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Method Development and Validation of Amoxicillin Trihydrate by HPTLC in Bulk and Pharmaceutical Dosage Form

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Abstract: ***Bacterial Infections are diseases that affect your skin, lungs, brain, blood, and other parts of the body. Common symptoms of bacterial infection include-Fever, Chills, Fatigue, Headache, etc can occur, other symptoms include Redness of the skin, Ulcers, Diarrhoea, and Stomach pain produced by Bacteria, In such treatment Amoxicillin is the best therapeutic and efficacious treatment given in the last decades. Various method is used for the analytical separation of Amoxicillin by HPLC (High-Pressure Liquid Chromatography), and RP-HPLC (Revers Phase High-Pressure Liquid Chromatography) and provides good technical assistance, but current scenario the HPTLC method is used for the detection of many compounds. The HPTLC method is a powerful and accurate method for the detection of Amoxicillin Trihydrate, this method is very less costly and provides highly précised results. By HPTLC various Validation parameters are produced like Specificity, Robustness, Ruggedness, linearity, Accuracy, Precision LOD (Limit of Detection) and LOQ (Limit of Quantification) is precisely obtained by the HPTLC method.***

Keywords: HPTLC, HPLC, Amoxicillin Trihydrate, Validation, Antimicrobial

1. Introduction

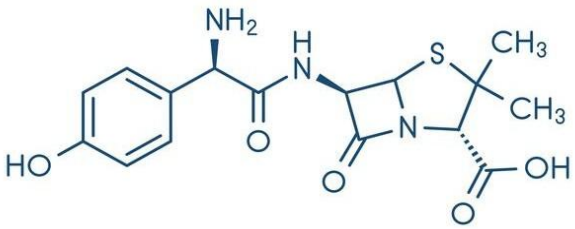
The term antibiotic was taken from the word "antibiosis" which means "against life". In the past, antibiotics were considered to be organic compounds produced by one microorganism which are toxic to other microorganisms (Russell, 2004). The first antibiotic, penicillin, which was first discovered and reported in 1929 by Alexander Fleming was later found to be among some other antibiotic compounds called the penicillins. (McGeer et al., 2001). The members of the Penicillin class include Penicillin G, Penicillin V, Oxacillin (dicloxacillin), Methicillin, Nafcillin, Ampicillin, Amoxicillin, Carbenicillin, Piperacillin, Mezlocillin and Ticarcillin (Boundless, 2016). Penicillin G was the first to be produced amongst this group of antibiotics, in fact of all antibiotics. Although penicillin G was discovered by Alexander Fleming in the 1920s, it took the efforts of several other workers such as Ernst Chain, Edward Abraham, Norman Heatley, and Howard Florey in 1945 to understand the cultural requirements of the fungus and its clinical effectiveness.

Amoxicillin Trihydrate

Amoxicillin the most commonly used antibiotics in the primary care establishment. It is an amino-penicillin, design by adding an extra amino group to penicillin, to fight antibiotic resistance. Amoxicillin covers a extensive variety of gram-positive bacteria, with some added gram-negative content compared to penicillin. Identical to penicillin, it covers major Streptococcus species and has be rectified coverage of Listeria monocytogenes and Enterococcus. It also has distribution over Haemophilus influenzae, few Escherichia coli, Actinomyces, Clostridial species, Salmonella, Shigella, and Corynebacteria.

Drug Profile

A semi-synthetic antibiotic, an analog of ampicillin, with a broad spectrum of bactericidal activity, against many Gram-positive and Gram-negative microorganisms Chemically it is **(2S, 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**. It may be represented structurally as:



amoxicillin

The molecular formula of amoxicillin is $C_{16}H_{19}N_3O_5S•3H_2O$, and its molecular weight is **419.45.**

Synonyms of Amoxicillin

Amox, Amoxicilina, Amoxicillin, Amoxicilline, Amoxicillinum, Amoxycillin, pHydroxympicillin

Brand Names

Augmentin 625, Moxatag, Prevpac, Amoxil, Clavum 625, Talicia, Voquezna 14 day Dualpak 20; 250; 500

Capsules:

Each capsule of AMOXIL, with a royal blue opaque cap and pink opaque body, contains 250 mg or 500 mg amoxicillin in the trihydrate form. The cap and body of the 250-mg capsule are printed with the product name AMOXIL and 250; the cap and body of the 500-mg capsule are printed with AMOXIL and 500. Inactive ingredients: D&C Red No.28, FD&C Blue No.1, FD&C Red No.40, gelatin, magnesium stearate, and titanium dioxide.

Tablets:
Every tablet contains 500 mg or 875 mg of amoxicillin as the trihydrate. Each film-coated, capsule-shaped, pink tablet is debossed with AMOXIL centered over 500 or 875, respectively. The 875-mg tablet is scored on the reverse side. Inactive ingredients: Colloidal silicon dioxide, cross povidone, FD&C Red No.30 Aluminum Lake, hypromellose, magnesium stearate, microcrystalline cellulose, polyethylene glycol, sodium starch glycolate, and titanium dioxide.

Powder for Oral Suspension:
Every 5 ml of reconstituted suspension contains 125 mg, 200 mg, 250 mg, or 400 mg amoxicillin as the trihydrate.

Mode of Action (MOA)
It acts by inhibiting the bacterial cell wall Synthesis, It is Penicillate sensitive and is effective against gram-positive organisms and gram-negative microorganisms but it is inactive against beta-lactamase-producing organisms. This is an analog of Ampicillin.

Penicillins and other beta-lactam antibiotics work by interfering with inter-peptides linking of peptidoglycans, it is a strong, structural molecule found specifically in bacterial cell walls. Cell walls without intact peptidoglycan cross-links are structurally weak and prone to collapse and disintegrate when the bacteria attempt to divide. The eukaryotic cells of humans do not have cell walls; Human cells are not damaged by penicillins.

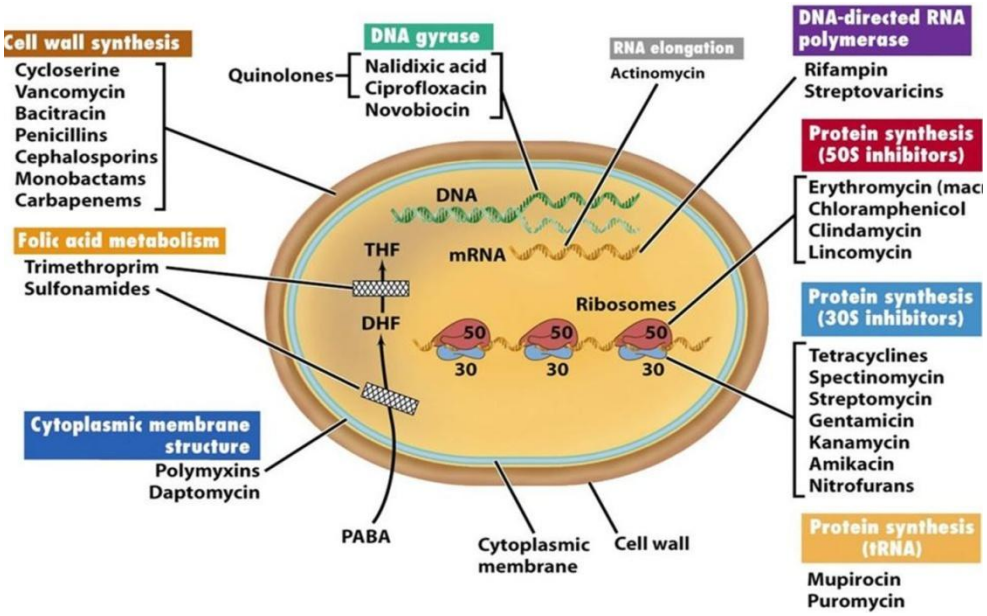


Figure: Mode of Action of various Penicillins

Current Indications of Amoxicillin
According to its current label, amoxicillin is indicated in the treatment of infections due to susceptible non-beta-lactamase-producing strains of the designated microorganisms in the following conditions: Infections of the ear, nose, and throat: due to Streptococcus species (alpha-beta-hemolytic strains only), S. pneumoniae, Staphylococcus species, or H. influenzae. Infections of the genitourinary tract: due to E. coli, P. mirabilis, or E. faecalis.

Infections of the skin and skin structure: due to Streptococcus species. (alpha-and beta-hemolytic strains only), Staphylococcus spp., or E. coli. Infections of the lower respiratory tract: due to Streptococcus species. (alpha- and beta-hemolytic strains only), S. pneumoniae, Staphylococcus spp., or H. influenzae. Gonorrhea, acute or uncomplicated genital, and urethral infections, due to N. gonorrhoeae (males and females). Amoxicillin is also recommended for use in the following conditions: Gastritis and peptic ulcer disease caused by H. pylori (as adjunct treatment agent-in combination with metronidazole and bismuth subsalicylate/macrolide) Lyme disease caused by B. burgdorferi Typhoid fever caused by S. typhi. Amoxicillin is used in combined form vonoprazan with clarithromycin as co-packaged triple treatment or in combination with vonoprazan a co-packed dual therapy to treat H. pylori infection in adults.

- Infection of Ear, Nose, and Throat infections
- Infection of the UTI
- Infection of the skin and skin structure
- Infection of the lower respiratory tract
- Treatment of gonorrhea and Anthrax
- Treatment of infective Endocarditis
- Treatment of Lyme disease

Contraindication

History of any Penicillin hypersensitivity is contraindicated. Kidney and Liver functions should be monitored.

Adverse Effect
- Nausea and Vomiting
- Diarrhea
- Serum sickness reaction
- Pseudomonas colitis
- Anemia and Urticaria

- Hypersensitivity vasculitis
- Steven-Johnsons syndrome.
- Toxic epidermal necrosis and Skin Rashes.

HPTLC (High-Performance Thin Layer Chromatography)

HPTLC (high-performance thin-layer chromatography) is a sophisticated form of TLC, which provides superior separation efficiency. The HPTLC concept includes validated methods for qualitative and quantitative analysis/and fulfills all quality requirements for use in fully regulated environments.

The HPTLC technique is an automated and sophisticated form of thin-layer chromatography with superior and advanced separation efficiency and detection limits and is often an exceptional alternative to high-performance liquid chromatography (HPLC) and gas chromatography (GC). High-performance thin-layer chromatography is also known as flat-bed chromatography or as planar chromatography.

The HPTLC technique is often used in the pharmaceutical sector for process development, adulterant detection in herbal goods, and standardizing herbal medications. Additionally, HPTLC has demonstrated that technology can assess pharmaceuticals without allowing auxiliary compounds to skew the results. For the estimate of numerous active ingredients like Losartan, acetaminophen, diclofenac, and famotidine, researchers have created accurate and user-friendly HPTLC methodologies throughout time. Confirming the presence of active ingredients (API) or excipients is the primary goal. For a quick parallel screening of several samples, HPTLC is an excellent choice. In about an hour, up to 10 samples may be examined. The created procedure is straightforward and uses a minimal amount of solvents and chemicals. Straightforward and uses a minimal amount of solvents and chemicals.

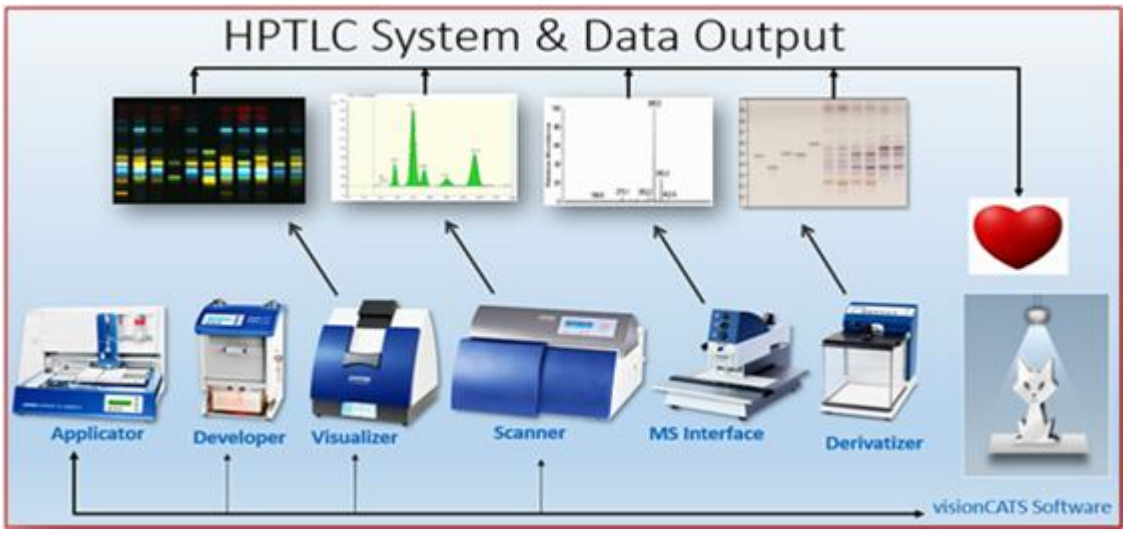


Figure: HPTLC device components

Principle

The HPTLC works on the same principles as TLC such as the principle of separation is adsorption. The mobile phase or solvent flows through the capillary action. The analytes move according to their affinities towards the stationary phase (adsorbent). The higher affinity component travels slower toward the stationary phase. A low-affinity component travels rapidly toward the stationary phase. On a chromatographic plate, then, the components are separated.

Applications of HPTLC

- High-performance thin-layer chromatography is used to analysis of molecules in both qualitative and quantitative terms.
- HPTLC can estimate the concentration of components although TLC can only separate components.
- HPTLC can analyze a complex structure or a very small amount of compounds.
- This method is used in the food industry to evaluate nutrients, beverages, vitamins, and pesticides in fruit, vegetables, and other foodstuffs.
- HPTLC is useful in the forensic detection of substances, including adulteration, overdose, counterfeit drugs, and drug misuse.
- To identify the substances including drug abuse, overdose, adulteration, and counterfeit drugs it is used forensic dept.
- HPTLC is used in pharmaceuticals for quality control.
- HPTLC is used for The analysis of forced degradation studies, stability testing, and checking the presence of impurities in the drug.

Advantage of HPTLC

- More than one analyst works on the system simultaneously.
- HPTLC can be sharable, as it is not devoted to any sample.
- The pre-coated plates of HPTLC are available at low prices.
- There is less maintenance cost as compared to other equipment.
- HPTLC has a wide range of stationary phases.

- HPTLC has no risk of contamination, since the use of the freshly prepared mobile phase and stationary phase.
- Mobile phases are not required for filtration and degassing such as HPLC.
- It is highly sensitive, reproducible, and precise as compared with a thin layer chromatography

Disadvantage of HPTLC

- Short separation bed is a major disadvantage of HPTLC
- A limited number of samples per plate can be tested.
- Sometimes silica gel is present during detection.

Experimental procedure for HPTLC

1) **Sample Preparation:**
   This requires a highly concentrated solution since much less sample quantity needs to be applied. The plate's solvents must be non-polar of the volatile type. Polar solvents are commonly used to dissolve samples for reversed-phase chromatography.
2) **Selection of Chromatographic Layers:**
   The layer of HPTLC is available in the form of very fine particle size silica gel pre-coats which is widely used as adsorbent.
3) **Pre Washing:**
   To water vapor or volatile impurities, the plates must be cleaned. It may be cleaned with a suitable solvent such as methanol.
4) **Conditioning:**
   Plates are placed in an oven at 120 ° C for 15 to 20 minutes to perform conditioning.
5) **Sample Application:**
   The size of the sample spot is not greater than 1 mm in diameter. There are various methods for spotting samples in HPTLC. One is a self-loading capillary in which small quantities of samples can apply on the HPTLC plate.
6) **Pre-Conditioning:**
   Saturation is necessary for highly polar mobile phases although there is no need for saturation for low polarity mobile phases.
7) **Mobile Phase of HPTLC:**
   Through trial and error, the mobile phase of the suitable solvents is to be selective.
8) **Chromatographic Development:**
   The linear development method in high-performance thin-layer chromatography is the most common technique here the plate is positioned vertically in an appropriate container with a solvent or mobile phase. The mobile phase is generally fed by capillary action and both sides may produce chromatograms.
9) **Detection of spot and Scanning:**
   The HPTLC instrument has attached to computer and data recording devices. The development of spots is viewed as peaks at wavelengths of selected UV regions. The height and the area of the peaks are determined by the instrument and recorded as a percentage.

Validation

Validation is the documented act of providing that any procedure, process, equipment, material, activity, or system leads to the expected results.

ISO defines validation as the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended are fulfilled.

According to the Food and Drug Administration (FDA), the goal of validation is "Establish documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specification and quality attributes. "

Needs of Validation

Before the introduction of a new method into routine use, When the condition change for which a method has been validated and when the method is changed, and the change is outside the original scope of the method.
To accept the individual sample as a member of a population under study, and
To admit the sample to the measurement process

Validation Parameters

Validation parameters defined by ICH guidelines are discussed below:

Accuracy

In analytical procedures, accuracy is defined as the closeness of agreement between true value and accepted value. The results of the accuracy study are expressed as percent recovery. The accuracy range between (98 %-102 %) is accepted.

Precision

Precision is an analytical procedure expressed as how closes the measurement to each other. In other words, precision is the description of random errors.

Precision may be considered at three stages:

Repeatability:

Repeatability shows the precision under the same operating conditions over short intervals of time, It is also termed as Intra assay precision.

Intermediate Precision

It expresses the precision within a laboratory, different days, different equipment, reagents, etc.

Reproducibility:

It explains the precision between different labs.

Specificity

Specificity is the capability to evaluate the analyte in the presence of components. which is expected to be present. Specificity is the algorithm used by browsers to determine the CSS declaration that is the most relevant to an element, which in turn, determines the property value to apply to the element.

$$\text{Specificity} = [d/ (b+ d)] \times 100$$

Limit of Detection and Limit of Quantification (LOD, LOQ)

Limit of Detection

The limit of detection (LOD) is the lowest concentration level of analyte that can be determined during an analytical run. . Typically, the LOD is determined to be the region where the "signal to noise ratio" is greater than 5. Chromatic limit detection is the injected amount that results in a peak with a height at least twice or three times as high a baseline noise level.

S/N= 2/1 or 3/1

Where, N= Noise, S= Signal

This may be calculated based on the standard deviation (SD) of the response and slope of the curve (S).

LOD= 3.3 (SD) /S

Where, SD = Standard Deviation, S = Slope

Limit of Quantification

LOQ is the parameter of quantitative analysis for the trace level of the compound in the sample matrix, which is quantitatively determined with precision and accuracy. LOQ is expressed as the concentration of the selective signal-to-noise ratio.

The quantification limit of chromatography is the injected amount of sample that results in a peak height 10 times greater than the baseline.

S/N= 10/1

Where, S = Signal N = Noise

It is calculated based on the standard deviation of the response vs. the slope of the curve (S).

LOQ = 10 (SD) / S

Where, SD = Standard deviation, s = Slope

Linearity

Linearity is the analytical process and it can obtain test results, which are directly proportional to the concentration of analyte in a sample. (In Linearity the correlation coefficient study must be, R>0.999).

Range

The range is the analytical process that is an interval between the upper and lower concentration of analyte in a sample.

Robustness

Robustness is the method of analytical science that measures the capacity to remain unaffected by small quantity, but intentionally variation in technical parameters an indication of its credibility during normal application.

Ruggedness

Ruggedness is the process to measure the degree of reproducibility of testing results found by analysis of the same sample beneath the discrepancy of normal test conditions in different laboratories. Testing of ruggedness normally, when the procedure is to be used in more than one laboratory.

2. Conclusion

This review concludes that the method development and validation of Amoxicillin trihydrate may be good because the HPTLC procedure is very reliable and cost-effective. This method is very sophisticated and easy for the detection of various compounds in the mixture or individual API. In the current scenario, the HPTLC method is very useful for the detection of various drugs with mobile phases.

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