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Coordinatore: Chiar.mo Prof. Mauro Freccero

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Tutore

Chiar.mo Prof. Mauro Freccero

Tutore Aziendale

Dr Alessandro Donadelli

Tesi di Dottorato di

Giovanni Fogliato

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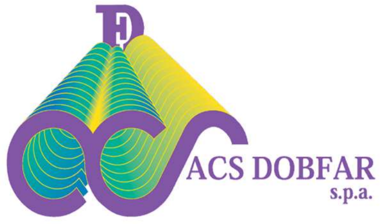

This thesis is the result of an executive PhD which relevant experimental work has been performed at R&D Laboratories of ACS-DOBFAR SpA.

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Abstract

Carbapenems are highly effective β-lactam antibiotics, typically administered by intravenous infusion in a hospital setting. Ertapenem (**1**) is a prominent member of the carbapenem class of antibiotics and is used broadly.

Figure 1: Ertapenem Sodium structure

Ertapenem was discovered at Zeneca Pharmaceuticals (now AstraZeneca) and was licenced to Merck. Merck developed Ertapenem as medicine, which is on the market under the trade name Invanz®, since 2001. Zeneca/Merck patents expired in 2017, therefore Ertapenem can be developed for generic markets.

Carbapenems are of total synthesis origin and their preparation is a long synthetic chain which requires many different types of chemical reactions.

Since Meropenem synthesis is already in place on commercial scale at ACS-DOBFAR and Meropenem and Ertapenem share the same 1-β-methylcarbapenem core, the development of Ertapenem synthesis exploited this similarity.

For that reason, the advanced intermediate enolphosphate **2** produced over Meropenem synthesis was employed as a starting point for Ertapenem manufacturing process, see figure 2.

*Figure 2: Ertapenem synthesis from advanced intermediate **2***

Ertapenem synthesis has been therefore developed starting from advanced intermediate **2**.

Ertapenem analytical method was developed starting from available information retrieved in literature. Identification of impurities has been done by LC-MS studies and comparison with literature. A few impurities were isolated or synthetized and full characterization was performed. Setting specification was achieved by comparison of commercial product Invanz®, sourced from the market.

Few scale up experiences were necessary to address a final robust manufacturing process that consistently delivers Ertapenem Sodium with suitable quality. Developed manufacturing process is constituted of a telescopic process that starts from advanced intermediate **2** and produces Ertapenem Sodium crude. This latter by a recrystallization leads to Ertapenem Sodium.

Ertapenem sodium and related compounds have shown to be particularly unstable and such property challenged the manufacturing process and it was necessary to achieve a good final powder morphology in order to get a quick filtration and drying, even if this latter action reduced the process yield.

The following table resumes the evolution of manufacturing process over development:

Table 1. Ertapenem Sodium Crude manufacturing process evolution

STAGE	PROCESS A	PROCESS B	PROCESS C	PROCESS D
Coupling to Ertapenem TMSAllyl (4)	ACN/-50°C	Same	Same	Same
Hydrolysis to ErtapenemAllyl (5)	Aq. HCl	Same	Same	Same
Work-up	Carbamate and HP20 column	Neither carbamate nor column	DCM solution freezing to Water <1.0%	Same
Isolation ErtapenemAllyl (5)	Precipitation from aqueous solution	No, telescoped in next stage	Same	Same
Deblocking	DCM/Pdtetrakis/N-MeAniline	Same	Same	Same
Work-up	Carbamate and HP20 column	Same	Carbamate and no column	Same
Isolation Ertapenem Sodium Crude (1)	H_2O:MeOH:IPA	Same	H_2O:MeOH:1-Propanol	H_2O:MeOH:1-Propanol (different operations order)
Overall yield	33%	49%	49%	40%
SCALE-UP	Pilot batch up to ErtapenemAllyl	Pilot batch	Pilot batch (failure)	Kilo-LAB

A robust manufacturing process D, suitable for commercial manufacturing on 10-100 kg scale was finally outlined. Material obtained from kilogram scale laboratory manufacturing employing developed process, produced Ertapenem Sodium of desired quality.

CHAPTER 1 : Introduction

1.1 β-Lactam Antibiotics

Antibiotics are a type of antimicrobial substances active against bacteria and are the most important one of antibacterial agents for fighting bacterial infections. Antibiotics are commonly classified based on their mechanism of action, chemical structure, or spectrum of activity. Most antibacterial antibiotics target bacterial functions or growth processes. There are many classes of antibiotics, whose the main are: Glycopeptides, β-lactams, Macrolides, Tetracyclines, Aminoglycosides and Quinolones. Among the various antibiotic classes, β-lactams are arguably the most successful class of compounds in the history of antibiotics therapies, due mostly to their long-standing record of safety and efficacy[1].

β-lactam antibiotics are bactericidal, and act by inhibiting the biosynthesis of the peptidoglycan layer of bacterial cell wall. Peptidoglycan layer is in fact important for cell wall structural integrity. The final transpeptidation step in the synthesis of the peptidoglycan is facilitated by transpeptidase enzyme. β-lactams antibiotics share a basic chemical structure that includes a three-carbon, one-nitrogen cyclic amido structure known as β-lactam ring. The structural similarity between β-lactam antibiotics and D-alanyl-D-alanine facilitates their binding to the active site of transpeptidase enzyme. The β-lactam nucleus of the molecule irreversibly binds to the Ser_{403} residue of the transpeptidase enzyme active site (Figure 3).

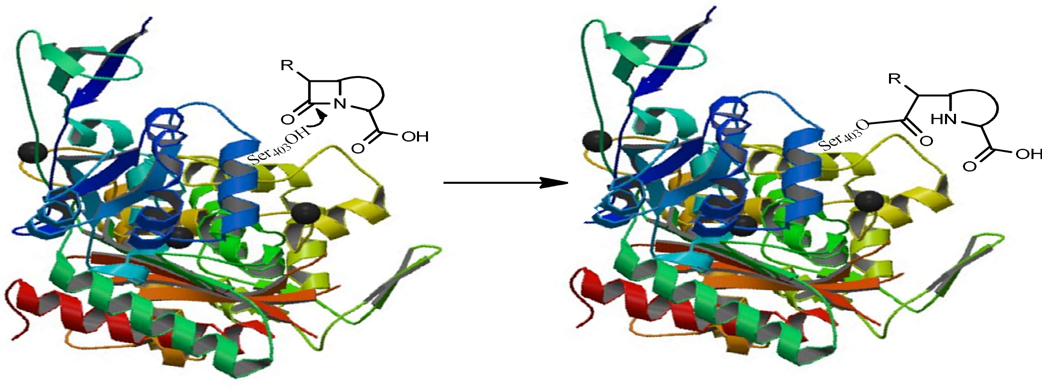

Figure 3: Mechanism of action of a beta lactam antibiotic

First β-lactam antibiotic discovered, penicillin, was isolated by Alexander Fleming in 1929, from *Penicillium chrysogenum*[2]. After such discovery penicillin was made available for widespread use, since year 1940.

β-lactam antibiotics are nowadays categorized into 5 subclasses:

1. **Penicillins**
2. **Cephalosporins**
3. **Penems**
4. **Carbacephem**
5. **Monobactams**

β-lactam antibiotics and in particular penicillins, are known to be highly sensitizing compounds, since they cause severe allergic reactions in few subjects. Allergic reactions constitute the most common and significant side-effect of penicillins. Allergic reactions are assumed to be the result of an antigen-antibody-sensitized human cell interaction.

The categorization was therefore introduced by regulatory agencies like FDA since strict guidelines were emitted[3] for β-lactams manufacturing. FDA guidance recommends that the manufacturing of each sensitizing β-lactam subclass (from the 5 above) should be structurally isolated from areas in the facility in which other products or another beta-lactam subclass product are manufactured to avoid cross contamination and reduce the risk of allergic reactions.

1.1.1 Penicillins

R
H
N
S
O
N
O
COOH

Figure 4: Penicillin general structure

As already mentioned, penicillins were the first β-lactam compounds to be discovered and used as antimicrobial drugs. They are structurally constituted by β-lactam ring fused with thiazolidine five-member ring, see figure 4. Penicillins differ from each other for the nature of R acid residue. Penicillins are produced by fermentation, with which Penicillin G (**6**) is produced. From Pen G, 6-APA (**7**) can be obtained by amide hydrolysis which can be performed either chemically or enzymatically. From 6-APA (**7**) all Penicillins can be obtained by re-acylation with suitable acid residue R, see figure 5.

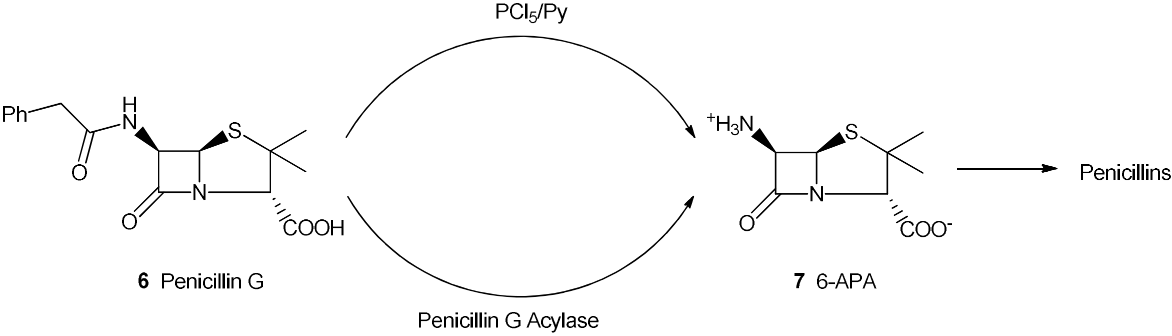

Figure 5: General Penicillin synthetic pathway

Amoxicillin (**8**), Ampicillin (**9**) and Piperacillin (**10**) are the main blockbusters in this subclass, see figure 6.

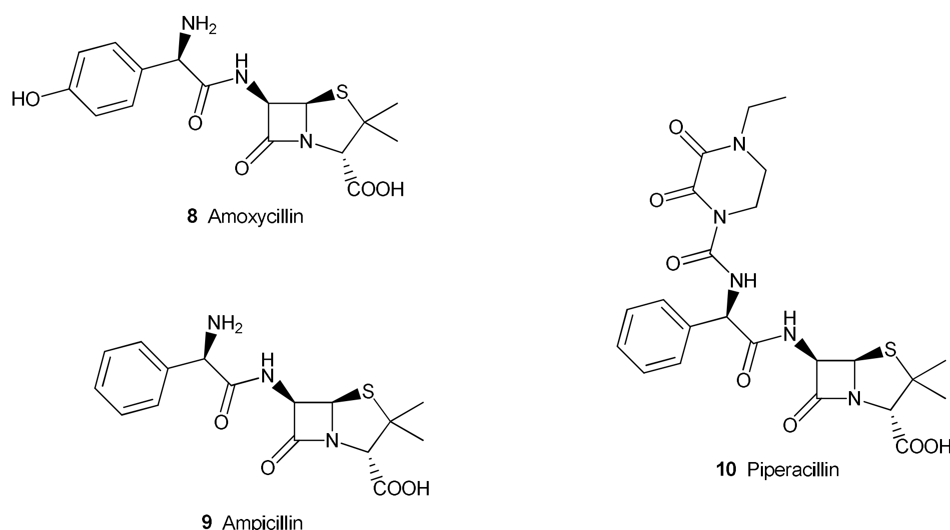

Figure 6: Penicillin blockbusters

New Updates

There are no new chemical entities under development in this subclass.

1.1.2 Cephalosporins

Figure 7: Cephalosporins general structure

Cephalosporins are structurally constituted by β-lactam ring fused with dihydrothiazine six-member ring, see figure 7, and were first discovered by Brotzu in 1948[4]. Cephalosporins differ from each other for the nature of R_1 and R_2.

Cephalosporins can be produced from Cehalosporin C (**11**) which is obtained by fermentation. From Cehalosporin C (**11**), 7-ACA (**13**) can be obtained either chemically or by enzymatic steps, see figure 8.

Figure 8: General Cephalosporins synthetic pathway through 7-ACA

From 7-ACA (**13**) different cephalosporins can be obtained in two steps which involve the introduction of the two substituents R_1 and R_2. Typically, only cephalosporins bearing a CH_2X substituent in position 3 are produced through this strategy. Ceftriaxone (**14**), Ceftazidime (**15**), Cefepime (**16**) and Cefuroxime (**17**) are between the main cephalosporins produced through 7-ACA way, see figure 9.

Ceftobiprole Medocaril (**18**) is a recently approved fourth generation cephalosporin which is also produced from 7-ACA by a multistep synthesis, see figure 9.

Figure 9: Cephalosporins blockbusters from 7-ACA

Cephalosporins may also be produced from penicillins, exploiting the thermal rearrangement of sulfoxides discovered by Morrin at Ely-Lilly[5], in which an intramolecular proton transfer accompanies elimination to form a new carbon-carbon double bond. Therefore, penicillin sulfoxides **19** by heating generate intermediate sulfenic derivative **20** which can lead to different compounds depending from the trapping reagent which is used. Typically, penicillin sulfoxides and trapping reagent are heated into refluxing toluene to lead the desired compound as per figure 10.

Figure 10: Penicillin sulfoxide rearrangements

Compound **21** is the precursor of 7-ADCA (**27**) from where cephalosporins such as cephalexin (**28**) and cefadroxil (**29**) are obtained, see figure 11.

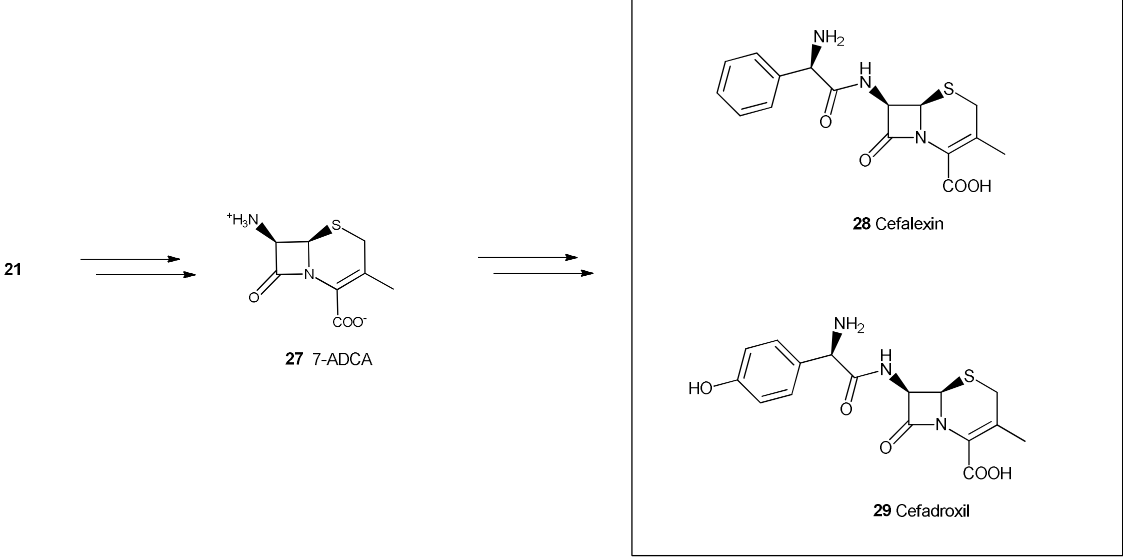

Figure 11: Cephalosporins from 7-ADCA

Compound **22** is the precursor of GCLE (**30**) from where cephalosporins such as Ceftolozane (**31**) and Ceftiderocol (**32**) are produced, see figure 12.

Figure 12: Cephalosporins from GCLE

Compound **23** is the precursor of GHYH (**33**) from where cephalosporins such as Ceftibuten (**35**) and Ceftaroline Fosamil (**34**) are produced, see figure 13.

Figure 13: Cephalosporins from GHYH

Compound **25** is the precursor of Cefaclor and compound **24** can also lead to GCLE (**30**).

New Updates

1) Ceftaroline Fosamil[6] (**34**) a fifth generation cephalosporin active against *Methicillin Resistant Staphylococcus Aureus* (*MRSA*) bacteria, which was approved in 2010 in US and 2012 in EU

2) Ceftobiprole Medocaril[6] (**18**) from fifth generation active against *MRSA* which was approved in 2013 for EU market and it is now under development for US market.

3) Ceftolozane[7] (**31**) a fifth-generation cephalosporin, active against *Pseudomonas Aeruginosa* which was approved in 2014 US and 2015 EU markets.

4) Ceftiderocol[8] (**32**), is under development (phase 3) by Shionogi. Ceftiderocol is interesting since it is the first siderophore cephalosporin. Siderophore linked to an antibiotic is a very innovative[9] approach which works on active transport to facilitate the delivery of antibiotic through the barrier of gram negative bacteria membrane. In fact, to develop an infection, bacteria require iron which is essential for physiological processes. Thus, bacteria synthesize and excrete iron chelating agents called siderophores that bind ferric iron with high affinity. The resulting complex is then recognized by specific outer membrane proteins that initiate internalization by an active transport of the ferric siderophore. Therefore, a "Trojan horse antibiotic" like Ceftiderocol consists of a siderophore directly attached to a β-lactam antibiotic. The presence of siderophore allows the antibiotic to easily pass through the bacteria membrane and therefore increase antibacterial activity.

1.1.3 Carbacephems

NH_2
H
N
O
O
N
Cl
COOH

36 Loracarbef

Figure 14: Loracarbef a carbacephem

Carbacephems are structurally constituted by β-lactam ring fused with tetrahydropyridine six-member ring, see figure 14, and are of total synthesis origin. It is practically an empty/dead class in which there is only Loracarbef (**36**) developed by Ely Lilly[10] which had a poor success on the market.

1.1.4 Monobactams

37 Aztreonam

Figure 15: Aztreonam a monobactam

Monobactams are structurally constituted by a monocyclic β-lactam ring, see figure 15, and were first discovered by Sykes and Imada in 1981[11]. This class is also constituted by a single approved drug, Aztreonam (**37**). However, many companies are strongly trying to develop novel monobactam antibiotics, therefore the class could be expected to be more populated in few years. However, development of monobactam APIs is problematic due to the lack of regulated facilities where to produce API under GMP for clinical use.

1.1.5 Penems

For this class all regulatory agencies, FDA in primis incorrectly defines penem class the class in which carbapenem are categorized. In reality penem and carbapenem have two chemically different structures.

Penem

Figure 16: Penem general structure

Penems are structurally constituted by β-lactam ring fused with a thiazoline five member ring, see figure 16. Penems differ from each other for the structural moiety of R. None of the penem is currently approved on regulated markets other than Faropenem (**38**) in Japan, see figure 17.

38 Faropenem

39 Sulopenem

40 Sulopenem Etzadroxil

Figure 17: Penem approved and under development

New Updates

Sulopenem[12] (**39**) and Sulopenem etzadroxil (**40**), see figure 17, are currently in development (phase 3) for three indications. Such development pursued by Iterum Pharmaceuticals is particularly interesting since involves two parallel trains of development: Sulopenem (**39**) for hospital parenteral administration and Sulopenem etzadroxil (**40**), for oral administration. It is in fact a common practice

to start treatment of a serious infection in hospital with a carbapenem since they are superior vs other subclasses, however when acute phase of infection is overcome patient is discharged and physicians are obliged to change the antibiotic for post-acute treatment, since there are no oral carbapenem on the market up to date. The Iterum development program targets to cover this gap. Therefore, hospital treatment is designed to start with Sulopenem (**39**) and home treatment is designed to continue with Sulopenem etzadroxil (**40**), which is a prodrug delivering same API Sulopenem (**39**) in vivo.

1.1.6 Carbapenems

Carbapenem

Figure 18: Carbapenem general structure

Carbapenems are structurally constituted by β-lactam ring fused with a pyrroline five-member ring, see figure 18, and were first discovered by Brown and co-workers in 1976[13]. Different carbapenems differ only for the chemical nature of R1 and R2, R1 could be H or Me.

Of the several subclasses of β-lactams, the carbapenems possess the broadest spectrum of activity and exhibit better β-lactamase stability compared to other β-lactams[14]. These exclusive properties have resulted in carbapenems often being used as antibiotics of last resort, particularly for the treatment of serious and life-threatening Gram-positive and Gram negative infections known or suspected to be caused by multi-drug resistant (MDR) bacteria[15].

The first discovered carbapenem antibiotic was thienamycin [16,17] (**41**), a naturally derived product of *Streptomyces cattleya*. Thienamycin (**41**) is poorly stable therefore it has never been developed as a drug. First marketed carbapenem was Imipenem[18] (**42**) (1980), discovered by Merck, which is a more stable version of thienamycin (**41**). In imipenem (**42**) in fact the free primary amino group of thienamycin (**41**) is masked by an amidino group which provides an enhanced stability vs thienamycin.

In vivo, imipenem (**42**) is hydrolyzed by renal dehydropeptidase (DHP). Cilastatin is an inhibitor of DHP that stabilizes imipenem in vivo by preventing its hydrolysis. Subsequently, it was discovered[19] that introduction of a β-methyl group at C-1 of carbapenem renders them resistant to DHP while retaining the antibiotic activity and spectrum. Because of these useful properties, all subsequent carbapenem antibiotics, excluding panipenem (**43**), that were developed contain a β-methyl group at C-1.

There have been six carbapenem antibiotics approved after imipenem, figure 19. Panipenem (**43**) was approved in Japan in 1993, and that was followed by the U.S. FDA approval of Meropenem (**44**) (1996). Meropenem (**44**) is on the World Health Organization's list of essential medicines, the most effective and safe medicines needed in a health system. Biapenem (**45**) was approved only in Japan (2001). Ertapenem (**1**) was approved by U.S. FDA in 2001, followed by Doripenem (**46**) which was approved by the U.S. FDA 2007. Tebipenem pivoxyl (**47**), was the last carbapenem antibiotic that was approved by Japanese regulators in 2009.

Despite that thienamycin (**41**) was initially obtained from natural origin, all marketed carbapenems are produced through total synthesis.

New Updates

Tebipenem pivoxyl (**47**), see figure 19, is currently in development (phase 3) in US and EU markets.

41 Thienamycin

42 Imipenem

43 Panipenem

44 Meropenem

45 Biapenem

1 Ertapenem

46 Doripenem

47 Tebipenem Pivoxyl

Figure 19: Carbapenem antibiotics

1.2 Antimicrobial Resistance (AMR) and β-Lactamase Inhibitors (BLIs)

Antimicrobial resistance (AMR) is the ability of a microorganism like bacteria to stop an antimicrobial substance, like an antibiotic, from working against it. As a result, standard treatments become ineffective, infections persist and may spread to others. Antimicrobial resistance occurs naturally over time, usually through genetic changes. However, the misuse and overuse of antimicrobials is accelerating this process. In many places, antibiotics are overused and misused in people and animals, and often given without professional oversight. Examples of misuse include when they are taken by people with viral infections like colds and flu, and when they are given as growth promoters in animals or used to prevent diseases in healthy animals. Antimicrobial resistant-microbes are found in people, animals, food, and the environment (in water, soil and air). They can spread between people and animals, including from food of animal origin, and from person to person. Poor infection control, inadequate sanitary conditions and inappropriate food-handling encourage the spread of antimicrobial resistance.

Resistance can occur by multiple mechanisms, however resistance to β-lactam antibiotics is mediated primarily through the expression of β-lactamases, which degrade the β-lactam[20]. β-lactamases are enzymes produced by both Gram-positive and Gram-negative bacteria that hydrolyze the β-lactam amide[21].

β-Lactamase-mediated resistance can in principle be addressed through the development of novel β-lactams with increased stability against β-lactamases or by co-dosing with a β-lactamase inhibitor (BLI). Beta-lactamase inhibitors are a class of compounds that block the activity of beta-lactamase enzymes, irreversibly binding to enzyme active site, preventing the degradation of beta-lactam antibiotics and restoring therefore the API antibiotic activity.

Penicillins are particularly sensible to β-lactamases therefore penicillin were the first β-lactams that were combined with a Beta Lactamase Inhibitor (BLI). The first generation BLIs which were used in combination with penicillins were: Clavulanic Acid (**48**), Sulbactam (**49**) and Tazobactam (**50**), see figure 20.

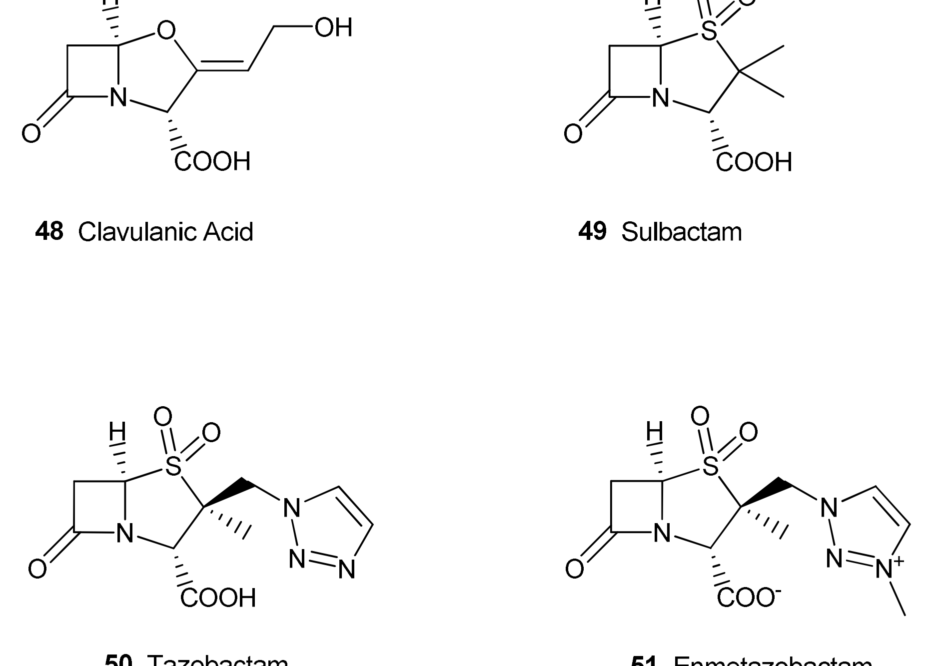

Figure 20: First generation β-lactamase inhibitors

The combinations Amoxycillin (**8**)/Clavulanic Acid (**48**) (Augmentin®), Ampicillin (**9**)/Sulbactam (**49**) (Unasyn®) and Piperacillin (**10**)/Tazobatam (**50**) (Tazocin®) quickly became some of the most widely prescribed antibacterial agents.

Antibiotic resistance is an issue of great concern that has attracted the attention of health agencies, media, and global leaders. It has a high social and economic burden, and it has been estimated that failure to treat drug-resistant infections, in 2009 had a cost of $20 billion in the United States[22]

More recently, there has been renewed interest in the field of β-lactamase inhibitor discovery to counter the threat from newer β-lactamases, which are not inhibited by clavulanic acid or tazobactam, such as the extended spectrum β-lactamases (ESBLs) and carbapenemases.

Extended spectrum serine β-lactamases (ESBLs) are spreading and cause resistance to penicillins, cephalosporins, and monobactams. Physicians are therefore relying increasingly on carbapenems, which are stable to ESBLs, but this now appears to be driving the global spread of organisms producing carbapenemases[23].

This has led to the development of two new synthetic classes of inhibitors[24], namely the diazabicyclooctane (DBO) series, as exemplified by Avibactam (**52**) and cyclic boronates exemplified by Vaborbactam[25] (**57**), see figures 21, 22.

52 Avibactam **53** Relebactam **54** Nacubactam

55 Zidebactam **56** Durlobactam

Figure 21: Novel generation DBO β-lactamase inhibitors

57 Vaborbactam **58** VNRX-5133

59 QPX-7728

Figure 22: Novel generation cyclic boronates β-lactamase inhibitors

New Updates

As far as novel combinations appeared to the market, the recent outcome are:

1) Zerbaxa® which is the combination of Ceftolozane (**26**) with Tazobactam (**50**), which was approved by US FDA in 2014. Zerbaxa is the combination of a novel cephalosporin with a first generation BLI. Zerbaxa represents the first combination of a cephalosporin + BLI.
2) Avycaz® which is the combination of Ceftazidime (**15**) with Avibactam (**52**), which was approved by US FDA in 2015. Avycaz is the combination of a known cephalosporin with a novel BLI.
3) Vabomere® which is the combination of Meropenem (**44**) with Vaborbactam (**57**), which was approved by US FDA in 2017. Vabomere is the combination of a known carbapenem with a novel BLI. Vabomere represents the first combination of a carbapenem + BLI.
4) Recarbrio® which is the combination of Imipenem (**42**) with Cilastatin and Relebactam (**53**), which was approved by US FDA in 2019. Recarbrio is also the combination of a known carbapenem with a novel BLI.
5) Cefepime (**16**) + Zidebactam (**55**) which is in clinical Phase 1 development
6) Meropenem (**44**) + Nacubactam (**54**) which is in clinical Phase 1 development
7) Sulbactam (**49**) + Durlobactam (**56**) which is in clinical Phase 2 development
8) Cefepime (**16**) + Enmetazobactam (**51**) which is in clinical Phase 3 development
9) Cefepime (**16**) + VNRX-5133 (**58**) which is in clinical Phase 1 development
10) QPX-7728 (**59**) has been recently presented on June, 23rd, 2019 at Interscience Conference of Antimicrobial Agents and Chemotherapy (ICAAC)[26], to be an ultrabroad spectrum inhibitor of Serino and Metallo Beta-lactamases (Class B). It is remarkable important announcement since Metallo Beta-lactamases represents currently the challenge of new antibiotics development and up to date still there are no combinations available on the market that can inhibit emerging Metallo beta-lactamases. Therefore, it could be expected that QPX-7728 will soon start a Drug Development program in combination with a β-lactam antibiotic.

This above extensive list shows as now antibiotic development is focused on novel β-lactam inhibitors and their combination with either new chemical entities and already existing antibiotics.

Aim of the Thesis

As noted, carbapenems are highly effective antibiotics, typically administered by intravenous infusion in a hospital setting. Ertapenem (**1**) is a prominent member of the carbapenem class of antibiotics and is used broadly. Importantly, ertapenem is the only carbapenem antibiotic administered once daily[15,27,28], compared to other carbapenems which typically require intravenous infusions every 6-8 hours. This unique feature of Ertapenem is also important in view of hospitalization costs, in fact when the acute phase of infection is reduced the patient is sent home and called daily for a single antibiotic infusion. Ertapenem was discovered at Zeneca Pharmaceuticals (now AstraZeneca) and was licenced to Merck. Merck developed Ertapenem as medicine and it is on the market under the trade name Invanz® since 2001. Zeneca/Merck patents were expired in 2017 therefore Ertapenem can be developed for generic markets.

The key of this thesis is therefore analytical and manufacturing process development with the purpose to outline a robust and scalable process suitable for commercial manufacturing on 10-100 kg scale. The desired output of this project is therefore Ertapenem Manufacturing Process applicable on industrial scale, on which a registration manufacturing campaign can be smoothly executed. Then upon submission of a relevant dossier and its approval by regulatory authorities generic Ertapenem can be launched into the market and Ertapenem can be manufactured on routine basis at ACS-DOBFAR. The stages involved in process development to reach the target to outline a robust process require that once the main parameters of a chemical reaction are outilined, a long list of activities have to take place. A comprehensive process understanding is fundamental and particularly the knowledge of the link between impurities formation vs process parameters is crucial. Process development experiments typically occours at 20-30 g scale, which are followed by 3/4 DEMO batches which are laboratory trials performed at increased scale (usually in the range of 100 g), run with the purpose to test a first scale-up and reproducibility of the process. An intermediate scale up on a kilogram scale manufacturing may also take place, always on laboratory, otherwise the natural pathforward of process development, is a pilot scale, where a few kilograms manufacturing can be achieved on equipments which are very similar to those employed in a commercial scale facility.

The core structure of ertapenem as other β-methyl antibiotics, including Meropenem (**44**), is common. They are differentiated only by the structure of their side chain at C-2. Total synthesis of carbapenem

is a long synthetic chain which requires many different types of chemical reactions, therefore the first challenge in carbapenen synthesis is the choice of carboxyl protecting group. β-lactam ring is instable to basic pH and in particular carbapenem are also poorly stable in acidic conditions. Such restrictions limit the choice of carboxyl protecting group to those that can be removed in mild conditions. Therefore, the most employed protecting group in carbapenem synthesis are ParaNitroBenzyl ester (PNB) and Allyl ester. In fact the first can be removed by a final hydrogenolysis and the second by a Pd(0) mediated hydrolysis, both of them operate in very mild conditions. Every carbapenem synthesis starts from a common building block 4-Acetoxyazetidinone, called AOSA (**60**), which is also obtained by chemical synthesis, see figure 23.

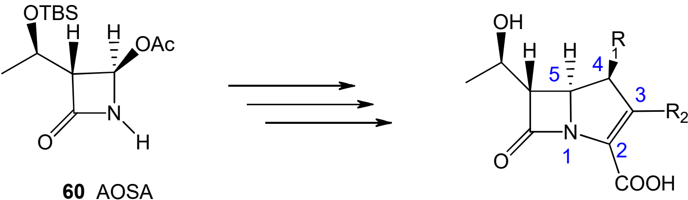

Figure 23: Carbapenems starting material

From AOSA (**60**) to form carbapenem skeleton there are two known approaches:

1) Merck way which employs carbene insertion to form nitrogen (1) carbon (2) bond of 5-member ring[19,29], see figure 24.

Figure 24: Merck disconnection approach

2) Dieckmann condensation to form carbon (2) carbon (3) double bond of 5-member ring[30,31], see figure 25

Figure 25: Dieckmann disconnection approach

Ertapenem synthesis has been developed by Merck and there are many publications that describe Ertapenem synthesis through PNB route and carbene insertion approach[19, 32]. On other hand Meropenem synthesis is already in place at commercial scale in ACS-DOBFAR and follows the allyl route and Dieckmann condensation approach[31]. Both carbene insertion approach and Dieckmann condensation approaches have in common a versatile enolphosphates **2, 61** which can be used to access to different carbapenems:

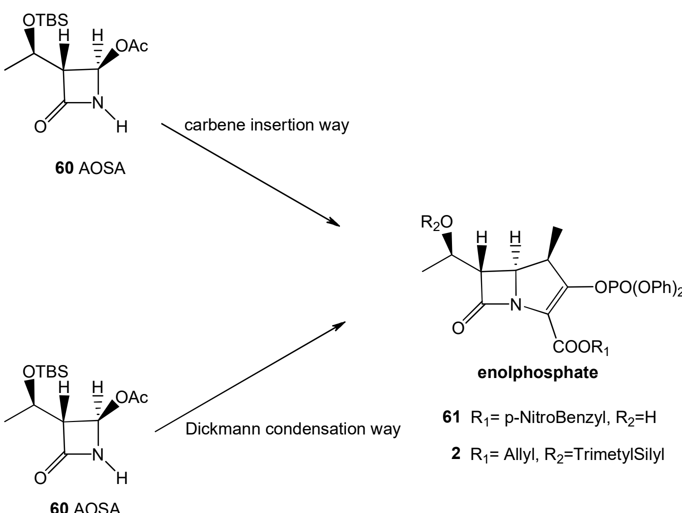

Figure 26: Carbapenem enolphosphate a versatile intermediate

Enolphosphates **2, 61** are particular advanced intermediates from where different carbapenems can be easily prepared in few synthetic steps.

For instance, Meropenem (**44**) is prepared from enolphosphate allyl protected **2** in 3 steps:

Figure 27: Meropenem synthesis from enolphosphate ***2***

Ertapenem is prepared by Merck from enolphosphate PNB protected **61** in 2 steps:

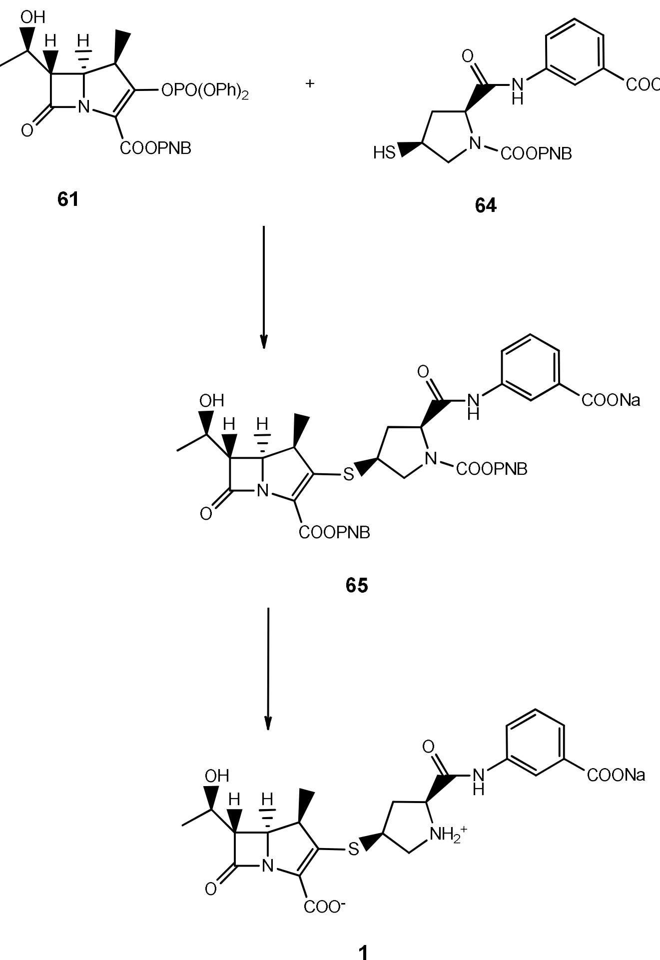

Figure 28: Ertapenem synthesis from enolphosphate ***61***

Enolphosphate PNB **61** is a crystalline solid, therefore it can be isolated as a solid intermediate. On other hand enolphosphate allyl ester **2** is not isolated and is available in solution as an in-process intermediate.

Differently from what described by Merck, ACS-DOBFAR is going to develop Ertapenem manufacturing process through the allyl route for the following reasons:

1) Enolphosphate allyl protected **2** is already routinely manufactured at ACS-DOBFAR
2) PNB route requires hydrogenation in final step and ACS-DOBFAR does not have hydrogenation capability in its facilities.

CHAPTER 2 : Analytical Development

2.1 Related Substances HPLC Test Method Development

2.1.1 Test Method Development

Figure 29. Drug substance molecular structure

MERCK and Co.Inc. is the inventor of Ertapenem drug product which is sold as Invanz® trade name, therefore Invanz is the Reference Listed Drug (RLD) product available on the market, containing Ertapenem Sodium drug substance **(1)**.

Currently there is neither EP nor USP analytical monograph for Ertapenem sodium. Therefore, starting from scientific literature[33], a suitable HPLC analytical method for related substances has been developed. In the HPLC method developed by Merck, the mobile phase A was only phosphate buffer solution at pH 8.0 and the mobile phase B was only acetonitrile. It is known that the use of sole aqueous solutions may damage the shelf life of column and the sole use of organic phase such as acetonitrile may result in irregular pressure in HPLC pump. In order to avoid these complications, the mobile phases were changed keeping the gradient profile unchanged in order to maintain the same solvent strength and to have similar relative retention times and same order of elution of main impurities.

Finally, an appropriate column has been selected in order to have a higher robustness at pH 8.0. Two columns have been evaluated:

- Inertsil phenyl 250 x 4.6 mm, 5 µm (reported in literature[33])

- Kromasil Eternity Phenylhexyl 250 x 4.6 mm, 5µm

Inertsil phenyl stationary phase is constituted of silica gel functionalized with phenyl groups (phenylsilane).

On other hand Kromasil stationary phase is from same USP category as Interstil and is constituted by silica gel functionalized with phenylhexyl groups (phenylhexylsilane).

For detailed information about analytical test methods, see experimental section.

Kromasil Eternity Phenylhexyl has showed higher robustness at pH 8.0 since provided better reproducibility of the analysis over time. Kromasil Eternity Phenylhexyl column has therefore been selected.

The following lot of Ertapenem sodium has been used:
- Ertapenem sodium batch 201211003 purchased from Zhejiang Hisoar Pharmaceutical Co., Ltd, China.

The following lots of Invanz have been used:
- Invanz® lot 0928770
- Invanz® lot 2049750

In Figure 30 is reported the pattern of impurity profile reported in literature[33]. In figures 31 and 32 the impurity profile of Invanz is reported respectively with Inertsil phenyl and Kromasil Eternity phenylhexyl column.

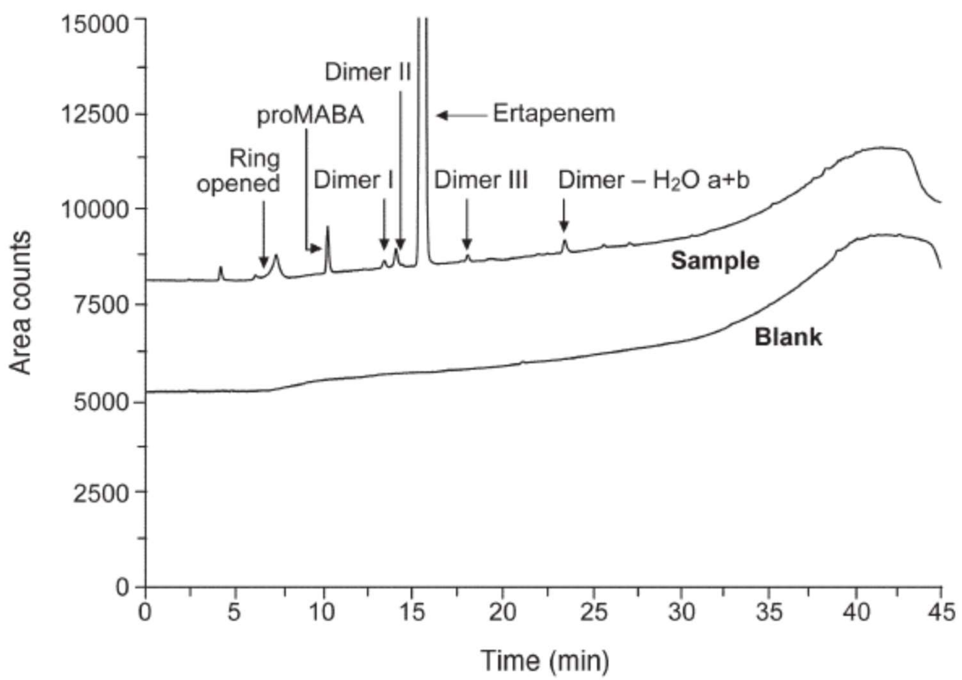

Figure 30. Invanz® typical impurity profile from literature[33], Oxford University Press license number 4673750764708

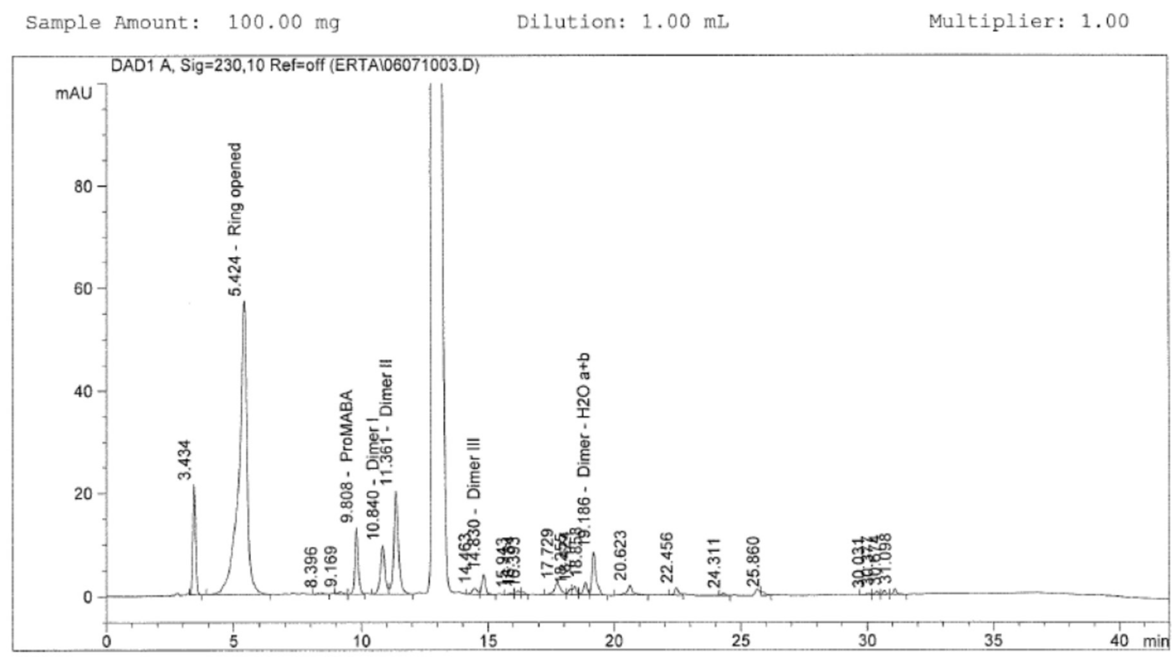

Figure 31. Impurity profile of Invanz lot 0928770 with Inertsil phenyl, see section 4.1.1

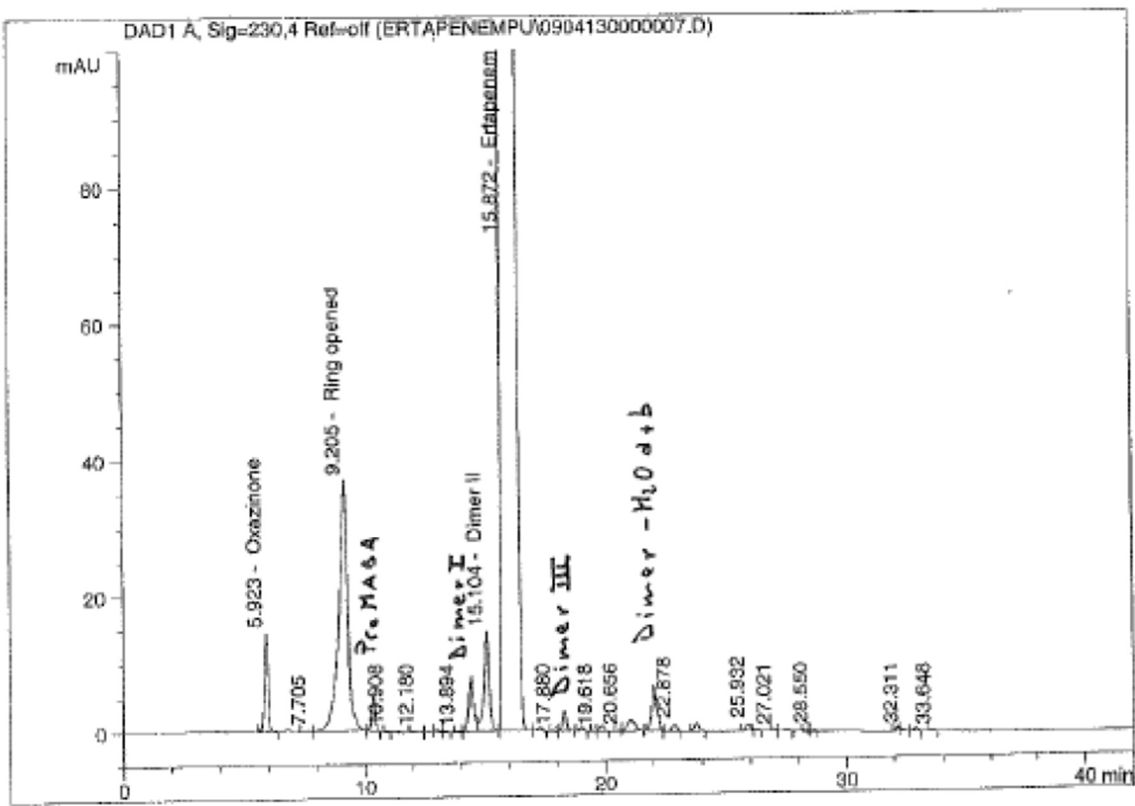

Figure 32. Impurity profile of Invanz lot 2049750 with Kromasil Eternity Phenylhexyl, see section 4.1.2

The comparison between figures 30, 31 and 32 proved that the same pattern of impurity profile of Invanz have been maintained and then a peak attribution of the impurities was possible in association with a LC MS investigation.

It can be noticed that the impurity profile reported in literature, figure 30, lacks of the first impurity which is on the contrary retrieved in figures 31 and 32. This discrepancy can be explained by the fact that in figure 30 is reported the impurity profile of Ertapenem Drug Substance and in figures 31 and 32 are reported the impurity profile of commercial Invanz Drug Product. In fact as explained later first impurity observed in figures 31 and 32 has been identified as oxazinone (**66**), which is an impurity which is formed in the step from Drug Substance to Drug Product.

Therefore, the resolution between peaks is maintained and is adequate for the purpose.

Table 2. Straightforward comparison between HPLC charts in figure 31 and figure 32

Elution Order	Inertsil column[a] Retention time	Kromasil column[b] Retention time	Peak Identification
1	3.4 min	5.9 min	Oxazinone (**66**)
2	5.4 min	9.2 min	Ring Opened (**67**)
3	9.8 min	10.9 min	ProMaba (**68**)
4	10.8 min	14.5 min	Dimer I (**69**)
5	11.4 min	15.1 min	Dimer II (**70**)
6	13.0 min	15.9 min	Ertapenem (**1**)
7	14.8 min	18.3 min	Dimer III (**71**)
8	17.7 min	21.0 min	Unknown
9	19.2 min	22.0 min	Dehydrodimer a+b (**72**)+(**73**)

[a]*From HPLC chart shown in figure 31.* [b]*From HPLC chart shown in figure 32*

As listed in table 2, the Kromasil column retention times of all the impurities are systematically higher than those attained by Inertsil column.

2.1.2 Impurities Identification

In order to confirm the impurity identification, a couple of LC MS investigations have been performed.

2.1.2.1 First LC-MS Investigation

A first preliminary LC MS study has been performed on Invanz (RLD) lot 0928770 using Inertsil phenyl as column for HPLC method for related substances and for LC MS method, see section 4.1.3. The peaks of eight impurities found in Invanz were collected (Figure 33) and injected into LC-MS method and the molecular mass was determined. Table 3 summarizes the data obtained and the proposed structures.

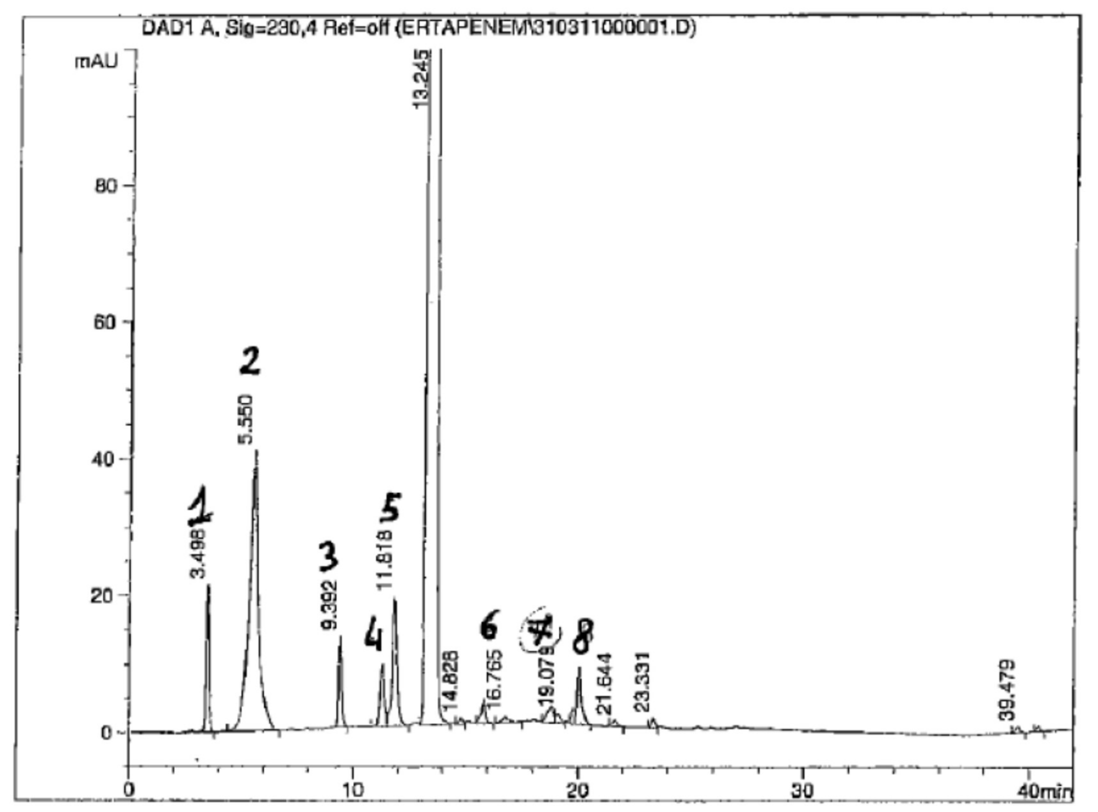

Figure 33. Impurities collected from Inertsil method and injected in LC-MS.

Table 3. Mass determination of impurities Ertapenem detected with Inertsil method

Peak N.	Retention time	m/z $[M+H]^+$	Proposal Impurity
1	3.50 min.	520	Oxazinone **66**
2	5.55 min.	494	Ring Opened **67**
3	9.39 min.	235	Pro MABA **68**
4	11.27 min	951	Dimer I **69**

Peak N.	Retention time	m/z $[M+H]^+$	Proposal Impurity
5	11.82 min	951	Dimer II **70**
6	15.82 min	951	Dimer III **71**
7	18.80 min	458	Unknown

Peak N.	Retention time	m/z $[M+H]^+$	Proposal Impurity
8	20.04 min	933	Dehydrodimer a **72** Dehydrodimer b **73**

Oxazinone 66

Mass of Peak 1 was consistent with Oxazinone (**66**) degradation product reported by A.Vailaya et al.[34] LC-MS is consistent with Oxazinone structure reported in literature.

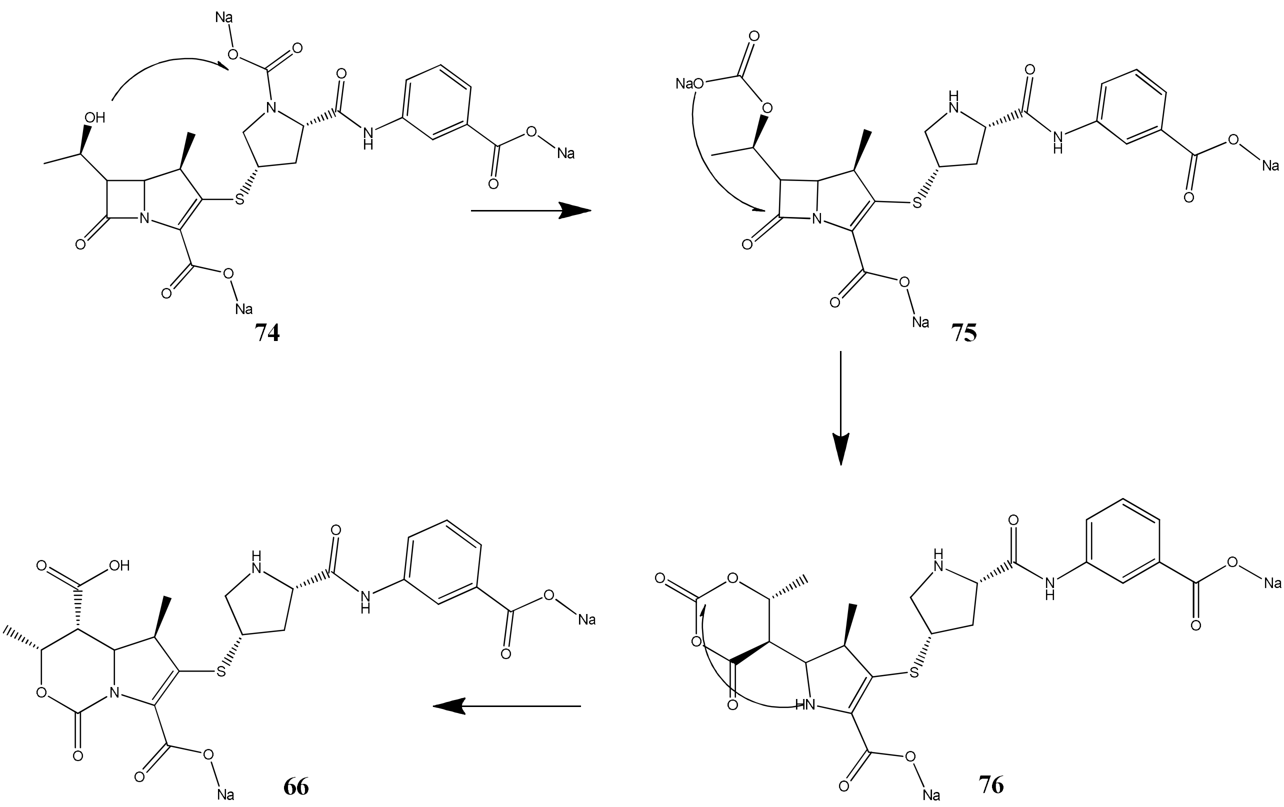

Figure 34. Oxazinone degradation pathway

Oxazinone **66**, figure 34 is formed by degradation which is initiated by carbon dioxide, in fact oxazinone **66** is an impurity which is not retrieved in Ertapenem drug substance **1**. Ertapenem drug product **74** is a carbamate derivative of Ertapenem **1**, therefore as described in literature[32] oxazinone impurity **66** formation is through migration of carbon dioxide from pirrolidine ring to hydroxyl group of ertapenem to form intermediate **75** which attacks labile betalactam to form intermediate **76** which then rearranges to form oxazinone **66**.

An authentic sample of Oxazinone **66** has been prepared by reaction of Ertapenem with carbon dioxide following a procedure described in literature[32]. The absorption of acquired ^{1}H-NMR and ^{13}C-NMR chemical shifts are consistent with published[32] NMR data for Oxazinone (**66**).

Ring Opened 67

Mass of Peak 2 was consistent with Ring opened (**67**) degradation product reported by P. Sajonz et al[33].

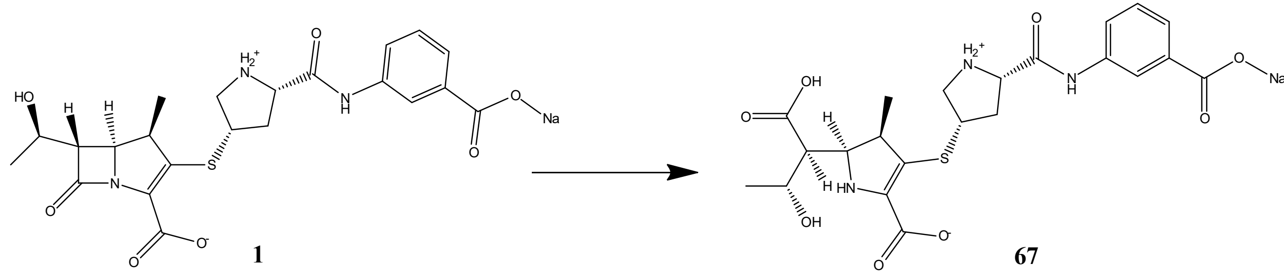

Figure 35. Ring Opened reasonable degradation pathway

Ring Opened impurity **67** is formed by hydrolytic degradation of Ertapenem labile betalactam ring. An authentic sample of Ring Opened **67** impurity has been prepared by hydrolytic degradation of Ertapenem, followed by chromatographic purification. The acquired NMR spectra are consistent with Ring Opened **67** structure. In particular, the chemical shifts of protons of formerly β-lactam prove that Ertapenem is hydrolysed, as shown in the following figures:

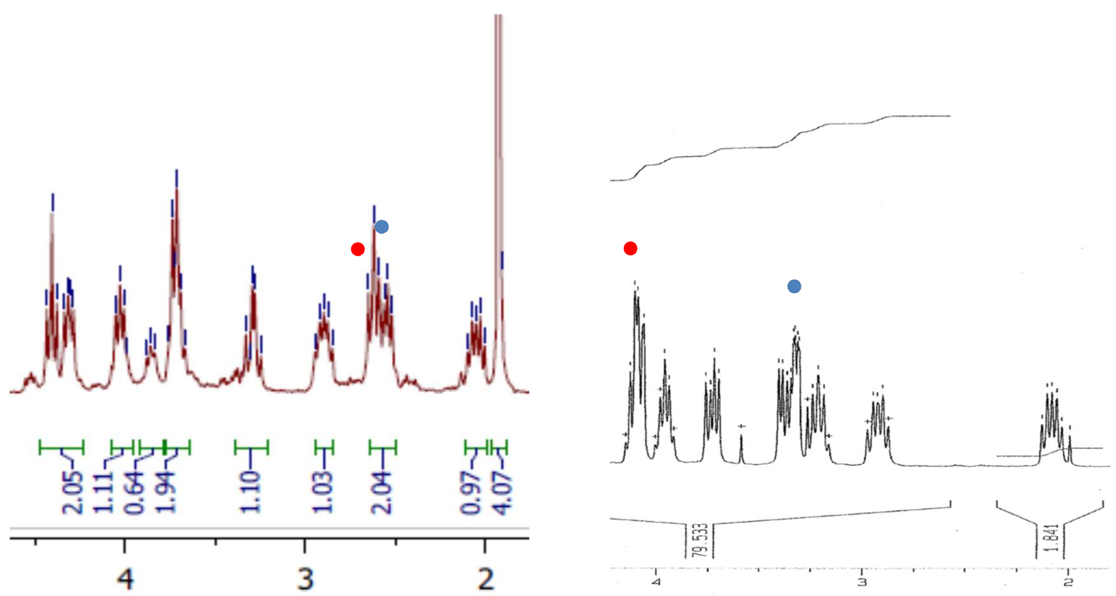

Figure 36. Ring Opened ^{1}H-NMR spectra *Figure 37. Ertapenem ^{1}H-NMR spectra*

ProMaba 68

Peak 3 was confirmed to be ProMABA **68**, an impurity product of side chain as described by *Brends et al*.[35] Mass of peak 3 was consistent with ProMaba **68**. In addition, an authentic sample was prepared following a procedure described in literature[35]. The acquired NMR spectra are consistent with ProMaba **65** structure and with literature[35] spectroscopic data.

Dimers 69, 70 and 71

Peaks 4, 5 and 6 are isomers corresponding to dimers as confirmed by mass spectrometry, therefore the structure assignment was performed by comparison of chromatographic profile defined by HPLC method reported by Merck[33].

Figure 38. Dimer I+II degradation pathway

Dimer I and Dimer II are constitutional isomers differing for the double bond position (see table 3) on the pyrroline ring (1-pyrroline and 2-pyrroline). As depicted in literature for Ertapenem[36] and for similar 1β-Methylcarbapenem antibiotics[37], the dimer bearing 2-pyrrolidine moiety is formed by intermolecular aminolysis, a reaction between the amino group of pyrrolidine ring of the first molecule with the β-lactam ring of the second molecule. 1-pyrroline and 2-pyrroline dimers are believed to exist as a mixture of double bond isomers in aqueous solution.

They are impurities that grow in Ertapenem Sodium drug substance stability. On other hand Dimer I **69** and Dimer II **70** do not grow in Ertapenem drug Product stability, since in drug product pyrrolidine ring is protected as carbamate.

Figure 39. Dimer III supposed degradation pathway

The formation of Dimer III **71** has been proposed to pass through intermediate anhydride **77** as per a similar mechanism reported in literature[38] for Biapenem. Therefore, carboxyl group of one Ertapenem molecule attacks labile β-lactam ring of a second Ertapenem molecule to form anhydride **77** which then rearranges to form Dimer III **71**.

Unspecified impurity

Peak 7 with relative retention time of 1.4, was the major unspecified impurity and the mass value found was 458. At the moment no chemical structure is proposed.

Dehydrodimers a+b (72) and (73)

For the peak 8, a probable co-elution of two dimers occurs as observed in figure 30.

Figure 40. *Dehydrodimer a+b degradation pathway*

Structures of both dehydrodimers have been described in literature[36], their formation involves a dehydration step, however a clear mechanism for their formation has not been reported.

2.1.2.2 Second LC-MS Investigation

The second LC MS study, see figure 41, has been performed on Invanz (RLD) lot 2049750 using Kromasil Eternity Phenylhexyl as column for HPLC method for related substances and for LC MS method, see section 4.1.4. In this stage Invanz was injected in the LC MS method and the masses of the main impurities reported in table 3 were confirmed. Table 4 summarizes the mass found and the peak attribution of impurities showed in figure 32.

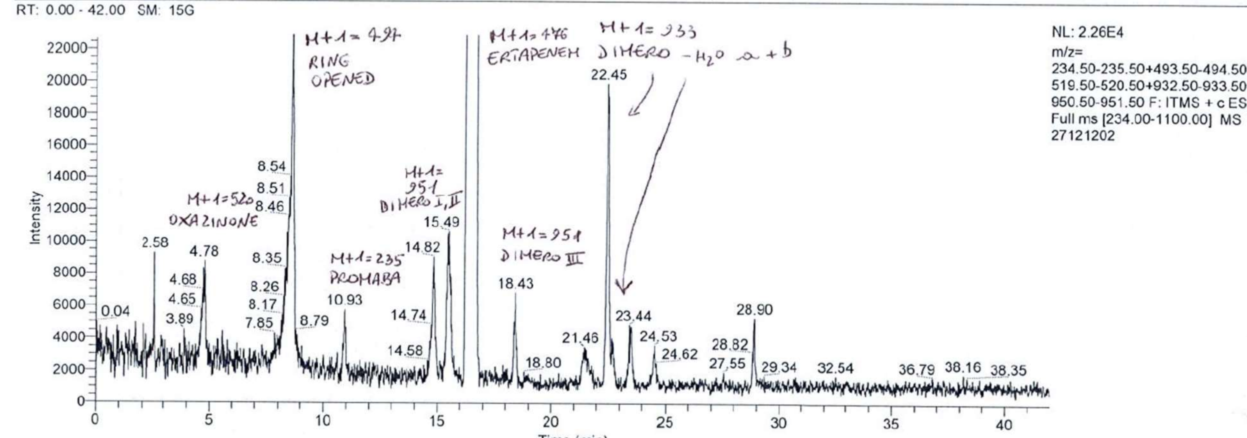

Figure 41. LC-MS of Invanz lot 2049750 analyzed by Kromasil

Table 4. LC MS study of Invanz lot 2049750

Rt[a] in HPLC method	Rt[a] in LC MS method	m/z $[M+H]^+$	Peak attribution
5.9	4.8	520	Oxazinone (**66**)
9.2	8.6	494	Ring opened (**67**)
10.9	10.9	235	ProMABA (**68**)
14.5	14.8	951	Dimer I (**69**)
15.1	15.5	951	Dimer II (**70**)
18.3	18.4	951	Dimer III (**71**)
22.0	22.5	933	Dehydrodimer a+b (**72**)+(**73**)

[a]*Retention time*

2.1.3 Chemical and Thermal Degradations in Solution and Solid State (Forced Degradations)

In order to confirm the suitability of developed Ertapenem test method and to assess its stability indicating performance, a preliminary forced degradation study was performed. Ertapenem sodium lot. 201211003 was used for this study. Forced degradation is also known as stress testing and a drug is degraded forcefully by applying artificial methods. It is useful tool to know the impurities developed during storage in various environmental conditions. The forced degradation study should have acid and base hydrolysis, photolysis, thermal degradation and oxidation. No regulatory guideline mentions the pH conditions for acid and base hydrolysis, the temperature for thermal degradation or type or concentration of oxidizing agent.

2.1.3.1 Basic degradation

Basic degradation has been simulated by treatment of an aqueous Ertapenem solution with NaOH 0.1 N for 2 minutes. Over exposure of Ertapenem Sodium to basic condition the impurity Ring Opened was observed to grow from initial 0.6% up to 25.0%, all the other impurities remain substantially unchanged.

2.1.3.2 Oxidative degradation

Oxidative degradation has been simulated by treatment of an aqueous Ertapenem solution with H_2O_2 30% and sample injected immediately. Over exposure of Ertapenem Sodium to oxidative condition the following impurity were observed to form/grow:

- Relative Retention time (RRt) 0.64 up to 2.2%
- RRt 0.82 up to 2.4%
- Dimer II from 0.6 up to 2.6%

2.1.3.3 Acid degradation

Acid degradation has been simulated by treatment of an aqueous Ertapenem solution with HCl 0.1 N for 5 minutes. Over exposure of Ertapenem Sodium to acidic condition the impurity Ring Opened was observed to grow from initial 0.6% up to 21.7%. All the other impurities remain substantially unmodified.

2.1.3.4 Thermal stress degradation at the solid state

Exposure to 40°C and Relative Humidity (RH) 75%:

The thermal stress was performed in an oven suitable for the purpose where both temperature and RH were set. Ertapenem powder is exposed in an open container for 2 hours. Many impurities are observed to grow and to form, from which the most relevant are as following:

- Ring Opened from 0.6% up to 3.7%
- Dimer II from 0.6% up to 1.5%
- Dimer III from 0.16% up to 1.3%
- Dehydrodimer a+b from 0.15% up to 2.7%
- RRt 0.49 up to 0.9%
- RRt 1.47 up to 0.6%
- RRt 1.54 up to 0.9%
- RRt 1.76 up to 0.2%

Data above indicate that 40°C and RH 75% are the worst conditions for Ertapenem stability since the exposure is both thermal and hydrolytic.

Exposure to 60°C in dry condition without control of humidity:

The thermal stress was performed in a standard laboratory oven. Ertapenem powder is exposed in a closed container for 2 hours.

The same impurities observed to grow in 40°C/75% RH were also observed however at lower level. Therefore:

- Ring Opened from 0.6% up to 3.7%
- Dimer II from 0.6% up to 1.2%
- Dimer III from 0.16% up to 0.6%
- Dehydrodimer a+b from 0.15% up to 0.7%
- RRt 0.49 up to 0.5%
- RRt 1.47 up to 0.12%
- RRt 1.54 up to 0.6%
- RRt 1.76 up to 0.13%

2.1.3.5 Thermal stress degradation in solution

Exposure to 25°C:

Thermal stress of an aqueous Ertapenem solution was performed at 25°C for 4 hours.

The following impurity were observed to grow:

- Ring Opened from 0.6% up to 1.4%
- Dimer II from 0.6% up to 0.8%

Exposure to 60°C:

Thermal stress of an aqueous Ertapenem solution was performed at 60°C for 1 hour.

The following impurity were observed to form/grow:

- Ring Opened from 0.6% up to 2.8%
- Dimer II from 0.6% up to 2.8%
- Dimer III from 0.16% up to 1.5%
- RRt 1.19 up to 1.4%

2.1.3.6 UV exposure

UV exposure of an aqueous Ertapenem solution was performed at 365 nm for 3 hours.

The following impurity were observed to grow:
- Ring Opened from 0.6% up to 1.3%
- Dimer II from 0.6% up to 2.0%
- Dimer III from 0.16% up to 0.6%

The above impurities are formed by aqueous degradation in solution, therefore no UV degradative effect was observed.

2.1.3.7 Forced degradation studies result

The analytical work performed can be summarized in the enhancement of few impurities that can therefore be defined Ertapenem degradation impurities. Such impurities were evaluated through chemical/physical/thermal stress and their determination/verification of molecular weight by LC-MS analysis. In table 5 the key aspects of forced degradation studies are summarized.

Table 5. Qualitative abstract of forced degradation study for Ertapenem Sodium

Forced degradation study	**Main Degradation impurities observed**	**LC MS investigation**	**Conclusion**
Basic degradation	Ertapenem Open Ring	Open Ring $[M+H]^+$= 494; mass confirmed	Increasing of Ertapenem Open Ring
Oxidative degradation	2 impurities at RRt 0.64 and RRt 0.82. Also Dimer II was observed but it could be linked to stress in solution	$[M+H]^+$= 492 for both impurities assigned to ertapenem sulphoxides	Increasing of Ertapenem sulfoxides.
Acid degradation	Ertapenem Open Ring	Open Ring $[M+H]^+$= 494; mass confirmed	Increasing of Ertapenem Open Ring
Thermal solid state **2 hrs, +40°C/RH75%**	Ertapenem Open Ring Dimer I, Dimer II, Dimer III, Dimer –H_2O a+b. Others at RRt: 0.49, 1.47, 1.54 and 1.76	Ertapenem Open Ring Dimer I, Dimer II, Dimer III, Dimer –H_2O a+b masses confirmed	Increasing of Ertapenem Open Ring Dimer I, Dimer II, Dimer III, Dimer –H_2O a+b
Thermal solid state **2 hr, +60°C**	Ertapenem Open Ring Dimer II, Dimer III, Dimer–H_2O a+b. Others at RRt: 0.49, 1.54, 1.76	Ertapenem Open Ring Dimer II, Dimer III, Dimer –H_2O a+b masses confirmed	Increasing of Ertapenem Open Ring, Dimer II, Dimer III, Dimer –H_2Oa+b
Thermal in solution **RT for 4 hr.**	Slightly increase of Ertapenem Open Ring and Dimer II.	Ertapenem Open Ring and Dimer II masses confirmed	Increasing of Ertapenem Open Ring and Dimer II.
Thermal in solution **60°C for 1 hr.**	Ertapenem Open Ring Dimer II, Dimer III. Other at RRt: 1.19	Ertapenem Open Ring Dimer II, Dimer III masses confirmed	Increasing of Ertapenem Open Ring Dimer II, Dimer III
UV exposure	Ertapenem Open Ring Dimer II, Dimer III. which are formed by aqueous degradation in solution, no UV degradative effect observed	Not done	No specific degradation

Forced degradation studies showed that the main degradation impurities are those reported in literature and confirmed by LC MS study. Only in the oxidation study we observed the increase of two new degradation impurities that are not reported in literature. Anyway, these impurities are not present in any significant levels in Invanz reference product.

2.1.4 CONCLUSIONS

The HPLC method for related impurities has been developed starting from the information reported in literature. The main specified identified impurities reported in literature have been confirmed and identified by two separated LC MS studies. An improved HPLC test method has been outlined by replacement of Inertsil column with Kromasil column which is known to be more suitable for the pH working condition of mobile phases.

Following to Kromasil test method development a full validation was performed according to ICH Q2 guideline. From validation work the precision results have been reported in table 6, since they are relevant to the intrinsic variability of test method.

Table 6. Kromasil Test Method Variability as per Precision Validation exercise

Component	**Statistical Data**	
	St. Dev. (σ)	**Relative St. Dev (RSD%)**
Oxazinone	0.0007	4.7%
Ring Opened	0.0086	0.3%
ProMABA	0.0013	0.3%
Dimer I	0.0090	2.7%
Dimer II	0.0098	1.0%
Dimer III	0.0013	0.3%
Dehydrodimers (a+b)	0.0068	2.5%
Ertapenem	0.0275	0.0%

2.2 Ertapenem Setting Specifications

With the purpose to set specification either for Ertapenem Drug Product and Drug Substance, marketed Ertapenem Drug Products Invanz® vials from different markets (EU-US) were sourced and analyzed with analytical test methods developed for the purpose.

Proposed specification will represent a reference for analytical data comparison.

2.2.1 ANALYTICAL DATA ACQUISITION AND TREATMENT

Eight batches of Invanz® from relevant markets (EU-US) have been dedicated to this study as depicted in table 7. Samples are listed depending on the market.

Table 7. Invanz batches sourced from the market

Batch	Market
2029670	EU (Italy)
2042520	EU (Italy)
2044720	EU (Italy)
2040900	EU (Switz)
2062830	EU (Italy)
2049750	US
2075480	US
2075450	US

Results of analysis of those 8 Invanz batches are presented in the following tables.

Table 8. Invanz analysis results for water content, pH and assay

Batch (Market)	New Water Content	pH	Assay
	KF-Oven		as Ertap. Acid
2029670 (Switz-EU)	1.7	7.6	74.6
2042520 (Italy-EU)	1.5	7.6	74.2
2044720 (Italy-EU)	1.5	7.8	73.7
2040900 (Switz-EU)	1.7	7.6	73.5
2049750 (USA)	1.4	7.6	75.5
2062830 (Italy-EU)	1.7	7.6	75.4
2075480 (USA)	1.7	7.6	75.9
2075450 (USA)	1.5	7.6	75.4
Average	1.6	7.6	74.8
Standard Deviation	0.125	0.071	0.904
Average + 3 std. dev	2.0	7.8	77.5
Average - 3 std. dev	1.2	7.4	72.1
RSD%	7.9	0.9	1.2
Max	1.7	7.8	75.9
Min	1.4	7.6	73.5

Table 9. Invanz analysis results for related substances

Batch (Market)	Related Substances								
	Oxazinone	Ring Opened	ProMABA	Dimer I	Dimer II	Dimer III	Dehydro dimers a+b	Total Purity	Total Imp.
2029670 (Switz-EU)	0.49	3.34	0.24	0.36	0.65	0.11	0.37	93.9	6.1
2042520 (Italy-EU)	0.62	3.83	0.23	0.38	0.69	0.12	0.37	93.1	6.9
2044720 (Italy-EU)	0.56	3.51	0.23	0.38	0.68	0.12	0.37	93.5	6.5
2040900 (Switz-EU)	0.56	3.42	0.35	0.44	0.81	0.11	0.38	93.4	6.6
2049750 (USA)	0.52	3.32	0.20	0.42	0.75	0.12	0.36	93.7	6.3
2062830 (Italy-EU)	0.49	3.08	0.23	0.39	0.71	0.12	0.33	94.1	5.9
2075480 (USA)	0.49	3.18	0.26	0.37	0.62	0.09	0.25	94.4	5.6
2075450 (USA)	0.47	2.95	0.26	0.33	0.54	0.09	0.25	94.7	5.3
Average	0.53	3.33	0.25	0.38	0.68	0.11	0.34	93.9	6.2
Standard Deviation	0.051	0.273	0.045	0.034	0.082	0.013	0.055	0.535	0.535
Average + 3 std. dev	0.68	4.15	0.38	0.49	0.93	0.15	0.50	95.5	7.8
Average - 3 std. dev	0.37	2.51	0.12	0.28	0.44	0.07	0.17	92.2	4.5
RSD%	9.7	8.2	17.9	8.9	12.0	11.9	16.3	0.6	8.7
Max	0.62	3.83	0.35	0.44	0.81	0.12	0.38	94.7	6.9
Min	0.47	2.95	0.20	0.33	0.54	0.09	0.25	93.1	5.3

Table 10. Invanz analysis results for unspecified impurities ≥0.04%

Batch (Market)	Rel.Subst	Rel.Subst	Rel.Subst	Rel.Subst
	RRt 1.25	RRt 1.32	RRt 1.44	RRt 1.50
2029670 (Switz-EU)	0.04	0.14	0.04	0.05
2042520 (Italy-EU)	0.05	0.14	0.05	0.07
2044720 (Italy-EU)	0.04	0.13	0.05	0.06
2040900 (Switz-EU)	0.05	0.13	0.05	0.05
2049750 (USA)	0.04	0.13	0.05	0.06
2062830 (Italy-EU)	0.08	0.12	0.05	0.06
2075480 (USA)	< 0.04	0.09	< 0.04	< 0.04
2075450 (USA)	< 0.04	0.10	0.04	< 0.04
Average	NA	NA	NA	NA
Standard Deviation	NA	NA	NA	NA
Average + 3 std. dev	NA	NA	NA	NA
Average - 3 std. dev	NA	NA	NA	NA
RSD%	NA	NA	NA	NA
Max	NA	NA	NA	NA
Min	NA	NA	NA	NA

All batches showed good data homogeneity (i.e. low % Relative Standard Deviation) and were treated statistically as unique data set.

Maximum % Relative Standard Deviation found for related substances is 17.9%. Due to low concentrations of related substances, a %RSD in this range is to be considered low.

For each data set, average, standard deviation, average + 3 x standard deviation, average – 3 x standard deviation, standard deviation percentual (RSD%), max value and min value were calculated. Where applicable specification limit were set as average ± 3 times standard deviation, as this is a procedure generally accepted by all regulatory agencies.

Statistical data treatment of water content, pH, assay is described in table 8

Statistical data treatment of related substances is described in table 9

Dimer I and Dimer II are constitutional isomers differing for the double bond position (see table 3) on the pyrroline ring (1-pyrroline and 2-pyrroline). As depicted in literature for Ertapenem[36] and a similar 1β- Methylcarbapenem antibiotics[37], the dimer bearing 2-pyrrolidine moiety is formed by intermolecular aminolysis, a reaction between the amino group of pyrrolidine ring of the first molecule with the β-lactam ring of the second molecule. 1-pyrroline and 2-pyrroline dimers are believed to exist as a mixture of double bond isomers in aqueous solution. The isomers are two tautomeric forms enamine/imine and as such they should exist in equilibrium. Therefore, it has been proposed the approach of having unique specification for the sum of 2 dimers

In order to confirm the chemical rationale proposed, stability in solution of US batch 2045750 was investigated. Dimer I showed a decreasing trend of -22% (21%-43%) over 10 injections in the refrigerated autosampler whereas Dimer II showed an increasing trend of +22% (79% - 57%). The sum of Dimer I + Dimer II (Dimers I + II) is stable over ten HPLC runs. Largest unknown impurity was monitored in order to exclude further degradation beyond the conversion of Dimer I into Dimer II generating a single unknown impurity. Total impurities were monitored to have the total degradation under control. The analytical study is summarized in table 11**.**

Table 11. Dimer I and Dimer II study over time

	1st Injection	2nd injection	3rd injection	4th injection	5th injection
Dimer I	0.42	0.39	0.34	0.31	0.29
Dimer II	0.56	0.60	0.64	0.68	0.70
Dimers I + II	0.98	0.99	0.98	0.99	0.99
Largest Unspecified Impurity	0.14	0.13	0.13	0.12	0.12
Total Impurities	6.8	6.9	6.8	7.0	7.0
Dimer I / (Dimers I + II) x 100	43%	39%	35%	32%	29%
Dimer II / (Dimers I + II) x 100	57%	61%	65%	68%	71%

Table 11. Dimer I and Dimer II study over time ,Cont.

	6th injection	7th injection	8th injection	9th injection	10th injection
Dimer I	0.28	0.25	0.24	0.22	0.21
Dimer II	0.73	0.75	0.76	0.78	0.79
Dimers I + II	1.01	1.00	0.99	1.00	1.00
Largest Unspecified Impurity	0.12	0.11	0.11	0.11	0.11
Total Impurities	7.0	7.1	7.0	7.2	7.2
Dimer I / (Dimers I + II) x 100	28%	25%	24%	22%	21%
Dimer II / (Dimers I + II) x 100	72%	75%	76%	78%	79%

Table 12. Dimer I + II study statistical analysis

Batch (Market)	Rel.Subst	Rel.Subst	Rel.Subst
	Dimer I	Dimer II	Dimers I + II
2029670 (Switz-EU)	0.36	0.65	1.01
2042520 (Italy-EU)	0.38	0.69	1.07
2044720 (Italy-EU)	0.38	0.68	1.06
2040900 (Switz-EU)	0.44	0.81	1.25
2049750 (USA)	0.42	0.75	1.17
2062830 (Italy-EU)	0.39	0.71	1.10
2075480 (USA)	0.37	0.62	0.99
2075450 (USA)	0.33	0.54	0.87
Average	0.38	0.68	1.07
Standard Deviation	0.034	0.082	0.115
Average + 3 std. dev	0.49	0.93	1.41
Average - 3 std. dev	0.28	0.44	0.72
RSD%	8.9	12.0	10.8
Max	0.44	0.81	1.25
Min	0.33	0.54	0.87
New Specification Proposal			1.4

A unique specification for Dimers I + II was proposed as per statistical treatment depicted in table 12 based upon average + 3 standard deviation of experimental values treated as a sum of Dimers. Unspecified impurities ≥ 0.04% are listed in table 10

Unspecified impurities at a level of < 0.04% were not considered as representative. Four unspecified impurities ≥ 0.04% were assigned RRts (RRt 1.25, RRt 1.32, RRt 1.44, RRt 1.50) but no statistical treatment was performed since all impurities were below 0.2%.

2.2.2 Drug Product Setting specifications

Data presented in tables 8-12 were relevant to Drug Product, therefore any reasoning gathered could lead to setting specification of Ertapenem Drug Product. A justification of specifications for each test follows. At this stage of development, some of the specification have been slightly widened, where appropriate. Some of the specifications have been slightly tightened based upon available data review. For each test a rationale is proposed. Proposed specification will be considered as a reference until tested by feasibility studies.

2.2.2.1 *Water Content*

Proposed specification: ≤ 2.5 %

Rationale: Average + 3 std. dev. evaluation would justify a specification of ≤ 2.0 %, however, at this stage of development, the specification has been tentatively set at this value in view of collecting sound data to determine feasibility.

2.2.2.2 *pH*

Proposed specification: 7.1 – 8.1

Rationale: Average ± 3 std. dev. evaluation would justify a specification range of 7.8-7.4, however the specification range has been judged too tight for this stage of development and tentatively widened to average ± 0.5 pH units in view of collecting data to determine feasibility.

2.2.2.3 *Potency*

Proposed specification: 71.1 - 78.6 %

Rationale: Average ± 3 std. dev. evaluation would justify a specification range of 72.1 – 77.5%, however the specification range has been judged too tight for this stage of development and widened to 71.1 - 78.6 % (average ± 5%) in view of collecting data to determine feasibility. Specification is expressed as %w/w Ertapenem Acid and not as label claim to keep consistency between API and DP. In addition to this, powder availability represents a constrain to dedicate 1 entire vial to express potency as label claim at this stage of development.

2.2.2.4 *Related Substances*

2.2.2.4.1 *Oxazinone*

Proposed specification: ≤ 0.70 %

Rationale: Based upon average + 3 std. dev. this related substance would be justified until 0.68%. The specification was slightly widened to 0.70%. Maximum value found experimentally is 0.62%.

2.2.2.4.2 *Ring Opened*

Proposed specification: ≤ 6.0 %

Rationale: Based upon average + 3 std. dev. this related substance would be justified until 4.15%. However, specification was loosened up to ≤ 6.0 % since it is a known major metabolite.

2.2.2.4.3 ***ProMABA***

Proposed specification: ≤ 0.40 %

Rationale: Based upon average + 3 std. dev. this related substance would be justified until 0.38%. The specification was slightly widened to 0.40%. Maximum value found experimentally is 0.35 %.

2.2.2.4.4 ***Dimers I+II***

Proposed specification: ≤ 1.4 %

Rationale: Based upon average + 3 std. dev. this related substance would be justified until 1.41%. The specification was round to 1.4%. Maximum value found experimentally is 1.25%.

2.2.2.4.5 ***Dimer III***

Proposed specification: ≤ 0.20 %

Rationale: This is a degradation product; taking into account a daily dose of 1g/day, according to ICH guideline[5], the qualification threshold is 0.2%. The specification was slightly tightened to ≤ 0.20 % for consistency with decimals proposed (2) for other impurities. Maximum value found experimentally is 0.12%.

2.2.2.4.6 ***Dehydro dimers (a+b)***

Proposed specification: ≤ 0.45 %

Rationale: Based upon average + 3 std. dev. this related substance would be justified until 0.50%. Specification was slightly tightened to ≤ 0.45 % since the maximum value experimentally found is 0.38 %.

2.2.2.4.7 ***Largest unspecified impurity***

Proposed specification: ≤ 0.20 %

Rationale: Taking into account a daily dose of 1g/day, according to ICH guideline[39], the qualification threshold for degradation products is 0.2%. The specification was slightly tightened to ≤ 0.20% for consistency with decimals proposed (2) for other impurities. All unspecified impurities are below this level.

2.2.2.4.8 ***Total impurities***

Proposed specification: $\leq$ 9.5 %

Rationale: This value is the sum of the maximum value allowed for each specified impurity and largest unspecified impurity, see calculation below:

Total imp. = (0.70 + 6.0 + 0.40 + 0.50 + 0.90 + 0.20 + 0.45 + 0.20) % = 9.35 %

2.2.3 DRUG SUBSTANCE SETTING SPECIFICATIONS

Ertapenem Sodium specifications have been set with appropriate limits so that Drug Substance can be transformed in finished product that complies Drug product specifications and therefore is comparable with Invanz commercial product.

2.2.3.1 ***Water Content***

Proposed specification: $\leq$ 19.0%

It has been set on process capabilities rationale, supported by EMA scientific discussion paper[40], where the upper limit of water content is NMT 19.0%. It does not interfere with drug product since the manufacturing process involves compounding in aqueous solution and lyophilization. The limit for water in drug product will be assured by lyophilization process.

2.2.3.2. ***pH***

Proposed specification: 4.5 – 6.5

According to literature[32] pH of Ertapenem monosodium is 5.5, therefore a range of 4.5-6.5 has been set.

2.2.3.3. ***Assay***

Proposed specification: ≥ 72.5% as Ertapenem Acid and ≥ 95.5% as Ertapenem Sodium Anhydrous basis

Limits have been developed using mass balance criteria as following:

Assay as Ertapenem Sodium = [100 - KF- total solvents - Total Impurities]=

= [100 - 19.0 - 1.8 – 3.0]= 76.2%

Assay as Ertapenem Acid = Assay as Ertapenem Sodium x (acid molecular mass/sodium molecular mass) = 76.2 x (475.52/497.50) = 72.8%

72.8% is rounded down to 72.5%

Assay as Ertapenem Sodium Anhydrous basis = (Assay as Ertapenem acid x sodium molecular mass x 100) / acid molecular mass x (100 - KF- total solvents) =

= (72.5 x 497.50 x 100) / 475.52 (100 - 19 - 1.8) =95.8% is rounded down to 95.5%

2.2.3.4. ***Related Substances***

Drug product process is constituted of a lyophilization, Ertapenem monosodium drug substance (**1**) is compounded in water together with 1 equivalent of $NaHCO_3$ and adjusting pH at 7.5-7.8 by NaOH addition. At end of lyophilization process Ertapenem is retrieved as trisodium carbamate **(74)**.

$NaHCO_3$ / NaOH

1 → **74**

Figure 42: Ertapenem Drug Product scheme

Therefore, for related substances a couple of reference lyophilization trials have been reported below to see the evolution of related substances over lyophilization from Drug Substance (DS) to Drug Product (DP).

Table 13: *Related substances evolution over lyophilization*

Related Substances	Drug Substance		Drug Product	Drug Substance		Drug Product	Average (%)
Oxazinone	n.d.	⇨	0.15%	0.04%	⇨	0.21%	+0.16
Ring Opened	0.48%		2.2%	0.52%		2.2%	+1.7
ProMABA	0.18%		0.18%	0.05%		0.07%	+0.01
Dimer I+II	0.37%		1.3%	0.34%		1.0%	+0.8
Dimer III	0.13%		0.15%	0.09%		0.12%	+0.03
Dehydro dimer a+b	0.12%		0.19%	0.13%		0.19%	+0.07

2.2.3.4.1. ***Oxazinone***

Proposed specification: ≤ 0.20%

Drug product setting spec provides for oxazinone a limit of 0.70%. Since it is a degradation impurity and increases in the process from DS to DP a conservative limit of 0.20% has been set in Drug Substance.

2.2.3.4.2. ***Ring Opened***

Proposed specification: ≤ 1.5%

Drug product setting spec provides for Ring opened a limit of 6.0%. Since it is a degradation impurity and increases in the process from DS to DP a conservative limit of 1.5% has been set in Drug Substance.

2.2.3.4.3. ***ProMABA***

Proposed specification: ≤ 0.40%

Drug product setting spec provides for ProMABA a limit of 0.40%. Since it is a process impurity and does not increase over Drug product process, the same limit has been set for Drug Substance.

2.2.3.4.4. ***Dimer I+II***

Proposed specification: ≤ 0.70%

These two dimers are evaluated as sum since dimer I converts to dimer II. Drug product setting spec provides for Dimer I+II a limit of 1.4%. Since they are degradation impurities and increase in the process from DS to DP a conservative limit of 0.70% has been set in Drug Substance.

2.2.3.4.5. ***Dimer III***

Proposed specification: ≤ 0.20%

It is a degradation impurity of Drug Substance. A limit of 0.20% has been set for Drug Product based on analysis and statistical treatment of commercial product Invanz data. Considering that does not increase over Drug product process, the same limit has been set for Drug Substance.

2.2.3.4.6. ***Dehydrodimers (a+b)***

Proposed specification: ≤ 0.20%

Drug product setting spec provides for Dehydrodimers (a+b) a limit of 0.45%. Since it is a degradation impurity and increases in the process from DS to DP a conservative limit of 0.20% has been set in Drug Substance.

2.2.3.4.7. ***Largest unspecified impurity***

Proposed specification: ≤ 0.10%

A limit of 0.10% has been set in compliance with ICH guideline[41].

2.2.3.4.8. ***Total impurities***

Proposed specification: ≤ 3.0%

Rationale: This value is the sum of the maximum value allowed for each specified impurity and largest unspecified impurity, see calculation below:

Total imp. = (0.20 + 1.5 + 0.40 + 0.70 + 0.20 + 0.20 + 0.10) % = 3.3 %, rounded down to 3.0%.

2.2.3.5. ***Residual solvents***

Drug product process is constituted of a lyophilization therefore there is the possibility for solvents to be further reduced from Drug Substance to Drug Product.

2.2.3.5.1. ***Methanol***

Proposed specification: $\leq$ 0.30%

The limit has been set in compliance with ICH guideline Q3C, option 1.

2.2.3.5.2. ***Isopropanol***

Proposed specification: $\leq$ 0.50%

The limit has been set in compliance with ICH guideline Q3C, option 1.

2.2.3.5.3. ***Methyl Acetate***

Proposed specification: $\leq$ 1.0%

The limit has been set based on process capabilities rationale, which is also supported by ICH Q3C, option 2, considering that Ertapenem daily dosage is 1.0 g.

CHAPTER 3 : Manufacturing Process Development

3.1 Manufacturing Process Development

Main topic of this thesis is manufacturing process development, therefore few chemical reactions will be reproduced several time with the purpose to outline a process that is scalable at commercial scale and delivers a product with consistent yield and quality. The main stages of such type of work are the following:

1) Development of main conditions to produce a certain item
2) Test process robustness and reproducibility at lab scale through DEMO batches manufacturing
3) Perform use test prior to every scale up

Use test is a laboratory trial which is performed before a plant scale up, which employs all materials (reagents and solvents) of same quality as the one afterwards employed at scale. It is usually run to minimize scale up risks linked to a different quality of raw materials

4) Perform a kilogram scale laboratory test or a pilot manufacturing.

Kilogram scale laboratory test is a scale-up trial performed with glass reactor in the lab with a scale ranging from few hundred grams to few kilograms. Pilot manufacturing is a small trial performed on plant using stainless steel or glass lined reactors. Pilot manufacturing scale ranges from few kilograms to few tenths of kilograms.

5) Use the learning of kilolab/pilot experience to further improve the manufacturing process
6) Repeat the stages from point 1 going forward.

Ertapenem manufacturing process developed by Merck[32] involves coupling between carbapenem enolphosphate and ertapenem side chain, see figure 43.

Carbapenem enolphosphate **61** is the p-nitrobenzyl (PNB) crystalline intermediate which is stable and easily available. Side chain **64** was p-nitrobenzyloxycarbonyl (PNZ) protected at pirrolidine amino group in first generation process[32], which produces after coupling Ertapenem di-protected **78** that is subjected to Hydrogenolysis at medium pressure to afford Ertapenem Sodium.

In Merck second generation process[32] carbapenem enolphosphate is reacted with side chain **3** where pirrolidine amino group is the free HCl salt. After coupling mono-PNB Ertapenem **79** is produced, which is then subjected to Hydrogenolysis at medium pressure to afford Ertapenem Sodium.

61 **64** R=PNZ **3** R=H **78** R=PNZ **79** R=H **1**

Figure 43. Merck Ertapenem synthetic scheme

On other hand ACS-DOBFAR SpA produces on ordinary basis Meropenem employing carbapenem enolphosphate allyl protected **2** (POCA). Such intermediate is not isolated but is produced in situ through the Dieckmann approach from isolated intermediate **80**.

pTSA/Toluene TMCS/TEA Toluene **80** **81** **82**

1)NaH, TMSONa, /Toluene/THF 2)Diphenylchlorophosphate/Toluene/THF **2 POCA** **83**

Figure 44. ACS synthetic scheme for carbapenem enolphosphate (POCA)

Carbapenem enolphosphate allyl protected **2** is produced in situ, from open intermediate **82** where one carboxyl group is activated as thioester with p-Chlorothiophenol. Upon deprotonation of carbon atom in α position to the other carboxyl group, the formed carbanion attacks the activated carboxyl group, releasing the good leaving group p-Chlorothiophenol, which is trapped by allylbromide to form a stable allylthioether **83**. β-keto intermediate is therefore activated as enolphosphate by reaction with DiphenylChloroPhosphate (DCP). The HPLC chart of this intermediate is in fact pretty clean and contains only POCA (**2**) and inert allylthioether **83** byproduct.

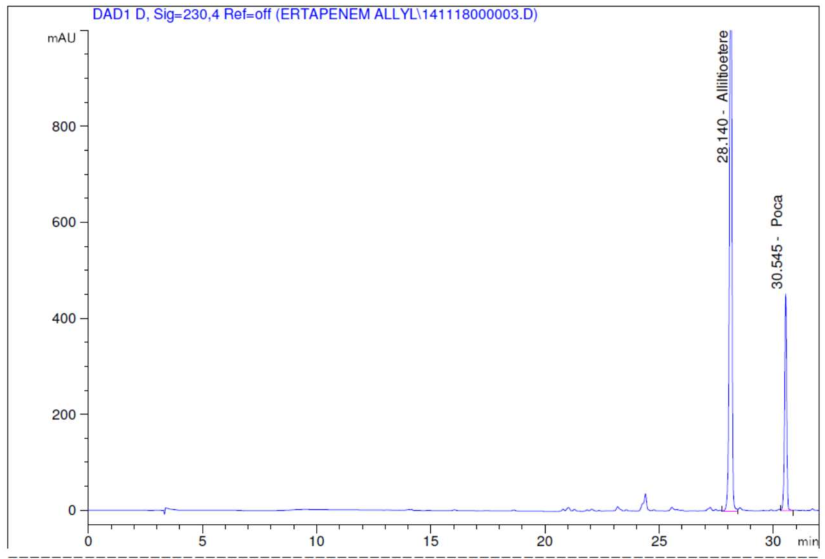

*Figure 45. In Process Control (IPC) HPLC chart[a] of not isolated POCA **2**, see section 4.1.5*

[a]*run time 32 min*

3.2 Ertapenem Allyl Development

Starting from POCA (**2)** solution it has been evaluated the development of ACS process to produce Ertapenem. Therefore, POCA (**2)** have been reacted with Ertapenem side chain **3** in the form of Hydrochloridrate, which is commercially available, see figure 46.

Figure 46. TMS Ertapenem Allyl synthetic scheme

Formally such coupling appears as a vinyl S_N where side chain thio group displaces the good leaving group diphenylphosphate. Actually, most likely, such reaction proceeds through a Micheal addition on α,β insatured carboxylate, followed by β-elimination of diphenylphosphate, see figure 47

Figure 47. Enolphosphate-Thiol coupling supposed mechanism

This hypothesis has been consolidated by two facts:

1) A recent publication[42] where Michael addition/β-elimination is reported for the synthesis of 4-selenocoumarins (**87**), see figure 48

Figure 48. Proposed reaction sequence toward 4-selenocoumarins[42]

The article[42] reports the hypothesis that alkene bearing electron withdrawing groups can react with nucleophiles in a conjugate fashion, generating a stabilized anion. If the β-carbon carries a suitable leaving group, its displacement can further stabilize the system by regenerating the conjugated system.

2) During Ceftaroline Fosamil (**34**) (US approval in 2010) process development the same type of reaction was studied on cephalosporins. Therefore, as per figure 49 enol **88** was converted in mesylate **89**. However due to the presence of base for the mesylation reaction a not negligible amount of Δ3 mesylate **90** is formed.

Figure 49. Ceftaroline Fosamil synthetic steps that involve Michael addition/β-elimination

Compound **90** is not anymore a conjugated alkene and was found unreactive to nucleophile **91**. This observation further proves that such reaction proceeds through Michael addition/β-elimination since only the conjugated system **89** is reactive to nucleophile **91**.

In Merck literature[32], the side chain **3** is reacted with carbapenem enolphosphate PNB protected **61** in N-EthylPirrolidone (NEP) in presence of a catalytic amount of tributylphosphine at -55°C/-60°C

with tetramethylguanidine. Similar coupling conditions were applied on POCA (**2)** (allyl carbapenem enolphosphate).

Therefore, a screening of main parameters was performed for coupling reaction described on figure 46 in order to optimize reaction performance.

SOLVENT

POCA is produced as MeCN solution, therefore it was evaluated the use of this solvent in place of NEP, in order to avoid solvent switch. Use of MeCN provided a suitable reaction rate and did not introduce any particular issue on the workup.

BASE

Role of base in coupling reaction is to neutralize hydrochloride salt providing soluble side chain free base, neutralize side chain carboxylic acid and to generate thiolate anion to attack the α,β system on POCA, so base should be strong enough to accomplish all tasks. Base was maintained tetramethylguanidine since provided acceptable performances (solubility of Side chain). Attempts to use diisopropylamine were failure since the reaction rate was too slow, most likely due to side chain solubility concerns.

TEMPERATURE

Temperature was kept -45/-50°C, very similar to what described in Merck literature[3], to ensure the right chemoselectivity. In fact, side chain contains three nucleophilic groups (carboxyl, amino and thio) and low temperature assures that the strongest nucleophile is reacting with carabpenem enolphosphate.

TRIBUTYLPHOSPHINE

An addition of 2.5% equivalents of Bu_3P is maintained as suggested by Merck literature[32] to reduce the side chain thiol oxidation to form related disulfide.

Coupling takes place in about 4 hours after which the consumption of POCA (**2**) and formation of Ertapenem allyl ester silylated (**4**) can be observed, see figure 50.

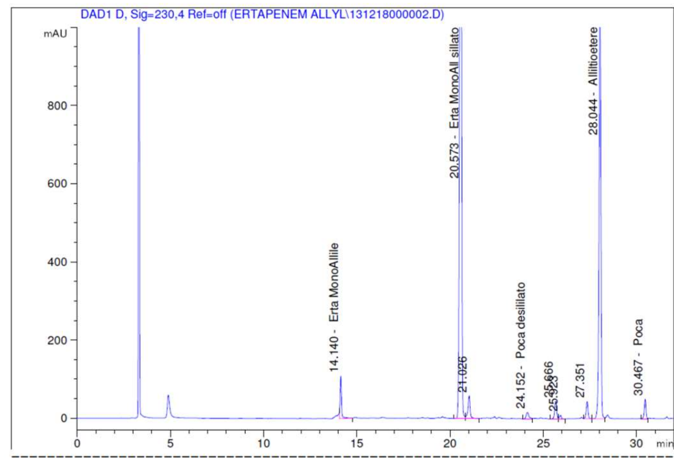

Figure 50. In Process Control (IPC) HPLC chart at end of coupling, see section 4.1.5

The following deprotection of TMS hydroxyl protecting group (see figure 51) is achieved by direct end of coupling treatment with diluted HCl at pH 3.0.

TMSO H H N S H N O OH O O O O

aq. HCl

HO H H N S H N HCl O OH O O O O

4 5

Figure 51. Ertapenem Allyl synthetic scheme

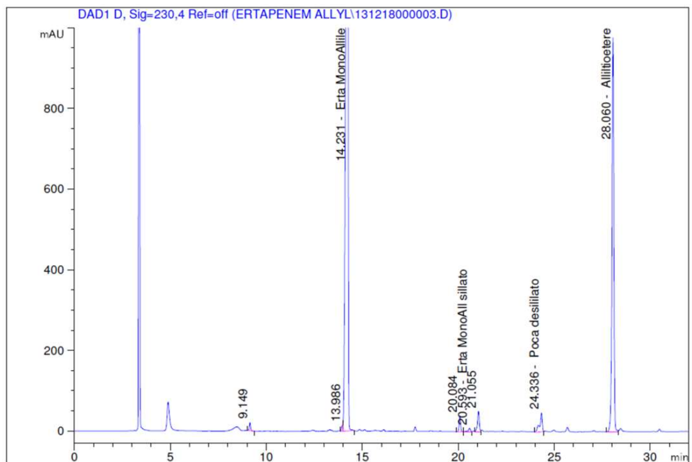

Figure 52. In Process Control (IPC) HPLC chart at end of hydrolysis, see section 4.1.5

Once silyl ether hydrolysis is accomplished, work-up is initiated by phase separation. Ertapenem allyl ester is retrieved in organic layer.

PURIFICATION STEP

It has been discovered that Ertapenem allyl ester (**5**) provides a unique behavior, in fact despite its structure contains a lipophilic ester, it could be transformed in a highly ionized species, soluble in water, by treatment with a carbonic acid salt. It is indeed a known[43] feature of either Meropenem and Ertapenem to form carbamate on side chain pyrrolidine ring by treatment with a carbon dioxide donor at basic pH.

Therefore, as per figure 53, Ertapenem allyl ester (**5**) at acidic pH is soluble in organic solvents and insoluble in water, on other hand treating Ertapenem allyl ester with Sodium Carbonate to adjust pH to neutrality the carbamate derivative (**93**) is formed and desired product becomes soluble in water. In addition, carbamate establishes a transient protection on pyrrolidine ring providing a higher stability of Ertapenem allyl ester in aqueous solution. In fact, pyrrolidine ring is a strong nucleophilic center causing oligomerization of Ertapenem, in the absence of the above mentioned temporal protection, therefore increasing the impurity load of crude mixture.

$NaHCO_3$ / HCl

5
Soluble in organic solvents

93
Soluble in water

Figure 53. Ertapenem Allyl - Ertapenem Allyl carbamate interconversion

This unique feature of Ertapenem Allyl ester is used to purify it from most of organic soluble impurities, by extracting Ertapenem Allyl ester in aqueous solution as carbamate derivative. Once Ertapenem Allyl ester is brought in aqueous solution it can be noticed that still contains a consistent impurity load, see figure 54.

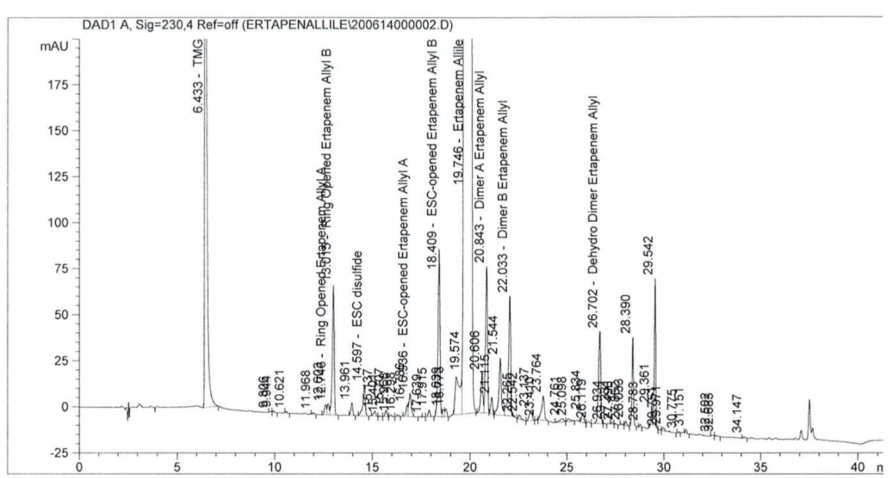

Figure 54. Related substances HPLC chart of Ertapenem allyl, prior purification, see section 4.1.6

In order to reduce the impurity load a filtration over HP20 resin is performed on column. HP20 is a divinylbenzene/styrene copolymer based resin, therefore working for Hydrophobic Interaction Chromatography (HIC), which has affinity for lipophilic impurities. Performing such purification as filtration through column removes lipophilic impurities from Ertapenem Allyl ester carbamate solution by absorption of the latter onto resin. Ratio between Ertapenem Allyl ester vs amount of resin has been optimized in order to balance yield drop with purity enhancement.

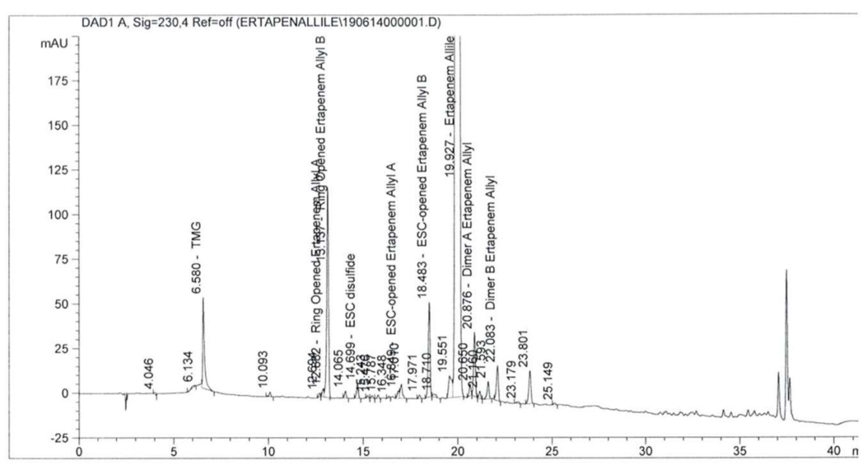

Figure 55. Related substances HPLC chart of Ertapenem allyl, after purification, see section 4.1.6

As it can be observed from comparison of figures 54-55 resin column treatment removes, partially TetraMethylGuanidine (TMG) (by removing head fractions) and removes lipophilic impurities eluting at a retention time higher than 25 min.

Once Ertapenem Allyl ester carbamate (**93**) solution is purified by HIC, Ertapenem Allyl ester (**5**) is crashed out of solution, simply breaking the carbamate by pH adjustment down to 5.0 with diluted HCl. Ertapenem Allyl ester (**5**) formed is not anymore soluble in water therefore it precipitates out of solution as an amorphous solid.

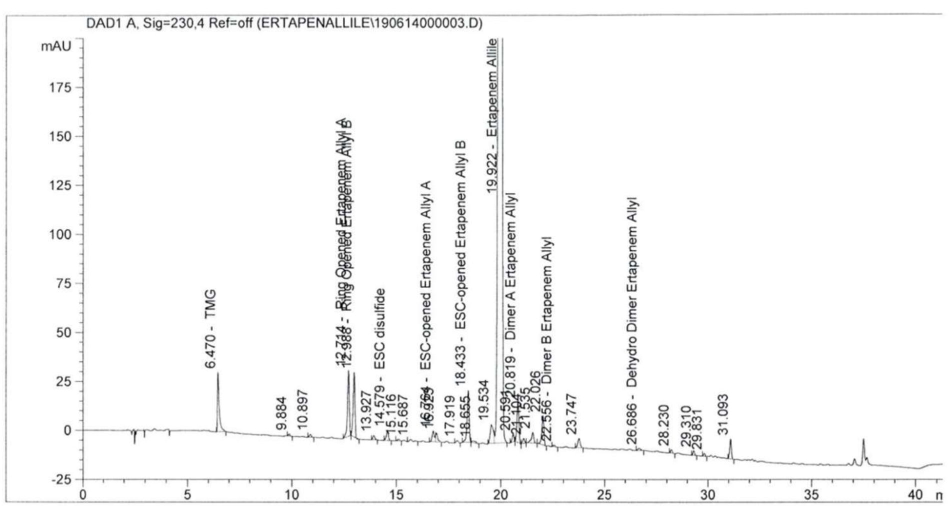

Figure 56. Related substances HPLC chart of Ertapenem allyl, solid, see section 4.1.6

As it can be observed from comparison of figures 55-56 Ertapenem Allyl ester (**5**) precipitation does not introduce any benefit in purification, since no effective impurity purge is achieved. Ertapenem Allyl ester (**5**) precipitation is only performed with the purpose to isolate an intermediate, store it in cold room and have a solid intermediate to proceed in next step.

As it can be observed in table 14, purity of Ertapenem Allyl (**5**) solid is similar to pre-column solution other than removal of TMG. For few impurities, such as Open ring Erta-Allyl and Dimer A Erta-Allyl the solid is even worse than pre-column solution. This behavior opens the possibility to use such solution telescopically in next step. To telescope means that such solution is progressed into the next step without any further purification isolation stages. However, for the sake to facilitate development of the deprotection step the isolation of Ertapenem Allyl (**5**) solid has been considered beneficial.

Table 14. Ertapenem Allyl typical Quality in-process

Item	Before purification	After purification	Precipitated Solid at pH 5
Related Substances			
-TMG	5.6%	1.4%	n.d.
- Open ring Erta-Allyl	0.90%	3.3%	1.9%
- ESC disulfide	0.25%	0.23%	0.25%
- ESC opened Erta-Allyl	1.3%	1.6%	1.6%
- Dimer A Erta-Allyl	0.94%	0.98%	1.6%
- Dimer B Erta-Allyl	0.91%	0.61%	1.0%
- Dehydrodimer Erta-Allyl	0.68%	n.d.	0.61%
- Largest unsp.	0.47%	0.66%	0.42%
- Total impurities	14.4%	11.0%	9.8%

3.3. Ertapenem Allyl Impurities

The following impurities have been tentatively characterized in Ertapenem allyl:

Table 15. Ertapenem allyl impurity characterization[a]

Peak N.	Retention time	ID	Proposal Impurity
1	6.4 min.	Standard injection	Tetramethylguanidine
2	13.0 min.	m/z $[M+H]^+=534$	Ring Opened Ertapenem Allyl A (**94**)
3	13.3 min.	m/z $[M+H]^+=534$	Ring Opened Ertapenem Allyl B (**95**)
4	14.9 min	m/z $[M+H]^+=531$	ESC-disulfide (**96**)
5	17.2 min	m/z $[M+H]^+=782$	ESC-Opened Ertapenem Allyl A (**97**)
6	18.8 min.	m/z $[M+H]^+=782$	ESC-Opened Ertapenem Allyl B (**98**)

Peak N.	Retention time	ID	Proposal Impurity
7	20.2 min.	m/z $[M+H]^+=516$	Ertapenem Allyl (Main constituent) (**5**)
8	21.3 min.	m/z $[M+H]^+=1031$	Dimer A Ertapenem Allyl (**99**)
9	22.5 min	m/z $[M+H]^+=1031$	Dimer B Ertapenem Allyl (**100**)
10	27.1 min	m/z $[M+H]^+=1013$	Dehydro Dimer Ertapenem Allyl (**101**)

[a] *For method reference see section 4.1.7*

Impurities structural identification has been tentatively performed by LC-MS analysis of Ertapenem allyl (**5**) solution at end of synthesis. In addition, analogy with impurities retrieved in RLD drug product has also been considered to draft hypothetical structures.

3.3.1 ***TetraMethylGuanidine (TMG)***

It is a process impurity, a reagent actually, which carries over during work-up since it is soluble either in organic solvents and water. TMG is reduced by fractioning of head fractions over HP20 column purification step.

3.3.2 ***Ring Opened Ertapenem Allyl A + B*** (**94**) + (**95**)

Ring Opened Ertapenem Allyl A (**94**) and Ring Opened Ertapenem Allyl B (**95**) are tautomers in equilibrium. By only LC-MS it is not possible to distinguish between the two compounds. They are degradation impurities which are formed in aqueous media, since ring opening is essentially a hydrolysis.

3.3.3 ***ESC-disulfide*** (**96**)

It is a process impurity which is formed by oxidation of thiol side chain ESC. It is described in literature[35]. It can be limited by addition of tributylphosphine in reaction mixture.

3.3.4 ***ESC-Opened Ertapenem Allyl A + B*** (**97**) +(**98**)

ESC-Opened Ertapenem Allyl A (**97**) and ESC-Opened Ertapenem Allyl B (**98**) are tautomers in equilibrium. By only LC-MS it is not possible to distinguish between the two compounds. They are process impurities which arise from reaction of Ertapenem allyl **(5)** with a second molecule of thiol side chain ESC **(3)**. Thiol group in side chain is in fact a strong nucleophile, therefore it should be avoided to accumulate high concentration of ESC (**3)** that may attack the labile β-lactam ring. In this respect a dual addition of ESC (**3)** is performed over the coupling reactive step, in order to limit ESC (**3)** concentration and limit ESC opened related impurities.

3.3.5 ***Dimer A Ertapenem Allyl*** **(99)**

It is a degradation impurity which takes place from attack of pyrrolidine ring of one molecule to β-lactam moiety of a second molecule. Very common pathway in Ertapenem chemistry. It can be limited by formation of carbamate derivative which protects pyrrolidine ring. Dimer A Ertapenem Allyl **(99)** is structurally related and is the precursor of Ertapenem dimer I+II, (**69**) **+** (**70**).

3.3.6 ***Dimer B Ertapenem Allyl* (100)**

It is a degradation impurity which takes place from attack of side chain carboxylate of one molecule to β-lactam moiety of a second molecule. Dimer B Ertapenem Allyl (**100)** is structurally related and is the precursor of Ertapenem dimer III, (**71**).

3.3.7 ***Dehydro Dimer Ertapenem Allyl* (101)**

It is a degradation impurity which takes place from attack of side chain carboxylate of one molecule to pyrrolidine ring nitrogen atom of a second molecule. Dehydro Dimer Ertapenem Allyl (**101)** is structurally related and is the precursor of Ertapenem dehydro dimer (**72**) + (**73**).

3.4. Ertapenem Allyl Manufacturing Process (A)

3.4.1. PROCESS DESCRIPTION

The knowledge acquired in separated stages allowed us to outline a full manufacturing process that starts from POCA (**2**) and produces ErtapenemAllyl (**5**). Manufacturing process is high level described here by synthetic scheme of figure 57, flow chart of figure 58 and following process narrative. For a more detailed description see experimental part.

2 POCA

3

4

aq HCl

5

Figure 57. Ertapenem Allyl synthetic scheme

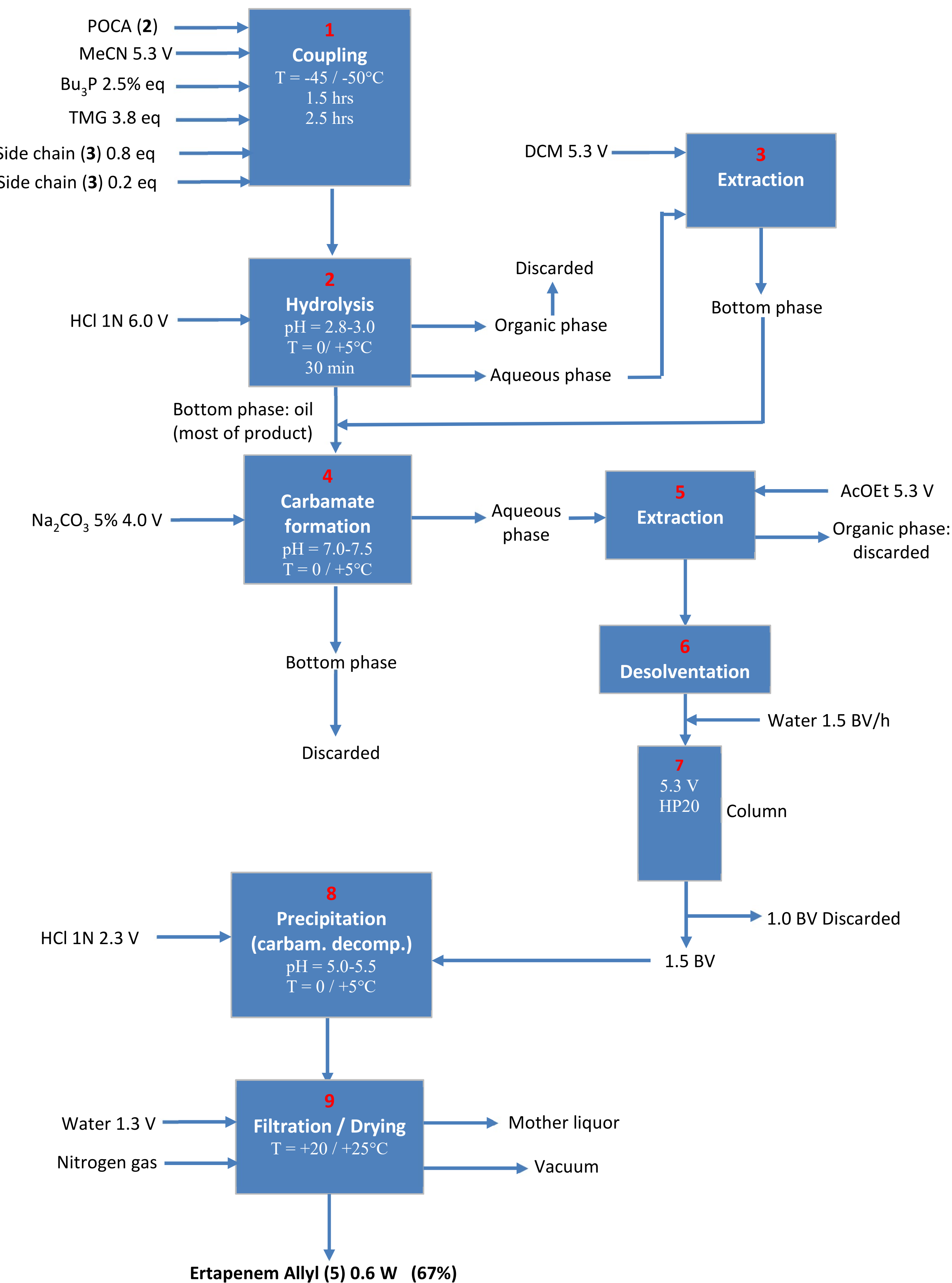

Figure 58. Ertapenem Allyl manufacturing process flow diagram

Manufacturing process high level narrative

For references to manufacturing stages, see flow diagram in figure 58

Stage 1 flow diagram

Coupling was performed in MeCN at about -50°C under Nitrogen atmosphere, Bu_3P (2.5% eq) was used to reduce Side chain thiol dimerization. Side chain was added in powder form in two different portions in order to reduce extent of ESC-Opened Ertapenem Allyl formation. Coupling completion was checked by means of HPLC.

Stage 2 flow diagram

Once coupling was achieved diluted HCl was added to hydrolyze silyl ether protecting group. Hydrolysis completion was checked by means of HPLC. Then upon settling three phases were obtained. Bottom oily phase contains most of the product, top organic layer was exhaust and therefore discarded (it removes p-ChlorophenylAllylthioether, and trimethylsilyl byproducts).

Stage 3 flow diagram

Intermediate aqueous layer was back extracted with DCM.

Stage 4 flow diagram

Oily phase and DCM layer from above back extraction were combined and treated with Sodium Carbonate aqueous solution at about 0°C, to form Ertapenem Allyl Carbamate. Upon settling lower exhaust organic layer was discarded.

Stage 5 flow diagram

Upper aqueous layer was washed with AcOEt to remove residual organic soluble impurities.

Stage 6 flow diagram

Rich aqueous solution was kept under vacuum to remove trace of organic solvents.

Stage 7 flow diagram

Aqueous solution was passed through a column containing a hydrophobic resin. Head fractions were discarded (to remove most of TMG). Column was eluted with demineralized water and a single main fraction was collected.

Stage 8 flow diagram

pH was adjusted to about 5 by diluted HCl addition to crash out Ertapenem Allyl.

Stage 9 flow diagram

Amorphous material was collected by filtration, washed with demineralized water and dried by nitrogen flow and vacuum.

67% molar yield of Ertapenem Allyl was obtained.

3.4.2 REPRESENTATIVE LABORATORY DEMO TRIALS

With the purpose to demonstrate robustness of above described manufacturing process, few representative laboratory batches were performed on a scale of 75 g of POCA.

Table 16. Ertapenem Allyl DEMO laboratory batches

Laboratory batch	**Starting POCA**	**Ertapenem allyl Output**	**Yield (%)**	**Purity**
FN 0414	75 g	46 g	68.0	93.8%
FN 0494	75 g	42 g	62.1	91.6%
FN 0504	75 g	44 g	65.0	92.3%

Table 17. Ertapenem Allyl DEMO batches quality

Item	FN 0414	FN 0494	FN 0504	Average	Dev Std
Related Substances					
-TMG	0.46%	0.35%	0.63%	0.48%	0.1
- Open ring Erta-Allyl	0.63%	0.82%	0.66%	0.70%	0.1
- ESC disulfide	0.17%	0.22%	0.23%	0.21%	0
- ESC opened Erta-Allyl	0.70%	1.5%	1.8%	1.3%	0.6
- Dimer A Erta-Allyl	0.90%	2.0%	1.4%	1.4%	0.6
- Dimer B Erta-Allyl	1.4%	1.2%	1.0%	1.2%	0.2
-Dehydrodimer Erta-Allyl	0.85%	0.57%	0.51%	0.64%	0.2
- Largest unsp.	0.35%	0.48%	0.41%	0.41%	0.1
- Total impurities	6.2%	8.4%	7.7%	7.4%	1.1

DEMO laboratory manufacturing evidenced a variability of Ertapenem Allyl process mainly in terms of quality. Ertapenem Allyl DEMO manufacturing has however provided an overall quality superior to that evidenced over small scale development, see table 14. This unexpected outcome was attributed to process variability.

Even if process was demonstrated not to be really robust and consistent it was decided to proceed into a pilot manufacturing with the purpose to acquire process experience and accumulate material for further processing.

3.4.3 ERTAPENEM ALLYL PILOT MANUFACTURING

Based on reproducibility observed over laboratory DEMO batches a pilot manufacturing was performed for Ertapenem Allyl scale up. Pilot manufacturing was performed on 8.1 Kg POCA scale employing 150 L stainless reactors coupled with a 60 L column.

Table 18. Ertapenem Allyl Pilot manufacturing performances

Batch	Type	Starting POCA	Ertapenem allyl Output	Yield (%)	Purity
FN 0554	Laboratory use test	75 g	45 g	66.5	90.2%
FN 0584	Pilot manufacturing	8.1 Kg	5.0 Kg	68.4	83.0%

As it appears in above table, Ertapenem Allyl pilot manufacturing provided yield performances very similar to that observed in laboratory DEMO batches and confirmed in laboratory use test. Use test is a laboratory run performed right before manufacturing, using all materials employed in manufacturing scale up.

On other hand, purity of Ertapenem Allyl from pilot manufacturing resulted lower than that observed at laboratory scale. In order to analyze the reasons of such discrepancy, quality observed in-process over pilot batch is shown in the following table.

Table 19. Ertapenem Allyl Quality in-process Pilot manufacturing vs Use test[a]

Item	Before column		After column		Solid	
	Use test	*Pilot*	*Use test*	*Pilot*	*Use test*	*Pilot*
Related Substances						
-TMG	5.6%	4.4%	1.4%	0.71%	n.d.	0.19%
- Open ring Erta-Allyl	0.90%	1.8%	3.3%	4.1%	1.9%	3.8%
- ESC opened Erta-Allyl	1.3%	1.5%	1.6%	1.8%	1.6%	1.6%
- Dimer A Erta-Allyl	0.94%	0.79%	0.98%	0.86%	1.6%	4.4%
- Dimer B Erta-Allyl	0.91%	0.66%	0.61%	0.66%	1.0%	1.2%
- Dehydrodimer Erta-Allyl	0.68%	1.0%	n.d.	n.d.	0.61%	1.4%
- Largest unsp.	0.47%	0.91%	0.66%	0.57%	0.42%	0.70%
- Total impurities	14.4%	14.8%	11.0%	10.8%	9.8%	17.0%

[a]the items with discrepancy between use test and pilot have been highlighted in yellow

As it appears in above table use test and pilot run provided similar performances up to the solution post column. On other hand in final solid it can be observed a consistent discrepancy between use test

and pilot material, this is particularly observed for Open ring Erta-Allyl, Dimer A Erta-Allyl and Dehydrodimer Erta-Allyl. Since those impurities are degradation byproducts most likely they have grown over drying.

To further analyze pilot manufacturing, the final drying stage is therefore focused in below table.

Table 20. Ertapenem Allyl pilot Quality over drying Pilot manufacturing vs Use test

Item	**Use Test**	**Pilot Manufacturing**		
		12 hrs drying	*48 hrs drying*	*End of drying (72 hrs)*
Related Substances				
- Open ring Erta-Allyl	1.9%	1.8%	2.6%	3.8%
- ESC opened Erta-Allyl	1.6%	1.7%	1.4%	1.6%
- Dimer A Erta-Allyl	1.6%	1.5%	3.3%	4.4%
- Dimer B Erta-Allyl	1.0%	1.2%	1.2%	1.2%
- Dehydrodimer Erta-Allyl	0.61%	0.5%	1.3%	1.4%
- Largest unsp.	0.42%	0.5%	0.6%	0.70%
- Total impurities	9.8%	10.4%	13.2%	17.0%

As it can be observed in above table up to 12 hrs drying the quality of pilot batch was very similar to that of Use test. Discrepancy between Use test and Pilot manufacturing is therefore due to degradation experienced during prolonged drying. In fact, in laboratory trials drying takes place overnight, on other hand at manufacturing scale drying took 3 days.

In conclusion first manufacturing experience was positive, even if the quality of the material obtained from pilot scale is lower than expected. Pilot manufacturing provided a substantial amount of Ertapenem intermediate valid for development of next step, involving deprotection of allyl ester and isolation of Ertapenem Sodium.

3.5 Ertapenem Allyl Deprotection Development

3.5.1 Deprotection Overview

Starting from isolated Ertapenem Allyl ester (**5**), we have studied and optimized the deprotection step to afford Ertapenem API.

$(Ph_3P)_4Pd$

5

Ertapenem Sodium 1

Figure 59. Ertapenem Allyl deptrotection synthetic scheme

Allyl ester deprotection is a well-known procedure working under very mild conditions which are suitable for acid- and base-sensitive molecules such as carbapenems[44,45]. Such deprotection procedure involves Pd^0 catalytic pathway where Pd forms π-Allyl complex.

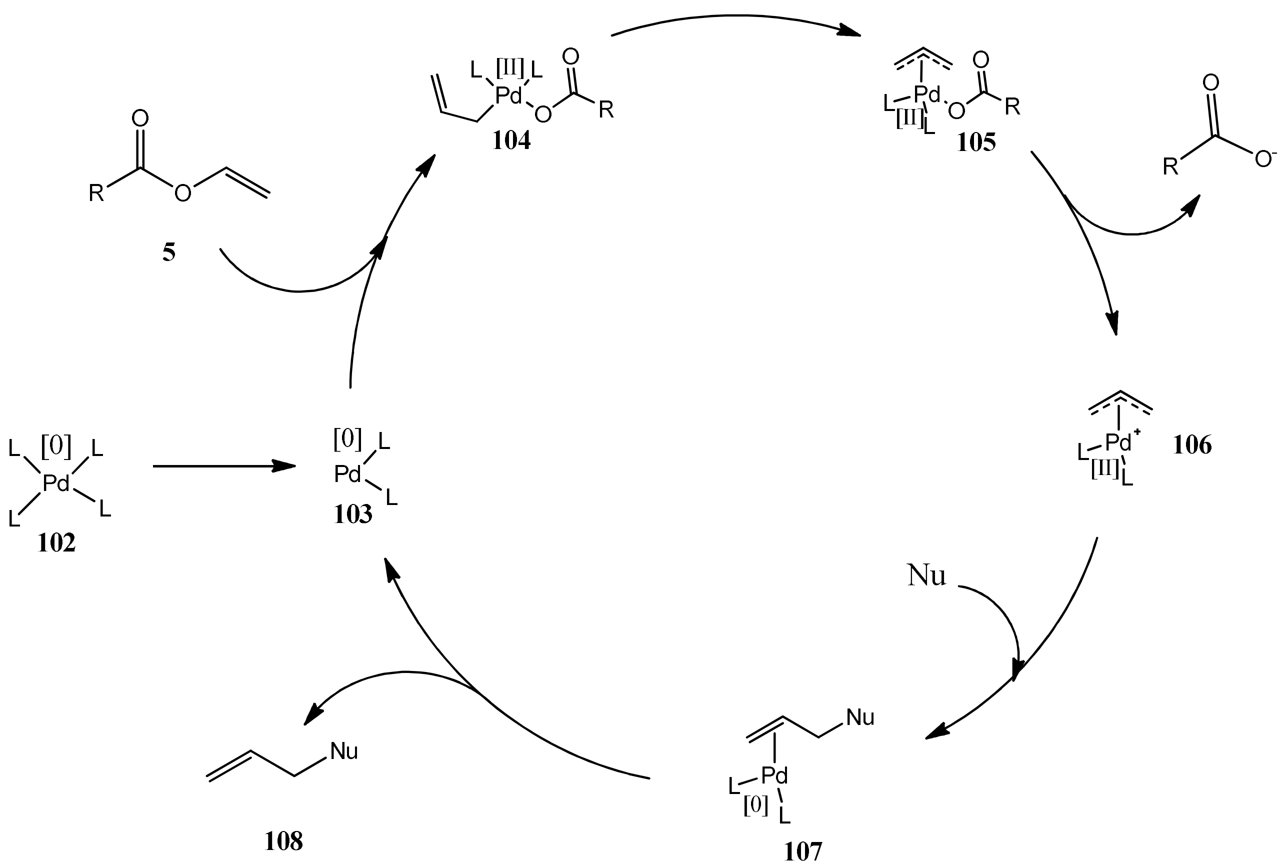

Figure 60. Allyl ester deprotection mechanism

Pd^0tetrakis **(102)** is a stable complex with 18 electrons, to initiate a catalytic cycle Pd^0tetrakis releases two ligands generating a reactive Pd^0 complex (**103**) with 14 electrons. Complex (**103**) undergoes oxidative addition of Ertapenem allylester (**5**) to provide unstable Pd^{+2} σ-allyl complex (**104**) with 16 electrons, which immediately rearranges to provide Pd^{+2} π-allyl complex (**105**) with 18 electrons. Complex (**105**) releases Ertapenem carboxylate to form Pd^{+2} π-allyl complex (**106**) with 16 electrons. Complex (**106**) undergoes S_N2 attack of a nucleophile to form Pd^0 π- complex (**107**) with 16 electrons. Finally, complex (**107**) releases allylated nucleophile (**108**) by decomplexation to re-form reactive complex (**103**).

Such deprotection is already implemented on industrial scale at ACS-DOBFAR in the Meropenem (**44**) synthesis. In fact, removal of allyl ester and allyloxycarbonyl protecting groups is involved, in final stage of Meropenem synthesis. Meropenem di-allyl precursor (**63**) (Figure 61) is therefore treated with tetrakis(triphenylphosphine)palladium(0) [$(Ph_3P)_4Pd$] in presence of a large excess of N-MethylAniline.

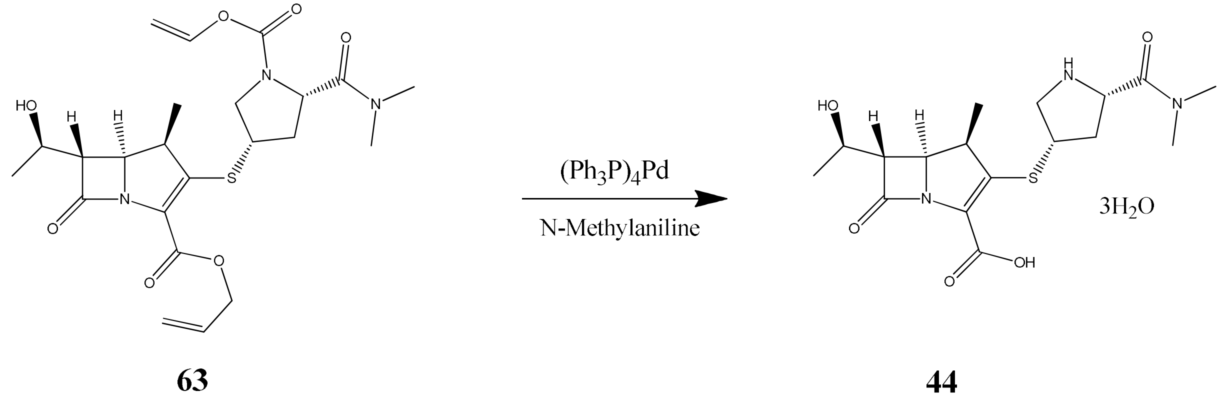

Figure 61. Meropenem Allyl ester ALLOC deptrotection

The development of Ertapenem allyl ester was therefore facilitated by Meropenem know-how acquired by ACS Dobfar.

Several methods have systematically been developed for deprotection of the allyl group. In this context particularly useful are the mild deprotections of allyl esters explored by Nagakura[46] for unstable penems, which are nucleophile sensitive. In order to find the best experimental conditions to achieve Ertapenem Allyl ester deprotection, we have investigated several allyl scavengers including sulfinic salt in several solvents or mixture of solvents. The results are listed as following:

3.5.2 SOLVENT AND ALLYL SCAVENGER SCREENING

SOLVENT

As solvent for this step were tested, THF, THF/H_2O, THF/CO_2 and DCM. DCM was selected since provided precipitation of Ertapenem while deprotection occurs with a good morphology.

TRAPPING NUCLEOPHILE

Role of trapping nucleophile is to react with π-Allyl Pd complex (**106**), to capture Allyl cation and release Pd^0 complex (**103**) to restart a new cycle, see figure 60.

Many different nucleophiles were tested as per below table:

Table 21. Trapping nucleophile screening for allyl removal

Allyl Scavenger	Yield[a]
Aniline	78%
N-Methylaniline	81%
Imidazole	Degradation
Sodium 2-ethylexanoate	Not reacted
Indole	Not reacted
Triethylsylane	Not reacted
Morpholine	Degradation
4-aminopyridine	Not reacted
Morpholine acetate buffer	22%
Anisole	Not reacted
Benzimidazole	Degradation
6-aminoindazole	Degradation
2-benzylaminopyridine	2%
2-aminopyridine	9%
2,6-dimethylaniline	39%
Sodium p-toluensulfinate	49%
Thiosalicylic acid	76%
Pyrrolidine	Degradation
Dimedone	Not reacted
p-chlorothiophenol	Not reacted
Tetramethylguanidine	Degradation
Trimethylsilylpyrrolidine	Degradation
Trimethylsilyldiethylamine	60%

[a]*measured in solution by HPLC*

The best results were observed with Aniline, N-methylaniline and thiosalycilic acid. N-methylaniline was selected since provided the best results and is less toxic than other two nucleophiles. A large excess (> 4 eq) of trapping nucleophile is required since Ertapenem also intrinsically contains a strong nucleophile in pyrrolidine ring which can be alkylated by π-allyl Pd^0 complex (**106**) to form N-allyl Ertapenem (**52**), as depicted in figure 62. An excess of trapping nucleophile reduces the formation of such byproduct.

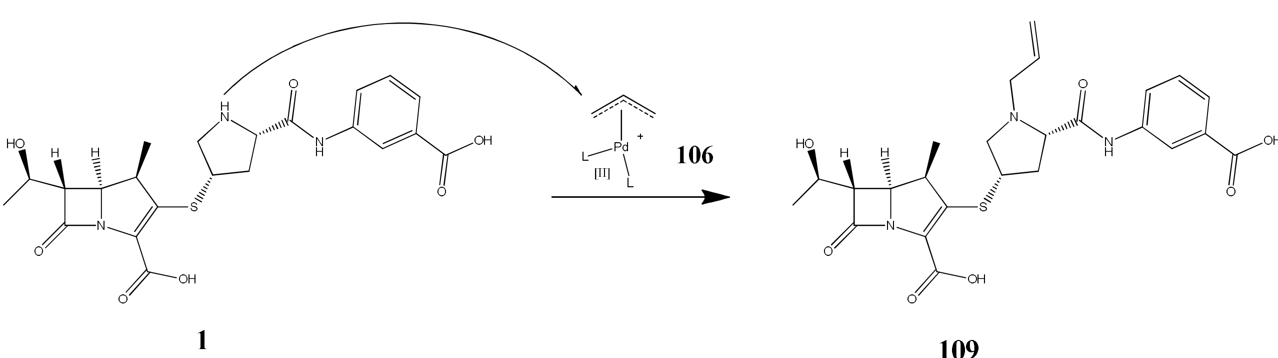

Figure 62. Ertapenem deprotection byproduct

3.5.3 DIBUTYLPHOSPHATE EFFECT

An addition of 0.3 equivalents of Bu_2HPO_4 has been found beneficial to reduce Ertapenem N-Allyl byproduct (**109**) formation.

The generation of the N-allyl Ertapenem (**109**) as byproduct was not negligible, as it reached systematically at least 3% of the deprotected substrate. Therefore, following the strategy used for Meropenem case we decided to add Bu_2HPO_4. In fact, it is believed that organic soluble phosphate acts as a weak inorganic and DCM soluble, protonating the ertapenem pyrrolidine nitrogen atom, with the generation of an ion pair (**110**) that keeps pyrrolidine protected from allyl cation addition (figure 63).

110

Figure 63. Ertapenem/Bu_2HPO_4 hypothetical adduct

The addition of a sub stoichiometric amount of Bu_2HPO_4 implemented the efficiency of the deprotection step, reducing the byproduct below 1% (table 21).

Table 22. Bu_2HPO_4 role in Ertapenem N-Allyl byproduct formation

Bu_2HPO_4	**Ertapenem N-Allyl**
No addition	$> 3\%$
0.3 eq	$< 1\%$

3.5.4 DEPROTECTION PERFORMANCE

Deprotection takes place in about 2.5 hours after which the consumption of Ertapenem Allyl and formation of Ertapenem can be observed, see figure 64

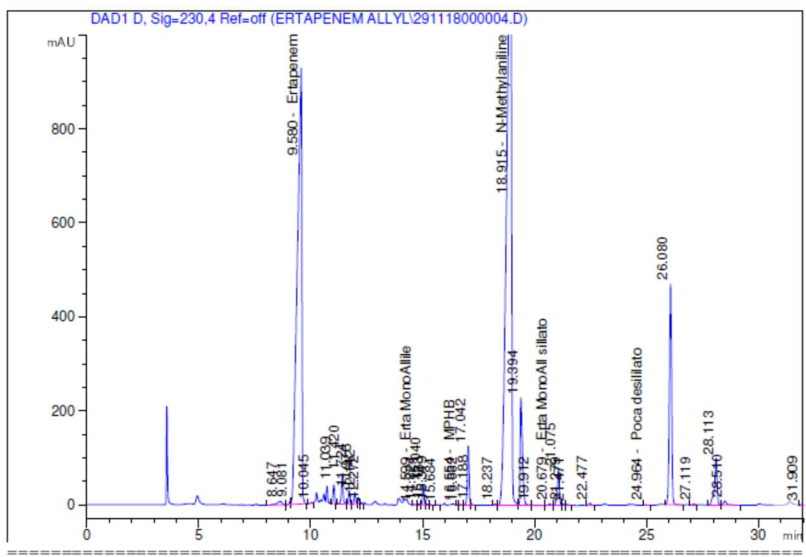

Figure 64. In Process Control *(IPC) HPLC chart at end of deprotection, see section 4.1.5*

In above IPC deprotection HPLC chart it can be noticed disappearance of Ertapenem Allyl ester (**5**) (Rt =14.6 min) and formation of Ertapenem (**1**) (Rt =9.6 min) together with the peaks of N-MethylAniline (with a retention time Rt =18.9 min) and N-Methyl, N-AllylAniline (Rt 26.1 min).

3.5.5 ERTAPENEM RECOVERY

Ertapenem is observed to come out of solution while deprotection proceed, therefore it has been tried to recover Ertapenem by simple filtration. The quality of Ertapenem isolated in such way shows several out of specification which do not purge even with a couple of crystallizations, see below table.

Table 23. Quality of Ertapenem recovered by filtration and further recrystallizations[a]

Test	Ertapenem Sodium Specifications	Ertapenem precipitated at end of reaction FN 1103	Ertapenem Sodium (first re-crystallization) BG 0054	Ertapenem Sodium (second re-crystallization) BG 0104
Related Substances				
Specified Identified Impurities:				
-Oxazinone	NMT 0.20%	n.d.	0.03%	0.01%
-Ring Opened	NMT 1.5%	0.90%	0.73%	0.38%
-ProMABA	NMT 0.40%	n.d.	0.01%	0.03%
-Dimer I+II	NMT 0.70%	**1.6%**	**1.2%**	0.63%
-Dimer III	NMT 0.20%	**1.1%**	**0.68%**	**0.53%**
-Dehydro dimer a+b	NMT 0.20%	**0.92%**	**0.89%**	**0.22%**
Unspecified Impurities:				
- Single Unspecified impurity	NMT 0.10%	**1.9% (RRT2.3)**	**0.36% (RRT1.5)**	**0.27% (RRT 1.2)**
- Total Impurities	NMT 3.0%	**14.1%**	**6.2%**	**3.3%**

[a]*item in red are out of specifications*

Ertapenem directly precipitated out of reaction mixture have proved to be particularly impure, the main impurities that are far away from specification are dimer I+II (**69, 70**), dimer III (**71**), dehydrodimers (**72, 73**) and an impurity eluting at Relative Retention Time (RRt) 2.3. Two consecutive re-crystallizations were not effective to get Ertapenem Sodium within specifications.

3.5.6 ALTERNATIVE WORK-UP

Alternatively, to direct filtration it has been exploited the approach already employed for Ertapenem Allyl (**5**) to extract Ertapenem in aqueous phase, by treatment with a carbonic acid salt. Once Ertapenem is extracted in water at neutral pH, excess of N-methylaniline and its allylated form are washed away in the organic layer. It is a known[43] feature of either Meropenem and Ertapenem to form carbamate on side chain pyrrolidine ring by treatment with a carbon dioxide donor at basic pH (pH>7.5).

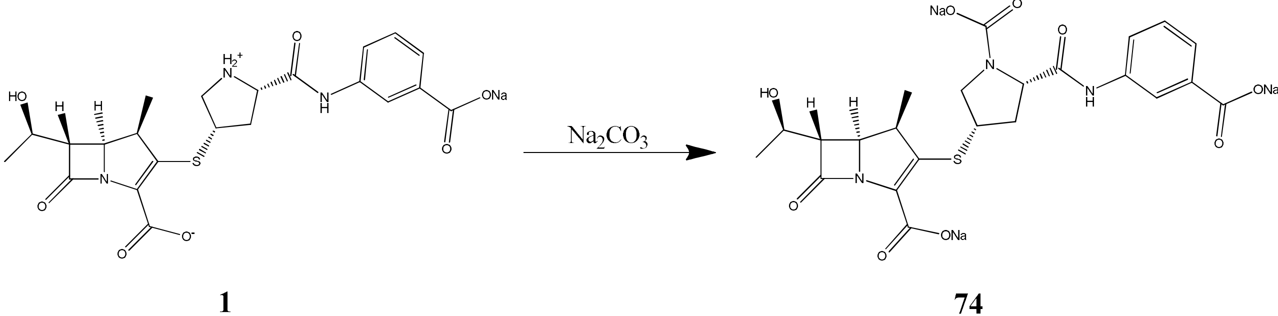

Figure 65: Ertapenem carbamate formation

Therefore, the suspension at the end of deprotection step was treated with sodium carbonate solution to adjust pH to neutrality, the carbamate derivative (**74**) is formed and solubilized in water, see figure 65. In addition, the carbamate establishes a transient protection of pyrrolidine ring providing a higher stability of Ertapenem in aqueous solution. In fact, pyrrolidine ring is a strong nucleophile center that causes oligomerization of Ertapenem, therefore increasing the impurity load of crude mixture. To prove this concept few Ertapenem solution stability studies were performed.

Table 24. Stability of aqueous solution of Ertapenem at pH 7.5 by Sodium Bicarbonate[a]

Related Substances	**t0**	**2h**	**6h**	**24h**	**24h (+25C)**[b]
Specified Identified Impurities:					
-Oxazinone	0.10%	0.11%	0.15%	0.25%	2.2%
-Ring Opened	3.0%	3.1%	3.2%	3.8%	8.3%
-ProMABA	0.11%	0.10%	0.13%	0.11%	0.86%
-Dimer I	0.12%	0.55%	0.98%	1.7%	3.7%
-Dimer II	0.28%	0.78%	2.1%	7.1%	19.5%
-Dimer III	0.72%	0.73%	0.73%	0.74%	0.89%
-Dehydrodimer a+b	0.66%	0.66%	0.68%	0.66%	0.53%
Unspecified Impurities:					
- Single Unspecified impurity	1.0%	1.9%	2.0%	1.7%	5.7%
- Total Impurities	9.1%	10.9%	13.1%	19.9%	53.8%

[a]120 mg/mL at 5°C b: after 24 hours at +5°C the sample was left additional 24 hours at +25°C

Table 25. Stability of aqueous solution of Ertapenem at pH 5.5[a]

Related Substances	**t0**	**2h**	**6h**	**24h**	**24h (+25C)[b]**
Specified Identified Impurities:					
-Oxazinone	0.10%	0.15%	0.26%	0.50%	Fully degraded
-Ring Opened	2.6%	2.8%	3.6%	6.5%	Fully degraded
-ProMABA	0.10%	0.10%	0.11%	0.11%	Fully degraded
-Dimer I	0.11%	0.41%	0.96%	2.0%	Fully degraded
-Dimer II	0.31%	1.5%	4.6%	10.7%	Fully degraded
-Dimer III	0.8%	1.0%	1.9%	3.9%	Fully degraded
-Dehydrodimer a+b	0.64%	0.72%	0.86%	1.2%	Fully degraded
Unspecified Impurities:					
- Single Unspecified impurity	0.61%	0.59%	1.4%	3.3%	Fully degraded
- Total Impurities	8.2%	10.7%	18.5%	38.0%	Fully degraded

[a]120 mg/mL at 5°C b: after 24 hours at +5°C the sample was left additional 24 hours at +25°C

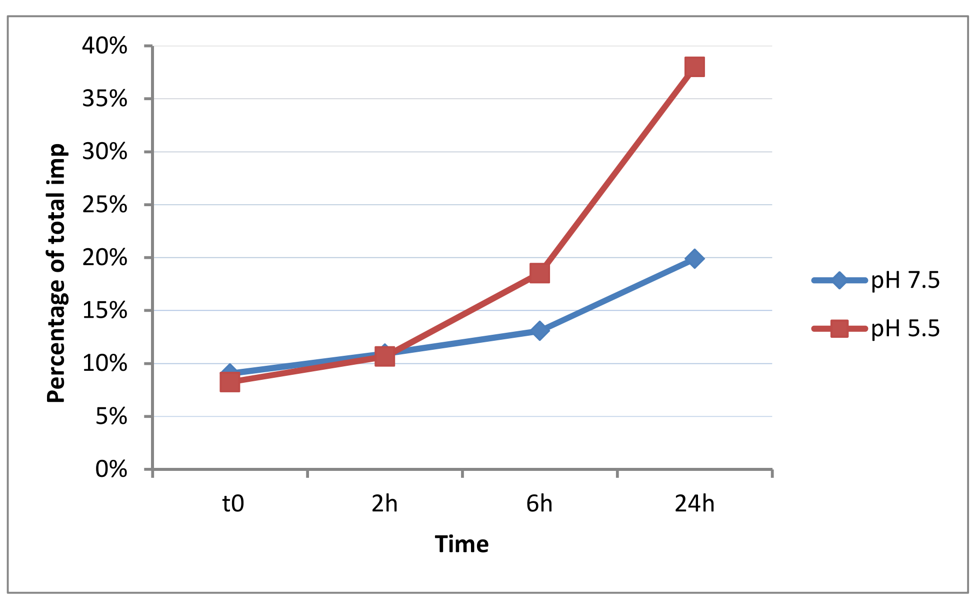

Figure 66. Total impurities behavior in solution 120 mg/mL at 5°C

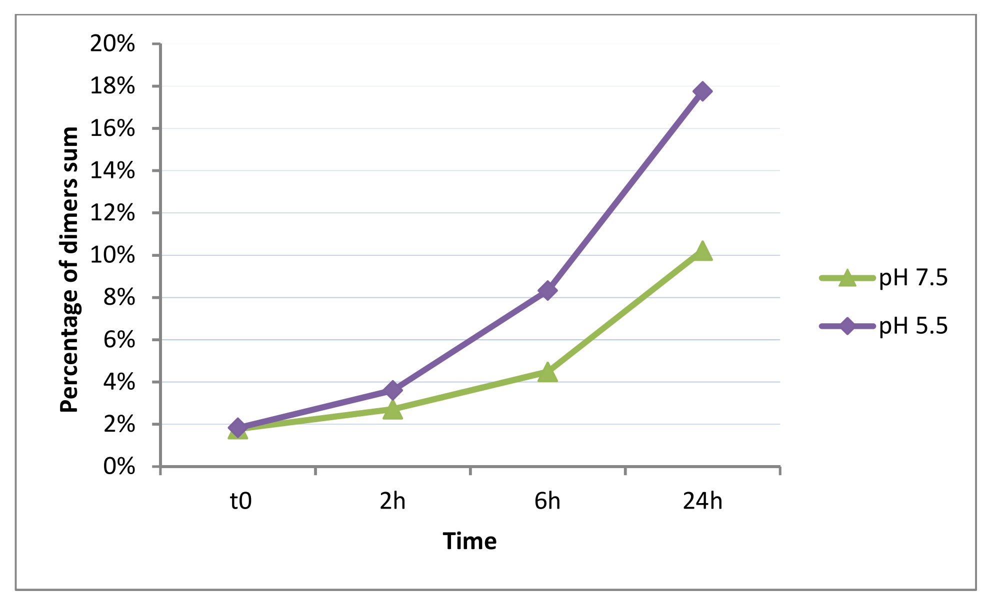

Figure 67. Dimers behavior in solution 120 mg/mL at 5°C

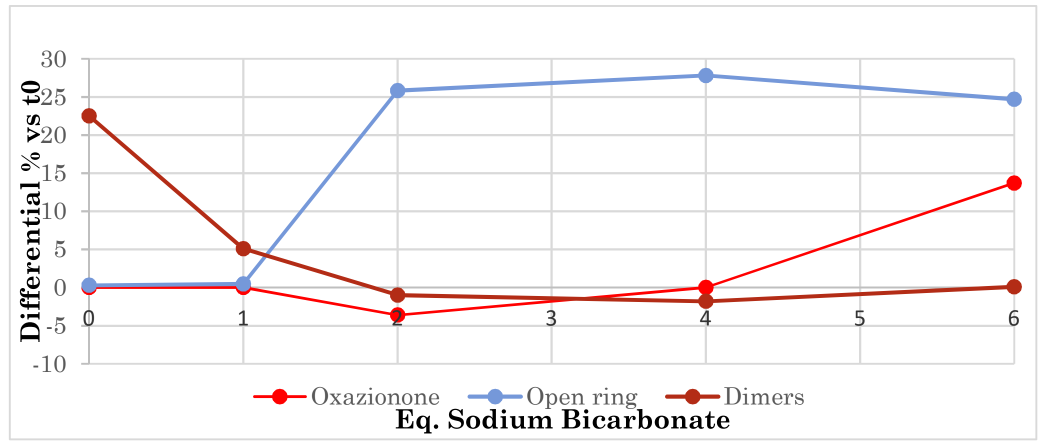

Figure 68. Ertapenem solution pH 7.5, stability vs eq $NaHCO_3$ 2hrs 3°C

NOTE: Points 0 and 1 eq $NaHCO_3$ were adjusted to pH 7.5 with NaOH

From acquired stability studies it is clear that Ertapenem is more stable in solution at pH 7.5 when this is achieved by addition of 1 eq carbonate. Below 1 eq $NaHCO_3$ extensive formation of dimers is observed, above 1 equivalent open ring and oxazinone degradations becomes important (figure 68).

Once Ertapenem is extracted in water as carbamate (**74**), phases are separated and Ertapenem Sodium (**1**) is recovered by pH adjustment (5.5) with AcOH/MeOH and precipitation with isopropanol/MeOH addition.

3.5.7 ERTAPENEM INCOMPATIBILITY WITH ACETONE

After recovery of the Ertapenem Sodium by filtration, it was noticed that a rinse with acetone of wet paste was apparently beneficial since the powder took a good morphology and dried quickly. Unfortunately, it has been noticed that acetone reacts with Ertapenem forming a related substance which is an adduct of Ertapenem and acetone. When Ertapenem is put in contact with Acetone it has been noticed a new impurity eluting at RRT 1.10 which has the mass of Ertapenem + 58.

111

Figure 69. Ertapenem related impurity RRt 1.10 formed by reaction with Acetone

The hypothesis of RRt 1.10 structure, see figure 69, have been drawn in analogy with Hetacillin (**112**) which is produced by reaction of Ampicillin (**9**) with Acetone[47], see figure 70. Ampicillin has the same amino-amido groups pattern as Ertapenem.

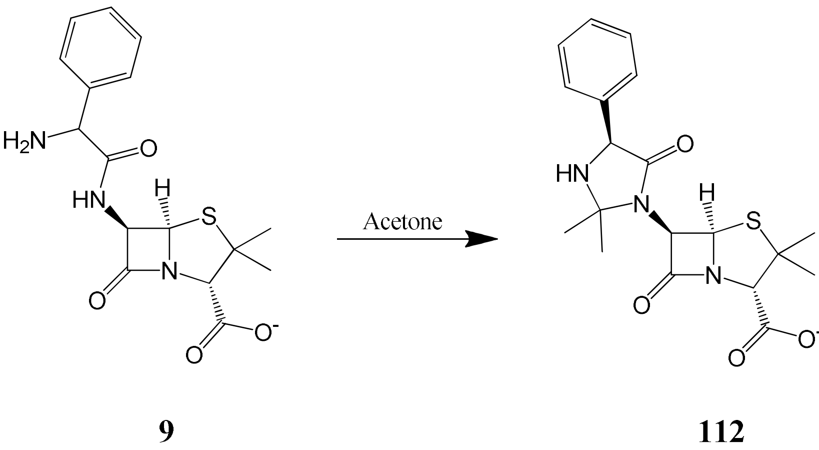

Figure 70. Hetacillin formation from Ampicillin

Since acetone is not suitable for a final wash on Ertapenem sodium wet paste, methyl acetate was found a good alternative.

3.5.8 ERTAPENEM SODIUM QUALITY FROM AQUEOUS WORK-UP

Quality of isolated Ertapenem Sodium was found definitely improved in comparison to those achieved by direct filtration, as the total impurities were 2-3% *vs* 14%. Quality was further improved by Ertapenem Sodium recrystallization. As it can be observed in below table, only one impurity remains out of specification, RRt 1.61, which is N-Allyl impurity identified by LC-MS (**109**) already described in figure 62.

Table 26. Ertapenem Sodium quality from aqueous work up[a]

Related Substances	**Ertapenem sodium Specifications**	**FN 0104** *Erta Na crude*	**FN 0144** *Erta Na crude*	**FN 0344** Ertapenem Sodium[b]
Specified Identified Impurities:				
-Oxazinone	NMT 0.20%	0.09%	0.04%	0.02%
-Ring Open	NMT 1.5%	0.43%	0.57%	0.44%
-ProMABA	NMT 0.40%	nd	<0.03%	nd
-Dimer I +II	NMT 0.70%	0.28%	0.54%	0.34%
-Dimer III	NMT 0.20%	0.13%	0.18%	0.09%
-Dehydro dimer a+b	NMT 0.20%	0.20%	**0.60%**	0.04%
Unspecified Impurities:				
- Single Unspecified impurity	NMT 0.10%	**0.22%**[c]	**0.25%**[c]	**0.19%**[c]
- Total Impurities	NMT 3.0%	2.1%	**3.3%**	1.4%

[a]item in red are out of specifications [b]FN 0104 + FN 0144 mix 1:1, recrystallized
[c]RRt 1.61

3.5.9 RESIN TREATMENT

In order to remove impurity RRt 1.61, which is the only out of specification in Ertapenem Sodium re-crystallized, a resin treatment was introduced on aqueous solution before isolation.

Table 27. Ertapenem Sodium quality from aqueous work up and resin treatment[a]

Test	**Ertapenem Sodium crude FN0624**	**Ertapenem Sodium recryst. after resin treatment FN0634**	**Specifications**
Related Substances			
- Oxazinone	n.d.	n.d.	NMT 0.20%
- Open Ring	0.57%	0.41%	NMT 1.5%
- Promaba	0.08%	n.d.	NMT 0.40%
- Dimer I+II	0.45%	0.14%	NMT 0.70%
- Dimer III	**0.33%**	0.10%	NMT 0.20%
- Dehydrodimer a+b	**0.56%**	n.d.	NMT 0.20%
- Largest unspecified	**0.19%**	n.d.	NMT 0.10%
- Total impurities	3.0%	0.65%	NMT 3.0%

[a]*item in red are out of specifications*

As it can be observed introducing a resin treatment on Ertapenem aqueous solution prior to isolation led to isolation of Ertapenem Sodium quality that meets specification by a single re-crystallization.

Resin treatment in between Ertapenem Sodium crude and Ertapenem Sodium reduces the recrystallization yield of about 5%, due to Ertapenem retains on the resin.

3.5.10 ERTAPENEM SODIUM YIELD OVERVIEW

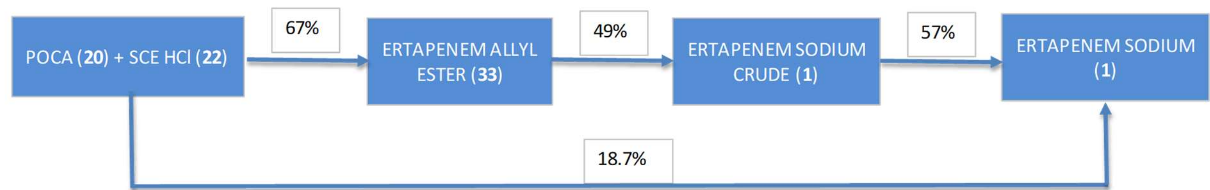

Figure 71. Whole Ertapenem Sodium yield overview

The overall yield for the synthesis of the Ertapenem Sodium is very low, as highlighted in Figure 43.

There are two main factors that drops the yield down:

1) The use of HIC resin (HP20L) for both Ertapenem Allyl and on Ertapenem Sodium purification, reduces the purification yields as the resin retains part of the products

2) The high solubility of Ertapenem Sodium in aqueous media causes a high loss of Ertapenem Sodium in mother liquor. Additionally, two crystallizations are involved in whole process.

According to data from tables 26-27 it appears that avoiding a recrystallization from Ertapenem Crude to Ertapenem Sodium may be very difficult. On other hand a possible approach to improve process yield is to avoid isolation of Ertapenem Allyl. In fact, in table 14 it was noticed that from Ertapenem Allyl end of synthesis to isolated Ertapenem Allyl there were no substantial improvement, in the impurity profile after crystallization. Therefore, Ertapenem Allyl solution seemed adequate for a sequential one-pot synthesis without isolation, synthesising directly Ertapenem Sodium (**telescoping** synthesis). HIC resin treatment on Ertapenem Allyl it may avoided, as well.

The experience gained in deprotection starting from solid Ertapenem Allyl was applied on a direct process that starts from POCA, produces Ertapenem Allyl as DCM solution which is immediately converted to Ertapenem crude. Such direct process provided a yield of 49% which is a breakthrough compared to 33% provided by the previous process which involved Ertapenem Allyl isolation.

3.6. ERTAPENEM SODIUM CRUDE MANUFACTURING PROCESS (B)

3.6.1. PROCESS DESCRIPTION

The knowledge acquired in separated stages allowed to outline a telescopic manufacturing process that starts from POCA (**2**) and produces Ertapenem Sodium (**1**) crude. Manufacturing process is high level described here by synthetic scheme of figure 72, flow chart of figure 73 and following process narrative. For a more detailed description see experimental part.

Figure 72. Ertapenem Sodium Crude synthetic scheme

This scheme represents the telescopic direct process for the synthesis of Ertapenem Sodium crude from POCA (**2**) and is therefore the combination of schemes in figure 46, 51 and 59.

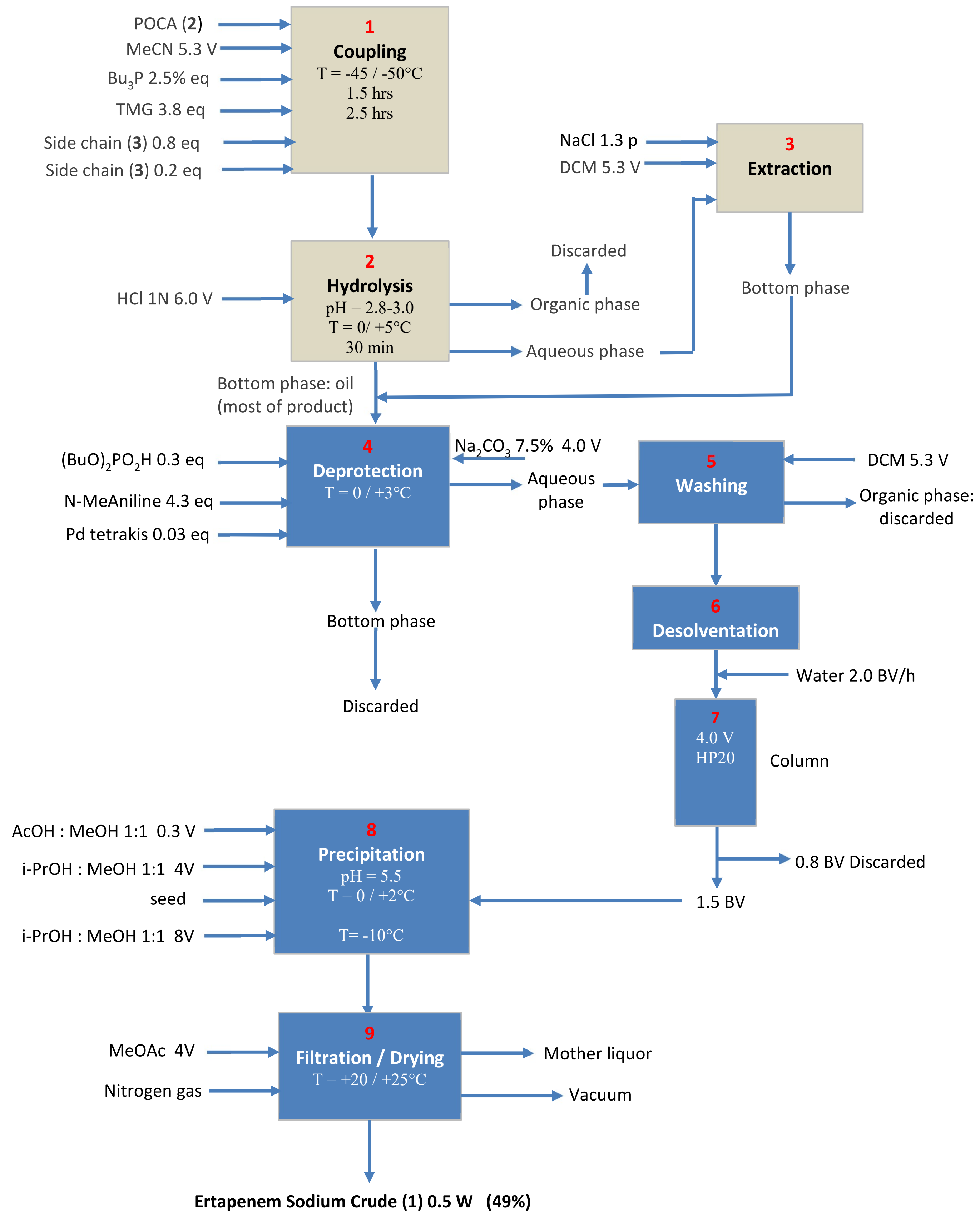

Figure 73. Ertapenem Sodium crude manufacturing process flow diagram[a]

[a]boxes in grey represents stages unchanged vs manufacturing process A

Manufacturing process high level narrative

For references to manufacturing stages, see flow diagram in figure 73. Text in grey represents stages which are unchanged vs manufacturing process A

Stage 1 flow diagram

Coupling was performed in MeCN at about -50°C under Nitrogen atmosphere, Bu_3P (2.5% eq) was used to reduce Side chain thiol dimerization. Side chain was added in powder form in two different portions in order to reduce extent of ESC-Opened Ertapenem Allyl formation. Coupling completion was checked by means of HPLC.

Stage 2 flow diagram

Once coupling was achieved diluted HCl was added to hydrolyze silyl ether protecting group. Hydrolysis completion was checked by means of HPLC. Then upon settling three phases were obtained. Bottom oily phase contains most of the product, top organic layer was exhaust and therefore discarded (it removes p-ChlorophenylAllylthioether, and trimethylsilyl byproducts).

Stage 3 flow diagram

Intermediate aqueous layer was back extracted with DCM with NaCl aid.

Stage 4 flow diagram

Oily phase and DCM layer from above back extraction were combined and cooled down to 0°C. Dibutylphosphate, N-Methylaniline and Pdtetrakis were loaded under Nitrogen atmosphere. Deprotection completion was checked by means of HPLC. Once deprotection was achieved, reaction mixture was treated with Sodium Carbonate aqueous solution, to form Ertapenem Carbamate. Upon settling lower exhaust organic layer is discarded.

Stage 5 flow diagram

Upper aqueous layer was washed with DCM to remove residual organic soluble impurities (N-Methylaniline and N-Allyl N-Methylaniline).

Stage 6 flow diagram

Rich aqueous solution was kept under vacuum to remove trace of organic solvents.

Stage 7 flow diagram

Aqueous solution was adjusted to pH 7.9 and passed through a column containing a hydrophobic resin. Head fractions were discarded, then column was eluted with demineralized water and a single main fraction was collected.

Stage 8 flow diagram

Temperature was adjusted to about 0°C and pH corrected to 5.6 by MeOH:AcOH addition. Precooled IPA:MeOH were added and crystallization initiated by seeding. Additional precooled IPA:MeOH were added and stirring was maintained for 15/20 hrs at -10°C.

Stage 9 flow diagram

Ertapenem Sodium was collected by filtration, washed with MeOAc and dried by nitrogen flow and vacuum.

49% molar yield of Ertapenem Sodium was obtained.

3.6.2 REPRESENTATIVE LABORATORY DEMO TRIALS

With the purpose to demonstrate robustness of above described manufacturing process, few representative laboratory batches were performed on a scale of 150 g of POCA.

ERTAPENEM SODIUM CRUDE

Table 28. Ertapenem Sodium Crude DEMO laboratory batches

Laboratory batch	Starting POCA	Ertapenem Sodium crude		Yield (%)	Purity
		Output	Potency[a]		
FN 0105	150 g	75.0 g	84.0%	50.5	97.8%
FN 0115	150 g	74.0 g	82.1%	48.7	97.4%
FN 0125	150 g	73.5 g	83.0%	48.9	97.1%

[a]*assay as Ertapenem* $C_{22}H_{25}N_3O_7S$ *vs external standard*

Table 29. Ertapenem Sodium Crude DEMO batches quality

Item	Ertapenem Sodium Crude FN0105	Ertapenem Sodium Crude FN0115	Ertapenem Sodium Crude FN0125	Average	Std Dev σ
Related Substances					
- Oxazinone	n.d.	n.d.	n.d.	-	-
- Ring Opened	0.21%	0.25%	0.20%	0.22%	0.03
- PROMABA	n.d.	n.d.	n.d.	-	-
- Dimer I+II	0.29%	0.34%	0.22%	0.28%	0.06
- Dimer III	0.18%	0.21%	0.20%	0.20%	0.02
-Dehydrodimer a+b	0.27%	0.30%	0.27%	0.28%	0.02
- Largest unsp.	0.18%[a]	0.35%[a]	0.53%[b]	0.35%	0.18
- Total imp.	1.3%	2.6%	2.9%	2.3%	0.85

[a]RRt 1.65 [b]RRt 1.79

DEMO laboratory manufacturing evidenced a good reproducibility of Ertapenem Sodium Crude process either in terms of yield and most items of quality. A certain variability was observed for largest unspecified impurity and total impurities. However, in general quality of Ertapenem Sodium Crude as reported in table 29, was good and it was almost complying specifications for Ertapenem Sodium. The items which were slightly out of specification (Dimer III, Dehydrodimers and single unspecified impurity) purged efficiently by a simple recrystallization from Ertapenem Sodium Crude to Ertapenem Sodium.

ERTAPENEM SODIUM

Ertapenem Sodium Crude produced in laboratory DEMO manufacturing was further recrystallized to produce Ertapenem Sodium. The following tables reports data achieved.

Table 30. Ertapenem Sodium DEMO laboratory batches

Laboratory batch	Starting Ertapenem Sodium Crude		Ertapenem Sodium		Yield (%)	Purity
	Input	Batch	Output	Potency[a]		
FN 0135	70 g	FN 010/5	50.0 g	88.0%	74.8	98.3%
FN 0145	70 g	FN 011/5	51.0 g	85.5%	75.9	98.9%
FN 0155	70 g	FN 012/5	54.5 g	85.3%	80.0	99.1%

[a]*assay as Ertapenem $C_{22}H_{25}N_3O_7S$ vs external standard*

Table 31. Ertapenem Sodium DEMO batches quality[a]

Item	Ertapenem Sodium Crude FN0105	Ertapenem Sodium FN0135	Ertapenem Sodium Crude FN0115	Ertapenem Sodium FN0145	Ertapenem Sodium Crude FN0125	Ertapenem Sodium FN0155	Specifications Ertapenem Sodium
Related Substances							
- *Oxazinone*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	NMT 0.20%
- *Ring Opened*	0.21%	0.12%	0.25%	0.18%	0.20%	0.11%	NMT 1.5%
- *PROMABA*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	NMT 0.40%
- *Dimer I+II*	0.29%	0.62%	0.34%	0.34%	0.22%	0.26%	NMT 0.70%
- *Dimer III*	0.18%	0.12%	0.21%	0.12%	0.20%	0.09%	NMT 0.20%
- *Dehydrodimer a+b*	0.27%	0.13%	0.30%	0.10%	0.27%	0.06%	NMT 0.20%
- *Largest unsp.*	0.18%	0.07%	0.35%	0.09%	0.53%	0.05%	NMT 0.10%
- *Total imp.*	1.3%	1.7%	2.6%	1.1%	2.9%	0.9%	NMT 3.0%

[a]*item in red are out of specifications*

Recrystallization of Ertapenem Sodium Crude has produced Ertapenem Sodium fully complying with required specifications. Therefore, Ertapenem Sodium Crude manufacturing process has been considered suitable for scale up.

3.6.3 ERTAPENEM SODIUM CRUDE PILOT MANUFACTURING

Based on consistency and reproducibility observed over laboratory DEMO batches a pilot manufacturing was performed for Ertapenem Sodium Crude scale up. Pilot manufacturing was performed on 8.1 Kg POCA scale employing 150 L stainless reactors coupled with a 60 L column.

Table 32. Ertapenem Sodium Crude Pilot manufacturing performances

Batch	Type	Starting POCA	Ertapenem Sodium Crude Output	Yield (%)	Purity
FN 0955	Laboratory use test	150 g	67 g	45.4	96.8%
FN 0965	Pilot manufacturing	8.1 Kg	3.8 Kg	50.4	89.4%

As it appears in above table, Ertapenem Sodium Crude pilot manufacturing provided yield performances very similar to that observed in laboratory DEMO batches. As far as quality is concerned purity of pilot batch in comparison with use test is provided in the following table:

Table 33. Ertapenem Sodium Crude PILOT batch quality[a]

Item	Ertapenem Sodium Crude Use test FN0955	Ertapenem Sodium Crude PILOT FN0965	Specifications Ertapenem Sodium
Related Substances			
- *Oxazinone*	n.d.	n.d.	NMT 0.20%
- *Ring Opened*	0.33%	0.80%	NMT 1.5%
- *PROMABA*	n.d.	n.d.	NMT 0.40%
- *Dimer I+II*	0.40%	2.0%	NMT 0.70%
- *Dimer III*	0.27%	1.3%	NMT 0.20%
- *Dehydrodimer a+b*	0.33%	0.89%	NMT 0.20%
- *Largest unsp.*	0.50%	0.94%	NMT 0.10%
- *Total imp.*	3.2%	10.6%	NMT 3.0%

[a]*item in red are out of specifications*

Purity of Ertapenem Sodium Crude from pilot manufacturing resulted far away from that observed at laboratory scale. In order to analyze the reasons of such discrepancy, quality observed in-process over pilot batch manufacturing is shown in the following graphs in comparison with the same observed over use test performance.

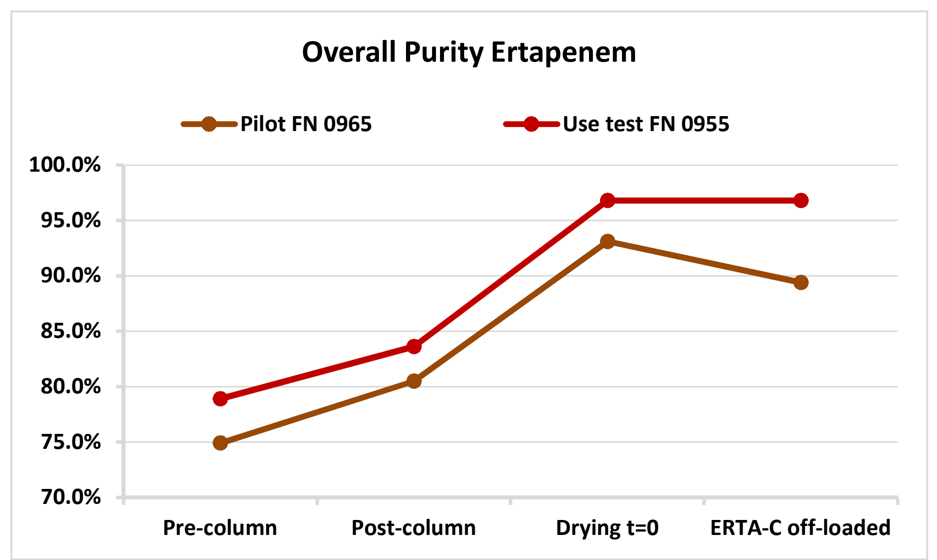

Figure 74. Ertapenem Sodium Crude overall purity over progress of process

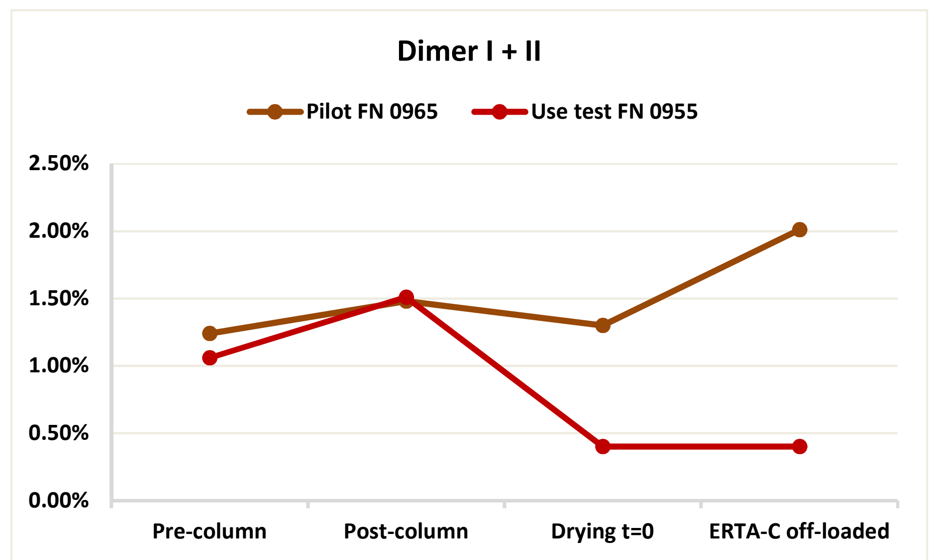

Figure 75. Ertapenem Sodium Crude Dimer I+II over progress of process

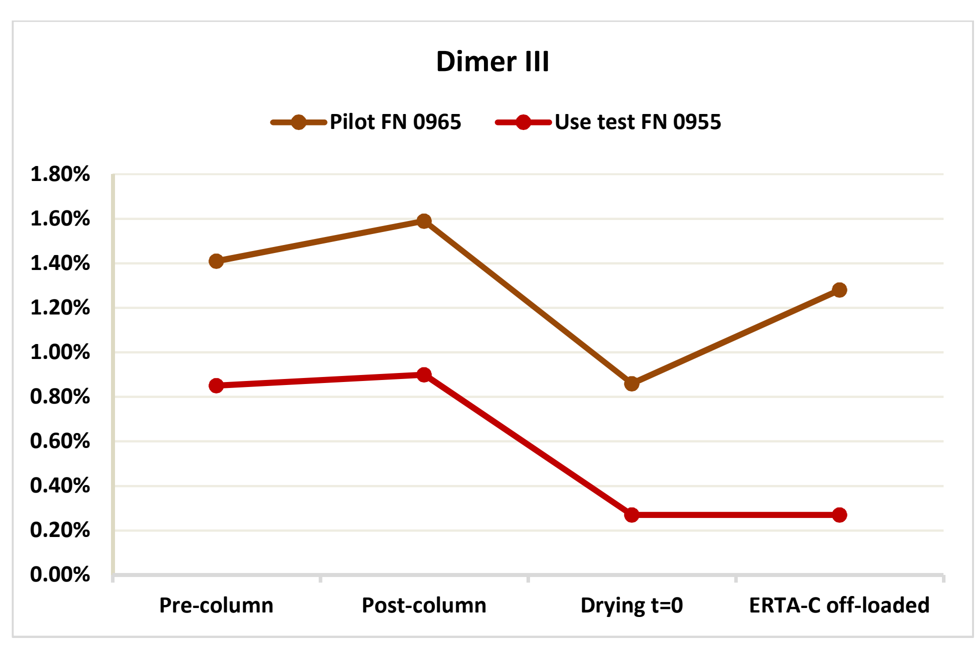

Figure 76. Ertapenem Sodium Crude Dimer III over progress of process

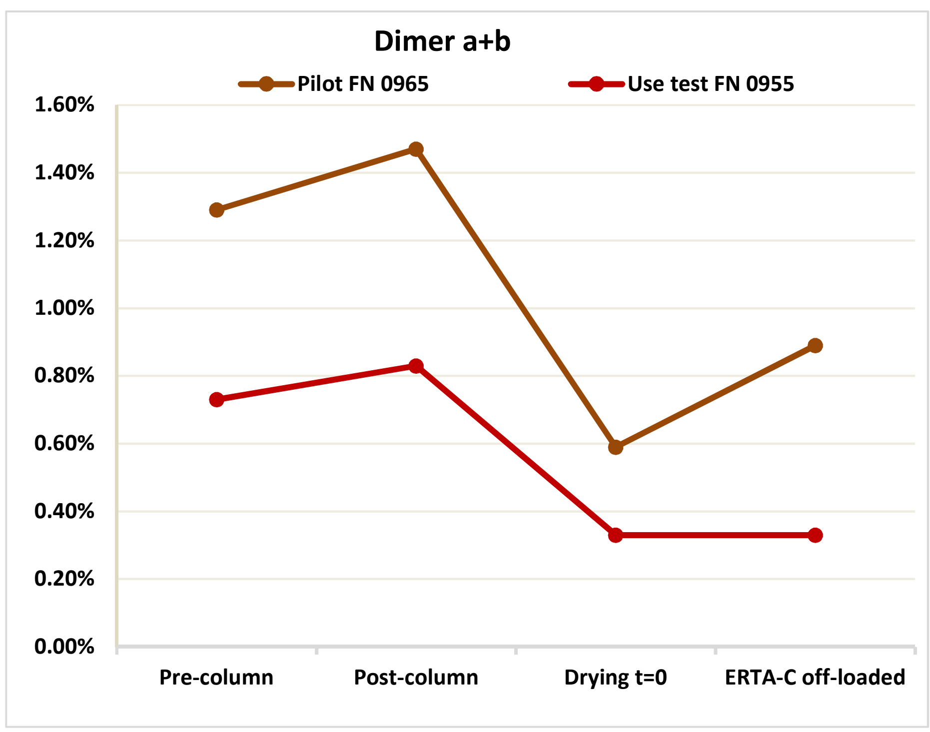

Figure 77. Ertapenem Sodium Crude Dehydrodimer a+b over progress of process

As it can be observed from above graphs pilot manufacturing provided an overall purity slightly lower than use test at end of synthesis (pre-column data point). However, after that point, the pilot batch

behavior of purity run similarly to use test up to the beginning of drying as it can be observed in figure 74 slopes. Upon drying the two batches definitely diverge each other, with the pilot batch loosing purity over drying. Same behavior can be observed for Dimer III and dehydrodimers a+b in figures 75-76. On other hand the behavior of Dimer I+II in figure 75 evidenced another issue, since in addition to what already observed for other impurities, it can be even observed a deviation in the purge over precipitation. In fact, data points post column and drying time zero, have in between the precipitation of Ertapenem Sodium from aqueous solution. In pilot batch, such precipitation has provided essentially no purge of Dimer I+II, compared to use test were conversely an efficient purge is observed (from 1.5 to 0.4%).

In addition to that, the drying step has been further investigated evaluating the change in purity as a function of the progress of the drying process. The latter has been evaluated measuring the water content by Karl Fisher test (K.F.). The results are summarized in figure 78.

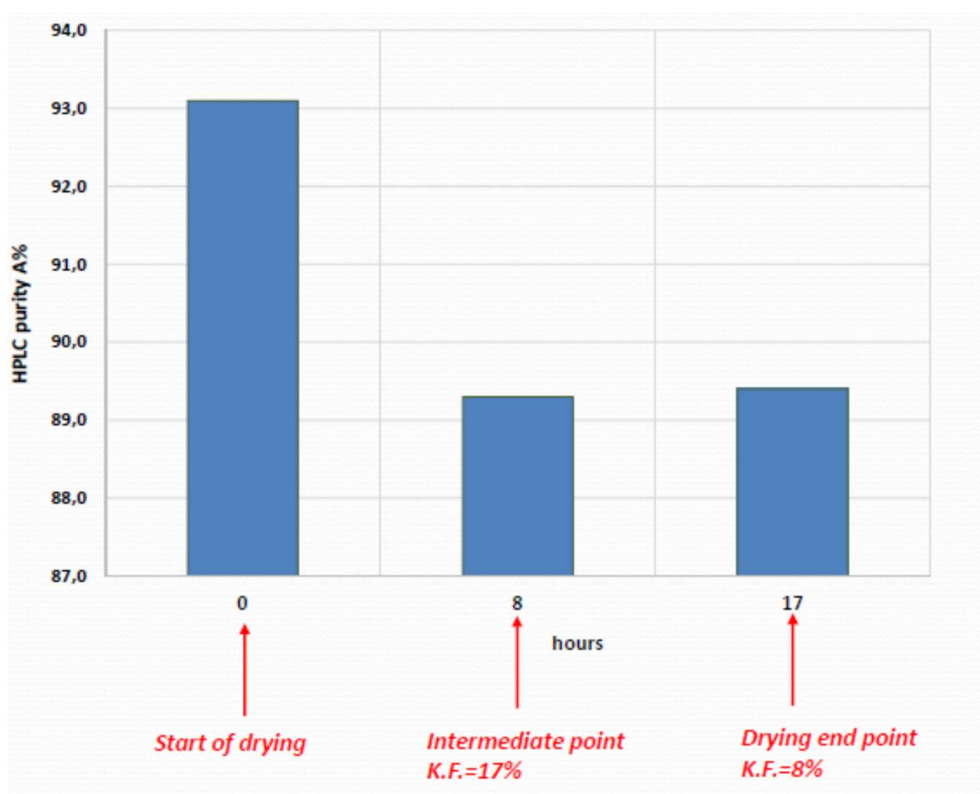

Figure 78. *Ertapenem Sodium Crude Pilot drying profile*

As it appears in above graph Ertapenem Sodium purity have drop over the first 8 hours of drying, when water content was observed 17%. After that point overall purity was maintained essentially unchanged.

In conclusion second manufacturing experience was positive, since demonstrated the manufacturing feasibility of Ertapenem Sodium Crude (**1**) by a telescopic process from POCA (**2**). Precipitation of Ertapenem Sodium from aqueous solution and subsequent drying should be improved to avoid the issues experienced in this pilot manufacturing.

To improve drying and avoid degradation is necessary to reduce the time to remove residual water down below 15-17%. In order to achieve such target there are two possible areas of action:

1) To dimension drying equipment to reach such capacity, which may be challenging from technological point of view.
2) To improve the morphology of Ertapenem Sodium crude (**1**) solid to facilitate removal of liquid constituted by water and organic solvents, in order to have a solid that at the end of filtration retains less liquid phase, therefore facilitating drying step.

3.7. ERTAPENEM SODIUM TELESCOPIC PROCESS IMPROVEMENTS

3.7.1. EVALUATION OF MOISTURE IN ERTAPENEM ALLYL DEPROTECTION

Over development of manufacturing process and in particular when telescopic approach is considered, the DCM solution containing Ertapenem Allyl is coming from aqueous work up, see figure 79.

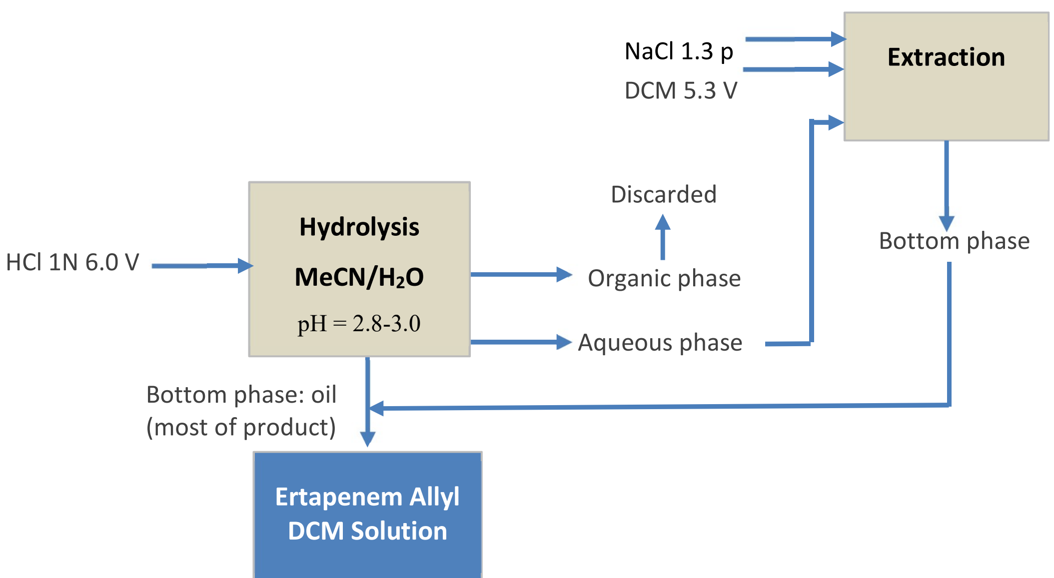

Figure 79. Ertapenem Allyl Process Flow Diagram Extract

It has been noticed that Ertapenem Allyl DCM solution provides a water content of about 3%. Stability studies, see section 3.5.6, indicate that Ertapenem degrades in aqueous solution especially at low pH. Based on such observations it has been considered to reduce water content in Ertapenem Allyl DCM solution prior to proceed to remove allyl ester protecting group.

The rationale of this thought is to generate Ertapenem API in almost dry DCM to preserve Ertapenem stability. Once ester deprotection is achieved, aqueous work up is performed. However, in that case the use of Sodium Carbonate produce Ertapenem carbamate in aqueous solution which has been observed to be sufficient stable at 0/+5°C, see section 3.5.6.

To proof this concept a couple of twin trials were performed. A single preparation of Ertapenem Allyl DCM solution was performed, according to the process described in section 3.6.1. At that point Ertapenem Allyl DCM solution was split in two equal portions, one has been processed directly into the deprotection step, the other has been partially dried, prior to proceed into deblocking step. Both trials were completed up to the isolation of Ertapenem Sodium Crude. Differently from what described in section 3.6.1, in both trials the chromatographic purification step was omitted. Most relevant data of this twin trials have been reported in the following table.

Table 34. Effect of Ertapenem Allyl DCM solution moisture, over deblocking step

TRIAL ID	Ertapenem Allyl DCM solution Water Content	Ertapenem Sodium Crude Quality			
		Purity	Dimer I+II	Dimer III	Dehydro Dimers a+b
FN 0026	3%	90.0%	1.4%	1.1%	2.2%
MBE 0016	0.8%	96.7%	0.39%	0.19%	0.84%

As it appears from above table, moisture content into the deprotection step has a remarkable effect on the quality of Ertapenem Sodium Crude, mainly on dimers impurity profile. Therefore, the hypothesis to reduce impurities by reducing water content from the Ertapenem allyl deprotection step has been verified on experimental basis and this finding opened the way for skipping the column purification in Ertapenem Sodium manufacturing process.

3.7.2. EVALUATION OF ANTISOLVENTS COMPOSITION ON ERTAPENEM SODIUM PRECIPITATION

At the end of manufacturing process Ertapenem Sodium is recovered by precipitation from the aqueous solution by addition of antisolvents.

Among water soluble solvents acetone was not considered since found incompatible with Ertapenem, see figure 69, therefore alcohols were tested as follows:

1) MeOH : 2-Propanol 1:1 as described in section 3.6.1
2) MeOH : 1-Propanol 1:1 as described in Merck literature[32]

Mixture with 2-propanol provides a higher recovery since Ertapenem Sodium is less soluble.

Two twin trials were completed. A single preparation of Ertapenem Sodium was performed, according to the process described in section 3.6.1. Once Ertapenem Sodium was extracted in aqueous solution by Sodium Carbonate the trials were split in two equal portions, one was precipitated by MeOH: 2-Propanol, the other was precipitated by MeOH : 1-Propanol. Differently from what described in section 3.6.1 in both trials the column purification step was omitted. Most relevant data of these trials have been reported in the following table.

Table 35. Effect of Antisolvents composition, over Ertapenem Sodium precipitation

TRIAL ID	Antisolvents	Ertapenem Sodium Crude Quality			
		Purity	**Dimer I+II**	**Dimer III**	**Dehydro Dimers a+b**
FN 004/6	MeOH : 2-Propanol	91.9%	0.75%	0.47%	2.1%
MBE 002/6	MeOH : 1-Propanol	97.6%	0.13%	0.11%	0.58%
MBE 003/6	MeOH : 2-Propanol	92.4%	1.9%	1.1%	0.72%
FN 005/6	MeOH : 1-Propanol	97.2%	0.48%	0.39%	0.30%

As it appears from above table, antisolvents composition may influence the quality of Ertapenem Sodium Crude, mainly on dimers impurity profile. MeOH : 1-Propanol antisolvents have proved to be more efficient in purging dimers related substances.

Therefore, this further finding open the way to the removal of column purification in Ertapenem Sodium manufacturing process, as well.

These two improvements outlined here above:

1) Effect of moisture over allylester deprotection step
2) Effect on antisolvents composition on the Ertapenem Sodium purity

In conjunction to the already existing manufacturing process set up, allowed to point out an improved Ertapenem Sodium manufacturing process which did not involve a HIC column purification.

In particular, moisture reduction in Ertapenem Allyl DCM solution was achieved by a freezing step where DCM solution is cooled to -30°C. Most of the water is therefore forming ice, which is removed simply by a vessel to vessel transfer, very similar as per phases separation. This straightforward operation allows to reduce moisture <1%, from a starting moisture of about 3%, resulting in an improved purity at end of deprotection from about 90 to about 97%.

3.8. ERTAPENEM SODIUM CRUDE MANUFACTURING PROCESS (C)

3.8.1. PROCESS DESCRIPTION

Ertapenem Sodium Crude (1) manufacturing process has been previously described in section 3.6.1. On the basis of those additional data acquired in section 3.7 were addressed in an improved manufacturing process C that starts from POCA (**2**) and produces Ertapenem Sodium (**1**) crude, without use of HIC purification in the work-up. Manufacturing process is high level described here by synthetic scheme of figure 80, flow chart of figure 81 and following process narrative. For a more detailed description see experimental part.

Figure 80. Ertapenem Sodium Crude synthetic scheme

This scheme represents the telescopic direct process for the synthesis of Ertapenem Sodium crude which is identical to the scheme shown in figure 72, with exception that HP20 resin is removed.

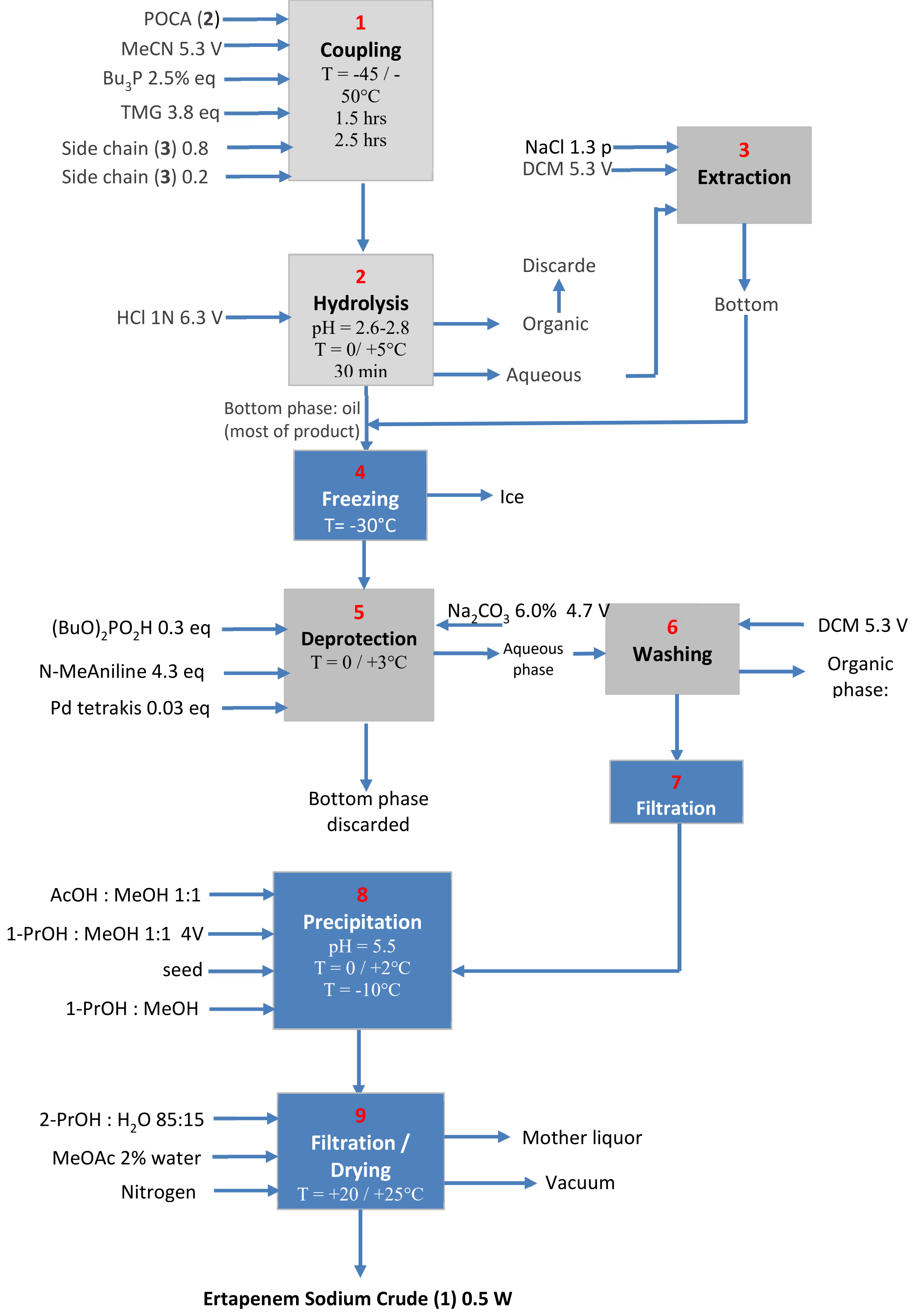

Figure 81. Ertapenem Sodium crude manufacturing process flow diagram[a]

[a]*boxes in grey represents stages unchanged vs manufacturing process B*

Manufacturing process high level narrative

For references to manufacturing stages, see flow diagram in figure 81. Text in grey represents stages which are unchanged vs manufacturing process B

Stage 1 flow diagram

Coupling was performed in MeCN at about -50°C under Nitrogen atmosphere, Bu_3P (2.5% eq) was used to reduce Side chain thiol dimerization. Side chain was added in powder form in two different portions in order to reduce extent of ESC-Opened Ertapenem Allyl formation. Coupling completion was checked by means of HPLC.

Stage 2 flow diagram

Once coupling was achieved diluted HCl was added to hydrolyze silyl ether protecting group. Hydrolysis completion was checked by means of HPLC. Then upon settling three phases were obtained. Bottom oily phase contains most of the product, top organic layer was exhaust and therefore discarded (it removes p-ChlorophenylAllylthioether, and trimethylsilyl byproducts).

Stage 3 flow diagram

Intermediate aqueous layer was back extracted with DCM with NaCl aid.

Stage 4 flow diagram

Oily phase and DCM layer from above back extraction were combined and cooled down to -30°C and hold for few hours. DCM layers was transferred into another vessel to remove ice.

Stage 5 flow diagram

Therefore, temperature was adjusted at about 0°C and Dibutylphosphate, N-MethylAniline and Pdtetrakis were loaded under Nitrogen atmosphere. Deprotection completion was checked by means of HPLC. Once deprotection was achieved, reaction mixture was treated with Sodium Carbonate aqueous solution, to form Ertapenem Carbamate. Upon settling lower exhaust organic layer is discarded.

Stage 6 flow diagram

Upper aqueous layer was washed with DCM to remove residual organic soluble impurities (N-Methylaniline and N-Allyl N-Methylaniline).

Stage 7 flow diagram

Rich aqueous solution was cartridge filtered to remove particulate.

Stage 8 flow diagram

Temperature was adjusted to about 0°C and pH corrected to 5.6 by MeOH:AcOH addition. 1-Propanol:MeOH were added maintaining 0°C and crystallization initiated by seeding. Additional precooled 1-Propanol:MeOH were added and stirring is maintained for 15/20 hrs at -10°C.

Stage 9 flow diagram

Ertapenem Sodium was collected by filtration, washed with 2-Propanol : water and MeOAc in sequence. Dried by nitrogen flow and vacuum.

49% molar yield of Ertapenem Sodium was obtained.

3.8.2 REPRESENTATIVE LABORATORY DEMO TRIALS

With the purpose to demonstrate robustness of above described manufacturing process, few representative laboratory batches were performed on a scale of 150 g of POCA.

Table 36 Ertapenem Sodium Crude DEMO laboratory batches

Laboratory batch	Starting POCA	Ertapenem Sodium crude		Yield (%)	Purity
		Output	Potency[a]		
FN 0086	150 g	72.0 g	84.1%	48.5	97.6%
FN 0096	150 g	78.0 g	84.8%	53.0	97.8%
MBE 0056	150 g	70.0 g	87.0%	48.8	96.9%

[a]*assay as Ertapenem $C_{22}H_{25}N_3O_7S$ vs external standard*

Table 37. Ertapenem Sodium Crude DEMO batches quality

Item	**Ertapenem Sodium Crude FN0086**	**Ertapenem Sodium Crude FN0096**	**Ertapenem Sodium Crude MBE0056**	**Average**	**σ**
Related Substances					
- Oxazinone	n.d.	n.d.	n.d.	-	-
- Ring Opened	0.25%	0.18%	0.32%	0.25%	0.07
- PROMABA	n.d.	n.d.	n.d.	-	-
- Dimer I+II	0.30%	0.25%	0.29%	0.28%	0.03
- Dimer III	0.11%	0.09%	0.15%	0.12%	0.03
- Dehydrodimer a+b	0.41%	0.32%	0.47%	0.40%	0.08
- Largest unsp.	0.25%[a]	0.18%[a]	0.28%[b]	0.24%	0.05
- Total imp.	2.4%	2.2%	3.1%	2.6%	0.47

[a]RRt 1.45 [b]RRt 2.19

DEMO laboratory manufacturing evidenced a good reproducibility of Ertapenem Sodium Crude process either in terms of yield and quality. In general quality of Ertapenem Sodium Crude was good and it was almost complying specifications for Ertapenem Sodium. The items which were slightly out of specification (dehydrodimers and largest unspecified impurity) purge efficiently by a simple recrystallization from Ertapenem Sodium Crude to Ertapenem Sodium. Therefore Ertapenem Sodium Crude manufacturing process was considered suitable for a further scale up.

3.8.3 ERTAPENEM SODIUM CRUDE PILOT MANUFACTURING

This pilot manufacturing experience was something different from the previous since a deviation occurred over manufacturing. Coupling and subsequent hydrolysis to produce Ertapenem Allyl was regular, on other hand after work-up at the anhydrification step the resulting water content resulted 1.4% vs the desired <1.0%. By mistake, it was decided not to repeat the anydrification step, by

freezing at -30°C and phase separation. The batch was moved into the deprotection step, where it was observed the formation of a hard-gummy solid that has even compromised the vessel stirring mechanism. Once deprotection was deemed complete the treatment with Sodium Carbonate solution was not efficient to dissolve the gummy solid, and a lower yield was retrieved in aqueous solution. Part of the batch was therefore lost, therefore antisolvents volume were adjusted accordingly and Ertapenem Sodium Crude was recovered.

Table 38. Ertapenem Sodium Crude Pilot manufacturing performances

Batch	**Type**	**Starting POCA**	**Ertapenem Sodium Crude Output**	**Yield (%)**	**Purity**
FN 0186	Pilot manufacturing	8.1 Kg	1.2 Kg	12.9	94.2%

As far as quality is concerned it can be observed a consistent improvement in comparison to the previous pilot manufacturing.

Table 39. Quality comparison of two Ertapenem Sodium Crude Pilot manufacturing

Items	**FN 0965**	**FN 0186**
Related Substances	***Pilot Process B***	***Pilot Process C***
- Oxazinone	0.03%	n.d.
- Ring Open	0.80%	0.73%
- proMABA	n.d.	n.d.
- Dimer I + Dimer II	2.01%	0.99%
- Dimer III	1.28%	0.48%
- Deydro Dimers a+b	0.89%	0.83%
- Largest Unspecified	0.94%	0.43%
- Total Impurities	10.1%	5.80%
Assay as is	N/A	72.2%
Water Content	N/A	12%

3.8.4. ROOT CAUSE INVESTIGATION

Pilot manufacturing FN0186 resulted failure and the main cause was thought to be the water content over deprotection. Therefore, in this respect an investigation was performed, deprotection step was conducted at small scale varying water content of ErtapenemAllyl DCM solution. Results showed that when water content is intermediate between 1.0% and 2.5% a gummy solid is formed over deprotection proceed, as per the following table:

Table 40. Quality comparison of two Ertapenem Sodium Crude Pilot manufacturing

Deprotection Moisture Content	**Deprotection reaction mixture Appearance**	**Quality impact**
< 1.2%	Ertapenem precipitates as dry solid	Control on formation of Dimers
1.2%<Water Content<2.5%	Ertapenem solid forms a sticky gummy solid that compromises stirring	-
>2.5%	Ertapenem precipitates as fluid gummy solid	Higher formation of dimers

Data showed in above table explains what happened during last pilot manufacturing.

When water is sufficiently high (>2.5%) over deprotection it forms a fluid gummy solid in which Ertapenem is partially dissolved. No issues are encountered for plant feasibility, however the elevated amount of water causes Ertapenem degradation.

When water is sufficiently low (<1.2%) Ertapenem which is formed over reaction remains a dry solid that does not provide any issue for plant feasibility and in addition lower degradation is observed.

When water is between 1.2% and 2.5% provides a very dangerous scenario since the water takes all formed Ertapenem in a sticky ball that may damage the stirring system at plant manufacturing.

It is therefore mandatory for that stage to achieve a water content lower than 1.2%.

3.9. ERTAPENEM SODIUM SOLID FORM IMPROVEMENT

3.9.1. ERTAPENEM SODIUM STABILITY STUDY

Since pilot manufacturing of Ertapenem Sodium Crude evidenced a consistent degradation over drying, see section a stability study at different temperatures and water contents was performed.

Table 41. Ertapenem Sodium with 12% water content Stability

Time Station	0	t1 1 week +25°C	t2 1 month -20°C	t2 1 month +5°C	Specs
TESTS					
Water Content	12.4%	12.0%	12.0%	12.1%	≤ 19.0
Related Substances					
- Oxazinone	0.04%	0.06%	0.04%	0.05%	≤ 0.20
- Ring Opened	0.97%	**3.6%**	0.99%	**2.0%**	≤ 1.5
- ProMABA	0.12%	0.12%	0.12%	0.12%	≤ 0.40
- Dimer I + II	0.48%	**1.1%**	0.49%	**0.70%**	≤ 0.70
- Dimer III	**0.22%**	**0.53%**	**0.22%**	**0.31%**	≤ 0.20
- Dehydro Dimer a+b	**0.20%**	**0.38%**	**0.20%**	**0.24%**	≤ 0.20
- Largest unspec. Impurity	0.09%	**0.33%**	0.08%	**0.13%**	≤ 0.10
- Total impurities	2.4%	**6.9%**	2.4%	**3.9%**	≤ 3.0
Unspecified imp ≥ 0.04%					
Other (RRT 0.82)		0.08%			≤ 0.10
Other (RRT 1.09)		0.04%			≤ 0.10
Other (RRT 1.18)	0.05%	0.09%	0.05%	0.06%	≤ 0.10
Other (RRT 1.22)		0.06%			≤ 0.10
Other (RRT 1.32)		0.06%			≤ 0.10
Other (RRT 1.38)		0.04%			≤ 0.10
Other (RRT 1.45)		0.04%			≤ 0.10
Other (RRT 1.52)	0.09%	**0.33%**	0.08%	**0.13%**	≤ 0.10
Other (RRT 1.72)		0.05%			≤ 0.10

Table 42. Ertapenem Sodium with 15% water content Stability

Time Station	0	t1 1 week +25°C	t2 1 month -20°C	t2 1 month +5°C	Specs
TESTS					
Water Content	14.5%	13.7%	14.2%	14.2%	≤ 19.0
Related Substances					
- Oxazinone	0.04%	0.05%	0.04%	0.04%	≤ 0.20
- Ring Opened	0.91%	**4.0%**	0.97%	**2.0%**	≤ 1.5
- ProMABA	0.12%	0.12%	0.12%	0.12%	≤ 0.40
- Dimer I + II	0.46%	**1.3%**	0.47%	**0.73%**	≤ 0.70
- Dimer III	**0.22%**	**0.62%**	**0.22%**	**0.33%**	≤ 0.20
- Dehydro Dimer a+b	**0.19%**	**0.46%**	0.19%	**0.24%**	≤ 0.20
- Largest unspec. Impurity	0.07%	**0.39%**	0.07%	**0.15%**	≤ 0.10
- Total impurities	2.2%	**7.8%**	2.3%	**4.0%**	≤ 3.0
Unspecified imp ≥ 0.04%					
Other (RRT 0.76)		0.04%			≤ 0.10
Other (RRT 0.83)		0.08%			≤ 0.10
Other (RRT 0.93)		0.04%			≤ 0.10
Other (RRT 1.09)		0.04%			≤ 0.10
Other (RRT 1.18)	0.05%	0.10%	0.05%	0.06%	≤ 0.10
Other (RRT 1.22)		0.08%			≤ 0.10
Other (RRT 1.25)		0.04%			≤ 0.10
Other (RRT 1.32)		0.06%			≤ 0.10
Other (RRT 1.38)		0.05%			≤ 0.10
Other (RRT 1.46)		0.06%			≤ 0.10
Other (RRT 1.52)	0.07%	**0.39%**	0.07%	**0.15%**	≤ 0.10
Other (RRT 1.72)		0.05%			≤ 0.10

Table 43. Ertapenem Sodium with 19% water content Stability

Time Station	0	t1 1 week +25°C	t2 1 month -20°C	t2 1 month +5°C	Specs
TESTS					
Water Content	19.0%	17.5%	18.6%	17.6%	≤ 19.0
Related Substances					
- Oxazinone	0.04%	0.04%	0.04%	0.03%	≤ 0.20
- Ring Opened	0.69%	**3.9%**	0.81%	**1.6%**	≤ 1.5
- ProMABA	0.10%	0.08%	0.10%	0.10%	≤ 0.40
- Dimer I + II	0.53%	**1.3%**	0.57%	**0.71%**	≤ 0.70
- Dimer III	**0.21%**	**0.74%**	**0.22%**	**0.31%**	≤ 0.20
- Dehydro Dimer a+b	**0.28%**	**0.81%**	**0.26%**	**0.33%**	≤ 0.20
- Largest unspec. Impurity	0.09%	**0.53%**	0.08%	**0.16%**	≤ 0.10
- Total impurities	2.2%	**9.1%**	2.3%	**3.6%**	≤ 3.0
Unspecified imp ≥ 0.04%					
Other (RRT 0.76)		**0.21%**			≤ 0.10
Other (RRT 0.82)		0.09%			≤ 0.10
Other (RRT 0.93)		0.05%			≤ 0.10
Other (RRT 1.09)		**0.30%**			≤ 0.10
Other (RRT 1.18)	0.05%	**0.11%**	0.04%	0.06%	≤ 0.10
Other (RRT 1.21)		0.08%		0.04%	≤ 0.10
Other (RRT 1.25)		0.04%			≤ 0.10
Other (RRT 1.26)		0.08%			≤ 0.10
Other (RRT 1.32)		0.05%			≤ 0.10
Other (RRT 1.38)		0.10%			≤ 0.10
Other (RRT 1.45)	0.09%	**0.14%**	0.08%	0.08%	≤ 0.10
Other (RRT 1.52)	0.07%	**0.53%**	0.08%	0.16%	≤ 0.10
Other (RRT 1.72)		0.08%			≤ 0.10

Stability studies indicate that Ertapenem is poorly stable, major degradants are ring opened and dimers. It is stable at -20°C for more than 1 month, however it is not stable either at +5°C and +25°C. Stability trends depend on temperature and are particularly serious at +25°C, moreover they are worst when water content is higher. This behavior is obvious for open ring but also dimers trend is worst when water content is higher.

In this connection the degradation observed over drying in previous Ertapenem Sodium crude pilot run is easily explained. It is therefore mandatory for Ertapenem Sodium Crude to achieve a solid state morphology that filters quickly and dries efficiently. Once obtained Ertapenem Sodium Crude is obtained it should be stored in a -20°C cold room, right away.

3.9.2. IMPROVEMENT OF ERTAPENEM SODIUM PRECIPITATION DEPROTECTION

Since previous pilot manufacturing was failure, before moving into a further scale-up experience, a further development was performed with the aim to improve the manufacturing process performance. In particularly a change of final precipitation mode to improve Ertapenem Sodium morphology was studied.

Current Ertapenem Sodium manufacturing process involves precipitation of Ertapenem Sodium from aqueous solution by the following operations:

1) aqueous solution 1V
2) addition of 0.35 V MeOH and 0.35 V 1-Propanol at 0°C
3) seeding
4) nucleation, then addition of further 0.8 V MeOH and 1.1 V 1-Propanol
5) cooling at -10°C and growing for 12 hours.

By this approach Ertapenem Sodium quickly precipitates out of solution as antisolvents are added.

On other hand it was discovered that reducing total amount of antisolvents and changing addition order, precipitation is slower and provides a much better morphology. Therefore, improved precipitation procedure is as follows:

1) aqueous solution 1V
2) addition of 1.0 V MeOH and 1.0 V 1-Propanol at 0°C
3) cooling at -10°C
4) seeding
5) nucleation and growing for 24-40 hours

Ertapenem Sodium precipitation is slower and takes longer time, however provides Ertapenem Sodium with a much better morphology which filters and dries much faster than previous procedure.

3.10. Ertapenem Sodium Crude Manufacturing Process (D)

3.10.1. PROCESS DESCRIPTION

Ertapenem Sodium Crude (1) manufacturing process has been previously described in section 3.8.1. On the basis of additional data acquired in section 3.9, an improved manufacturing process D that starts from POCA (**2**) and produces Ertapenem Sodium (**1**) crude was outlined.

Manufacturing process is high level described here by synthetic scheme of figure 82, flow chart of figure 83 and following process narrative. For a more detailed description see experimental part.

Figure 82. Ertapenem Sodium Crude synthetic scheme

This scheme represents the improved direct process for the synthesis of Ertapenem Sodium crude from POCA (**2**) therefore is identical to the scheme shown in figure 80.

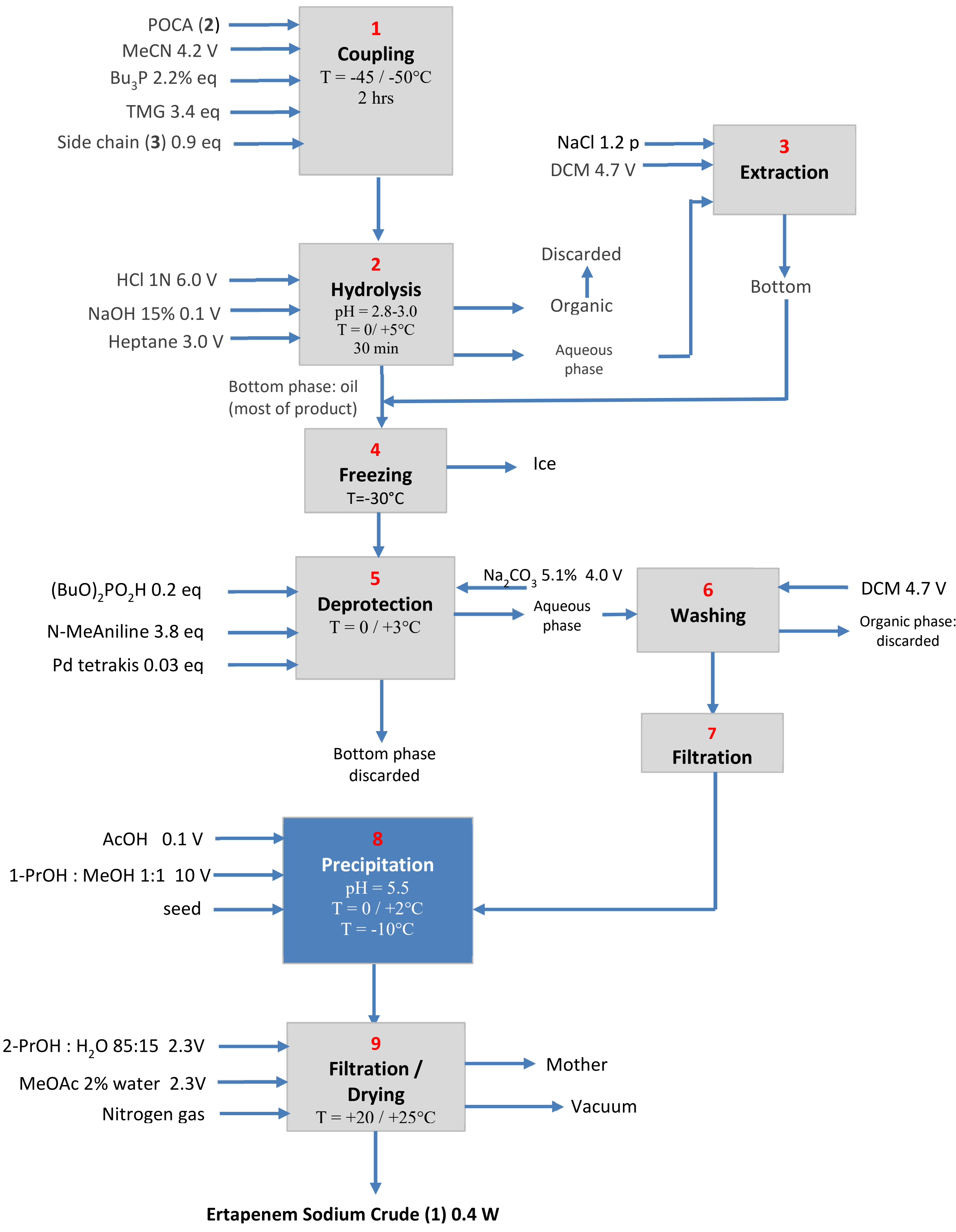

Figure 83. Ertapenem Sodium crude manufacturing process flow diagram[a]

[a]*boxes in grey represents stages unchanged vs manufacturing process C*

Manufacturing process high level narrative

For references to manufacturing stages, see flow diagram in figure 83. Text in grey represents stages which are unchanged vs manufacturing process C.

Stage 1 flow diagram

Coupling was performed in MeCN at about -50°C under Nitrogen atmosphere, Bu_3P (2.2% eq) was used to reduce Side chain thiol dimerization. Side chain (0.9 eq) was added in powder form in four consecutive portions. Coupling completion was checked by means of HPLC.

Stage 2 flow diagram

Once coupling was achieved diluted HCl was added to hydrolyze silyl ether protecting group. Hydrolysis completion was checked by means of HPLC. pH was adjusted to 4.8 with diluted NaOH. Heptane was added to the mixture. Then upon settling three phases were obtained. Bottom oily phase contains most of the product, top organic layer was exhaust and therefore discarded (it removes p-ChlorophenylAllylthioether, and trimethylsilyl byproducts).

Stage 3 flow diagram

Intermediate aqueous layer was back extracted with DCM with NaCl aid.

Stage 4 flow diagram

Oily phase and DCM layer from above back extraction were combined and cooled down to -30°C and hold for few hours. DCM layers was transferred into another vessel to remove ice.

Stage 5 flow diagram

Therefore, temperature was adjusted at about 0°C and Dibutylphosphate, N-MethylAniline and Pdtetrakis were loaded under Nitrogen atmosphere. Deprotection completion was checked by means of HPLC. Once deprotection was achieved, reaction mixture was treated with Sodium Carbonate aqueous solution, to form Ertapenem Carbamate. Upon settling lower exhaust organic layer is discarded.

Stage 6 flow diagram

Upper aqueous layer was washed with DCM to remove residual organic soluble impurities (N-Methylaniline and N-Allyl N-Methylaniline).

Stage 7 flow diagram

Rich aqueous solution was cartridge filtered to remove particulate.

Stage 8 flow diagram

Temperature was adjusted to about 0°C and pH corrected to 5.5 by AcOH addition. 1-Propanol:MeOH 1:1 were added. Temperature was adjusted to -10°C and crystallization initiated by seeding. Stirring was maintained for 24/40 hrs at -10°C.

Stage 9 flow diagram

Ertapenem Sodium was collected by filtration, washed with 2-Propanol : water and MeOAc in sequence. Dried by nitrogen flow and vacuum.

40% molar yield of Ertapenem Sodium was obtained.

3.10.2 REPRESENTATIVE LABORATORY DEMO TRIALS

With the purpose to demonstrate robustness of above described manufacturing process and in particular of modified precipitation mode, few representative laboratory batches were performed on a scale of about 100-160 g of POCA.

Table 44 Ertapenem Sodium Crude DEMO laboratory batches

Laboratory batch	Starting POCA	Ertapenem Sodium crude		Yield (%)	Purity
		Output	Potency[a]		
MRU 0089	104 g	43.1 g	81.9%	40.8	97.9%
MRU 0159	104 g	41.2 g	84.9%	40.4	98.1%
MRU 0169	166 g	65.6 g	82.4%	39.1	98.1%

[a]*assay as Ertapenem* $C_{22}H_{25}N_3O_7S$ *vs external standard*

Table 45. Ertapenem Sodium Crude DEMO batches quality

Item		Ertapenem Sodium Crude MRU 008/9	Ertapenem Sodium Crude MRU 015/9	Ertapenem Sodium Crude MRU 016/9	Average	σ
Related Substances						
- *Oxazinone*		n.d.	n.d.	n.d.	-	-
- *Ring Opened*		0.24%	0.31%	0.17%	0.24%	0.07
- *PROMABA*		0.01%	n.d.	n.d.	-	-
- *Dimer I+II*		0.30%	0.23%	0.36%	0.30%	0.07
- *Dimer III*		0.10%	0.13%	0.19%	0.14%	0.05
- *Dehydrodimer a+b*		0.35%	0.48%	0.35%	0.39%	0.08
- *Largest unsp.*		0.26%[a]	0.20%[a]	0.31%[a]	0.26%	0.06
- *Total imp.*		2.1%	1.9%	1.9%	2.0%	0.12

[a]*RRt 2.0*

DEMO laboratory manufacturing evidenced a good reproducibility of Ertapenem Sodium Crude process, in particular variability was even reduced if compared to previous process, as shown in table 37. In general quality of Ertapenem Sodium Crude was good and it was almost complying specifications for Ertapenem Sodium. The items which were slightly out of specification are dehydrodimers and largest unspecified impurity. Yield was observed very consistent between batches, even if was lower vs the previous process as a result of modified crystallization procedure. This lower performance was accepted since the improved solid state morphology is expected to be easily reproduced at scale without suffering for any scale-up effect.

3.10.3 ERTAPENEM SODIUM CRUDE KILOGRAM LAB MANUFACTURING

Based on consistency and reproducibility observed over laboratory DEMO batches a couple of kilogram scale manufacturing were performed on laboratory for Ertapenem Sodium Crude scale up. Kilogram manufacturing were performed on about 600 g POCA scale employing 10 L jacketed glass cylindric reactor.

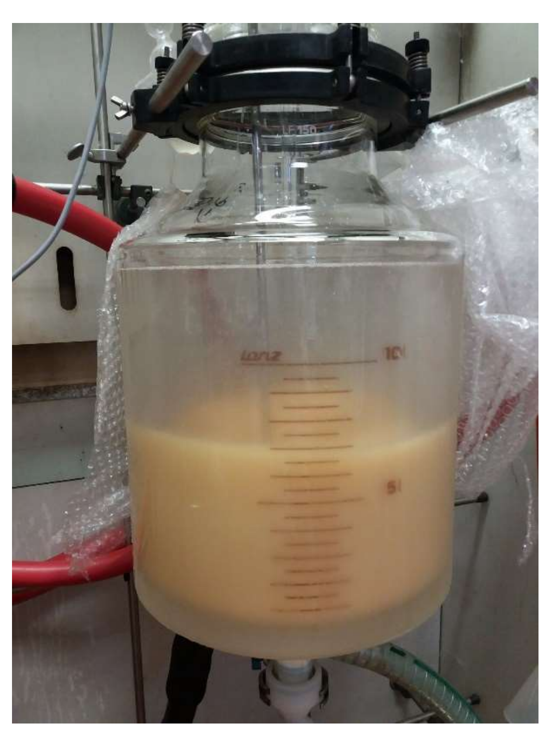

Figure 84. 10 L jacketed glass cylindric reactor over Ertapenem Allyl deblocking stage

Table 46. Ertapenem Sodium Crude Kilogram Lab Scale manufacturing performances

Batch	Type	Starting POCA	Ertapenem Sodium Crude Output	Yield (%)	Purity
MRU 0399	Kilogram Lab Scale	623 g	190 g	28.2	98.1%
MRU 0839	Kilogram Lab Scale	623 g	253 g	39.5	97.8%
-	DEMO batches	-	-	40.1	98.0%

As it appears in above table, first Ertapenem Sodium Crude Kilogram scale manufacturing provided yield performances much lower to that observed in laboratory DEMO batches. This unexpected result was due to an inconvenient experienced during final precipitation stage, in fact over first 23 hour the behavior was normal and similar to DEMO batches, however over last night the chiller that controlled temperature at -10°C has stopped to work and temperature of the mixture reached +8°C. as a result of that much of product already precipitated out of solution was re-dissolved causing therefore a lower recovery.

Therefore, Kilogram scale manufacturing was repeated, and second run resulted exactly as expected.

As far as quality is concerned purity of both kilogram scale batches in comparison with average of DEMO batches is provided in the following table:

Table 47. Ertapenem Sodium Crude Kilogram Lab Scale quality

Item	KILO LAB Ertapenem Sodium Crude MRU 0399	KILO LAB Ertapenem Sodium Crude MRU 0839	Average of DEMO batches
Related Substances			
- Oxazinone	n.d.	0.02%	-
- Ring Opened	0.09%	0.21%	0.24%
- PROMABA	<0.01%	<0.01%	-
- Dimer I+II	0.36%	0.38%	0.30%
- Dimer III	0.15%	0.10%	0.14%
- Dehydrodimer a+b	0.51%	0.47%	0.39%
- Largest unsp.	0.35%[a]	0.11%	0.26%[a]
- Total imp.	1.9%	2.2%	2.0%

[a]RRt 2.0

As it appears in above table, overall quality is similar to that of DEMO batches, even if it can be observed a slightly lower performance for dimer I+II and dehydrodimers a+b. First batch showed also an higher amount of impurity eluting at RRt 2.0, most likely as a result of temperature excursion over last night of aging.

Both Ertapenem Sodium crude from Kilogram scale manufacturing were considered adequate to produce Ertapenem Sodium complying specifications and it were therefore subjected to recrystallization work, see section 3.12.

Overall, the scale up of Ertapenem Sodium crude up to Kilogram scale, was considered successful.

3.11 ERTAPENEM SODIUM CRUDE PROCESS CHANGES HISTORY

Over the manufacturing process development described in this thesis four processes were outlined and tested over scale up. The following table resumes changes and evolution between said processes.

Table 48. Ertapenem Sodium Crude manufacturing process evolution

STAGE	PROCESS A	PROCESS B	PROCESS C	PROCESS D
Coupling to Ertapenem TMSAllyl (4)	ACN/-50°C	Same	Same	Same
Hydrolysis to ErtapenemAllyl (5)	Aq. HCl	Same	Same	Same
Work-up	Carbamate and HP20 column	Neither carbamate nor column	DCM solution freezing to Water <1.0%	Same
Isolation ErtapenemAllyl (5)	Precipitation from aqueous solution	No, telescoped in next stage	Same	Same
Deblocking	DCM/Pdtetrakis/N-MeAniline	Same	Same	Same
Work-up	Carbamate and HP20 column	Carbamate and HP20 column	Carbamate and no column	Same
Isolation Ertapenem Sodium Crude (1)	H_2O:MeOH:IPA	H_2O:MeOH:IPA	H_2O:MeOH:1-Propanol	H_2O:MeOH:1-Propanol (different operations order)
Overall yield	33%	49%	49%	40%
SCALE-UP	Pilot batch up to ErtapenemAllyl	Pilot batch	Pilot batch (failure)	Kilo-LAB

PROCESS A

In Process A isolation of Ertapenem Allyl (**5**) was made possible through carbamate formation, extraction in water, purification by HIC and subsequent precipitation by acidification. Ertapenem allyl (**5**) deblocking requires again HIC purification to lead Ertapenem Sodium crude (**1**).

PROCESS B

In Process B, coupling and hydrolysis to form Ertapenem Allyl (**33**) are same as process A. However, Ertapenem Allyl is telescoped in deblocking step, without any purification, avoiding therefore carbamate formation, HIC purification and precipitation. The rest of operation to lead Ertapenem Sodium crude (**1**) are essentially the same as per process A. One HIC purification is therefore removed and one isolation (Ertapenem Allyl (**33**)) is avoided. Yield results consistently improved versus process A.

PROCESS C

In Process C, coupling and hydrolysis to form Ertapenem Allyl (**33**) are same as process A/B. However Ertapenem Allyl DCM solution is dried before to be subjected to deblocking reaction. Final precipitation of Ertapenem Sodium crude (**1**) is performed by 1-Propanol:MeOH, instead of 2-Propanol:MeOH used in process B. These two changes allowed to remove HIC purification of Ertapenem Sodium solution. Removal of any HIC purification from whole process is an important breakthrough, considered the low stability of Ertapenem solution. Yield remains same as process B.

PROCESS D

In Process D, all the process is essentially the same as process C, with exception of precipitation stage in which a different ratio of antisolvents and different operations order allowed to obtain an improved Ertapenem Sodium crude (**1**) solid state morphology that consistently improves filtration and drying performances. Yield is 20% lower than process C however it is believed that process D is much more suitable for scale up.

3.12 ERTAPENEM SODIUM DEVELOPMENT

Once Ertapenem Sodium crude process was optimized the second minor focus was to apply a re-crystallization to get Ertapenem Sodium, as per figure 85.

Figure 85. Ertapenem Sodium overall process

Therefore, the knowledge learned over development of crude process was applied for a crystallization process development. Ertapenem Sodium crude was therefore dissolved in water at the same concentration applied in crude precipitation and addition of antisolvents was performed with the same conditions developed for method D, as follows:

1) aqueous solution 1V

2) addition of 1.0 V MeOH and 1.0 V C_3-alcohol at 0°C

3) cooling at -10°C

4) seeding

5) nucleation and growing for 24-40 hours

As C_3-alcohol either 1-Propanol and 2-Propanol were tested. Main results are showed in following table.

Table 49. Ertapenem Sodium re-crystallization from crude performances

Batch	Antisolvent	Ertapenem Sodium Crude input		Ertapenem Sodium output		
		Purity	Single unk imp.	Purity	Single unk imp.	Yield
MRU 0429	1-Propanol	98.1%	0.31%[a]	99.0%	0.23%[a]	59%
MRU 0439	2-Propanol	98.1%	0.31%[a]	98.6%	0.25%[a]	76%

[a]RRt 2.0

From data of above table, it appears that 2-Propanol provides a yield much superior than 1-Propanol and it is clear that a simple recrystallization is not enough to purge the impurity eluting at RRt 2.0 below the specification limit of not more than (NMT) 0.10% as per the setting specification from section 2.2.3.

In order to improve this purge a use of hydrophobic interaction chromatography (HIC) resin was investigated, as per following table:

Table 50. Ertapenem Sodium re-crystallization with employ of HIC resin

Batch	HIC resin	Ertapenem Sodium Crude input		Ertapenem Sodium output		
		Purity	Single unk imp.	Purity	Single unk imp.	Yield
MRU 0439	without	98.1%	0.31%[b]	98.6%	0.25%[b]	76%
MRU 0539	1V HP20 in batch mode[a]	98.1%	0.35%[b]	99.1%	0.07[b]	65%

[a]1V HP20 resin vs activity Ertapenem loaded into the solution and kept under stirring for 30 min
[b]RRt 2.0

From data of the table above, it appears that including HIC resin in batch mode was very effective in removal of impurity eluting at RRt 2.0 which was obtained well below the specification limit (NMT 0.10%).

3.13 ERTAPENEM SODIUM MANUFACTURING PROCESS

3.13.1 PROCESS DESCRIPTION

Above short development allowed to outline a manufacturing process that starts from Ertapenem Sodium Crude and produces Ertapenem Sodium. Manufacturing process is high level described here by synthetic scheme of figure 86, flow chart of figure 87 and following process narrative. For a more detailed description see experimental part.

H_2O

2-PrOH/MeOH

Ertapenem Sodium Crude
1

Ertapenem Sodium
1

Figure 86. Ertapenem Sodium synthetic scheme

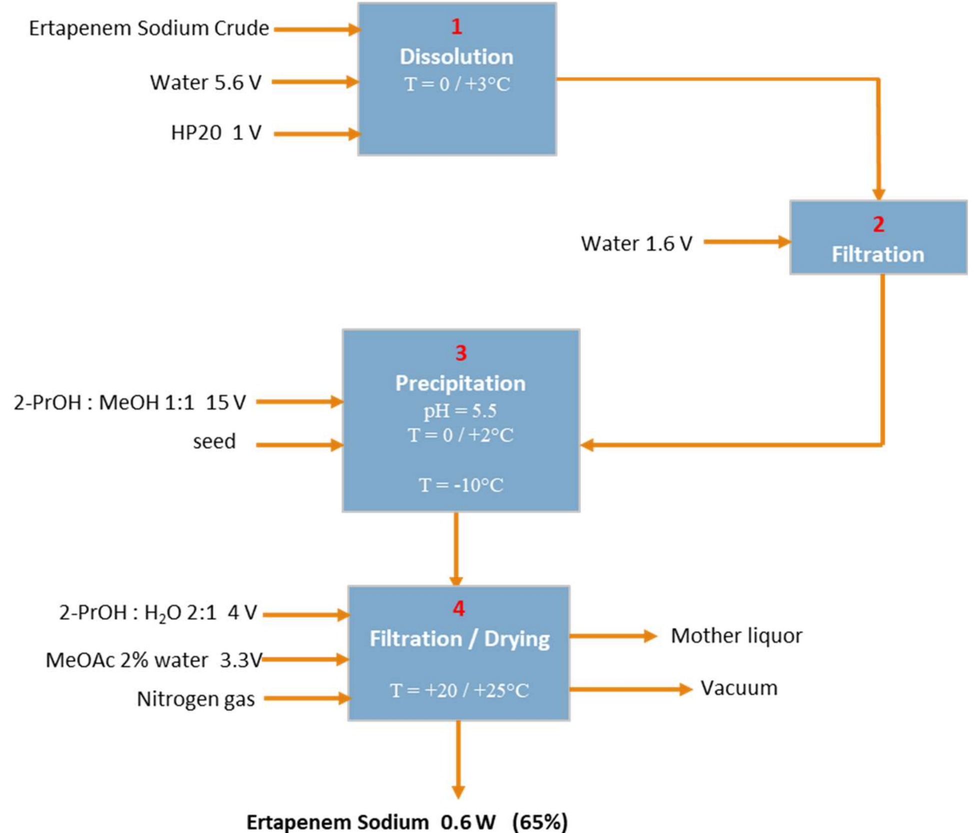

Figure 87 Ertapenem Sodium manufacturing process flow diagram

Manufacturing process high level narrative

For references to manufacturing stages, see flow diagram in figure 87.

Stage 1 flow diagram

Ertapenem Sodium Crude is dissolved in water at 0/+3°C. HP20 is loaded and kept under stirring for 30 min.

Stage 2 flow diagram

Resin was filtered off and resin was washed with water.

Stage 3 flow diagram

Temperature was adjusted to about 0°C and 2-Propanol:MeOH 1:1 were added. Temperature was adjusted to -10°C and crystallization initiated by seeding. Stirring was maintained for 24 hrs at -10°C.

Stage 4 flow diagram

Ertapenem Sodium was collected by filtration, washed with 2-Propanol : water and MeOAc in sequence. Dried by nitrogen flow and vacuum.

65% molar yield of Ertapenem Sodium was obtained.

3.13.2 ERTAPENEM SODIUM CRUDE KILOGRAM LAB MANUFACTURING

Based on results observed over development a kilogram scale re-crystallization was performed on laboratory to purify Ertapenem Sodium Crude. Kilogram manufacturing was performed on 150 g Ertapenem Sodium crude from process D scale up, employing 10 L jacketed glass cylindric reactor.

Table 51. Ertapenem Sodium Kilogram Lab Scale manufacturing performances

Batch	Type	Starting Ertapenem Sodium Crude	Ertapenem Sodium Output	Yield (%)	Purity
MRU 0549	Kilogram Lab Scale	150 g	87 g	64.0	98.7%
MRU 0539	Representative small scale trial	12.5 g	7.5 g	64.7	99.1%

As it appears in above table, Ertapenem Sodium Crude recrystallization scale up proceeded similarly to lower scale, providing Ertapenem Sodium.

Table 52. Ertapenem Sodium Kilogram Lab Scale quality

Item	KILO LAB Ertapenem Sodium MRU 0549	Specifications
Related Substances		
- Oxazinone	n.d.	≤ 0.20%
- Ring Opened	0.12%	≤ 1.5%
- PROMABA	< 0.01%	≤ 0.40%
- Dimer I+II	0.44%	≤ 0.70%
- Dimer III	0.11%	≤ 0.20%
- Dehydrodimer a+b	0.20%	≤ 0.20%
- Largest unsp.	0.08%[a]	≤ 0.10%
- Total imp.	1.3%	≤ 3.0%

[a]*RRt 1.4*

As it appears in above table, Ertapenem Sodium produced from Kilogram Scale complies with specification and it is therefore suitable to be transformed into Drug Product.

This means that whole manufacturing process from POCA to Ertapenem Sodium is suitable for commercial scale purpose since produces Ertapenem Sodium of desired quality.

3.14 ERTAPENEM DRUG PRODUCT

Ertapenem Sodium material obtained in Process D scale up experience (see section 3.13.2) was transformed into Drug Product to verify its comparability with Invanz innovator product. Therefore, Ertapenem Sodium MRU0549 was compounded with $NaHCO_3$ and NaOH according to the batch formula described in Invanz package information, see table 53.

Figure 88: Ertapenem Drug Product scheme

Table 53. Ertapenem Drug Product Batch Formula

Material	mg/vial
Ertapenem Sodium	1000[a]
Sodium bicarbonate	175
Sodium Hydroxide	q.b to adjust pH 7.5

[a]*As Ertapenem* $C_{22}H_{25}N_3O_7S$

Compounded solution was therefore lyophilized in vial to afford Ertapenem Drug Product.

Figure 89 Ertapenem Drug Product BG0939

Table 54. Ertapenem Drug Product quality and comparison with Invanz

Item	KILO LAB Ertapenem Sodium MRU 0549	DRUG PRODUCT Prototype	Average Invanz[a]	Specifications
Related Substances				
- Oxazinone	n.d.	0.51%	0.53% (σ 0.05)	≤ 0.70%
- Ring Opened	0.12%	4.0%	3.3% (σ 0.27)	≤6.0%
- PROMABA	n.d.	<0.01%	0.25% (σ 0.05)	≤ 0.40%
- Dimer I+II	0.44%	1.1%	1.1% (σ 0.08)	≤ 1.4%
- Dimer III	0.11%	0.14%	0.11% (σ 0.01)	≤ 0.20%
- Dehydrodimer a+b	0.20%	0.35%	0.34% (σ 0.06)	≤ 0.45%
- Largest unsp.	0.08%	0.11%	-	≤ 0.20%
- Total imp.	1.3%	6.9%	6.2% (σ 0.54)	≤ 9.5%

[a]see section 2.2.1, table 9

As it appears in the table above, Ertapenem Drug Product produced from Ertapenem Sodium from Kilogram Scale complies with specification of Drug Product and is comparable to innovator product Invanz.

Conclusions and Remarks

This thesis was focused on the development of Ertapenem Sodium Manufacturing Process. A Process that delivers consistently and reproducibly the desired quality has been outlined: Manufacturing Process D. Ertapenem Sodium obtained from Process D it was tested into Drug product process delivering a Drug product which was comparable with innovator product Invanz. Unfortunately to achieve desired quality without any scale-up effect, a lower yield was experienced. Cost analysis of Process D was performed and still found to be competitive with market target price of generic Ertapenem Drug Product.

Next to these activities, ACS-DOBFAR will perform further scale up experiences with the target to produce registration batches. Data from registration batches will be used to file Abbreviated New Drug Application (ANDA). Once regulatory dossier will be approved Ertapenem Sodium manufacturing will be part of ACS-DOBFAR pipeline and Ertapenem Drug Product will be launched on the market.

CHAPTER 4 : Experimental Section

4.1 Analytical Test Methods

4.1.1 HPLC Method 1 (Inertsil)

It is the method described in literature[33] for Ertapenem related substances analysis, which was used to start analytical development.

5.1.1.1 Equipment

Agilent Technologies 1200 HPLC provided with a diode array UV-vis detector.

5.1.1.2 Reagents

Water, HPLC grade

Acetonitrile, HPLC grade

Disodium hydrogen phosphate for analysis

Orthophosphoric acid, 85%, for analysis

5.1.1.3 Operative Conditions

Column:	Inertsil Phenyl 250 mm x 4.6 mm, 5 µm
Injection Volume:	10 µL
Autosampler Temperature:	4°C
Flow:	1.0 mL/min
Oven Temperature:	20°C
Wavelength:	230 nm, 4
Stop Time:	45 min
Equilibration Time:	10 min

Gradient Profile:

Time	% Mobile Phase A	% Mobile Phase B
0.0	98%	2
3.0	95%	5%
25.0	85%	15%
35.0	75%	25%
45.0	75%	25%

5.1.1.4 Mobile Phases

- Mobile Phase A

Accurately weigh and transfer about 2.0 g of disodium hydrogen phosphate in 2000 mL of water, adjust pH 8.00 ± 0.02 with orthophosphoric acid.

- Mobile Phase B

Acetonitrile

5.1.1.5 Injection solutions

- Blank Solution

Water

- Sample Preparation

Accurately weigh and transfer about 50 mg of sample into a 25 mL volumetric flask. Dissolve in and dilute to volume with water. Sonicate if necessary. Inject immediately after dissolution.

5.1.1.6 Retention times

Peak	**Relative Retention time**
Oxazinone	~ 0.26
Ring opened	~ 0.42
ProMABA	~ 0.75
Dimer I	~0.83
Dimer II	~ 0.88
Ertapenem	1.0 (about 13 min)
Dimer III	~ 1.14
Dehydro Dimers (a+b)	~ 1.48

4.1.2. HPLC Method 2 (Kromasil)

It is the method currently used for Ertapenem related substances analysis.

4.1.2.1. Equipment

Agilent Technologies 1200 HPLC provided with a diode array UV-vis detector.

4.1.2.2. Reagents

Water, HPLC grade

Acetonitrile, HPLC grade

Disodium hydrogen phosphate for analysis

Orthophosphoric acid, 85%, for analysis

4.1.2.3. Operative Conditions

Column: Kromasil Eternity PhenylHexyl 250 mm x 4.6 mm, 5 µm

Injection Volume: 10 µL

Autosampler Temperature: 4°C

Flow: 1.0 mL/min

Oven Temperature: 20°C

Wavelength: 230 nm, 4

Stop Time: 42 min

Gradient Profile:

Time	% Mobile Phase A	% Mobile Phase B
0.0	100	0
22.0	43.5	56.5
32.0	0	100
42.0	0	100
42.1	100	0
50.0	100	0

4.1.2.4. Mobile Phases

- Sodium Phosphate Buffer in water (pH 8.0)

Accurately weigh and transfer about 2.0 g of disodium hydrogen phosphate in 2000.0 mL of water, adjust pH 8.00 ± 0.02 with orthophosphoric acid.

- Mobile Phase A

Add 20 mL of acetonitrile to 980 mL of sodium phosphate buffer pH 8.0 and mix well.

- Mobile Phase B

Add 250 mL of acetonitrile to 750 mL of sodium phosphate buffer pH 8.0 and mix well.

4.1.2.5. Injection solutions

- Blank Solution

Water

- Sample Preparation

Accurately weigh and transfer about 35 mg of sample into a 25 ml volumetric flask. Dissolve in and dilute to volume with water. Sonicate if necessary. Inject immediately after dissolution.

4.1.2.6. Retention times

Peak	**Relative Retention time**	**Method Variability**	
		Std Dev (σ)	**Relative Std Dev (RSD%)**
Oxazinone	~ 0.33	0.0007	4.7%
Ring opened	~ 0.55	0.0086	0.3%
ProMABA	~ 0.64	0.0013	0.3%
Dimer I	~0.91	0.0090	2.7%
Dimer II	~ 0.96	0.0098	1.0%
Ertapenem	1.0 (about 16 min)	0.0013	0.3%
Dimer III	~ 1.13	0.0068	2.5%
Dehydro Dimers (a+b)	~ 1.40	0.0275	0.0%

4.1.3. HPLC Method 3 (Inertsil LC-MS)

It is a LC-MS method developed with Inertsyl column, used for Ertapenem related substances identification purposes.

4.1.3.1. Equipment

Agilent Technologies 1200 HPLC coupled with MS Thermo LXQ.

4.1.3.2. Reagents

Water, HPLC grade

Acetonitrile, HPLC grade

Ammonium acetate for analysis

Ammonia solution 32% extra pure

4.1.3.3. Operative Conditions

Column:	Inertsil Phenyl 250 mm x 4.6 mm, 5 µm
Injection Volume:	10 µL
Autosampler Temperature:	5°C
Flow:	1.0 mL/min
Oven Temperature:	25°C
Stop Time:	50 min
Gas temperature:	200°C
Scan:	Positive (Acquisition: 234-1100)

Gradient Profile:

Time	% Mobile Phase A	% Mobile Phase B
0.0	100%	0
22.0	87%	13%
32.0	77%	23%
42.0	77%	23%
42.1	100%	0
50.0	100%	0

4.1.3.4. Mobile Phases

- Diluted Ammonia (3%)

In a 100 mL volumetric flask, dilute 10 mL of ammonia solution 32% to volume with water.

-Mobile Phase A

Dissolve 880 mg of ammonium acetate in 980 mL of water. Add 20 ml of acetonitrile and take pH 8.0 with ammonia 3%.

-Mobile Phase B

Acetonitrile

4.1.3.5. Sample solution

2 mg /mL in water

4.1.4. HPLC Method 4 (Kromasil LC-MS)

It is a LC-MS method developed with Kromasyl column, used for Ertapenem related substances identification purposes.

4.1.4.1. Equipment

The analytical investigation has been carried out using Agilent Technologies 1200 HPLC coupled with MS Thermo LXQ.

4.1.4.2. Reagents

Water, HPLC grade

Acetonitrile, HPLC grade

Ammonium acetate for analysis

Ammonia solution 32% extra pure

4.1.4.3. Operative Conditions

Column:	Kromasil Eternity Phenylhexyl 250 x 4.6 mm, 5μm
Flow:	1.0 mL/min (Ertapenem Retention Time about 15 minutes)
Wavelength:	230 nm
Injection volume:	10 μL
Autosampler:	5°C
Column temperature:	20°C
Stop time:	50 minutes
Gas temperature:	350°C
Scan:	Positive (Acquisition: 234-1100)

Gradient profile

Time (min)	% Mobile Phase A	% Mobile Phase B
0	100	0
22	87	14
32	77	25
42	77	25
42.1	100	0
50	100	0

4.1.4.4. Mobile Phases

- Diluted Ammonia (3%)

In a 100 mL volumetric flask, dilute 10 mL of ammonia solution 32% to volume with water.

-Mobile Phase A

Dissolve 810 mg of ammonium acetate in 980 ml of water. Add 20 ml of acetonitrile and take pH 8.0 with ammonia 3%.

-Mobile Phase B

Acetonitrile

Sample solution

2 mg /mL in water

4.1.5. HPLC Method 5 (IPC)

It is the In-Process Control (IPC) method, used to follow reaction conversions from POCA (**2**) to Ertapenem Sodium.

4.1.5.1. Equipment

Agilent Technologies 1100 HPLC provided with UV-vis detector.

4.1.5.2. Reagents

Water, HPLC grade

Acetonitrile, HPLC grade

Methanol, HPLC grade

Sodium di-hydrogen phosphate (NaH_2PO4), for analysis

4.1.5.3. Operative Conditions

Column:	Kromasil Eternity PhenylHexyl 250 mm x 4.6 mm, 5 µm
Injection Volume:	20 µL
Autosampler Temperature:	4°C
Flow:	1.0 mL/min
Oven Temperature:	20°C
Wavelength:	230 nm, 4
Stop Time:	40 min

Gradient Profile:

Time	% Mobile Phase A	% Mobile Phase B
0.00	100.0	0.0
28.00	10.0	90.0
32.00	10.0	90.0
32.10	100.0	0.0
40.00	100.0	0.0

4.1.5.4. Mobile Phases

- Mobile Phase A

Accurately weigh and transfer about 1 g of sodium dihydrogen phosphate in 970 mL of water, add 30 ml of acetonitrile. Record the pH (about 4.7 $\pm$ 0.05).

- Mobile Phase B

Acetonitrile

Injection solutions

Diluent: Methanol as is

Blank preparation: Diluent as is

SCE Standard preparation

In a 100.0 mL volumetric flask dissolve about 25 mg of SCE WS standard, exactly weighed, and dilute to volume with water (Sol A). Dilute 1 mL of solution A to 100 mL with methanol.

Erta-MonoAllyl Standard preparation

In a 100.0 mL volumetric flask dissolve about 20 mg of FN 058/4 (WS standard: purity 83%, assay 78%), exactly weighed, and dilute to volume with diluent

POCA Sample Preparation

In a 100.0 mL volumetric flask dilute about 70 mg of POCA solution, exactly weighed, to volume with diluent

Sample Preparation (end of reaction):

In a 50.0 mL volumetric flask, dilute about 125 mg of end-of-reaction solution to volume with acetonitrile.

4.1.5.5. Retention times

Peak	**Retention time**
Tetramethylguanidine	3.4 min
SCE (**3**)	8.1 min
Ertapenem (**1**)	9.6 min
Ertapenem Allyl (**5**)	14.6 min
N-Methylaniline	18.9 min
TMS Ertapenem Allyl (**4**)	20.6 min
N-Allyl-N-Methylaniline	26.1 min
Allylthioether (**83**)	28.1 min
POCA (**2**)	30.5 min

4.1.6. HPLC Method 6 (Ertapenem Allyl Analysis)

It is the method currently used for Ertapenem Allyl (**5**) related substances analysis.

4.1.6.1. Equipment

Agilent Technologies 1200 HPLC provided with a diode array UV-vis detector.

4.1.6.2. Reagents

Water, HPLC grade

Acetonitrile, HPLC grade

Methanol, HPLC grade

Ammonium acetate for analysis

Ammonia solution 32% extrapure

4.1.6.3. Operative Conditions

Column:	Kromasil Eternity PhenylHexyl 250 mm x 4.6 mm, 5 µm
Injection Volume:	20 µL
Autosampler Temperature:	4°C
Flow:	1.0 mL/min
Oven Temperature:	20°C
Wavelength:	230 nm, 4
Stop Time:	50 min

Gradient Profile:

Time	% Mobile Phase A	% Mobile Phase B
0.0	100	0
23.0	64.0	36.0
37.0	10	90
42.0	10	90
42.1	100	0
50.0	100	0

4.1.6.4. Mobile Phases

- Diluted ammonia (3%)

In a 100 mL volumetric flask, dilute 10 mL of ammonia solution 32% to volume with water.

-Mobile Phase A

Dissolve 810 mg of ammonium acetate in 980 mL of water. Add 20 mL of acetonitrile and take pH 8.0 with ammonia 3%.

-Mobile Phase B

Acetonitrile

4.1.6.5. Injection solutions

- Blank Solution

In a 100 mL volumetric flask, dilute 5.0 mL MeOH with water

- Sample solution

In a 100 mL volumetric flask, accurately weigh 35 mg of Ertapenem Allyl and dissolve in 5.0 mL MeOH. Bring to volume with water

4.1.6.6. Retention times

Peak	**Relative Retention time**
Tetramethylguanidine	~ 0.32
Ertapenem Side Chain (**3**)	~ 0.58
Ring Opened Ertapenem Allyl A	~ 0.64
Ring Opened Ertapenem Allyl B	~ 0.65
ESC disulfide	~ 0.73
ESC-opened Ertapenem Allyl A	~ 0.84
ESC-opened Ertapenem Allyl B	~ 0.93
Ertapenem Allyl (**5**)	1.0 (about 20 min)
Dimer A Ertapenem Allyl	~ 1.04
Dimer B Ertapenem Allyl	~ 1.11
Dehydrodimer Ertapenem Allyl	~ 1.34
Allyl thio ether (**83**)	~ 1.85
POCA (**2**)	~ 1.95

4.1.7. HPLC Method 7 (Ertapenem Allyl LC-MS)

It is a LC-MS method used for Ertapenem Allyl, related substances identification purposes.

4.1.7.1. Equipment

The analytical investigation has been carried out using Agilent Technologies 1200 HPLC coupled with MS Thermo LXQ.

4.1.7.2. Reagents

Water, HPLC grade

Acetonitrile, HPLC grade

Ammonium acetate for analysis

Ammonia solution 32% extra pure

4.1.7.3. Operative Conditions

Column:	Kromasil Eternity Phenylhexyl 250 x 4.6 mm, 5µm
Flow:	1.0 mL/min (Ertapenem Allyl Retention Time about 20 minutes)
Wavelength:	230 nm, 4
Injection volume:	10 µL
Autosampler:	5°C
Column temperature:	20°C
Stop time:	50 minutes
Gas temperature:	350°C
Scan:	Positive (Acquisition: 150-1200)

Gradient profile

Time	% Mobile Phase A	% Mobile Phase B
0	100	0
32	50	50
42	50	50
42.1	100	0
50	100	0

4.1.7.4. Mobile Phases

Mobile Phase A: Dissolve 800 mg of ammonium acetate in 1000 mL of water. Add 20 mL of acetonitrile and take pH 8.0 with ammonia.

Mobile Phase B: Acetonitrile

Sample solution

1.4 mg /mL in water:acetonitrile 75:25

4.2. Chemical and Thermal Degradations in Solution and Solid State (Forced Degradations)

4.2.1 Basic degradation

In a 25 mL round-bottomed flask add about 35 mg of Ertapenem sodium, dissolve in 6 mL of water, add 1 mL of NaOH 0.1N, let react for 2 minutes, add 1 mL of HCl 0.1N and bring to volume with water.

4.2.2 Oxidation degradation

In a 25 mL round-bottomed flask add about 35 mg of Ertapenem sodium, dissolve in 6 mL of water, add 0.5 mL of H_2O_2 30%, bring to volume with water and inject immediately.

4.2.3 Acid degradation

In a 25 mL round-bottomed flask add about 35 mg of Ertapenem sodium, dissolve in 6 mL of water, add 1 mL of HCl 1N, let react for 5 minutes, add 1 mL of NaOH 1N and bring to volume with water.

4.2.4 Thermal stress degradation at the solid state

Exposure to 40°C and RH 75%: Ertapenem sodium was exposed to a 40°C/75%RH in an open vial for 2 hours. 35 mg were weighed in a 25 mL round bottomed flask and dissolved to volume with water.

Exposure to 60°C: Ertapenem sodium was exposed to a 60°C in a closed vial for 2 hours. 35 mg were weighed in a 25 mL round bottomed flask and dissolved to volume with water.

4.2.5 Thermal stress degradation in solution

Exposure to room temperature: 35 mg of Ertapenem sodium were dissolved in 25 mL of water and the solution was left at room temperature for 4 hours.

Exposure to 60°C: 350 mg of Ertapenem sodium were dissolved in 25 mL of water and the solution was left at 60°C for 1 hour. This mother solution was diluted 1:10 with water.

4.2.6 UV exposure

UV Exposure: Sample preparation: 350 mg of Ertapenem sodium were dissolved in 25 mL of water and the solution was left exposed for 3 hours at 365 nm. This mother solution was diluted 1:10 with water.

4.3. SYNTHETIC PROTOCOLS

4.3.1. SYNTHESIS OF OXAZINONE (66)

Ertapenem Sodium **1** (12 g, 24.1 mmol) was dissolved in water (300 mL), temperature adjusted to 0/+5°C and added with $NaHCO_3$ (6.1 g, 72.6 mmol, 3.0 eq). The resulting solution was charged to an autoclave together with dry ice (8.0 g, 182 mmol, 7.5 eq) sealed and left under stirring at ambient temperature for 24 hours.

Oxazinone formation was checked by HPLC method 2.

Reaction mixture was concentrated to about 50 mL under reduced pressure. HCl 15% was added to adjust pH=4.0. The resulting solution was loaded on a column (7 cm internal diameter) containing HP20SS resin (1000 mL) with a flow of 1.0 BV/h (17 mL/min). Elution was performed by water at 2.0 BV/h flow (34 mL/min). First BV (1000 mL) was discarded, then 20 fractions (50 mL each) were collected. Fractions 8-9 were combined and lyophilized to obtain the title product (3.9 g).

Oxazinone (66): White solid (yield 31%). MS $[M+H]^+$=520. **^{1}H-NMR** (300 MHz, D_2O, 25°C, TMS) δ (ppm) 7.89 (s, 1H), 7.69 (m, 2H), 7.49 (t, J=7.9 Hz, 1H), 4.73 (m, 1H), 4.55 (dd, J=9.7, 5.2 Hz, 1H), 4.42 (t, J=8.3 Hz, 1H), 3.83 (m, 1H), 3.61 (dd, J=12.0, 6.6 Hz, 1H), 3.29 (dd J=12.1, 5.8 Hz, 1H), 3.20 (m, 1H), 2.88-2.93 (m, 2H), 2.11 (m, 1H), 1.47 (d, J=6.6 Hz, 3H), 1.18 (d, J=7.2 Hz, 3H). **^{13}C-NMR** (75 MHz, D_2O, 25°C, TMS) δ (ppm) 178.1, 174.6, 169.5, 168.6, 151.3, 140.6, 137.3, 136.1, 129.0, 125.9, 124.1, 121.7, 119.6, 77.5, 60.1, 58.0, 52.4, 44.5, 42.6, 41.9, 35.2, 19.6, 12.9.

4.3.2. PREPARATION OF RING OPENED (67)

Ertapenem Sodium **1** (10 g, 20.1 mmol) was dissolved in water (100 mL), added with Na_2CO_3 10% (25 g, 23.6 mmol, 1.2 eq) to adjust pH = 9.0 and stirred at 22/23°C for 19 hours. To the resulting solution NaOH 30% (0.5 g, 3.8 mmol, 0.2 eq) was added and let to react for 4 hours.

Ertapenem hydrolysis completion was checked by HPLC method 2.

To reaction mixture acetic acid (0.5 g, 8.3 mmol, 0.4 eq) was added to adjust pH = 5.5. The resulting solution was loaded on a column (6 cm internal diameter) containing HP20L resin (500 mL) with a flow of 1.5 BV/h (12.5 mL/min). Elution was performed by water at 1.5 BV/h flow (12.5 mL/min). First BV (500 mL) was discarded, 20 fractions (50 mL each) were collected. Fractions 7-16 were combined and lyophilized to obtain the title product (5.5 g).

Ring Opened (67): White solid (yield 55%). MS $[M+H]^+$=494. **^{1}H-NMR** (300 MHz, D_2O, 25°C, TMS) δ (ppm) 7.77 (s, 1H), 7.52-7.62 (m, 2H), 7.39 (t, J=7.9 Hz, 1H), 4.29 (t, J=8.2 Hz, 1H), 4.21 (dd, J=9.0, 6.1 Hz, 1H), 3.93 (m, 1H), 3.61 (m, 2H), 3.17 (m, 1H), 2.79 (m, 1H), 2.52 (m, 1H), 1.95 (m, 1H), 1.16 (d, J=6.2 Hz, 3H), 0.95 (d, J=7.1 Hz, 3H). **^{13}C-NMR** (75 MHz, D_2O, 25°C, TMS) δ (ppm) 179.9, 174.3, 172.3, 169.5, 136.9, 135.7, 128.6, 125.6, 123.9, 121.5, 72.5, 67.6, 59.6, 55.4, 51.9, 43.5, 41.2, 36.4, 22.8, 19.9, 19.3, 13.4.

4.3.3. SYNTHESIS OF PROMABA (68)

To a solution of BOC-L-proline (20.0 g, 92.9 mmol) in THF (400 mL, 20V) was added carbonyldiimidazole (16.0 g, 98.7 mmol, 1.1 eq). Reaction mixture was stirred at +20/+25°C for 3 hours, then 3-aminobenzoic acid (14 g, 102.1 mmol, 1.1 eq) was added. The resulting mixture was stirred at +20/+25°C for 15 hours. Water was added (1000 mL, 50V), pH adjusted to 2.0 using phosphoric acid and solution extracted with ethyl acetate (3 x 400 mL). The combined organic phases were dried (Na_2SO_4) and concentrated to afford crude Boc-PROMABA. The residue was dissolved in a 1N solution of HCl in acetic acid (200 mL, 200 mmol, 2.2 eq). After 3 hours at +20/+25°C, ethyl acetate (200 mL, 10 V) was slowly added to the slurry. The solids were filtered and washed with ethyl acetate. Upon drying 19 g of title compound was obtained.

PROMABA (68): White solid (yield 87%). MS $[M+H]^+$=235. **^{1}H-NMR** (300 MHz, D_2O, 25°C, TMS) δ (ppm) 7.86 (s, 1H), 7.67 (d, J=7.7 Hz, 1H), 7.54 (d, J=8.0 Hz, 1H), 7.37 (t, J=7.9 Hz, 1H), 4.44 (m, 1H), 3.41 (m, 2H), 2.46 (m, 1H), 2.01-2.12 (m, 3H). **^{13}C-NMR** (75 MHz, D_2O, 25°C, TMS) δ (ppm) 170.0, 167.8, 136.4, 131.0, 129.3, 126.4, 125.6, 121.8, 60.2, 46.5, 29.6, 23.7.

4.3.4. SYNTHESIS OF ERTAPENEM ALLYL (5) PROCESS A

2 POCA 3 4 aq HCl 5

To a solution of POCA (**2**) (75 g, 0.131 mol) in acetonitrile (400 mL, 5.3V), tributyl phosphine (0.66 g, 3.2 mmol, 0.025 eq) was added. Temperature was adjusted to -50/-45°C and tetramethylguanidine (58 g, 0.504 mol, 3.8 eq) was added, followed by Ertapenem Side Chain (**3**) (32.4 g, 0.107 mol, 0.8 eq). Reaction mixture was aged at -50/-45°C for 90-100 min, then a second portion of Ertapenem Side Chain (**3**) (9.3 g, 0.031 mol, 0.2 eq) was loaded. Reaction mixture was aged at -50/-45°C for other 2.5 hrs.

End of coupling was checked by HPLC method 5

When coupling was deemed complete, HCl 1N was added (450 mL, 6V) to adjust pH=2.8-3.0, allowing temperature to rise up to 0/+5°C. Reaction mixture was aged at 0/+5°C for 40 min.

End of hydrolysis was checked by HPLC method 5

Reaction mixture was settled at +10°C for 20-30 min, where 3 phases were observed. The lower oily phase was separated and collected. The upper organic phase was discarded, and intermediate aqueous phase was extracted with DCM (400 mL, 5.3V). After separation the organic phase was combined with oily phase, previously collected.

Combined organic solution was therefore treated with Na_2CO_3 5% solution (300 mL, 4V) at 0/+5°C to adjust pH=7.0-7.5. Biphasic mixture was then settled for 20-30 min. The lower

organic phase was discarded, and aqueous phase was washed with ethyl acetate (400 mL, 5.3V). Biphasic mixture was settled for 20-30 min. The upper organic phase was discarded, and aqueous phase was subjected to vacuum stripping for 20 min.

Aqueous solution was therefore loaded on a column (6 cm internal diameter) containing HP20L resin (400 mL, 5.3V) with a flow of 1.5 BV/h (10 mL/min). Elution was performed with water at 1.5 BV/h flow (10 mL/min). First BV (400 mL) was discarded, following 1.5 BV (600 mL) were collected.

To main fraction collected, HCl 1N (170 mL, 2.3V) was added in 20-30 min at 0/+5°C, to adjust pH=5.0-5.5.

Precipitated Ertapenem Allyl was then collected by filtration and wet paste washed with water (100 mL, 1.3V). Wet cake was dried by vacuum and nitrogen stream to obtain the title product (44 g).

Ertapenem Allyl (5): White solid (yield 65%). MS $[M+H]^+$=516. **^{1}H-NMR** (300 MHz, DMSO, 25°C, TMS) δ (ppm) 10.75 (s, 1H), 8.41 (s, 1H), 7.95 (aromatic, 1H), 7.75 (aromatic, 1H), 7.52 (aromatic, 1H), 7.07 (aromatic, 1H), 5.97 (m, 1H), 5.48 (d, J=15 Hz, 1H), 5.27 (d, J=12 Hz, 1H), 4.77 (AB, 1H), 4.69 (AB, 1H), 4.32 (m, 1H), 4.21 (m, 1H), 4.09 (m, 1H), 3.88 (m, 1H), 3.60 (m, 2H), 3.35 (m, 1H), 3.00 (m, 1H), 2.82 (m, 1H), 1,90 (m, 1H), 1.25 (d, J=6 Hz, 6H). **^{13}C-NMR** (75 MHz, DMSO, 25°C, TMS) δ (ppm) 173.9, 170.1, 167.2, 160.0, 138.6, 132.2, 131.7, 128.9, 124.4, 124.0, 123.5, 120.3, 119.9, 117.7, 64.7, 64.3, 60.1, 59.6, 55.4, 54.3, 43.1, 41.5, 35.6, 21.7, 16.9.

4.3.5. SYNTHESIS OF ERTAPENEM SODIUM CRUDE (1) PROCESS A

$(Ph_3P)_4Pd$

5

Ertapenem Sodium 1

To a suspension of Ertapenem Allyl (**5**) (100 g, 0.194 mol) in DCM (1200 mL, 12.0V), dibutylphosphate (11.9 g, 0.056 mol, 0.3 eq) and N-methylaniline (97.2 g, 0.907 mol, 4.7 eq) were added. Temperature was adjusted to 0/+5°C and palladium-tetrakis(triphenylphosphine) (6.2 g, 5.4 mmol, 2.7% eq) was added. Reaction mixture was aged at 0/+5°C for 2.5 hrs. During reaction course it was observed Ertapenem Allyl dissolution, followed by reprecipitation of Ertapenem.

End of deprotection was checked by HPLC method 5

At completion, reaction mixture was poured into Na_2CO_3 7.5% solution (300 mL, 3V) at 0/+5°C to adjust pH=7.0-7.5. Biphasic mixture was then settled for 20-30 min. The lower organic phase was discarded.

Aqueous solution was therefore loaded on a column (5 cm internal diameter) containing HP20L resin (400 mL, 4V) with a flow of 1.5 BV/h (10 mL/min). Elution was performed with water at 1.5 BV/h flow (10 mL/min). First BV (400 mL) was discarded, following 1.5 BV (600 mL) were collected.

A mixture of AcOH/MeOH 1:1 (30 mL, 0.3V) was added to adjust pH=5.0-5.5 at 0/+5°C.

To the resulting solution a mixture isopropanol/MeOH 1:1 (400 mL, 4V) was added in 60 min. Ertapenem Sodium seed was added (1g, 1%) and batch stirred for 60 min at 0/+5°C.

To the slurry a mixture of isopropanol/MeOH 1:1 (800 mL, 8V) was added in 120 min and the batch was stirred overnight at 0/+5°C.

Slurry was filtered, and wet paste washed with AcOMe (400 mL, 4V). Wet cake was dried with vacuum and nitrogen stream to obtain the title product (53.9 g).

Ertapenem Sodium Crude (1): White solid (yield 49%).

4.3.6. RECRYSTALLIZATION TO ERTAPENEM SODIUM (1)

H_2O

2-PrOH/MeOH

Ertapenem Sodium Crude
1

Ertapenem Sodium
1

Ertapenem Sodium crude (32 g, 56.5 mmol) was dissolved in Na_2CO_3 7.5% solution (180 mL, 5.6V) at 0/+5°C. The solution was treated with HP20L resin (140 mL, 4.4V) and stirred at 0/+5°C for 1 hour. The resin was filtered off and washed with water (100 mL, 3V). To filtrate a mixture of AcOH/MeOH 1:1 (10 mL, 0.3V) was added to adjust pH=5.0-5.5 at 0/+5°C.

To the resulting solution a mixture isopropanol/MeOH 1:1 (150 mL, 4.7V) was added in 30 min. Ertapenem Sodium seed was added (0.5g, 1.5%) and batch stirred for 60 min at 0/+5°C.

To the slurry a mixture isopropanol/MeOH 1:1 (450 mL, 14V) was added in 120 min and the batch was stirred overnight at 0/+5°C.

Slurry was filtered, and wet paste washed with AcOMe (100 mL, 3V). Wet cake was dried with vacuum and nitrogen stream to obtain the title product (18.2 g).

Ertapenem Sodium (1): White solid (yield 57%). MS $[M+H]^+$=476, $[M+Na]^+$=498, fragments m/z 432, 388, 345, 267.

In order to fully characterize Ertapenem Sodium (**1**), the following NMR acquisitions were performed: ^{1}H-NMR, ^{13}C-NMR, DEPT135, COSY and HSQC. The following tables summarize data achieved.

Table 55: ^{1}H-NMR (D_2O) 300 MHz

Chemical shift (δ), ppm	Integral and Molteplicity	Proton assignments
1.06	3H, d (7.1 Hz)	CH_3(C4)
1.16	3H, d (6.3 Hz)	CH_3CHOH (C8)
2.08	1H, m	1H (CH_2 3a)
2.92	1H, m	1H (CH_2 3a)
3.21	1H, m	CH-CH_3 (4)
3.31	1H, dd (6.0 Hz, 2.4 Hz)	1H (β-lactam ring, C_6-H)
3.37	1H, dd (12.1 Hz, 5.6 Hz)	1H (CH_2N) (5a)
3.72	1H, dd (12.1 Hz, 6.8 Hz)	1H (CH_2N) (5a)
3.96	1H, m	CH-S (4a)
4.00-4.12	2H, om	CH-OH (7), 1H (β-lactam ring, C_5-H)
4.55	1H, t (8.4 Hz)	CH (2a)
7.35	1H, t (8.0 Hz)	(aromatic proton)
7.53	1H, d (8.0 Hz)	(aromatic proton)
7.60	1H, d (8.0 Hz)	(aromatic proton)
7.77	1H, s	(aromatic proton)

Table 56: ^{13}C-NMR (D_2O) 75 MHz

^{13}C-NMR signal	Chemical shift (δ), ppm	Carbon assignments
1	15.7	C_{10}
2	19.9	C_9
3	34.5	$C_{3'}$
4	40.3	$C_{4'}$
5	42.5	C_4
6	52.3	$C_{5'}$
7	55.7	C_5
8	58.5	C_6
9	59.9	$C_{2'}$
10	64.9	C_8
11	121.5	-
12	123.9	-
13	126.0	-
14	129.0	-
15	133.3	-
16	136.0	-
17	137.0	-
18	137.6	-
19	166.6	-
20	167.4	-
21	174.2	-
22	176.3	-

Table 57: HSQC correlation (D_2O)

^{1}H-Chemical shift (δ), ppm	^{1}H-Assignments	^{13}C- Chemical shift (δ), ppm
1.06	CH_3(C4)	15.7 (C_{10})
1.16	CH_3CHOH (C8)	19.9 (C_9)
2.08	1H (CH_2 3')	34.5 ($C_{3'}$)
2.92	1H (CH_2 3')	34.5 ($C_{3'}$)
3.21	CH-CH_3 (4)	42.5 (C_4)
3.31	1H (β-lactam ring, C_6-H)	58.5 (C_6)
3.37	1H (CH_2N) (5')	52.3 ($C_{5'}$)
3.72	1H (CH_2N) (5')	52.3 ($C_{5'}$)
3.96	CH-S (4')	40.3 ($C_{4'}$)
4.00-4.12	CH-OH (8), 1H (β-lactam ring, C_5-H)	55.7 (C_5), 64.9 (C_8)
4.55	CH (2')	59.9 ($C_{2'}$)
7.35	(aromatic proton)	129.0
7.53	(aromatic proton)	123.9
7.60	(aromatic proton)	126.0
7.77	(aromatic proton)	121.5

4.3.7. SYNTHESIS OF ERTAPENEM SODIUM CRUDE (1) PROCESS B

2 POCA + 3 → (TMG, MeCN) → 4

aq HCl → 5 → ($(Ph_3P)_4Pd$, N-methylaniline, aq. Na_2CO_3, HP20L, IPA/MeOH) → 1

To a solution of POCA (**2**) (150 g, 0.262 mol) in acetonitrile (800 mL, 5.3V), tributyl phosphine (1.31 g, 6.5 mmol, 0.025 eq) was added. Temperature was adjusted to -50/-45°C and tetramethylguanidine (116 g, 1.007 mol, 3.8 eq) was added, followed by Ertapenem Side Chain (**3**) (64 g, 0.212 mol, 0.8 eq). Reaction mixture was aged at -50/-45°C for 90-100 min, then a second portion of Ertapenem Side Chain (**3**) (18 g, 0.060 mol, 0.2 eq) was loaded. Reaction mixture was aged at -50/-45°C for other 2.5 hrs.

End of coupling was checked by HPLC method 5

When coupling was deemed complete, HCl 3.7% was added (900 mL, 6.0V) to adjust pH=2.8-3.0, allowing temperature to rise up to 0/+5°C. Reaction mixture was aged at 0/+5°C for 40 min.

End of hydrolysis was checked by HPLC method 5

Reaction mixture was settled at +10/+15°C for 20-30 min, where 3 phases were observed. The lower oily phase was separated and collected. The upper organic phase was discarded, and intermediate aqueous phase was treated with NaCl (200 g) and extracted with DCM (800 mL, 5.3V). After separation the organic phase was combined with oily phase, previously collected.

The resulting organic solution was cooled to 0/3°C under nitrogen inert atmosphere, then dibutylphosphate (14.4 g, 0.069 mol, 0.3 eq), N-methylaniline (120 g, 1.120 mol, 4.3 eq) and

palladium-tetrakis(triphenylphosphine) (7.6 g, 6.6 mmol, 2.5% eq) were added. Reaction mixture was aged at 0/+3°C for 2.5 hrs. During reaction course it was observed Ertapenem Allyl dissolution, followed by reprecipitation of Ertapenem.

End of deprotection was checked by HPLC method 5

At completion, reaction mixture was quickly treated with Na_2CO_3 7.5% w/v solution (600 mL, 4.0V) allowing temperature to rise up to +10/+15°C to adjust pH=6.8-7.0. Biphasic mixture was then settled for 20-30 min. The lower organic phase was discarded.

Rich aqueous solution was then washed with DCM (800 mL, 5.3V). The biphasic system was stirred at +8/+12°C for 5-10 min, then separation was performed.

Aqueous solution was then stripped at +10/+15°C under vacuum for 20-30 min, then cooled down to 0/+5°C and adjusted to pH = 7.8-8.0 with Na_2CO_3 10% w/v.

Aqueous solution was therefore loaded on a column (6 cm internal diameter) containing HP20L resin (600 mL, 4V) at 0/+5°C with a flow of 2.0 BV/h (20 mL/min). Elution was performed with water at 2.0 BV/h flow (20 mL/min). First 0.8 BV (500 mL) were discarded, following 1.5 BV (900 mL) were collected.

A mixture of AcOH/MeOH 1:1 (45 mL, 0.3V) was added to adjust pH=5.5-5.7 at 0/+5°C.

To the resulting solution a mixture isopropanol/MeOH 1:1 (600 mL, 4V) was added. Ertapenem Sodium seed was added (2g, 1%) and batch stirred for 60 min at 0/+5°C.

To the slurry a mixture isopropanol/MeOH 1:1 (1200 mL, 8V) was added in 240 min and the batch was stirred overnight at -10°C.

Slurry was filtered, and wet paste washed with AcOMe (2 x 300 mL, 4V). Wet cake was dried with vacuum and nitrogen stream to obtain the title product (74 g).

Ertapenem Sodium Crude (1): White solid (yield 50%).

4.3.8. SYNTHESIS OF ERTAPENEM SODIUM CRUDE (1) PROCESS C

To a solution of POCA (**2**) (150 g, 0.262 mol) in acetonitrile (800 mL, 5.3V), tributyl phosphine (1.31 g, 6.5 mmol, 0.025 eq) was added. Temperature was adjusted to -50/-45°C and tetramethylguanidine (116 g, 1.007 mol, 3.8 eq) was added, followed by Ertapenem Side Chain (**3**) (64 g, 0.212 mol, 0.8 eq). Reaction mixture was aged at -50/-45°C for 90-100 min, then a second portion of Ertapenem Side Chain (**3**) (18 g, 0.060 mol, 0.2 eq) was loaded. Reaction mixture was aged at -50/-45°C for other 2.5 hrs.

End of coupling was checked by HPLC method 5

When coupling was deemed complete, HCl 3.7% was added (950 mL, 6.3V) to adjust pH=2.6-2.8, allowing temperature to rise up to 0/+5°C. Reaction mixture was aged at 0/+5°C for 30 min.

End of hydrolysis was checked by HPLC method 5

Reaction mixture was settled at +4/+8°C for 20-30 min, where 3 phases were observed. The lower oily phase was separated and collected. The upper organic phase was discarded, and intermediate aqueous phase was treated with NaCl (200 g) and extracted with DCM (800 mL, 5.3V). After separation the organic phase was combined with oily phase, previously collected.

The resulting organic solution was cooled at -30°C for 15 hours, then ice is filtered off.

Water content of organic solution was checked (K.F <1.2%).

The resulting dry organic solution was adjusted at 0/3°C under nitrogen inert atmosphere, then dibutylphosphate (14.4 g, 0.069 mol, 0.3 eq), N-methylaniline (120 g, 1.120 mol, 4.3 eq) and palladium-tetrakis(triphenylphosphine) (7.6 g, 6.6 mmol, 2.5% eq) were added. Reaction mixture was aged at 0/+3°C for 2.5 hrs. During reaction course it was observed Ertapenem Allyl dissolution, followed by reprecipitation of Ertapenem.

End of deprotection was checked by HPLC method 5

At completion, reaction mixture was quickly treated with Na_2CO_3 6.4% w/v solution (700 mL, 4.7V) allowing temperature to rise up to +10/+15°C. Biphasic mixture was then settled for 20-30 min. The lower organic phase was discarded.

Rich aqueous solution was then washed with DCM (800 mL, 5.3V). The biphasic system was stirred at +8/+12°C for 5-10 min, then separation was performed.

Aqueous solution was then clarified by filtration and a mixture of AcOH/MeOH 1:1 (50 mL, 0.3V) was added to adjust pH=5.4-5.6 at 0/+5°C.

To the resulting solution a mixture 1-propanol/MeOH 1:1 (600 mL, 4V) was added. Ertapenem Sodium seed was added (1.5 g, 1%) and batch stirred for 60 min at 0/+5°C.

To the slurry a mixture of 1-propanol (925 mL, 6.2V) and MeOH (700 mL, 4.7V) was added in 480 min and the batch was stirred over night at -9/-11°C.

Slurry was filtered, and wet paste washed with 2-propanol/MeOH 85:15 (400 mL, 2.7V), followed by AcOMe 2%wet (400 mL, 2.7V). Wet cake was dried by vacuum and nitrogen stream to obtain the title product (75 g).

Ertapenem Sodium Crude (1): White solid (yield 48%).

4.3.9. SYNTHESIS OF ERTAPENEM SODIUM CRUDE (1) PROCESS D

TMSO H H OP(OPh)2 N O O O
2 POCA
+ HS NH2+ Cl- H N O OH O
3
TMG MeCN
TMSO H H S N H N O OH O N O O O
4
aq HCl
HO H H S N H HCl N O OH O N O O O
5
(Ph3P)4Pd NH aq. Na2CO3 1-PrOH/MeOH
HO H H S N H2+ N O ONa O N O O O-
1

To a solution of POCA (**2**) (127.8 g, 0.224 mol) in acetonitrile (530 mL, 4.2V), tributyl phosphine (0.98 g, 4.9 mmol, 0.022 eq) was added. Temperature was adjusted to -50/-45°C and tetramethylguanidine (87 g, 0.755 mol, 3.4 eq) was added, followed by Ertapenem Side Chain (**3**) (61.5 g, 0.203 mol, 0.9 eq) in four portions. Reaction mixture was aged at -50/-45°C for 2 hrs.

End of coupling was checked by HPLC method 5

When coupling was deemed complete, HCl 3.7% was added (740 mL, 5.8V) to adjust pH=2.6-2.8, allowing temperature to rise up to 0/+5°C. Reaction mixture was aged at 0/+5°C for 30 min.

End of hydrolysis was checked by HPLC method 5

pH was adjusted to 4.7-4.9 with NaOH 15% and heptane (370 mL, 2.9V) was added, mixture was settled at +4/+8°C for 20-30 min, where 3 phases were observed. The lower oily phase was separated and collected. The upper organic phase was discarded, and intermediate aqueous phase was treated with NaCl (150 g) and extracted with DCM (600 mL, 4.7V). After separation the organic phase was combined with oily phase, previously collected.

The resulting organic solution was cooled at -30°C for 15 hours, then ice was filtered off.

Water content of organic solution was checked (K.F <1.2%).

The resulting dry organic solution was adjusted at 0/3°C under nitrogen inert atmosphere, then dibutylphosphate (10.8 g, 0.051 mol, 0.2 eq), N-methylaniline (90 g, 0.840 mol, 3.8 eq) and palladium-tetrakis(triphenylphosphine) (6.8 g, 5.9 mmol, 2.6% eq) were added. Reaction mixture was aged at 0/+3°C for 2.5 hrs. During reaction course it was observed Ertapenem Allyl dissolution, followed by reprecipitation of Ertapenem.

End of deprotection was checked by HPLC method 5

At completion, reaction mixture was treated with Na_2CO_3 5.4% w/v solution (500 mL, 3.9V) allowing temperature to rise up to +10/+15°C. Biphasic mixture was then settled for 20-30 min. The lower organic phase was discarded.

Rich aqueous solution was then washed with DCM (600 mL, 4.7V). The biphasic system was stirred at +8/+12°C for 5-10 min, then separation was performed.

Aqueous solution was then clarified by filtration and AcOH (12 g) was added to adjust pH=5.4-5.6 at 0/+5°C.

To the resulting solution a mixture 1-propanol/MeOH 1:1 (1250 mL, 10V) was added over 1 hour.

Temperature was adjusted at -9/-11°C and Ertapenem Sodium seed was added (0.7 g, 0.5%) and batch stirred for 24-40 hours at -9/-11°C.

Slurry was filtered, and wet paste washed with 2-propanol/MeOH 85:15 (205 mL, 2.3V), followed by AcOMe 2% wet (290 mL, 2.3V). Wet cake was dried with vacuum and nitrogen stream to obtain the title product (50 g).

Ertapenem Sodium Crude (1): White solid (yield 40%).

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List of Abbreviations

ACN	acetonitrile
DCM	dichloromethane
DIPEA	diisopropyl ethyl amine
HPLC	high performance liquid chromatography
IPC	In Process Control
MS	mass spectroscopy
NMR	nuclear magnetic resonance
TEA	triethylamine
THF	tetrahydrofuran
PNB	ParaNitroBenzyl