PQM Promoting the Quality of Medicines

PRODUCT INFORMATION REPORT

AMOXICILLIN

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About PQM

The Promoting the Quality of Medicines (PQM) program is a cooperative agreement between the U.S. Agency for International Development (USAID) and the U.S. Pharmacopeial Convention (USP). The PQM program provides technical assistance to strengthen medicines regulatory authorities and quality assurance systems and supports manufacturing of quality-assured priority essential medicines for malaria, HIV/AIDS, tuberculosis, neglected tropical diseases, and maternal and child health.

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Executive Summary

Amoxicillin is one of the several semisynthetic derivatives of 6-aminopenicillanic acid (6-APA) developed at Beecham, England in the 1960s.

Amoxicillin is used to treat certain infections caused by gram-positive bacteria, such as pneumonia, bronchitis, and gonorrhea and infections of the ears, nose, throat, urinary tract, and skin, as well as some gram-negative bacteria. Amoxicillin belongs to a class of medications called penicillin-like (beta-lactam) antibiotics.

This product information report provides expert scientific analysis of the physicochemical, biopharmaceutics and toxicological properties, API synthesis, analytical, formulation, and manufacturing of amoxicillin. It is expected that the PIR will provide critical information and guidance to manufacturers, as well as stakeholders concerned with access and supply of priority essential medicines.

The information presented in this report is based extensive literature review of data available in the public domain and the opinion of several experts in the field. The authors have taken great care to appropriately cite all references used in this report and provide proper attribution where necessary.

Information provided includes: chemical structure/formula, IUPAC name, physico-chemical properties, moisture sorption, and solubility related data. Amoxicillin has been characterized through various spectroscopic techniques such as FTIR, NMR, Mass, and UV Visible. These have been summarized in the document. The 6-amino penicillanic acid is key material for the synthesis of amoxicillin. Different synthetic and enzymatic routes and their mechanism to synthesize API are also discussed. This PIR also highlights the stability in aqueous and solid states and gives the mechanism of degradation and the degradation products formed. Amoxicillin has been reported to exist as amoxicillin base, sodium salt, and trihydrate. However, amoxicillin trihydrate is the most stable amongst them.

Amoxicillin is included in the penicillin group of compounds which are well documented as sensitizing agents, causing anaphylaxis type allergic responses in susceptible patients. Dedicated manufacturing equipment and manufacturing sites are therefore recommended for amoxicillin production. Qualitative formulae for amoxicillin dispersible tablet formulations, DisperMox® and Flemoxin Solutab®, are described with proposed functions of the excipients along with US Food and Drug Administration

(FDA) inactive ingredient database (IID) limits for the individual excipient. Amoxicillin trihydrate dispersible tablets can be manufactured by using dry granulation process. Requirements for manufacturing equipment along with the proposed manufacturing process have been outlined. Possible scale-up recommendations are included in accordance with US FDA's *Scale Up and Post Approval Changes Guidance for Immediate Release* formulations.

The report also provides toxicology information. Acute toxicity values published for amoxicillin include oral LD50 in mice of 25 g/kg and in rats of 15 g/kg. Amoxicillin is not listed as a known carcinogen in any groups of the International Agency for Research on Cancer (IARC). Amoxicillin is an FDA pregnancy category B drug. The occupational exposure limit (OEL) value and acceptable daily exposure (ADE) of amoxicillin were calculated to be 320 µg/m3 and 2.8 mg/day, respectively. Based on the OEL value, amoxicillin falls in category 2 (100-1000 µg/m3) of the 4-band control banding system. Precautions for safe handling include avoiding contact with skin and eyes. It is also recommended to avoid the formation of dust and aerosols and to provide appropriate exhaust ventilation at places where dust is formed. Normal measures for preventive fire protection are recommended.

Key Manufacturing Challenges

The table below summarizes the key challenges associated with the manufacture of amoxicillin.

Challenges	Description of the challenges and solution
Inherent properties	Amoxicillin is an aminobenzyl penicillin and a beta lactam antibiotic which is associated with penicillin-induced anaphylactic reactions. The manufacture of amoxicillin API and FPP therefore require that manufacturing operations be conducted in a dedicated facility with the proper containment plan and controls to prevent cross contamination and exposure to operators. The need for dedicated facilities and the establishment of controls can be expensive and technically challenging for manufacturers given the limited profit margins for products such as amoxicillin. See section on Formulation Barriers to Entry (page 8) for additional discussion.
Product development	Manufacturers, particularly those that may be less experienced, have difficulty properly formulating amoxicillin dispersible tablets (DT) to pass quality control tests, with particular challenges in meeting the criteria for dispersibility. To pass dispersibility criteria, amoxicillin DT must disintegrate in water within three minutes before administration to produce homogeneous dispersion. The resulting dispersion must be smooth and able to passes through a sieve screen with a nominal mesh aperture of 710 µm (No. 25 sieve).
Manufacturing and controls	Careful monitoring and control of humidity during manufacturing and packaging operation is required to produce quality-assured amoxicillin DT, but this is a challenge for many generic manufacturers that lack adequate/qualified air handling units (AHU) system. Amoxicillin DT must also be packaged properly to protect product from exposure to humidity which can result in degradation. For this purpose, Alu-Alu blister is normally used. This is considered impermeable and highly protective.

Challenges	Description of the challenges and solution
Bioequivalence and biowaiver	WHO TRS 937 (biowaiver guideline) classifies amoxicillin as BCS class I (i.e. a highly soluble/highly permeable drug). Also, in August 2017, FDA issued bioequivalence guidance on amoxicillin oral suspension and revised DS profile acceptance criteria for amoxicillin oral suspension. However both the FDA and WHO Prequalification Team have not issued bioequivalence or biowaiver guidance specifically for amoxicillin DT, leading to the assumption that the guidance for oral suspension can be applied in general context. Refer to section on Bioavailability and Pharmacokinetics (page 9) for additional discussion. There is no guidance as to whether or not palatability study is a requirement for amoxicillin DT. Furthermore, except for the general guidance available for zinc sulfate palatability study, there is no specific guidance on conducting amoxicillin DT palatability study. Guidance available for zinc sulfate palatability study and taste masking evaluation are the best reference currently.

Solid Dosage Form

General Summary

The drug is one of the several semisynthetic derivatives of 6-aminopenicillanic acid (6-APA) developed at Beecham, England in the 1960s. It became available in 1972 under the original trade name Amoxil, and was the second aminopenicillin to reach the market (after ampicillin in 1961). Despite being one of the oldest antibiotics it is still the most frequently prescribed antibiotic in the world. After the patent expired in 1998, many pharmaceutical companies started manufacturing amoxicillin. The drug is now marketed under a substantial number of generic and trade names. Amoxicillin dispersible tablet (DT) is listed as one of the USP PQM GMP Portfolio - Current USAID Priority Medicines. The dispersible tablet of amoxicillin has been included in the list of priority medicines for child survival, by The United Nations Children's Fund (UNICEF) [4]. The World Health Organization (WHO) recommends administering amoxicillin for childhood pneumonia in the form of 250 mg dispersible tablets [5].

Amoxicillin is an antibiotic with broad spectrum antimicrobial activity. It is currently marketed by multiple pharmaceutical companies in US in various dosage forms. There are a number of active drug master files (DMF) filed with the US FDA for amoxicillin trihydrate API, which are listed in Table 1 [1], below. The table also includes manufacturers with certificates of suitability (CEPs) issued by the European Directorate for the Quality of Medicines (EDQM).

Table 1. List of active DMF for amoxicillin trihydrate API as of August 06, 2017

DMF#*	Submit Date	Holder	Subject	CEP from EDQM
30725	7/6/2016	ANTIBIOTICOS DE LEON SLU	AMOXICILLIN TRIHYDRATE	Y
13776	9/1/1998	FERSINSA GB SA DE CV	AMOXICILLIN TRIHYDRATE	Y
16950	11/14/2003	DSM SINOCHEM PHARMACEUTICALS NL	AMOXICILLIN TRIHYDRATE (PURIMOX(TM))	Y
20517	5/9/2007	ZHUHAI UNITED LABORATORIES CO LTD	AMOXICILLIN TRIHYDRATE USP	Y

DMF#*	Submit Date	Holder	Subject	CEP from EDQM
25875	3/16/2012	DSM SINOCHEM PHARMACEUTICALS NETHERLANDS BV	AMOXICILLIN TRIHYDRATE (PURIMOX)	Y
26066	5/15/2012	NORTH CHINA PHARMACEUTICAL GROUP SEMISYNTECH CO LTD	AMOXICILLIN TRIHYDRATE (NON STERILE, API)	Y
13207	4/30/1998	SMITHKLINE BEECHAM CORP DBA GLAXOSMITHKLINE	AMOXICILLIN TRIHYDRATE	Y
13518	9/1/1998	TEVA PHARMACEUTICAL INDUSTRIES LTD	AMOXICILLIN TRIHYDRATE USP	Y

*All of them are type-II DMFs, which include drug substance and their allied compounds

Formulation Barriers to Entry

Cross Contamination

Amoxicillin is an amino penicillin and is associated with penicillin induced hypersensitivity reactions. FDA and European Medicines Agency (EMA) recommend having a dedicated manufacturing facility to avoid any cross contamination by this class of agents into other classes of products [2, 3].

Reverse Engineering

The drug is included in WHO's List of Essential Medicines in liquid and solid oral dosage forms. Amoxicillin dispersible tablet is listed as one of the USP/PQM's GMP current priority medicines. The dispersible tablet of amoxicillin has been included in the list of priority medicines for child survival, by UNICEF [4]. WHO recommends administering amoxicillin for childhood pneumonia in the form of 250 mg dispersible tablets [5]. WHO has also recommended it for use as a short course of treatment for newborn sepsis [6]. The 250 mg strength is not marketed in USA, however, it is marketed in Europe's Flemoxin Solutab® [7]. Limited information is available for Flemoxin Solutab®. However a larger body of information is available for 200 mg strength-DisperMox, which was approved in 2003 in the USA [8], but is currently discontinued. Therefore, information for both products is included in this report.

A qualitative formula for marketed formulations of DisperMox® and Flemoxin Solutab® was retrieved from the review documents of respective formulations available on US FDA and Medicine Evaluation Board (MEB, Netherlands) websites, respectively.

DisperMox® was available in the US as 200 mg to 600 mg tablets. Flemoxin Solutab® is available in the Netherlands as tablets from 250 mg to 1000 mg.

Discussion of Excipients

A list of excipients with their proposed function in DisperMox® and Flemoxin Solutab® tablet is provided in Tables 2 and 3, respectively [7, 8]. FDA's Inactive Ingredient Database (IID) can be accessed for individual inactive ingredients. IID provides the dosage forms the excipient is approved for and the maximum concentration approved for that dosage form. Quantitative limits for excipients were checked for oral dispersible tablets [9].

Table 2. List of excipients and their proposed function with IID limits for DisperMox®

Ingredients	Function	Reference (Page number of reference)	IID Limit	Usual recommended concentration
Aspartame	Sweetening agent	[10] pp.48-51	2.5 mg	-
Colloidal silicon dioxide	Glidant	[10] pp.185-189	20 mg	0.1-1 %
Cross carmellose sodium	Disintegrant	[10] pp. 206-208	8 mg	0.5-5 %
FD&C Red No.40 aluminum lake	Coloring agent	[11]	21.25 mg(tablet)	-
Magnesium stearate	Lubricant	[10] pp. 404-408	16 mg	0.25-5 %
Microcrystalline cellulose	Diluent and binder	[10] pp. 129-134	340 mg	5-15 % (binder); 20-90 (diluent)
Strawberry guarana flavor	Flavor	[12]	-	-

Table 3. List of excipients and their proposed function with IID limits for Flemoxin Solutab®

Ingredients	Function	Reference (Page number of reference)	IID Limit	Usual recommended concentration
Dispersible cellulose	Dispersing agent	[10] pp. 134-135	200 mg(tablet)	-
Microcrystalline cellulose	Diluent and binder	[10] pp. 129-134	340 mg	5-15 % (binder); 20-90 (diluent)
Crospovidone	Disintegrant	[10] pp. 208-210	340 mg	2-5 %
Flavor vanillin	Flavor	[13]	-	-
Flavor mandarin	Flavor	[13]	-	-
Flavor lemon	Flavor	[13]	-	-
Saccharine	Sweetening agent	[10] pp. 605-608	0.5 mg	0.02-0.5 %
Magnesium stearate	Lubricant	[10] pp. 404-408	16 mg	0.25-5 %

Formulation Challenges

Amoxicillin sodium is very hygroscopic in nature, while trihydrate is non-hygroscopic. Among free base, sodium salt, and trihydrate, amoxycillin trihydrate is the most stable solid form [14]. Still, amoxicillin trihydrate is sensitive to temperature and humidity. It also shows degradation in alcohol and polyols. Solution degradation can be catalyzed by phosphate, Mono- and Di-hydrogen citrate ions. It has optimum stability in pH 5.8-6.5 citrate buffer [8]. It is recommended to use compacted or directly compressible API for manufacturing of dispersible tablet.

Efficacy, ADME, and Adverse Effects

Amoxicillin works by killing sensitive bacteria. The drug is a moderate-spectrum, bacteriolytic, β-lactam antibiotic in the aminopenicillin family that is used to treat bacterial infections caused by susceptible Gram-positive and Gram-negative microorganisms. It is typically the drug of choice within the penicillin class, because it is better absorbed following oral administration than other β-lactam antibiotics. Amoxicillin has been reported to be more active *in vitro* than ampicillin against *Enterococcus faecalis, Helicobacter pylori, and Salmonella spp*., but less active against

Shigella spp. Amoxicillin is inactivated by beta lactamases. Complete cross-resistance has been reported between amoxicillin and ampicillin. Amoxicillin's spectrum of activity may be extended if used with a beta-lactamase inhibitor such as clavulanic acid. Potassium clavulanate is also reported to increase activity of amoxicillin against other species not considered sensitive to amoxicillin. These include *Bacteroides, Legionella, and Nocardia spp., Haemophilus influenzae, Moraxella catarrhalis (Branhamella catarrhalis), and Burkholderia pseudomallei (Pseudomonas pseudomallei).* However, *Ps. aeruginosa, Serratia marcescens*, and many other Gram-negative bacteria remain resistant. Transferable resistance has been reported in *H. pylori* [8, 14].

Amoxicillin is widely distributed at varying concentrations in body tissues and fluids. It crosses the placenta and small amounts are distributed into breast milk. Low concentrations of amoxicillin pass into the cerebrospinal fluid unless the meninges are inflamed. Amoxicillin is metabolized to a limited extent to penicilloic acid and is excreted in the urine. About 60% of an oral dose of amoxicillin is excreted unchanged in the urine within 6 hours by glomerular filtration and tubular secretion. Urinary concentrations above 300 micrograms/mL have been reported after a dose of 250 mg. Amoxicillin is removed by hemodialysis. High concentrations have been reported in bile and some amount may be excreted in the feces. The pharmacokinetics of amoxicillin and clavulanic acid are broadly similar and neither appears to affect the other to any great extent [8, 14].

Hypersensitivity reactions are more likely in patients with a history of allergy, asthma, hay fever, or urticarial [15]. Hypersensitivity reactions have been reported in up to 10% of patients and have included anaphylaxis, urticarial rash, erythematous maculopapular rash, serum sickness-like reactions, erythema multiforme, urticaria, edema, hypotension, fever, eosinophilia, exfoliative dermatitis, toxic epidermal necrolysis, acute generalized exanthematous pustulosis, hypersensitivity vasculitis, angioedema, Stevens-Johnson syndrome, and dyspnoea [15, 16].

Dermatologic side effects have included rash, fixed drug eruption, and bullous pemphigoid. Erythematous maculopapular rashes occur frequently in patients with infectious mononucleosis who take amoxicillin. These rashes may be due to hypersensitivity. Three out of four patients with infectious mononucleosis and an amoxicillin-associated rash displayed hypersensitivity to amoxicillin and ampicillin by skin tests and lymphocyte transformation tests. Two of these patients had side-chain-specific sensitization [15-17].

Gastrointestinal side effects have included diarrhoea, nausea, vomiting, generalized abdominal cramps, colitis, haemorrhagic colitis, pseudomembranous colitis (Clostridium difficile associated diarrhoea), and black hairy tongue. Abdominal pain has

also been reported. Amoxicillin has been associated with haemorrhagic, sometimes inflammatory colitis, which typically affects the ascending colon. Renal side effects have included crystalluria and acute interstitial nephritis, often associated with fever, rash, and eosinophilia. A patient undergoing dental extraction and receiving warfarin anticoagulation therapy had prolonged bleeding times (PT and INR), and decreased haemoglobin and haematocrit. The bleeding might have happened due to vitamin K deficiency as a result of depletion of intrinsic vitamin K-producing gut flora from use of amoxicillin for prophylaxis of subacute bacterial endocarditis.

Hematologic side effects associated with penicillin have included thrombocytopenia, anaemia, leukopenia, thrombocytopenic purpura, agranulocytosis, haemolytic anaemia, eosinophilia, and granulocytopenia. These effects are usually reversible and are believed to be due to hypersensitivity reactions [15-17].

Bioavailability and Pharmacokinetics

Amoxicillin is mostly absorbed orally achieving peak concentrations in 1.5 hours (approx.), with 74-92% oral bioavailability and 20% bound to plasma proteins. The half-life of amoxicillin is 61.3 minutes and approximately 60% of an orally administered dose of amoxicillin is excreted in the urine within 6 to 8 hours. The oral dosage at which half of experimental animals are estimated to die is >100 times and >60 times the human dose for antibacterial use (250 mg/kg) in mice and rats respectively. Amoxicillin is resistant to inactivation by gastric acid. It is more rapidly and better absorbed than ampicillin when given orally. Peak plasma amoxicillin concentrations of about 5 micrograms/mL have been observed 1 to 2 hours after a dose of 250 mg, with detectable amounts present for up to 8 hours. Doubling the dose can double the plasma concentration. The presence of food in the stomach does not appear to diminish the total amount absorbed. Concentrations of amoxicillin after intramuscular injection are similar to those achieved with oral doses. About 20% of the drug is bound to plasma proteins. A plasma half-life of 1 to 1.5 hours has been reported. The half-life may be prolonged in neonates, the elderly, and patients with renal impairment. In severe renal impairment, the half-life may be 7 to 20 hours [8, 14].

US FDA has established a Biopharmaceutics Classification System (BCS) for immediate release (IR) solid oral dosage forms for the purposes of requesting a waiver from having to perform a bioequivalence (BE) study in support of an abbreviated new drug application (ANDA). The BCS is a scientific framework for classifying drug substances based on their aqueous solubility and intestinal permeability. According to the BCS, drug substances are classified as follows:

Class 1: High Solubility – High Permeability

Class 2: Low Solubility – High Permeability

Class 3: High Solubility – Low Permeability

Class 4: Low Solubility – Low Permeability

According to the US FDA BCS guidance [100], when the SDF is rapidly dissolving demonstration of in vivo bioavailability (BA) or BE may not be necessary for drug products containing Class 1 and Class 3 drug substances. The ANDA manufacturer/applicant could request a biowaiver as long as the inactive ingredients used in the dosage form do not significantly affect absorption of the active ingredients.

There has been a farrago regarding the biopharmaceutical classification of amoxicillin. Recently, a published review on the biowaiver monograph of amoxicillin classified it as BCS class 1 compound [19]. A bioequivalence study has been reported for amoxicillin in an FDA review document for DisperMox® [8]. It was an open label, two part, and single dose cross-over study where 24 healthy human volunteers participated. The dispersed mixture of DisperMox® tablet, 400 mg, produced blood levels similar to those achieved with corresponding doses of conventional amoxicillin oral suspension. Orally administered conventional suspension, 400 mg/5 mL, resulted in average blood levels in the range of 3.3 mcg/ml to 11.5 mcg/ml 1 to 2 hours after administration. Orally administered DisperMox® 400 mg tablets result in average blood levels in the range of 3.2 mcg/ml to 11.5 mcg/ml 1 to 2 hours after administration. Pharmacokinetic data retrieved from the FDA review document for DisperMox® is tabulated in Table 4.

Based on the assumption that the posology for the tablets in clinical use will include that they be dispersed in water prior to administration, it is recommended that proposed products be compared against Amoxil powder for oral suspension (GlaxoSmithKline) or Clamoxyl powder for oral suspension (GSK). Amoxil powder for oral suspension is available in the UK. Clamoxyl power for oral suspension is available in Spain, France, and Belgium. Please note it should be highlighted that comparators must be sourced from the market of an ICH-associated country as this assures the quality of the comparator [18].

The generic product manufacturer of the amoxicillin dispersible tablets is recommended to request a bio-waiver from WHO and/or US FDA based on available literature cited above. *In-Vitro* dissolution profile comparison against the comparator product (listed above) is recommended to be performed for the generic drug product application.

Table 4. Mean pharmacokinetic data (n=24) for DisperMox® bioequivalence study

	Conventional suspension (400 mg/5 mL)	DisperMox, 400 mg
C_{max}(mcg/mL)	8.4	7.5
T_{max}(hour)	1-2	1-2
$AUC_{0\text{-}inf}$(mcg-hr/mL)	18.5	17.9

Process Equipment Selection

Amoxicillin trihydrate can be formulated using dry granulation followed by tablet compression. Major unit operations involved in the dry granulation process include milling, sieving, blending, dry granulation, de-sizing, lubrication, compaction, and coating. A V-blender, double-cone blender, bin blender, or octagonal blender can be used for blending. A roller compactor can be used for generation of compacts. The latter can then be converted to granules of required size using a multi-mill or oscillatory granulator. Granules are then mixed with extragranular excipients using a suitable blender (see above). The lubricated granules are then compressed using a rotary tablet press. The tablet cores can then be film coated using an appropriate film coating pan.

Manufacturing Process

Based on the knowledge regarding the API and the excipients utilized for DisperMox® and Flemoxin Solutab® products, the following manufacturing process guideline is provided to the generic drug product manufacturer.

Dry granulation process allows manufacturing in a solvent free environment. The first step is milling the API. However, this is an optional process based on particle size of supplied API raw material. Milled powder is then subjected to sieving to achieve uniform particle size. Bulk powder obtained after sieving is mixed with appropriate inactive ingredients and blended for a sufficient amount of time to ensure homogeneity of the blend. The properly mixed homogeneous blend is then processed by roller compaction to obtain compacts, which are broken down to get granules. The granules are mixed with extragranular inactive ingredients and then compressed in a tablet press to obtain the tablet cores, followed by film coating to obtain the final formulation.

Scale-Up Challenges

Scale up of amoxicillin dispersible tablets shall require higher capacity conventional equipment for dry granulation that includes: a blender (double-cone/V/octagonal/bin), dry granulator (roller compactor), tablet press, and a film coating pan. Best practices for the development, scale-up, and post-approval change control of IR dosage form are documented in a recently published white paper [20]. Criterion for required data for proposed scale up and post approval changes (SUPAC) have been well documented in FDA guidance published in 1995 [21]. SUPAC documentation for equipment used in individual unit operation has also been addressed by recent FDA guidance [22]. SUPAC-IR requirement for 10x scale up is specified in section V.B of "Scale-Up and Post-Approval Changes: Chemistry, Manufacturing, and Controls, In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation". Level 1 changes have been recommended for 10x scale up, in FDA 'question-answer document' for SUPAC-IR [3]. Level 1 changes include - change in batch size, up to and including a factor of 10 times the size of the pilot/bio-batch, where: 1) the equipment used to produce the test batch(es) is of the same design and operating principles; 2) the batch(es) is (are) manufactured in full compliance with current good manufacturing practices (CGMPs); and 3) the same standard operating procedures (SOPs) and controls, as well as the same formulation and manufacturing procedures, are used on the test batch(es) and on the full-scale production batch(es). Level 1 changes require submission of annual report with a long-term stability study of at least one production batch, while no in vivo bioequivalence study is required.

Toxicology Information

Toxicity

Serious and occasionally fatal hypersensitivity (anaphylactic) reactions have been reported in patients on penicillin therapy including amoxicillin. Although anaphylaxis is more frequent following parenteral therapy, it has also occurred with oral penicillins. These reactions are more likely to occur in individuals with a history of penicillin hypersensitivity and/or a history of sensitivity to multiple allergens. There have been reports of individuals with a history of penicillin hypersensitivity who have experienced severe reactions when treated with cephalosporins. Before initiating therapy with amoxicillin, careful inquiry should be made regarding previous hypersensitivity reactions to penicillins, cephalosporins, or other allergens. If an allergic reaction occurs, amoxicillin should be discontinued and appropriate therapy instituted.

Acute toxicity values published include oral LD_{50} in mice of 25 g/kg and in rats of 15 g/kg [23].

Overdose of amoxicillin may result in interstitial nephritis resulting in oliguric renal failure and crystalluria, which in some cases leads to renal failure. Acute ingestion of large doses of amoxicillin may cause nausea, vomiting, diarrhoea, and abdominal pain.

Carcinogenic, Reproductive, and Developmental Hazards

Studies that detect mutagenic potential of amoxicillin alone have not been conducted; however, the following information is available from tests on a 4:1 mixture of amoxicillin and potassium clavulanate. The combination of amoxicillin and potassium clavulanate was non-mutagenic in the Ames bacterial mutation assay, and the yeast gene conversion assay. It was weakly positive in the mouse lymphoma assay, but the trend toward increased mutation frequencies in this assay occurred at doses that were also associated with decreased cell survival. It was negative in the mouse micronucleus test and in the dominant lethal assay in mice. Long-term studies in animals have not been performed to evaluate carcinogenic potential. Amoxicillin is not listed as a known human carcinogen in any IARC groups. Amoxicillin is an FDA pregnancy category B drug meaning that pregnant rats and mice given Augmentin® (2:1 ratio formulation of amoxicillin: clavulanate) at oral doses up to 1200 mg/kg/day revealed no evidence of harm to the fetus due to Augmentin® and there are no adequate and well-controlled studies in humans. Based on body surface area, this dose of amoxicillin is

approximately 4 times the maximum recommended adult human oral dose (875 mg every 12 hours).

Reproduction studies have been performed in mice and rats at doses up to 2000 mg/kg (3 and 6 times the highest human dose (3 g), based on body surface area). There was no evidence of harm to the foetus due to amoxicillin. Reproductive studies in animals have shown no evidence of impaired fertility or harm to the foetus. Amoxicillin is not a human teratogen. Amoxicillin has been shown to be excreted in human milk. Amoxicillin use by nursing mothers may lead to sensitization of infants [8, 23, 24].

OEL Calculations

Utilizing the NOAEL [8] and uncertainty/safety factor for determining occupational exposure limits as presented by Robert [24], with consideration to uncertainty factors discussed by Naumann and Weideman [25], an OEL for amoxicillin can be calculated as follows:

OEL = NOEL (mg/kg/day) x BW (kg) / V (m^3/day) x S x UF x MF x α

OEL = 2450 mg/kg/day x 70 kg / 10 m^3/day x 2 x 900 x 10 x 3 = 0.317 mg/m^3 = 320 µg/m^3

NOAEL= No Observable Adverse Effect Level

UF=uncertainty factors (6 for rat to human extrapolation, 10 for inter-human variation, 3 for sub-chronic to chronic extrapolation, 5 for available pre-clinical toxicity data)

MF= Modifying factor of 10 for fatal anaphylactic reactions that may happen due to penicillins.

S= steady state based on elimination half-life = 2

α = pharmacokinetic factor based on bioavailability = 3

V = volume of air breathed in a shift = 10 m^3

This OEL is designed to be an 8-hour a day, 40-hour a week airborne concentration which nearly all workers may be repeatedly exposed to day-after-day without adverse health effects, based on currently available information. It does not take into account hyper-sensitive or otherwise unusually responsive individuals or persons with hypersensitivity to amoxicillin, which may be exacerbated by exposure to this drug.

Control Band Assignment

Amoxicillin has been assigned as a Category 2 (100-1000 µg/m^3) substance in the 4-band control banding system [26].

Table 5. Band system for hazardous chemicals

Band No	Target range of exposure concentration	Hazard Group	Control
1	>1 to 10 mg/m^3 dust >50 to 500 ppm vapour	Skin and eye irritation	Use good industrial hygiene practice and general ventilation
2	>0.1 to 1 mg/m^3 dust >5 to 50 ppm vapour	Harmful on single exposure	Use local exhaust ventilation
3	>0.01 to 0.1 mg/m^3 dust >0.5 to 5 ppm vapour	Severely irritating and corrosive	Enclose the process
4	<0.01 mg/m^3 dust <0.5 ppm vapour	Very toxic in single exposure, reproductive hazard, sensitizer	Seek expert advice

Industrial Hygiene Sampling and Analytical Methods

- Precautions for safe handling: Avoid contact with skin and eyes. Avoid formation of dust and aerosols. Provide appropriate exhaust ventilation in places where dust is formed. Normal measures for preventive fire protection.
- Analytic laboratory methods: Method is applicable to amoxicillin. Method involves reaction with 0.1 N NAOH producing stable fluorescent products. Concentrations as low as 0.01 µg/mL were detected [27].

ADE Calculations

Utilizing the uncertainty/modifying factor method for determining acceptable daily exposure (ADE) values with consideration to the methods discussed by Sergant, et al. [28] and the EMA [29], an ADE for amoxicillin can be calculated as follows:

ADE = (POD mg/day) / UF_C x MF x PK

ADE = 750 mg / 90 x 1 x 3 = 2.8 mg/day

Where:

POD = Point of Departure

BW = Body-weight (kg)

$UF_C = (UF_A \times UF_H \times UF_S \times UF_E \times UF_R)$

UF_A = Interspecies

UF_H = Intraspecies variability

UF_S = Length of study

UF_E = Severity of effect

UF_R = Reference effect level

MF = Modifying Factor

PK = Pharmacokinetic Factor

The ADE is the daily dose of a substance below which no adverse effects are anticipated by any route, even if the exposure occurs over a lifetime.

Choice of Uncertainty and Modifying Factors

In calculating the ADE value for Amoxicillin, a composite UF_C of 100 was used. The choice was made to account for the following factors:

1. The lowest daily therapeutic dose (250 mg x 3) was selected as the point of departure, and this dose is based on human data; therefore, a factor of 1 was applied to UF_A.
2. In the absence of specific intraspecies variability of data, a conservative default factor of 10 is applied to UF_H to extrapolate from the general human population to sensitive subgroups, such as children and geriatrics [30].
3. The data reviewed was based on studies less than 26-weeks; therefore, an uncertainty factor of 3 was applied to UF_S [25].
4. Based on market data, the adverse health effects are usually moderate and a considerable number of people have experienced hypersensitive reactions due to amoxicillin; therefore, an uncertainty factor of 3 was applied to UF_E.
5. A minimum daily therapeutic dose has been established; and an uncertainty factor of 10 was already applied in UF_H to protect sensitive subgroups, therefore, an uncertainty factor of 1 was applied to UF_R.
6. The database of information was complete; therefore, a modifying factor of 1 was used to account for mild adverse effects other than hypersensitivity produced by amoxicillin.
7. A composite PK factor of 3 was used to account for variable human pharmacokinetics [31].

Analytical Profile

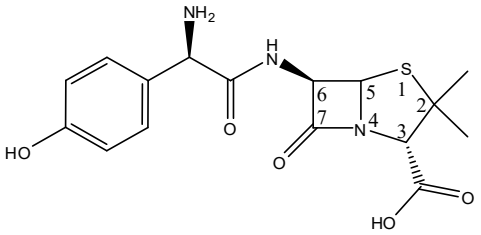
Amoxicillin is a penicillin antibiotic. It is susceptible to degradation by β-lactamase-producing bacteria, which are resistant to a narrow spectrum of β-lactam antibiotics, such as penicillin.

The Active Pharmaceutical Ingredient (API)

The API is available in anhydrous, trihydrate, and sodium salt forms. Even monohydrate, dihydrate, and trihydrate forms have been reported; however, the trihydrate is the most stable hydrated form. Amoxicillin trihydrate has a good oral bioavailability that is not affected by the concomitant ingestion of food. Amoxicillin anhydrous is a white powder with a sulphurous odor. Amoxicillin trihydrate is crystalline and off-white in color.

International Pharmacopoeia (Ph. Int.) lists it as an odorless white or almost white, crystalline powder. Amoxicillin sodium is said to be a white, almost white, very hygroscopic, powder [32]. Depending on the type of dosage form to be produced, amoxicillin API comes in two forms: i) Sterile sodium amoxicillin for injectable medicinal products (IM/IV); ii) Amoxicillin trihydrate for oral medicinal products [33].

Chemical Structure/Formula



Name	CAS No.	Formula	Molecular Weight
Amoxicillin	26787-78-0	$C_{16}H_{19}N_3O_5S$	365.41 g/mol
Amoxicillin trihydrate	61336-70-7	$C_{16}H_{19}N_3O_5S.3H_2O$	419.45 g/mol
Amoxicillin sodium	34642-77-8	$C_{16}H_{18}N_3O_5S.Na$	387.39 g/mol

Stereochemistry

Amoxicillin has the S, R, R configuration at C2 (equivalent to C3 in conventional penicillin numbering), C5 and C6, respectively, that is common to all penicillins. The side-chain configuration at C10 is R in some of the literature. This is referred to as D (-).

IUPAC Name

Amoxicillin: (2S,5R,6R)-6-[[(2R)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid

Amoxicillin trihydrate: (2S,5R,6R)-6-[[(2R)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid; trihydrate

Amoxicillin sodium: sodium;(2S,5R,6R)-6-[[(2R)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate

USP 40 (USAN):

Amoxicillin: 4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6-[[amino(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl- 7-oxo-, trihydrate [2S-[2α,5α,6 β (S*)]]-;

Amoxicillin trihydrate: 4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6-[[amino(4-hydroxyphenyl)acetyl] amino]-3,3-dimethyl-7-oxo-, trihydrate [2*S*-[2a,5a,6b(*S**)]]-;

(2*S*,5*R*,6*R*)-6-[(*R*)-(–)-2-Amino-2-(*p*-hydroxyphenyl)-acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid trihydrate

Amoxicillin sodium: Sodium [2S-[2α,5α,6β(S*)]]-6-[[amino(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate

EP:

Amoxicillin trihydrate: (2S,5R,6R)-6-[[(2R)-2-Amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate

Amoxicillin sodium: Sodium [2S-[2α,5α,6β(S*)]]-6-[[amino(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate

Ph. Int.:

Amoxicillin trihydrate: (-)-6-[2-Amino-2-(p-hydroxyphenyl)acetamido]-3,3- dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0]heptane-2- carboxylic acid trihydrate; (2S,5R,6R)-6-[(R)-2-amino-2-(4-hydroxyphenyl)acetamido]-3,3- dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate; 6-[[amino(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1- azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate

Other Names:

Amoxicillin trihydrate: D-(-)-alpha-amino-p-hydroxybenzyl penicillin trihydrate; (-)-6-[2-amino-2-(p-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabi; (2s-(2alpha,5alpha,6beta(s*)))-acetyl)amino)-3-dimethyl-7-oxo-trihydrate

MESH SYNONYMS: Actimoxi, Amoxicillin, Amoxicillin Anhydrous, Amoxicillin Clariana Brand, Amoxicillin monopotassium salt, Amoxicillin monosodium salt, Amoxicillin Sodium, Amoxicillin trihydrate, Amoxicillin, (R*)-isomer, Amoxicilline, Amoxil, Amoxycillin, etc.

Physical Properties

Crystal Forms

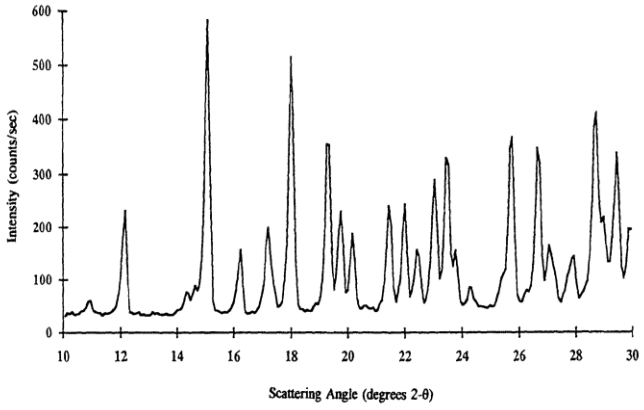
A single crystal X-ray diffraction study showed that the crystals of amoxicillin trihydrate were orthorhombic, in space group P2, 2, 2, with four molecules in the unit cell [34]. The thiazolidine ring conformation had the sulphur atom out of the plane formed by the other four atoms. Comparison with the crystal structure of ampicillin trihydrate showed that the molecular packing and conformation were similar, but an additional element of three-dimensional rigidity was provided by hydrogen bonding of the p-OH group to the carboxylate of a neighboring molecule [35]. This may explain the observation that when amoxicillin trihydrate was dehydrated (e.g. over P_2O_5), it retained some crystallinity, shown by X-ray powder diffractometry, and on absorption of water vapor reverted to the trihydrate, shown by IR and X-ray powder diffractometry. By contrast, dehydration of ampicillin trihydrate gave an amorphous hygroscopic material, which remained amorphous on absorption of water vapor to well above the trihydrate level. Comparison of the crystal structure of amoxicillin trihydrate with that of ampicillin anhydrate showed that the p-OH group would reduce the benzene ring overlap that contributes to the stability of the anhydrate crystal form. This was consistent with the failure of amoxicillin to crystallize under the conditions used to prepare ampicillin anhydrate [36]. However, a hygroscopic crystalline anhydrate of

amoxicillin was obtained by solid state removal of methanol from a crystalline monomethanolate. Unfortunately, little information is available about this last form. Amoxicillin sodium salt is normally prepared in an amorphous form. An anhydrous crystalline form can be obtained by removal of solvent from various solvates, either in the solid state or by displacement in solution with a solvent of lower dielectric constant.

X-Ray Powder Diffractogram

The powder diffractogram of amoxicillin trihydrate obtained with copper Kα radiation is shown below in Figure 1. Diffraction lines with a relative intensity greater than 10% are listed below, with their d spacing and relative intensities as shown in Figure 1.

Figure 1. X-Ray powder diffractogram of amoxicillin trihydrate



Diffraction angle (°2θ)	d (A°)	Relative intensity (%)
12.17	7.27	35
15.13	5.85	100
16.25	5.45	22
17.20	5.15	30
18.04	4.91	91
19.33	4.59	64
19.77	4.49	36
20.20	4.39	27
21.48	4.13	39
22.01	4.04	37
22.46	3.96	23
23.05	3.86	46
23.49	3.79	58
23.82	3.73	21
25.75	3.46	65
26.69	3.34	61
27.10	3.29	23
27.90	3.20	20
28.71	3.11	73
29.47	3.03	54

The diffraction angles are close to those given in more limited data sets [37], but some of the relative intensities are significantly different. Such differences can arise from differences in the size and orientation of particles in the sample. Amoxicillin sodium salt, in its usual amorphous form, gives a featureless diffractogram.

Melting Point (°C) [38]

194 °C

Optical Rotation [32]

Specific optical rotation ranges of +280 to +305° and +240 to +290° were calculated on the anhydrous basis for the trihydrate (0.2% in water) and the sodium salt (0.25% in 0.4% potassium hydrogen phthalate) respectively.

Log P [38].

0.87

Pka

pKa1 = 3.2 (acid); pKa2 = 11.7 (primary amine) additional dissociation data also available in reference [39].

Solubility

BP/EP [32]

Amoxicillin is very soluble in water, sparingly soluble in anhydrous ethanol, and very slightly soluble in acetone. Amoxicillin trihydrate is slightly soluble in water, very slightly soluble in ethanol (96%), and practically insoluble in fatty oils. It dissolves in dilute acids and dilute solutions of alkali hydroxides.

Ph. Int.

Amoxicillin trihydrate is slightly soluble in water and methanol R; very slightly soluble in ethanol (~750 g/l) TS, ether R, and fatty oils; and soluble in dilute acids and dilute solutions of alkali hydroxides.

Other references for Amoxicillin Solubility

The solubility of amoxicillin trihydrate in water varies with pH. In a study at 37°C in aqueous potassium chloride (μ = 0.5) over a pH range of 2 to 8 showed a minimum solubility of about 0.013 M in the pH range of 4 to 6. The calculated intrinsic solubility at the isoelectric point was 0.013M (5.45 mg/ml) [37]. For the trihydrate, at an unspecified temperature, the solubility is about 4, 7.5, 3.4 and 1.3 mg/ml in water, methanol, ethanol, and acetone respectively [37]. Water solubility is reported to be 3430 mg/ml [24]. 1 g of amoxicillin trihydrate is soluble in about 370 mL water, about 2000 mL alcohol, about 290 mL phosphate buffer (1%, pH 7), and about 330 mL methanol [40]. Amoxicillin is soluble in 1 M ammonium hydroxide (50 mg/ml), yielding a clear, colorless to light yellow solution [38].

Bulk and Tapped Density

A patent reported that the amoxicillin trihydrate they handled had an average volume-based grain size of 10 μm to 30 μm and a bulk density of 0.15 g/ml to 0.45 g/ml. There was no specific upper limit for the tapped density that may be less than 1.2 g/ml. An increased tapped density improved the flow properties. According to the invention, the

crystalline powder had a bulk density and tapped density such that the ratio d_t/d_b was less than 1.7, preferably less than 1.45, wherein d_t=tapped density and d_b=bulk density. This resulted in improved flowability. There was no specific lower limit for the ratio d_t/d_b. The ratio d_t/d_b may be higher than 1.05, for instance higher than 1.1 [41].

Additional Characterization by Various Techniques

UV Spectrum

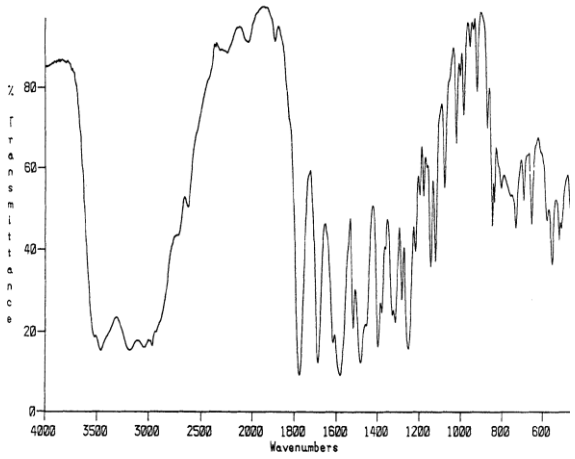
λmax: 230 nm, 274 nm (ethanol)

Extinction coefficient: E^{mM} = 10.85 (230 nm), 1.4 (274 nm) (ethanol) [38].

Infrared Spectra

The frequencies and assignments of significant bands, and IR spectra of amoxicillin trihydrate and amoxicillin sodium salt in a potassium bromide disc are given in figures 2 and 3.

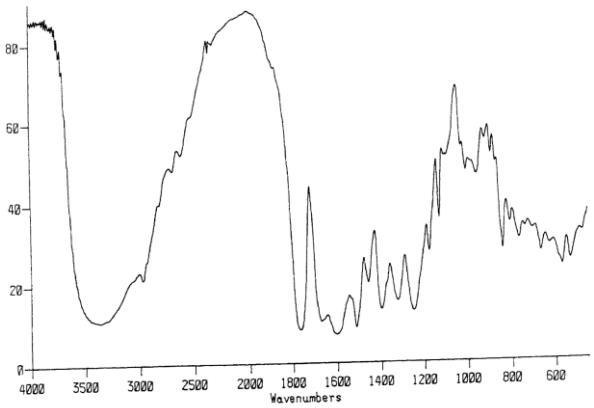
Figure 2. Infrared spectrum of amoxicillin trihydrate



Amoxicillin trihydrate

Frequency (cm^{-1})	Assignment
3520	Water of crystallisation, OH stretch
3458, 3175	Amide NH and phenol OH stretch
3046	Benzene ring CH stretch
2964	Methyl CH stretch
1775	β-Lactam CO stretch
1686	Amide I, CO stretch
1580	COO^- asymmetric stretch and NH_3^+ asymmetric deformation
1517	Benzene ring C=C stretch
1482	Amide II, NH bend CN stretch combinatio band and NH_3^+ symmetric deformation
1396, 1378	Gem dimethyl CH deformation and phenol OH combination band
1327	COO^- symmetric stretch
1314	Fused thiazolidine β-lactam skeletal mode
1283	Amide III, NH bend CN stretch in plane combination band
1250	Phenol CO combination band
1143, 1120	Benzene ring CH in plane deformation

Figure 3. Infrared spectrum of amoxicillin sodium salt



Amoxicillin sodium salt

Frequency (cm^{-1})	Assignment
3366 (v. broad)	Phenol and water OH, amine and amide NH stretches
2969	Methyl CH stretch
1764	β-Lactam CO stretch
1671	Amide I, CO stretch
1601	COO^- asymmetric stretch
1513	Amide II, NH bend CN stretch combination band and benzene ring C=C stretch
1457	Gem dimethyl CH deformation
1398	COO^- symmetric stretch and phenol OH combination band
1321	Fused thiazolidine β-lactam skeletal mode
1248	Phenol CO and Amide III, NH bend CN stretch in plane combination bands
1173,1126	Benzene ring CH in plane deformation

The water contributing to the very broad band at 3366 cm-' is partly present in the sample but also comes from water uptake during grinding with potassium bromide [35].

Proton Nuclear Magnetic Resonance Spectrum

400 MHz spectrum of amoxicillin trihydrate in D_2O adjusted to pD 8 with NaOD is shown in Figure 4. Chemical shifts relative to external sodium dimethylsilapentane- l-sulphonate and assignments are listed in figure 4, using the numbers in the following structure.

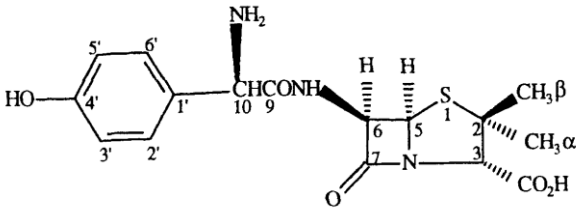
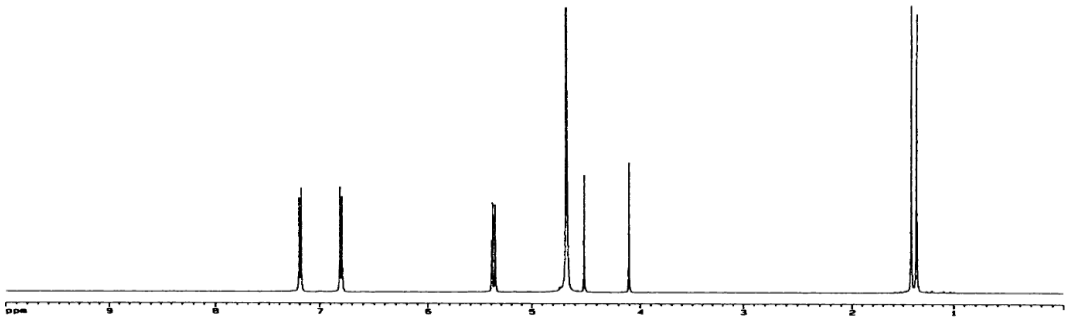


Figure 4. 400 MHz Proton NMR spectrum of amoxicillin in D_2O at pD 8



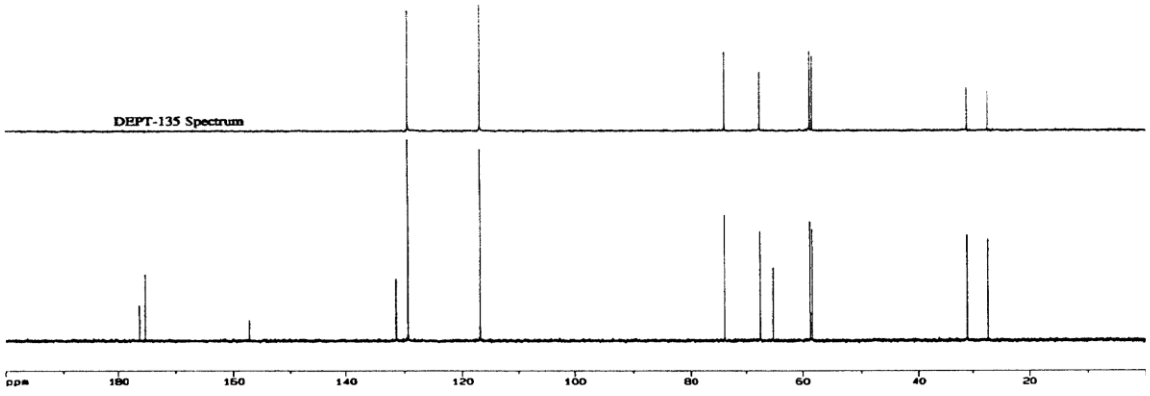
Chemical shift (ppm)	Multiplicity	Assignment
1.37	Singlet	α-CH_3
1.42	Singlet	β-CH_3
4.11	Singlet	3-H
4.52	Singlet	10-H
4.68	Singlet	HOD
5.36, 5.39	Quartet, J=3.9Hz	5-H,6-H
6.80; 7.19	Double doublet	3'-H,5'H; 2'-H,6'-H

The α- and β- methyl were assigned from Nuclear Overhauser effect experiments relative to 3-H. The HOD signal is due to exchange of the water, carboxy, amine, amide, and phenol protons. The chemical shifts are pH dependent. In D_2O at the natural Pd [42], or with addition of a small amount of DCI [37] or trifluoroacetic acid, the 5-H and 6-H protons were not separated and the methyl signals were less well separated than at pD 8.

Carbon - 13 Nuclear Magnetic Resonance Spectrum

The 100 MHz C- 13 spectrum of amoxicillin trihydrate in D_2O at pD 8 is shown in Figure 5. The lower trace is the full proton decoupled spectrum and the upper trace is the DEPT 135 spectrum in which signals from the quaternary carbons are suppressed.

Figure 5. 100 MHz C-13 NMR spectrum of amoxicillin in D_2O at pD 8



Chemical shift (ppm)	Assignment
27.2	α-CH_3
31.1	β-CH_3
58.3	C10
58.7	C6
65.0	C2
67.4	C5
73.9	C3
116.8	C3', C5'
129.4	C2', C6'
131.4	C1'
157.3	C4'
175.2	C7 and COOH
176.3	C9

The assignments are based on 2D COSY and COLOC experiments, as well as the chemical shifts and results of the DEPT spectrum. All the assignments, except C7 and C9, which are interchanged, are consistent with published values for amoxicillin trihydrate dissolved in dimethyl sulphoxide [43], despite some chemical shift differences due to solvent effects.

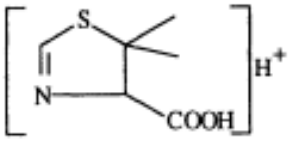
Solid state C-13 NMR spectra of amoxicillin trihydrate and other penicillins have been used to compare thiazolidine ring conformations in the solution and in the solid state [44]. The results indicated rapid interconversion between the S out of plane and the C3 out of plane conformations in solution, with an equilibrium ratio of 74 to 26 for solutions of amoxicillin sodium salt.

Mass Spectrum

The positive and negative ion spectra obtained from amoxicillin trihydrate by the fast atom bombardment ionization technique with a glycerol matrix are shown in Figures 6 and 7 respectively. The glycerol ions have been subtracted. Protonated and deprotonated pseudomolecular ions are seen at 366 and 364 m/z in the positive and negative ion spectra respectively. Significant fragments in the positive ion spectrum arise by loss of the amino group (349) with cleavage across the amide (134), cleavage across the β-lactam (160) and α- to the amide (122). The only significant fragment in the negative ion spectrum, at 223, comes from a complex cleavage of the S-C_2, C_5-N and C_6-C_7 bonds. These fragmentations occur with other penicillins [45]. The structures of the fragment ions are:

349 $[M - NH_2]^+$

223 $[p\text{-}OHC_6H_4CH(NH_2)CONHCH{=}CHS]^-$

160 

134 $[p\text{-}OHC_6H_4CHCO]^+$

122 $[p\text{-}OHC_6H_4CHNH_2]^+$

Figure 6. Positive ion mass spectrum of amoxicillin trihydrate

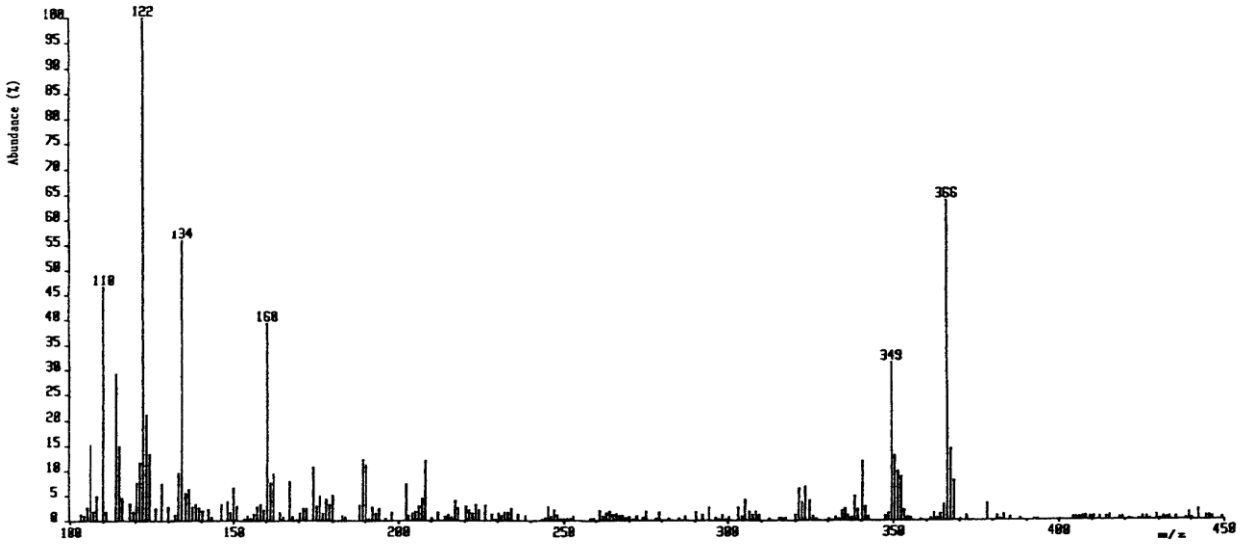
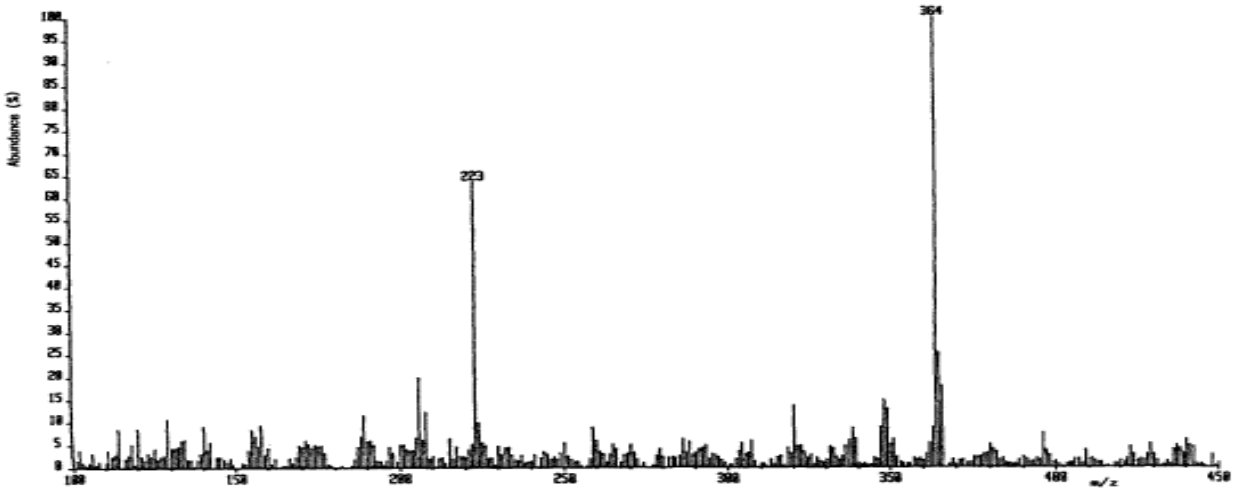


Figure 7. Negative ion mass spectrum of amoxicillin trihydrate



Amoxicillin trihydrate does not give a useful mass spectrum by the conventional electron impact ionization technique. Even with a specialized in-beam procedure, the technique gave spectra from several other penicillins [46], Laser desorption [47] and desorption chemical ionization [48] both gives pseudo-molecular ions and the latter technique also gives significant fragmentation.

Synthetic Routes

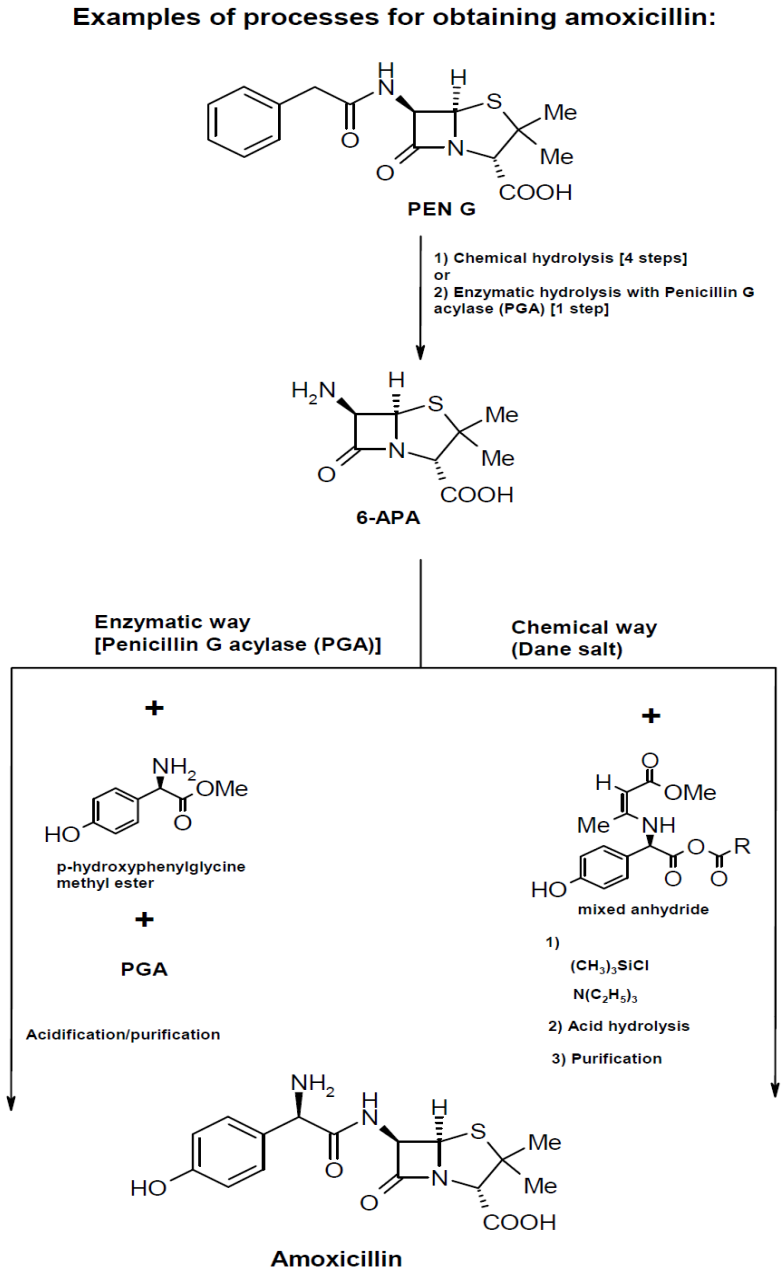
In terms of the industrial production described in the literature, the processes for obtaining amoxicillin trihydrate from the key intermediate 6-APA are chemical or enzymatic schemes [49]. The 6-APA is obtained from Penicillin G (PEN G/benzyl-penicillin) after breaking the amide bond [-CONH-] using enzymatic or chemical methods, as shown in Figure 8. Numerous patents also exist and describe different routes of synthesis, or variations on the existing synthesis of amoxicillin.

The conventional methods (using Dane salt for chemically obtaining) amoxicillin 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 non-recyclable waste.

In contrast, enzymatic methods 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.

Figure 8. Synthesis of amoxicillin through enzymatic and chemical routes



The literature also describes "one pot" trials for obtaining amoxicillin directly from PEN G. Numerous synthetic routes for sodium amoxicillin from amoxicillin trihydrate are described in the literature (e.g. treatment with sodium hydroxide, sodium 2-ethylhexanoate, or sodium diethyl oxaloacetate).

Different routes for the preparation of amoxicillin are also reported [46, 50, 51].

Limitation of Yield and Contamination Control During Synthesis

Semi-synthetic β-lactam antibiotics are the most prescribed class of antibiotics in the world. Chemical coupling of a β-lactam moiety with an acyl side chain has dominated the industrial production of semi-synthetic β-lactam antibiotics since their discovery in the early 1960's. Enzymatic coupling of a β-lactam moiety with an acyl side chain can be accomplished in a process that is much more environmentally benign but also results in a much lower yield. The lower yield in the enzymatic synthesis can be attributed to the fact that the enzymes that catalyze the reaction, Penicillin G Acylase (PGA) or α-amino ester hydrolase (AEH), have the ability to catalyze the undesired primary hydrolysis of the side chain acyl donor and the secondary hydrolysis of the antibiotic, in addition to the desired synthesis reaction.

The enzymatic synthesis of β-lactam antibiotics can be improved via reaction engineering, medium engineering, and data-driven protein engineering.

The one-pot, one-step system, a batch process in which all substrates and enzymes are present at the beginning of the reaction, achieved 39% yield, but could be advantageous due to its operational ease and faster cycle times.

The one-pot, two step system, in which the PGA catalyzed hydrolysis of penicillin G was allowed to proceed prior to the addition of the acyl donor and AEH catalyzed synthesis, achieved 47% yield. Secondary hydrolysis was minimized by optimizing relative enzyme loadings.

Both configurations present a promising alternative to the current two-pot set-up, which requires intermittent isolation of the intermediate, 6-aminopenicillanic acid (6-APA). Medium engineering is primarily of interest in β-lactam antibiotic synthesis as a means to suppress the undesired primary and secondary hydrolysis reactions.

The applicability of PGA and AEH for the synthesis of semi-synthetic β-lactam antibiotics has been explained. It was shown that the two enzymes could be combined in a novel one-pot cascade, which had the potential to eliminate an isolation step in the current manufacturing process. Furthermore, the previously reported *exsitu* mixed donor synthesis of ampicillin for PGA could also occur *insitu* in the presence of a suitable side chain acyl donor and co-solvent. Finally, significant progress has been made towards obtaining a selective PGA that was capable of synthesizing diastereomerically pure semi-synthetic β-lactam antibiotics from racemic substrates [52].

Contamination Caused by Solvents

Traditionally, amoxicillin is prepared from penicillin G or penicillin V, following complex chemicals conversions that involve the intermediate formation of 6- amino penicillanic acid. This requires hazardous and environmentally harmful chemicals such as butyl acetate, dichloromethane, dimethyl acetamide, isopropanol, and/or pyridine. Furthermore, auxiliary chemicals such as pivalic acid and triethylamine, end up as unwanted contaminants in the final product. Dichloromethane, a well-known contaminant, may often occur in ranges from 1000 to 5000 ppm [53].

Impurity Profile

Amoxicillin is a widely used semi-synthetic penicillin. The high molecular weight impurities (polymers) of amoxicillin are generated easily during production and storage of the material. Some RP-HPLC methods and HPLC-MS techniques have been reported in the literature. For routine analysis it is difficult to judge the polymer peaks without using the reference substances. However, the high molecular weight impurity peaks could be easily confirmed by a gel filtration chromatography (GFC) system. A novel GFC system based on the interaction between cephalosporins and SephadexG-10 has been developed, but there are no GFC methods reported using an amoxicillin polymer control. One study used three GFC methods to examine the separation of high molecular weight impurities in amoxicillin. The result showed that GFC was a rather simple separation mode as compared to RP-HPLC. Separation on the super-dex peptide column was advantageous for determining amoxicillin polymers over other gel filtration chromatographic methods [54].

Degradation Products

Similar to other penicillins, amoxicillin hydrolyses in aqueous alkali to a penicilloic acid, V. This has been isolated and characterized as the monosodium salt [55], which was subsequently shown to have retained the 5R configuration of the parent penicillin. Epimerization of the penicilloic acid in aqueous solution has been studied [56] and shown to occur at C5, rather than at C6 as stated, without evidence [57]. Penicilloic acid can also be obtained by hydrolysis with β-lactamases.

Degradation of amoxicillin in aqueous solution containing phosphate sorbitol and zinc sulfate or diethanolamine and zinc sulphate [58] gave a product which was assumed to be the piperazine-2, 5-dione, VI, from its spectroscopic properties, and by analogy with ampicillin degradation. This was subsequently confirmed by characterizing the isolated product, which was shown to form in significant amount on degradation of concentrated aqueous solutions of amoxicillin sodium salt. The epimerization of VI is analogous to that of the penicilloic acid [59]. The facile epimerization of these

compounds is characteristic of substituted thiazolidines which are not N-acylated. This is significant for HPLC analysis because it produces diastereoisomers, which may be resolved as separate components, thus giving a misleading impression of the number of different structural types present.

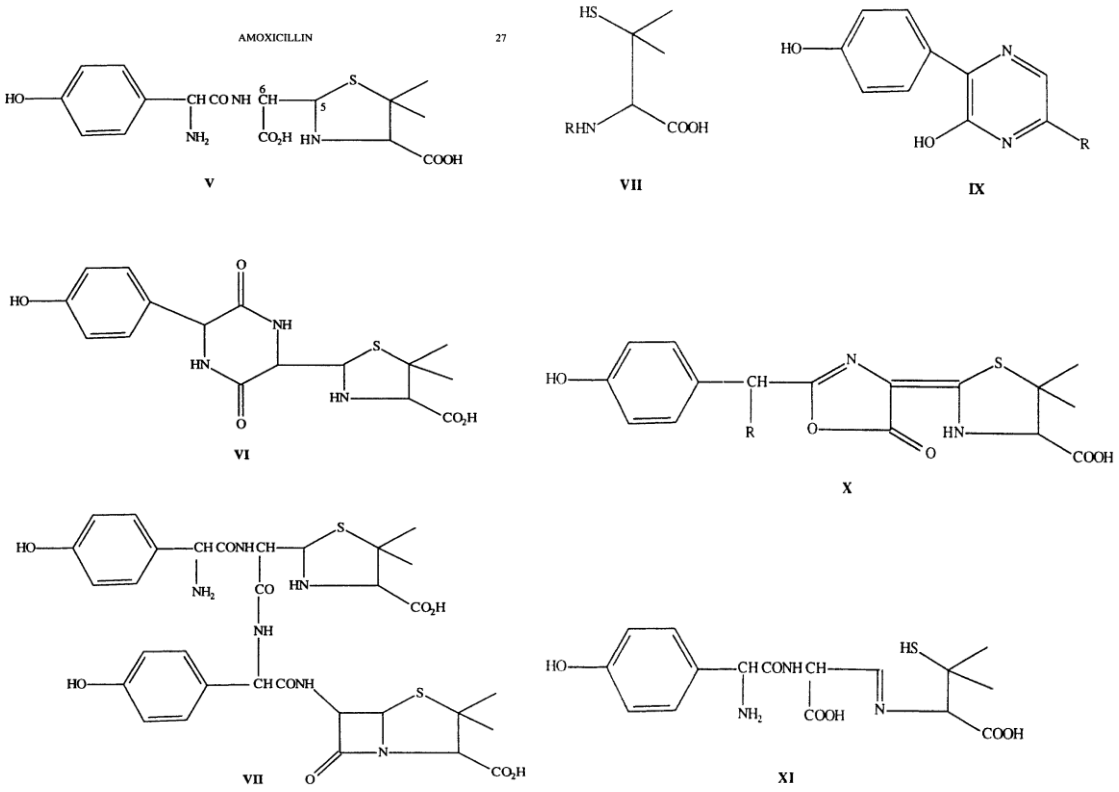
Amoxicillin dimer, present as an impurity in amoxicillin sodium salt API, was isolated and characterized as VII [60]. A more detailed characterization, together with data on amoxicillin trimer, was given in both compounds that were isolated from an aged 20% solution of Amoxicillin sodium salt. Chromatographic analysis also indicated the presence of a tetramer and the penicilloates corresponding to these three oligomers.

Little information is available about the structure of degradation products formed in neutral or acidic solution or in the solid state. The penicilloic acid, small amounts of penicillamine (VIII, R=H), and a pyrazine (assumed to be M, R=CH_3, but see below) were found [61] under gastrointestinal pH conditions, 1.5 increased stepwise to 8. A small amount of N-formylpenicillamine (VIII, R=CHO) was formed at pH 7 [62].

A fluorescent compound which slowly formed in amoxicillin solutions at pH 1 to 4 was identified as the pyrazine, IX, R=CH_3. However, the identification, which was subsequently confirmed [63], was done on a product formed by degradation in the presence of added formaldehyde. Identity of this material with the product from degradation of amoxicillin alone was established only by thin layer chromatography. However, the methyl group in the analogous pyrazine from ampicillin has been proven to come from the formaldehyde [64]. Furthermore, ampicillin without formaldehyde gives a pyrazine with H in place of the methyl [65]. Consequently, the pyrazine reported as being formed from amoxicillin in the absence of formaldehyde is probably M, R=H rather than M, R= CH_3. This is supported by the finding of an HPLC peak in amoxicillin extensively degraded at pH 2 to 7 with the same retention time and UV spectrum as an authentic sample of IX, R=H [66]. Other peaks detected in this study were assigned to amoxicillin penicillenic acid (X, R=NH_2) and amoxicillin penamaldic acid (XI).

Solid state degradation of amoxicillin trihydrate and sodium salt gave qualitative increases in the piperazine-2, 5-dione (VI) and in unidentified peaks which were tentatively ascribed to amoxicillin oligomers and their penicilloates [67]. Amoxicillin penicilloic acid increased in the trihydrate but not in the sodium salt.

Figure 9. Degradation products



Degradation products may be present as impurities in amoxicillin trihydrate and sodium salt APIs. Several samples of the trihydrate API contained the penicilloic acid and some also contained the piperazine-2, 5-dione [68]. Both these compounds were present in samples of the sodium salt API, which also contained the dimer [69].

Investigation of a New Amoxicillin Sodium Impurity Unstable in Solution

A study investigating new amoxicillin sodium impurity has been reported [59]. It was detected by reversed-phase HPLC in commercial injectable preparations only when examined very soon after the drug was dissolved in the solvent vial (within about 10 min). The stability of this impurity was investigated by the degradation kinetic of its aqueous solutions. Ionspray mass spectrometry with flow-injection analysis and HPLC-MS methods were used to establish its nature. Some hypotheses concerning its chemical structure were formulated. The most likely assumption referred to the (5S,6R) amoxicillin piperazinedione diasteroisomer. The presence of the amoxicilloic acid methyl ester, an intermediate of the Amoxicillin degradation process, was also hypothesized [70].

Stability Studies

Stability in Aqueous Solution

Kinetic studies of the degradation of amoxicillin in dilute aqueous solution (0.4 to 4 mg/ml) have been carried out over the following pH range: 1 to 10 at 35°C [69], 1.1 to 10.8 at 35°C [59], 8.2 to 12.6 at 35°C [62], 2 to 7 at 30, 40, 50 and 60°C [71], and 1.5 to 9 at 37°C [68]. At constant pH, degradation followed first order or pseudo first order kinetics, with a minimum rate at about pH 6 [70]. Degradation was subject to general acid base catalysis by citrate and phosphate buffers with a 10-fold increase in rate being ascribed to phosphate in one study. Increasing ionic strength was reported to have a positive effect on degradation rate in alkali and a negative effect in acid [35]. Degradation at higher amoxicillin concentrations (25 to 125 mg/ml) at pH 8.6 to 10 and 35°C gave non first order kinetics, indicative of a dimerization reaction [48]. Interpretation of the results indicated that dimerization proceeds through attack by the NH_2 group of one molecule on the 3-lactam carbonyl of another and is subject to general base catalysis by the NH_2 and phenolic O^- groups in other molecules. The rate of dimerization of amoxicillin at pH 9 is greater than that of other amino penicillins at least partly due to this effect of the phenolic group [71].

The stability of amoxicillin sodium salt has been studied in various intravenous infusion fluids. Degradation was faster at the higher amoxicillin concentrations and particularly in fluids containing dextrose, dextran, or sorbitol [72]. Other studies also showed a deleterious effect of carbohydrates and alcohols on the stability of amoxicillin in solution.

Stability in the Solid State

Kinetic studies of the degradation of amoxicillin trihydrate and sodium salt APIs in sealed containers at 37, 50, 80, 90, 100, and 110°C have been reported [73]. Results for the sodium salt were interpreted as indicating a sequential two step degradation. The trihydrate showed first order kinetics at 37 and 50°C but at higher temperatures its degradation rate was consistent with formation of a solid plus a gas.

Results consistent with sequential two step degradation were found for both amoxicillin trihydrate and sodium salt APIs in open containers at 80 to 140°C [35]. The same author found that under controlled humidity conditions, degradation was first order at 23 to 90% relative humidity (RH) and 64 to 90°C for trihydrate and at 50 to 90% RH and 40 to 70°C for sodium salt. Although, at 23% RH, sequential reactions occurred with the sodium salt [35]. The logarithm of the first order rate constants at a fixed temperature increased linearly with RH or with the logarithm of the vapor pressure confirming the importance of water for the degradation of these compounds.

Methods of Analysis

It is common knowledge that the polymers of β-lactam antibiotics are important with respect to the quality of the product.

For quality control purposes, the separation of amoxicillin and its related substances containing dimer and trimer amoxicillin is recommended to be done by a gradient elution on a C18 column. The identification of the polymer peaks in comparison to their relative retention times between amoxicillin and polymers are described in the BP/USP/EP. Their profiles are as given below.

Literature Review on HPLC High Performance Liquid Chromatography Methods

Bird et al. summarized HPLC methods which have been published until 1994 for the analysis of amoxicillin, its impurities, and degradation products in drug substances and formulated products. Most of these methods use the reversed phase mode on C18 columns, with UV detection and a mobile phase containing a small amount of methanol or acetonitrile in phosphate buffer at pH 4 to 6. More complex conditions, including ion pairing and post column derivatization, have been used in a few studies. But simpler conditions were also used in the few methods for normal assay purposes. A vast number of methods have been reported since the Bird et al. review. These are listed in Table 6 [74].

Pharmacopeial Methods

The pharmacopoeias (USP/EP/BP) give an HPLC method for assay of the drug substance for both the trihydrate and sodium salt monograph [32, 36].

IP: IP2014 only gives the method for Assay by HPLC; test for related substances is not therein.

BP/EP gives the similar HPLC method for the determination of related substances and assay for amoxicillin sodium and trihydrate form.

In BP/EP, the limits are same for assay and related substances but different in USP40 [36] . Refer to table 7 given below.

Table 6. HPLC methods for amoxicillin, its impurities, and degradation products

Sample type	Method type and mobile phase	Comments	Ref.
Assay of amoxicillin content			
API, FP	RP/C18/UV MeCN/pH 5.0 PO_4	USP method	[75]
FP	RP/C18/UV MeOH/ pH 4.4 PO_4	USP, with clavulanate	[75]
API, FP	RP/C18/UV MeOH/1.25%Ac	Heat degraded samples Correlation with bioassay	[76]
API, FP	RP/C18/UV MeOH/ pH 6.0 PO_4	With clavulanate which is assayed simultaneously	[77]
API, FP	RP/C18/UV & EC MeOH/H_2O/Ac	UV preferred to EC for routine assay	[78]
API, FP	RP/C18/UV MeCN/pH 4.1 PO_4	Penicillines in presence of degraded products	[79]
API, FP	RP/C8/UV MeOH/ pH 5.0 PO_4	Comparison with chemical methods	[80]
API,	RP/C18/post column NaOH/HgCl/EDTA/UV MeOH/PO_4	Method for 6 penicillins	[81]
API	RP/C18/UV MeOH/ pH 2.5 PO_4	To measure hydrolysis rate, pH 2 to 7.	[82]
API	RP/C18/UV MeOH/ pH 6.5 PO_4	Stability in simulated gastric juice	[83]
API	RP/C18/IP/UV MeCN/Bu_4NOH/PO_4	Stability in intravenous solutions	[84]
API	RP/C8/gradient/UV MeOH/ pH 7.0 PO_4	Penicilloic, DKP and oligomers	[85]
API	RP/C8/gradient/UV MeCN/PO_4	Penicilloic, dimer and polymers	[86]
API	RP/C18/UV MeOH/ PO_4	Penicilloic measured, other degradation products detected	[87]
API	RP/C18/gradient/UV MeOH/PO_4	Solid state stability, qualitative method for degradation products	[88]
FP	RP/C18/gradient/UV 2 columns in series MeOH/MeCN/PO_4	Penicilloic,6-APA and HPG	[89]

Sample type	Method type and mobile phase	Comments	Ref.
Miscellaneous studies			
API	RP/C8/UV MeOH/ pH 7.0 PO_4	Resolution from the side chain diastereoisomer	[90]
API	RP/C18/IP/UV and RP/Ph/UV various mobile phases	Investigation of retention mechanism	[91]
API	RP/C18/UV i-PrOH/pH 7.25 PO_4	Effect of temperature on separation of 6- penicillins.	[92]
API	RP/C18,CN and Polymer/UV NP/SiO_2/UV Various mobile phases	Comparison of different column types	[93]

API – Active Pharmaceutical Ingredient; FP – Finished product

Table 7. The pharmacopeial limits for assay of amoxicillin

		IP 2014		USP	BP	EP
Assay for active pharmaceutical ingredient						
Amoxicillin (on anhydrous basis by HPLC)		85.0-100.5%		90.0-100.5%	89.0-102.0%	89.0-102.0%
Amoxicillin trihydrate (on anhydrous basis by HPLC)		95.0-100.5%		90.0-100.5%	95.0-102.0%	95.0-102.0%
Assay for finished product						
Amoxicillin capsules		90.0-110.0%		90.0-120.0%	92.5 to 110.0%	NA
Amoxicillin tablets for oral suspension (Dispersible Tablets)		90.0-120.0%		90.0-110.0%	NA	NA
Amoxicillin oral suspension (by HPLC)		90.0-120.0%		90.0-120.0-%	80.0-120.0%	NA
Amoxicillin and potassium clavulanate injection	amoxicillin clavulanic acid	90.0-107.5% 90.0-107.5%		NA	NA	NA
Amoxicillin and potassium clavulanate oral suspension	amoxicillin clavulanic acid	90.0-120.0% 90.0-125.0%	amoxicillin clavulanic acid	90.0-120.0% 90.0-125.0%	80.0-120.0%	NA
Amoxicillin and potassium clavulanate tablets	Amoxicillin clavulanic acid	90.0-120.0% 90.0-120.0%	Amoxicillin clavulanic acid	90.0-120.0% 90.0-120.0%	90.0-105.0%	NA
Amoxicillin injection (By HPLC)		90.0-120.0%		90.0-120.0%	95.0-105.0%	NA

Table 8. Assay of related substance and degradation products

Related Substances	USP	BP		EP	
Amoxicillin (by HPLC)	NA	Impurity J	NMT 3.0%	Impurity J	NMT 3.0%
		Any other impurity	NMT 2.0%	Any other impurity	NMT 2.0 %
		Total impurities	NMT 9.0%	Total impurities	NMT 9.0 %
Amoxicillin Injection (by HPLC)	NA	Amoxicillin dimer	NMT 3.0%	NA	
		Any other secondary peak	NMT 2.0%		
		Sum of the areas of all the secondary peaks	NMT 9.0%		
Amoxicillin trihydrate (by HPLC)	Individual impurities: **see Impurity Table 9***	Any other impurity	NMT 1.0 %	NA	
	Total impurities: NMT 5.0%				
Amoxicillin capsules	No Test for RS	Any secondary peak	NMT 1.5%	NA	
		Area of not more than one secondary peak	NMT 1.0%		
		Any other secondary peak	NMT 1.0%		
Amoxicillin and potassium clavulanate oral suspension	NA	Amoxicillin dimer	NMT 2.0%	NA	
		Any other secondary peak	NMT 1.0%		
Amoxicillin and potassium clavulanate tablets	NA	Amoxicillin dimer	NMT 2.0%	NA	
		Any other secondary peak	NMT 1.0%		

Table 9. Impurity table*

Name	Relative retention time	Acceptance criteria NMT (%)
Amoxicillin related compound I[a] (D-hydroxyphenylglycine)	0.32	1.0
Amoxicillin related compound D[b,c] (amoxicillin open ring)	0.53	1.0
	0.68	1.0
Amoxicillin related compound A[d] (6-aminopenicillanic acid)	0.78	0.5
Amoxicillin related compound B[e,f] (L-amoxicillin)	0.87	--
Amoxicillin	1.0	--
Amoxicillin related compound G[9] (D-hydroxyphenyl glycylamoxicillin)	2.9	1.0
Amoxicillin related compound E[h,i] (amoxicillin penilloic derivative)	4.5	1.0
Amoxicillin related compound MI (N-(penicillin-6-yl) open ring amoxicillinamide)	6.0	1.0
Amoxicillin related compound F[e,k] (phenylpyrazinediol)	6.3	--
Amoxicillin related compound C[l] (amoxicillin rearrangement product)	6.4	1.0
Amoxicillin related compound E[h,i] (amoxicillin penilloic derivative)	6.7	1.0
Amoxicillin related compound J[m] (amoxicillin open ring dimer)	8.8	1.0
Amoxicillin related compound L[n] (N-(penicillin-6-yl) amoxicillinamide)	9.0	1.0
Any unspecified individual impurity	--	1.0

[a](R)-2-Amino-2-(4-hydroxyphenyl)acetic acid.

[b]The chromatographic system resolves two penicilloic acids from each other.

[C](4S)-2-{{(R)-2-Amino-2-(4-hydroxyphenyl)acetomido](carboxy)methyl)-5,5-dimethylthiazolidine-4-carboxylic acid.

[e]These compound are listed for information only and are not to be reported.

[l](4S)-2-{[R)-2-Amino-2-(4-hydroxyphenyl)acetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[l](2S,5R,6R)-6-(2-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]-2-((4S)-4-carboxy-5,5-dimethylthiazolidin-2-yl)acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

[k]3-(4-hydroxyphenyl)pyrazin-2-ol.

[l](4S)-2-[5-(4-hydroxyphenyl)-3,6-dioxopiperazin-2yl]-5,5-dimethylthiazolidine-4-carboxylic acid.

[m](2S,5R,6R)-6-((2R)-2-{2-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamidol]-2-{(4S)-4-carboxy-5,5-dimethylthiazolidin-2yl]actamidol}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]-heptane-2-carboxylic acid.

[n](2S,5R,6R)-6-{(2S,5R,6R)-6-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1azabicyclo{3.2.0]heptane-2-carboxamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

Recent Development for the Quantification of Amoxicillin

Fast and straightforward methods of determination are generally preferred because amoxicillin suffers degradation in solution. Therefore, sensitive and specific methods for the quantification in numerous matrices have been published for analysis of amoxicillin sodium/trihydrate and its formulations.

A few references reported in literature are:

- Development of analytical method for simultaneous estimation of amoxicillin and Carbocisteine in solid dosage form by RP-HPLC [64, 94]
- Fast HPLC-MS/MS method for determining penicillin antibiotics in infant formulas using molecularly imprinted solid-phase extraction [95]
- Advances in the determination of β-lactam antibiotics by liquid chromatography [96]

Gas Chromatography (GC)

Amoxicillin is too involatile and thermally unstable to be analyzed via gas chromatography without derivatization. Many attempts to prepare a silyl derivative for GC resulted in mixtures of compounds with different numbers of silyl residues. Successful GC of the methyl ester of the N-benzoyl derivative has been reported [35].

Bioanalytical Methods of Assay of Drug and its Metabolites

Detection of Metabolites

No antibacterially active metabolites of amoxicillin were detected in human urine by TLC with bioautographic detection using bacillus subtilis on agar gel [96].

Amoxicillin penicilloic acid V was detected in human urine by TLC with iodine vapor detection and identified by co-chromatography with the product of alkaline and β-

lactamase hydrolysis. The identification was confirmed by other workers using TLC and HPLC [64].

A new metabolite detected in human urine by HPLC was identified as amoxicillin penamaldic acid (xi) on the basis of spectroscopic investigations of material, obtained by *in-vitro* treatment of Amoxicillin penicilloic acid, which was shown to co-elute with the metabolite on HPLC [61]. More detailed UV and NMR studies on material prepared in the same way showed that the assignment of (xi) was incorrect and that the new metabolite was the 5S diastereoisomer of the penicilloic acid V produced by epimerization of the 5R isomer formed by hydrolysis of Amoxicillin [61]. The presence of both isomers in rat and human urine has recently been confirmed by high field proton NMR.

Amoxicillin piperazine-2,5-dione vi was identified as a metabolite in human urine by co-elution of an HPLC peak with an authentic sample and by NMR in rat and human urine [97]. The amount represented about 0.8% of the oral dose of Amoxicillin [98].

NMR signals indicating the presence of the amoxicillin dimer and amoxicillin carbamate (amoxicillin with NHCOOH instead of NH_2) were seen in some samples of rat urine [99]. The latter compound is believed to be formed by reversible reaction of amoxicillin with bicarbonate present at unusually high levels in some urine samples.

Assay in Biological Samples

Following the Bird et al. review, a vast number of HPLC methods for the determination of an assay of amoxicillin and its metabolites in biological fluids have been reported [35]. The methods are nearly all reverse phase chromatography, similar to assays of drug substance and formulated products. Although, methods with biological fluids more commonly use the ion pairing technique to aid with the separation from endogenous components. Also, pre- or post- column derivatization is quite widely used to improve sensitivity and/or separation from endogenous components [35].

Conclusion

Amoxicillin is a broad-spectrum antibiotic used for the treatment of a variety of bacterial infections including the treatment of bacterial pneumonia in children. Amoxicillin's potential for causing anaphylaxis type allergic responses in susceptible patients necessitates dedicated manufacturing equipment and manufacturing sites for its production. Additionally, monitoring and control of humidity during manufacturing and packaging operations presents manufacturing challenges, especially for some generic manufacturers that may have outdated facilities without the necessary air handling units or appropriate packaging equipment to effectively control for humidity. These manufacturing considerations, in addition to others described in this document, combined with a lack of guidance for bioequivalence and palatability, particularly for amoxicillin dispersible tablet formulation, represent significant barriers for amoxicillin manufacturers and directly impact the global supply and access to this life-saving product.

This product information report summarizes the available literature and provides expert scientific analysis of the physicochemical, biopharmaceutics, pharmacokinetics and toxicological properties, API synthesis, analytical, formulation, and manufacturing of amoxicillin. It is expected that the report will provide critical information and guidance to manufacturers, as well as stakeholders concerned with access and supply of priority essential medicines.

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