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SPIKED FORCE DEGRADATION ASSAY METHOD EVALUATION FOR ESTIMATION OF AMOXYCLAV IN ORAL DOSAGE FORM

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ABSTRACT

A specific, precise, accurate ultra pressure liquid chromatography (UPLC) method is developed for estimation of amoxicillin and potassium clavulanate in market 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. The method was validated in terms of linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) etc. in accordance with ICH guidelines. Linear regression analysis data for the calibration plot showed that there was good linear relationship between response and concentration in the range of 250-750 µg/ml amoxicillin and 62.5-187.5 µg/ml for potassium clavulanate respectively. The LOD and LOQ values for were found to be 0.0029 (µg/ml) and 0.0091 (µg/ml) for amoxicillin and 0.0052 (µg/ml) and 0.0160 (µg/ml) for potassium clavulanate respectively. No chromatographic interference from excipients and degradants were found. The proposed method was successfully used for estimation of amoxicillin and potassium clavulanate in market 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 AMOXYCLAV with the aim of determining the extent of the influence of different stress conditions on the stability of the dry powder inhaler product.

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	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.

Table. Drug Profile of Clavulanic acid.

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.

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

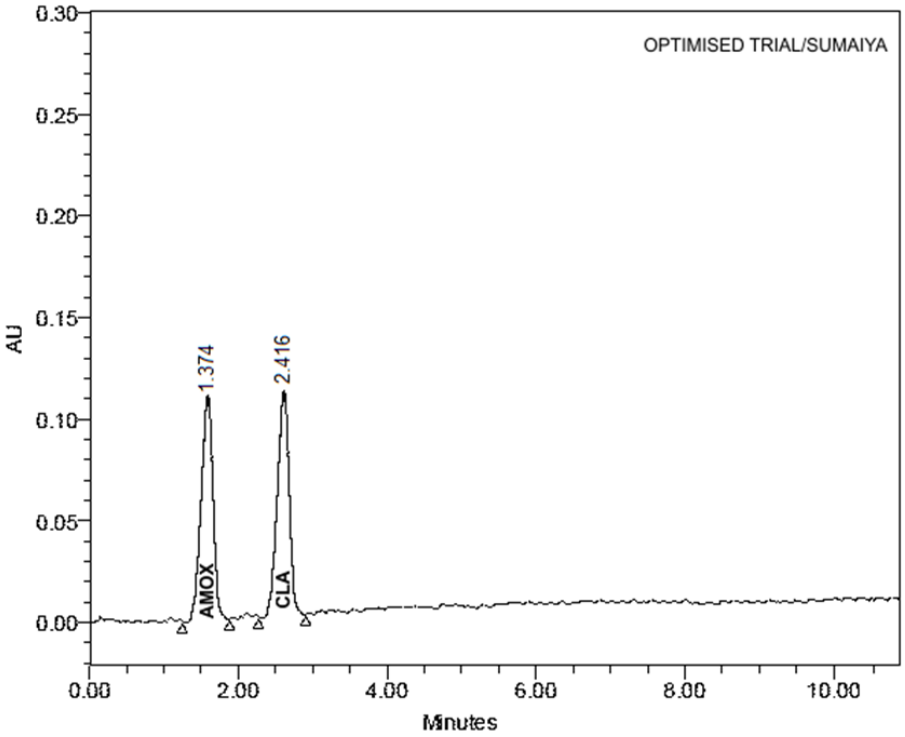
Mobile phase: KH2PO4: Methanol (80:20)

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.

A. Analysis of the marketed formulation.

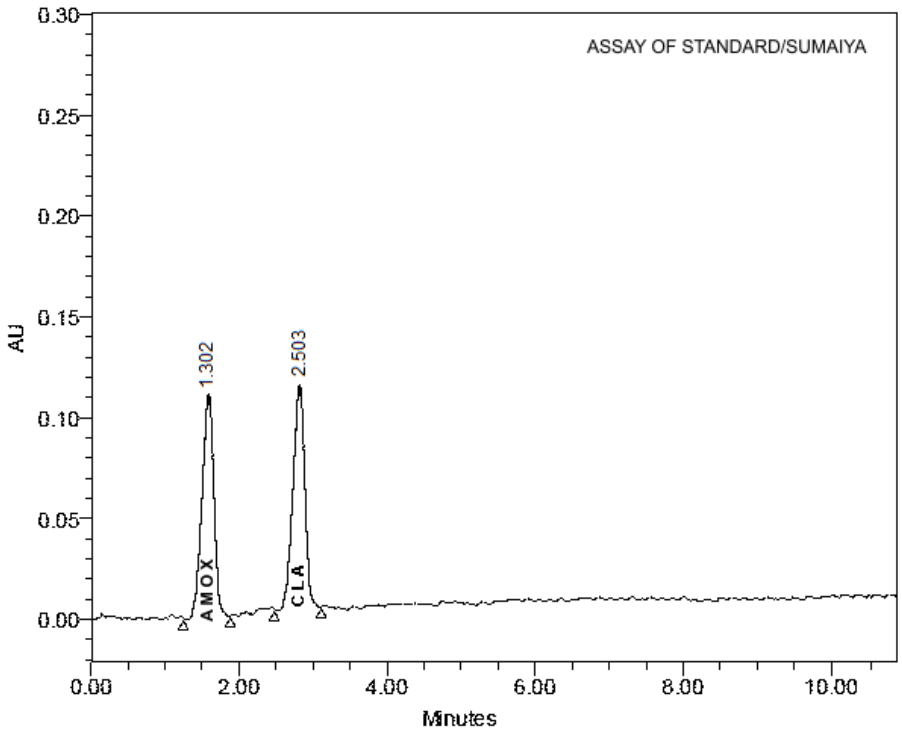
Twenty tablets (AMOXYCLAV) were weighed and crushed to fine powder. The tablet powder equivalent to 50 mg of Amoxicillin trihydrate and 12.5 mg of Potassium Clavulanate was transferred to a 10 ml volumetric flask and dissolved in 5ml of methanol. The suspension was sonicated for 15minutes. Finally, the volume was made up to the mark with methanol. The solution was filtered through 0.4 µm membrane filter paper. Transfer 5ml of the above solution into 50ml volumetric flask and make up the volume with methanol. The clear solution obtained was diluted with the same solvent systems as used for calibration graphs to give a final concentration within the range of linearity. The solution was analyzed for the estimation of drug by proposed method.



Chromatogram of standard preparation of AMOXYCLAV (KH2PO4: Methanol (80:20))

Stability Indicating Assay

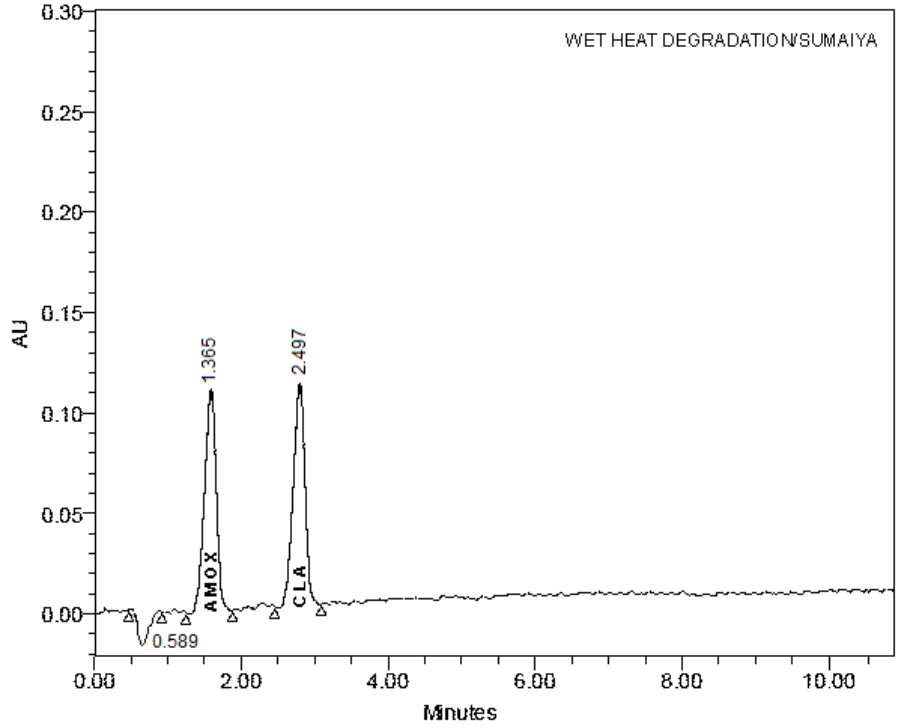
Sample Control: An accurate 10 ml of the prepared pure drug stock solution of working standard was transferred to a clean and dry RBF. The concentration of the sample was 625 µg/ml. It was injected into the UPLC system against a blank of KH2PO4: Methanol (80:20)v/v after optimizing the mobile phase composition,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



Chromatogram no: Assay of (Sample Control)

a. Wet heat degradation

Accurate 10 ml of pure drug sample was transferred to a clean and dry RBF. 30ml of HPLC grade water was added to it. Then, it was refluxed in a water bath at 60°C for 6 hours uninterruptedly. After the completion of reflux, the drug became soluble and the mixture of drug and water was allowed to cool at room temperature. Final volume was made up to 100 ml with HPLC grade water to prepare 100 ppm 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 Wet heat degradation.

b. Photolytic Degradation

The photochemical stability of the drug was also studied by exposing the drug solution (4ml) to sunlight for 72 h. Twenty microlitres of the resultant solutions were injected onto column and the chromatograms were run as described.

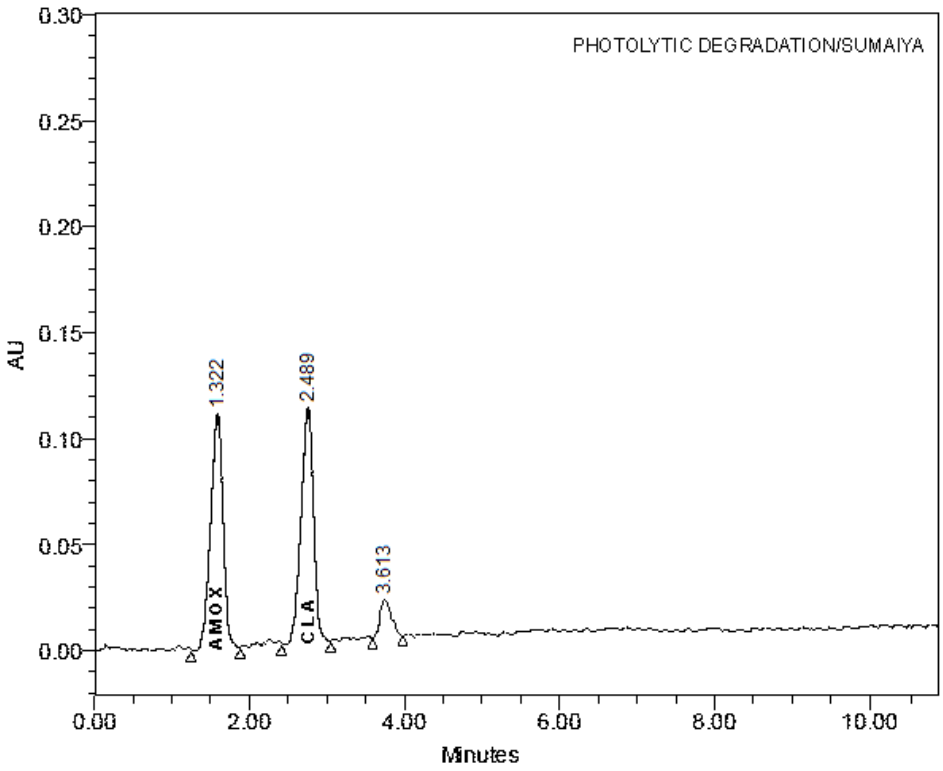


Table 200: Summary of Forced Degradation Studies (AMOXYCLAV).

Nature of Stress	Degradation condition	Time(h)	Number of degradation products (Rt)	Relative Retention Time
Wet Heat	105°C	24	1 (0.589)	0.1525
Photolytic	AT	72	1(3.613)	0.9480

CONCLUSION

A specific, precise, accurate ultra pressure liquid chromatography (UPLC) method is developed for estimation of AMOXYCLAV 625MG TAB in market 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 AMOXYCLAV indicated that the developed method is specific for the estimation of AMOXYCLAV 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 AMOXYCLAV in commercial formulations.

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