

Research Article

SIMULTANEOUS ESTIMATION OF AMOXICILLIN, AMPICILLIN, PENICILLIN V POTASSIUM AND CLAVULANATE POTASSIUM IN REVERSE PHASE CHROMATOGRAPHY BY HPLC

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Abstract

A rapid sensitive reversed phase HPLC method has been developed for simultaneous residue determination of Amoxicillin, Ampicillin, Penicillin V Potassium and Clavulanate potassium from beta-lactum production effluent waste sample. This chromatographic separation of these four Active pharmaceutical ingredients was carried out on a Atlantis C18 reversed phase column (150 x 4.6 mm, 5 μ) with a simple gradient program for 15 minutes with mobile phase –A as 0.025M monobasic potas-

sium phosphate pH adjusted to 3.0 and mobile phase-B as Acetonitrile and mobile phase flow rate used constantly at 1.0 mL / minute. The chromatography analysis was monitored at 215nm with column oven temperature at 40° and injection volume as 100 μ L. All the components were separated with good resolution in less than 10 minutes. Validation of method showed it to be robust, precise, accurate and linear over a range of analysis.

Keywords: Liquid chromatography, Amoxicillin, Ampicillin, Penicillin.V.Potassium and Clavulanate potassium. Active pharmaceutical ingredients.

Introduction

Amoxicillin¹ is an analog of Ampicillin, derived from the basic penicillin nucleus, 6-aminopenicillanic acid. The amoxicillin molecular formula is $C_{16}H_{19}N_3O_5S \cdot 3H_2O$, and Chemically amoxicillin is (2S,5R,6R)-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 with molecular weight 419.45. Amoxicillin used in treatment of prevent infections that are in ear, nose, throat, genitourinary tract, lower respiratory tract and skin and skin structure. The Ampicillin trihydrate² is a semisynthetic penicillin derived from the basic penicillin nucleus, 6 Amino penicillanic acid. Ampicillin is designated chemically as (2S, 5R, 6R)-6-[(8)-2-Amino-2-phenylacetamido]-3,3-dimethyl-t-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid. The chemical formula is $C_{16}H_{19}N_3O_5S \cdot 3H_2O$ and its molecular weight is 403.45. Ampicillin is used for genitourinary or gastrointestinal tract infections other than gonorrhea in men and women. Penicillin V potassium¹ is a potassium salt form of penicillin v, is an semi synthetic antibiotic used to treat certain infections caused by bacteria such as pneumonia, scarlet fever, and ear, skin, and throat infections. It also is used to prevent recurrent rheumatic fever and chorea. Penicillin V potassium is designated chemically as Mono potassium (2S,5R,6R)-3,3-dimethyl-7-oxo-6-(2-phenoxyacetamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate. The chemical formula is $C_{16}H_{17}KN_2O_5S$ and molecular weight is 388.48. Clavulanate Potassium¹ is produced by the fermentation of *Streptomyces clavu-*

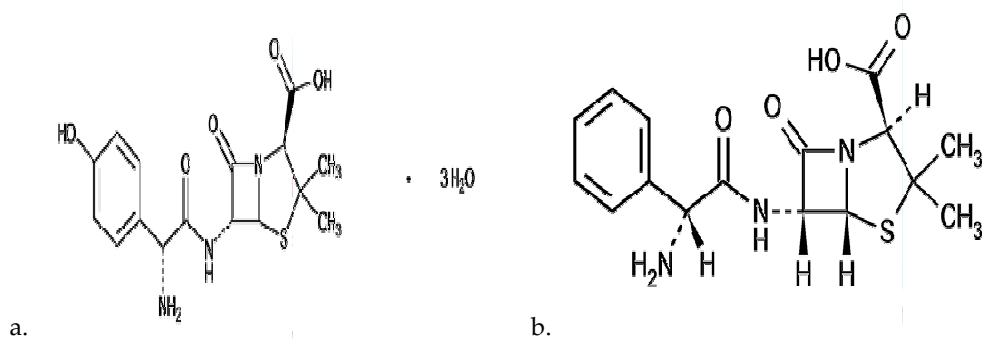
ligerus. It is a β -lactam structurally related to the penicillins and possesses the ability to inactivate a wide variety of β -lactamases by blocking the active sites of these enzymes. Clavulanic acid in clavulanate potassium is particularly active against the clinically important plasmid-mediated β -lactamases frequently responsible for transferred drug resistance to penicillins and cephalosporins. The clavulanate potassium molecular formula is $C_8H_8KNO_5$, and the molecular weight is 237.25. Chemically, clavulanate potassium is potassium (Z)-(2R,5R)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylate.

The structures of all the four drugs were shown in figure 1. All though penicillin does not have characteristic absorption their UV spectrometry emerged as a uniform analytical method for their determination. The British pharmacopeia (1980) recommends UV analysis of Oral suspension. The United States pharmacopeia and code of federal regulation permit the application of UV-VIS for several penicillin derivatives at particular wave lengths between 230nm and 280nm. Several authors have been reported HPLC methods for the analysis of these drug substances in single or combination. Estimated, Amoxicililn alone by HPLC ^{3,7} and by UV spectrophotometer ⁴, Ampicillin alone by UV spectrophotometer ⁵ and by HPLC ⁷, Penicillin V Potassium alone by HPLC ^{6,7} and Clavulanate Potassium alone by HPLC ^{6,7}. Estimated in combi-

nation, Amoxicililn and Ampicillin in Bovine milk ^{8,9,11} Cow milk ¹⁰ and all penicillins in penicillinase reactor and idometric determination ¹², by HPLC ¹³. A literature survey showed that no direct method have been previously reported for the analysis of these four drugs in combination, in Antibiotics production effluent waste which is connected to public drainage system. It is very important to prevent contamination of these antibiotic drugs in public sewage and Environment. The aim of the present study is the development of cost-effective, rapid, sensitive, specific and validated HPLC method for the determination of Amoxicillin, Ampicillin, Penicillin V potassium and Clavulanate Potassium in production effluent waste connected to public drainage system in 'ppm' level. Developed method was validated ^{14,16} for linearity, accuracy precision and robustness. The 215nm was selected for the simultaneous determination of these drugs.

EXPERIMENTAL

Chemicals and reagents: Active pharmaceutical ingredients Amoxicillin, Ampicillin, Penicillin V. Potassium and Clavulanate Potassium were provided by American Antibiotics Inc (Baltimore, USA). HPLC grade of Acetonitrile is from Acrose (New jersey, USA), Monobasic potassium phosphate is from Alfa Aesar (UK) and phosphoric acid is from Labchem (Pittsburgh, PA, USA). HPLC grade water was used for the mobile phase preparation.



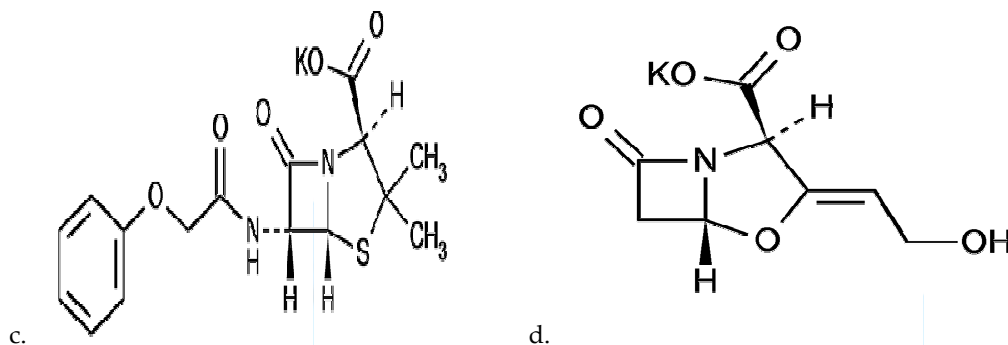


Figure 1. Structures of each Analytes a) Amoxicillin Trihydrate b) Ampicillin c) Penicillin V Potassium d) Clavulanate Potassium

Table 2. Linearity of the method

Parameter	Clavulanate potassium	Amoxicillin	Ampicillin	Penicillin V
Slope (b)	0.000104	0.000147	0.000138	0.000109
Intercept (a)	0.881216	0.303005	0.251523	1.018747
Correlation coefficient	0.9997	0.99998	0.99994	0.99967

Table 2. LOQ precision

Name	Clavulanate potassium	Amoxicillin	Ampicillin	Penicillin V
% RSD (n=6)	2.58	1.35	2.55	2.87

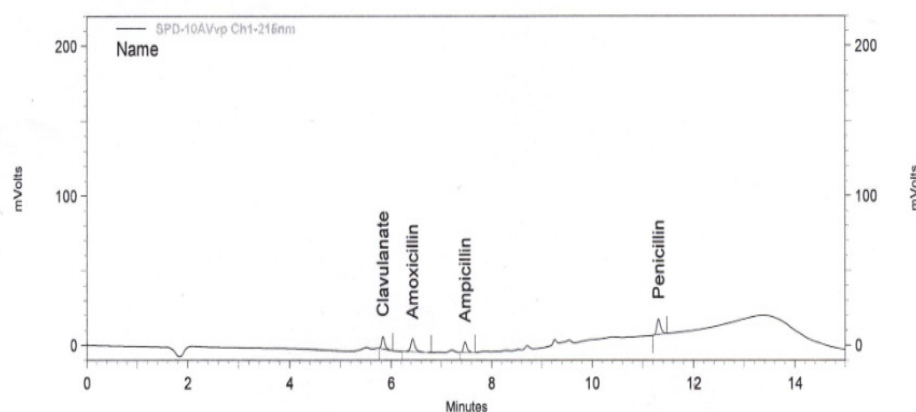


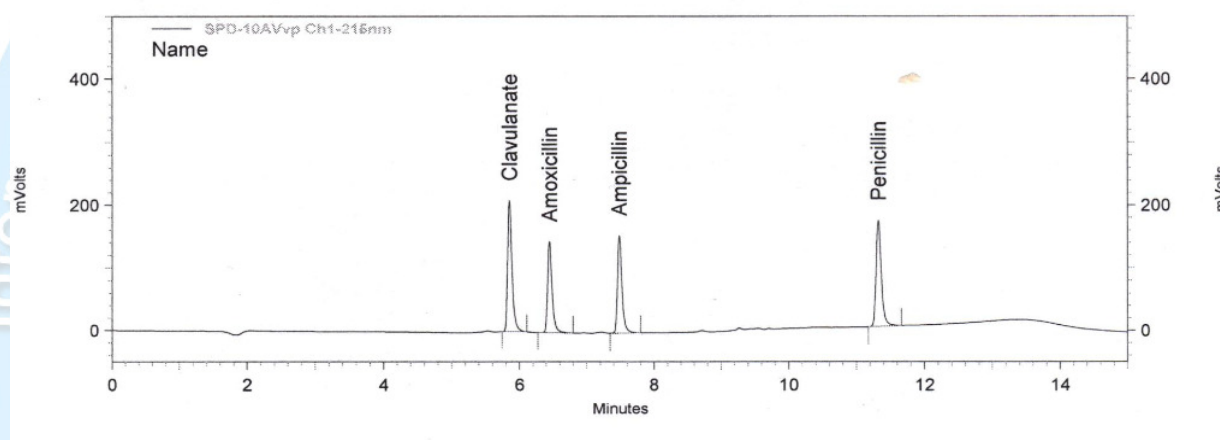
Figure-2 : Typical chromatogram at Limit of Quantification level

Table 3. Accuracy of the method

Drug name	mean Recovery	% RSD(n=15)
Amoxicillin	99.58	1.27
Ampicillin	99.63	1.20
Penicillin V Potassium	101.04	0.73
Clavulanate Potassium	100.28	0.87

Table 4. Precision of the method

Parameter	Clavulanate potassium	Amoxicillin	Ampicillin	Penicillin V
% RSD(n=6)	0.14	0.83	0.56	0.30

**Figure 3. Typical chromatogram for sample**

Samples and solutions: Standard solution preparation: Standard solutions at a concentration of about $5\mu\text{g} / \text{mL}$ of Amoxicillin, Ampicillin, Penicillin.V.Potassium and Clavulanate Potassium were prepared by dissolving the appropriate amount of standard in the water¹⁷. Standard stock solutions were stored at room temperature for 72 hours at most and their stability was checked over this period by daily preparing and injecting a diluted solution of each analyte. The working standard solutions at the concentrations in the linear range were prepared immediately before analysis by appropriate dilution of the stock solutions. Sample solutions were prepared by collecting the production waste drainage 5mL in every two hours interval for 24 hours, the representative 20 mL of sample taken and diluted to 100mL with water and used for regular testing¹⁸.

Equipments and experimental conditions: The HPLC system was Shimadzu LC-10 (Kyoto, Japan) equipped with quaternary gradient pump LC-10Avp, auto-sampler Sil-10Avp and a UV visible Detector SPD -10Avp was used to make sample injections¹⁹. A reversed phase Atlantis C18 column (150 x 4.6 mm i.d, $5\mu\text{m}$ particle size) was used for analysis column temperature at 40°C . The Mobile phase-A consisted of 0.025M potassium phosphate adjusted to pH 3.0 with orthophosphoric acid, Acetonitrile used as Mobile phase-B and delivered at 1.0mL/Min. The Sample injection volume used as $100\mu\text{L}$ and monitored at Detector wavelength was 215nm. The Pump Gradient program used is given in table 1.

Results and Discussion:

Linearity: Calibration curves were performed by analysis of working standard solutions of

Amoxicillin, Ampicillin, Penicillin V potassium and Clavulanate potassium with seven different concentration in the range of 0.5 ppm to 12.5 ppm. Each concentration was injected and measured in triplicate. The retention times of Amoxicillin, Ampicillin, Penicillin V potassium and Clavulanate potassium were approximately 6.5, 7.5, 11.3 and 5.9 minutes respectively. The Linearity of each component expressed with correlation coefficient of the calibration curve (r) for Amoxicillin, Ampicillin, Penicillin V potassium and Clavulanate Potassium are 0.99998, 0.99990, 0.99948 and 0.99997 respectively. The results as shown in Table 1 are within the concentration indicated there was an excellent correlation between peak area and concentration of each drug component.

Table: 1 List of Mobile Phase A & B with various concentration

Time	Mobile phase-A	Mobile phase-B	Curve
0.5	100	0	0
10	30	70	1
2	100	0	1
3	100	0	0

Limit of Quantification: The limit of Quantification (LOQ) was estimated from the signal to noise ratio (S/N). This limit was defined as the lowest concentration level that provided a peak area with signal to noise ratio higher than 10. The LOQ of Amoxicillin, Ampicillin, Penicillin V potassium and Clavulanate potassium were determined experimentally by analysis of six injection of each drug at LOQ concentration ²¹. The LOQ concentration of Amoxicillin, Ampicillin, Penicillin V potassium and Clavulanate potassium was selected as 0.1ppm, which is very low concentration and Signal to Noise ratio found more than 10. The precision of the method at LOQ found satisfactory with % RSD less than 2.9%, for details as shown in table-2.

Accuracy: Accuracy was verified by applying the standard addition method to synthetic mixture of diluent (99.5:0.5 DI water: IPA) to which known quantities of each drug substance corresponding to 10 % to 250 % of 2 ppm level added. The accuracy was expressed as the percentage of analytes recovered by assay. Mean recoveries are shown in Table

3. The results in table indicate that the method is highly accurate for simultaneous determination of the four drugs.

Precision: Method precision was determined from results of six independent determinations of 100% standard of Amoxicillin, Ampicillin, Penicillin V potassium and Clavulanate potassium spiked to tap water collected where effluent waste vent connected to public sewage. The relative standard deviation (RSD) of each peak response was < 0.8 % for the four drug substances. Results provided in Table-4, a typical Chromatogram for sample solution provided in figure 3.

Robustness: The robustness of a method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters. Since the described method was designed for future application in the routine analysis the robustness of the proposed method was evaluated by changing the pH of the buffer, Flow, mobile phase-B, temperature of oven, different columns. The most critical parameter was found to be the pH value that must be measured with an uncertainty not larger than ± 0.10 units to achieve good peak shapes and separation between Clavulanate potassium and Amoxicillin. Column to column and different temperatures $\pm 2.0^\circ\text{C}$ results indicated that there were no significant differences between the columns and the separation of the drug substances is achievable under the given conditions using the method developed.

Solution stability: The stability of standard and sample solutions was determined by monitoring the peak area ratio of solutions over a period of 24 hours. The results shown the standard is stable upto 18 hours and sample is stable upto 6 hours.

Method application: The HPLC method is rapid and sensitive for the quantitative determination of Amoxicillin, Ampicillin, Penicillin V potassium and Clavulanate potassium in Antibiotics production drainage waste or Production cleaning waste.

Conclusions: A cost-effective, rapid, specific gradient HPLC method has been developed for the simultaneous determination of Amoxicillin, Ampicillin, Penicillin V potassium and Clavulanate potassium using UV-VIS detector. The method has

validated for accuracy, precision, linearity, robustness and stability. The method uses a simple mobile phase composition, easy to prepare. The rapid run time of 15 minutes and relatively low flow rate (1.0mL/Minute) allows the analysis of large number of samples with less mobile phase that proves to be cost effective. Hence this UV-VIS method can be used for the routine production drainage waste samples in order to prevent contamination to county public sewage water per environment regulations.

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