ejpmr, 2020,7(9), 320-325

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

CHEMICAL FORCE DEGRADATION ASSAY METHOD EVALUATION FOR SIMULTANEOUS ESTIMATION OF AMOXICILLIN AND POTASSIUM CLAVULANATE IN ORAL DOSAGE FORM

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Article Received on 03/07/2020 **Article Revised on 23/07/2020** **Article Accepted on 13/08/2020**

ABSTRACT

A specific, precise, accurate ultra pressure liquid chromatography (UPLC) method is developed for estimation of amoxicillin and potassium clavulanate in bulk dosage form. The method employed, with Xterra RP-8 (150mm x 4.6 mm i.d., particle size 5 µm) in a gradient mode, with mobile phase of KH2PO4: Methanol (80:20). The flow rate was 0.5 ml/min and effluent was monitored at 248 nm.

During the stability analysis of the drug product, all known impurities were detected by the above stability-indicating method. No chromatographic interference from excipients and degradants were found. The proposed method was successfully used for estimation of amoxicillin and potassium clavulanate in bulk dosage form.

KEYWORDS: amoxicillin, potassium clavulanate, oral dosage form, UPLC method.

INTRODUCTION

Regulatory agencies recommend the use of stability indicating methods (SIMs) for the analysis of stability samples. This requires stress studies in order to generate the potential related impurities under stressed conditions, method development and validation. With the evident of the International Conference on Harmonization (ICH) guidelines, requirements for the establishment of SIMs have become more clearly mandated.[14] Environmental conditions including light, heat and the susceptibility of the drug product towards hydrolysis or oxidation can play an important role in the production of potential impurities. Stress testing can help identifying degradation products and provide important information about intrinsic stability of the drug product. Therefore, herein we report the results of stability study of amoxicillin and potassium clavulanate with the aim of determining the extent of the influence of different stress conditions on the stability of the dry powder inhaler product.

Therapeutic category	Anti-Infective Agents
CAS Registry number	26787-78-0
Chemical name	(2S,5R,6R)-6-[(2R)-2-amino-2-(4-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid
Molecular formula	“$C_{16}H_{19}N_3O_5S$
Molecular Weight	365.404
Solubility	“0.958 mg/mL
pka	3.23
λ_{max}	247 nm
Pharmacology	Amoxicillin is given with clavulanic acid to treat acute bacterial sinusitis, community acquired pneumonia, lower respiratory tract infections, acute bacterial otitis media, skin and skin structure infections, and urinary tract infections.

Nevertheless, it is frequently used in the quantitative and qualitative determinations of different β-lactam antibiotics being considered today a useful alternative and also a complementary technique to the more frequently used HPLC methods (Garcia-Ruiz, Marina, 2006; Baillon-Perez *et al*., 2009; Garcia-Campana *et al*., 2009).

Our aim was the development of a new alternative method for the simultaneous separation of AMX and CLA, the optimization and also to verify the applicability of the newly developed method in the determination of the two β-lactam derivatives from pharmaceutical preparations.

Table. Drug profile of amoxicillin.

Therapeutic category	Anti-Infective Agents
CAS Registry number	58001-44-8
Chemical name	(2R,3Z,5R)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylic acid
Molecular formula	"$C_8H_9NO_5$
Molecular Weight	199.1608
Solubility	"337.0 mg/mL
pka	3.32
λ_{max}	258 nm
Pharmacology	Clavulanic acid combined with other antibiotics is indicated to prevent the development of drug-resistant strains of bacteria and promotes their therapeutic antibacterial effects.

Table. Drug profile of clavulanic acid.

Experimental

MATERIALS

EQUIPMENTS	**SOURCE**
Ultra Pressure Liquid Chromatography (UPLC)	Acquity UPLC Systems, Waters Laboratories
Electrospray ionization and MS-MS	Mass Spectrometer PE Sciex Model: API 3000
Chromatographic data software	Empower
Column	C18 column (250 ×4.6 mm id)—ACE Generix
Detector	PDA
Injector	Automated
Electronic Balance	Eagle
Sonicator	Band Line Sonerex
p^H Meter	Lab India p^H meter

METHODOLOGY

Method validation

The analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: the sample, the reference standard and the reagents preparations, use of the apparatus, generation of the calibration curve, use of the formulae for the calculation, etc. The described method extensively validated in terms of specificity, system suitability, linearity, accuracy, precision, limit of detection, limit of quantification and robustness.

- **Forced degradation studies of our selected pharmaceutical drugs.**

In order to establish the analytical method for a stability indicating method, the drugs are subjected to various stress conditions to conduct forced degradation studies. Stress studies were carried out under the conditions of acid/base hydrolysis, oxidation, reduction, in accordance with ICH Q1A (R2). Several trials with different severity of each stressed condition are to be conducted, so that upto 10-30% degradation is to be achieved.

RESULTS

Preparation of Standard Stock Solution

The pure drug of Amoxicillin trihydrate and Potassium Clavulanate were injected into the UPLC system and run in different solvent systems. Different mobile phases like acetonitrile and water; methanol and water; methanol and buffer were tried in order to find the best conditions for the separation of Amoxicillin trihydrate and Potassium Clavulanate. It was found that Methanol and Potassium dihydrogen phosphate gives satisfactory results as compared to other mobile phases. This mobile phase system was tried with different proportions and using different flow rates. A mixture of Buffer and Methanol in the ratio of 80:20 was prepared and pH = 3.0 was maintained, at which they showed better separation. Hence 80:20 v/v ratio of mobile phase was considered to be the optimal composition and pH of mobile phase is maintained to 3.0.

1. Preparation of mobile phase

Mobile phase: KH2PO4: Methanol (80:20)

2. Preparation of standard stock solution

A standard stock solution was prepared by accurately weighing about 100 mg of Amoxicillin trihydrate and 25 mg of Potassium Clavulanate standard and is transferred into 20 ml volumetric flask; add 5ml of methanol and

sonicate for 10min, make up the volume to 20 with methanol (stock solution-I). From this, transfer 5ml of above solution to 50ml volumetric flask and make up the volume with methanol labeled as stock solution-II.

1. Selection of analytical wavelength

By appropriate dilution of standard stock solution with mobile phase, various concentrations of Amoxicillin trihydrate and Potassium Clavulanate were prepared separately. The solution was scanned using double beam UV-visible spectrophotometer 1700 in the "Spectrum mode" between the range of 400 to 200 nm and their spectra was overlaid. From the overlaid spectra of Amoxicillin trihydrate and Potassium Clavulanate, 248 nm was selected as analytical wavelength for multi component analysis using UPLC method.

2. Chromatographic conditions

The mobile phase containing mixture of Methanol and Potassium dihydrogen phosphate buffer whose ratio was 20:80 was selected as the optimum composition of mobile phase, of which pH was maintained to 3.0, because it was found that this solvent system resolved both the components ideally. The flow rate was set to 0.5 ml/min and UV detection was carried out at 248 nm. The mobile phase and samples were degassed by ultrasonication for 20 min and filtered through 0.4 µm membrane filter paper.

3. Selection of analytical concentration range and preparation of calibration curve for amoxicillin trihydrate and potassium clavulanate.

Amoxicillin trihydrate

Appropriate aliquots were pipetted out from the standard stock solution (500 µg/ml) in to a series of 50 ml volumetric flasks. The volume was made up to the mark with the mobile phase to get a set of solutions having the concentration range, ranging from 250, 375, 500, 625 and 750 µg/ml of Amoxicillin trihydrate.

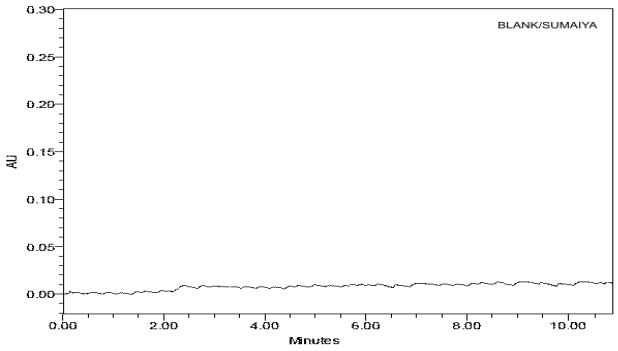
Triplicate dilutions of each of the above mentioned concentrations were prepared separately and from these triplicate solutions, 20 µl of each concentration of the drug were injected into the UPLC system two times separately and their chromatograms were recorded under the same chromatographic conditions as described above. Peak areas were recorded for all the peaks and a standard calibration curve of Area against concentration was plotted.

Potassium clavulanate

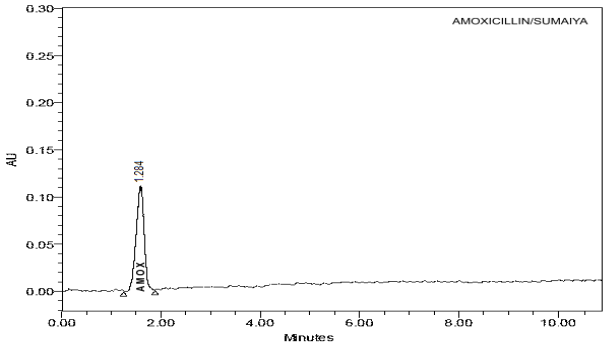
Appropriate aliquots were pipetted out from the standard stock solution (125 µg/ml) in to a series of 50 ml volumetric flasks. The volume was made up to the mark with the mobile phase to get a set of solutions having the concentration range, ranging from 62.5, 93.75, 125, 156.25 and 187.5 µg/ml of Potassium Clavulanate.

Triplicate dilutions of each of the above mentioned concentrations were prepared separately and from these triplicate solutions, 20 µl of each concentration of the drug were injected into the UPLC system two times separately and their chromatograms were recorded under the same chromatographic conditions as described above. Peak areas were recorded for all the peaks and a standard calibration curve of Area against concentration was plotted.

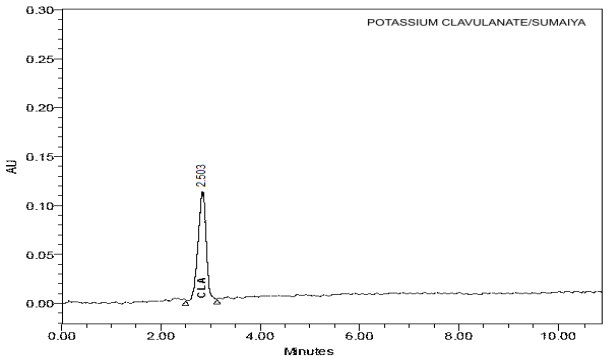
Both the drugs follow the Beer's & Lambert's law in the concentration range of 250 to 750µg/ml for Amoxicillin trihydrate and 62.5 to 187.5 µg/ml for Potassium Clavulanate. The linearity of calibration curves and adherence of the system to Beer's & Lambert's law was validated by high value of correlation coefficient and less than 2% relative standard deviation (R.S.D.) for the intercept value.



Chromatogram of Blank Chromatogram.



Chromatogram of Amoxicillin.

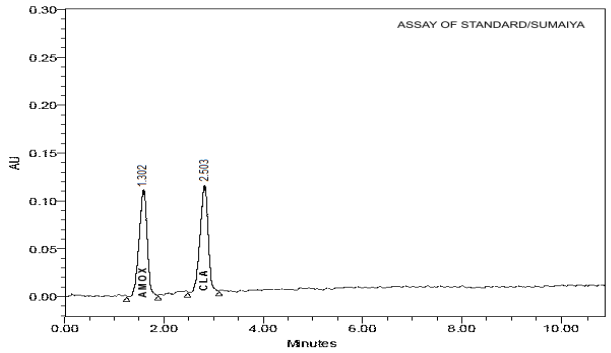


Chromatogram of potassium clavulanate.

Chromatogram of standard preparation of amoxicillin and potassium clavulanate. (KH2PO4: Methanol (80:20))

Stability indicating assay

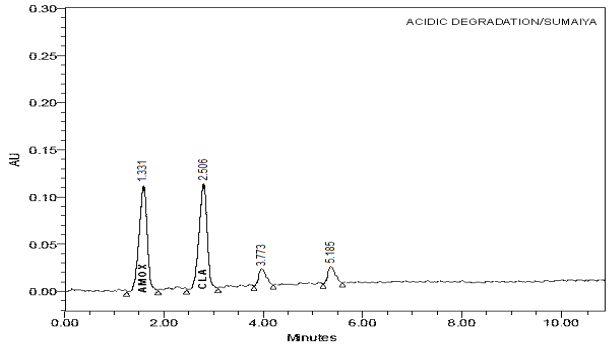
Sample Control: An accurate 10 ml of the prepared pure drug stock solution of working standard was transferred to a clean and dry RBF. The concentration of the sample was 625 µg/ml. It was injected into the UPLC system against a blank of KH2PO4: Methanol (80:20)v/v after optimizing the mobile phase composition, chromatogram was recorded.



Chromatogram no: Assay of (Sample Control).

a. Acidic degradation

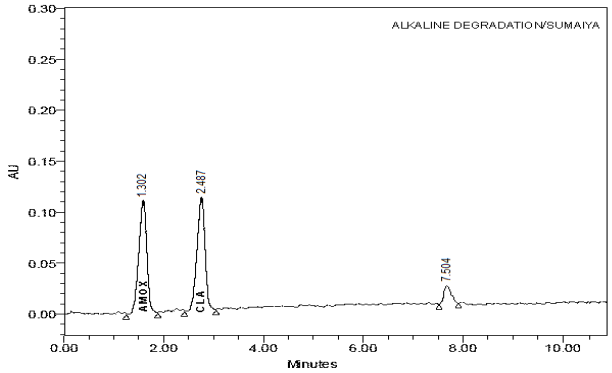
An accurate 10 ml of pure drug sample solution was transferred to a clean and dry round bottom flask (RBF). 30 ml of 0.1 N HCl was added to it. It was refluxed in a water bath at 60°C for 4 hours. Drug became soluble after reflux which was insoluble initially. Allowed to cool at room temperature. The sample was then neutralized using 2N NaOH solution and final volume of the sample was made up to 100ml with water to prepare 100ppm solution. It was injected into the UPLC system against a blank of KH2PO4: Methanol (80:20) v/v after optimizing the mobile phase composition, chromatogram was recorded.



Chromatogram no: Chromatogram showing the degraded products in Acidic degradation.

b. Alkaline degradation

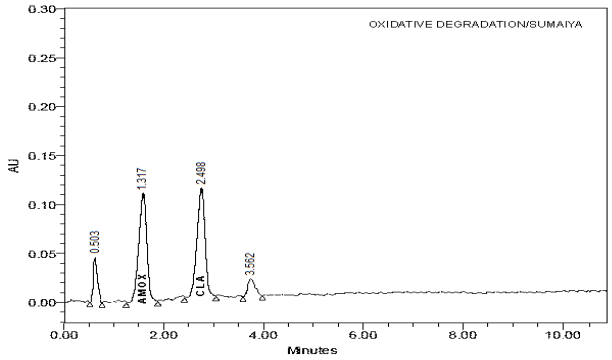
An accurate 10 ml of pure drug sample solution was transferred to a clean and dry RBF. 30 ml of 0.1N NaOH was added to it. It was refluxed in a water bath at 60°C for 4 hours. Drug became soluble after reflux which was insoluble initially. It was allowed to cool at room temperature. The sample was then neutralized using 2N HCl solution and final volume of the sample was made up to 100ml with water to prepare 100ppm solution. It was injected into the UPLC system against a blank of KH2PO4: Methanol (80:20) v/v after optimizing the mobile phase composition, chromatogram was recorded."



Chromatogram no: Chromatogram showing the degraded products in Alkaline degradation.

c. Oxidation with (3%) H_2O_2

Approximately 10 ml of pure drug sample was transferred in a clean and dry 100 ml volumetric flask. 30 ml of 3% H_2O_2 and a little methanol was added to it to make it soluble and then kept as such in dark for 24 hours. Final volume was made up to 100 ml using water to prepare 100 ppm solution. The above sample was injected into the UPLC system. The chromatogram was recorded.



Chromatogram no: Chromatogram showing the degraded products in oxidative degradation.

Table No: 200. Summary of Forced Degradation Studies (Amoxicillin and Potassium Clavulanate).

Nature of Stress	Degradation condition	Time(h)	Number of degradation products (Rt)	Relative Retention Time
Acidic	60°C	3	2 (3.773, 5.185)	0.4283
Alkaline	60°C	9	1 (7.504)	0.5049
Oxidative	RT	48	2 (0.503, 3.562)	0.9384

CONCLUSION

A specific, precise, accurate ultra pressure liquid chromatography (UPLC) method is developed for estimation of amoxicillin and potassium clavulanate in bulk dosage form. The method employed, with Xterra RP-8 (150mm x 4.6 mm i.d., particle size 5 µm) in a gradient mode, with mobile phase of KH2PO4: Methanol (80:20) and effluent was monitored at 248 nm.

Based on peak purity results, obtained from the analysis of stability indicating studying samples using described method, it can be concluded that the presence of co-eluting peak along with the main peaks of amoxicillin and potassium clavulanate indicated that the developed method is specific for the estimation of amoxicillin and potassium clavulanate in presence of degradation products. Further the proposed UPLC method has excellent precision, sensitivity and reproducibility. Even though no attempt has been made to identify the degraded products, proposed method is appropriate to be used as physical stability indicating method for assay of amoxicillin and potassium clavulanate in commercial formulations.

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