C	-8 °C	HIGH	MEDIUM	HIGH	CPP	In case of deviation from PAR (above -3 °C), the quality of the API could be affected. Considered as critical parameter
Maintain reaction temperature	-3 °C to -12 °C	-8±3 °C	-8 °C	HIGH	MEDIUM	HIGH	CPP	In case of deviation from PAR (above -3 °C), the quality of the API could be affected. Considered as critical parameter
Reaction time	10-20.5 h	12-14 h	13 h	LOW	LOW	LOW	NKPP	Experimentally was demonstrated that the reaction crude is stable at -5 °C (NOR limit)

[2]Note2: Yield, safety and costing are not quality attributes, but are also considered as relevant responses for the study.

[3]Note3: The experimental references used to justify the PAR-NOR values proposed are collected in PEL-YY-XXXX/ZZ

Using the information obtained from the CPPA tool (PAR, NOR and target values) were prepared the MBRs for the validation of the different steps of the **FH0317** process. In this phase, the MBRs already created for the execution of the pilot batches were used as base. As part of the PQRA, a manufacturing process risk assessment (MPRA) study of each step of the process was conducted. The main goal was to detect gaps in the prepared MBRs and, to confirm the suitability of the industrial equipment to be used during the validation. In Attachment 3 is included a fragment of the MPRA performed for the 2nd step of the **FH0317** process.

1.6.2.4.3. Attachment 3

Example of the MPRA FMEA tool

Unit Operation	Operation Nr.	Activity or Material or Equipment	NOR	PAR[1]	Potential Failure Mode(s)	Potential Effect(s) of Failure Related CQA	SEV	Potential Cause(s)/ Mechanism(s) of Failure	OCC	Current Design Controls	DET	RPN	Critical process parameter	Recommended Actions	Responsibility & Target Completion Date	Action Results Actions Taken	New SEV	New OCC	New DET	New RPN	Residual Risk and Residual Risk Acceptance
Step 5; Obtention of FH-3 (intermediate from reduction under Luche conditions)																					
Reference Process (FHG MBR): 03170																					
Reduction reaction																					
01	05	Cool reactor content to -8 °C ± 3 °C	-8 °C ± 3 °C	-11 °C to -3 °C	Higher temperature	Temperature at this point is considered a critical parameter. Relevant effect is expected in case of deviation at this point.	25	Human error/Temperature sensor failure	1	MBR. Manually operated with automatic control. Manual and automatic record. Calibrated temperature sensor (periodic verification)	3	**75**	**YES**	Include a warning in the MBR indicating the criticality of the reaction temperature for the product quality	Already implemented by Farmhispania pilot plant office Feb-2019	Record the inclusion	N/A	N/A	N/A	**N/A**	N/A

Unit Operation	Operation Nr.	Activity or Material or Equipment	NOR	PAR[1]	Potential Failure Mode(s)	Potential Effect(s) of Failure Related CQA	SEV	Potential Cause(s)/ Mechanism(s) of Failure	OCC	Current Design Controls	DET	RPN	Critical process parameter	Recommended Actions	Responsibility & Target Completion Date	Action Results Actions Taken	New SEV	New OCC	New DET	New RPN	Residual Risk and Residual Risk Acceptance
					Lower temperature	Temperature at this point is considered a critical parameter. Relevant effect is expected in case of deviation.	25	Human error/Temperature sensor failure	1	MBR. Manually operated with automatic control. Manual and automatic record. Calibrated temperature sensor (periodic verification)	3	75	Yes	Precipitation is observed during the cooling, comment could be included in the next MBR	Already implemented by Farmhispania pilot plant office Feb-2019	Record the inclusion	N/A	N/A	N/A	N/A	N/A
	06	Check that the RV-02 scrubber connection is open	N/A	N/A	No connection of local exhaust	It could promote a safety problem	4	Human error	1	MBR. Manual control	5	20	No	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
		Stirring rate check to ensure that the formed slurry is efficiently mixed	N/A	N/A	No proper stirring check	Inefficient stirring. The test SBG-1 failed and the issue was related with the inefficient stirring.	25	Human error	1	Not controlled	9	225	No	Pending to include a stirring control.	Already implemented by Farmhispania pilot plant office Feb-2019	MBR. Manually operated. Calibrated agitator	25	1	3	75	D: Rescored from no detectability to good detectability because in-situ supervisor verification is included in the MBR The residual risk is low **No additional actions needed**
		Load through the solid loading system NaBH4 in RV-02.	-8 °C ± 3 °C 0.11	-11 °C to -3 °C 0.1	More quantity	No effect is expected if minor deviation.	4	Human error/Scales failure	3	MBR. Manually operated. Calibrated scales with daily verificatio	1	12	No	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Unit Operation	Operation Nr.	Activity or Material or Equipment	NOR	PAR[1]	Potential Failure Mode(s)	Potential Effect(s) of Failure Related CQA	SEV	Potential Cause(s)/ Mechanism(s) of Failure	OCC	Current Design Controls	DET	RPN	Critical process parameter	Recommended Actions	Responsibility & Target Completion Date	Action Results Actions Taken	New SEV	New OCC	New DET	New RPN	Residual Risk and Residual Risk Acceptance
		Load in N portions of 0,5 kg maintaining temperature at -8 °C ± 3 °C.	kg/kg ± 1 % 80 rpm	1 kg/kg ± 10 % N/A						n. FHG SOP (GP-13/**)											
					Less quantity	No effect is expected if minor deviation.	1	Human error/Scales failure	3	MBR. Manually operated. Calibrated scales with daily verification. FHG.	3	9	No	N/A	N/A	N/A	N/A	N/A	N/A	**N/A**	N/A
					Starting material out of specifications	No relevant impact expected if minor deviation.	4	Human error/Analytical error	1	FHG SOP (CG-15/**). A non-released batch can not be used.	1	4	No	N/A	N/A	N/A	N/A	N/A	N/A	**N/A**	N/A
					Higher temperature	Temperature at this point is considered a critical parameter. Relevant effect is expected in case of deviation at this point.	25	Human error/Temperature sensor failure	1	MBR. Manually operated with automatic control. Manual and automatic record. Calibrated temperature sensor (periodic verification)	3	75	Yes	Include a warning in the MBR indicating the criticality of the reaction temperature for the product quality	Already implemented by Farmhispania pilot plant office Feb-2019	Record the inclusion	N/A	N/A	N/A	**N/A**	N/A

Unit Operation	Operation Nr.	Activity or Material or Equipment	NOR	PAR[1]	Potential Failure Mode(s)	Potential Effect(s) of Failure Related CQA	SEV	Potential Cause(s)/ Mechanism(s) of Failure	OCC	Current Design Controls	DET	RPN	Critical process parameter	Recommended Actions	Responsibility & Target Completion Date	Action Results Actions Taken	New SEV	New OCC	New DET	New RPN	Residual Risk and Residual Risk Acceptance
					Lower temperature	Temperature at this point is considered a critical parameter. Relevant effect is expected in case of deviation.	25	Human error/Temperature sensor failure	1	MBR. Manually operated with automatic control. Manual and automatic record. Calibrated temperature sensor (periodic verification)	3	**75**	**Yes**	Include a warning in the MBR indicating the criticality of the reaction temperature for the product quality	Already implemented by Farmhispania pilot plant office Feb-2019	Record the inclusion	N/A	N/A	N/A	**N/A**	N/A
	07	Maintain reaction conditions for 13 h.	13 h	N/A (IPC)	Longer reaction time	No expected effect if minor deviation. In JSR-90 reaction was left to evolve for 20.5 h and no relevant impact in the impurity profile was observed.	4	Human error	3	MBR. Manual control	5	**60**	**No**	N/A	N/A	N/A	N/A	N/A	N/A	**N/A**	N/A

Unit Operation	Operation Nr.	Activity or Material or Equipment	NOR	PAR[1]	Potential Failure Mode(s)	Potential Effect(s) of Failure Related CQA	SEV	Potential Cause(s)/ Mechanism(s) of Failure	OCC	Current Design Controls	DET	RPN	Critical process parameter	Recommended Actions	Responsibility & Target Completion Date	Action Results Actions Taken	New SEV	New OCC	New DET	New RPN	Residual Risk and Residual Risk Acceptance
					Shorter reaction time	It may lead to uncomplete reaction. However, no relevant effect is expected as the reaction is not stopped until meeting the IPC limit	1	Human error	1	MBR. Manual control	5	5	No	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Note 1: The source of the Target, NOR and PAR values listed in this table is the Farmhispania report: CPPA-XX-XXX/XX.
Note 2: Cells containing N/A correspond to parameters not considered critical in terms of quality. PAR not studied.

As a result of the MPRA, a series of small modifications were applied over the **FH0317** MBRs. The implemented changes allowed to reduce the risk in terms of quality related to potential cross contaminations, operating errors and safety issues. The MBRs resulting of the PQRA were successfully used in the **FH0317** validation. As it is mentioned above the process was validated after the completion of three consecutive batches without relevant issues.

1.6.2.5. Process scale-up conclusions

- An industrial manufacturing process for the obtention of **FH0317** was successfully developed and validated. The mentioned process allowed to obtain 8.3 kg of **FH0317** per batch with a yield and purity of 32 %, 98.8 % respectively. The small differences on process performance compared with the results obtained after the initial optimization phase (28 % yield and 99.0 % purity; described in section 1.6.1.6) were mainly attributed to the modifications implemented over the final purification step which, as it is mentioned in section 1.6.2.1, was adapted to include a polish filtration.
- During the execution of the pilot batches performed before the validation, a series of problems associated to the scale increase were found. The corresponding investigations were performed in order to determine the source of the issues observed. The process was modified in order to cover the observed gaps and eventually a manufacturing method that could be successfully validated was obtained.
- A new purification process based on a recrystallization was designed. The developed purification allowed to include a polish filtration operation that reduced the risk of foreign matter contaminations in the final product.
- The step 2 w.up was optimized in order to avoid the phase separation problems observed during the first pilot batches. The corresponding scale down experiments were performed to evaluate the suitability of the proposed optimizations before its implementation.
- The step 2 crystallization was studied and modified in order to avoid the unloading issues observed during the performed industrial batches.
- The PQRA of the **FH0317** process was successfully performed. The mentioned studies allowed to reach the process knowledge, control and robustness levels required for its validation.

1.7. FH0317 project conclusions

- A suitable method for the preparation at industrial scale of **FH0317** has been developed and validated within the requested timelines. The obtained API, which is a new chemical entity, had the quality requested by the customer and could be used to perform the clinical trials. Therefore, the main goal of this project has been achieved.
- The **FH0317** manufacturing method initially received presented several drawbacks (low robustness, the purity of the obtained product was poor, low yields and operations complex

to be performed at industrials scale). The synthesis of **FH0317** was optimized to solve the initially observed weaknesses. Eventually, a suitable process for the obtention of the desired API at large scale was developed.

- Through a series of small scale experiments the reaction conditions of the steps 1 to 4 were studied and optimized. These studies allowed to reduce the formation of impurities and reduce reaction times. The w.up of the mentioned synthetic steps were also modified to adapt them to the limitations of the large scale equipment, reduce product loses and increase the purity of the isolated intermediates. The final purification step was totally redesigned in order to increase the purity of the obtained API and reduce its residual solvent content. The purification process was also optimized to obtain **FH0317** with the desired PSD.

- Compared with the original process, the optimized process allowed to reach higher purities, yields and to obtain a product within the residual solvent and PSD specifications.

- The developed **FH0317** manufacturing method was successfully scaled-up to 8.5 kg scale. Seven pilot batches were carried out in order to study the robustness of the process and, to detect problems derived from the scale increase and from the limitations of the industrial equipment used. During the execution of the industrial batches, some minor problems were found. The corresponding investigations and laboratory scale experiments were performed in order to determine the root causes of the mentioned incidences. Derived from the mentioned troubleshooting activities, a series of modifications were proposed and applied over the initial industrial process. The implemented changes allowed to adapt the process to the industrial scale and to correct its weaknesses.

- In the context of the **FH0317** project, a complete process quality risk assessment methodology was developed (see Annex 1). The developed methodology was designed to reduce the risk associated to the manufacturing processes developed at Farmhispania Group and, to increase its robustness in a systematic way. The process quality risk assessment performed allowed to define the criticality associated to each process parameter/material attribute of the process, stablish suitable control strategies and detect/correct gaps in the process MBRs. The implementation of the developed methodology allowed to reach the process knowledge and process robustness levels required for the process validation.

- The **FH0317** industrial process was eventually validated in the Farmhispania S.A. plant. Three 8.5 kg validation batches were completed without relevant incidences demonstrating that the developed process was suitable for the obtention of **FH0317** at commercial scale.

CHAPTER 6

GENERAL CONCLUSIONS

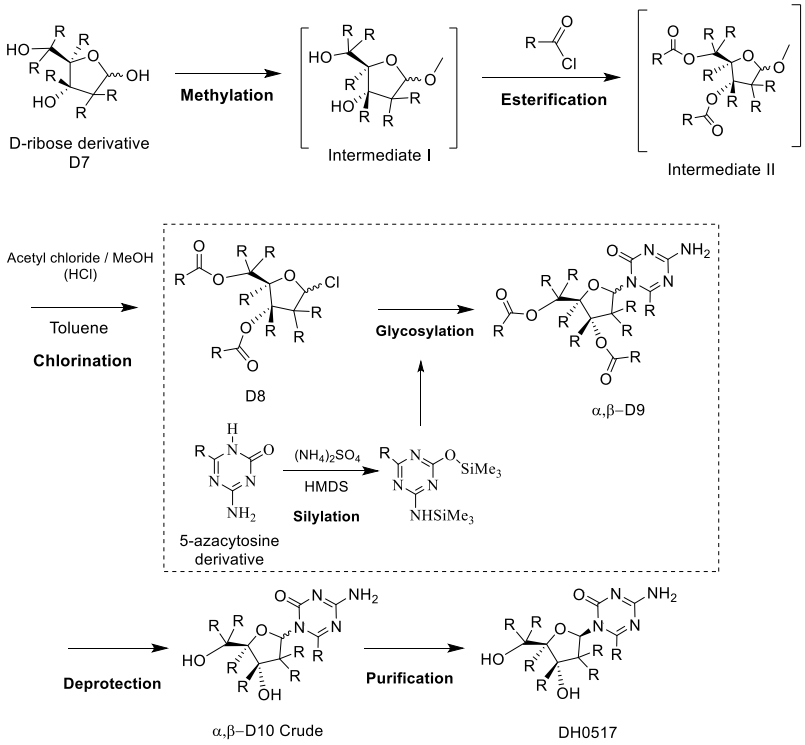
INDUSTRIAL PhD THESIS

PROCESS DEVELOPMENT FOR THE SYNTHESIS AT INDUSTRIAL SCALE OF ACTIVE PHARMACEUTICAL INGREDIENTS

Chapter 6

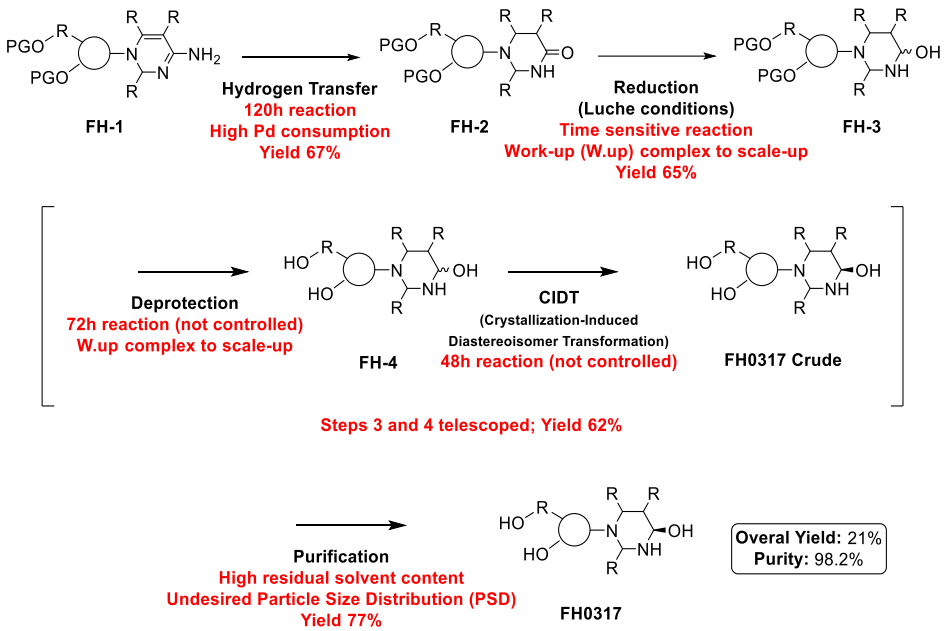
1. General conclusions

- Based in information from the literature, a manufacturing method for the obtention at commercial scale of a highly potent active pharmaceutical ingredient (**DH0517**) has been developed (Scheme 42). The manufacturing method has been validated and scaled-up successfully at a 17 kg scale (118 kg at the glycosylation step). The optimization of the glycosylation, which is the key step of the synthesis, leaded to a process that allowed to reach at industrial scale yields (regarding the **D9** β-anomer) equivalent to the highest ones reported in the examined literature.



Scheme 42. Synthetic route developed at Farmhispania S.A. for the DH0517 preparation.

- Different process impurities and intermediates of the **DH0517** process were successfully isolated and characterized. The obtained samples were used to create the analytical standards required to guarantee an accurate control of the synthesis. The mechanism of formation of the different studied substances was evaluated. These studies served to demonstrate that Farmhispania S.A. has a deep knowledge and control of the synthesis, increasing its commercial attractive and the chances of obtaining the approval from any regulatory authority around the world for the **DH0517** commercialization.
- From a preliminary process that presented several deficiencies in terms of robustness, quality and time scale (Scheme 43) a manufacturing method for the obtention at industrial scale of an active pharmaceutical ingredient (**FH0317**) has been developed. The developed process has been scaled-up and validated successfully at an 8.5 kg scale.



Scheme 43. FH0317 RoS including the main drawbacks detected for each step.

- A process quality risk assessment (PQRA) methodology based on the FMEA and the QbD principles, has been developed and implemented during the **FH0317** process validation. Quality risk assessment methodologies are well known and have been used in many different industries including many examples within the pharmaceutical area and especially in the development and manufacturing of pharmaceutical specialities.
- The developed methodology allows to define the criticality associated to each process parameter/material attribute of the process, to stablish suitable control strategies and detect/correct gaps in the process MBRs. Its utilization allows to obtain in a systematic way, robust and well controlled processes complying consistently with the desired and targeted quality standards.
- By the time this thesis was started, the Farmhispania Group did not have any specific methodology supporting process characterisation and process validation. The work carried out in this thesis and specially the methodology developed and set up, constitutes a significant and valuable legacy to the Farmhispania Group as, since its implementation after FH0317 process validation, it has been used in many other projects among the different teams and sites of the Group.
- As part of the experimental work carried out in this thesis different *in-silico* tools such as the Dynochem software have been used to better simulate, predict and understand physico-chemical process features. A series of Dynochem applications and utilities have been used in processes developed and, general protocols have been created allowing any Process Chemist at the Farmhispania Group to take advantage of the tool and generate the required process knowledge.
- By the time this thesis was started, there were no examples of the utilization of *in-silico* simulations for the study of the unit operations part of the processes developed at the Farmhispania Group. Again, the implementation of Dynochem through the FH0317 and the DH0517 projects, has led to a completely new area of knowledge adding significant value and robustness to the processes developed at Farmhispania.

CHAPTER 7

EXPERIMENTAL SECTION

INDUSTRIAL PhD THESIS

PROCESS DEVELOPMENT FOR THE SYNTHESIS AT INDUSTRIAL SCALE OF ACTIVE PHARMACEUTICAL INGREDIENTS

Chapter 7

1. General methods

Disclaimer: the information generated during the **DH0517** and the **FH0317** projects is property of Farmhispania S.A. In order to protect the trade secret and the interests of the company this chapter have been censored.

If considered appropriate, further information would be made available under request to the author.

BIBLIOGRAPHY

INDUSTRIAL PhD THESIS

PROCESS DEVELOPMENT FOR THE SYNTHESIS AT INDUSTRIAL SCALE OF ACTIVE PHARMACEUTICAL INGREDIENTS

Bibliography

(1) What does an industrial doctorate project involve? http://doctoratsindustrials.gencat.cat/en/contents/view/6 (accessed Jul 8, 2020).

(2) Group, F. Farmhispania Group – Active pharmaceutical ingredients http://www.farmhispaniagroup.com/ (accessed Jun 12, 2019).

(3) Walsh, R. A history of the pharmaceutical industry - Pharmaphorum https://pharmaphorum.com/articles/a_history_of_the_pharmaceutical_industry/ (accessed Jun 6, 2019).

(4) Sullivan, T. A Tough Road: Cost To Develop One New Drug Is $2.6 Billion; Approval Rate for Drugs Entering Clinical Development is Less Than 12% https://www.policymed.com/2014/12/a-tough-road-cost-to-develop-one-new-drug-is-26-billion-approval-rate-for-drugs-entering-clinical-de.html (accessed Jun 10, 2019).

(5) Onawole, H. Huawei join Samsung, Alphabet in top 5 R&D investors for 2018 - Gizmochina https://www.gizmochina.com/2019/01/02/huawei-join-samsung-alphabet-in-top-5-rd-investors-for-2018/ (accessed Apr 20, 2020).

(6) Food and drug administration (FDA). Development & Approval Process (Drugs) | FDA https://www.fda.gov/drugs/development-approval-process-drugs (accessed Jun 6, 2019).

(7) Roser, M. Life Expectancy https://ourworldindata.org/life-expectancy (accessed Jul 26, 2020).

(8) MacKinnon III, G. E. *Understanding Health Outcomes and Pharmacoeconomics*, First.; Jones & Bartlett Publishers, 2011.

(9) Jänne, P. A.; Kim, G.; Shaw, A. T.; Sridhara, R.; Pazdur, R.; McKee, A. E. Dose Finding of Small-Molecule Oncology Drugs: Optimization throughout the Development Life Cycle. *Clin. Cancer Res.* **2016**, *22* (11), 2613–2617. https://doi.org/10.1158/1078-0432.CCR-15-2643.

(10) Markolin, P. P. Dynamic Undocking, a Structure Guided Tool for Virtual Drug Discovery. *Advances in biological science*. 2017.

(11) Robertson, A. Flexible Japanese robot could replace human lab assistants using 3D “virtual lab bench” - The Verge https://www.theverge.com/2012/7/9/3145062/mahoro-japanese-lab-assistant-robot (accessed Jul 2, 2020).

(12) Hughes, J. P.; Rees, S.; Kalindjian, S. B.; Philpott, K. L. Principles of Early Drug Discovery. *Br. J. Pharmacol.* **2011**, *162* (6), 1239–1249. https://doi.org/10.1111/j.1476-5381.2010.01127.x.

(13) Thomsen, R. P. The Role of Patents in the Pharmaceutical Industry https://slideplayer.com/slide/4737288/ (accessed Jul 10, 2019).

(14) Clinical Trial | Definition | AIDSinfo https://aidsinfo.nih.gov/understanding-hiv-aids/glossary/144/clinical-trial (accessed Jul 2, 2020).

(15) Center for Drug Evaluation and Research; Food and Drug Administration; Department of Health and Human Services. Drug Master Files: Guidelines | FDA. Food and Drug administration (FDA): Rockville, Maryland 2017.

(16) Rabah Kamal, Cynthia Cox, D. M. What are the recent and forecasted trends in prescription drug spending? - Peterson-Kaiser Health System Tracker https://www.healthsystemtracker.org/chart-collection/recent-forecasted-trends-prescription-drug-spending/#item-start (accessed Jun 10, 2019).

(17) Watson, W.; Knight, J. Chemical Development and Scale-Up in He Fine Chemical and Pharmaceutical Industrial Principles and Practice. Scientific Update Ltd: Nice (France) 2017, pp 56–67.

(18) Enamel, Z. T. I. Glass-Lined Reactor | Glass Lined Equipment | Taiji http://taijiglasslined.com/1-glass-lined-reactor/232311/ (accessed Sep 2, 2019).

(19) Swetha, M. Unit operation , centrifugation https://www.slideshare.net/medishettiswetha/unit-operation-centrifugation?from_action=save (accessed Jul 8, 2019).

(20) Drying, I. T. Tips for Drying Active Pharmaceutical Ingredients https://www.process-heating.com/articles/86271-tips-for-drying-active-pharmaceutical-ingredients (accessed Jul 9, 2019).

(21) Italvacuum. Vacuum dryer / rotary / batch / for the chemical industry http://www.directindustry.com/prod/italvacuum-srl/product-27975-106867.html (accessed Jul 8, 2019).

(22) Packed Tower Wet Scrubbers | Aircon Systems | Manufacturer in Shahadra,, Delhi | ID: 3688617433 https://www.indiamart.com/proddetail/packed-tower-wet-scrubbers-3688617433.html (accessed Jul 9, 2019).

(23) Adam, F. M.; Bish, G.; Calo, F.; Carr, C. L.; Castro, N.; Hay, D.; Hodgson, P. B.; Jones, P.; Knight, C. J.; Paradowski, M.; Parsons, G. C.; Proctor, K. J. W.; Pryde, D. C.; Rota, F.; Smith, M. C.; Smith, N.; Tran, T.-D.; Hitchin, J.; Dixon, R. Development of a Practical Synthesis of Toll-like Receptor Agonist PF-4171455: 4-Amino-1-Benzyl-6-Trifluoromethyl-1,3-Dihydroimidazo [4,5- *c*] Pyridin-2-One. *Org. Process Res. Dev.* **2011**, *15* (4), 788–796. https://doi.org/10.1021/op200021a.

(24) Occupational Safety and Health Administration (OSHA). 1910.119 - Process safety management of highly hazardous chemicals. | Occupational Safety and Health Administration https://www.osha.gov/laws-regs/regulations/standardnumber/1910/1910.119 (accessed Jul 13, 2019).

(25) Ezidock. Ezidock - Pharmapure AS Chargebag on Vimeo https://vimeo.com/248313175 (accessed Jul 14, 2019).

(26) Chemical Safety Board. Imperial Sugar Company Dust Explosion and Fire https://www.csb.gov/imperial-sugar-company-dust-explosion-and-fire/ (accessed Jul 16, 2019).

(27) Land, C. M. van 't. Product Quality and Safeguarding Drying. In *Drying in the Process Industry*; John Wiley & Sons, Inc.: Hoboken, New Jersey, 2011; pp 289–312.

(28) Explosion Testing for dust gases & vapours. Testing for Minimum Ignition Energy test (MIE) https://www.explosiontesting.co.uk/mie_15.html (accessed Jul 13, 2019).

(29) Explosion Testing for dust, gases & vapours. Testing for Minimum Ignition Temperature test (MIT) https://www.explosiontesting.co.uk/ignition_temp_9.html (accessed Jul 13, 2019).

(30) Ball F., J. Specification of Rupture Disk Burst Pressure. National Board of Boiler and Pressure Vessel Inspectors: Columbus 2011, p 2.

(31) CSB. Pesticide Chemical Runaway Reaction Pressure Vessel Explosion https://www.csb.gov/assets/recommendation/bayer_report_final.pdf (accessed Jul 15, 2019).

(32) Adiabatic Accelerating Rate Calorimeter http://www.thermalhazardtechnology.com/products/accelerating+rate+calorimeter (accessed Jul 21, 2019).

(33) Fauske, A.; Knoechel, D. J. Reaction Calorimetry vs. Adiabatic Calorimetry: Which Method is Right? https://www.fauske.com/blog/-reaction-calorimetry-vs.-adiabatic-calorimetry-which-method-is-right-for-me (accessed Jul 21, 2019).

(34) Sparktech. Inner Coil Reaction Vessels, Capacity: 20-100 L | ID: 5731381162 https://www.indiamart.com/proddetail/inner-coil-reaction-vessels-5731381162.html (accessed Sep 3, 2019).

(35) Hall, A.; Muller, F.; Carpenter, K. Dynamic Modeling for Batch Process Simulation: A Case Study and Software Tool Development. *Chimia (Aarau).* **2003**, *57* (1–2), 49–50.

(36) Fox, S. Improving Pharma Process Operations https://www.genengnews.com/magazine/70/improving-pharma-process-operations/ (accessed Sep 4, 2020).

(37) ScienView. DynoChem http://www.scienview.com/DynoChem.html (accessed Aug 1, 2019).

(38) American society for testing and materials (ASTM). Chetah. ASTM international 2009.

(39) Glodek, M.; Liebowitz, S.; Squibb, B.-M.; McCarthy, R.; Plough, S.; McNally, G.; Oksanen, C.; Schultz, T.; Sundararajan, M.; Vorkapich, R.; Vukovinsky, K.; Watts, C. Process Robustness—A PQRI White Paper. *Pharm Eng* **2006**, *26*.

(40) Andrews, U. of S. Pressure - Temperature nomograph http://chemistry.st-andrews.ac.uk/labs/misc/nomo.html (accessed Sep 2, 2019).

(41) VEGA instrumentos, S. A. Level measurement of liquids | Radar level sensors from VEGA https://www.vega.com/en/radar/liquids (accessed Sep 3, 2019).

(42) I.C.T, S. L.; Instrumentación Científica Técnica, S. L. Placa porosa, vidrio PYREX http://www.ictsl.net/productos/vidrio/01d63695d409bbe2a.html (accessed Sep 2, 2019).

(43) McConville, F. X. *The Pilot Plant Real Book: A Unique Handbook for the Chemical Process Industry*, 2nd ed.; Fxm Engineering & Design: Worcester MA 01602, 2007. https://doi.org/10.1021/ed080p1260.

(44) Angstadt, N, C. Purines and pyrimidines https://library.med.utah.edu/NetBiochem/pupyr/pp.htm (accessed Sep 3, 2018).

(45) Garcia-Manero, G.; Odenike, O.; Amrein, P. C.; Steensma, D. P.; DeZern, A. E.; Michaelis, L. C.; Faderl, S.; Kantarjian, H. M.; Lowder, J. N.; Taverna, P.; Oganesian, A.; Zhang, X.; Azab, M.; Savona, M. R. Successful Emulation of IV Decitabine Pharmacokinetics with an Oral Fixed-Dose Combination of the Oral Cytidine Deaminase Inhibitor (CDAi) E7727 with Oral Decitabine, in Subjects with Myelodysplastic Syndromes (MDS): Final Data of Phase 1 Study. *Blood* **2016**, *128* (22).

(46) Institute, N. C. *Myelodysplastic Syndromes Treatment (PDQ®): Patient Version*; PDQ Adult Treatment Editorial Board, 2002.

(47) Institute, N. C. *Adult Acute Myeloid Leukemia Treatment (PDQ®): Patient Version*; PDQ Adult Treatment Editorial Board, 2002.

(48) Jabbour, E.; Issa, J.-P.; Garcia-Manero, G.; Kantarjian, H. Evolution of Decitabine Development: Accomplishments, Ongoing Investigations, and Future Strategies. *Cancer* **2008**, *112* (11), 2341–2351. https://doi.org/10.1002/cncr.23463.

(49) Lin, K. T.; Momparler, R. L.; Rivard, G. E. High-Performance Liquid Chromatographic Analysis of Chemical Stability of 5-Aza-2'-Deoxycytidine. *J. Pharm. Sci.* **1981**, *70* (11), 1228–1232.

(50) Beisler, J. A. Isolation, Characterization, and Properties of a Labile Hydrolysis Product of the Antitumor Nucleoside, 5-Azacytidine. *J. Med. Chem.* **1978**, *21* (2), 204–208. https://doi.org/10.1021/jm00200a012.

(51) Rogstad, D. K.; Herring, J. L.; Theruvathu, J. A.; Burdzy, A.; Perry, C. C.; Neidigh, J. W.; Sowers, L. C. Chemical Decomposition of 5-Aza-2'-Deoxycytidine (Decitabine): Kinetic Analyses and Identification of Products by NMR, HPLC, and Mass Spectrometry. *Chem. Res. Toxicol.* **2009**, *22* (6), 1194–1204. https://doi.org/10.1021/tx900131u.

(52) Chan, K. K.; Giannini, D. D.; Staroscik, J. A.; Sadee, W. 5-Azacytidine Hydrolysis Kinetics Measured by High-Pressure Liquid Chromatography and 13C-NMR Spectroscopy. *J. Pharm. Sci.* **1979**, *68* (7), 807–812. https://doi.org/10.1002/jps.2600680705.

(53) Larroca, M. A. Prospecto Dacogen (Decitabina; Polvo Liofilizado Para Inyectables). Janssen Cilag Farmacéutica: Swensweg (Holland) 2011, pp 1–30.

(54) Liu, Y.; Lambeth, G. H.; Nixon, B. W. Processes for Producing Decitabine Which Minimal or No Purification of Intermediates., September 30, 2010.

(55) Mao, L.; Wen, S.; Mao, H.; Wang, Y.; Ji, X.; Zhang, L.; Wang, Y.; Chen, L.; Yan, J.; Yin, H. Process for Preparation of β-Isomer Enriched Decitabine Precursors., August 7, 2013.

(56) Fan, J.; Song, B.; Xue, W.; Tao, S.; Chen, P. Process for Synthesis of Decitabine., November 19, 2008.

(57) Henschke, J. P.; Zhang, X.; Yu, J.; Hu, K.; Mei, L. Improved Process for the Synthesis and Purification of Decitabine., April 8, 2010.

(58) Sun, C.; Ma, S.; Li, W. Method for Preparing Decitabine., August 5, 2009.

(59) Zhang, Q.; Wu, X.; Yi, B. Preparation of 2-Deoxy-D-Ribose Derivatives as Intermediate of Decitabine., November 26, 2008.

(60) Jiang, L.; Chu, G.; Wang, Z.; Huang, H.; Li, W. Process for Preparation of Decitabine and Its Intermediate., November 4, 2009.

(61) Vujjini, S. K.; Varanasi, G.; Arevelli, S.; Kandala, S. C.; Tirumalaraju, S. R.; Bandichhor, R.; Kagga, M.; Cherukupally, P. An Improved and Scalable Process for the Synthesis of 5-Azacytidine: An Antineoplastic Drug. *Org. Process Res. Dev.* **2013**, *17* (2), 303–306. https://doi.org/10.1021/op300192e.

(62) Jungmann, O.; Kraut, N. Method of Producing Nucleosides. US20100210833A1, 2010.

(63) Jungmann, O.; Kraut, N. Industrial Process for the Production of Nucleosides by Direct Glycosylation of the Nucleoside Base. EP2048151A1, 2007.

(64) Jungmann, O.; Kraut, N. Industrial Process for the Production of Nucleosides by Direct Glycosylation of the Nucleoside Base., 2009.

(65) Jungmann, O.; Kraut, N. Method of Producing 2'-Deoxy-5-Azacytidine (Decitabine) via Metal Triflate-Catalyzed Coupling Reaction of 5-Azacytosine with Chloro Sugar., April 22, 2009.

(66) Niedballa, U.; Vorbruggen, H. A General Synthesis of Pyrimidine Nucleosides. *Angew. Chem. Int. Ed. Engl.* **1970**, *9* (6), 461–462. https://doi.org/10.1002/anie.197004611.

(67) Johnson, T. B.; Hilbert, G. E. The Synthesis of Pyrimidine-Nucleosides. *Science* **1929**, *69* (1796), 579–580. https://doi.org/10.1126/science.69.1796.579.

(68) Nishimura, T.; Shimizu, B.; Iwai, I. A New Synthetic Method of Nucleosides. *Chem. Pharm. Bull. (Tokyo).* **1963**, *11* (11), 1470–1472. https://doi.org/10.1248/cpb.11.1470.

(69) Wang, Z. Hilbert-Johnson Reaction. In *Comprehensive Organic Name Reactions and Reagents*; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2010; pp 1408–1414. https://doi.org/10.1002/9780470638859.conrr314.

(70) Lunato, A. J.; Wang, J.; Woollard, J. E.; Anisuzzaman, A. K. M.; Ji, W.; Rong, F.-G.; Ikeda, S.; Soloway, A. H.; Eriksson, S.; Ives, D. H.; Blue, T. E.; Tjarks, W. Synthesis of 5-(Carboranylalkylmercapto)-2'-Deoxyuridines and 3-(Carboranylalkyl)Thymidines and Their Evaluation as Substrates for Human Thymidine Kinases 1 and 2. *J. Med. Chem.* **1999**, *42* (17), 3378–3389. https://doi.org/10.1021/jm990125i.

(71) Kissman, H. M.; Pidacks, C.; Baker, B. R. Puromycin. Synthetic Studies. XI. D-Ribofuranosyl Derivatives of 6-Dimethylaminopurine. *J. Am. Chem. Soc.* **1955**, *77* (1), 18–24. https://doi.org/10.1021/ja01606a005.

(72) Wang, Z. Vorbrüggen Glycosylation. In *Comprehensive Organic Name Reactions and Reagents*; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2010; pp 2915–2919. https://doi.org/10.1002/9780470638859.conrr652.

(73) Agrofoglio, L. A.; Gillaizeau, I.; Saito, Y. Palladium-Assisted Routes to Nucleosides. **2003**. https://doi.org/10.1021/CR010374Q.

(74) Vorbrüggen, H.; Bennua, B. Nucleoside Syntheses, XXV1) A New Simplified Nucleoside Synthesis. *Chem. Ber.* **1981**, *114* (4), 1279–1286. https://doi.org/10.1002/cber.19811140407.

(75) Seela, F.; Peng, X. Regioselective Synthesis of Indazole N 1- and N 2-(B-d-Ribonucleosides). *Nucleosides, Nucleotides and Nucleic Acids* **2004**, *23* (1–2), 227–237. https://doi.org/10.1081/NCN-120027830.

(76) Hocek, M.; Šilhár, P.; Shih, I.; Mabery, E.; Mackman, R. Cytostatic and Antiviral 6-Arylpurine Ribonucleosides. Part 7: Synthesis and Evaluation of 6-Substituted Purine l-Ribonucleosides. *Bioorg. Med. Chem. Lett.* **2006**, *16* (20), 5290–5293. https://doi.org/10.1016/J.BMCL.2006.07.092.

(77) Rico-Gómez, R.; Rodríguez-González, A.; Ríos-Ruíz, J.; Nájera, F.; López-Romero, J. M. Synthesis of Theophylline and 6-Thiotheophylline 7-Ribosyl Nucleosides. *European J. Org. Chem.* **2003**, *2003* (20), 4023–4032. https://doi.org/10.1002/ejoc.200300377.

(78) Co, E.; Henschke, J. The Development and Manufacture of Azacitidine, Decitabine, and Cladribine: Stereoselective Ribonucleoside Drug Synthesis Using the Vorbrüggen Glycosylation. In *Comprehensive Accounts of Pharmaceutical Research and Development: From Discovery to Late-Stage Process Development. Volume 2*; Vaidyanathan, R., Abdel-Magid, A. F., Jaan, P., Eds.; American Chemical Society, 2016; pp 271–321. https://doi.org/10.1021/bk-2016-1240.ch009.

(79) Holbrook, M. T. Chloroform. In *Kirk-Othmer Encyclopedia of Chemical Technology*; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2003. https://doi.org/10.1002/0471238961.0308121508151202.a01.pub2.

(80) Birkofer, L.; Wegner, P. 1.3-Cycloadditionen Mit Trimethylsilyl-Azid; Über IsomereN-Acetyl-1.2.3-Triazole. *Chem. Ber.* **1966**, *99* (8), 2512–2517. https://doi.org/10.1002/cber.19660990817.

(81) Murayama, M. Exposure Apparatus and Air-Conditioning Apparatus for Use with Exposure Apparatus. US6535270 (B1), 2003.

(82) Johnson Matthey. *Process for the Preparation of N-(Trimethylsilyl)-4-(Trimethylsilyloxy)-1,3,5-Triazin-2-Amine*; 2010.

(83) Shaterian, H. R.; Doostmohammadi, R.; Ghashang, M.; Rahmani, M. A Mild, Simple, Efficient, and Selective Protection of Hydroxyl Groups Using Silica-Supported Sodium Hydrogen Sulfate as a Heterogeneous Catalyst. *Phosphorus. Sulfur. Silicon Relat. Elem.* **2008**, *183* (12), 3127–3135. https://doi.org/10.1080/10426500802066054.

(84) Murray, P. M.; Bellany, F.; Benhamou, L.; Bučar, D.-K.; Tabor, A. B.; Sheppard, T. D. The Application of Design of Experiments (DoE) Reaction Optimisation and Solvent Selection in the Development of New Synthetic Chemistry. *Org. Biomol. Chem.* **2016**, *14* (8), 2373–2384. https://doi.org/10.1039/C5OB01892G.

(85) Bach, T.; Brummerhop, H. Trimethylsilyl Trifluoromethanesulfonate (TMSOTf) and N,N-Di-Iso-Propyl Ethyl Amine (Hünig Base): An Effective Reagent Combination for Selective Silylation and Elimination Reactions. *J. für Prakt. Chemie* **1999**, *341* (4), 410–413. https://doi.org/10.1002/(sici)1521-3897(199905)341:4<410::aid-prac410>3.0.co;2-4.

(86) Vorbrüggen, H.; Krolikiewicz, K.; Bennua, B. Nucleoside Synthesis with Trimethylsilyl Triflate and Perchlorate as Catalysts. *Chem. Ber.* **1981**, *114* (4), 1234–1255. https://doi.org/10.1002/cber.19811140404.

(87) Henschke, J. P.; Zhang, X.; Huang, X.; Mei, L.; Chu, G.; Hu, K.; Wang, Q.; Zhu, G.; Wu, M.; Kuo, C.; Chen, Y. A Stereoselective Process for the Manufacture of a 2'-Deoxy-β-D-Ribonucleoside Using the Vorbrüggen Glycosylation. *Org. Process Res. Dev.* **2013**, *17* (11), 1419–1429. https://doi.org/10.1021/op4002005.

(88) Beutner, G. L.; Bultman, M. S.; Cohen, B. M.; Fan, J.; Marshall, J.; Sfouggatakis, C. Follow the Silyl Cation: Insights into the Vorbrüggen Reaction. *Org. Process Res. Dev.* **2019**, *23* (9), 2050–2056. https://doi.org/10.1021/acs.oprd.9b00304.

(89) Vorbrüggen, H.; Höfle, G. On the Mechanism of Nucleoside Synthesis. *Chem. Ber.* **1981**, *114* (4), 1256–1268. https://doi.org/10.1002/cber.19811140405.

(90) Kennedy, C. R.; Lin, S.; Jacobsen, E. N. The Cation-π Interaction in Small-Molecule Catalysis. *Angew. Chem. Int. Ed. Engl.* **2016**, *55* (41), 12596–12624. https://doi.org/10.1002/anie.201600547.

(91) Graczyk, P. P.; Mikołajczyk, M. Anomeric Effect: Origin and Consequences; 2007; pp 159–349. https://doi.org/10.1002/9780470147306.ch2.

(92) Sasaki, K.; Hashimoto, Y. 2,6-Lactones as a New Entry in Stereoselective Glycosylations. *Synlett* **2017**, *28* (10), 1121–1126. https://doi.org/10.1055/s-0036-1588722.

(93) Hosoya, T.; Takano, T.; Kosma, P.; Rosenau, T. Theoretical Foundation for the Presence of Oxacarbenium Ions in Chemical Glycoside Synthesis. *J. Org. Chem.* **2014**, *79* (17), 7889–7894. https://doi.org/10.1021/jo501012s.

(94) Sweeney, J.; Perkins, G.; Aguilar, E.; Fernández-Rodríguez, M. A.; Marquez, R.; Amigues, E.; Lopez-Gonzalez, R. Trimethylsilyl Trifluoromethanesulfonate. In *Encyclopedia of Reagents for Organic Synthesis*; Wiley, 2018; pp 1–36. https://doi.org/10.1002/047084289X.RT338.PUB3.

(95) Piťhová, P.; Pískala, A.; Piťha, J.; Šorm, F. Nucleic Acids Components and Their Analogues. LXVI. Hydrolysis of 5-Azacytidine and Its Connection with Biological Activity. *Collect. Czechoslov. Chem. Commun.* **1965**, *30* (8), 2801–2811. https://doi.org/10.1135/cccc19652801.

(96) Labortecnic. Dust-Free production GMP design; Sieves http://www.labortecnic.com/images/content/Tamizadoras_Ing.pdf (accessed Jul 26, 2020).

(97) Supply, S. 3M Micro-Klean RT Series Filter Cartridge RT40Y16G20NN, 1 Micron, 25 Per Case 3M Micro-Klean RT Series Filter Cartridge RT40Y16G20NN, 1 Micron, 25 Per Case https://www.strobelssupply.com/catalog/product/view/id/135542/ (accessed Sep 12, 2019).

(98) Guideline, I. C. H. H. T. Impurities in New Drug Substances Q3A (R2). In *Proceedings of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Geneva, Switzerland*; ICH, 2006; pp 1–15.

(99) EMA. Guideline on Active Substance Master File Procedure. In *Active substance master file procedure*; European Medicine Agency: London, 2018; pp 1–23.

(100) FDA. How Drugs are Developed and Approved | FDA https://www.fda.gov/drugs/development-approval-process-drugs/how-drugs-are-developed-and-approved (accessed Sep 1, 2019).

(101) Guideline ICH, H. T. Impurities: Guideline for Residual Solvents Q3C (R5). In *International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use*; ICH, 2018; pp 1–25.

(102) Tester, R. F.; Karkalas, J. Carbohydrates | Classification and Properties. In *Encyclopedia of Food Sciences and Nutrition*; Elsevier, 2003; pp 862–875. https://doi.org/10.1016/B0-12-227055-X/00166-8.

(103) Mackie, W.; Perlin, A. S. Pyranose–Furanose and Anomeric Equilibria: Influence of Solvent and of Partial Methylation. *Can. J. Chem.* **1966**, *44* (17), 2039–2049. https://doi.org/10.1139/v66-304.

(104) Chris P Schaller. Pyranose and Furanose Forms - Chemistry LibreTexts https://chem.libretexts.org/Core/Organic_Chemistry/Reactions/Addition_Reactions/Addition_to_Carbonyls/Pyranose_and_Furanose_Forms (accessed Mar 5, 2018).

(105) Chen, L.; Zhao, J.; Pradhan, S.; Brinson, B. E.; Scuseria, G. E.; Zhang, Z. C.; Wong, M. S. Ring-Locking Enables Selective Anhydrosugar Synthesis from Carbohydrate Pyrolysis. *Green Chem.* **2016**, *18* (20), 5438–5447. https://doi.org/10.1039/C6GC01600F.

(106) Lemieux, R. U.; Anderson, L.; Conner, A. H. The Mutarotation of 2-Deoxy-β-d-Erythro-Pentose ("2-Deoxy-β-d-Ribose") : Conformations, Kinetics, and Equilibria. *Carbohydr. Res.* **1971**, *20* (1), 59–72. https://doi.org/10.1016/S0008-6215(00)84949-6.

(107) Furberg, S. The Structure of 2-Deoxyribose. *Acta Chem. Scand* **1960**, *14* (6), 9.

(108) Lemieux, R. U.; Stevens, J. D. The Proton Magnetic Resonance Spectra and Tautomeric Equilibria of Aldoses in Deuterium Oxide. *Can. J. Chem.* **1966**, *44* (3), 249–262. https://doi.org/10.1139/v66-037.

(109) Deriaz, R. E.; Overend, W. G.; Stacey, M.; Teece, E. G.; Wiggins, L. F. Deoxy-Sugars. Part V. A Reinvestigation of the Glycal Method for the Synthesis of 2-Deoxy-D- and -L-Ribose. *J. Chem. Soc.* **1949**, *0* (0), 1879. https://doi.org/10.1039/jr9490001879.

(110) Pfeffer, P. E.; Parrish, F. W.; Unruh, J. Effects of Carbohydrate-Structure Changes on Induced Shifts in Differential Isotope-Shift 13C-N.M.R. *Carbohydr. Res.* **1980**, *84* (1), 13–23. https://doi.org/10.1016/S0008-6215(00)85425-7.

(111) Angyal, S. J. The Composition of Reducing Sugars in Solution. *Adv. Carbohydr. Chem. Biochem.* **1984**, *42*, 15–68. https://doi.org/10.1016/S0065-2318(08)60122-5.

(112) Kabayama, M. A.; Patterson, D. The Thermodynamics of Mutarotation of Some Sugars: II. Theoretical Considerations. *Can. J. Chem.* **1958**, *36* (3), 563–573. https://doi.org/10.1139/v58-079.

(113) Aruoma, O. I.; Grootveld, M.; Halliwell, B. The Role of Iron in Ascorbate-Dependent Deoxyribose Degradation. Evidence Consistent with a Site-Specific Hydroxyl Radical Generation Caused by Iron Ions Bound to the Deoxyribose Molecule. *J. Inorg. Biochem.* **1987**, *29* (4), 289–299. https://doi.org/10.1016/0162-0134(87)80035-1.

(114) Deriaz, R. E.; Overend, W. G.; Stacey, M.; Wiggins, L. F. 597. Deoxy-Sugars. Part VI. The Constitution of β-Methyl-2-Deoxy- L -Ribopyranoside and of Aβ-Methyl-2-Deoxy- L -Ribofuranoside. *J. Chem. Soc.* **1949**, *0* (0), 2836–2841. https://doi.org/10.1039/JR9490002836.

(115) Mathlouthi, M.; Seuvre, A. M.; Koenig, J. L. F.T.-I.R. and Laser-Raman Spectra of d-Ribose and 2-Deoxy-d-Erythro-Pentose ("2-Deoxy-d-Ribose"). *Carbohydr. Res.* **1983**, *122* (1), 31–47. https://doi.org/10.1016/0008-6215(83)88404-3.

(116) Zinner, H.; Nimz, H.; Wittenburg, E. Benzoate Der 2-Desoxy-D-Ribose. *Chem. Ber.* **1960**, *93* (2), 340–346. https://doi.org/10.1002/cber.19600930211.

(117) Daniel Heinrich; Thomas Wagner, and; Diederichsen*, U. Synthesis and DNA Incorporation of an Ethynyl-Bridged Cytosine C-Nucleoside as Guanosine Surrogate. **2007**. https://doi.org/10.1021/OL7025334.

(118) Wang, Z.-X.; Duan, W.; Wiebe, L. I.; Balzarini, J.; De Clercq, E.; Knaus, E. E. Synthesis of 1-(2-Deoxy-β-d-Ribofuranosyl)-2,4-Difluoro-5-Substituted-Benzene Thymidine Mimics,* Some Related α-Anomers, and Their Evaluation as Antiviral and Anticancer Agents. *Nucleosides, Nucleotides and Nucleic Acids* **2001**, *20* (1–2), 11–40. https://doi.org/10.1081/NCN-100001435.

(119) Chargaff, Erwin; Davidson, J. N. (James N. *The Nucleic Acids: Chemistry and Biology. Volume 1.*, Third.; Academic Press INC.: New York, 1955.

(120) Pretsch, Erno; Bühlmann, P.; Affolter, C; Herrera, A; Martinez, R. *Determinación Estructural de Compuestos Orgánicos*; Elsevier Masson: Barcelona, España, 2002.

(121) Ben-Hattar, J.; Jiricny, J. An Improved Synthesis of 2'-Deoxy-5-Azacytidine by Condensation of an 9-Fluorenylmethoxycarbonyl-Protected Sugar onto the Silylated Base. *J. Org. Chem.* **1986**, *51* (16), 3211–3213. https://doi.org/10.1021/jo00366a028.

(122) Purushothaman, M.; Viswanath, V.; Rao, B. N.; Begum, S. I.; Purushothaman, M., Viswanath, V., Rao, B. N., & Begum, S. I. Preparation and Evaluation of Decitabine Liposmoes. *Indo Am. J. Pharm. Sci.* **2015**, *2* (9), 1264–1273.

(123) Suzhou Bichal Biological Technology Co., L. Decitabine|Suzhou Bichal Biological Technology Co.,LTD http://www.bichal.com/pview/B-5218-1405311002495770-view.html (accessed Mar 15, 2018).

(124) VWR. 5-Aza-2'-Deoxycytidne. Crystalline Powder | VWR https://us.vwr.com/store/product/14513099/5-aza-2-deoxycytidne-crystalline-powder (accessed Mar 15, 2018).

(125) Wepplo, P. A Guide to Organic Chemistry Mechanisms : A Guided Inquiry Workbook Easy to Follow Curved Arrows; CurvedArrowPress, 2008; p 54.

(126) Ester to Acid - Common Conditions http://commonorganicchemistry.com/Rxn_Pages/Ester_to_Acid/Ester_to_Acid_Index.htm (accessed Jan 24, 2019).

(127) Meier, L.; Monteiro, G. C.; Baldissera, R. A. M.; Sá, M. M. Simple Method for Fast Deprotection of Nucleosides by Triethylamine-Catalyzed Methanolysis of Acetates in Aqueous Medium. *J. Braz. Chem. Soc.* **2010**, *21* (5), 859–866. https://doi.org/10.1590/S0103-50532010000500013.

(128) Bruice, T. C.; Mayahi, M. F. The Influence of the Leaving Tendency of the Phenoxy Group on the Ammonolysis and Hydrolysis of Substituted Phenyl Acetates. *J. Am. Chem. Soc.* **1960**, *82* (12), 3067–3071. https://doi.org/10.1021/ja01497a023.

(129) Ilieva, S.; Galabov, B.; Musaev, D. G.; Morokuma, K.; Schaefer, H. F. Computational Study of the Aminolysis of Esters. The Reaction of Methylformate with Ammonia. *J. Org. Chem.* **2003**, *68* (4), 1496–1502. https://doi.org/10.1021/jo0263723.

(130) Montgomery, J. A.; Hewson, K. 1-(Adenm-9-Yl)-2,5-Anhydro-1-Deoxy-d-Allitol, a Homolog of Adenosine. *J. Heterocycl. Chem.* **1970**, *7* (2), 443–445. https://doi.org/10.1002/jhet.5570070239.

(131) Batoux, N.; Granet, R.; Zerrouki, R.; Krausz, P. 3-Substituted Pyrazinone Nucleosides—A New Family of D4T Analogues. *Nucleosides, Nucleotides and Nucleic Acids* **2009**, *28* (9), 866–873. https://doi.org/10.1080/15257770903169999.

(132) Clemens, R. T.; Jennings, M. P. An Efficient Total Synthesis and Absolute Configuration Determination of Varitriol. *Chem. Commun.* **2006**, *0* (25), 2720. https://doi.org/10.1039/b603931f.

(133) Watanabe, K. A.; Harada, K.; Zeidler, J.; Matulic-Adamic, J.; Takahashi, K.; Ren, W. Y.; Cheng, L. C.; Fox, J. J.; Chou, T. C. Synthesis and Anti-HIV-1 Activity of 2'-"up"-Fluoro Analogs of Active Anti-AIDS Nucleosides 3'-Azido-3'-Deoxythymidine (AZT) and 2',3'-Dideoxycytidine (DDC). *J. Med. Chem.* **1990**, *33* (8), 2145–2150. https://doi.org/10.1021/jm00170a016.

(134) Nishino, S.; Yamamoto, H.; Nagato, Y.; Ishido, Y. Partial Protection of Carbohydrate Derivatives. Part 19. Highly Regioselective 5'- *O* -Aroylation of 2'-Deoxyribonucleosides in Terms of Dilution - Drop-by-Drop - Addition Procedure. *Nucleosides and Nucleotides* **1986**, *5* (2), 159–168. https://doi.org/10.1080/07328318608068670.

(135) Hadj-Bouazza, A.; Zerrouki, R.; Krausz, P.; Laumond, G.; Aubertin, A. M.; Champavier, Y. New Acyclonucleosides: Synthesis and Anti-HIV Activity. *Nucleosides, Nucleotides and Nucleic Acids* **2005**, *24* (8), 1249–1263. https://doi.org/10.1081/NCN-200067423.

(136) Institute, N. C. What Is Cancer? https://www.cancer.gov/about-cancer/understanding/what-is-cancer (accessed Sep 5, 2019).

(137) Kara Rogers. Plasma cell https://www.britannica.com/science/plasma-cell (accessed Sep 5, 2019).

(138) Newman, T.; Biggers, A. What is a cell? https://www.medicalnewstoday.com/articles/320878.php (accessed Sep 5, 2019).

(139) Felman, A.; Chun, C. What to know about lymphoma https://www.medicalnewstoday.com/articles/146136.php (accessed Sep 13, 2019).

(140) Parascandola, J. *King of Poisons: A History of Arsenic*; Potomac Books, I., Ed.; 2012.

(141) Ward, D. Fowler's Solution https://www.flickr.com/photos/daveward/338346821 (accessed Sep 12, 2019).

(142) Baguley, B. C. A Brief History of Cancer Chemotherapy. In *Anticancer Drug Development*; Baguley, B. C., Kerr, D. J., Eds.; Academic Press, 2002; pp 1–11. https://doi.org/10.1016/B978-012072651-6/50002-4.

(143) Howes, M.-J. R. The Evolution of Anticancer Drug Discovery from Plants. *Lancet Oncol.* **2018**, *19* (3), 293–294. https://doi.org/10.1016/S1470-2045(18)30136-0.

(144) Dilda, P. J.; Hogg, P. J. Arsenical-Based Cancer Drugs. *Cancer Treat. Rev.* **2007**, *33* (6), 542–564. https://doi.org/10.1016/J.CTRV.2007.05.001.

(145) Worldpress. Mice and Mustard Gas: A History of Chemotherapy – Speaking of Research https://speakingofresearch.com/2013/11/20/mice-and-mustard-a-history-of-chemotherapy/ (accessed Sep 12, 2019).

(146) DeVita, V. T.; Chu, E. A History of Cancer Chemotherapy. *Cancer Res.* **2008**, *68* (21), 8643 LP – 8653. https://doi.org/10.1158/0008-5472.CAN-07-6611.

(147) UCLA; Hardinger, S. Mechlorethamine; chlormethine http://www.chem.ucla.edu/~harding/IGOC/M/mechlorethamine.html (accessed Sep 13, 2019).

(148) OncoLink Team. Mechlorethamine (Mustargen®, Nitrogen Mustard) | OncoLink https://www.oncolink.org/tratamiento-del-cancer/oncolink-rx/mechlorethamine-mustargen-r-nitrogen-mustard (accessed Sep 13, 2019).

(149) Kim, K.-W.; Roh, J. K.; Wee, H.-J.; Kim, C. Alkylating Anticancer Drugs. In *Cancer Drug Discovery*; Springer Netherlands: Dordrecht, 2016; pp 71–94. https://doi.org/10.1007/978-94-024-0844-7_4.

(150) Rocha, C.; Silva, M.; Quinet, A.; Cabral-Neto, J.; Menck, C.; Rocha, C. R. R.; Silva, M. M.; Quinet, A.; Cabral-Neto, J. B.; Menck, C. F. M. DNA Repair Pathways and Cisplatin Resistance: An Intimate Relationship. *Clinics* **2018**, *73* (Suppl 1). https://doi.org/10.6061/clinics/2018/e478s.

(151) Moudi, M.; Go, R.; Yien, C. Y. S.; Nazre, M. Vinca Alkaloids. *Int. J. Prev. Med.* **2013**, *4* (11), 1231–1235.

(152) National Cancer Institute. Hormone Therapy for Breast Cancer https://academic.oup.com/jnci/article-lookup/doi/10.1093/jnci/djv048 (accessed Oct 2, 2019).

(153) Conlon, K. C.; Miljkovic, M. D.; Waldmann, T. A. Cytokines in the Treatment of Cancer. *J. Interf. Cytokine Res.* **2019**, *39* (1), 6–21. https://doi.org/10.1089/jir.2018.0019.

(154) Wishart DS, Feunang YD, Guo AC, Lo EJ, Marcu A, Grant JR, Sajed T, Johnson D, Li C; Sayeeda Z, Assempour N, Iynkkaran I, Liu Y, Maciejewski A, Gale N, Wilson A, C. L.; Cummings R, Le D, Pon A, Knox C, W. M. Interferon alfa-2b https://www.drugbank.ca/drugs/DB00105 (accessed Oct 3, 2019). https://doi.org/10.1093/nar/gkx1037.

(155) Scott, A. M.; Allison, J. P.; Wolchok, J. D. Monoclonal Antibodies in Cancer Therapy. *Cancer Immun.* **2012**, *12*, 14.

(156) Staff, M. C. Monoclonal antibody drugs for cancer: How they work https://www.mayoclinic.org/diseases-conditions/cancer/in-depth/monoclonal-antibody/art-20047808 (accessed Oct 8, 2019).

(157) Pento, J. T. Monoclonal Antibodies for the Treatment of Cancer. *Anticancer Res.* **2017**, *37* (11), 5935–5939. https://doi.org/10.21873/anticanres.12040.

(158) Gary Kobs. Using Pepsin to Prepare F(ab')2 Fragments and Determine Deuterium Exchange | Promega Connections https://www.promegaconnections.com/using-pepsin-to-prepare-fab2-fragments-and-determine-deuterium-exchange/ (accessed Oct 10, 2019).

(159) Drug Price Information https://www.drugs.com/price-guide/ (accessed Oct 14, 2019).

(160) Fookes, C. Antineoplastic Combinations https://www.drugs.com/drug-class/antineoplastic-combinations.html?condition_id=&generic=1&sort=rating&order=desc (accessed Oct 15, 2019).

(161) Studley, J. Deuterium in organic chemistry and drug development https://www.scientificupdate.com/webinar_events/deuterium-in-organic-chemistry-and-drug-discovery/20181114/ (accessed Jul 26, 2020).

(162) Studley, J. Organofluorine Chemistry Past, Present and Future; Scientific Update Ltd: Lisbon (Portugal), 2019; p 66.

(163) Reich, H. Bordwell pKa Table (Acidity in DMSO) https://www.chem.wisc.edu/areas/reich/pkatable/ (accessed Nov 3, 2019).

(164) Ranu, C. ; Sarkar, A. ; Guchhait, S. K. ; Ghosh, K.; Ranu, B. C.; Sarkar, A.; Guchhait, S. K.; Ghosh, K. Catalytic Hydrogen Transfer Reductions Using Ammonium Formate - A Review. *J. Indian Chem. Soc.* **1998**, *75*, 690–694.

(165) Tsuji, M.; Hattori, M.; Shimamoto, D. Hydrogen Production from Formic Acid Decomposition at Room Temperature: AgPd@Pd/TiO2 Core-Shell Nanocatalyst Prepared by Microwave Heating. *Chim. Oggi/Chemistry Today* **2016**, *34* (3), 55–58.

(166) Hu, C.; Pulleri, J. K.; Ting, S.-W.; Chan, K.-Y. Activity of Pd/C for Hydrogen Generation in Aqueous Formic Acid Solution. *Int. J. Hydrogen Energy* **2014**, *39* (1), 381–390. https://doi.org/10.1016/J.IJHYDENE.2013.10.067.

(167) Barakat, M.; Mahmoud, M.; Mahrous, Y. Recovery and Separation of Palladium from Spent Catalyst. *Appl. Catal. A Gen.* **2006**, *301* (2), 182–186.

(168) Edwards, J. K.; Hutchings, G. J. Palladium and Gold–Palladium Catalysts for the Direct Synthesis of Hydrogen Peroxide. *Angew. Chemie Int. Ed.* **2008**, *47* (48), 9192–9198.

(169) Sarioğlan, Ş. Recovery of Palladium from Spent Activated Carbon-Supported Palladium Catalysts. *Platin. Met. Rev.* **2013**, *57* (4), 289–296.

(170) Luche, J. L. Lanthanides in Organic Chemistry. 1. Selective 1,2 Reductions of Conjugated Ketones. *J. Am. Chem. Soc.* **1978**, *100* (7), 2226–2227. https://doi.org/10.1021/ja00475a040.

(171) Gemal, A. L.; Luche, J. L. Lanthanoids in Organic Synthesis. 6. Reduction of .Alpha.-Enones by Sodium Borohydride in the Presence of Lanthanoid Chlorides: Synthetic and Mechanistic Aspects. *J. Am. Chem. Soc.* **1981**, *103* (18), 5454–5459. https://doi.org/10.1021/ja00408a029.

(172) Ward, D. E.; Rhee, C. K. Chemoselective Reductions with Sodium Borohydride. *Can. J. Chem.* **1989**, *67* (7), 1206–1211. https://doi.org/10.1139/v89-182.

(173) Flick, E. W. *Industrial Solvents Handbook, Noyes Data Corp*, Fifth.; W.Flick, E., Ed.; Noyes data corporation: Westwood, New Yersey, U.S.A, 1991.

(174) Gmehling, J. *Azeotropic Data*; Azeotropic Data; VCH, 1994.

(175) NIST. Solubility System: Dichloromethane with Water https://srdata.nist.gov/solubility/sol_detail.aspx?goBack=Y&sysID=60_242 (accessed Nov 23, 2019).

(176) Zhao, M. M.; McNamara, J. M.; Ho, G. J.; Emerson, K. M.; Song, Z. J.; Tschaen, D. M.; Brands, K. M. J.; Dolling, U. H.; Grabowski, E. J. J.; Reider, P. J.; Cottrell, I. F.; Ashwood, M. S.; Bishop, B. C. Practical Asymmetric Synthesis of Aprepitant, a Potent Human NK-1 Receptor Antagonist, via a Stereoselective Lewis Acid-Catalyzed Trans Acetalization Reaction. *J. Org. Chem.* **2002**, *67* (19), 6743–6747. https://doi.org/10.1021/jo0203793.

(177) Brands, K. M. J.; Payack, J. F.; Rosen, J. D.; Nelson, T. D.; Candelario, A.; Huffman, M. A.; Zhao, M. M.; Li, J.; Craig, B.; Song, Z. J.; Tschaen, D. M.; Hansen, K.; Devine, P. N.; Pye, P. J.; Rossen, K.; Dormer, P. G.; Reamer, R. A.; Welch, C. J.; Mathre, D. J.; Tsou, N. N.; McNamara, J. M.; Reider, P. J. Efficient Synthesis of NK1 Receptor Antagonist Aprepitant Using a Crystallization-Induced Diastereoselective Transformation. *J. Am. Chem. Soc.* **2003**, *125* (8), 2129–2135. https://doi.org/10.1021/ja027458g.

(178) IKA. IKA Wet milling https://www.ikaprocess.com/en/Wet-milling-appl-5.html (accessed Feb 22, 2020).

(179) Manufacturing, S. Rotor stator| Schold Manufacturing https://schold.com/product/rotor-stator/ (accessed Feb 22, 2020).

(180) IKA works mixer parts, UTL 2000/5, DR 2000/5 Rotor and Stator, Generator set| PicClick https://picclick.com/IKA-Works-mixer-parts-UTL-2000-5-DR-2000-5-352498180381.html (accessed Feb 22, 2020).

(181) Biermann, C. J. *Handbook of Pulping and Papermaking*, Second.; Academic Press, 1996.

(182) Wiercigroch, E.; Szafraniec, E.; Czamara, K.; Pacia, M. Z.; Majzner, K.; Kochan, K.; Kaczor, A.; Baranska, M.; Malek, K. Raman and Infrared Spectroscopy of Carbohydrates: A Review. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* **2017**, *185*, 317–335. https://doi.org/10.1016/J.SAA.2017.05.045.

(183) DiMasi, J. A.; Grabowski, H. G.; Hansen, R. W. Innovation in the Pharmaceutical Industry: New Estimates of R&D Costs. *J. Health Econ.* **2016**, *47*, 20–33. https://doi.org/10.1016/J.JHEALECO.2016.01.012.

(184) Woodcock, J. The Concept of Pharmaceutical Quality. *Am. Pharm. Rev.* **2004**, *7*, 10–15.

(185) Lionberger, R. A.; Lee, S. L.; Lee, L.; Raw, A.; Yu, L. X. Quality by Design: Concepts for ANDAs. *AAPS J.* **2008**, *10* (2), 268–276. https://doi.org/10.1208/s12248-008-9026-7.

(186) Guideline. Pharmaceutical Development Q8 (R2). *ICH Harmon. Tripart.* **2009**, *4*.

(187) Mohammed, A. Q.; Sunkari, P. K.; Srinivas, P.; Roy, A. K. Quality by Design in Action 1: Controlling Critical Quality Attributes of an Active Pharmaceutical Ingredient. *Org. Process Res. Dev.* **2015**, *19* (11), 1634–1644. https://doi.org/10.1021/op500295a.

(188) Christine M. V. Moore. Quality by Design –FDA Lessons Learned and Challenges for International Harmonization. In *International Conference on Drug Development*; FDA: Austin, TX, 2012; pp 1–28.

(189) Kane, A. Phase-Appropriate Formulation and Process Design. *Pharm. Technol.* **2016**, *40* (1), 42–45.

(190) Guideline ICH, H. T. ICH Guideline Q9 on Quality Risk Management. In *International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use*; ICH, 2015; p 20.

(191) Stamatis, D. H. *Failure Mode and Effect Analysis : FMEA from Theory to Execution*, Second.; ASQ Quality Press: Milwaukee, 2003.

(192) Datalyzer. FMEA | Introduction to Failure Mode and Effects Analysis https://www.datalyzer.com/knowledge/fmea/ (accessed Nov 4, 2018).

(193) Joshi, G.; Joshi, H. FMEA and Alternatives v / s Enhanced Risk Assessment Mechanism. *Int. J. Comput. Appl.* **2014**, *93* (14).

(194) Rakesh A. Chaudhari, Ashok P. Pingle, Chetan S. Chaudhari, Chetan Yewale, K. P. QbD an Emerging Paradigm for Development of Pharmaceuticals. *Int. J. Pharm.* **2014**, *4* (2), 138–146.

(195) Maguire, J.; Peng, D. How to Identify Critical Quality Attributes and Critical Process Parameters. In *Office of Process and Facility (OPF) OPQ/CDER/FDA, FDA/PQRI 2nd conference, North Bethesda, MD*; 2015.

(196) Ganzer, W. P.; Materna, J. A.; Mitchell, M. B.; Wall, L. K. Current Thoughts on Critical Process Parameters and API Synthesis. **2005**, *29*, 46–66.

(197) Stangler, D. T. What to Control? CQAs and CPPs. In *BWP Workshop on Setting Specifications*; European Generic medicines Association: London, 2011; pp 1–19.

(198) Sparks, C. Solid-Liquid Separations - ppt video online download http://slideplayer.com/slide/4537232/ (accessed Sep 2, 2019).

(199) Shiel, W. C. J. Medical Definition of Body surface area https://www.medicinenet.com/script/main/art.asp?articlekey=39851 (accessed Sep 15, 2019).

ANNEX 1

PROCESS QUALITY RISK ASSESSMENT

INDUSTRIAL PhD THESIS

PROCESS DEVELOPMENT FOR THE SYNTHESIS AT INDUSTRIAL SCALE OF ACTIVE PHARMACEUTICAL INGREDIENTS

Annex 1

1. Process quality risk assessment (PQRA)

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