A review on analytical method development and validation of amoxicillin.

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Abstract

Amoxicillin is a penicillin antibiotic to treat antibacterial infections including ear infection, strep throat, pneumonia, skin infection and urinary tract infection. The mechanism of action like absorption, distribution, metabolism and excretion mechanism of resistance. The analytical method for analysis of amoxicillin, high-performance liquid chromatography, chromatographic separation. A simple sensitive and rapid isocratic rp-hplc method has been developed simultaneous in estimation of Amoxicillin (AMX) in synthetic mixture, as per the other referred articles.

Keywords: Amoxicillin, HPLC, Antibiotic.

Introduction

Amoxicillin (α-amino-p-hydroxy-benzyl penicillin) was one of several semisynthetic derivatives of 6-aminopenicillonic acid. It was discovered in 1958 and Come into medical used in 1972. Amoxicillin is in the beta-lactam family of antibiotics.

Generic Name-amoxicillin, Brand Name-Amoxil, Drug class-penicillin, Amino.

It was to be effective against a wide range of infections caused by Gram tue and Gram-ue bacteria in both human and animals (Figure 1).

IUPAC name : (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.

Molecular formula: $C_{16}H_{19}N_3O_5S$

Molar mass: 365.40g/mol

Mechanism of action

Absorption

Amoxicillin is stable in presence of gastric acid and after oral administration it is rapidly absorbed. The effect of food on absorption of Amoxicillin from tables of AMOXIL has been 400mg and 875mg was partially investigated. Orally administered doses of Amoxicillin suspension is 125mg/5ml and 250mg/ml, result in average peak blood levels 1to 2 hours after administration in range of 1.5mch/ml to 3.0mcg/ml to 5.0 MCG/ml respectively. Single doses of 400mg chewable tablets and 400mg/5ml suspension of AMOXIL (Table 1).

Distribution

In blood serum, Amoxicillin is approximately 20% protein bound. Metabolism and excretion: The half-life of an orally administered dose of Amoxicillin is excreted in urine within 6 to 8 hours.

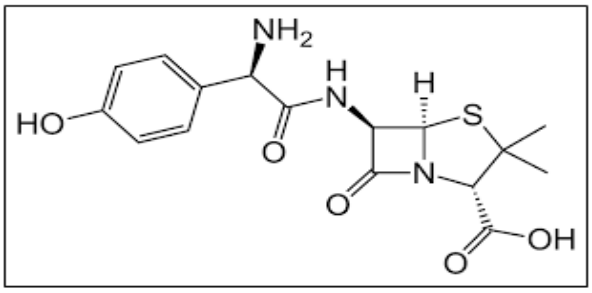

Figure 1. *Amoxicillin structure.*

Table 1. *Mean Pharmacokinetic Parameters of Amoxicillin (400 mg chewable tablets and 400 mg/5ml suspension) in Healthy Adults.*

Dose	AUC0-∞ (mcg.hr/mL)	Cmax (mcg/mL)
Amoxicillin	Amoxicillin (± S.D.)	Amoxicillin (± S.D.)
400mg (5ml of suspension)	17.9(2.4)	5.92(1.62)
400mg (1chewable tablet)	17.9(2.4)	5.18(1.64)

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- ✓ Mechanism Resistance: Resistance to Amoxicillin is mediated primarily through enzyme called beta-lactamases that cleave the beta-lactam ring of Amoxicillin, rendering it inactive. Inactiveness the Amoxicillin has been shown to be active against most isolates of bacteria.
- ✓ Gram Positive Bacteria
- ✓ *Enterococcus faecalis*
- ✓ *Staphylococcus spp*.
- ✓ *Streptococcus pneumoniae*
- ✓ *Streptococcus spp*. (Alpha and beta hemolytic)
- ✓ Gram negative bacteria
- ✓ *Escherichia coli*
- ✓ *Hemophilus influenzas*
- ✓ *Helicobacter pylori*
- ✓ *Proteus mirabilis* [1]

Literature survey

The HPLC method was developed and validated to measure simultaneously the amount of amoxicillin at single wavelength (254nm). The sample was injected into C18 column with buffered mobile phase (pH 4.0) and UV detection at 254nm. The linearity for concentration between 0.15 and 600µg/ml. The Intra and inter-day precision less than 2.5%. The Limits of Detection (LOD) and quantification were 0.05 and 0.15g/ml author conclude that sample were stable in the release media (37°c) and the HPLC injector at least for 12h [2].

The study was to develop and validate a simple and sensitive RP-HPLC method for simultaneously determination. The chromatographic system LC-10 AT VP Pump, SPD-10 AUP UV/Visible detector. Separation was achieved from Hibarpurospher Star RP-18e (5µm, 250x4.6mm) Column, a mobile phase Consist 0.02 M phosphate buffer: Acetonitnie (93:7 V/V, PH 3.0). Flow rate is detected 1.0ml/min. They conclude the retention time about 8min. at 230nm peak separation detected. Linear Concentration range is 0.15µg/ml to 20µg/ml. The author concludes that their method is accurate, precise and reproducible in desired range. The limit of detection was 0.039µg/ml and limit quantification was 0.078µg/ml respectively also this method used as analysis of amoxicillin in pharmaceutical dosage form, as well as this method can give help in pharmacokinetic study [3].

The RP-HPLC method was simple, accurate rapid, economical. Amoxicilin and probencide in combined tablet dosage form. The method is carried out by isocratic technique on a reversed phase in C18 Column (250x4.6mm, 5µm particle size) kept at ambient temperature. Mobile phase containing a mixture of buffer (pH 4.0) methanol (40-60%v/v). The flow rate is 0.1ml/min. Average retention time were 3.447min, linear concentration range is 5-15µm/ml. The mean assay was found to be 99.95 % [4].

The HPLC method was Rapid and sensitive drugs and it was developed for routine analysis of amoxicillin trihydrate in bulk drugs and pharmaceutical formulations. UV detection at 229nm chromatographic separation injected on a capacel Pak C18 type MG column. The mobile phase containing mixture of phosphate buffer and methanol (50:50, %V/V). Rate of flow is 1.0 ml/min and pH adjusted to 3.0. The author conclude that the method was validated for linearity (r^2=0.999), accuracy, precision, sensitivity and robustness testing [5].

The Hassouna mem and team developed a new RP-HPLC method and evaluated for determination of Amoxicillin (AMO) residues in Nicomas Coating machine using Batabasic-C18 (4.6mmx250mm) serum or equivalent. The mobile phase is a mixture of 0.05M sodium dihydrogen phosphate: methanol cgs.sv/v). The pH is adjusted to 4.4 with help of Orthophosphonic acid. The flow rate is 1.5ml/min UV detection at 230 nm and injection volume is 100µl. They conclude the retention time of AMO is 6.292 minutes and total run time is 7.0 minutes linear relationship range is obtained 0.03 to 6ppm or correlation coefficient of 0.9989. The author concludes that validation method according to ICH guidelines and USP requirements for new methods [6].

The stability indicating High Pressure Liquid Chromatography (HPLC) method and has been developed for estimation of Amoxicillin from injectable dosage form. The separation was obtained using a mobile phase composition at ratio of 95:5 (v/v) of pH 5.0 buffers and methanol on injected C18 column (250x4.0mm, 4µm) with UV detection at 220nm. The flow rate of Iml/minutes linear calibration range was 79.51 to 315. 32µg/ml. The author concluded that this method validation data show excellent result for precision, linearity, specificity, limit of detection, limit of quantification and robustness. This method is successfully used for routine quality control and stability studies [7].

The Atiah H Almalki and co. workers developed high-performance liquid chromatography mass spectrometry method for analysis of amoxicillin trihydrate. The chromatographic separation carried out by the sample injected on C18 Column (3.5 µmps, 100mmx4.6mm id) using Acetonitrile: water (65:35 by volume) as a mobile phase in min. The retention times were concluding 1.61min. The method is validated in linear range of 2-28µgml from result it suggest that HPLC/MS were statistically compared with those obtained from the reported HPLC method. The Author concludes that no significant difference appeared respecting accuracy and precision [8].

The objective of the work is to develop and validates an HPLC method. For the determination of the combination. This Combination of amoxicillin and enrofloxacin is a well-known mixture of veterinary drugs. The Nidal batrawi and co-workers was developed new Simple and efficient reversed phase HPLC method for determination of qualitative and quantitative of amoxicillin and enrofloxacin. It is an injectable preparation with a mixture of inactive excipient has been developed and validated. The method is performed by using reversed-phase (RP)-C18e (250mm x 4.0mm, 5µm) column at room temperature. The gradient mobile phase of acetonitrile and phosphate buffer contain methanol at pH 5.0,

a flow rate of 0.8ml/ min and UV detection at 267nm. This method is validated in accordance with the Food and Drug Administration (FDA) and the international conference on Harmonisation guidelines and showed excellent limeanity, accuracy, precision, specificity, robustness, ruggedness and suitability. The author concluded there method can also be used for purity and degradation evaluation of these formulations [9].

The method was developed for the estimation of Amoxicillin in bulk and pharmaceutical formulations. The sample is injected in hypersil C18 column (250x 4.6mm.I.D.particle size µm). The mobile phase contain mixture of potassium dihydrogen phosphate and methanol in ratio 95: 05 v/v. It pumped at a flow rate of 1.0ml/min. It was detected of 283nm. It response was linear in the concentration of 20-100µg/ml .The intra and inter-variation found by team less than 2%. Author concluded that the proposed method is simple, fast accurate, precise and reproducible. It can be applied for routine quality Control analysis of Amoxicillin in bulk and pharmaceutical formulations [10].

The High Performance Liquid Chromatographic (HPLC) method for the quantitation of amoxicillin in human plasma using cefadroxil as an Internal Standard (IS) has been developed and validated. The drug and the IS were eluted from symmetry C18 stainless steel column (5µm,150x4.6mm I.D) at room temperature with a mobile phase consist mixture of methanol: 75mM potassium dihydrogen phosphate buffer solution C10: 90, v/v) PH adjusted to 3.0 with phosphoric acid, at a flow rate of 1.5ml min/1. Ultra violet detection at 228 nm. Each analysis required no longer or more than 10 min. They concluded that the quantification was achieved by measurements of the peak area ratio of the drug of the internal standard, and the limit of quantification of ampicillin in plasma was 0.5µgml/1[11].

Conclusion

Amoxicillin is a pencillin antibiotic to treat antibacterial infections, including ear infection, strep throat, pneumonia, skin infection and urinary tract infection. The mechanism of action like absorption, distribution, metabolism of excretion mechanism of Resistance. The Analytical method for analysis of amoxicillin, high-performance liquid chromatography, chromatographic separation.

A simple, sensitive and rapid isocratic RP-HPLC Method has been developed for simultaneous estimation of Amoxicillin (AMX) in synthetic mixture also the run time under optimum chromatographic condition was found to be less than 7min as per referred articles.

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