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The production process of antibiotics: amoxicillin

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Dedication

This thesis is wholeheartedly dedicated to my beloved parents, sisters, and brother, who have always been a huge source of inspiration to me and who have continually supported and encouraged me throughout my life. I also dedicate this project to my lovely nieces and nephews, who are extremely precious to my heart.

It was a pleasure to share this meaningful journey with my good friend and colleague, Meryem.

Sara

I would like to heartily and proudly dedicate my humble work and effort: to my parents, who were extremely encouraging and helpful all throughout my studies, to my supportive friend and colleague, Sara, with whom I had the pleasure to work, to my two brothers, little sister and friends who did not hesitate to cheer me up at every obstacle I encountered on my journey.

Meryem

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PRACTICAL PART

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List of abbreviations

A.K.A: Also known as.

AMOX: Amoxicillin.

APA: Aminopenicillanic acid.

ATB: Antibiotic.

API: Active Pharmaceutical ingredient.

BP: British Pharmacopoeia

BPCRS: British Pharmacopeia chemical reference substance.

CASO: Casein Soybean Digest Agar.

CDD: Centers for Disease Control and Prevention.

CGMP: Current Good Manufacturing Practice.

CSF: Cerebrospinal fluid.

DNA: Deoxyribonucleic acid.

D.T: Dispersible Tablets.

E. coli: Escherichia coli.

EPE: Economic Public Enterprise.

FDA: United States Food and Drug Administration.

FPPs: Finished Pharmaceutical Products.

FTM: Fluid Thioglycollate Medium.

GMP: Good Manufacturing Practice.

HPLC: High performance liquid chromatography.

IM: Intramuscular.

IV: Intravenous.

ISO: International Organization of Standardization.

L.A.L: Limulus amebocyte lysate.

MAC: MacConkey Agar.

MAF: Marketing Authorization File.

MEA: Middle East and Africa.

MSDS: Material Safety Data Sheet.

N: Nitrogen.

NDA: New Drug Application.

ODE: 1-Octadecene.

ODS: Octadecylsilyl.

ODT: Orally disintegrating tablets.

ONS: Office National des Statistiques.

ORL: Otorhinolaryngology.

PBPs: Penicillin-binding proteins.

PCA: Pharmacie Centrale Algérienne.

PG: Peptidoglycan.

PGA: Penicillin G acylase.

PH: Potential of hydrogen.

Ph. Eur: European Pharmacopeia.

PHPG: p-hydroxyphenyl glycine.

PHPGME: p-hydroxyphenyl glycine methyl ester.

P.O: Per os.

POS: Powders for Oral Suspensions.

PVC: Polyvinyl chloride.

PVP: Polyvinylpyrrolidone.

RNA: Ribonucleic acid.

ROS: Reactive oxygen species.

RPM: Revolutions per minute.

SC: Subcutaneous.

SCDM: Soybean Casein Digest Medium.

SDA: Sabouraud Dextrose Agar.

SNIC: Société Nationale des Industries Chimiques.

TAMC: Total aerobic microbial count.

TGA: Therapeutic Good Administration.

TLC: Thin Layer Chromatography.

TSB: Tryptic Soy Broth.

TYMC: Total yeast mold count.

USP: United States Pharmacopeia.

UV/VIS: Ultraviolet/visible.

V/V: Volume per volume.

WHO: World Health Organization.

W/V: Weight per volume.

INTRODUCTION

For countless centuries, human beings were vulnerable to various diseases that are caused by bacteria; and because bacterial infections are highly contagious, this led to the widespread of these diseases on a massive scale, resulting in many instances in pandemics and epidemics such as plague, cholera, syphilis, tuberculosis, and typhoid fever just to name a few, which eventually led to the loss of millions of lives.

However, the origin of these vicious diseases remained a mystery to humankind mostly because of a general lack of knowledge, until the second half of the nineteenth century, when French chemist Louis Pasteur and German microbiologist Robert Koch were able to develop the "germ theory of disease" by proving that microorganisms were in fact responsible for these contaminations.

In 1928, Scottish microbiologist Alexander Fleming serendipitously discovered penicillin, which is an antibiotic derived from Penicillium mold. This discovery is considered to be a tremendous breakthrough in microbiology and marked the beginning of the antibiotics' era as it paved the way for the discovery of a multitude of other antibiotics, which ultimately led to an impactful revolution in the pharmaceutical industry.

Antibiotics have since then reigned supreme as the cure of a plethora of infectious diseases. According to the Centers of Disease Control and Prevention (CDC), over 156 million antibiotics are prescribed each year in the United States alone, hence why the pharmaceutical industry, which is responsible for the development, the production, and the distribution of medications, has had to relentlessly find better and more efficient methods of production, adopt new technologies, and upgrade its facilities in order to adapt to the ever-growing market and to fulfill the colossal demand.

Over the last fifteen years, the Algerian pharmaceutical industry has known a significant growth, and it is now considered to be the biggest market in the Middle Eastern and African (MEA) region. It has recorded an 8% increase every year in average, mainly because of the rapid growth of the population, which is estimated at nearly 45 million in 2021 by the National Bureau of Statistics (ONS), and also because of the remarkable efforts that the government has undertaken with the aim of attracting more investments to the local pharmaceutical market.

The production of antibiotics is a long, complicated process that necessitates a large amount of money, time, and resources. The production methods chosen differ from one antibiotic to another, and from dosage form to another. But on which basis do we determine the most adequate and most efficient production process?

The objectives of this thesis are to describe the different steps of production of three different dosage forms of AMOXYPEN® that are produced in the SAIDAL group's Medea production complex, to explain why certain methods of production are chosen over others, and also to document the rigorous methods of quality control used all throughout the production process.

Our thesis is divided into three different sections:

- The first section is the theoretical part that is subdivided into four chapters:
  - ✓ Chapter one introduces the different antibiotics, with amoxicillin as its main focus.
  - ✓ Chapter two describes the production process of amoxicillin, which is the active pharmaceutical ingredient used in AMOXYPEN®.
  - ✓ Chapter three lists the different administration routes as well as the various dosage forms of drugs.
  - ✓ Chapter four explains the manufacturing and quality control methods of three dosage forms (dispersible tablets, powders for oral suspensions, and powders for injectable solutions) in detail.

- The second section of this thesis is the practical part:

It encompasses all the information that we have gathered during our time at SAIDAL's Medea production complex, where we followed the manufacturing and quality control methods of three dosage forms of AMOXYPEN®, from raw materials to finished pharmaceutical products.

In this section, we have described the different physicochemical properties of the active ingredient (amoxicillin) and the excipients used during the production of the three dosage forms, as well as the packaging materials and the equipment. We have also noted all the manufacturing and quality control steps of AMOXYPEN® that we had observed, in order to conduct a comparative study between the different manufacturing processes used.

❖ The last section:

It is devoted to the results of all the manufacturing and quality control processes used for the raw materials, the semi-finished products and the finished products. We provided commentaries and explained the results obtained thoroughly.

Chapter 1

Chapter 1: Antibiotics

I. Antibiotics:

1. Definition of Antibiotics:

According to the FDA: "Antibiotics are drugs that fight infections caused by bacteria, and not viruses. Antibiotics are not effective against viral infections like the common cold, most sore throats, and the flu." [1]

An antibiotic is a chemical substance that is produced by a living organism, generally a microorganism, and that has the capacity, in dilute solution, to either selectively inhibit the growth of other microorganisms, or to destroy them completely.

Antibiotics are commonly produced by soil microorganisms, and they probably represent a mechanism by which organisms in a complex environment can control the growth of competing microorganisms. [2]

2. History of antibiotics:

Antibiotics have been used for millennia to treat infections, although until the last century or so, people did not know that these infections were in fact caused by bacteria. Various molds and plant extracts were used to treat infections by some of the earliest civilizations – the ancient Egyptians, for example, applied moldy bread to infected wounds.

Ever since Robert Koch and Louis Pasteur proved in the late nineteenth century that diseases can be caused by germs, scientists have been searching for efficient ways to kill these germs. Pasteur later on had an approach to use harmless bacteria to cure diseases caused by harmful ones.

In the late of the 19th century, scientists began to observe antibacterial chemicals in action. Paul Ehrlich, a German physician, noted that certain chemical dyes colored some bacterial cells but not others. He concluded that, according to this principle, it must be possible to create substances that can kill certain bacteria selectively without harming other cells. [3] In 1909, Paul discovered that a chemical called arsphenamine was an effective treatment for syphilis; this became the first modern antibiotic, although Ehrlich himself referred to his discovery as 'chemotherapy'. The word 'antibiotics' was first used over 30 years later by the Ukrainian-

American inventor and microbiologist Selman Waksman, who in his lifetime discovered over 20 antibiotics.

In 1910, an arsenical drug, named “Salvarsan”, had been discovered. This new drug was effective against the bacteria that causes syphilis. Despite the fact that this drug was toxic, it remained the drug of choice for the next several decades until it was replaced by penicillin in the 1940s. [4]

Alexander Fleming serendipitously discovered penicillin upon returning from a holiday in Suffolk in 1928, when he noticed that a fungus, *Penicillium notatum* (later re-identified as *P. rubens*), had contaminated a culture plate of *Staphylococcus* bacteria that he had accidentally left uncovered. The fungus had created bacteria-free zones wherever it grew on the plate. Later on, Fleming isolated and grew the mold in a pure culture. He found that *P. notatum* proved extremely effective even at very low concentrations, preventing *Staphylococcus* growth even when diluted 800 times, and was less toxic than the disinfectants used at the time. [5]

After early successful trials in treating human wounds, collaborations with British pharmaceutical companies ensured that the mass production of penicillin was possible. During the fire in Boston, Massachusetts, USA, in which nearly 500 people died, many survivors received skin grafts which are susceptible to infection by *Staphylococcus*. Treatment with penicillin was largely successful, and the US government began supporting the mass production of the drug. By 1944, penicillin was being widely used to treat troops for infections both in the field and in hospitals throughout Europe and by the end of World War II, penicillin was nicknamed 'the wonder drug' because it had already saved many lives. [6]

In 1939, the French-born American microbiologist René Dubos discovered two other antibiotics: gramicidin and tyrocidin, which are produced by bacteria of the genus Bacillus. Both were proven to be valuable in treating superficial infections, but alas, they were too toxic for internal use.

Tubercle bacillus was one of the bacteria that was unaffected by penicillin. However, it was highly sensitive to streptomycin, an antibiotic that was later on isolated from *Streptomyces griseus* in 1943. As well as being effective against tuberculosis, streptomycin was highly active against many other kinds of bacteria, including the typhoid fever bacillus.

3. Classification of antibiotics:

Antibiotics can be classified based on different characteristics: their origin, physicochemical structure, type of action, and mechanism of action.

3.1. Classification based on the origin of antibiotics:

Antibiotics are classified according to their origin or source into three different classes:

- Natural antibiotics: they are obtained and extracted naturally from microorganisms; like cephalosporin, cephamycin, penicillin and gentamicin. Natural antibiotics are highly toxic compared to synthetic ones.
- Semi-synthetic antibiotics: they are derived from natural antibiotics with different advantageous characteristics, like having a larger spectrum of activity or causing minimal side effects compared to the original ones. It is also noted that they can act against the bacteria that shows resistance to the antibiotic they are derived from. In this class, we can mention **amoxicillin**, ampicillin and amikacin.
- Synthetic antibiotics: these antibiotics are usually chemically similar to the natural antibiotics but they are not extracted from a microorganism. In the early ages, bacteriologists used to classify them as chemotherapeutic agents like dyes. This class includes: sulphonamides, cotrimoxazole, quinolone, and many more. [7]

3.2. Classification based on the structure of antibiotics:

Antibiotics can also be classified based on their physical and chemical properties. They can be differentiated on the basis of their water solubility, or solubility in organic solvents, on whether they have acidic, basic, or amphoteric character, on whether they contain N or other elements. Bacteriologists ranged antibiotics according to these physicochemical characteristics, using chromatographic paper or thin-layer chromatographic data, to facilitate their screening and identification.

The chemical structure determines all the physical, chemical, microbiological, pharmacological and clinical properties of an antibiotic. Antibiotics of similar structural skeleton exhibit similar microbiological activity, and have the same side effects and toxicity.

Some common classes of antibiotics based on chemical or molecular structures include Beta-lactams, Macrolides, Tetracyclines, Quinolones, Aminoglycosides, Sulphonamides, Glycopeptides and Oxazolidinones. [8]

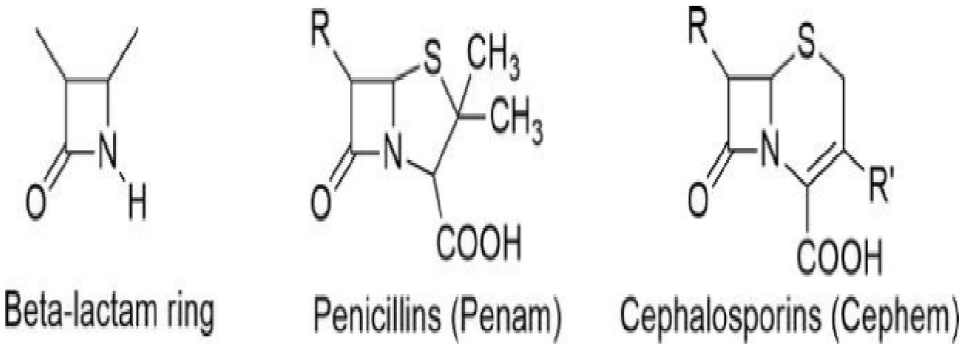


Figure 1: Structure of Beta-lactams

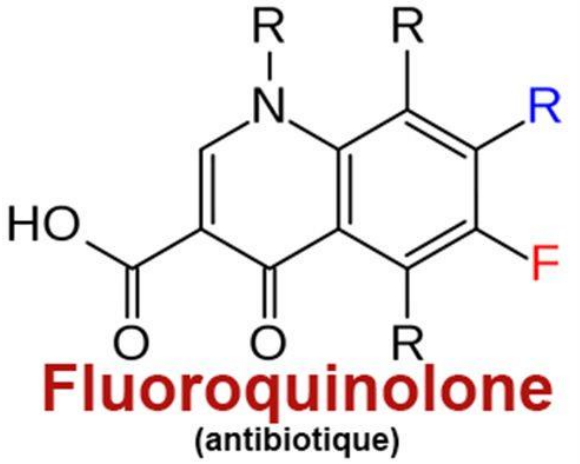


Figure 2: structure of fluoroquinolone

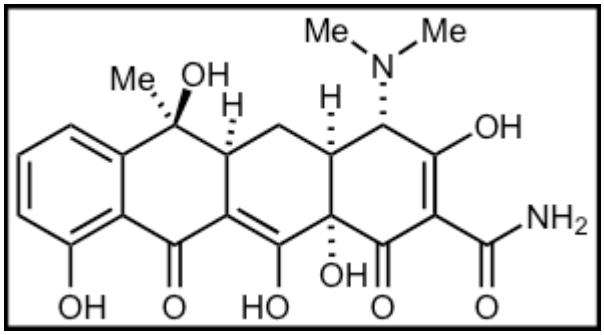


Figure 3: Structure of tetracycline

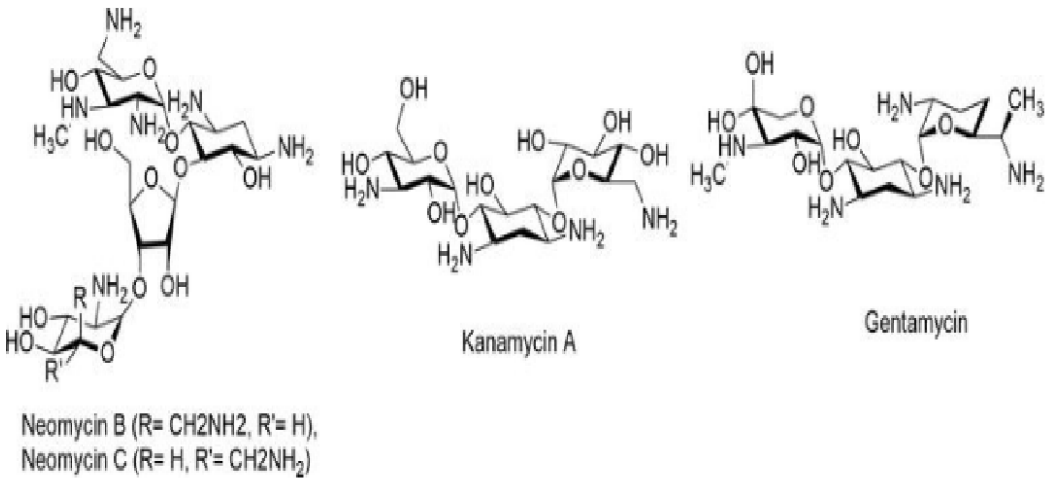


Figure 4: Structures of famous aminoglycosides

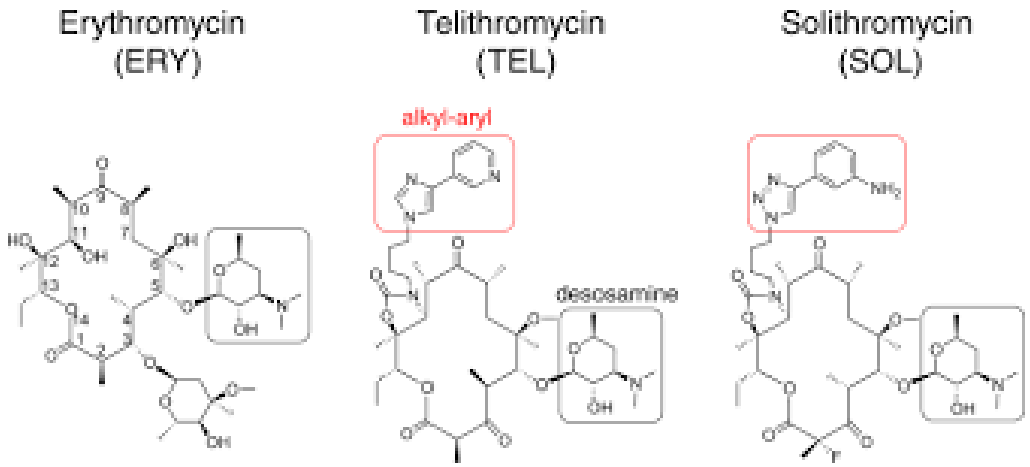


Figure 5: Structures of well-known macrolides

3.3. Classification based on the mechanism of action of antibiotics:

One of the most important factors used to classify antibiotics is their function or mode of action. The mechanism of action of antibiotics consists of targeting the essential processes for bacterial growth; therefore, antibiotics can be divided into five different groups. These mechanisms of action are [9]:

- Inhibition of cell wall synthesis.
- Breakdown of cell membrane structure or function.
- Inhibition of the structure and function of nucleic acids.
- Inhibition of protein synthesis.
- Blockage of key metabolic pathways.

a. Cell wall synthesis inhibitors:

Bacteria are encased by a rigid layer of peptidoglycan, which protects them from the osmotic pressure and the harsh conditions of the environment. In order to survive, bacteria use transglycosylases and transpeptidases to synthesize the peptidoglycans necessary for the cell wall. These two enzymes play very crucial roles by adding disaccharide pentapeptides to extend the glycan strands of existing peptidoglycan molecules and also cross-link strands of immature peptidoglycan units.

Antibiotics that belong to this class inhibit bacterial growth by inhibiting the synthesis of peptidoglycans. They inhibit the synthesis of PG by binding themselves to PG units, as well as blocking transglycosylase and transpeptidase activity. [10]

b. Inhibitors of membrane function:

The cytoplasmic membrane covers the cytoplasm, and serves as a barrier to control the composition of the cell. If by any means the functional role of the membrane is interrupted or disturbed, macromolecules and ions will flow inside; which results in the termination of the cell. Each cell membrane contains specific types of lipids that differentiate it from other cell membranes, hence why antibiotics of this class are specific for each microbial group. [11]

c. Inhibition of nucleic acid synthesis:

The nucleic acid synthesis is one of the most important targets for antibiotics. A slight difference in the enzyme that stimulates DNA and RNA synthesis between eukaryotic and prokaryotic cells helps the antibiotics attain a selective toxicity.

The antibiotics of this class are divided into DNA inhibitors and RNA inhibitors. RNA inhibitors block the bacterial transcription process; like rifampin, a famous example of the rifamycin family, which is able to create a barrier that inhibits elongation of RNA. On the other hand, DNA inhibitors disrupt the replication of the DNA by interfering in the function of the helicase enzyme responsible for unwinding the double helix structure of DNA. [12]

d. Inhibitors of protein synthesis:

Proteins are responsible for the structural composition, as well as the metabolic and physiological processes of bacteria, amongst other vital roles. Therefore, it is one of the most important targets for antibiotics to cure diseases. Protein synthesis inhibitors disturb any stage of protein synthesis, such as initiation and elongation stages. They are considered as one of the broadest classes and can be divided into two subclasses: the 50S inhibitors and the 30S inhibitors. [13]

Table 1: Examples of antibiotics and their binding sites [14]

Drug type	Binding site, pathway disturbed
Aminoglycoside	Bind to the 30S ribosomal subunit. This affects all steps in the protein synthesis, such as initiation of translation, blocking of the elongation and peptide formation.
Macrolides	Bind to the 50S ribosomal subunit, blocking peptidyl transfer.
Tetracycline and glycycline	Bind to the 30S ribosomal subunit and disturbs protein translation.
Streptogramine	Bind to the 50S ribosomal subunit.
Phenicols	Bind to the 50S ribosomal subunit.
Oxazolidinone	Bind to the 50S ribosomal subunit and block the initiation stage.

e. Inhibitors of key metabolic pathways:

It has been observed that some antibiotics like sulphonamides and trimethoprim mimic a substrate needed for cellular metabolism of bacteria. This deception causes bacterial enzymes to attach themselves to the antibiotic rather than the bacterial substrate. For example: sulphonamides mimic tetrahydrofolate, which is essential for the synthesis of folic acid in bacterial cells. [15]

3.4.Classification based on the type of action of antibiotics:

Generally, antibiotics can be also classified on the basis of their types of action into: bacteriostatic or bactericidal.

- **Bacteriostatic antibiotics:** mainly inhibit the growth of bacteria without eliminating the agent completely at a safe and achievable concentration. They function by stopping an essential metabolic process, like inhibiting protein synthesis. This definition could raise the assumption that after removing the antibiotic, the bacteria can resume their growth; but keep in mind that these antibiotics' true purpose is giving sufficient time for the immune system to kill the bacteria.

- **Bactericidal antibiotics:** eliminate the bacterium at a safe and practically achievable concentration. In some cases, these antibiotics do not eliminate 100% of the bacteria, but by taking down the majority, they help the immune system to finally eradicate the

problem. Bactericidal antibiotics function by causing irreversible damage to their targets, however, some bacteriologists in the recent studies proposed an alternative mechanism for the action of bactericidal antibiotics. They suggested that toxic reactive oxygen species (ROS) are produced in the presence of antibiotics, leading to cell death. This theory has been challenged recently, stating that toxic reactive oxygen has no impact on the vitality of bacteria. [2]

Table 2: Examples of bacteriostatic antibiotics [14]

Bacteriostatic antibiotics	Function
Sulphonamides	Inhibit folate synthesis in early stages.
Amphenicoles	Inhibit protein synthesis.
Spectomycin	Bind with a thethe p30S unit of ribosomes.
Trimethoprim	Disturb the tetrahydrofolate synthesis pathway.
Tigecycline	Inhibit protein synthesis by binding to the 30S ribosomal subunit.
Erythromycin, Clarithromycin, azithromycin	Inhibit protein synthesis.
Linezolid	Inhibit protein synthesis.
Doxycycline, Tetracycline, Minocycline	Inhibit protein synthesis.

Table 3: Examples of bactericidal antibiotics [14]

Bactericidal antibiotics	Function
Penicillin	Inhibit cell wall synthesis.
Carbapenems	Interfere with the synthesis of the cell wall.
Gentamycin, Tobramycin, Amikacin	Inhibit protein synthesis.
Quinolones and Fluoroquinolones	Block bacterial DNA replication.
Vancomycin	Inhibits cell wall synthesis.
Polymyxin B and colistin	Disrupt cell membrane.

On an important note, it is difficult to mark a clear boundary between bacteriostatic and bactericidal antibiotics. If a high concentration of bacteriostatic agents is used, then they can behave like bactericidal agents. Similarly, bactericidal antibiotics can also behave as bacteriostatic agents, if the concentration used is too low. [2]

4. Antibiotics classes:

Generally speaking, the most frequent type of classification of antibiotics is the one based on their chemical structure, because the antibiotics that share the same chemical structure exhibit similar patterns of antibacterial activity, effectiveness, modes of action, toxicity, and allergic potential. The different classes of antibiotics are represented in the table down below:

Table 4: The classes of antibiotics

ATB Classes		Examples	Mode of action
Beta-Lactams	**Penicillins**	Natural: Penicillin G, Penicillin V	Inhibit bacterial cell wall biosynthesis
		Semi-synthetic: Methicillin, Ampicillin, **Amoxicillin**, Nafcillin, Oxacillin, Cloxacillin	
	Cephalosporins	1st generation: Cefazolin, Cefadroxil, Cephalothin, Cephalexin; Cephapirin	
		2nd generation: Cefaclor, Cefuroxime, Cefoxitin, Cefprozil	
		3rd generation: Ceftriaxone, Ceftibuten, Cefotaxime, Ceftazidime	
		4th generation: Cefepime, Cefpirome	
		5th generation: Ceftaroline, Ceftobiprole	
	Carbapenems	Ertapenem, Imipenem, Tomopenem	
	Monobactams	Aztreonam	
Glycopeptides		Vancomycin, Teicoplanin, Telavancin	
Phosphonates		Fosfomycin	
Lipopetides		Daptomycin, Surfactin	Disrupt multiple cell membrane functions
Aminoglycosides		Streptomycin, Neomycin, Kanamycin, Paromomycin	Inhibit the synthesis of proteins by bacteria
Tetracyclines		Tetracycline, Doxycycline, Limecycline, Oxytetracycline	
Macrolides		Erythromycin, Clarithromycin, Azithromycin	
Streptogramins		Pristinamycin IIA, Pristinamycin IA	
Oxazolidinones		Linezolid, Posizolid, Tedizolid, Cycloserine	
Sulfonamides		Prontosil, Sulfanilamide, Sulfadiazine, Sulfisoxazole	Inhibit the synthesis of folic acid
Quinolones		1st generation: Nalidixic acid, Cinoxacin	Inhibit the synthesis of nucleic acids
		2nd generation: Norfloxacin, Ciprofloxacin,	
		3rd generation: Levofloxacin, Sparfloxacin,	
Ansamycins		Geldanamycin, Rifamycin, Naphthomycin	

4.1.Beta-lactam antibiotics:

4.1.1. Chemical structure and classification:

The Beta-lactam antibiotics are a vast group of compounds that feature a four-membered beta-lactam ring in their chemical structure, which is a highly strained and reactive cyclic amide and is a key element to the mode of action of this group of antibiotics. The subclasses of beta-lactams differ from one another with regard to their side chains and the presence of other ring structures. [16]

There are four notable members of the beta-lactam group: the penicillins, the cephalosporins, the carbapenems, and the monobactams.

The penicillins' beta-lactam ring is fused to a thiazolidine ring and they only have one side chain (R1 group at 6-position), whereas the cephalosporins' beta-lactam ring is fused to a dihydrothiazine ring, and they have two side chains (R1 and R2 at the 3- and 7-positions, respectively). Monobactams contain a monocyclic ring structure, while carbapenems contain a bicyclic nucleus composed of a beta-lactam ring with an associated five membered ring. [17]

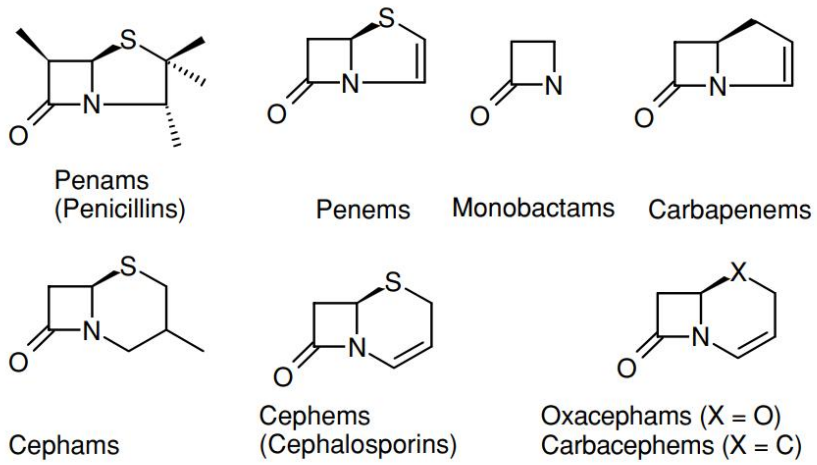


Figure 6: Beta-lactam antibiotics

4.1.2. Mode of action:

Beta-lactams are a family of bactericidal antibiotics. Most of them work by inhibiting the cell wall biosynthesis in the bacterial organism, which is why they are mainly active against bacteria that reproduce and divide at a rapid rate. [18]

The antibiotics that belong to this group act primarily as inhibitors of the formation of peptidoglycan by targeting the penicillin binding proteins (PBPs). PBPs are a group of enzymes embedded in the bacterial cell membrane; they are indispensable during the bacterial cell wall biosynthesis due to their involvement in the cross-linking of the bacterial cell wall. When the beta-lactam ring part binds to multiple PBPs, they form stable covalent complexes that lead to the inactivation of the PBPs, and subsequently to the inhibition of the cell wall formation. [19]

4.2.Penicillins:

Alexander Fleming first noticed the antibacterial nature of Penicillin in 1928, when he observed a contaminated culture of Staphylococcus aureus with the mold *Penicillium rubens*, which was originally identified as *P. rubrum*, *P. notatum*, and *P. chrysogenum* (Houbraken et al. 2011). He was able to isolate the compound around the mold and named it Penicillin. However, it wasn't until 1940 that the first clinical trial with penicillin was undertaken against a streptococci infection in a mice model. It was then that the first beta-lactam antibiotic was discovered.

Penicillin can be divided into two categories, the natural and the semisynthetic penicillins. Natural penicillins are produced from the fermentation of the fungus *Penicillium rubens*, whereas the semisynthetic ones are made from the 6-Aminopenicillanic Acid (6-APA). [20]

4.2.1. Chemical Structure and classification:

Penicillins are part of the penams subclass; therefore, they possess a basic bicyclic structure, known as the 6-aminopenicillanic acid or 6-APA. This structure is composed of an enclosed dipeptide formed by the condensation of L-cystein and D-valine, resulting in the beta-lactam ring and in the thiazolidinic ring as well. The antibacterial activity of the penicillins lies within the Beta-lactam ring, meaning that any modification in this ring structure forms penicilloic acid and leads to the loss of the antibacterial activity. The side chain differs from one penicillin compound to the other, and it determines not only the spectrum of activity but also the pharmacokinetic properties of each compound. [21]

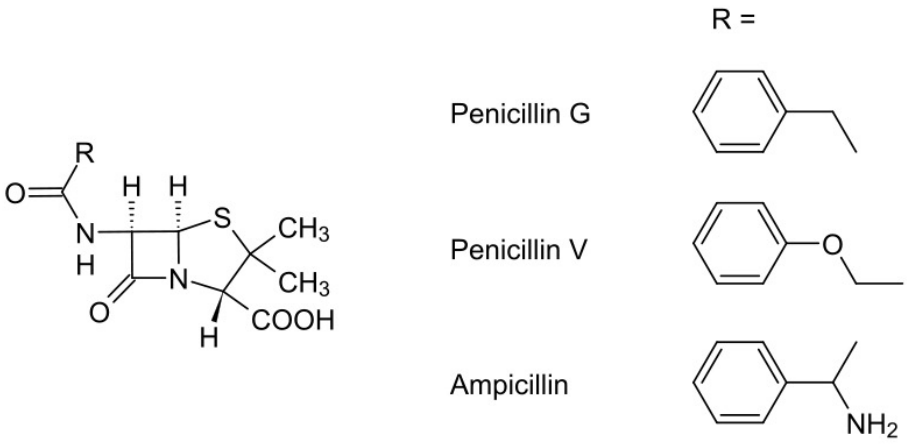


Figure 7: Chemical structures of some penicillins

4.2.2. Spectrum of activity:

Penicillins are known for their rather broad antibacterial spectrum, hence why they are among the most commonly used medications and are recommended for many indications. They can be divided into four groups based on their spectrum of activity, as shown in the table hereafter. [22]

Table 5: The spectrum of activity of the different Penicillin groups [21]

Penicillins	Spectrum of activity
Natural Penicillins: Penicillin G, Penicillin V	All streptococci, minimal activity against staphylococci, meningococci, most Gram+ anaerobes.
Beta-Lactamase resistant Penicillins: Nafcillin, Methicillin, Oxacillin, Cloxacillin, Dicloxacillin	As above, plus enhanced activity against staphylococci (except MRSA).
Extended-spectrum penicillins: Ampicillin, **Amoxicillin**, Carbenicillim, Ticarcillin, Mezlocillin, Piperacillin	Gram+ cocci and some Gram- bacilli.
Beta-Lactam with Beta-Lactamase inhibitor: Amoxicillin/Clavulanic acid, Ampicillin sulbactam, Ticarcillin/Clavulanic acid, Piperacillin/Tazobactam	Improved activity against Beta-Lactamase producing bacteria (staphylococci and selected Gram-bacilli) But not all Beta-lactamase are inhibited.

4.3. Amoxicillin:

4.3.1. Definition of Amoxicillin:

Amoxicillin is the most common bactericidal antibiotic in primary care settings. It belongs to the amino-penicillin family, and is created by adding an amino group to the penicillin to fight antibiotic resistance. It is widely used because of its fast absorption, large spectrum of activity, and low cost. [23]

Amoxicillin was discovered in 1958, and was first used for medical purposes in 1972. It is considered to be the most prescribed antibiotic for children, and it appears on the World Health Organization's list of "Essential Medicines". [24] It is used to treat a variety of bacterial infections. Among these infections we can list a few: middle ear infection, strep throat, pneumonia, skin infections, and urinary tract infections. [25]

This antibiotic is often used with clavulanic acid, a beta-lactamases inhibitor. This combination's purpose is to block the inactivation of amoxicillin by the beta-lactamases; thus, terminating the resistant germs. [25]

4.3.2. FDA approved indications:

The Food and Drug Administration (FDA) recommends the use of Amoxicillin in these cases [23]:

- **Ear, nose and throat infections:** treatment of tonsillitis, pharyngitis and otitis in adults and pediatric patients≥12 years old.
- **Helicobacter pylori eradication:** triple therapy (amoxicillin with lansoprazole and clarithromycin) reduces the risk of duodenal ulcer recurrence.
- **Lower respiratory tract infections:** treatment of lower respiratory tract infections due to streptococcus species.
- **Acute bacterial sinusitis:** treating infections due to streptococcus species.
- Infections of the skin and skin structure.
- **Urinary tract infections:** treatment of the genito-urinary tract infections due to one of these organisms: *Escherichia coli, Proteus mirabilis, or Enterococcus faecalis*

The Center for Disease Control and Prevention (CDC) recommends using it for post-exposure prophylaxis for anthrax.

4.3.3. Administration:

Amoxicillin can be given orally, intravenously (IV) or intramuscularly (IM). It also comes in immediate release or extended-release tablets. Its absorption is not affected by other nutrients that are present. [23]

Figure 8: Amoxicillin pills

Figure 9: Injectable amoxicillin

4.3.4. Recommended doses:

The optimal doses of amoxicillin recommended in "The pharmacokinetic-pharmacodynamic interface" are [23]:

- In adults, 750-1750 mg/day in divided doses.
- On pediatric patients≥3 months, 20-45 mg/kg/day in divided doses.
- Dosing for H. pylori Infection: Triple therapy: 1 gram amoxicillin, 500 mg clarithromycin, and 30 mg lansoprazole, all given twice daily (every 12 hours) for 14 days.
- Dual therapy: 1 gram amoxicillin and 30 mg lansoprazole, each given three times daily.

Generally, patients with impaired renal function do not need a dose reduction unless it is a severe case.

Amoxicillin is a pregnancy category B drug under the old FDA classification system, which means that it does not demonstrate a clear risk. The dose used for pregnant and postpartum women is the same used for a nonpregnant adult. [26]

4.3.5. Pharmacokinetic parameters of amoxicillin:

Amoxicillin has an excellent oral absorption. This absorption is not influenced by the presence of nutrients in the digestive tract.

Effective levels of this drug can be found in almost all tissues and fluids inside the human body. The total penetration into the cerebrospinal fluid (CSF) is about 6%, however it is poorly penetrated into the sputum.

It has been observed that this drug is mostly eliminated without being metabolized. 50 to 70% of amoxicillin is found intact inside the urine. The renal excretion occurs in the first 6 hours after administration and is not affected by the probenecid, which is a drug used in the treatment of chronic gout. [27]

Table 6: Pharmacokinetic parameters of amoxicillin [27]

Pharmacokinetic parameters	Amoxicillin
Oral absorption	90%
Plasma half-life	1.3 hours
Plasma protein binding	18%

4.3.6. Side Effects:

- **Common side effects:** some gastrointestinal complaints such as nausea, diarrhea, and vomiting. [28]
- **Nephrotoxicity:** crystalluria, interstitial nephritis. [29]
- Allergic reaction: amoxicillin can cause hypersensitivity reactions type I, II, III, IV. [30]
- **Hepatotoxicity:** it has been observed in some cases that amoxicillin can lead to some idiosyncratic liver injuries. [31]
- **Non-allergic rash:** 3 to 10% of children taking amoxicillin show a late developing rash; called sometimes “Amoxicillin rash”. [32]

Chapter 2

Chapter 2: Production of amoxicillin.

I. Discovery and development:

1. Origin of amoxicillin:

The restricted spectrum of activity of the penicillins, and the resistance developed by bacteria against penicillin G led to the search for derivatives of penicillin that could treat a wider range of infections.

Scientists from the Beecham Research Laboratories found a way to obtain 6-APA from penicillin, and realized that other beta-lactam antibiotics can be synthesized by attaching different side-chains to this nucleus.

6-APA or 6-aminopenicillanic acid is the central component of the penicillium generated beta-lactam antibiotics. 6-APA is formed from penicillin G after being catalyzed by penicillin acylases. This chemical compound is used as an intermediate in the synthesis of various semi-synthetic beta-lactam antibiotics, such as ampicillin and amoxicillin. [33]

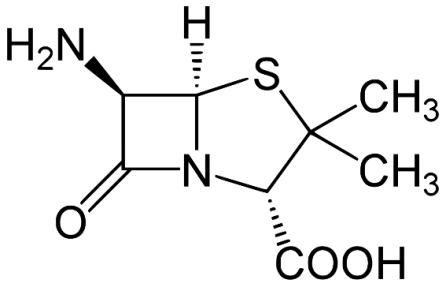


Figure 10: 6-aminopenicillanic acid chemical structure

The development of ampicillin was an important stepping stone, as it had a broader spectrum of activity compared to the original penicillins. Ampicillin made it possible for doctors to treat a larger range of both Gram-positive and Gram-negative infections. [34]

After further research, amoxicillin was finally discovered in 1958. It was noted that amoxicillin has a better bioavailability and is almost completely absorbed from the gut. Amoxicillin has a better duration of action as well, meaning that it kills bacteria at a faster rate than ampicillin does. The main reason attributed to this fact is the difference in their structure, as amoxicillin has an additional hydroxyl group on the benzene ring, which led to amoxicillin being more lipid soluble. This characteristic allows amoxicillin to cross cell membranes more quickly. [35]

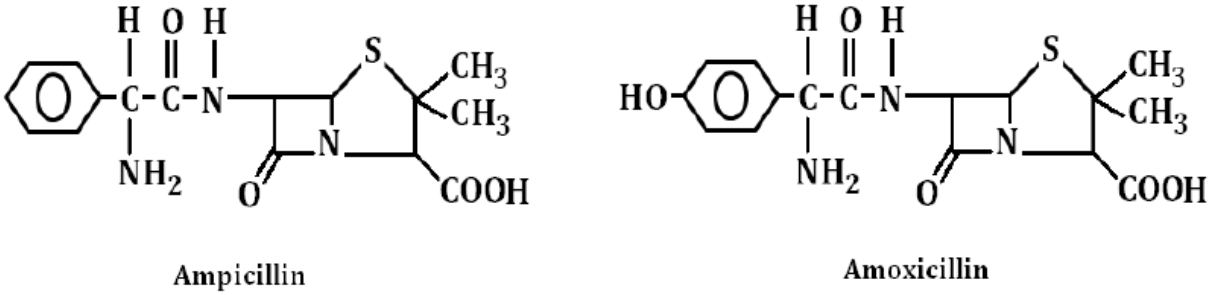


Figure 11: The chemical structure of ampicillin and amoxicillin.

II. Preclinical and clinical research:

1. Preclinical research:

According to the FDA, preclinical studies are usually not very large, however, these studies must provide detailed information on dosing and toxicity levels. After preclinical testing, researchers review their findings and decide whether the drug should be tested in people.

The specific activities conducted in this preparation would vary but would include assessment of toxicity in two species, assessment of genotoxicity, more extensive in vivo efficacy profiling, and assays to ensure appropriate delivery of drugs. [36]

Despite being discovered in 1958, amoxicillin finally came into medical use in 1972, after a series of in vitro and in vivo experiments proved its effectiveness. [37]

2. Clinical research:

According to the FDA, preclinical research is not a substitute for the studies of ways the drug will interact with the human body. “Clinical research” refers to studies, or trials, that are done in people.

The antibiotics studies, during the clinical research phase, were amoxicillin, ampicillin, and cephalothin. The clinical pharmacological studies were conducted in 11 normal volunteers ranging in age from 20 to 36 years old and weighing from 106 to 182 lbs. Informed consent was obtained from each subject according to institutional policies. [38]

After testing amoxicillin in vivo, it was discovered that its antibacterial spectrum was similar to ampicillin, that it produces substantially higher serum levels than the same dose of ampicillin, and that it is far more effective for the treatment of infections compared to ampicillin. [38]

3. New Drug Application:

If a drug developer has evidence from its early tests and preclinical and clinical research that a drug is safe and effective for its intended use, the company can file an application to market the drug. The FDA review team thoroughly examines all submitted data on the drug and makes a decision to approve or not to approve it. [39]

A New Drug Application (NDA) tells the full story of a drug. Its purpose is to demonstrate that a drug is safe and effective for its intended use in the population studied.

A drug developer must include everything about a drug (from preclinical data to Phase 3 trial data) in an NDA. Developers must include reports on all studies, data, and analyses. Along with clinical results, developers must include:

- Proposed labeling
- Safety updates
- Drug abuse information
- Patent information
- Any data from studies that may have been conducted outside the United States
- Institutional review board compliance information
- Directions for use

In the early 1970's, the SmithKline Beecham Pharmaceuticals submitted a New Drug Application to the FDA, and other supplements in the following years, in order to receive an approval to commercialize Amoxicillin. (Appendix 1)

4. FDA Review:

Once the FDA receives an NDA, the review team decides if it is complete. If it is not complete, the review team can refuse to file the NDA. If it is complete, the review team has 6 to 10 months to make a decision on whether to approve the drug or not. The process includes the following:

- Each member of the review team conducts a full review of his or her section of the application. For example, the medical officer and the statistician review clinical data, while a pharmacologist reviews the data from animal studies. Within each technical discipline represented on the team, there is also a supervisory review.
- FDA inspectors travel to clinical study sites to conduct a routine inspection. The Agency looks for evidence of fabrication, manipulation, or withholding of data.
- The project manager assembles all individual reviews and other documents, such as the inspection report, into an "action package." This document becomes the record for FDA review. The review team issues a recommendation, and a senior FDA official makes a decision.

5. FDA approval:

In cases where FDA determines that a drug has been shown to be safe and effective for its intended use, it is then necessary to work with the applicant to develop and refine prescribing information. This is referred to as "labeling." Labeling accurately and objectively describes the basis for approval and how best to use the drug. [39]

Often, though, remaining issues need to be resolved before the drug can be approved for marketing. Sometimes the FDA requires the developer to address questions based on existing data. In other cases, the FDA requires additional studies. At this point, the developer can decide whether or not to continue further development. If a developer disagrees with an FDA decision, there are mechanisms for formal appeal.

On January 18th, 1974, SmithKline Beecham Pharmaceuticals received approval for the use of Amoxil® (amoxicillin) in the treatment of gram-positive and gram-negative infections due to certain susceptible organisms. [40]

III. Industrial production of amoxicillin:

1. Introduction:

In order to meet the demand for the major antibiotic penicillin G, during and after World War II, a submerged fermentation process was developed.

Penicillin G is typically produced by feeding the mold *P. chrysogenum* phenylacetic acid during the fermentation process, and the amide that is generated on the beta-lactam ring gives the penicillin its characteristic structure. However, this approach is restricted to a limited range of organic acids and there are limitations in the ability of the producing organism to substitute desirably more complex precursors into this portion of the penicillin molecule. This is due to the inability of the acylating enzyme to accept these donors. [41]

A different, semisynthetic approach was developed in order to overcome the limitations of the fermentation process. This new method resulted in the production of new penicillins with a better chemical stability, broader antibacterial spectra, including coverage of both Gram-negative and Gram-positive activity. [42]

Among the earliest second-generation penicillins, we could find ampicillin and amoxicillin. These antibiotics not only had activity against Gram-positive bacteria, but showed significant activity against Gram-negative species that are clinically important, such as *Haemophilus influenzae, Escherichia coli* and *Proteus mirabilis*.

2. Penicillin G synthesis pathway:

As mentioned earlier, the main source of 6-APA is natural penicillin G, therefore, in order to produce semi-synthetic beta-lactam antibiotics, including amoxicillin, it is necessary to produce penicillin G (also known as benzyl-penicillin) beforehand.

Penicillin G is not a typical fermentative antibiotic, so despite the fact that the majority of antibiotics are produced by fermentation using bacteria, penicillin G is actually made by a fungus called *Penicillium chrysogenum*. [43]

The biosynthetic pathway for the formation of penicillin G in the *Penicillium chrysogenum* cell through the formation of intermediates occurs in the form of amino acids such as α-aminoadipate, L-cysteine, and L-valine. These intermediates are formed from glucose due to

enzymes. The formation of 6-APA is an amino acid combination of L-cysteine and L-valine, a step that is part of the formation of antibiotic penicillin G in *P. chrysogenum* cells. [44]

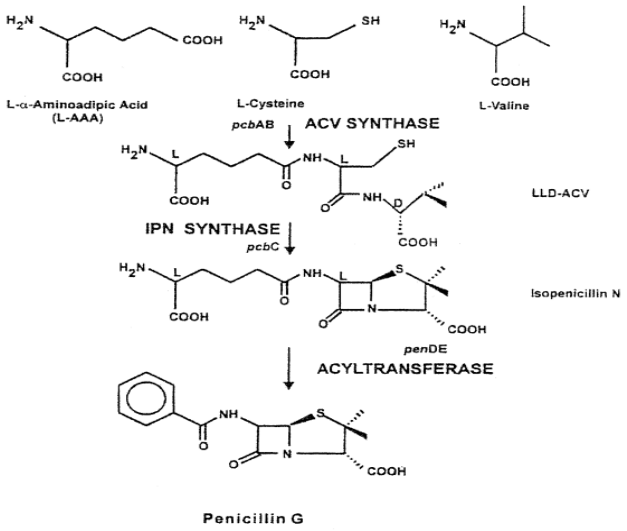


Figure 12: Biosynthetic pathway of Penicillin G [43]

3. Production process of penicillin G:

The industrial production of penicillin has known a tremendous evolution over the years. Nowadays, unlike the methods used in the 1940's, the manufacturing processes are highly computerized and automated. These upgrades have resulted in an increase in the yield and a decrease in the production costs. [44]

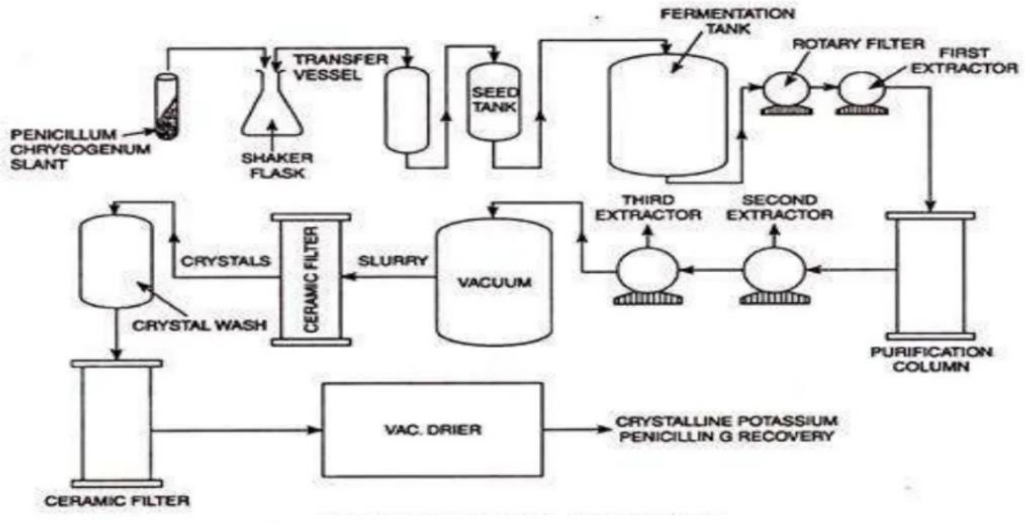


Figure 13: Commercial production of penicillin G.

3.1. Fermentation process:

The fermentation production of penicillin G is a fed-batch process carried out aseptically in stainless steel tank reactors.

First, a volume of sterile medium in a vessel is inoculated. The broth is fermented for a defined period. The tank is then emptied and the products are separated to obtain the antibiotic. The vessel is then recharged for batch operation with the medium and the sequence repeated, as often as required. [45]

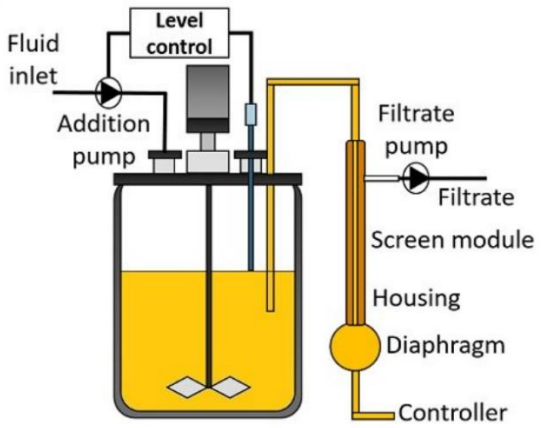


Figure 14: Fed-batch fermenter.

3.1.1. Preparation of inoculum:

An inoculum can be defined as the population of microorganisms or cells that is introduced in the fermentation medium or any other suitable medium. Inoculum preparation involves obtaining the organisms in an optimal state that is compatible with inoculation into cell culture, tissue culture, media, and fermenters. The main goal is to achieve a high level of viable biomass in an adequate physiological state for use as an inoculum. [45]

The inoculum prepared is a suspension of conidia of *Penicillium chrysogenum*. The spores of *P. chrysogenum* that are taken from working stocks cultures, are suspended in water or non-toxic lauryl sulphonate, then added to the flask containing wheat bran and a nutrient solution. A 4 days old shake flask culture is then inoculated into a seed tank for 3 days. [46]

3.1.2. Preparation of the growth medium:

Media for cell-mass build-up are designed to provide fast growth in a fed-batch mode with minimal changes in pH levels. They need to provide a readily available carbohydrate, such as glucose or sucrose, and a soluble source of nitrogen, such as corn steep liquor, or yeast extract. Ammonium sulphate can also be used to provide additional nitrogen. Calcium carbonate or phosphates can be added if buffering is required, which is often the case due to organic acids that can be produced by the rapid metabolism of sugars.

In 1958, Jackson prepared a media for penicillin production. The major constituents of the medium included [47]:

- Fermentable carbohydrate: Corn steep liquor (3.5%), lactose (3.5%), glucose (1%).
- Potassium dihydrogen phosphate: 0.4%.
- Organic nitrogen source.
- Phenyl acetic acid precursor.
- Edible oil: 0.25%.
- Calcium carbonate (which acts as a buffer): 1%.
- PH after sterilization: 5.5 to 6.0.

The most suitable media are the ones that use inexpensive raw materials in combinations that lead to maximal productivity. The production stage fermentations are fed-batch, which provides the chance to optimize the fermentation in order to provide the fine balance between controlled cell growth and maximum biosynthesis. Raw materials for use in the initial batch phase have to provide both immediate utilizable soluble nutrients as well as longer lasting and, therefore, less-soluble sources. [48]

3.1.3. Fed-batch fermentation:

During fed-batch fermentations, intermittent or continuous feeding of nutrients is used to supplement the reactor contents and provide control over the substrate concentration. By starting with a relatively dilute solution of substrate and adding more nutrients as the conversion proceeds, high growth rates are avoided. Fed-batch process is often used for penicillin production but is not common practice in the antibiotics industry as a whole. [47]

Crude sugar and the precursor are fed throughout the cycle. The precursor substance used for penicillin G is phenyl acetic acid. Meanwhile, the pH levels, temperature, carbon dioxide, dissolved oxygen, sugar precursor, and ammonia are closely monitored and controlled for optimal antibiotic production. (Waites et al. 2001)

- Aeration (oxygen supply): supply of oxygen in a bioreactor is the limiting factor in penicillin biosynthesis. Aeration speed is between 1.5 to 3.0.
- Temperature: plays an important role in penicillin production, it should be maintained at 28° C.
- Biomass production: production of penicillin depends upon the biomass production. Therefore, it is recommended to have a high biomass concentration in the vessel. It is achieved by increasing the agitation rate and power.
- PH: it is maintained by calcium and magnesium carbonate in the medium by phosphate buffer. It is also controlled by adding sodium hydroxide or sulphuric acid in the medium.

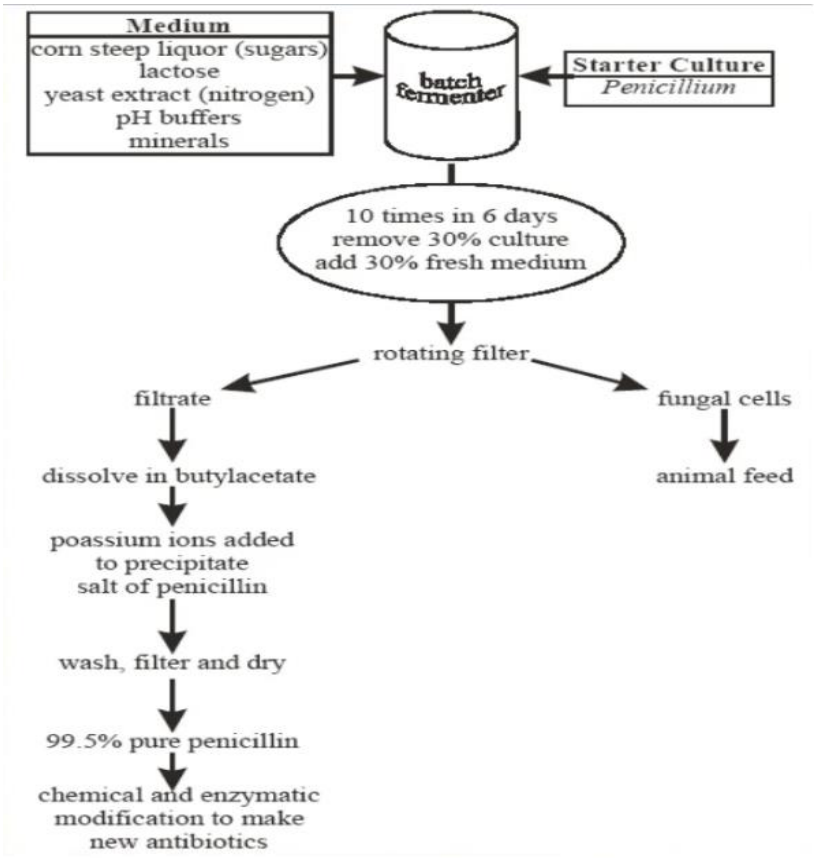


Figure 15: Penicillin G fermentation process.

3.1.4. Recovery and post recovery processing:

The downstream recovery procedures include: the filtration, extraction, and purification of the penicillin.

The recovery process needs to be tailored to the specific compounds at hand, therefore, in the case of penicillin G, solvent recovery is the favored option. The whole broth is acidified and the antibiotic is then extracted using organic solvents, this step is followed by a thorough cleaning process. Furthermore, recent processes that use penicillin G in order to produce 6-APA ring nucleus, use back-extraction from the solvent into an aqueous phase, which is employed as the mother liquor for the enzymatic process that converts the penicillin G into 6-APA.

It has been noted that the recovery and post-processing of the antibiotic is a method that frequently has substantial production costs, in addition to the environmental costs associated with the techniques used. For example, improved solvent and precursor recovery have reduced the cost of producing penicillin, and the regeneration and recycling of resins used in other antibiotic recovery processes have improved economics for production of these compounds. [48]

The **figure 16** attached down below summarizes both the recovery and the purification processes of penicillin. The key element to this method is the organic solvent extraction. In order to produce active, high-purity penicillin G, a special centrifugal extractor, called Podbielniak, is used for the extraction, which results in a short extraction time and little penicillin degradation in the organic solvent. [46]

The removal of mold mycelium is done with the help of a rotary vacuum filter. This step is done under conditions that avoid the contamination of the filtrate with enzymes, which may lead to the destruction of the antibiotic. Calcium chloride and polyelectrolyte are added to the mycelium to form large particles known as flocs. The penicillin is then extracted from the filtrate into an organic solvent (amyl acetate). After that, the penicillin obtained is transferred into a pH-neutral aqueous solution. These steps increase the penicillin concentration about one hundred times. Activated carbon is used after the extraction mainly to remove impurities. [49]

Then, filtration removes this carbon and prepares the penicillin for precipitation as a sodium or potassium salt. Precipitation is induced by acetone and followed by washing with an alcohol to remove any remaining impurity. [46]

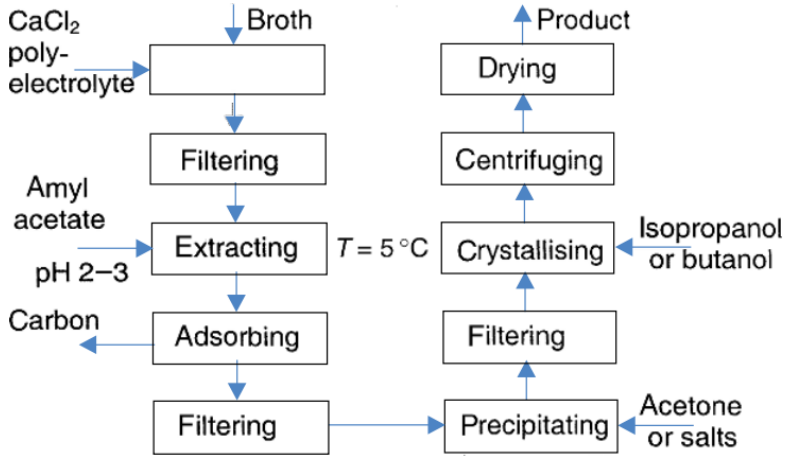


Figure 16: Downstream recovery in the production of penicillin. [48]

3.2. Comparison between old and modern production methods:

During the 1950s, Penicillin G production habitually involved a single batch process, meaning that on top of the fact that the fermentation process itself was a single batch process, the process of media sterilization was an in-situ process in the fermenter as well. The media used was lactose, cycle times were around 120 hours and minimal process control was employed. The morphology of the organism was filamentous. Removal of the mycelium was by batch filtration and there were many subsequent extraction stages. Tank volumes were 50-100 m3, titers were 0.5--1.0 g/l, process efficiency was 70--80% and costs were US$275/350 kg. [49]

In contrast, modern fermentations are highly efficient processes. Cheaper and more readily available carbons sources such as glucose/sucrose mixtures are fed continuously to the fermentation in a controlled manner, first to promote growth and then to sustain maximum production phases. The media are sterilized continuously and the operational mode is semicontinuous, with part of the fermentation broth being drawn off and continuously processed. Fermentation variables such as pH and aeration are computer controlled. Growth is now in the form of pellets, and downstream processing is based on whole broth recovery (no removal of the biomass) and continuous extraction with recovery of solvents and precursor after splitting. Fermentation tank volumes are larger (100--200

m3) and titers are now >40 g/l. Efficiencies are over 90%. These factors have decreased costs to US$15/20 kg. [49]

These improvements in the manufacturing process not only led to an improvement in the scale of production, but also helped reduce the cost of antibiotics and precursors to semisynthetic derivatives.

Table 7: Modification in the Production strategies of penicillin. [45][50]

Fermentation	Conventional Methods	Modern Approach
Media constituents	Lactose/glucose	Sucrose/nonconventional substrates/organic wastes
Sterilization process	Batch	Continuous
Morphological alterations	Filamentous	Pellets
Mode of operation	Batch	Fed-batch
Total cycle	120 h	200 h
Analytical methods	In vitro assay	High performance liquid chromatography
Operational control	Semi-controlled/manual	Fully automated
Production (g/l)	1-2.5	50
Downstream recovery	Filtration	Whole broth
Extraction stages	Many	Few
Precursor extraction	Discarded	Recovered
Process efficiency	70%	90%
Environmental issues	Less	Several
Cost approximation	Approx. US$350/kg	Approx. US$20/kg

4. Production of 6-APA:

More than 60% of 6-APA is produced enzymatically [51], and in order to do so, immobilized enzymes, such as penicillin G acylase or PGA, have been used as catalysts in the pharmaceutical industry [52]. Penicillin G acylase is a microbial enzyme that catalyzes the deacylation of penicillin with formation of side chain acid and 6-Aminopenicillanic acid (6-APA). It can be immobilized either in the form of isolated enzyme or whole cell enzyme by different techniques [53].

The production of 6-APA is a multi-step process involving fermentation, acylation, extraction, concentration, crystallization, filtration, washing, and drying in which penicillin acylases may be used as purified enzymes or in immobilized form. [54]

The steps involved in the enzymatic conversion of penicillin G into 6-APA include isolation process comprising an enzyme reaction mixture incubation in a rotary shaker set for 50 rpm for 4 h at 28°C, centrifugation of reaction mixture, acidic pH adjustment of supernatant, supernatant extraction for phase separation, pH adjustment of aqueous phase containing 6-APA, supernatant concentration under vacuum, concentrate treatment with methanol and finally 6-APA crystallization by pH adjustment to 4.3. White crystals of 6-APA were obtained at the completion of this process. [55] [56]

5. Production process of amoxicillin:

5.1. Amoxicillin synthesis pathway:

Amoxicillin (AMOX) is synthesized from the reaction of p-hydroxyphenyl glycine methyl ester (PHPGME) and 6 amino penicillanic acid (6-APA). Two side reactions occur: PHPGME hydrolysis to p-hydroxyphenyl glycine (PHPG) and amoxicillin hydrolysis to 6-APA and PHPG. [47]

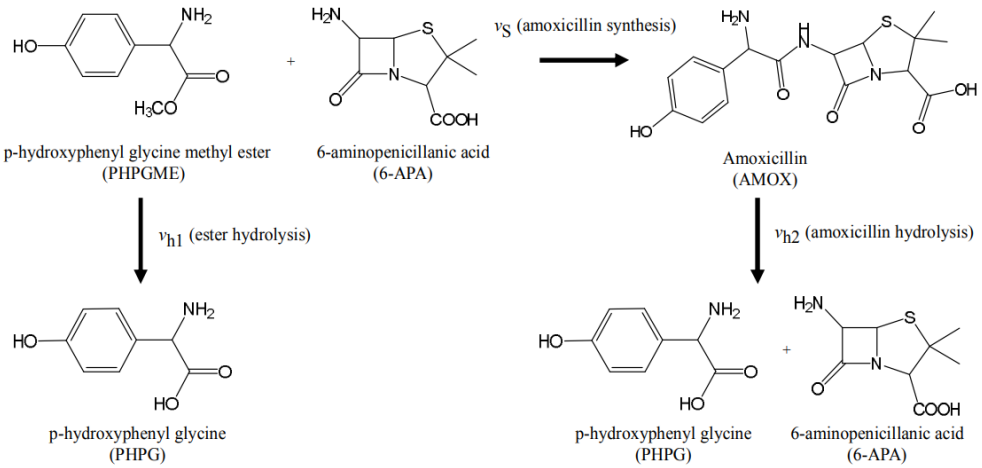


Figure 17: Simplified amoxicillin synthesis pathway [47]

5.2. Amoxicillin synthesis kinetic model:

Equations (1)-(8) describe the kinetic model for the reaction pathways shown in **Figure 17**.

Here:

C = the species concentration at time t;

vi = the species rate of formation;

vh1 = the rate of PHPGME hydrolysis;

vh2 = the rate of AMOX hydrolysis;

vS = the rate of AMOX synthesis;

k = the species inhibition constant;

kEN = the 6-APA adsorption constant;

kcat = the reaction rate constant;

KM = the reaction empirical rate constant;

Xmax is the maximum conversion ratio of the enzyme reagent complex into AMOX.

Subscripts i and j denote species and reactions, respectively.

The system of dynamic ODEs is solved simultaneously using the built-in MATLAB ODE solver ode15s. [47]

Equations (1)-(8) are:

$$\frac{dC_i}{dt} = v_i \tag{1}$$

$$v_{AMOX} = v_S - v_{h2} \tag{2}$$

$$v_{6\text{-}APA} = v_{h2} - v_S \tag{3}$$

$$v_{PHPG} = v_{h1} + v_{h2} \tag{4}$$

$$v_{PHPGME} = \frac{k_{cat,2} C_E C_{PHPGME}}{K_{M1}\left(1 + \frac{C_{AMOX}}{k_{AMOX}} + \frac{C_{PHPG}}{k_{PHPG}}\right) + C_{PHPGME}} \tag{5}$$

$$v_{h1} = v_{PHPGME} - v_S \tag{6}$$

$$v_{h2} = \frac{k_{cat,1} C_E C_{AMOX}}{K_{M2}\left(1 + \frac{C_{PHPGME}}{k_{PHPGME}} + \frac{C_{6\text{-}APA}}{k_{6\text{-}APA}} + \frac{C_{PHPG}}{k_{PHPG}}\right) + C_{AMOX}} \tag{7}$$

$$v_S = \frac{k_{cat,2} C_E C_{PHPGME}}{K_{M1}\left(1 + \frac{C_{AMOX}}{k_{AMOX}} + \frac{C_{PHPG}}{k_{PHPG}}\right) + C_{AMOX}} \frac{C_E}{k_E + C_E} X_{max} \tag{8}$$

5.3. Synthesis of amoxicillin through enzymatic and chemical routes:

Amoxicillin has been reported to exist as amoxicillin base, sodium salt, and trihydrate. However, amoxicillin trihydrate is the most stable amongst them, hence why amoxicillin is usually produced as amoxicillin trihydrate. [57] During the crystallization of amoxicillin from its solution form, it picks up three molecules of water as water of crystallization, although anhydrous amoxicillin can also be made and used. It is a fine, white to off- white crystalline powder, which is sparingly soluble in water. [58]

Numerous patents exist and describe different routes of synthesis, or variations on the existing synthesis of amoxicillin. The conventional methods using Dane salt to chemically obtain amoxicillin (Appendix 2), which typically involve more than 10 steps, require low-reaction temperatures (-30°C), and use toxic solvents like methylene chloride and sialylation reagents. It is reported that the production of one kilogram of amoxicillin generates up to about 70 kg of nonrecyclable waste. In contrast, enzymatic methods (Appendix 3) require far fewer steps, use milder reaction conditions, and generate less waste. The latter approach is being implemented for industrial production: enzymatic synthesis has been used by multinationals since 2006. [59]

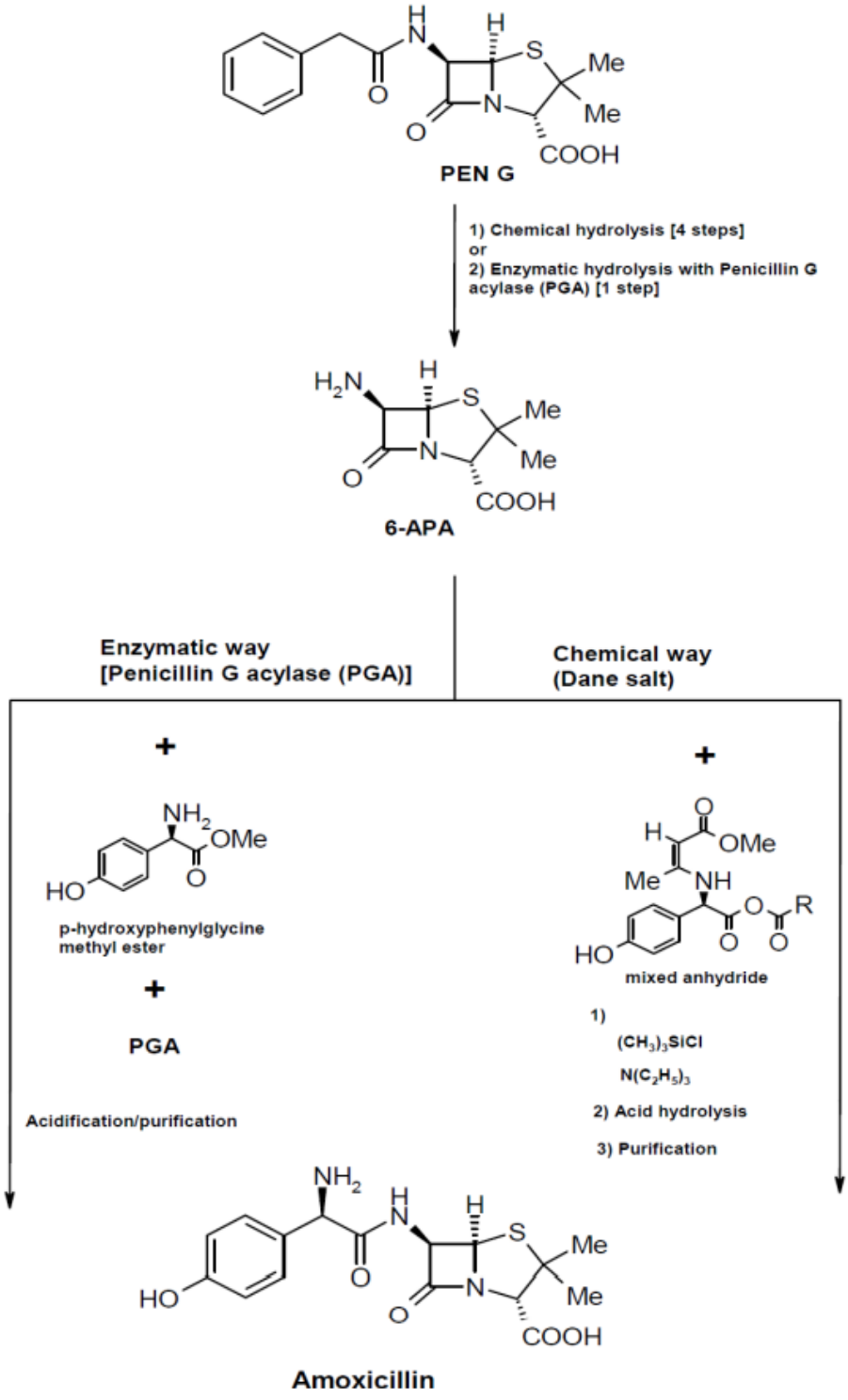


Figure 18: Examples of processes for obtaining amoxicillin. [60]

Chapter 3

Chapter 3: Dosage forms and their administration routes

I. Administration routes and dosage forms:

A route of administration in pharmacology is the means by which a drug, fluid, poison or other substance enters the human body. It is classified by the location at which the drug is administered. Many parameters related to the convenience and compliance influence the choice of the administration route. The administration route is also chosen based on the pharmacokinetic and pharmacodynamic profiles of the drug. Therefore, it is extremely important to understand the characteristics of these routes and the techniques associated with them. [1]

The different dosage forms of amoxicillin are shown in the table down below:

Table 8: Dosage forms of each administration route

Administration route	Dosage form	Action
Oral route	Dry forms: tablets, capsules, powders.	The active pharmaceutical ingredient's liberation depends on the dosage form and the pharmacokinetic of the active ingredient. Some drugs exercise their action on the digestive tract and others need to reach the blood circulation.
	Wet forms: suspensions, syrups.	
Parenteral route (IM, IV, SC, through a catheter)	Injections, perfusions, implants.	Rapid or sometimes immediate response to the drug. A professional is necessary for the administration of these dosage forms.
Rectal route (through the rectum)	Suppositories and local creams.	The active ingredient is quickly released to exercise its action in the rectum or to pass to the bloodstream with a high bioavailability.
Ocular route	Eye drops, ointments.	Treatment of ophthalmic and allergic pathologies.
Respiratory route	Inhalators.	A local treatment for respiratory and ORL pathologies.
Transcutaneous route	Ointments, gels, patches and creams.	Local treatment.

1. Oral route:

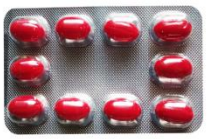
1.1. Definition of the oral route:

Oral route, or in other words, oral administration, is one of the most common administration routes used to take drugs. In this route, the substance is taken through the mouth. Sometimes, we refer to medication taken orally as medication per os, abbreviated to P.O. Usually, drugs administered through this route have a systematic effect; they reach different body parts via the bloodstream. [61]

1.2. Solid dosage forms:

The table down below represents all solid dosage forms administered via the oral route:

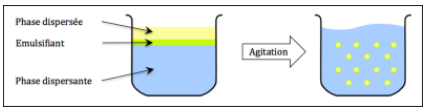
Table 9: Solid dosage forms of the oral route

Dosage form	Definition	Figure
Tablet	Compressed solid dosage form containing one or several active ingredients, with or without excipients.	 **Figure 19: Tablets**
Soft gelatin capsule	A type of capsule that is used to contain medicine in the form of liquid or powder. It dissolves quickly in the digestive tract.	 **Fig. 20: Soft gelatin capsules**
Hard gelatin capsule (a.k.a: two pieces capsule)	Solid form in which the drug is enclosed within a small shell.	 **Fig. 21: Hard gelatin capsules**
Granule	Solid, dry aggregates of powder particles sufficiently resistant to withstand handling.	 **Figure 22: Granules**

1.3. Liquid dosage forms:

The liquid dosage forms administered orally are mentioned in the table down below:

Table 10: Liquid dosage forms of the oral route

Dosage form	Definition	Figure
Syrup	A medicine in the form of a thick liquid containing a sugar solution.	 **Figure 23: Syrup**
Suspension	A coarse dispersion containing finely divided insoluble material suspended in a liquid medium.	 **Figure 24: Suspension**
Emulsion	A liquid preparation consisting of two completely immiscible liquids, one of which can be minute globules coated by gum, dispersed in the other.	 **Figure 25: Emulsion**
Ampule of oral solution	A small sealed vial, which is used to contain a solution to be administered orally.	 **Fig. 26: Ampule of oral solution**

2. Parenteral route:

2.1. Definition of the parenteral route:

Parenteral is a word divided into two parts “para” which means beside and “enteron” which means intestine. This name is given to this route to explain that this administration does not go by the intestines. Although parenteral drug administration means any non-oral administration route, it is usually interpreted to inject the drug directly into the body. [62]

Parenteral is a sterile preparation for injection. It bypasses one or all skin layers and mucous membranes. The most common parenteral routes are the intramuscular route (IM), the subcutaneous route (SC) and the intravenous route (IV). [63]

Parenteral route is the fastest route to administer drugs, with immediate action in seconds for the intravenous route and in minutes for the intramuscular and subcutaneous routes. They possess a 100% bioavailability and they are used especially for drugs that are poorly absorbed or have a notorious effect on the digestive tract. [64]

2.2. Preparations' properties:

The preparation administered by the parenteral route needs to be: limpid, neutral, isotonic, **sterile** and **pyrogen-free**. The following table represents the different preparations of this route:

Table 11: Different preparations of the parenteral route

	Type of the preparation	Administration route
Preparations for injection.	Solution	IM, IV, SC
	Emulsion	IV
	Suspension	IM, SC
Preparations for perfusion.	Solution (≥100ml)	IV
	Emulsion (≥100ml)	IV
Powders for injectable preparations.	**Solution**	**IM**, IV, SC
	Suspension	IM, SC
Implants.	Implants under the skin	

3. Rectal route:

3.1. Definition of the rectal route:

This administration route uses the rectum for administering drugs. These drugs pass into the body's circulation system through the rectum's blood vessels, to be distributed to different organs. A drug administered rectally has a faster onset, higher bioavailability, shorter peak and shorter duration than oral administration. [65]

3.2. Dosage forms of the rectal route:

The different dosage forms are presented in the table down below:

Table 12: Dosage forms of the rectal route

Dosage form	Definition	Figure
Suppository	A form of medicine contained in a small piece of solid material, such as cocoa, butter or glycerin, that melts at body temperature. [66]	 **Figure 27: Suppository**
Rectal capsule	Soft gelatin capsule with an elongated shape that contains a drug dispersed in liquid or pasty excipients.	 **Figure 28: Rectal capsules**
Enema or clyster	An injection of fluid into the lower bowel by the way of the rectum. [67]	 **Figure 29: Enema**

4. Transcutaneous route:

4.1. Definition of the transcutaneous route:

According to the dictionary's definition, transcutaneous means: passing, entering or made by penetration through the skin. Based on this definition, the drugs administered using this administration route are applied directly on the unbroken skin.

4.2. Dosage forms of the transcutaneous route:

Table 13: Dosage forms of the transcutaneous route

Dosage form	Definition	Figure
Non-adhesive dressing.	A sterile dressing pad without an adhesive fixing. Absorbs exudates without sticking to the skin, therefore, causes minimal trauma to the skin.	 **Fig. 30: Non-adhesive dressing**
Ointment.	A smooth, oily substance that is rubbed on the skin to sooth or to heal wounds, burns, rashes, scraps, or other skin problems.	 **Figure 31: Ointment**
Cream	A water-soluble preparation applied to the skin to heal different skin diseases.	 **Figure 32: Cream**
Paste	Consists of a fatty base and a solid substance. It is usually thick and does not melt at body temperature. [68]	 **Figure 33: Paste**
Gel	A solid or semisolid system of multiple constituents. It contains a condensed mass enclosed and interpenetrated by a liquid.	 **Figure 34: Gel**

5. Ocular route:

5.1. Definition of the ocular route:

Ophthalmic drug administration is the administration of a drug to the eyes, to combat numerous diseased states of the eye. These states include bacterial infections, eye injury, glaucoma and dry eye. [64]

5.2. Dosage forms of the ocular route:

The dosage forms are presented in the following table:

Table 14: Dosage forms of the ocular route

Dosage forms	Definition	Figure
Eye drops.	Liquid drops applied to the surface of the eye, generally in small amounts like a single drop or few drops.	 **Figure 35: Eye drops**
Ocular ointment.	A drug in a greasy, semisolid form, which is melted by the body temperature. It is applied directly on the eye.	 **Figure 36: Ocular ointment**
Eyewash	A fluid, usually salineus, used to wash the contaminated eye by foreign materials or substances.	 **Figure 37: Eyewash**
Hydrogel	A crosslinked hydrophilic polymer that does not dissolve in water. It maintains a well-defined structure even though it is highly absorbent. [69]	 **Figure 38: Hydrogel**

Ocular insert.	Defined as a sterile, thin, multilayered, drug-impregnated, of solid or semisolid consistency device placed into the eye.	 **Figure 39: Ocular insert**

6. Respiratory route:

6.1. Definition of the respiratory route:

The respiratory route is the administration of drugs directly into the respiratory system. It includes nasal administration, inhalation and insufflations. The drugs administered through this route exercise their therapeutic action locally and quickly.

6.2. Dosage forms of the respiratory route:

The different dosage forms for drugs to be administered through the respiratory route are in the following table:

Table 15: Dosage forms of the respiratory route

Dosage form	Definition	Figure
Nebulization liquid.	Aqueous solutions, suspensions, or emulsions, which are converted into an aerosol by a nebulizer before administration.	 **Figure 40: Nebulizer**
Dry powder.	A dosage form specifically used to treat kids with asthma. It is administered using a dry powder inhaler, which is a device that delivers medication to the lungs. [70]	 **Figure 41: Inhaler**

Chapter 4

Chapter 4: Production processes of some dosage forms.

I. Tablets:

1. Definition of tablets:

According to the European pharmacopeia, "A tablet is an oral solid dosage form of medicament or medicaments with one or many active substances. It comprises a mixture of active substances and excipients generally in powder form, which is later on compacted or pressed into a solid, hard, smooth-coated pill that breaks down in the digestive tract."

In addition to the active substances and excipients, most tablets contain additives that contribute in holding the pill intact and improving the taste, texture, and appearance.

Some pills have a special coating that prevents them from crumbling in the stomach. This coating ensures that this tablet is not able to dissolve until it reaches the small intestine. Certain tablets are obtainable in a chewable form, while others are orally dissolving tablets (ODT); both forms dissolve in saliva. This type of pills is essential for people who have difficulty swallowing. In either case, the dissolved tablet ends up being absorbed into the bloodstream. [71]

2. Advantages and disadvantages of tablets:

The importance of this form could be explained by the following advantages [71]:

- Low cost: although it depends on the material used to manufacture them, generally speaking, tablets are still cheaper than other forms, which makes them more affordable for consumers.
- Durable and long-lasting: tablets are usually very stable and have a long shelf life.
- Tablets are easy to be administered and to be dispensed.
- Tablets are the lightest and most compact form.
- Packaging and transportation are cheaper and easier compared to any other form.
- They are the best suited for a large-scale production.
- Bitter and nauseous substances are easily introduced in tablets after a convenient coating.

- The possibility of modifying the location of the active ingredients' release.
- The multiple coating of tablets can solve incompatibility problems.
- It is a stable and important form for active substances with low solubility.

The disadvantages of this form are considerably low:

- The atmospheric nature and low-density character of some drugs do not allow them to be compressed into tablets.
- Bitter tasting drugs, unpleasant-smelling drugs, drugs that are sensitive to oxygen or moisture may need encapsulation or special coating, which can add to the cost of the final product.
- Substances with low wettability and slow dissolution are hard to convert into tablets with full drug bioavailability.
- Tablets are concentrated forms that can be harmful to the mucosa of the digestive tract if not absorbed fast enough.

3. Classification of tablets:

Tablets are formulated depending on the physicochemical properties of the substances used, on the site and the extent of drug absorption in the gastrointestinal tract, on the stability to heat, light, or moisture, on the biocompatibility with other ingredients, and on the solubility and the dose. [72]

The different types of tablets are:

- **Dispersible tablets**: also called effervescent tablets, they are tablets that disintegrate in water or other liquid. They are dissolved or dispersed in a liquid, moments before the administration.
- Swallowable tablets: they represent the most common type.
- Chewable tablets: they are used when a quicker rate of dissolution or buccal absorption is required.
- Buccal and sublingual tablets: they dissolve in the cheek pouch or under the tongue.
- Lozenges: are slow dissolving compressed pills that are deprived of disintegrants.
- Coated tablets.

- Enteric-coated tablets: the PH inside of the gastrointestinal tract increases from acidic to basic, from the stomach through the intestines to the colon. These changes are useful in terms of releasing a certain drug in a certain physiological location.
- Immediate release tablets.
- Controlled release tablets.

4. Formulation of tablets:

In order for a tablet to qualify as acceptable, it is important that its formulation ensures that the tablets [73]:

- Are strong and hard to withstand the mechanical shock that can be encountered during manufacturing, packing, shipping, dispensing and use.
- Are uniform in weight and in drug content.
- Are bioavailable according to indication requirements.
- Have a clear identity, and are free from any defect.
- Stay physiochemically stable over a long period of time.

4.1. Factors that influence the choice of the active pharmaceutical ingredient:

When selecting the appropriate active ingredient, we should consider:

- Its physicochemical characteristics.
- Its mechanism of action, and its fate in the body (absorption, transport, metabolism, and elimination).
- The recommended dosage.
- The economic aspect of the enterprise.

The main physicochemical properties that should be taken into consideration while choosing the active substance include: partition, the molecular weight and size of the drug molecule, its solubility, ionization state, and hydrogen bonding capacity. [74]

4.2. Factors that influence the choice of excipients:

In addition to the therapeutic agent, tablets also consist of some excipients that are necessary to ensure a satisfactory production process. These inert materials are added to the active

substance to increase its bulk and offer some desirable characteristics that the drug on its own lacks.

Tablet excipients can be subcategorized depending on the application into:

- Those that help provide satisfactory processing and compression properties to the formulation.
- Those that provide additional desirable physical properties to compressed tablets.

Many excipients used in tablet formulations are multifunctional, meaning they can perform more than one function inside of the drug; therefore, they can affect the properties of the tablet in various ways at different concentrations. [75]

Table 16: Some of the excipients used in the formulation of tablets [76]

Excipient	Function	Examples
Diluents	Provide bulk and enable accurate dosing of potent ingredients	Lactose, dextrin, glucose, sucrose, silicates, calcium and magnesium salt
Binders, compression aids, granulating agents	Bind the tablet ingredients together, give form and mechanical strength	Natural or synthetic polymers
Disintegrants	Aid dispersion of the tablet in the gastrointestinal tract, releasing the active ingredient and increasing the surface area for dissolution	Starch, cellulose derivatives, alginates
Glidants	Improve the flow of powders during tablet manufacturing	Colloidal anhydrous silicon and other silica compounds
Lubricants	Similar action to glidants	Stearic acid and its salts
Tablet coatings and films	Protect tablet from environment, increase mechanical strength, mask taste and smell, aid swallowing	Cellulose acetate phthalate
Coloring agents	Improve acceptability to patients, aid identification and prevent counterfeiting	Synthetic dyes and natural colors

Compatibility studies are conducted in order to choose the appropriate excipients. This selection process is based on: the excipients' stability and solubility properties, the regulatory restrictions, and the cost and marketing requirements.

4.3. Factors that influence the choice of the manufacturing process:

Usually, the choice the process employed during the manufacture of tablets depends on the following factors:

- Compression properties of the active pharmaceutical substance.
- Physicochemical stability of the active ingredient during the manufacturing process.
- The size of the ingredient particles used in the formulation.
- Accessibility of the necessary processing equipment.
- The cost of the formulation process.

5. Manufacturing of tablets:

The manufacturing of tablets needs to undergo many steps called “unified operations”. These unified operations depend on the type of the tablets, the mode of manufacturing, as well as the ingredients used.

There are three essential manufacturing procedures: wet granulation, dry granulation, and direct compression.

5.1. Direct compression:

As its name implies, it involves direct compression of powdered materials into tablets without changing the physical nature of the materials used. It is the most straightforward manufacturing option, with the fewest steps, making it the easiest and cheapest process. This process consists of only two primary steps: blending the active pharmaceutical ingredient (API) with excipients, and compressing the finished tablet. [77]

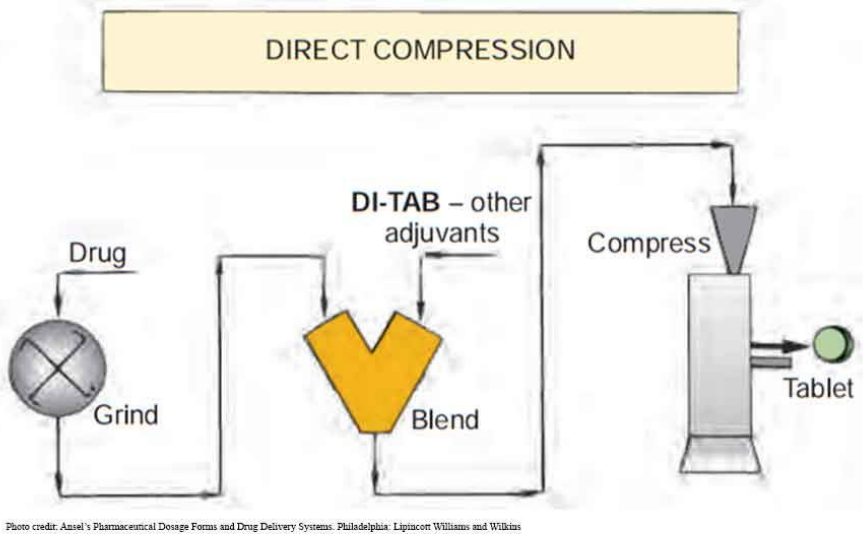


Figure 42: Steps of the direct compression [77]

Direct compression has various advantages and it helps avoid many problems associated with wet and dry granulation. Apart from the process's simplicity, the advantages include: lower capital, labor and energy costs for manufacturing, and avoidance of water, which is extremely useful when working with sensitive materials.

One of the essential requirements for direct compression is that the compression mix must have good flowability to ensure a consistent tablet weight, in order to maintain the safety and efficacy of the tablets over time.

During direct compression, it is important to take into consideration:

- The dose and size of the API.
- Final tablet size and compressibility.
- Binder and diluents particle size and bulk density.
- Powder blend adhesive and cohesive characteristics.
- Powder blend moisture content.
- Powder blend flow and compressibility.
- Type of lubricant and lubrication time.
- Manufacturing train design and segregation potentials.

Table 17: Examples of common excipients used in direct compression [78]

Functionality	Commonly used excipients
Diluent, flow and compression aid	Microcrystalline cellulose, lactose monohydrate, spray dried lactose, starch, dibasic calcium phosphate, Mannitol
Disintegrant and super disintegrant	Croscarmellose sodium, crospovidone, sodium starch glycolate, pregelatinized starch, low substituted hydroxypropyl cellulose, alginate
Glidant	Colloidal silicon dioxide, talc
Lubricant	Sodium stearyl fumarate, magnesium stearate, stearic acid
Coating	Low viscosity Hypromellose, pigment, iron oxide, aluminum flakes

5.2. Granulation technology:

Granulation technology is commonly used in the pharmaceutical industry during solid oral dosage form development, especially in tablet and capsule manufacturing (Parikh, 2010; Shanmugam, 2015). The process is habitually used for size enlargement, where small particles are produced by the addition of liquid to the powder mixture (excipient/API: active pharmaceutical ingredient) and the massing of the mix to produce granules. [79]

5.2.1. Dry granulation:

The dry granulation is the process of forming granules without adding any liquid to the powder blend or mixture, which makes it an ideal way to process moisture-sensitive materials. The fact that it is not necessary to dry the granules makes this process energy efficient. (Parikh, 2010; Shanmugam, 2015)

The granules are formed by compacting powder mixture into large pieces or compacts, which are broken down and sized into granules afterwards. This method is mostly used when excipients have sufficient inherent binding properties. [80]

The steps used to manufacture tablets when using the dry granulation process are the following:

1- Weighing and milling of formulation ingredients.
2- Mixing milled powder.
3- Compression of mixed powders into slugs.

4- Milling and sieving of slugs.
5- Mixing with disintegrant and lubricant.
6- Compression into tablets.

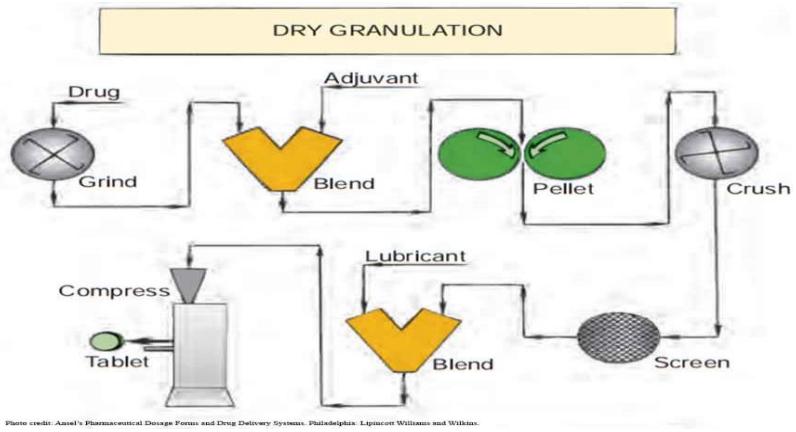


Figure 43: Process steps of dry granulation [80]

5.2.2. Wet granulation:

In the wet granulation process, granules are produced by the addition of a liquid or a dry binder with liquid to the powder blend or mixture (Parikh, 2010; Shanmugam,2015).

Granules are produced by combining the active ingredient and excipients with the solvent, which are later on dried and milled. The solvent binds the powder particles with strong bonds, therefore, in some cases, drying the mixture would fragment the powder. In such cases, adding a liquid solution of binder contributes to avoiding this issue. [81]

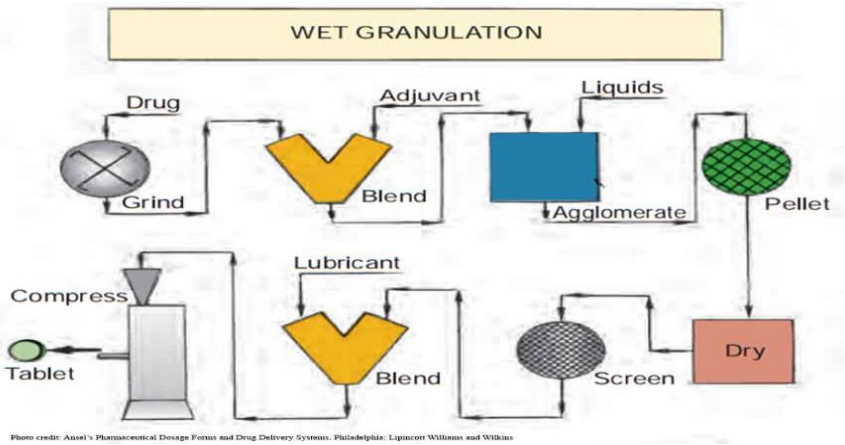


Figure 44: Process steps of wet granulation [81]

i. Low shear:

A traditional granulation process that uses low-speed planetary or trough mixers in which the drug and excipients are granulated with a binder solution. Later on, the wet mass is screened to form granules that are dried using a tray dryer. These granules are rescreened or milled to the size needed, blended with extra-granular excipients, lubricated, and compressed.

The struggles faced with this type of granulation are that the equipment used might not be a closed vessel, that soluble components can migrate during the tray drying, and the lack of in-process control. Low-shear (e.g., fluid-bed) granulation is favored over high-shear granulation when the granules are required to be porous and show fast disintegration and dissolution properties. [82]

ii. High shear:

High-shear granulators consist of two mixing blades: an impeller that rotates in the base of the mixer and a high-speed chopper that continually breaks up the wet mass (typically large agglomerates) during the granulation process. This supplies a more effective way in mixing materials and a smaller volume of water than the low shear granulation. These granulators have a closed vessel system that allows the granules to be transferred to a fluid-bed dryer under high containment. [83]

High-shear granulation is applicable on various formulations; however, the intensity of the mixing can lead to over-granulation that impacts granule tablet-ability.

iii. Fluidized bed granulation:

This process consists of three steps [84]:

- **Blending:** the API and excipients are blended with a low volume of fluidizing air to attain homogeneity and to preheat the dry powder blend. The particle size and density of the ingredients to be fluidized play a major role in achieving the effective fluidization.
- **Granulation:** in this particular step, an organic solvent or a binder solution is sprayed onto the fluidized bed.

- **Drying:** After the spraying has ended, the powder bed continues to be gently fluidized until the granulation is dry. The bed temperature and moisture content determine the end point for this step.

The advantages of this granulation are that a single piece of equipment is used for both granulation and drying, and that granules have a low density with better compressibility.

II. Powders for oral suspensions:

1. Definition of powders for oral suspensions:

Oral powders are preparations consisting of solid, loose, dry particles of varying degrees of finesse. They contain one or more active ingredients, with or without excipients and, if necessary, authorized coloring matter and flavoring substances. They are generally administered in or with water or another suitable liquid. They are presented as single-dose or multi-dose preparations. [85]

These solid substances have a diameter varying between 10nm and 1000µm, and are usually arranged through crushing or grinding. In this particular dosage form, the active pharmaceutical ingredient is processed and mixed into small amounts. [86]

In modern medicine, tablets and capsules have widely replaced the use of powders; however, they are still considered one of the oldest forms of dosage and possess some advantages that make them irreplaceable for pharmaceutical applications. The large use of this dosage form is due to the inherent physical instability of suspensions and the desire of having a long life-span. [86]

The lion's share of drugs prepared as a dry powder for oral suspensions are **antibiotics**.

2. Classification of powders for oral suspensions:

Powders for oral suspensions have been classified into two different types: unit-dose powders and multi-dose powders.

2.1. Unit-dose or single-dose powders for oral suspensions:

This dosage form is marketed within sachets and could be administered to the patient either by sprinkling it on top of a semisolid food like ice-cream or jelly, or by suspending it in a suitable vehicle such as water or other liquids. This mode of administration is favored for pediatric and geriatric populations that can present difficulty in swallowing; and for high dose compounds.

One of the essential requirements for this dosage form is the palatability of the medicine. Drugs which are extremely bitter or have an unpleasant taste are not appropriate to be formulated as powders for oral suspensions. [87]

2.2. Multi-dose powders for oral suspensions:

These multi-dose powders are marketed as powders in suitable-sized bottles for reconstitution with water by either the pharmacist, immediately before dispensing, or the patient, before consuming the drug. The powder dosage form has a huge physical stability which provides a long shelf of life for the commercial product at room temperature, especially considering that these drugs are very unstable in the presence of water.

It is important to note that the reconstituted suspension has a limited span of life under designated storage conditions, such as 14 days under refrigeration. [87]

3. Advantages and disadvantages of powders for oral suspensions:

Some of the advantages listed for oral powders are:

- Availability of the ingredients in a large range and the dose is effortlessly established to the patient.
- Powders can be easily dissolved in water or other liquids, which makes it easier to swallow, especially for children and older people.
- Powder dosage forms have a larger shelf life compared to other liquid forms. For example, powders for antibiotics have a life of 2 to 3 years, but they lose this shelf life once reconstituted with water.
- A large dose is impossible to be administered in any form other than the powder dosage form, which allows an effective administration. In some cases, it is not possible to manufacture tablets with doses between 1g to 5g.
- A water-soluble drug contained in an oral powder is easily and more rapidly dissolved than if it was contained in tablets or capsules, because the shells of tablets and capsules need to be disintegrated before dissolution.
- The cost of the production of oral powders is relatively low, which means the price of the product is cheap compared to other forms.
- Mixing powders allows a large amount of versatility.

Although oral powders have numerous advantages, their contribution in the pharmaceutical world is rather low due to some disadvantages that we have listed down below:

- In the case of drugs with unpleasant taste, powders are not the desirable dosage form, because masking the unpleasant quality can be challenging for this type of preparation.
- Powders are not the right form to dispense drugs that deteriorate quickly when exposed to the atmosphere or an acidic pH like the one present in the stomach. For example, salts

of ferrous iron are easily oxidized, thus they shouldn't be administered in the form of powders.

- Carrying powder can be bothersome due to their bulkiness and heaviness.
- Drugs that are deactivated in the stomach or can cause harm to the stomach, shouldn't be administered as powders.
- Using powders to deliver potent drugs requiring small doses is not appropriate (ex: bulk powders). This is because of variation in spoon fill, when individual doses are extracted from bulk powders using 5ml spoons.
- Hygroscopic or deliquescent drugs cannot be dispensed in the form of powders.

4. Formulation of powders for oral suspensions:

The formulation is a crucial step in the development and qualification of the production of powders for oral suspensions. The formulation needs to satisfy the demands of the different pharmacopeias in order to ensure:

- The uniformity of mass.
- The ability of the drug to be dispersible in liquids.
- The stability of the drug for a long period of time.
- The absence of any anomalies and defects.

4.1. Factors that influence the choice of the active ingredient:

Many studies have concluded that the parameters that should be taken into consideration when choosing the appropriate active ingredient are the same as the ones mentioned previously, with the addition of another critical factor.

This critical factor is the **particle size**. If by any means the particle size is smaller than the formulation's requirements, then the quantity of the active ingredient inside the drug is bigger, which presents a risk of overdosing. Same dilemma is faced if the particle size is bigger than the formulation's requirements, because the quantity of the active ingredient is minimal inside the drug, which puts the activity of the drug at risk. Another important role of the particle size is that it determines the rate of dissolution inside the human body. [88]

4.2. Factors that influence the choice of the excipients:

Excipients are mainly selected according to the dosage form and the requirements of the administration route. They are inert materials that have no adverse interaction with the active pharmaceutical ingredient.

A compatibility study is needed to provide useful information and reference for the selection of the excipient. Therefore, drug applicants should be fully aware of the interactions between the excipients and the active substance, and between the different excipients used.

Physical and chemical properties also play a major role in selecting the excipients because they can affect the quality of the preparation. [88]

4.3. Factors that influence the choice of the manufacturing process:

The choice of the manufacturing process depends on many factors related to the nature of the substances and/or to the economic situation of the enterprise. Some of these factors are the following [88]:

- The stability of the ingredients used in the manufacturing process.
- The particles' size of the ingredients used.
- The delicacy and the fragility of the ingredients.
- The equipment available to ensure the formulation process.

5. Manufacturing of powders for oral suspensions:

The manufacturing process for oral powders should fulfill the requirements of the Good Manufacturing Practice (GMP). These measures are taken into consideration during the manufacturing process in order to:

- Guarantee a suitable particle size range for the intended use.
- Minimize the microbial contamination risk.
- Minimize the cross-contamination risk.

Some force control agents are added to the mixture of APIs with the excipients, like magnesium stearate in order to reduce particle adhesion and improve powder flow. A range of blending approaches exist, which are characterized by the amount of shear applied during mixing. These shears are presented down below:

5.1. High shear mixing:

Shear forces are two forces where one pushes a part of the mixture while the other pushes a different part in the opposite direction in a parallel field. The higher the force is, the better the materials are incorporated.

High shear mixers have a high-speed rotor which forces the mixture outward against a stator to generate shear. These machines mix, rotate and agitate the batch. One of these mixers is called dispersion; it uses high levels of horsepower to create higher levels of shear. This procedure also ensures an even distribution of materials within the batch. [89]

5.2. Low shear mixing:

Low shear is a gentler and less forceful mixing process compared to the high shear mixing. This process is perfect for miscible materials that do not need big energy or force to combine.

It is mostly used to mix delicate materials such as adhesives and polymers, and large particle sizes' materials that shouldn't be broken during mixing. In this type of mixing a higher rate of shear mixing can cause material's degradation. [89]

III. Powders for injectable solutions:

1. Definition of powders for injectable solutions:

Powders for injectable solutions are solid sterile preparations consisting of one or more powders, including freeze-dried powders, intended to be dissolved in the specified liquid to obtain solutions for injection. [90]

Powders for injection are a famous parenteral dosage form for active substances or chemicals. The drugs used in this form cannot be marketed as ready to use injectables because they are unstable in an aqueous environment. They are relatively simple if the formulation and process development are respected. It is important to note also that their performance and stability are highly influenced by numerous parameters. [91]

2. Advantages and disadvantages of powders for injectable solutions:

This form has a lot of advantages compared to other orally administered forms:

- Diversity of the ingredients used.
- The drug's effect is quickly achieved.
- Extremely useful if the patient is vomiting or unconscious.
- The drug's action can be prolonged by modifying the formulation.
- Drugs that are unstable in a low pH can be administered through this route.
- High bioavailability.

Although this route of administration is extremely useful, it still has a number of disadvantages:

- The injection causes pain, and sometimes skin irritation, at the site of injection.
- This form requires a trained person to administer the drug.
- The administration of the drug through a wrong path of injection can be fatal.
- An overdose puts the patient's life at risk.
- The possibility of having a sensitivity reaction or an allergic reaction can lead to the death of the patient.

3. Formulation of powders for injectable solutions:

Pharmaceutical and analytical investigations are essential to precede and support formulation development for all dosage forms. These forms like other drug products must achieve certain specifications in order to be up for sale to the public. Injectable drugs have additional specifications because these products bypass the body's first defenses against microorganisms. These specifications are:

- Sterility: free from bacteria, viruses, molds… etc.
- Particle-free.
- Endotoxin free.

3.1. Factors that influence the choice of the active pharmaceutical ingredient:

In addition to the factors mentioned before, the powders for injectable solutions present numerous key factors that affect the formulation of these sensitive forms. The major factors that control the formulation are [92]:

- The crystal lattice arrangement of the molecules and their effect on the physicochemical properties such as the solubility, dissolution kinetics, hygroscopicity, and chemical stability.
- The particle size of the drug can modify the dissolution rate and the time required for the reconstitution.
- Particle distribution.

Table 18: Studies carried on some APIs [92]

Formulation study	Affected properties
Crystalline form, crystalline vs amorphous; polymorphism	Aqueous solubility, dissolution characteristics, chemical stability
Particle characteristics, particle size, crystal habit, particle shape	Flow properties
Particle-size distribution	Reconstitution time, blend uniformity, flow properties during filling operation
Bulk density and compatibility	Flow properties and compact formation in vacuum-based filling machines
Water content	Chemical stability during storage, flow properties
Hygroscopicity	Stability, environmental conditions for processing

3.2. Factors that influence the choice of the excipients:

The excipients that are used in this formulation can be bulking agents, buffering agents, tonicity modifiers, surfactant, co-solvents and antimicrobial agents.

They are employed to offer some characteristics that the active substance lacks but needs in the formulation, while maintaining the simplicity of the process and the optimal functionality. [80]

Table 19: Examples of excipients used in the formulation of powders for injection [93]

Excipient	Administration route	Percentages
Sucrose	Intravenous	90.00%
Sucrose	Intravenous	7.78%
Sucrose	Intravenous (infusion)	5.40%
Sucrose	Subcutaneous	6.84%
Sucrose	Subcutaneous	4.10%
Glycine	Intramuscular	2.76%
Glycine	Intravenous	25.00%
Glycine	Subcutaneous	2.76%
Dextran 40	Intravenous	30.00%
Anhydrous citric acid	Intravenous	42.18%
Citric acid	Intravenous	7.69%
Citric acid monohydrate	Intravenous	41.36%
Sodium citrate	Intracavitary	5.3-30%
Sodium citrate	Intramuscular	0.64%
Sodium citrate	Intravenous	16.35%
Sodium citrate	Subcutaneous	0.64%

4. Manufacturing of powders for injectable solutions:

Powders for injectable solutions are manufactured by dry mixing of the active ingredient with the excipients needed.

4.1. Dry mixing:

The sterile drug is mixed with the excipients, which can add a degree of complexity to the process in terms of verifying the uniformity of the blend, and the possibility of demixing during bulk-drug shipment and formulation process.

Some particle parameters, like differences in bulk densities between the APIs and the excipients, particle morphology, and flow properties, are of crucial importance. The bulk densities should be somewhat similar and the particles should have a smooth spherical surface, despite the fact that researchers recently found that the smooth spherical surface increases the chances of demixing in the post-mixing and pre-filling steps.

Two strategies are adopted to prevent the segregation of constituents with different particle sizes during bulk-drug and dosage form manufacturing. These strategies are the following [94]:

- **Bulk-drug manufacturing:** during shipment and storage of the drug, vacuum packing of the blend of API-excipients is used to prevent the relative movement and segregation of particles.
- **Dosage form manufacturing:** customizing the mixing terms based on the drug such as the mixing speed, the mixing time…etc.

IV. Quality control:

One of the most important functions in the pharmaceutical industry is the pharmaceutical quality control laboratory. The quality control laboratory is pertained by a remarkable portion of the Current Good Manufacturing Process (CGMP) regulations (21 CFR 211).

1. Definition of quality control:

By dictionary definition "quality control" means: checking and directing the degree or grade of excellence of process and products. In the pharmaceutical industry, it indicates an explicit system of inspection and control covering the production, evaluation and distribution of every drug before dispensing it into the market. The production of medications of superior efficacy, safety and elegance is the ultimate goal of these operations. Quality control provides assurance to the physician, pharmacist and the consumer that the given product presents uniformity and satisfies the purpose desired. [95]

According to the World Health Organization (WHO), the term quality control refers to the sum of all procedures undertaken to ensure the identity and purity of a particular pharmaceutical product.

2. Objectives of quality control:

According to the FDA, the main objective of quality control in the pharmaceutical industry is to test the drugs in their various stages of production, verifying that they are able to proceed to the next stage and release the manufacturing process in accordance with the regulations and specifications required for consumption.

Laboratory controls are an integral part of Good Manufacturing Practice and the requirements are described in the GMP regulations. The laboratory inspection may be limited to specific issues, or the inspection may encompass a comprehensive evaluation of the laboratory's compliance with CGMPs. As a minimum, each pharmaceutical quality control laboratory should receive a comprehensive GMP evaluation each two years as part of the statutory inspection obligation.

3. Quality control of a pharmaceutical product:

Quality control is responsible for many processes such as sampling, specifications and testing. It ensures that the materials are not used, and products are not dispensed, until their quality is confirmed to comply with the international standards. [96]

Different quality control tests can be used and are classified as the following:

- **Physicochemical control:** physicochemical properties are essential for determining the drug's pharmacokinetic and pharmacodynamic profiles. They also increase the success rate. It is then crucial to control these properties throughout the production. The physicochemical control includes: a control of the ingredients, the packaging and the final product.
- **Toxicological control:** the evaluation of drug safety and toxicology is a key element in the quality control of a drug. It is important to be well aware of the functional and anatomic consequences of the consecutive administration of a drug before dispensing it into the market.
- **Microbiological control:** for numerous reasons, microbial contamination is a challenge in the pharmaceutical industry. Quality control needs to ensure and maintain an excellent hygienic quality of its products to minimize the loss of materials and cost.

The quality control department functions for assuring the good quality of all products manufactured, at every stage of the process:

- Quality control of the raw materials
- Quality control of the intermediate product
- Quality control of the final product.

3.1. Quality control of the raw materials:

Quality control of raw materials include many tests such as identity, purity and content testing. These tests are carried out following the regulations of the pharmacopeia.

In the case of powders for injectable solutions another test is recommended which is the sterility test, to ensure that the ingredients are sterile and have no contamination whatsoever.

3.2. Quality control of intermediate product:

This quality control is carried out on the powder after the mixing, for both the oral powders and tablets, and during the compression for the tablets. But it is not applicable to the powders for injectable solutions.

3.2.1. On the powder:

During the production, the first step is the mixing of the active ingredient with the other raw materials. Quality control of the powder after the mixing is a key element in the qualification of a product. This control is executed in the following order: sampling, inspection and testing

as per specifications of in-process product for release or rejection for further processing and its documentation.

The most important tests performed on the powders are:

- **Homogeneity test:** the homogeneity of the mix should be certified and assured by dosing the active ingredient in the sample taken.
- **Humidity test:** in the case of wet granulation, the residual humidity needs to be dosed and verified to be under its limitation.
- **Fluidity test.**

3.2.2. On the tablet:

During the compression, it is important to control the outcome of tablets before advancing to other manufacturing steps. Numerous tests are carried out on tablets during this step but the most important of them all are these two:

- **The harshness of the tablet:** measured by a durometer
- **The mass of the tablet:** the tablets are weighted throughout the entire production process, and the average mass is calculated. The tablet's mass should be constant with a small range of variation.

3.3. Quality control of the final product:

The quality control of the final product is carried out based on the regulations and requirements of the GMP and the pharmacopeia. This control comprises physicochemical and microbial tests that are executed by the professional employees in the laboratory.

These following tests are performed on all the dosage forms studied:

- Identification assay and dosing different impurities.
- Microbial assay.
- Assay on the nature of the active ingredients.
- Verification of the packaging system.

Some additional tests are mentioned in the Table x down below. These tests are specific for each dosage form, because they are related to the manufacturing and the specific characteristics of each form.

Table 20: Additional specific quality control tests

Dosage form	Additional tests
Tablets	- Harshness test - Breakability test - Friability test - Mass uniformity essay - Content uniformity essay - Disintegration test - In vitro dissolution test
Powders for oral suspension	- Minimum fill test - Volatility test - Powder finesse
Powders for injectable solutions	- Uniformity of dosage units - Purity of the vehicle and added substances - Antimicrobial preservative - Water content - Completeness and clarity of solutions

Practical part

I. Introduction:

Our study took place in the SAIDAL group's Medea production complex during the month of March 2022.

This unit manufactures and controls approximately 215 pharmaceutical products in different dosage forms, such as capsules, tablets, injectable solutions, suspensions, creams… etc.

During our time there, we followed the production process and the quality control of three dosage forms of AMOXYPEN®: the dispersible tablets, powders for oral suspensions, and powders for injectable solutions, from the raw materials to the Finished Pharmaceutical Products (FPP).

The raw material used there is imported from foreign countries such as India, and placed in a storage warehouse in a waiting area where samples are taken to the Quality Control Laboratory to be tested. When the control is carried out, the raw material can be deemed as either: compliant or non-compliant. If the product is compliant, then it labelled with a green tag and transported to the area of compliant products.

Figure 45: Storage warehouse inside the SAIDAL production site.

The equipment and the methods used during the manufacturing and the control of the pharmaceutical products are described in the drug file in accordance with the monographs employed (USP, Ph. Eur., BP) and the Good Manufacturing Practices (GMP).

All operations are carried out under the responsibility of the production operators, managed and controlled by the production team leaders and the production managers according to a program validated by the factory management.

II. Presentation of the SAIDAL group:

The SAIDAL industrial group is a joint stock company with a share capital of 2,500,000,000 Algerian dinars, its main mission is to develop, produce and market pharmaceutical products for human and veterinary use. It is currently considered the leader of the pharmaceutical industry in Algeria with a large market share.

1. History and organization of the SAIDAL group:

1.1. History of the SAIDAL group:

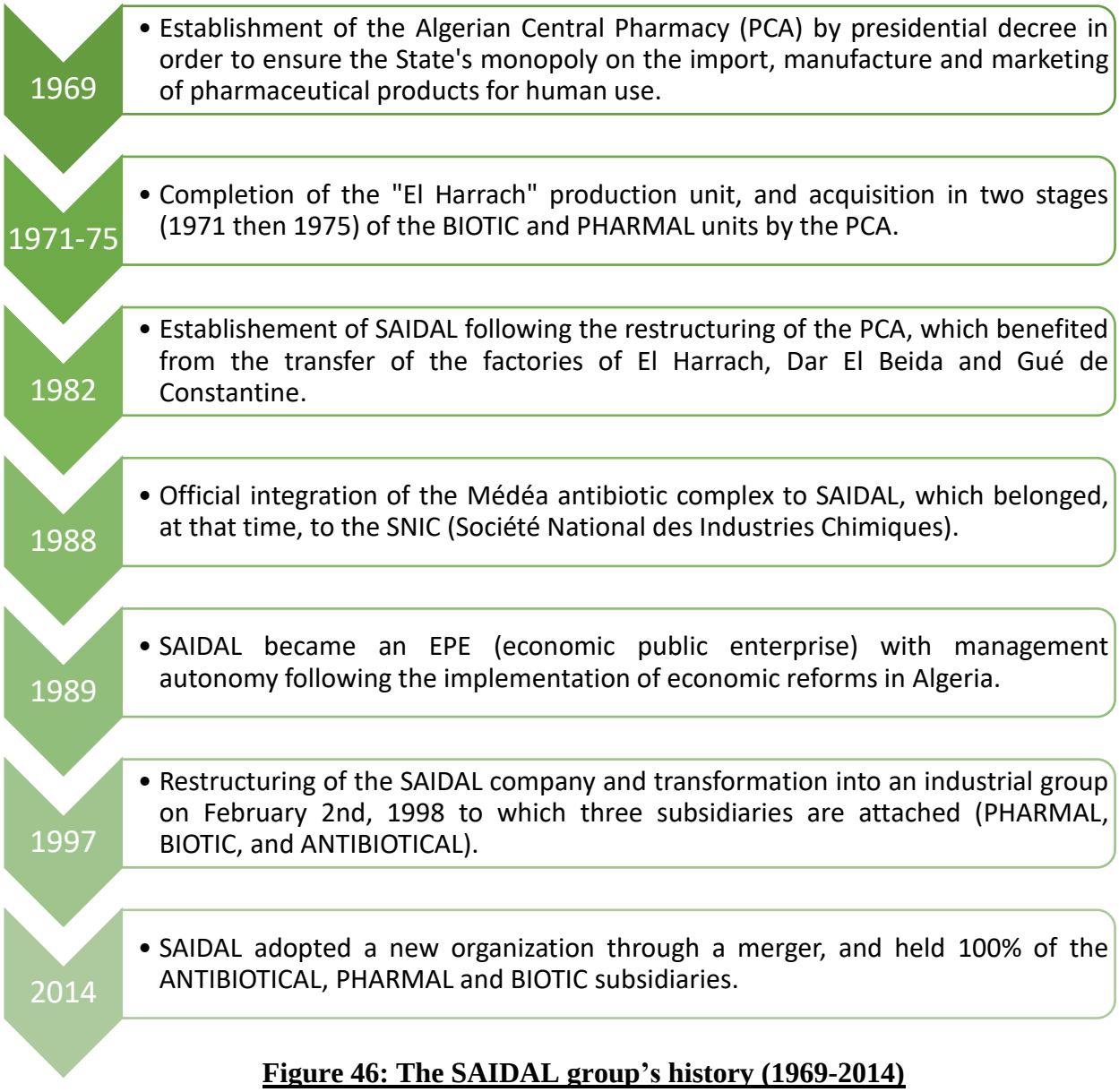


Figure 46: The SAIDAL group's history (1969-2014)

1.2. Organization of the SAIDAL group:

The SAIDAL Group proceeded, in January 2014, to a merger by absorption of the different subsidiaries: ANTIBIOTICAL, PHARMAL and BIOTIC. This decision was approved by its corporate bodies, and it gave rise to a new organization.

Figure 47: Logo of the SAIDAL group.

1.2.1. General management of the group:

It is a decision-making structure that brings together the Central Departments.

1.2.2. Production sites:

SAIDAL has 09 production sites situated in different parts of the country:

- Dar El Beida's production site.
- Medea's production site.
- Constantine's production site.
- Gué de Constantine's production site.
- El-Harrach's production site.
- Cherchell's production site.
- Batna's production site.
- Annaba's production site.
- Constantine's production site (Insulin unit).

Figure 48: SAIDAL group's production sites throughout Algeria

1.2.3. Distribution centers:

- A center Distribution Center.
- An eastern Distribution Center.
- A western Distribution Center.

2. Medea production site:

The Medea production site is specialized in the production of penicillin and non-penicillin antibiotics. It is equipped with the necessary facilities for the manufacture of pharmaceutical drugs, from raw materials to final pharmaceutical products.

Figure 49: SAIDAL Medea production complex

The Medea antibiotic production complex has:

3. Two semi-synthesis units for oral and sterile injectable products and an entity for pharmaceutical specialties.
4. Two production buildings for pharmaceutical specialties; one is dedicated to the production of penicillins and the other to the production of non-penicillins.
5. A production unit for packaging items (labels and vignettes printing).
6. A Quality Control laboratory.
7. General services necessary for the operation of these facilities.

The Antibiotics Complex produces the following pharmaceutical forms: injectables, capsules, syrups, ointments, creams, powders, and tablets.

The site is characterized by a significant production capacity in the manufacture of pharmaceutical specialties and by qualified analytical laboratories allowing full control over the quality of products.

III. Objectives:

The main objectives of this thesis are as follow:

- Describe the different steps of production of three different dosage forms (dispersible tablets, powders for oral suspensions, and powders for injectable solutions) of AMOXYPEN® that are produced in the SAIDAL group's Medea production complex.
- Explain the reason why certain methods of production are chosen over others.
- Document the rigorous methods of quality control used all throughout the production process.
- Establish a comparison between the production and the quality control processes of these three different dosage forms of the same antibiotic.

IV. Materials and methods:

1. Materials:

1.1. Presentation of the products:

1.1.1. AMOXYPEN® dispersible tablets:

AMOXYPEN® is the trade name of amoxicillin, which is an antibiotic that belongs to the amino-penicillin family. This trade name has been chosen by the pharmaceutical company SAIDAL.

AMOXYPEN® can be found in different dosage forms, one of these forms is the dispersible tablets form. Dispersible tablets are tablets that disintegrate in water in order to be administered orally. They are white and have a mint odor. They are marketed in boxes that contain 14 or 16 tablets, with a dose of 1g per tablet. It is prescribed to treat many infections like strep throat and pneumonia. It is relatively cheap, it costs around 360DA.

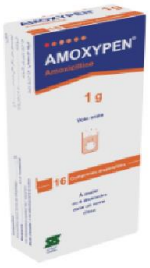
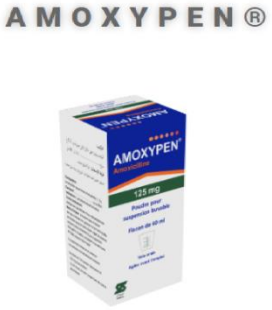


Figure 50: AMOXYPEN 14 pills' box

Figure 51: AMOXYPEN 16 pills' box

1.1.2. AMOXYPEN® powders for oral suspension:

One of the dosage forms of amoxicillin that are marketed under the name of AMOXYPEN® is the powders for oral suspension form. These powders are administered orally in or with water. This dosage form is presented as bottles of a white multi-dose powder with an odor of apricots. It is marketed in 60ml glass flacons with doses of 125mg, 250mg, and 500mg. It is sold for around 140 DA.



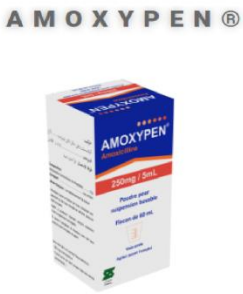


Figure 52: AMOXYPEN POS125mg

Figure 53: AMOXYPEN POS 250mg

1.1.3. AMOXYPEN® powders for injectable solutions:

Powders for injectable solutions are another dosage form for AMOXYPEN®. They are dissolved in a specific solvent to be injected later on through the intramuscular route. They are marketed in glass vials that contain a dose of 500mg or 1g, alongside with an ampule containing benzyl alcohol. It costs around 115 DA.

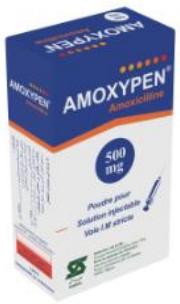


Figure 54: AMOXYPEN injectable powders

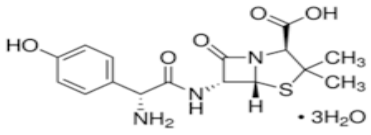
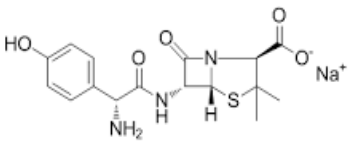
1.2. Raw materials:

1.2.1. Active pharmaceutical ingredients:

The active ingredient in AMOXYPEN® is amoxicillin, an antibiotic that belongs to the amino-penicillin family. Amoxicillin comes in two forms: sterile sodium amoxicillin for injectable medicinal products (IM); and non-sterile amoxicillin trihydrate for oral medicinal products.

Amoxicillin's physicochemical properties are demonstrated in the table down below:

Table 21: Physicochemical properties of the amoxicillin trihydrate and amoxicillin sodium

Physicochemical properties	Amoxicillin trihydrate	Amoxicillin sodium	References
Appearance	Amoxicillin trihydrate is a crystalline, odorless and off-white powder.	Sodium amoxicillin is an almost white and very hygroscopic powder	USP
Chemical structure			USP
Chemical formula	$C_{16}H_{19}N_3O_5S.3H_2O$	$C_{16}H_{19}N_3O_5S$ NA	USP
Stereochemistry	It has the S, R, R configuration at C2 (equivalent to C3 in conventional penicillin numbering), C5 and C6, respectively, that is common to all penicillin. The side-chain configuration at C10 is R in some of the literature. This is referred to as D (-)	It has the S, R, R configuration at C2 (equivalent to C3 in conventional penicillin numbering), C5 and C6, respectively, that is common to all penicillin. The side-chain configuration at C10 is R in some of the literature. This is referred to as D (-)	USP

Molecular weight	419.45 g/mol	387.39 g/mol	USP
Solubility	slightly soluble in water, very slightly soluble in ethanol (96%), and practically insoluble in fatty oils.	very soluble in water, sparingly soluble in anhydrous ethanol, and very slightly soluble in acetone.	British pharmacopeia
Bulk density	0.7 g/ml to 0.8 g/ml	0.7 g/ml to 0.8 g/ml	USP
Melting point	194 °C	194 °C	USP
Optical rotation	+240 to +290°	+240 to +290°	British pharmacopeia
Stability	The stability depends on the pH. It has high stability in an acidic pH	The stability depends on the pH. Presents high stability in acidic pH.	British pharmacopeia
Conservation	Sensitive to light and humidity, so it is conserved in a dark place with a temperature of 25°.	Sensitive to light and humidity, so it is conserved in a dark place with a temperature of 25°.	European pharmacopeia

1.2.2. Excipients:

The excipients used for the manufacturing of AMOXYPEN® in the three different dosage forms are presented in the tables down below:

Table 22: Excipient used in AMOXYPEN® dispersible tablets

Excipient	Role	Reference
Aerosol 200 Hydrophilic fumed silica	Improve the free flow and anti-caking characteristics of powders Anti-settling, anti-sagging and anti-thickening	USP
Aspartame	Artificial non-saccharide sweetener used to mask the unpleasant taste	USP
Mint aroma	Flavoring agent	USP
Polyplasdone Crospovidone PVP XL 10	Super disintegrant, helps the tablet disperse in water	USP
Avicel pH 102 microcrystalline cellulose	Texturizer, an anti-caking agent, a fat substitute, an emulsifier, an extender and a bulking agent in the production of tablets	USP
Magnesium stearate	A flow agent that keeps the ingredients of the tablet from sticking together. It forms a barrier between the drug and the equipment that manufacture them. It also slows the absorption and the breakdown of tablets	USP

Table 23: Excipients used in AMOXYPEN® powders for oral suspension

Excipient	Role	Reference
Aspartame	Artificial non-saccharide sweetener used to mask the unpleasant taste	USP
Sodium benzoate	A preservative that prolongs the shelf life	EMA/CHMP/ 508189/2013 9/10/2017
Maltodextrin	It is used as filler in sugar substitute such as aspartame	USP
Apricot aroma	Flavoring agent	USP

Table 24: Excipients used in AMOXYPEN® powders for injectable solutions

Excipient	Role	Reference
Sodium	It increases solubility. It has disintegration, lubrication, binding, emulsifying, and stabilizing properties.	EMA/CHMP/ 338679/2014 9/10/2017
Benzyl alcohol	It is used for its preservative properties, as a solubilizing agent or as a fragrance	EMA/CHMP/ 508188/2013 9/10/2017

1.3. Equipment:

1.3.1. Equipment used for the manufacturing of AMOXYPEN®:

All the equipment used during the production processes of the various dosage forms of AMOXYPEN® that we have observed in the production site are presented in the tables down below:

Table 25: Equipment used for the production of AMOXYPEN® dispersible tablets

Equipment	Principle of work	Figure
Convective mixer (XX4452A)	It consists of a vertical static shell or container, in which powders are circulated around by a rotating blade, paddle or screw. The rotation speed can go from 20 to 60 rpm for a tank capacity that goes from mere liters to tens of m^3	 **Figure 55: Convective Mixer**

Compactor (Alexenderwerk)	The powder comes through a feeding system to be shaped as tablets by a roller press. The horizontal feeding system, where the transport of the powder is ensured by an endless screw placed horizontally under the hopper.	 **Figure 56: Compactor**
Sieve shaker (WESTON)	Circular sieves are used in sieving pharmaceutical powders and solid forms in general. It uses an auto-shaker to assure a satisfying result.	 **Figure 57: sieve shaker**
Rotary Tablet press (IM A S250 SMART)	It is a mechanical device that has several tooling stations, which rotate to compress powder mixture into tablets of uniform size, shape and weight. The compaction force on the fill material is exerted by both upper and lower stamps leaving the powder granules to be compressed in the middle.	 **Figure 58: Rotary tablet press**

Table 26: Equipment used for the production of AMOXYPEN® powder for oral suspensions

Equipment	Principle	Figure
Ribbon mixer	It is a light duty mixer, mainly used for mixing powder components, which are pre-processed, like dried granules and pre-sieved powders. It achieves solid mixing when high shearing force is not desired.	 **Figure 59: Ribbon mixer**
Sieve shaker (WESTON)	Circular sieves are used in sieving pharmaceutical powders and solid forms in general. It uses an auto-shaker to assure a satisfying result.	 **Figure 60: Sieve shaker**

1.3.2. Sterilization equipment:

The manufacturing of the powder for injectable solutions is a complex process due to the fact that these dosage forms need a high level of sterilization for the materials and packaging used. This sterilization is assured by the equipment in the table down below:

Table 27: Equipment used in the sterilization of AMOXYPEN® powder for injectable solutions

Equipment	Principle	Figure
Autoclave (Telstar W4155 A)	The autoclave, also called steam sterilizer, uses steam under pressure to kill harmful microorganisms present on the vials placed inside a pressure vessel. The vials are heated to an appropriate heat sterilization temperature for a specific amount of time in order to destroy the protein structure of these microorganisms.	 **Figure 61: Autoclave**
Packaging washer (W4120)	This machine cleans the vials used in the packaging of AMOXYPEN powders for injectable solutions. It uses clean hot water to get rid of any particles present on the vials.	 **Figure 62: Packaging washer**

1.3.3. Equipment for the packaging of AMOXYPEN®:

Table 28: Equipment used for the primary packaging of AMOXYPEN®

Equipment	Principle	Figure
Thermo-forming machine FAMAR W4401C	The role of this machine is to make: - Transparent PVC blisters for AMOXYPEN® dispersible tablets - Aluminum foil	 **Fig. 63: Thermoforming machine**
Dosing machine hitimon	It is a machine used in the packaging of AMOXYPEN® powders for oral suspension, to distribute the powder inside flacons based on the information given. The powder is loaded in a superior hopper, while the caps are in the inferior one. Then the flacons are charged with powder and closed.	 **Fig. 64: Hitimon dosing machine**
Dosing machine HSUA W4115 A/B	This dosing machine is used in the packaging of AMOXYPEN® powders for injectable solutions. It charges the vials with the powder then seals them using rubber caps then aluminum caps.	 **Fig. 65: HSUA dosing machine**

Table 29: Equipment used for the secondary packaging of AMOXYPEN®

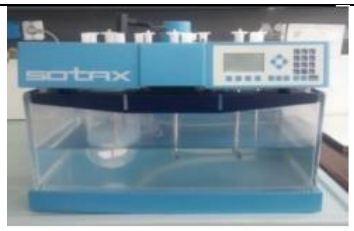
Equipment	Principle	Figure
Cartoner (ZANASI W4404C)	It is a packaging machine that forms the drug's box after placing the flacon or blister inside along with the leaflet.	 **Figure 66: Cartoner**
Vignette machine AVERY W4401C°	This machine places the adhesive vignette on the drug's box as it leaves the cartoning machine.	 **Figure 67: Vignette machine**

1.3.4. Equipment for quality control:

The quality control equipment that was used to ensure the quality of the pharmaceutical products is listed in the table down below:

Table 30: Equipment used in the quality control of AMOXYPEN®

Equipment	Description	Figure
HPLC (SHIMADZU)	It is used to separate the phases of solid or liquid regardless of its stability and volatility. The principle of HPLC focuses on what occurs when the analyte and a test solvent are pushed under pressure through a column within the HPLC equipment. It is used for identification of compounds, characterization of molecular bonds, purity testing and molecular quantification.	 **Figure 68: HPLC**
pH meter (Metrohm)	It measures the hydrogen ion activity in water-based solutions. It indicates the acidity or alkalinity of a solution by expressing it as pH. It measures the difference in electrical potential between a pH electrode and a reference electrode, hence why it is called sometimes "potentiometric pH meter"	 **Figure 69: pH meter**
TLC plates	It is a separation technique for quantitative and qualitative analysis. It uses a thin layer of a stationary phase coated on a glass, plastic or aluminum plate. A solvent, which represents the mobile phase, carries the sample and separates it as it moves across the plate.	 **Figure 70: TLC plates**
UV/VIS spectrometer (PerkinElmer precisely lambda 25)	It is used to measure how much the drug absorbs light, by measuring the intensity of light that passes through a sample, then compare it to the intensity of light through a reference sample or blank, using the same solvent in both cases.	 **Fig. 71: UV/VIS spectrometer**
Moisture determination balance (ERWEKA)	Quick and effective moisture analyzers. It measures the moisture of the powder after the mixing.	 **Figure 72: Moisture determination balance**

Dissolution tester (SOTAX)	The dissolution tester uses a defined setup with specific conditions to evaluate the performance of a product. It measures the release rate and total drug amount dissolved over time.	 **Fig. 73: Dissolution tester**
Friability meter (ERWEKA)	It measures the friability, which is the percentage of weight loss of powder from the surface of the tablets due to mechanical action. The rotation speed is 25 rpm and it lasts for 4 minutes.	 **Figure 74: Friability meter**
Disintegration' s equipment (OHAUS MB 45)	It determines whether a tablet would disintegrate within the time prescribed or not, when placed in a liquid medium under the prescribed conditions. The test is performed on 6 tablets placed in the liquid.	 **Figure 75: Disintegration's equipment**
Durometer (SOTAX HT1)	It is a standardized way to measure the tablets' hardness. It exercises a force on the tablet until the breakdown point. The tablet should have a diameter of 3 to 30mm and a thickness of 1.5 to 12mm.	 **Figure 76: Durometer**
Laminar Flow cabinet	It is an enclosed workstation that is used to create a contamination-free environment through filters to capture all particles entering the cabinet. It also has a UV germicidal lamp that sterilizes the interior of the cabinet and the content before the operation	 **Figure 77: Laminar flow cabinet**

2. Methods:

2.1. Manufacturing methods:

2.1.1. Manufacturing method of AMOXYPEN® dispersible tablets:

A. Principle:

The production process of AMOXYPEN® dispersible tablets employs granulation by dry process, which is a method that forms granules without using a liquid solution. This method is used when the product that needs to be granulated is moisture and heat sensitive or when it does not compress well. The production process goes through 5 stages: initial mixing, compacting, sieving, final mixing, compressing and packaging. Each one of these steps is followed by a thorough and precise control.

B. Pre-manufacturing steps:

Before starting the production, it is necessary to:

- Ensure the presence, in the preparation room, of the material safety data sheet (MSDS) corresponding to the specialty to be manufactured.
- Check the availability of utilities such as softened water and nitrogen.
- Check that the powder injection system is functional.
- Check that all the equipment (mixer, scale, granulator) is properly cleaned.
- Introduce, using a pallet truck, the raw materials from the corridor to the weighing area.
- Wear individual means of protection (masks and gloves).
- Check the presence, on each packaging of raw materials, of the green label which proves that the product used is compliant.
- Control the weight of each raw material according to the order form.
- Prepare the barrels by putting a polyethylene bag in each barrel to collect the mixture, and identify them by mentioning: the specialty, the date, the preparation phase, the batch number, the container number, the gross weight, and the name of the operator in charge of the operation.
- Granulate excipients that exhibit aggregates using the granulator on a 1-millimeter mesh screen.

C. Manufacturing steps:

a. Initial mixing:

In order to facilitates flow during compaction, amoxicillin powder is mixed with magnesium stearate for 5 minutes using a convective mixer.

Figure 78: Initial mix.

b. Compaction:

Compaction is a dry granulation technique in which a substance is compacted in order to obtain compacts of high density which will then be crushed and calibrated.

The type of compactor used during this compaction process is the roller compactor, which consists of three major units: the feeding unit, the compaction unit and the granulation unit. It is used to force fine powders between two counter rotating rolls and presses the raw materials into a solid compact.

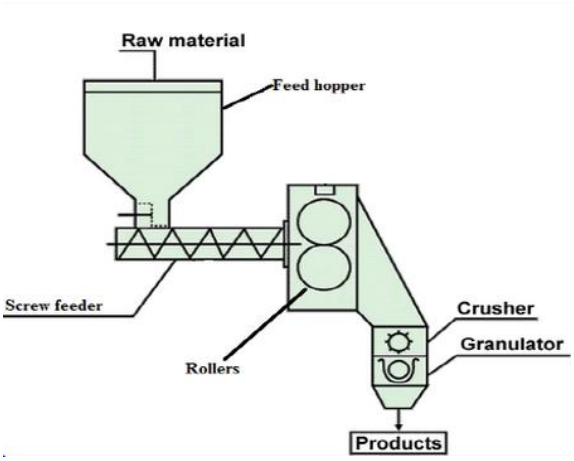


Figure 79: Roller compaction.

The powder compacting operation consists of three stages:

- Powder transport between the rollers.
- Powder compaction to form platelets.

- Platelets crushing to obtain granules.

The routing step can be either performed by gravity or by a feeding and a tamping auger (an endless screw). In this case, Archimedes' screws are used. After the powder has passed through the rollers, the powder then passes through a 1st grid, which diameter is 5mm, to be compacted in the form of platelets. After that, the powder passes through the mesh of the 2nd grid, with a 0.7mm diameter, to become a compacted powder.

Figure 80: Platelets formed from compaction.

The compact powder is collected in the barrels that were prepared beforehand, and then transferred to the sieving station.

c. Sieving:

- Definition:

Sieving is the passage of a solid product or a suspension through a sieve to carry out the separation and possibly the particle size analysis of certain elements.

- Method:

Once we fill the barrels with the compacted powder, we sift the content of each barrel on a 0.25mm grid.

We recover the sieved powder that is smaller than 0.25mm to recycle it gradually in the compactor, at the end, we take 50% of the sieved powder + 50% of the compacted powder that is greater than 0.25mm and put them in the barrels. The barrels will then be weighed.

d. Final mixing:

- **Pre-mix A:**

After completing the sieving step, we need to:

- Transfer the barrels of raw materials to the mixing room.
- Check the identity of the barrels according to the MSDS.
- Switch on the mixer.
- Make sure the mixer relief valve is closed.
- Open the mixer's powder injection valve halfway, the tap of the mixer safety valve, and the cooling valve.
- Open the manhole and then load into the mixer:
    - ✓ Compacted amoxicillin trihydrate.
    - ✓ Aerosil 200, also known hydrophilic fumed silica.
    - ✓ Aspartame.
    - ✓ Mint aroma.
    - ✓ Polyplasdone crospovidone or polyplasdone XL-10.
    - ✓ Avicel pH-102 (66% of the total quantity).
- Open the main nitrogen valve and adjust the flow to 20 L/min.
- Close the mixer's powder injection valve.
- Turn on the mixer and mix the active ingredient with these excipients for 20 minutes.
- Record the quantities of raw materials loaded, mixing time, and nitrogen flow in the batch record.

- **Pre-mix B:**

Once the pre-mix A is ready, the rest of the raw materials, which are magnesium stearate and Avicel pH-102 (the remaining 34%), are loaded into a double ethylene bag. The bag is slightly inflated with nitrogen and closed tightly with tape. The content of the bag is then manually agitated for 5 to 10 minutes.

Figure 81: Convective mixer.

- **Final mix:**

In order to obtain the final mix, we need to:

- Add premix B to premix A.
- Turn on the mixer and continue mixing for 30 minutes.
- Record quantities of materials added, mixing time and nitrogen flow in the batch record.
- When the mixing time is up, stop the mixer and close the main nitrogen and softened water valves.
- Wait for the sampling of the mixture for analysis.
- After sampling the mixture, discharge the mixer through the discharge valve into the previously prepared and identified barrels.
- If the analysis result is compliant, move on to the compression stage.

e. Compression (tablet formation):

- **Definition:**

Compression is an operation that allows the shaping of powders into tablets.

- **Before compression:**

First, we must:

- Transfer the barrels of the final mixture to the compression room.
- Raise the pressure to 5 bar and turn on the vacuum cleaner.
- Switch on the tablet press.
- Load the powder into the tablet press' feed hopper.

Figure 82: Rotary tablet press machine.

- **During compression:**

The aim of this step is to densify the powder and to put it in the form of a tablet. Pharmaceutical tablets are industrially produced from powders using rotary tablet presses.

- **Landfill:**

It is the withdrawal phase of the stamps that perform the compression.

During compression, the powder bed gains the energy from the various mechanisms that contributed to the densification.

- **Ejection:**

This operation is generally carried out by raising the upper stamp or lowering the mold plate.

- **Relaxation:**

Once the tablet has been ejected, it continues to expand to reach, after a certain time, a stable state. But if the powder is sensitive to humidity, the tablet might crack.

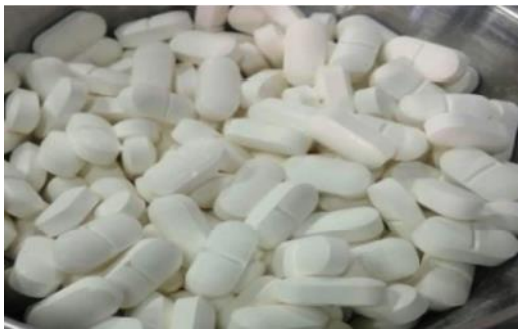


Figure 83: Amoxicillin dispersible tablets after compression.

f. Packaging:

The AMOXYPEN® dispersible tablets are packaged inside thermoformed transparent PVC blister strips that are thermo-sealed with aluminum foil. The blister package provides excellent protection from moisture, gas, and temperature, and it is easier for the patients to handle and store.

- **Blistering:**

Blistering is the process of making blisters or enclosing the tablets and capsules into preformed plastic packaging. It is done using a blister packaging machine.

The primary component of a blister pack is a cavity or pocket made, in our case, from transparent PVC, that has a lidding seal of aluminum foil. The formed pocket contains the product and the lidding seals the product in the package.

- **Blister Packing Machine:**

It is a high-quality machine that is suitable for handling automatic loading, filling or non-stop feeding. Blister packaging machines are used by the pharmaceutical industry to pack capsules and tablets.

Figure 84: Blister packing machine.

- **Packaging process:**

The packing process starts with the tablets being loaded in to a feeding hopper, then the unwinding station supplies the transparent PVC film at a rate corresponding to the speed of the packaging machine.

- **Thermoforming:**

PVC is a thermoplastic material; it softens under the action of heat and freezes in cold temperatures. The virgin PVC is brought under thermoforming plates which mold it in the form of pockets, which size corresponds to the size of the tablets to be packaged. The aluminum coils, which already have the drug information printed on their surface, are also transported to the sealing station. The aluminum is used to seal the PVC blisters containing the tablets using heat; this is called the heat-sealing stage.

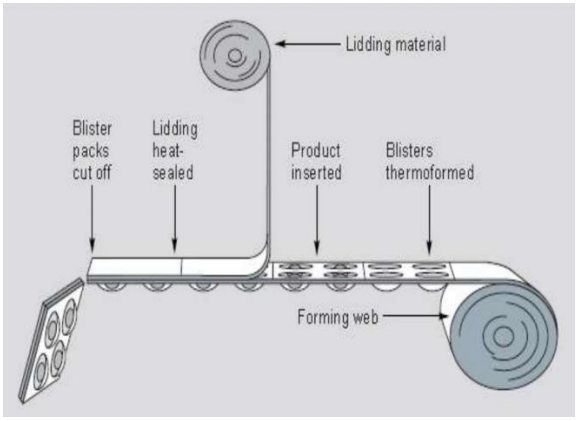


Figure 85: Simplified blistering process.

- **Tablet feeding:**

The tablets are brought to the packaging line after forming the PVC blisters but before the welding with aluminum happens. The arrival of these tablets to the PVC blister strips is carried out by a simple phenomenon of gravity by placing the tablets inside feeding hopper systems that connect the tablets to the formed PVC blister strips.

Figure 86: AMOXYPEN® tablets in the PVC blister strips.

- **Trimming:**

The sealed blister strips are cut into individual unit at the trimming station. These individual blisters are then transferred to the secondary packaging station.

- **Cartoning:**

The blisters are brought by the transfer belt into a blister container. This container will distribute a determined number of blisters in a location that is similar to a bucket. These stacked blisters then move forward on a conveyor belt. A detection cell will transmit a signal to the leaflet container when the blisters arrive. A folded leaflet is positioned opposite two blisters, and then they are inserted into the carton.

The cartons are delivered flat. The machine shapes the carton using suction cup arms while the cases are stacked in multiple layers. The bottom of the carton is folded then the cases are inserted inside of it. The filled carton continues its course, ready to be closed. It is then closed with sticky strips. Then the palletizing is represented by a mechanical arm which takes the cartons in their order of exit and places them on the pallet in the requested configuration.

Figure 87: Secondary packaging station.

D. Post-manufacturing steps:

At the end of production, we need to:

- Make sure that all the machines are switched off.
- Proceed to clean all the equipment used.
- Sweeping the floor and cleaning it with water and detergent.
- Washing and tidying all the cleaning materials inside the washing room.

2.1.2. Manufacturing method of AMOXYPEN® powders for oral suspension:

A. Principle:

The manufacturing of AMOXYPEN® powder for oral suspensions consists in a simple mixing of different ingredients. The principle of this manufacturing is to mix the powder of amoxicillin with some specific excipients, in the absence of any source of temperature or humidity due to the sensitivity of the active ingredient.

B. Pre-manufacturing steps:

Before starting the manufacturing or switching on the machines, we need to:

- Confirm the presence of the Material Safety Data Sheet (MSDS) specific of the product that should be manufactured inside the local.
- Check the availability of the utilities (soft water and nitrogen).
- Verify that the powder injection system is well functioning, otherwise the team manager needs to switch it on before starting the manufacturing.
- Double-check that all equipment and materials (the mixer, the balance, the sieve shaker, the endless screw, and the hopper) are properly cleaned.
- Verify the presence of the green label on all the raw materials' packages, which proves the good quality of the materials. Later on, these materials are transferred from the corridor to the inside of the weighting area to be weighted.
- Compare the weighting results of the materials with the order form.
- Notify the manufacturing responsible of any non-compliance concerning the packaging, the labels and the weight of the materials.
- Prepare the barrels by putting a polyethylene bag in each barrel to collect the mixture, and identify them by mentioning: the specialty, the date, the preparation phase, the batch number, the container number, the gross weight, and the name of the operator in charge of the operation.

C. Manufacturing steps:

The manufacturing of AMOXYPEN® powders for oral suspension comprises three important stages: the sieving, the mixing, and the packaging.

The excipients that will be mixed with the amoxicillin trihydrate are:

- ✓ Aspartame.
- ✓ Apricot aroma.
- ✓ Sodium benzoate.
- ✓ Maltodextrin.

a. Sieving:

Half of the weighted bags of the excipients are emptied inside of the sieve shaker, to be sieved on a grid of 0.25mm. Later on, the sieved powder of excipients is aspirated inside of the mixer.

The same operation is carried out for the whole quantity of the active ingredient, and then, for the rest of the quantity of the excipients.

Figure 88: Sieve shaker

b. Mixing:

The manufacturing of this drug consists of a low shear mixing due to the fact that the active ingredient of AMOXYPEN®, amoxicillin, is a sensitive ingredient when it comes to humidity and high temperature. This process comprises the following steps:

- Filling the mixer with the excipients and the active ingredient, by putting the API between the two portions of excipients like a sandwich.
- Opening the valve of the soft water and nitrogen.
- Turn on the mixer to mix all the ingredients together, using a low shear force.

- When the mixing has ended, we have to switch off the mixer; and then close the nitrogen and soft water valves.
- Opening the powder injection valve to take a sample to the laboratory in order for it to be analyzed and approved.
- If the analysis result is non-compliant, we have to redo the mixing for 10 minutes and take another sample for analysis. However, if the result is compliant, unload the powder inside the barrels that have been prepared and identified previously.
- In order to completely empty the mixer, we can use a stainless-steel spatula, along with moving the propeller of the mixer forward and backward from time to time.
- Weighting the mixing powder and registering the results in the batch record.
- Transporting the closed barrels using a trans-pallet to the storage area.

Figure 89: Ribbon mixer

c. Packaging:

In the case of AMOXYPEN® powders for oral suspension, the powder is enclosed in transparent flacons with a volume of 60ml, in different doses (125mg, 250mg, and 500mg).

- **Dosing:**

The packaging of these powders starts from the dosing machine that needs to be switched on empty at first for 2 to 4 minutes. After that, the barrels charged with powder are verified and emptied inside the inferior hopper, while aluminum caps are placed inside the capper's hopper using a clean shovel. Later on, we switch on the flacons' turntable and the dosing machine to be able to fill some flacons that are taken as a sample to verify the dose, the volume and the capping by the team manager.

After confirming that the capping is compliant, we complete filling all the flacons with the mixed powder. A tray is prepared for flacons that present some anomalies to be taken down from the process. The hoppers are refilled regularly until the whole quantity of the powder is finished.

Cleaning with a wet rag all throughout the dosing, and informing the team manager of any non-compliance or anomaly observed is important for the well-being of the process.

Figure 90: Hitimon dosing machine.

- **Cartoning:**

The flacons are labeled then transferred to the packaging station by a transfer belt, which notifies the detection cell that sends a signal to the leaflet container. By the time the flacons arrive, the leaflet is folded in line and positioned opposite to the flacon.

The cartons are generally delivered flat and stacked together. Later on, a machine shapes them using a suction arm and folds the bottom inside. After placing the flacon and the leaflet inside the carton, it is closed with an adhesive strip and a vignette is placed on it.

These small cartons are transferred by a belt to an employee, who is responsible for placing them in big cardboard boxes and stocking them in the storage room to be distributed later on.

Figure 91: Packaging station.

D. Post manufacturing steps:

After finishing all the manufacturing process, some steps need to be carried out:

- Switch off all the machines used.
- Deep cleaning of all the pieces of the following: the mixer, the sieve shaker and the dosing machine.
- Sweeping the floor and cleaning it with water and detergent.
- Washing and tidying all the cleaning materials inside the washing room.

2.1.3. Manufacturing method of AMOXYPEN® powders for injectable solutions:

A. Principle:

The manufacturing of AMOXYPEN® powder for injectable solution is a complex process because injectable products require the sterility of the drug due to their route of administration. Unlike most products, injectables have increased requirements because they can avoid our body's natural skin barriers and digestive tract barriers to toxins.

Injectable product manufacturers must ensure that the necessary quality parameters described within the pharmacopeial standards are met for patients' safety.

B. Manufacturing requirements:

- **Sterility:**

Achieving and maintaining products' sterility are among the greatest challenges manufacturers face for injectables since most rely on aseptic manufacturing. In order to meet regulators expectations for sterile production:

- Antibiotics must be kept dry, for reasons of stability, until the moment of use when they will be dissolved in a suitable solvent.
- The injectable powder to be distributed must be sterile and of well-defined particle size.
- The bottles, their caps, as well as all the equipment intended to be introduced into sterile air must be sterilized beforehand.

- The various steps of manufacturing of sterile drugs (preparing accessories, preparing the product and filling) must be carried out in controlled atmosphere areas, which should be maintained at an appropriate level of cleanliness, equipped with laminar air flow systems and supplied with air filtered by high efficiency air filters corresponding to the level of cleanliness required. Entry into these areas must be through airlocks reserved for personnel and/or material and substances.

- **Controlled atmosphere area:**

In controlled atmosphere areas, or aseptic manufacturing environments, product processing requires excluding microorganisms from the manufacturing methods. There are many validation steps to maintain sterility. Some of these validation steps include valid sterilization procedures for all components during manufacturing of the product, a proper method for sterile aseptic filtration, maintenance of ISO-certified clean rooms, validation of aseptic processes, training and application of good aseptic practices by contract manufacturing organizations, use of antimicrobial preservatives for multiple-dose products, proper testing of container-closure integrity, and proper testing for overall product sterility.

C. Manufacturing steps:

The steps to follow for the manufacturing and packaging of sterile AMOXYPEN® injectable powders ensure the compliance of the products with the required specifications.

a. Washing of primary packaging items:

It is important to wash the primary packaging items (rubber caps and glass bottles or vials) before the sterilization step. In order to do so we need to follow these instructions:

- Transfer the caps and bottles to the washing room on a stainless-steel trolley.

- Wash and rinse caps and bottles thoroughly with pyrogen-free distilled water.

- Rub, if necessary, with a single-ply cloth and suitable detergent.

- Group together the packaging items in a stainless-steel box.

- Introduce them into the controlled atmosphere area so that they are sterilized with formalin.

Figure 92: Washing system.

Figure 93: Stainless steel trolley.

b. Sterilization of primary packaging items:

The sterilization process is performed inside the autoclave TELSTAR W4155 A, following these steps:

- Check that the material is well washed.

- Make sure that the autoclave door on the sterile side is closed, then open the outer door.

- Check the cleanliness of the autoclave.

- Place the sterilization indicators on the trolley at the level of the three floors (top, middle, bottom).

- Insert the trolley into the autoclave and close the door from the outside.

- The instructions below are programmed beforehand on the autoclave software:

- ✓ Sterilization time = 30 minutes.
- ✓ Drying time = 90 minutes
- ✓ The sterilization temperature = 121°C.

Figure 94: Autoclave.

c. Passage to the controlled atmosphere area:

After the completion of the sterilization, it is finally possible to open the door of the autoclave on the sterile side and introduce the sterile raw materials and packaging items into the controlled atmosphere area.

d. Packaging:

In the case of AMOXYPEN® powders for injectable solutions, there are two types of packaging: 1gr in a transparent glass vial accompanied with an ampule of 5ml of solvent (benzyl alcohol), or a box of 50 glass vials filled with powder without accompanying solvent.

- **Dosing and capping:**

Once the material has been introduced into the controlled atmosphere area, the dosing system HSUA W 4115 is adjusted to the planned operating parameters and the distribution of the powder is carried out in the sterile vials. When the vials are filled with powder, they are then capped with rubber caps, and after leaving the controlled atmosphere area, the capped vials are sealed with aluminum seals.

Figure 95: AMOXYPEN® vials sealed with rubber caps

Figure 96: AMOXYPEN® vials sealed with aluminum caps

At first, only a few vials are filled and capped; those are samples that will help verify the dose, the volume, and the capping by the team manager. And after confirming that the dosing and capping processes are compliant, we complete filling all the vials with the mixed powder, and then we stick labels on each vial containing the necessary information about the product.

Figure 97: Dosing machine inside the controlled atmosphere area.

- **Cartoning:**

The vials are transferred to the packing station by a transfer belt, which notifies the detection cell that sends a signal to the leaflet container. By the time the vials arrive, the leaflet is folded in line and positioned opposite to the vial of powder and the solvent ampule.

Figure 98: Packaging station.

The cartons are generally delivered flat and stacked together. Later on, a machine shapes them using a suction arm and folds the bottom inside. After placing the vials and the leaflet inside the carton, it is closed with an adhesive strip and a vignette is placed on it.

These small cartons are transferred by a belt to an employee, who is responsible for placing them in big cardboard boxes and stocking them in the storage room to be distributed later on.

D. Post-production:

At the end of production, it is necessary to:

- Make sure that all the machines are switched off.
- Proceed to the cleaning of all the equipment used.

- Sterilize the equipment used inside the controlled atmosphere area.
- Sweeping the floor and cleaning it with water and detergent.
- Washing and tidying all the cleaning materials inside the washing room.

2.2. Quality control methods:

2.2.1. Sampling:

Sampling comprises the operations designed to select a portion of a pharmaceutical product for a defined purpose. The sampling procedure should be appropriate to the purpose of sampling, to the type of controls intended to be applied to the samples and to the material to be sampled. The procedure should be described in writing. All operations related to sampling should be performed with care, using proper equipment and tools. Any contamination of the sample by dust or other foreign material is liable to jeopardize the validity of the subsequent analyses. [97]

2.2.2. Quality control methods of AMOXYPEN®:

A. Quality control of raw materials:

a. Quality control of the active pharmaceutical ingredient:

The control of API is the first step in the quality control of any drug. This control consists of a large number of tests which are described in the USP, Ph. Eur., and BP. The results of these tests need to be compliant with physicochemical properties of the active ingredient.

i. Quality control of amoxicillin trihydrate:

The moment the substance arrives to the storage room, it is guided to the quarantine area and a sample is taken to the quality control laboratories to be analyzed.

❖ **Organoleptic control:**

In this control, the analyzer needs to check the appearance and solubility of the substance. The result of his control should be compliant with the properties described previously.

❖ **Identification:**

Amoxicillin trihydrate is identified using thin layer chromatography (TLC).

- Operating mode:

Two solutions are prepared: the first one contains 5µl of standard amoxicillin's solution with 0.1N of HCl, while the second contain 4mg/ml of the sample's aqueous dispersion with 0.1N of HCl.

Each solution is left to rest for 10 minutes on a 0.25mm layer of chromatographic silica gel mixture.

The solvent's system used comprises methanol, chloroform, pyridine and water.

Acceptation criteria: the reference factor value of the main spot of the sample's solution corresponds to that of the standard's solution.

- **Total moisture (Karl Fisher titration):**

It is determined on 100mg of amoxicillin trihydrate powder using the following equation:

$$\textbf{Water content (\%)} = \frac{V \times F}{W} \times 100$$

V: volume of KF's solution (ml).

F: Karl Fisher factor of the dosage solution (mg/ml).

W: sample's weight (mg).

The total moisture is limited between 11.5% and 14.5%.

ii. Quality control of amoxicillin sodium:

The first control is the organoleptic control; which is performed the same way as the one used for amoxicillin trihydrate.

- **Identification of amoxicillin sodium:**

Primary identification: A and D

Secondary identification: B, C and D

A: Infrared absorption spectrometry.

B: Thin layer chromatography.

C: Chemical reaction.

D: Sodium reaction a.

- **Infrared absorption spectrometry:**

First, 0.25 g of amoxicillin sodium is dissolved in 5 ml of water. Then, 0.5 ml of acetic acid is added and everything is mixed together. The mix is left to rest in an ice water bath for 10 minutes to form crystals. These crystals are filtrated and washed with 2 to 3ml of a mix, containing 1 volume of water with 9 volumes of acetone. Later on, they are put inside the oven to be dried at 60°C for 30 minutes.

The result is compared to the standard result in the system.

- **Thin layer chromatography:**

✓ **Chromatographic conditions:**

The plate used is the silanized silica gel plate.

✓ **Mobile phase:**

10 volumes of acetone and 90 volumes of ammonium acetate with a concentration of 154g/l adjusted to pH 5.0 are mixed with glacial acetic acid.

✓ **Operating mode:**

The solution to be examined: 25 mg of amoxicillin sodium is dissolved in 10ml of sodium bicarbonate's solution.

Control solution (a): 25mg of amoxicillin trihydrate BPCRS is dissolved in 10ml of sodium bicarbonate's solution.

Control solution (b): 25mg of amoxicillin trihydrate BPCRS with 25mg of ampicillin are dissolved in 10ml of sodium bicarbonate's solution.

1µl of each solution is applied and the plate is developed to 15cm, then the plate is dried by air and exposed to iodine vapor until spots appear. These spots are examined in daylight.

✓ **System suitability:**

The test is not valid unless the chromatogram obtained with control solution (b) shows two clearly separated spots.

✓ **Acceptation criteria:**

The main spot in the chromatogram obtained with the solution examined is similar in position, color and size to that in the chromatogram obtained with control solution (a).

- **Chemical reaction:**

Amoxicillin sodium is introduced inside a tube of 15mm diameter and 150 mm length. It is moistened with 0.05 ml of water, and then 2ml of sulfuric acid and formaldehyde is added. The content of the tube is blended by shaking the tube. At first, the solution is colorless, but after putting the tube in a water bath, it develops a yellow color.

- **Sodium reaction a:**

0.1g of amoxicillin sodium is dissolved in 2ml of water, and then 2ml of potassium bicarbonate with a concentration of 150g/l is added. The mix is boiled but it does not form any precipitate. 4ml of potassium pyroantimoniate solution is added and the mix is boiled. After that, the tube is cooled in iced water to form white and thick precipitate.

❖ **Total moisture:**

It is calculated the same way as the amoxicillin trihydrate with limits of ≤ 3.00%.

b. Quality control of the excipients:

The control of the excipients is mentioned in the drug master files described by the USP. First, we have the organoleptic tests, which include the appearance and solubility, and then the identification and dosage using spectrometry and HPLC.

c. Quality control of the packaging items:

The control of the packaging items is mostly the control of the appearance and quality of PVC, aluminum, flacons, vials, and caps used in the primary packaging of different dosage forms. This control comprises the identification and physicochemical tests.

B. In-process quality control:

During the production of powders for oral suspension and dispersible tablets, some quality control tests need to be performed.

a. Final mix control:

i. Active ingredient dosing:

The active ingredient is titrated using the high-performance liquid chromatography (HPLC).

✓ **Chromatographic conditions:**

- Mode: LC.
- Column: C18 4mm×25cm.
- Flow rate: 1.5ml/min.
- Detector: UV 230nm.
- Injection volume: 10µl.
- Temperature: ambient temperature.

✓ **Preparation of the mobile phase:**

It comprises diluent 96% with acetonitrile 4%. The two are mixed together and filtrated using a 0.45µm membrane filter and then degassed.

✓ **Preparation of the Buffer solution:**

6.8g of monobasic potassium phosphate is dissolved in 900ml of distilled water and shaken. The pH of the mix is adjusted to 5±0.1 by adding the potassium hydroxide 45%. After that, distilled water is added until we obtain 1000ml of volume.

✓ **Preparation of the standard solution:**

120 mg of standard amoxicillin trihydrate is weighted and put inside a 100ml volumetric vial.

✓ **Preparation of the sampled solution:**

180mg of the sample is weighted and put inside a 100 ml volumetric vial. Then, it is dissolved in a diluent and the same solvent is added until the 100ml line. After that the solution is filtrated using a 0.45µm syringe filter.

- ✓ **Calculation formula:**

Inject and read the areas of the standard and sample solution, then calculate the titer according to the following:

$$\mathbf{T\%} = \frac{\boldsymbol{Au \times Cs}}{\boldsymbol{As \times Cu}} \times \boldsymbol{P} \times \mathbf{100}$$

Au: Area presented for the sampled solution.

As: Area presented for the standard solution.

Cu: Concentration of the sampled solution.

Cs: Concentration of the standard solution.

P: Power of the standard solution.

The titer is limited between 60 and 73.33% of amoxicillin trihydrate.

b. Compression control:

This control is specific to AMOXYPEN® dispersible tablets.

- ❖ **Average mass:**

The average weight or mass is calculated using 20 tablets.

It is limited between 1425 and 1575 mg/tablet.

- ❖ **Mass uniformity:**

We weight 20 tablets, where their average weight represents a homogeneous production batch.

The difference between the individual weight of these 20 tablets and the average weight should not be superior to 5%.

❖ **Titer:**

$$\textbf{Titer} = \frac{\boldsymbol{T}(\%)\times\boldsymbol{AM}}{\boldsymbol{100}}$$

AM: Average mass (mg).

Each tablet should contain from 0.9 to 1.1g of amoxicillin.

❖ **Friability test:**

Friability test is used to test the durability of tablets during the packaging process and transport.

✓ **Conditions:**

- Speed: 25 rpm.
- Duration: 4 minutes.
- Number of tablets: 10.

✓ **Method:**

10 tablets are weighted and then placed inside the friability meter, which is switched on. Later on, these 10 tablets are reweighted.

✓ **Calculation formula:**

$$\textbf{FR (\%)} = \frac{\boldsymbol{W0-W1}}{\boldsymbol{W0}} \times \boldsymbol{100}$$

W0: Initial weight of the 10 tablets.

W1: Second weight of the 10 tablets.

FR% should be ≤ 1%

❖ **Hardness test:**

The hardness test is a method employed to measure the hardness of a material, which refers to the resistance of the tablet to permanent indentation.

10 tablets are placed inside the crushing part of the durometer, which is then switched on.

We take the measurements of each tablet and calculate its average. It should be limited between 60 to 160 Newton.

❖ **Disintegration test:**

The test determines whether a tablet disintegrates within the prescribed time under the prescribed experimental conditions. Disintegration does not imply a complete dissolution of the examined unit, but a defined state where no residue of the examined unit remains on the screen.

✓ **Conditions:**

- Volume: 600ml of distilled water.
- Temperature: 20±5°.
- Number of tablets: 6 tablets.

✓ **Method:**

- Introduce the water volume inside the vessel.
- Maintain the medium at a temperature of 20±5°.
- Place a tablet in each basket rack.
- Add a disc inside the 6 basket racks with tablets.
- Switch on the machine.
- Remove the basket rack door from the liquid to examine the condition of the units being tested.
- As soon as the tablets are disintegrated completely, we should switch off the machine and note the disintegration time.
- If one or two of these tablets are not disintegrated, we have to repeat the test on 12 units.

The results of this test are only satisfying if at least 16 units are tested and disintegrated, and the disintegration time should be ≤ 3minutes.

C. Quality control of the finished pharmaceutical products (FPPs):

a. Pharmaco-technical testing:

i. AMOXYPEN® 1g dispersible tablets:

❖ **Dissolution testing:**

This assay concerns the AMOXYPEN Dispersible Tablets; and it is done by HPLC as instructed by the USP 39.

✓ **Operating conditions:**

- Medium: 900 mL of distilled water
- Stirring speed: 75 rpm for 30 minutes.
- Temperature: 37±0.5.

✓ **Operating mode:**

- Diluent:

13.6 g of KH_2PO_4 are introduced into a 2000ml vial. It is dissolved with water, then the pH is adjusted to 5 by KOH 45%.

- Preparation of the standard solution:

- 50 mg of Amoxicillin Trihydrate Reference Standard is weighed into a vial judged to be 100ml.
- Dissolve and complete up to the gauge line with the solvent.
- 10ml is taken in a 100ml vial and completed with the diluent.

- Preparation of the sample solution:

The test is carried out on 6 tablets:

- We put a tablet in the dissolution test vessel containing 900ml of distilled water.
- After 30 minutes of stirring, a quantity of the solution is taken and filtered using a 0.45µm membrane filter.
- 4ml filtrate is put in a 100ml vial and completed to the gauge line with distilled water.
- Inject and read the areas of the standard and sample solution.

- Buffer preparation:

- Weigh 6.8gr of monobasic potassium phosphate, dilute it in 90ml of distilled water then shake it.
- Adjust the pH to 5 with 45% potassium hydroxide.

✓ **HPLC operating conditions:**

- Mode: LC.
- Column C18: 30cm*3.9mm, 5.9µm.
- Flow rate: 0.7 ml/min.
- Temperature: 40°C.
- Detector: UV 230nm.
- Injection volume: 20µl.
- Mobile phase: Diluent/acetonitrile: 97.5% /2.5% shake and filter using a 0.45µm membrane filter then degas.

✓ **Calculation formula:**

$$\boldsymbol{Q}(\%) = \frac{\boldsymbol{Au}}{\boldsymbol{As}} * \boldsymbol{C(s)} * \frac{\mathbf{900}}{\boldsymbol{Theoritical\ titer}\left(\frac{\boldsymbol{mg}}{\boldsymbol{tab}}\right)} * \frac{\mathbf{100}}{\mathbf{4}} * \boldsymbol{Ps} * \mathbf{100}$$

Q (%): amount of active ingredient released from the drug product.

Au: Area of the Sample solution.

As: Area of the Standard solution.

Cs: Concentration of the Standard solution, in mg/ml.

Ps: Power of the Standard solution.

Acceptance criteria: ≥80% in 30 minutes.

❖ **Disintegration testing:**

Identical to the disintegration testing performed during the in-process control.

❖ **Friability testing:**

Identical to the friability testing performed during the in-process control.

❖ **Hardness testing:**

Identical to the hardness testing performed during the in-process control.

b. Physicochemical control:

i. AMOXYPEN® 1g dispersible tablets:

❖ **Appearance:**

White oval-shaped dispersible tablets.

❖ **Identification by thin layer chromatography (TLC):**

Identical to the identification method used to identify the API.

❖ **Dosing by HPLC:**

Identical to the dosing performed during the in-process control.

ii. AMOXYPEN® 250mg powder for oral suspensions:

❖ **Appearance:**

Flacon filled with white powder.

❖ **Identification by TLC:**

Identical to the identification method used to identify the API.

❖ **Dosing by HPLC:**

Identical to the dosing method used during the in-process control, except for the calculation formula.

✓ **Calculation formula:**

$$C(\%) = \frac{Au\ x\ Cs\ x\ P\ x\ F\ x\ 100}{As\ x\ Cu}$$

C (%): Content of amoxicillin in the FPP.

Au: Area of the main peak obtained with the Sample.

As: Area of the main peak obtained with the Standard.

Cu: Concentration of the Sample solution.

Cs: Concentration of the Standard solution.

P: Power of the Standard.

F: Conversion factor.

iii. AMOXYPEN® 500mg powders for injectable solution:

- **Appearance:**

Transparent vial containing white powder. The reconstituted solution has to be limpid.

- **Identification by TLC:**

Identical to the identification method used to identify the API.

- **Dosing by HPLC:**

Carry out the method for liquid chromatography using the following solution:

- ✓ **Mobile phase:** A mixture of 8 volumes of mobile phase B and 92 volumes of mobile phase A.

- ✓ **Mobile phase A:** Mix 1 volume of acetonitrile and 99 volumes of a 25% v/v solution of 0.2M potassium dihydrogen orthophosphate adjusted to pH 5.0 with 2M sodium hydroxide.

- ✓ **Mobile phase B:** Mix 20 volumes of acetonitrile and 80 volumes of a 25% v/v solution of 0.2M potassium dihydrogen orthophosphate adjusted to pH 5.0 with 2M sodium hydroxide.

- ✓ **Operating mode:**

- (1) Add 80 mL of mobile phase A to a quantity of the mixed contents of the 10 vials containing the equivalent of 60 mg of amoxicillin and shake for 15 minutes. Mix with the aid of ultrasound for 1 minute, add sufficient mobile phase A to produce 100 mL, mix and filter.
- (2) 0.070% w/v of amoxicillin trihydrate BPCRS in mobile phase A.
- (3) 0.0004% w/v of cefadroxil BPCRS and 0.003% w/v of amoxicillin trihydrate BPCRS in mobile phase A.

✓ **Chromatographic conditions:**

- Column: stainless-steel column (25 cm × 4.6 mm) packed with octadecylsilyl silica gel for chromatography (5 µm) (Hypersil 5 ODS is suitable).
- Isocratic elution and the mobile phase: as described above.
- Flow rate: 1 mL per minute.
- Temperature: ambient.
- Detector: UV 254 nm.
- Injection volume: 50 µL of each solution.

✓ **System suitability:** The assay is not valid unless, in the chromatogram obtained with solution (3), the resolution factor between the peaks due to amoxicillin and cefadroxil is at least 2.0. If necessary, adjust the composition of the mobile phase to achieve the required resolution.

✓ **Determination of content:** Calculate the content of $C_{16}H_{19}N_3O_5S$ in a vial of average content weight from the chromatograms obtained and from the declared content of $C_{16}H_{19}N_3O_5S$ in amoxicillin trihydrate BPCRS.

✓ **Calculation formula:**

$$\boldsymbol{Q(mg/vial) = \frac{Au\ x\ Cs\ x\ AW\ x\ P}{As\ x\ Cu}}$$

Q: Quantity of amoxicillin in mg/vial.
Au: Area of the main peak obtained with the Sample solution.
As: Area of the main peak obtained with the Standard solution.
Cu: Concentration of the Sample in mg/ml.
Cs: Concentration of the Standard in mg/ml.
P: Power of amoxicillin trihydrate BPCRS.
AW: average weight in mg/vial.

✓ **Limits:** 450.00mg to 525.00mg per vial.

c. Microbiological control:

According to the European Pharmacopoeia (2008) the dispersible tablets and oral suspensions are classified among the pharmaceuticals product that are not necessarily sterile, but the injectable powders have to be sterile.

In order to identify any harmful causes or agents in order to reduce or eliminate these contaminations to ensure the safety, stability, purity, and in some cases, the sterility of the drug, microbiological quality controls are carried out.

i. Microbiological control of non-sterile AMOXYPEN® forms:

To assess the microbiological quality of AMOXYPEN® DT and powders for oral suspensions as finished products, the following controls are used:

- Total aerobic microbial count (TAMC).
- Total yeast mold count (TYMC).
- Enumeration of specified microorganisms: Escherichia coli, by the research method on agar medium.

✓ **Sample preparation:**

- Place the sample and the material under the hood and turn on the laminar flow,
- Turn on the UV lamp for 20 min for decontamination,
- Switch off the UV lamp after decontamination.
- Preparation of the 1/10 dilution “homogenized A”: put a quantity of 5g of the product to be examined in a bottle of 100 ml of peptone buffer solution with sodium chloride at pH 7.

❖ **Total aerobic microbial count (TAMC):**

✓ **Procedure:**

1 ml of the previously prepared sample was inoculated deeply into plates of Petri dishes containing the agar medium with casein and soya peptones (CASO agar) (Appendix 4). Incubation was made at 33°C for five days. After the incubation period, the colonies were counted using the colony counter.

✓ **Reading and interpretation:**

The presence of colonies on the plates after incubation does not necessarily indicate the non-conformity of the product, because the Ph. Eur. tolerates the presence of a limited number of bacterial colonies. The product is considered compliant if no colonies are observed on the incubated Petri dish or if the number of colony-forming units per gram has the following limit.

✓ **Limit:**

The number of colony forming units of TAMC ≤1000UFC/g.

❖ **Total Yeast Mold Count (TYMC):**

✓ **Procedure:**

1 ml of the sample was placed in deep culture in petri dishes containing the medium Sabouraud Dextrose Agar (SDA) (Appendix 4). Incubation was done at 33°C for seven days. After the incubation period, the colonies were counted using the colony counter.

✓ **Reading and interpretation:**

The presence of colonies on the plates after incubation does not necessarily indicate non-conformity of the product, because the Ph. Eur. tolerates the presence of a limited number of yeasts and mold. The product is considered compliant if no colonies are observed on the incubated plate or if the number of colony-forming units per gram presents the following limit.

✓ **Limit:** The number of colony forming units TYMC ≤100UFC/g.

❖ **Search for specified microorganisms:**

It is the search for certain pathogenic micro-organisms that require the use of highly selective specific methods. In the case of AMOXYPEN®, the pathogenic micro-organism researched is Escherichia coli only. And in order to avoid the ATB action of amoxicillin, penicillinases are added to inhibit the latter.

✓ **Procedure:**

To search for the pathogenic germ Escherichia coli, 10ml of the sample (equivalent to 1g of Amoxicillin) were transferred into 100ml of Tryptic Soy Broth (TSB) enrichment medium (Appendix 4), and incubated at 33°C for 24h, to ensure the revitalization of bacteria. Then, 1ml of the inoculum was mixed in 100 ml of MacConkey broth medium (Appendix 4) and incubated at 44°C for 48 hours, after the incubation period, a subculture was carried out in boxes of Petri dishes, previously containing MacConkey agar (MAC) medium (Appendix 4), then incubation was carried out at 33°C for 72 hours.

✓ **Reading and interpretation**

The growth of red, non-mucoid colonies of gram-negative rod bacteria indicates the possible presence of E. coli, to be confirmed by appropriate biochemical tests (like that of the indole).

The product satisfies the test if no colonies of the type described are observed or if the tests confirmatory biochemicals are negative.

ii. Microbiological control of sterile AMOXYPEN® FPPs:

As opposed to the non-sterile AMOXYPEN® products, AMOXYPEN® powders of injectable solutions is a sterile product, hence why it needs to comply with the harmonized pharmacopeial “Sterility testing” and “Bacterial Endotoxins Test”. These types of control aim to verify the absence of bacterial contamination in the product according to the Ph. Eur.

❖ **Sterility testing:**

It is a testing that confirms that the products are free from the presence of microorganisms. It is important for medical devices, pharmacy preparations, and other materials. It is a qualitative test that shows the quality of the samples tested.

The test for sterility is carried out under aseptic conditions. In order to achieve such conditions, the test environment has to be adapted to the way in which the sterility test is performed. The precautions taken to avoid contamination are such that they do not affect any micro-organisms which are to be revealed in the test. The working conditions in which the tests are performed are monitored regularly by appropriate sampling of the working area and by carrying out appropriate controls.

The sterility testing can be carried out by either membrane filtration or direct inoculation. In the case of AMOXYPEN® powders of injectable solutions, the latter is used.

- **Direct inoculation:**

Test articles are directly transferred into fluid thioglycollate medium (FTM) (Appendix 4) and soybean casein digest medium (SCDM). Both media are incubated for 14 days and checked every day for the presence of microbial growth.

The product is deemed sterile if no growth of micro-organisms occurs.

❖ **Bacterial endotoxin test:**

The test for bacterial endotoxins is designed to detect or quantify bacterial endotoxins of gram-negative bacterial origin that may be present in or on the sample to which the test is applied. It uses Limulus Amoebocyte Lysate (LAL) obtained from the aqueous extracts of circulating amoebocytes of horseshoe crab (Limulus polyphemus) which has been prepared and characterized for use as an LAL Reagent.

- ✓ **Limits:** Less than 2.5 IU/ml.

3. Results and discussion:

3.1. Results of the raw materials' quality control:

3.1.1. Results of the active ingredient's control:

The table down below represents the results of the two active ingredients' control used in different dosage forms of AMOXYPEN®.

Table 31: Quality control's results for AMOXYPEN's active ingredients

Parameters	Amoxicillin trihydrate	Reference	Sodium amoxicillin	Reference
Formula	$C_{16}H_{19}N_3O_5S.3H_2O$	USP	$C_{16}H_{19}N3O5S.Na$	USP
Appearance	Crystalline, off white powder.	Ph BP	Almost white powder	Ph BP
Molecular weight	419.45 g/mol	USP	387.39 g/mol	USP
Solubility	Slightly soluble in water	Ph BP	Very soluble in water	Ph BP
Hygroscopy	Non-hygroscopic.	USP	Very hygroscopic.	USP
Total moisture	12.9%	Ph Eur	2.5%	Ph Eur
Total Impurities	1.9%	Ph Eur	8.1%	Ph Eur
pH	4.6	Ph Eur	9.2	Ph Eur
N, N dimethylanilin	18ppm	Ph Eur	19 ppm	Ph Eur
Sterility	Non-sterile	Ph Eur	Sterile	Ph Eur
Quality	A satisfying visual control and the results are compliant			

3.1.2. Results of the excipients' quality control:

The control of the excipients used in the different dosage forms of AMOXYPEN® mentioned before, relies on the different documents presented from the USP. The results of these excipients' control are represented in the following tables:

Table 32: Quality control's results for the excipients used in the production of AMOXYPEN® dispersible tablets

Excipient	Appearance	Solubility	Reference
Magnesium stearate	It is a white powder with a slight odor.	Insoluble in water, slightly soluble in benzene and soluble in alcohol.	USP
Mint aroma	It has a sharp odor and taste that are cool and refreshing.	Slightly soluble in water, soluble in alcohol.	USP
Avicel	A white powder.	Insoluble in water	USP
Aspartame	White crystalline powder, with no odor and a sweet taste.	Soluble in water, slightly soluble in alcohol.	USP
Aerosil	White crystals or powder.	Insoluble in water and alcohol.	USP
PVP	A white powder with a light smell.	Insoluble in water.	USP
Quality	Compliant	Compliant	

Table 33: Quality control's results for the excipients used in the production of AMOXYPEN® powder for oral suspension

Excipient	Appearance	Solubility	Reference
Aspartame	White crystalline powder, with no odor and a sweet taste.	Soluble in water, slightly soluble in alcohol.	USP
Sodium benzoate	A white, almost odorless, crystalline powder or granules.	Freely soluble in water, sparingly soluble in ethanol.	USP
Maltodextrin	Hygroscopic powder with a white to yellow color.	Freely soluble in water, insoluble in alcohol.	USP
Apricot aroma	Apricot's odor and a sweet taste.	Slightly soluble in water	USP
Quality	Compliant	Compliant	

Table 34: Quality control's results for the excipients used in the production of AMOXYPEN® powders for injectable solutions

Excipient	Appearance	Solubility	Reference
Sodium powder	Very soft silvery-white powder.	Soluble in water and alcohol.	USP
Benzyl alcohol	Colorless liquid with a mild pleasant aromatic odor.	Moderate solubility in water.	USP
Quality	Compliant	Compliant	

3.1.3. Results of packaging items' quality control:

The packaging items used in our study for the manufacturing of the three dosage forms of AMOXYPEN® including the PVC, aluminum, flacons, vials and caps, need to be qualified first from the quality control laboratory. The results of their control are in the table down below:

Table 35: Quality control's results for packaging items

The packaging items	Appearance	Identification test	Physicochemical test	Reference
PVC	Compliant	Compliant	Compliant	USP
Aluminum	Compliant	Compliant	Compliant	USP
Glass flacons	Compliant	Compliant	Compliant	USP
Aluminum caps	Compliant	Compliant	Compliant	USP
Glass vials	Compliant	Compliant	Compliant	USP
Rubber caps	Compliant	Compliant	Compliant	USP
Aluminum seals	Compliant	Compliant	Compliant	USP

Commentary:

Based on the quality control's results, all the raw materials including the API, excipients and packaging items used in the manufacturing of the three dosage forms of AMOXYPEN®, are compliant according to the different pharmacopeias.

3.2. Comparison of the manufacturing processes of the different dosage forms:

The table below showcases the differences between the different manufacturing processes of AMOXYPEN® dispersible tablets, powders for oral suspensions, and powders for injectable solutions:

Table 36: The differences between the manufacturing processes of the three dosage forms.

Parameters	Dispersible Tablets	Powders for oral suspensions	Powders for injectable solutions
Active ingredient.	Non- sterile AMOX Trihydrate.	Non-sterile AMOX Trihydrate.	Sterile AMOX sodium.
Excipients used.	Magnesium stearate.	Sodium benzoate.	Sodium powder.
	Mint aroma.		
	Avicel.	Maltodextrin.	
	Aspartame.	Aspartame.	Benzyl alcohol.
	Aerosil.	Apricot aroma.	
	PVP		
Manufacturing process.	Initial mixing.	Sieving.	Washing.
	Compacting		
	Sieving	Dry mixing.	Sterilizing.
	Final mixing.		
	Compressing.	Packaging.	Packaging inside atmosphere controlled area.
	Packaging.		
Appearance of the final product.	White, oval-shaped breakable tablets.	White powder.	White powder.
Packaging used.	Transparent PVC blisters / Aluminum.	Transparent glass flacons, aluminum caps.	Transparent glass vials, rubber caps, aluminum seals.

Commentary:

The route of administration influences the choice of dosage forms as well as the manufacturing process requirements. The choice of the manufacturing process of the three different dosage forms depends not only on the physicochemical characteristics of the Active Pharmaceutical Ingredient, but also depends on whether the dosage forms need to be sterile or non-sterile.

AMOXYPEN® Dispersible Tablets and Powders for Oral Suspensions are non-sterile pharmaceutical products, which explains the use of a non-sterile API and the lack of the sterilization of the packaging materials and the production area during their respective manufacturing processes. Because both of these dosage forms are orally ingested, so they go through the digestive tract which is a natural barrier to toxins.

Meanwhile, AMOXYPEN® Powders for Injectable Solutions is a sterile pharmaceutical preparation, it necessitates the use of a sterile API and the thorough sterilization of the

packaging materials and the production area. The injectable solutions can avoid the body's natural skin barriers and digestive tract barriers to toxins, so it is important to ensure their sterility for the patient's safety.

Aspartame, which is a sweetener, and the mint and apricot aromas are used in both oral dosage forms to improve the taste of the drug and make it easier for the patients to ingest it.

The super-disintegrant Polyplasdone crospovidone (PVP) is used for the dispersible tablets to help them quickly break down into their primary particles, to facilitate their dispersion in water and the release of the active ingredient, but it is not used for the powders for oral suspensions due to the fact that these powders are already in their primary form, which makes their dispersion in water easier. The use of Aerosil 200 boosts the effect of the super-disintegrant.

Avicel pH-102 is used as a compression and flow aid, it gives the tablets an ideal compactibility and a uniform content, but it is not used in the production of both powders because they do not require compressing.

Penicillins are known for their instability with respect to variations in pH, temperature, pressure and humidity encountered during the manufacturing, which justifies the dry mixing process used in both AMOXYPEN® Dispersible Tablets and Powders for Oral Suspensions, and the dry granulation process used during the production of AMOXYPEN® Dispersible Tablets, as these two production processes use neither humidity nor temperature.

The PVC blisters and the glass flacons and vials used in the packaging of these three AMOXYPEN® dosage forms are all transparent because both AMOX trihydrate and AMOX sodium are not sensitive to light.

3.3. Results of semi-finished products' quality control:

The quality control of the semi-finished products is conducted on the dispersible tablets' form and the powder for oral suspensions only. While the powder for oral suspension's control consists of only dosing, the tablets' control comprises dosing and compression control as well.

Table 37: Quality control's results for the final mix

Parameter	Tablets	Quality	Reference	Powder for oral suspension	Quality	Reference
Titer	71.2%	Compliant	USP	70%	Compliant	USP

Table 38: Compression control's results for the dispersible tablets

Parameter	Result	Quality	Reference
Average mass	1500mg	Compliant	USP
Titer	1g	Compliant	USP
Friability	0.7%	Compliant	USP
Hardness	130 Newton	Compliant	USP
Disintegration ability	2 minutes	Compliant	USP

Commentary:

Based on the results of the different quality control tests, whether on the final mix or the tablet, the semi-finished products of AMOXYPEN® are all compliant according to the USP.

3.4. Results of the finished pharmaceutical products' quality control:

The quality control's results of AMOXYPEN® finished pharmaceutical products are represented in the table down below:

Table 39: Quality control's results of the FPPs

Parameter	Tablets	Quality	POS	Quality	INJ powder	Quality	Reference
Appearance	16 or 14 oval-shaped tablets	Compliant	Transparent flacons filled with white powder	Compliant	Transparent glass vials filled with white powder	Compliant	USP
Dissolution	90% in 30 minutes	Compliant	Not required	Compliant	Not required	Compliant	USP
Microbiological control	TAMC = 760UFC/g. TYMC =92UFC/g.	Compliant	TAMC = 820UFC/g. TYMC =73UFC/g.	Compliant	Absence	Compliant	USP

Sterility	Not required	Compliant	Not required	Compliant	Sterile	Compliant	USP
HPLC dosing	1g per tablet	Compliant	251mg per flacon	Compliant	487mg per vial	Compliant	USP

Commentary:

Based on the quality control's results, the final products of AMOXYPEN® are compliant according to the USP.

The dosage of the active ingredient in the final product is in the accepted limits for all dosage forms, which confirms the stability of the products.

The sterility of powders for injectable solutions is a key factor in the quality of the dosage form and its safety to be administered to humans.

Conclusion

Amoxicillin is a moderate-spectrum, bacteriolytic, beta-lactam antibiotic that is widely used to treat infections caused by gram-positive and gram-negative bacteria. It is usually the drug of choice within the beta-lactam class because it is better absorbed, following oral administration, than other beta-lactam antibiotics.

The Algerian pharmaceutical market is one of the main markets in the African-Middle Eastern (MEA) region thanks to vigorous and sustained growth for nearly 15 years, which reaches 8% per year on average.

In Algeria, the pharmaceutical industry is considered to be an important part of the health system. This industry has played a major role in the increase in quality and life expectancy. Algeria is also among the countries with the highest antibiotics consumption.

The manufacturing processes are chosen based on the physicochemical characteristics of the active ingredient, such as its sensitivity to the various physical agents (temperature, humidity, light…), and on the sterility requirements of the finished pharmaceutical products which depend on the administration route of the drug.

The production of AMOXYPEN® Dispersible Tablets and Powders for Oral Suspensions uses the Active Ingredient AMOX Trihydrate, which is sensitive to heat and humidity (according to the reference), and that is the reason why dry mixing and dry granulation is used during its manufacturing process.

AMOXYPEN® Dispersible Tablets and Powders for Oral Suspensions are non-sterile pharmaceutical products, which explains why their manufacturing process does not include a sterilization process as both of these dosage forms are administered orally, meaning that they go through the digestive tract which is a natural barrier to toxins.

Meanwhile, AMOXYPEN® Powders for Injectable Solutions is a sterile pharmaceutical preparation. It is injected directly into the muscle, avoiding the body's natural skin barriers and digestive tract barriers to toxins. It is important to ensure the sterility of this product all throughout the manufacturing process, which is why special equipment and an atmosphere-controlled area are used. If the sterility requirements are not met, there is a high risk for the patient's life.

All the manufacturing methods used for the three products were carried out in accordance with the Good Manufacturing Practices (GMPs), the Marketing Authorization File (MAF), and the different monographs (USP, Ph. Eur., and BP).

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APPENDIX

APPENDIX 1

SmithKline Beecham Pharmaceuticals
Attention: Ms. Sharon W. Shapowal
One Franklin Plaza
P.O. Box 7929
Philadelphia, Pennsylvania 19101-7929

Dear Ms. Shapowal:

Please refer to your supplemental new drug applications dated January 17, 1990 (Supplement-005); November 11, 1994 (Supplement-010); and October 3, 1997, received October 6, 1997 (Supplement-011), submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for Amoxil (amoxicillin) Capsules, Chewable Tablets, and Powder for Oral Suspension.

We note that this application is subject to exception provisions of Section 125(d)(2) of Title 1 of the FDA Modernization Act of 1997.

We also refer to our approvable letters to Supplement-005, dated June 8, 1993; and March 11, 1996; and Supplement-010, dated January 6, 1997.

In addition, we refer to your submissions to Supplement-005, dated February 10, 1995; and April 23, and July 30, 1997; and Supplement-010, dated April 23, and July 30, 1997.

Supplement-005 provides for changes to the **DESCRIPTION, CLINICAL PHARMACOLOGY, INDICATIONS AND USAGE, WARNINGS, PRECAUTIONS, ADVERSE REACTIONS, DOSAGE AND ADMINISTRATION,** and **HOW SUPPLIED** sections of the labeling.

Supplement-010 provides for changes to the **Microbiology** subsection of the **CLINICAL PHARMACOLOGY** section and the **REFERENCES** section of the labeling.

Supplement-011 provides for the addition of a new indication for Amoxil for use in combination with lansoprazole (with or without clarithromycin) in patients with duodenal ulcer (defined as an active ulcer or history of an ulcer within one year) to eradicate *Helicobacter pylori* and reduce the risk of duodenal ulcer recurrence. The User Fee Goal Date for this supplement is October 6, 1998.

Amoxicillin new drug application supplements submitted in 1997 [40]

CENTER FOR DRUG EVALUATION AND RESEARCH

Approval Package for:

Application Number: NDA 50-542/S-005, S-010, S-011

Trade Name: AMOXIL Capsules, Chewable Tablets, & Powder for Oral Suspension

Generic Name:(amoxicillin)

Sponsor: Smith Kline Beecham Pharmaceuticals

Approval Date: February 27, 1998

INDICATION: Provides for changes to the DESCRIPTION, CLINICAL PHARMACOLOGY, INDICATIONS AND USAGE, WARNINGS, PRECAUTIONS, ADVERSE REACTIONS, DOSAGE AND ADMINISTRATION, and HOW SUPPLIED sections of the labeling.

Amoxicillin approval package submitted to the FDA [40]

Medical Review of Supplement

NDA 50-542/S-011

OCT 28 1997

Date Submission Received: October 6, 1997

Applicant: SmithKline Beecham Pharmaceuticals

Drug Name: Amoxil® (amoxicillin) capsules, powder for oral suspension and chewable tablets

Category: β-Lactam

Date Review Started: October 15, 1997

Date Review Completed: October 20, 1997

Reviewer Note: The sponsor submitted this application as a "Special Supplement-Changes Being Effected." However, on October 7, 1997, the Division of Anti-Infective Drug Products responded in letter by stating, "Changes of the kind that you have proposed, in our opinion, are not the kind of changes permitted by regulation to be put into effect prior to approval of a supplement. An Approved supplement is required for the proposed changes; therefore, the supplement is being reviewed under 21 CFR 314.70(b)."

Purpose of Supplement:

The sponsor has submitted a labeling supplement to revise the labeling of Amoxil in accord with the approved labeling of PREVACID® (lansoprazole) Delayed-Release Capsules. The supplement adds a new therapeutic regimen to the Amoxil label and provides for the use of amoxicillin in combination with PREVACID (with or without clarithromycin) in patients with duodenal ulcer disease (defined as an active ulcer or history of an ulcer within one year) to eradicate *Helicobacter pylori* and reduce the risk of duodenal ulcer recurrence.

Material Submitted:

1. Letter of Authorization from Tap Pharmaceuticals Inc. allowing FDA to make reference to data contained in NDA 20-406 approved on June 17, 1997, in support of the labeling supplement
2. Draft labeling of the Amoxil package insert

Amoxicillin approval package submitted to the FDA [40]

APPENDIX 2

United States Patent [19]

Bender

[11] **4,231,954**

[45] **Nov. 4, 1980**

[54] **DANE SALT AND PROCESS FOR PREPARING AMINOPENICILLINS THEREFROM**

[75] Inventor: **Reinhold H. W. Bender**, Kennett Square, Pa.

[73] Assignee: **American Home Products Corporation**, New York, N.Y.

[21] Appl. No.: **31,890**

[22] Filed: **Apr. 20, 1979**

[51] **Int. Cl.**[3] **C07C 121/60**; C07D 499/00

[52] **U.S. Cl.** ..... **260/465 D**; 260/501.11; 260/501.12; 260/239.1; 562/426; 562/437

[58] **Field of Search** ..... 260/501.11, 501.12, 260/465 D; 562/437, 426

[56] **References Cited**

U.S. PATENT DOCUMENTS

3,316,247	4/1967	Fosker et al.	260/239.1
3,325,479	6/1967	Fosker et al.	260/239.1
3,406,185	10/1968	Patchett et al.	562/437
3,576,855	4/1971	Duonch et al.	562/437
3,654,266	4/1972	Robinson	260/239.1
3,868,364	2/1975	Ishimaru et al.	260/239.1
4,123,611	10/1978	Ishimaru et al.	562/561
4,128,547	12/1978	van der Drift et al.	260/239.1

FOREIGN PATENT DOCUMENTS

867414	9/1978	Belgium .
142416	11/1970	Netherlands .
476758	9/1969	Switzerland .
1339605	12/1973	United Kingdom .
1347979	2/1974	United Kingdom .

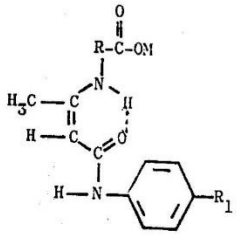
OTHER PUBLICATIONS

Ishimaru et al., Chem. Absts., 79, 78822(c), 1973.
Dane et al., Chem. Ber., 98, 789 (1965).
Laplex, Chem. Absts., 84, 44031(z), 1976.

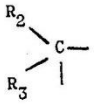
Primary Examiner—G. T. Breitenstein
Attorney, Agent, or Firm—George Tarnowski

[57] **ABSTRACT**

Amide-type Dane salts having the formula:



wherein R is a group of the formula:



wherein R_2 is hydrogen and R_3 is phenyl or substituted phenyl, R_1 is cyano or nitro, and M is hydrogen, an alkali metal or a triloweralkylamine are disclosed, as well as a process for preparing α-aminopenicillins from these salts and 6-APA.

5 Claims, No Drawings

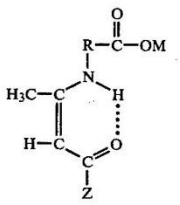
DANE SALT AND PROCESS FOR PREPARING AMINOPENICILLINS THEREFROM

BACKGROUND OF THE INVENTION

The α-aminopenicillins, such as for example ampicillin, amoxicillin and cyclacillin, are very useful antibiotics which are widely used against a large number of gram-positive and gram-negative micro-organisms.

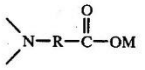
These semisynthetic penicillins have been prepared by various processes and there is a large body of literature dealing with these methods of preparation. A number of patent applications and patents disclose preparations in which 6-aminopencillanic acid is acylated with mixed anhydrides derived from the modified Dane salts of D-2-amino-(substituted)-acetic acid. Such methods of preparation are described in Netherlands Pat. No. 142,416; British Pat. No. 1,347,979 and U.S. Pat. Nos. 3,316,247, 3,325,479 and 4,123,611.

The Dane salts described in the literature can be either of the ester-type or the amide-type, i.e. in Dane salts having the general formula:



(I)

wherein



represents an amino acid residue and M is hydrogen or an alkali metal, and when Z is an alkoxy group they are of the ester-type, while when Z is an amino or substituted amino group they are of the amide-type.

The ester-type Dane salts have been widely used in preparing α-aminopenicillins and one process employing these salts is described in U.S. Pat. No. 4,128,547. In the general Dane salt/6-aminopenicillanic acid acylation process, the N-protected aminopenicillin which is formed during the acylation step is hydrolyzed to yield the desired α-aminopenicillin and, in the case of ester-type Dane salts, a β-ketoester. These β-ketoesters are generally liquids which are separated from the water-soluble α-aminopenicllin salts by extraction in an organic solvent. However, this is a significant disadvantage of the ester-type Dane salts since the β-ketoesters are not readily recovered from solution and so recycle of these β-ketoesters for the preparation of further starting Dane salts is not practicable on a commercial scale.

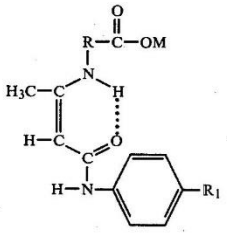
The amide-type Dane salts, in which Z is an amino or substituted amino group, are not as well-known as the ester-types and have not received as much attention in the literature. The amide-type Dane salts in which Z is the group NR_1R_2—, wherein R_1 is hydrogen and R_2 is o- or p-methoxyphenyl have been described in Chem. Ber., 98, 789 (1965) and Belgian Pat. No. 824,158. Those in which R_1 is hydrogen and R_2 is phenyl or halophenyl have been described in Swiss Pat. No. 476,758 and British Pat. No. 1,339,605. Those in which R_1 and R_2 are both alkyl or NR_1R_2 are morpholino have been described in Netherlands Pat. No. 142,416, British Pat. No. 1,339,605 and U.S. Pat. No. 4,123,611. Those in which R_1 and R_2 are both aryl or in NR_1R_2 form a piperidino ring have been described in U.S. Pat. No. 4,123,611. These known amide-type Dane salts, however, have the disadvantage that they generally give poor yields of the final product α-aminopenicillins.

The prior art also shows that it is advantageous to protect the carboxylic acid group or both the amino and carboxylic acid groups of 6-aminopenicillanic acid before it is reacted with the desired Dane salt. The proposed useful protecting groups include the trialkylhalosilanes, as in British Pat. No. 1,339,605 and U.S. Pat. No. 4,128,547; dialkyldihalosilanes, as in U.S. Pat. No. 3,654,266; and silanes having at least one C—O—Si bond in the molecule, such as in U.S. Pat. No. 3,868,364. However, the protection of the carboxylic group or carboxylic and amino groups of 6-aminopenicillanic acid has not produced an improvement in the overall prior art processes' economics, since their other disadvantages, such as low yield, complexity of procedure and low-purity of final product are not overcome thereby.

BRIEF DESCRIPTION OF THE INVENTION

It has been found now that the disadvantages of the prior art processes can be overcome by the novel amide-type Dane salts and the improved process of the present invention.

The novel amide-type Dane salts of the present invention, which have the general formula:



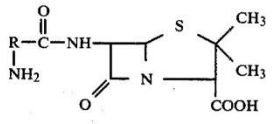
(II)

wherein R is a group of the formula:

(III)

wherein R_2 is a hydrogen atom and R_3 is phenyl or substituted phenyl; R_1 is cyano or nitro and M is hydrogen, an alkali metal or a triloweralkylamine are superior to the known ester-type and amide-type Dane salts for the preparation of α-aminopenicillins.

According to the improved process of the invention, α-aminopenicillins having the formula:



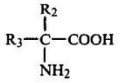
(IV)

where R is defined as hereinbefore, are prepared by reacting a derivative of 6-aminopenicillanic acid in a substantially anhydrous, inert, water-insoluble organic solvent at a temperature at or below −20° C. with at least a 0.8 molar amount of a mixed anhydride prepared by reacting an amide-type Dane salt (II) with an alkylchlorocarbonate in the presence of a catalyst in an inert, water-insoluble organic solvent, hydrolyzing the resulting N-protected aminopenicillin to yield an α-aminopenicillin and a β-ketoamide, and recovering the α-aminopenicillin and optionally, the β-ketoamide.

The term "lower alkyl" refers to groups in which the alkyl moiety has a carbon atom content of C_1-C_4.

DETAILED DESCRIPTION OF THE INVENTION

The novel amide-type Dane salts of Formula II include those in which the grouping >N—R—COOH represents an amino acid residue, especially that of an amino acid in which the amino group is at the α-position to the carboxyl group, which can be represented by the formula:



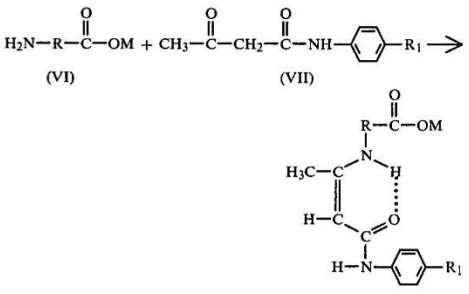
(V)

wherein R_2 is hydrogen and R_3 is methylthiophenyl, phenyl, nitrophenyl, aminophenyl, hydroxyphenyl, alkoxyphenyl, or halogenophenyl. The preferred amino acids and thus amino acid residues are those in which R_3 is phenyl, hydroxyphenyl or alkoxyphenyl. Most preferred are those in which R_3 is phenyl or p-hydroxyphenyl.

The R_1 substituents in Formula II include cyano and nitro, with nitro being especially preferred. It has been found that when R_1 is an electron-withdrawing group, such as the cyano and nitro groups, rather than an electron-donating group, such as alkoxy, under identical conditions the yields of final product α-aminopenicillin are greatly enhanced.

The Dane salts of the invention include those in which M is hydrogen, an alkali metal, or a triloweralkylamine. The most preferred being the sodium salt.

The novel Dane salts are conveniently prepared by condensing an α-amino acid (VI) or a salt thereof with a β-ketoamide (VII), one method of effecting this condensation being described by Dane et al. (Angew. Chem., 1962, 74, 873).



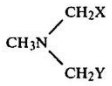
(VI) (VII)

The β-ketoamides used in the above condensation are commercially available or they can be conveniently prepared according to the diketene acetoacetylation reaction described by Zavialov et al. (Tetrahedron, 1966, 22, 2003).

In accordance with the improved process of the invention, 6-aminopenicillanic acid, in suitably protected form is reacted with a mixed anhydride formed from a Dane salt of the invention.

The mixed anhydride is prepared by reacting a Dane salt of Formula II, preferably the sodium salt, with an alkyl or aralkyl chlorocarbonate in the presence of a catalyst in a water-insoluble solvent. The useful chlorocarbonates include methyl chloroformate, ethyl chlorocarbonate, isobutyl chloroformate isopropyl chloroformate, benyl chloroformate and the like, with ethyl chloroformate being preferred.

The preferred catalysts have the formula:



(VIII)

where X is a hydrogen atom or an alkyl, substituted alkyl, phenyl, substituted phenyl, or carboxyl group; Y is a hydrogen atom or a lower alkyl group or X and Y together represent any one of the divalent radicals ethylene, substituted ethylene, trimethylene, substituted trimethylene, —CH_2OCH_2— or —$CH_2N(CH_3)CH_2$—. Examples of such catalysts are N-methylmorpholine and N,N-dimethybenzylamine with N-methylmorpholine being most preferred.

The water-insoluble solvent used in the mixed anhydride preparation may be methylene chloride to which dimethylformamide, sulfolane, tetrahydrofuran, N-methylpyrrolidone, 1,4-dioxane, acetonitrile, dimethylacetamide or tetramethylurea or a mixture thereof is added as a co-solvent or methylisobutylketone, to which one or more of the afore-mentioned co-solvents may optionally be added. The preferred solvent is methylene chloride with at least 10% by volume of a co-solvent. It is also preferable to avoid a mixture of solvents as the co-solvent.

The mixed anhydride preparation is preferably carried out at a temperature of −10° C. or below, most preferably at a temperature of about −20° to about −30° C.

The 6-aminopenicillanic acid (6-APA) is reacted with the mixed anhydride in the form of a derivative such as an alkali metal or alkaline earth salt, as a derivative of a substituted amine or as a silyl derivative in which the silyl group protects the 6-APA carboxylic acid group or both the carboxylic acid and amino groups. The preferred amine salts are the tertiary amine salts, especially triethylamine. However, since the β-lactam ring of 6-APA is prone to cleavage in an aqueous medium, and since such cleavage results in the need for complicated steps to separate and refine the final product, it is most preferable to carry out the acylation reaction in a non-aqueous solvent system and with the 6-APA suitably protected. Accordingly, in the process of the invention, the 6-APA is reacted with a silylating agent which provides good solubility in many organic solvents and at the same time protects the carboxylic acid group or both the carboxylic acid and amino groups of 6-APA. Moreover, after the acylation reaction, the silyl protecting group is readily removed.

VI

The useful silylating agents include halotrialkylsilanes, dihalodialkylsilanes, halotrialkoxysilanes, dihalodialkoxysilanes, halodialkylalkoxysilanes, halodialkoxyalkylsilanes or corresponding aryl or aralkyl silanes. The preferred silylating agents are dihalodialkylsilanes and halotrialkylsilanes having the formula:

(IX)

wherein R_1, R_2, and R_3 are each lower alkyl cycloalkyl, benzyl, or aryl such as methyl, ethyl, cyclopentyl, cyclohexyl, benzyl or phenyl, or one of R_1, R_2, or R_3 is defined as X, and X is any group readily displaced by a nucleophilic reaction involving a carboxylic acid or its salts or an amino group. X is preferably a halogen atom, most preferably chlorine, but good results can also be obtained with compounds such as trimethylsilylacetamide, bis(trimethylsilyl)acetamide, hexamethyldisilazene and bis-trimethylsilylurea. The most preferred silylating agents are trimethylchlorosilane or dimethyldichlorosilane.

The silylation is carried out in a dry, inert, water-insoluble solvent, preferably dry methylene chloride, and in the presence of a tertiary amine. The silylation is performed using about 2 equivalents of a tertiary amine, preferably triethylamine, with the amount of silylating agent varying with the type of agent employed. Thus, with trialkylchlorosilanes, it is preferable to use an amount between 1 and 3 equivalents, while with dialkyldichlorosilanes, it is preferable to use an amount between 0.5 and 1 equivalent. The silylation is carried out at a temperature between about 15° to about 40° C.

The acylation is carried out by cooling the mixed anhydride solution to a temperature of about −20° to about −35° C., and rapidly adding thereto a cooled solution of a 6-APA derivative, most preferably in the form of a silylated derivative. It is preferable to use the mixed anhydride in an amount of at least 0.8 equivalents based on the 6-APA derivative, the useful range being 0.8–1.2 equivalents. The addition is performed with stirring and the temperature is reduced to about −20° to −35° C. Stirring is continued for a further 0.5 to 5 hours. The intermediate N-protected aminopenicillin resulting from this acylation can then be hydrolyzed in situ.

The N-protected aminopenicillin is then hydrolyzed by mixing the solution with a dilute solution of an organic acid, or an inorganic acid, such as dilute aqueous hydrochloric acid, at a temperature of about 10° to about −5° C. and at a pH of about 0.9–2.0. The mixture is stirred at the same temperature for up to 2 hours.

The aqueous and organic layers are allowed to separate, the aqueous layer containing the desired final product, as its organic or inorganic salt, is washed with an inert, water-insoluble organic solvent, such as ethyl acetate, methylisobutyl ketone or methylene chloride. The organic layer, containing the β-ketoamide, liberated from the N-protected aminopenicillin during hydrolysis, is washed with water and the wash waters are extracted and added to the washed aqueous layer. The aqueous layer is adjusted to the isoelectric point of the αaminopenicillin, allowed to crystallize and the desired final product α-aminopenicillin recovered.

The organic layer and the organic solvent washes of the aqueous layer are combined, filtered and concentrated to dryness. The residue is stirred in water and concentrated hydrochloric acid at 30°–40° C. for 0.5 hour and then at a temperature of about 3° to about 5° C. for 2 hours. The liberated β-ketoamide crystallizes and is recovered. The latter, which is recovered at a high degree of purity and in quantitative yields, is readily recycled for the preparation of further starting amide-type Dane salts.

The improved process of the invention, using novel amide-type Dane salts, advantageously give high yields of α-aminopenicillins at the required high degree of purity with minimal losses of 6-APA due to β-lactam ring cleavage and any concomitant crystallization of 6-APA along with product α-aminopenicillin. The high concentrations of starting, intermediate, and final materials allows for a high throughput. Moreover, the absence of the hitherto usual organic solvent vacuum distillation step subsequent to acylation results in less product degradation and the elimination of the costs involved in vacuum distillation. The recyclable nature of the β-ketoamide liberated during hydrolysis provides a very significant advantage to process economics, as the readily recovered crystalline β-ketoamides are reused in further preparation of the amide-type Dane salts.

The following examples illustrate preferred embodiments of the invention, but the invention is not intended to be limited thereby.

EXAMPLE 1

D-2-(4-Hydroxyphenyl)-N-[1-methyl-2-(4-nitrophenylcarbamoyl)vinyl]glycine, sodium salt

A 5 L. 4-neck flask, fitted with a stirrer, thermometer, reflux condenser, nitrogen inlet and drying tube, is charged with 2.6 L. of methanol and 117 g. (2.88 moles) of sodium hydroxide pellets. The mixture is heated to reflux and stirred until all sodium hydroxide is dissolved. Then 457 g. (2.74 moles) of D(−)-p-hydroxyphenylglycine is added, followed by 640 g. (2.88 moles) of p-nitroacetoacetanilide. The reaction mixture is reheated and kept at reflux for 30 minutes. After removal of the heat source, the stirring is continued for 60 minutes and then the mixture is stirred for 3 hours at 3° C. The precipitate is collected by filtration and washed with 0.5 L. of methanol. The product is dried in an air oven at 4° C. overnight to obtain 927 g. (86.1% yield) of the title compound. Upon concentration of mother liquor and wash, a further 129 g. (12%) of product is isolated. Melting point: 260°–265° C. dec.

EXAMPLE 2

D-2-(4-Hydroxyphenyl)-N-[1-methyl-2-(4-nitrophenylcarbamoyl)vinyl]glycine, potassium salt

The title compound is prepared in 89.3% yield by a procedure similar to Example 1. Melting point: 220°–245° C. dec.

EXAMPLE 3

D-N-[2-(4-Cyanophenylcarbamoyl)-1-methylvinyl]-2-(4-hydroxyphenyl)glycine, potassium salt

The title compound is prepared in a similar manner as Example 1 in 78.8% yield using p-cycanoacetoacetanilide. When methanol is replaced by ethanol, the title compound is obtained in 92.3% yield. Melting point: 250°–255° C. dec.

EXAMPLE 4

D-N-[2-(4-Cyanophenylcarbamoyl)-1-methylvinyl]-2-(4-hydroxphenyl)glycine, sodium salt

The title compound is prepared in a similar manner as Example 1, using sodium hydroxide and methanol in 55.3% yield. Melting point: 230°–240° C. dec.

EXAMPLE 5

D-N-[1-Methyl-2-(4-nitrophenylcarbamoyl)vinyl]-2-phenylglycine, sodium salt

The title compound is prepared in a similar manner as Example 1 using D(−)phenylglycine, methanol and sodium hydroxide in 81.4% yield. Melting point: 220°–230° C. dec.

EXAMPLE 6

D-N-[1-Methyl-2-(4-nitrophenylcarbamoyl)vinyl]-2-phenylglycine, potassium salt

The title compound is prepared in a similar manner as as Example 1, using potassium hydroxide, in 86% yield. Melting point: 170°–178° C. dec.

EXAMPLE 7

6-[(−)-α-amino-p-hydroxyphenylacetamido]-penicillanic acid, trihydrate

A. Preparation of Mixed Anhydride

A 5 L. 4-neck flask, fitted with a stirrer, low temperature thermometer with "thermowatch", nitrogen inlet and drying tube, is charged with 600 ml. of methylene chloride, 120 ml. of dimethylacetamide (with a 4–5% H_2O content), 0.7 ml. of N-methylmorpholine and 100 g. (0.254 mole) of D-2-(4-hydroxyphenyl)-N-[1-methyl-2-(4-nitrophenylcarbamoyl)vinyl]glycine sodium salt prepared according to Example 1. The mixture is cooled, with stirring, to −30° C. and 28.4 g. (0.26 mole) of ethyl chloroformate is added all at once. The temperature is allowed to rise to −23° C. and the mixture is stirred at −23° C. for 1 hour.

B. Preparation of 6-APA Derivative

To a 1 L. 4-neck flask, fitted with a stirrer, thermometer, nitrogen inlet and dropping funnel, is charged 540 ml. of methylene chloride, 54 g. (0.25 mole) of 6-aminopenicillanic acid (6-APA) and 50.5 g. (0.5 mole) of triethylamine. 35.7 g. (0.33 mole) of trimethylchlorosilane is added, with vigorous stirring, over a period of 20 minutes, allowing the temperature to rise to 35° C. Stirring is continued, allowing the temperature to drop to room temperature.

C. Preparation of 6-[D(−)-α-amino-p-hydroxyphenylacetamido]-penicillanic acid, trihydrate

After cooling the mixed anhydride mixture of A above to −45° C. the silylated 6-APA mixture of B above is added all at once while the temperature rises to −30° C. The resulting mixture is stirred for 5 hours at −30° C. The mixture is allowed to warm to −10° C. and 700 ml. of water are added; the temperature rises to 5° C. The N-protected α-aminopenicillin in the resulting mixture is then hydrolyzed in situ. Thus, the pH of the mixture is ajusted to 1.5 with concentrated hydrochloric acid and the mixture is stirred for 15 minutes at 5° C. The layers are separated and the lower, organic layer is re-extracted with 100 ml. of water. The combined aqueous phases are washed with 250 ml. of ethyl acetate and then filtered through Celite.

The pH of the aqueous filtrate is adjusted to 5.4 at 5° C. with concentrated ammonia. The resulting thick slurry is stirred overnight at 0°–5° C. The product is filtered on a Buchner funnel, washed with aqueous acetone and dried to constant weight at 40° C. to afford 87.1 g. (83% of theory) of title compound. The K.F. analysis gave 14.2% against 12.9% H_2O of theory, iodometric assay, 855 mcg/mg. In another experiment the yield is 83.3 g. (79% of theory), K.F. 14.3% (theory 12.9% H_2O), iodometric assay, 828 mcg/mg.

D. Recovery of p-Nitroacetoacetanilide

The organic layer from C above is concentrated to dryness. The oily residue is stirred in 200 ml. water and 50 ml. concentrated hydrochloric acid at 30°–40° C. for 0.5 hour and then at 3°–5° C. for 2 hours. 53 g. (96.5% of theory) of crude p-nitroacetoacetanilide, having a melting point of 105°–110° C., is recovered. This crude material is used to prepare D-2-(4-hydroxyphenyl)-N-[1-methyl-2-(4-nitrophenylcarbamoyl)vinyl]glycine, sodium salt in the same way and with the same yield as in Example 1.

EXAMPLE 8

6-[D(−)-α-aminophenylacetamido]penicillanic acid, anhydrous

6-[D(−)-α-aminophenylacetamido]penicillanic acid (ampicillin) naphthalene sulfonic acid salt is prepared by a procedure similar to that in Example 7, except that 96 g. (0.25 mole) of D-N-[1-methyl-2-(4-nitrophenylcarbamoyl)vinyl]glycine sodium salt prepared according to Example 5 is used with 500 ml. of methylene chloride and 50 ml. of dimethylacetamide (with a 4–5% H_2O content). To the final aqueous ampicillin solution is added 100 ml. of ethyl acetate and 200 g. of aqueous β-naphathalene sulfonic acid solution (29% weight-/volume) while the pH is adjusted to 1.2 at 5°–10° C. by the concurrent addition of triethylamine. The resulting thick slurry is stirred overnight at 0°–5° C. The product is filtered on a Buchner funnel and washed with water and ethyl acetate. The yield is 203.8 g. wet ampicillin β-naphthaline sulfonic acid salt. Drying a sample indicates a yield of 123.5 g. or 89% of theory.

The wet filter cake is treated with one equivalent of triethylamine in 85% aqueous isopropanol at 65° C. for 30 minutes, filtered and dried to give 67.5 g. of anhydrous ampicillin for an overall yield of 77% based on 6-APA, iodometric assay, 1011 mcg/mg.

In another experiment the yield of anhydrous ampicillin is 65.6 g. for an overall yield of 75% based on 6-APA, iodometric assay, 999 mcg/mg.

EXAMPLE 9

6-[D(−)-α-amino-p-hydroxphenylacetamido]-penicillanic acid, trihydrate

The procedure of Example 7 is followed, except that the trimethylchlorosilane is replaced by 21.3 g. (0.165 mole) of dimethyldichlorosilane. The yield is 84.3 g. (80% of theory), K.F. 13.9% (12.9% H_2O of theory), iodometric assay, 862 mcg/mg.

EXAMPLE 10

6-[D(−)-α-aminophenylacetamido]-penicillanic acid, anhydrous

The procedure of Example 8 is followed, except that the trimethylchlorosilane is replaced by 21.3 g. (0.165 mole) of dimethyldichlorosilane. The yield of title compound is 69 g. for an overall yield of 79%, iodometric assay, 992 mcg/mg.

EXAMPLE 11

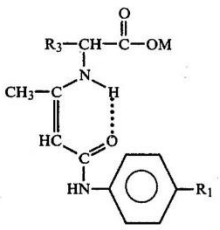
6-[D(−)-α-amino-p-hydroxyphenylacetamido]-penicillanic acid, trihydrate

The procedure of Example 7 is followed, except that the D-2-(4-hydroxyphenyl)-N-[1-methyl-2-(4-nitrophenylcarbamoyl)vinyl]glycine sodium salt is replaced with 97.5 g. of D-N-[2-(4-cyanophenylcarbamoyl)-1-methyl-vinyl]-2-(4-hydroxyphenyl)glycine, potassium salt prepared according to Example 3 and the trimethylchlorosilane is replaced with 21.3 g. (0.165 mole) of dimethyldichlorosilane. The yield of title compound is 65.5 g. (62.4% of theory).

The organic layer is treated as in Example 7D to yield 45 g. of p-cyanoacetoacetanilide (90% of theroy) having a melting point of 119°–120° C.

What is claimed is:

1. A compound having the formula:



wherein R_1 is cyano or nitro; R_3 is phenyl, methylthiophenyl, nitrophenyl, aminophenyl, hydroxyphenyl, alkoxyphenyl in which alkoxy is of 1–4 carbon atoms or halogenophenyl; and M is hydrogen, an alkali metal or a triloweralkylamine.

2. The compound of claim 1, wherein R_3 is phenyl.

3. The compound of claim 1, wherein R_3 is p-hydroxyphenyl.

4. The compound of claim 1, wherein R_1 is cyano.

5. The compound of claim 1, wherein R_1 is nitro.

* * * * *

APPENDIX 3

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(54) Title: ENZYMATIC PROCESS FOR THE PRODUCTION OF BETA-LACTAM ANTIBIOTICS IN THE PRESENCE OF PARTICULATE INOCULUM

(57) Abstract: The invention provides an improved process for producing β-lactam antibiotics catalyzed by an enzyme, which is immobilized onto a carrier, wherein the resulting β-lactam antibiotics are poorly soluble in the reaction media. The process according to the invention achieves particularly high yields and ensures that the enzyme immobilized onto a solid carrier retains its activity so that it remains stable for direct use in multiple further reaction cycles without the need for cost- and time-consuming reactivation.

ENZYMATIC PROCESS FOR THE PRODUCTION OF BETA-LACTAM ANTIBIOTICS IN THE PRESENCE OF PARTICULATE INOCULUM

Field of the Invention

The invention relates to the field of heterogeneous catalysis. The invention provides an improved process for producing β-lactam antibiotics catalyzed by an enzyme, which is immobilized onto a carrier, wherein the resulting β-lactam antibiotics are poorly soluble in the reaction media. The process according to the invention achieves particularly high yields and ensures that the enzyme immobilized onto a solid carrier retains its activity so that it remains stable for direct use in multiple further reaction cycles without the need for cost- and time-consuming reactivation.

Background of the Invention

The enzymatic β-lactam antibiotic synthesis is a suspension-to-suspension reaction. The substrates 6-aminopenicillanic acid (6-APA) or 7-aminodesacetoxycephalosporanic acid (7-ADCA), as well as amino acid derivatives constituting the side chains of the β-lactam antibiotics, e.g., phenylglycine (PG) or p-hydroxyphenylglycine (HPG) are used as solids suspended in water. The amino acid derivatives are typically used in an activated form, e.g., in the form of the corresponding ester or amide. The enzyme, e.g., penicillin G acylase, is typically immobilized onto a solid carrier, e.g., onto polymeric resin beads, and is also suspended in water so as to form a heterogeneous catalyst. The partly dissolved substrates and the respective activated amino acid derivatives are then converted to the desired β-lactam antibiotics in a reaction, which is catalyzed by said catalyst, e.g., by the penicillin G acylase immobilized onto a solid carrier.

The resulting β-lactam antibiotic products are poorly soluble in water and precipitate from the reaction media. However, it was observed that the conversion rate from the substrates and the activated amino acid derivatives to the desired β-lactam antibiotics decreases significantly over time, and in particular during the long-term or repeated use of the enzyme immobilized onto a solid carrier (heterogeneous catalyst) in consecutive cycles of the enzymatic reaction, which is particularly relevant for the large scale production of β-lactam antibiotics in an industrial setting. Consequently there is a need of process improvement.

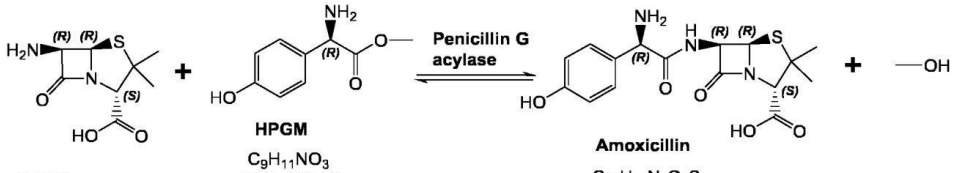
It has now been surprisingly found that when producing β-lactam antibiotics with poor solubility in the reaction media, using an enzyme immobilized onto a carrier as a heterogeneous catalyst, the products tend to precipitate onto the surface of the heterogeneous catalyst, thus inhibiting the enzyme and resulting in low yields of the product. The present invention provides a process to avoiding and reversing precipitation of the product onto the heterogeneous catalysts to allow the reactions to be more efficient in terms of time and yield, thus saving significant costs.

Description of the Invention

The present invention provides an improved process for the production of β-lactam antibiotics that have a poor solubility in the reaction media that proceeds *via* the use of a heterogeneous catalyst. In a preferred embodiment, it relates to an improved process for the production of amoxicillin *via* the use of an enzyme immobilized onto a solid carrier with an improved conversion rate from 6-APA and p-hydroxyphenylglycine methyl ester (HPGM) to amoxicillin.

The process according to the invention is principally suited for the production of β-lactam antibiotics having a β-lactam core structure such as a 6-aminopenicillanic acid (6-APA) core structure, or a 7-aminodesacetoxycephalosporanic acid (7-ADCA) core structure. The β-lactam antibiotics having a 6-APA core structure are typically acylated with an amino acid side chain in position 6. β-Lactam antibiotics having a 6-APA core structure are, for example, amoxicillin carrying a p-hydroxyphenylglycine (HPG) side chain, or ampicillin carrying a phenylglycine (PG) side chain. The β-lactam antibiotics having a 7-ADCA core structure are typically acylated with an amino acid side chain in position 7. β-Lactam antibiotics having a 7-ADCA core structure are, for example, cefadroxil having a p-hydroxyphenylglycine (HPG) side chain, or cephalexin having a phenylglycine (PG) side chain.

While the process according to the invention is principally suited for the production of any β-lactam antibiotics having a poor solubility in the reaction media, the invention will be described in more detail in the following using amoxicillin as an example. Amoxicillin, and in particular, amoxicillin trihydrate, is a known substance, and processes for its production are disclosed in the state of the art. Traditionally, the industrial scale production of semi-synthetic β-lactam derivatives such as amoxicillin, ampicillin, cefadroxil and cephalexin is performed by chemical methods under harsh conditions using reactive intermediates and organic solvents and processes that are environmentally unfriendly. Therefore, the synthesis of these β-lactam antibiotics catalyzed by enzymes constitutes a clear example of an enzymatic reaction of industrial importance and a more sustainable production process. The enzymatic synthesis of Amoxicillin is described, e.g., in WO 97/04086, WO 2004/082661 and WO 2010/072765. In a typical enzymatic procedure for producing amoxicillin, 6-APA is acylated with the aid of side chain p-hydroxyphenylglycine in the presence of an enzyme, e.g., penicillin G acylase, according to the reaction scheme below. p-Hydroxyphenylglycine is preferably used in an activated form, such as an ester or amide form thereof, for example, in the form of a p-hydroxyphenylglycine methyl ester (HPGM). The resulting amoxicillin may then be subjected to further down-stream processing, e.g., to obtain the common end product amoxicillin trihydrate.



6-APA

$C_8H_{12}N_2O_3S$

MW: 216,26

HPGM

$C_9H_{11}NO_3$

MW: 181,19

Amoxicillin

$C_{16}H_{19}N_3O_5S$

MW: 365,40

The substrates 6-APA and *p*-hydroxyphenylglycine methyl ester (HPGM) are used as solids suspended in water. The enzyme, e.g., penicillin G acylase, is immobilized onto a solid carrier, e.g. polymeric resin beads, and is also suspended in water so as to form a heterogeneous catalyst. The partly dissolved substrates 6-APA and HPGM are then converted to amoxicillin *via* the heterogeneous catalyst. The resulting amoxicillin is poorly soluble in water and precipitates from the reaction media. It was observed that the conversion rate from 6-APA and HPGM to amoxicillin significantly decreased over time, i.e., during the repeated or long-term use of said enzyme immobilized onto the solid carrier in consecutive cycles of the enzymatic reaction. The present inventors found that when producing β-lactam antibiotics with poor solubility in the reaction media via a heterogeneous catalyst, the products precipitate onto the surface of the heterogeneous catalyst, thus resulting in low yields of the product. It has now been surprisingly found that the decrease of the conversion rate can be avoided by performing the reaction in the presence of a particulate inoculum.

According to this invention, a process for the enzymatic production of β-lactam antibiotics with poor solubility in the reaction media is provided. If the reaction media is water, or an aqueous system, a solubility of about 0.1 to about 33 mg/mL in water is considered a "poor solubility". Suitable β-lactam antibiotics in this regard are, e.g., amoxicillin, ampicillin, cefadroxil or cephalexin. For example, amoxicillin*trihydrate has a water solubility of about 4.5 mg/mL, ampicillin*trihydrate of about 9.0 mg/mL, cefadroxil of about 0.399 mg/mL, or cephalexin*monohydrate of about 17.2 mg/mL. A preferred β-lactam antibiotic is amoxicillin.

In the process according to the invention, a suspension of the reactants is provided (step (a)). Further provided is an enzyme immobilized onto a solid carrier (step (b)). The reactants are then contacted in the presence of said enzyme in a reaction vessel, e.g., in a bioreactor, (step c).

It has been found that after a saturation of the β-lactam antibiotic in the reaction mixture is reached (i.e., during step (c)), the β-lactam antibiotic product crystallizes and precipitates on the surface of the solid carrier, onto which the enzyme is immobilized. Thus, the surface of the solid carrier, onto which the enzyme is immobilized, is increasingly covered with crystals of the β-lactam antibiotic product. This leads to the blocking of this heterogeneous catalyst. It is believed that said blocking results in a reduced total enzymatic activity of the heterogeneous catalyst, and leads to a significant decrease of the rate of enzymatic conversion of the compound having a β-lactam core structure, e.g., 6-APA or 7-ADCA, and the activated phenylglycine derivative to the desired β-lactam antibiotic product over time.

APPENDIX 4

Microbial culture media preparation:

- **Sabouraud Dextrose Agar SDA**

Dextrose	40.0 g
Peptic Digest of Animal Tissue	5.0 g
Pancreatic Digest of Casein	5.0 g
Agar	15.0 g
Purified water	1000 mL

The pH is adjusted to 5.6 ± 0.2 at 25°C after sterilization.

- **Tryptic Soy Broth TSB**

Tryptone (Pancreatic Digest of Casein)	17.0 g
Soytone (Peptic Digest of Soybean)	3.0 g
Dextrose	2.5 g
Sodium Chloride	5.0 g
Dipotassium Phosphate	2.5 g
Purified water	1000 mL

The pH is adjusted to 7.3 ± 0.2 at 25°C after sterilization.

- **Tryptic Soy Agar (Soybean-Casein Digest Agar – CASO agar)**

Enzymatic Digest of Casein	15.0 g
Enzymatic Digest of Soybean	5.0 g
Sodium Chloride	5.0 g
Agar	1.0 g
Purified water	1000 mL

The pH is adjusted to 7.3 ± 0.2 at 25°C after sterilization.

- **MacConkey Broth**

Peptic Digest of Animal Tissues	20.0 g/L
Lactose	10.0 g/L
Bile Salts	5.0 g/L
Sodium Chloride	5.0 g/L
Neutral Red	0.075 g/L

The pH is adjusted to 7.4 ± 0.2 at 25°C after sterilization.

- **MacConkey Agar MAC**

Peptone (Pancreatic Digest of Gelatin)	17.0 g
Proteose Peptone (Meat and Casein)	3.0 g
Lactose Monohydrate	10.0 g
Bile Salts	1.5 g
Sodium Chloride	5.0 g
Neutral Red	0.03 g
Crystal Violet	0.001 g
Agar	13.5 g
Purified water	1000 mL

The pH is adjusted to 7.1 ± 0.2 at 25°C after sterilization.

- **Fluid Thioglycollate Medium (FTM)**

L-Cystine	0.5g
Agar	0.75 g
Sodium chloride	2.5 g
Glucose monohydrate/anhydrous	5.5/5.0 g
Yeast extract (water-soluble)	5.0 g
Pancreatic digest of casein	15.0 g
Sodium thioglycollate or Thioglycollic acid	0.3 mL
Resazurin sodium solution (1 in 1000), freshly prepared	1.0 mL
Water	1000 mL

The pH is adjusted to 7.1 ± 0.2 at 25°C after sterilization.

- **Soybean Casein Digest Medium (SCDM)**

Casein peptone	17.0 g
Soybean peptone	3.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	2.5 g
Glucose monohydrate/anhydrous	2.5/2.3 g
Water	1000 mL

The pH is adjusted to 7.3 ± 0.2 at 25°C after sterilization.

Abstract:

The Algerian pharmaceutical industry experienced a significant growth, and it is now considered to be the biggest market in the Middle Eastern and African (MEA) region. It has recorded an 8% increase every year on average, as the manufacture of medicines in Algeria keep progressing in terms of both quality and methodology.

The objective of our study is to follow the manufacturing process and the quality control of three dosage forms of AMOXYPEN®: the dispersible tablets, powders for oral suspensions, and powders for injectable solutions, from raw materials to Finished Pharmaceutical Products (FPP), in order to explain why certain production methods are used for certain forms but not for others, and to understand how the administration route of drugs can influence their manufacturing processes.

Generally speaking, tablets and oral suspensions are considered to be non-sterile pharmaceutical preparations, whereas the injectable solutions are sterile, and in order to ensure their sterility throughout the entire manufacturing process, additional safety protocols are necessary.

Keywords: Pharmacy, pharmaceutical industry, amoxicillin, penicillin, beta-lactam, antibiotics' production, dosage forms, sterile pharmaceutical product, active pharmaceutical ingredient.

Résumé :

L'industrie pharmaceutique algérienne a connu une croissance importante ces 15 dernières années, et est désormais considérée comme l'un des principaux marchés de la région Afrique-Moyen Orient (MEA). Elle enregistre une augmentation de 8% chaque année en moyenne, vu que la fabrication des médicaments en Algérie ne cesse de progresser tant sur le plan qualitatif que méthodologique.

L'objectif de notre étude est de suivre le processus de fabrication et le contrôle qualité de trois formes galéniques d'AMOXYPEN® : les comprimés dispersibles, les poudres pour suspensions buvables, et les poudres pour solutions injectables, de la matières premières aux Produits Pharmaceutiques Finis (FPP), afin d'expliquer la raison pour laquelle certaines méthodes de production sont utilisées pour certaines formes galéniques mais pas pour d'autres, et afin de comprendre comment la voie d'administration des médicaments peut influencer leurs procédés de fabrication.

D'une manière générale, les comprimés et suspensions buvables sont considérés comme des préparations pharmaceutiques non stériles, alors que les solutions injectables sont stériles, et afin d'assurer leur stérilité tout au long du processus de fabrication, des protocoles de sécurité supplémentaires sont nécessaires.

Mots-clés : Pharmacie, industrie pharmaceutique, amoxicilline, pénicilline, bêtalactamines, production d'antibiotiques, formes galéniques, produit pharmaceutique stérile, principe pharmaceutique actif.