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RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF AMOXICILLIN AND CLAVULANIC ACID IN ORAL SUSPENSION

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ABSTRACT

RP-HPLC Method was developed and subsequently validated for the determination of Amoxicillin trihydrate and Potassium clavulanate in pharmaceutical formulation. Separation was achieved with an Inertsil ODS-3 C18, 5µ, (4.6×250 mm) and phosphate buffer pH 4.3 and methanol (60:40v/v) as flow rate 1.2ml/min at 245nm. The method was validated for its Linearity, Accuracy, Precision, Robustness and Ruggedness. The linearity range was 10-60µg/ml for Amoxicillin trihydrate and 1.425-8.55µg/ml for Potassium clavulanate and the correlation coefficient for both drugs were 0.999. The accuracy was performed and the % recovery was 99.69% and 99.85% for Amoxicillin trihydrate and Potassium clavulanate respectively. Forced degradation studies were conducted on two drugs by using stress conditions such as acid stress degradation, alkali stress degradation and Peroxide stress degradation. From these studies, it was observed that the proposed acceptance criteria met the requirements for acid, alkali degradation and peroxide degradation and found to be the drugs were stable and peak purity was passed.

Key words: RP-HPLC, Amoxicillin trihydrate and Potassium Clavulanate.

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INTRODUCTION

Analytic method development and validation are key elements of any pharmaceutical development program. HPLC analysis method is developed to identify, quantity or purifying compounds of interest. To know information concerning compound or analyte is worth its physical and chemical characteristics for example, molecular mass, structure and functionality, pKa values and UV spectra, solubility of compound(s) should be compiled. For pure compound, determine sample solubility whether it's organic soluble or water soluble, as this helps to select the best mobile phase

and column to be used in HPLC Method development Analytical methods are intended to establish the identity, purity, physical characteristics and potency of the drugs that we use. Methods are developed to support drug testing against specifications during manufacturing and quality release operations, as well as during long-term stability studies. Methods may also support safety and characterization studies or evaluations of drug performance. The stability-indicating assay is a method that is employed for the analysis of stability samples in pharmaceutical industry. With the advent of International Conference on Harmonization (ICH) guidelines, the requirement of establishment of stability-indicating assay method (SIAM) has become more clearly mandated. A stability-indicating method is defined as an analytical method that accurately quantitates the active ingredients without interference from degradation products, process impurities, excipients, or other potential impurities. A method that accurately quantitates significant degradants may also be considered stability-indicating. A proactive approach to developing a stability indicating HPLC method should involve forced degradation at the early stages of development with the key degradation samples used in the method development process (1-4).

The aim of the present analytical research is to develop a simple, precise, accurate, rapid, isocratic and economic RP-HPLC method for the assay of Amoxicillin and clavulanic acid in formulation and to validate it.

MATERIALS AND METHODS (5)

Optimized method

Analysis was carried out in RP-HPLC using column Inertsil ODS-3; 250 mm × 4.6 mm, 5µm, Mobile phase- phosphate buffer (pH 4.3): methanol (60:40) at a flow rate of 1.2 ml/min, injection volume-20µl with detection wavelength 245nm.

System Suitability

Prepare the standard solution as per the proposed test method and inject into the HPLC system. Record the USP tailing for Amoxicillin trihydrate and Potassium clavulanate peak from first injection and % RSD on replicate injections and the chromatograms were recorded.

Linearity concentrations

From the standard stock solution 10ml was taken and transferred to 100ml volumetric flask. From the above solution 1ml to 6ml were taken separately in 10 ml volumetric flask and make up the volume with mobile phase. Final concentration of Amoxicillin trihydrate and potassium Clavulanate was 10-60µg/ml and 1.425-8.55µg/ml respectively.

Precision

Weighed 100.0 mg of amoxicillin and 14.25 mg of Clavulanic acid working standard transferred to a 100 ml volumetric flask then it was dissolved in a 70 ml of methanol and the solution was sonicated for 5min. Then diluted up to the mark with methanol and mixed well. Further diluted 5 ml of above solution into 50 ml volumetric flask added upto the mark with methanol and mixed well. The solution was filtered through 0.45µm Nylon filter. Discard the first few ml of filtrate. Concentration of Amoxicillin trihydrate and Potassium Clavulanate about 100µg/ml and 14.25µg/ml. The precision of test method was evaluated by performing assay for prepared six individual test preparations of concentration was 40µg/ml and 5.7µg/ml for Amoxicillin trihydrate and Potassium Clavulanate respectively.

Procedure for accuracy

Accuracy at 50%

Accurately weighed and transferred 50 mg of Amoxicillin trihydrate and 7.125 mg of Potassium clavulanate and equivalent amount of placebo into a 100 ml volumetric flask, containing 70 ml of methanol and sonicated for 10min to dissolve, then diluted up to the mark with methanol and mixed well. Further diluted 5ml of above solution into 50 ml volumetric flask added upto the mark with mobile phase and mixed well. The solution was filtered through 0.45µm filter. Discarded the first few ml of filtrate. Concentration of Amoxicillin trihydrate and Potassium clavulanate about 50µg/ml and 7.125µg/ml.

Accuracy at 100%

Accurately weighed and transferred 100 mg of Amoxicillin trihydrate and 14.25 mg of Potassium clavulanate and equivalent amount of placebo into a

100 ml volumetric flask, containing 70 ml of methanol and sonicated for 10min to dissolve, then diluted up to the mark with diluent and mixed well. Further diluted 5ml of above solution into 50 ml volumetric flask added upto the mark with mobile phase and mixed well. The solution was filtered through 0.45µm filter. Discarded the first few ml of filtrate. Concentration of Amoxicillin trihydrate and Potassium clavulanate about 100µg/ml and 14.25µg/ml.

Accuracy at 150%:

Accurately weighed and transferred 150 mg of Amoxicillin trihydrate and 21.375 mg of Potassium clavulanate and equivalent amount of placebo into a 100 ml volumetric flask, containing 70 ml of diluent and sonicated for 10min to dissolve, then diluted up to the mark with diluent and mixed well. Further diluted 5ml of above solution into 50 ml volumetric flask added upto the mark with mobile phase and mixed well. The solution was filtered through 0.45µm filter. Discarded the first few ml of filtrate. Concentration of Amoxicillin trihydrate and Potassium clavulanate about 150µg/ml and 21.375µg/ml (6-9).

Forced degradation studies (5)

Forced degradation studies were carried out on the sample preparations of Amoxicillin trihydrate and Potassium Clavulanate and the degradation was evaluated by calculating the % degradation of Amoxicillin trihydrate and Potassium Clavulanate in comparison with unstressed sample preparation. The degradation between 10 % and 30% was tried by following stress conditions to prove that the method has indicating stability characteristics. Test preparation was subjected to stress degradation by treating the sample with hydrochloric acid, sodium hydroxide and hydrogen peroxide. The % degradation was evaluated by calculating the % assay and by comparing the assay results with the assay of unstressed sample.

RESULT AND DISCUSSION

Good reproducibility was obtained in mobile phase containing phosphate buffer pH 4.3 and methanol (60:40v/v). The flow rate was 1.2ml/min and detection wavelength was 245nm at ambient column temperature. The method was carried out on a Inertsil ODS-3 C18, 5µ, (4.6×250 mm) by using Waters Alliance-2690 HPLC system with EMPOWER software (Fig-1). Amoxicillin trihydrate and potassium Clavulanate was 10-60µg/ml and 1.425-8.55µg/ml respectively was used for linearity range. Correlation co-efficient for Amoxicillin trihydrate and Potassium Clavulanate were 0.999 and 0.999 respectively (Fig-2 and 3).

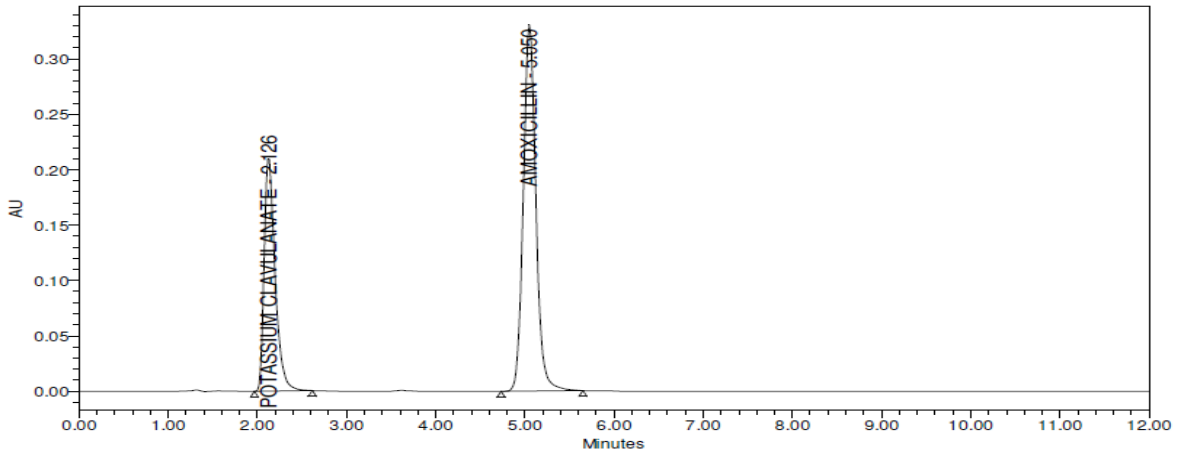

Fig-1 Chromatogram of Amoxicillin trihydrate and Potassium Clavulanate

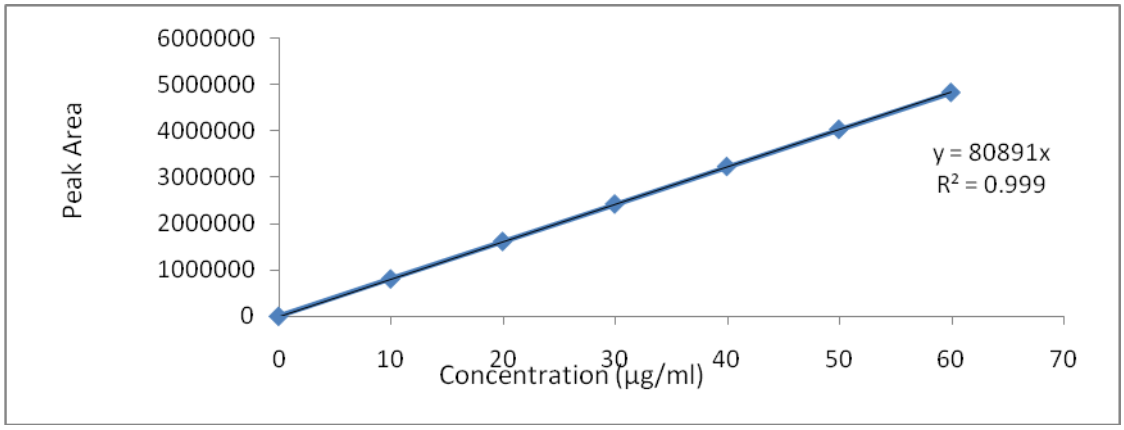

Fig-2 Linearity plot of Amoxicillin trihydrate

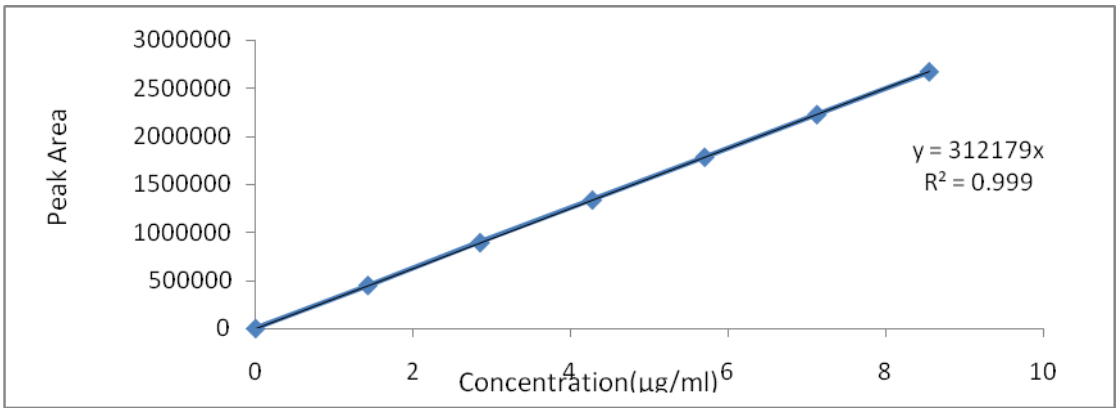

Fig-3 Linearity plot of Potassium clavulanate

The precision of test method was evaluated by performing assay for prepared six individual test preparations of concentration was 40µg/ml and 5.7µg/ml for Amoxicillin trihydrate and Potassium Clavulanate respectively. The results are given in table-1.

Table-1 Result of Method precision

Injection Number	%Assay for Amoxicillin trihydrate	%Assay for Potassium clavulanate	Acceptance Criteria
1	99.7	99.3	The % RSD for 6 replicates of Injections should be NMT 2.0
2	99.4	99.06	
3	99.8	99.3	
4	98.9	98.4	
5	99.5	99.18	
6	99.4	98.9	
Average	99.45	99.02	
%RSD	0.3	0.34	

Accuracy was performed in three different levels for Amoxicillin trihydrate and Potassium clavulanate. The known quantity of Amoxicillin trihydrate and Potassium clavulanate was spiked at 50%, 100% and 150% level into the placebo. The samples was analysed in triplicate for each level. From the results, % recovery was calculated. From the above results the obtained % Recovery was within the limit.

Forced degradation studies were carried out on the sample preparations of Amoxicillin trihydrate and Potassium Clavulanate and the degradation was evaluated by calculating the % degradation of Amoxicillin trihydrate and Potassium Clavulanate in comparison with unstressed sample preparation. Purity angle of Amoxicillin and potassium clavulanate for the stressed sample was less than purity threshold. So that the peak purity was passed for both drugs (Table-2 and 3).

Table-2 Degradation profile of Amoxicillin trihydrate

Stress conditions	% Degradation	Peak purity		Purity Flag	Acceptance Criteria
		Purity angle	Purity threshold		
Treated with 1N HCl (1ml) solution for 1hr at room temperature	10.86	0.017	0.277	No	The Purity angle should be less than Purity Threshold and no purity flag for Drug peak
Treated with 0.5N NaOH (1ml) solution for 1hr at room temperature	11.11	0.048	0.285	No	
Treated with 0.5% H_2O_2 (1ml) solution for 1hr at room temperature	12.1	0.057	0.265	No	

Table-3 Degradation profile of Potassium clavulanate

Stress conditions	% Degradation	Peak purity		Purity Flag	Acceptance Criteria
		Purity angle	Purity threshold		
Treated with 1N HCl (1ml) solution for 1hr at room temperature	12.57	0.080	0.281	No	The Purity angle should be less than Purity Threshold and no purity flag for Drug peak
Treated with 0.5N NaOH (1ml) solution for 1hr at room temperature	10.94	0.043	0.287	No	
Treated with 0.5% H_2O_2 (1ml) solution for 1hr at room temperature	3.5	0.050	0.277	No	

CONCLUSION

An analytical method was developed for the determination of Amoxicillin trihydrate and potassium clavulanate in oral suspension and the method was found to be simple, precise and accurate. This method was validated as per ICH guidelines and can be useful for the analysis of Amoxicillin trihydrate and potassium clavulanate. This work provides high degree of assurance that this method was suitable for routine analysis of Amoxicillin trihydrate and potassium clavulanate due to its sensitivity, simplicity and selectivity.

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BIBLIOGRAPHY

1. Sharma BK. Instrumental Methods of Chemical Analysis. 7th ed., Goel Publishing House, Meerut, 2000; 1-2.
2. Ranjit S. HPLC method development and validation- an overview. *J Pharm Educ Res* 2013; 4: 24-29.
3. Pranay W, Brijesh K. Bioanalytical Method Development- Determination of drugs in biological fluids by HPLC. *J Pharml Sci Technol* 2010; 2: 333-347.
4. Analytical Method Development and Validation; http://shodhganga.inflibnet.ac.in/bitstream/10603/8513/9/09_chapter%202.pdf
5. Method validation guidelines International Conference on Harmonization, Validation of Analytical Procedures: Text and Methodology, Q2 (R1). Geneva; 1996.
6. Beg S, Hasnain MS. Validated stability-indicating LC method for estimation of Amoxicillin Trihydrate in pharmaceutical dosage forms and time-dependent release formulations. *IJPSN* 2012; 4: 15-19.
7. Durga MT. Development and Validation of Stability Indicating HPLC Method for Simultaneous Estimation of Amoxicillin and Clavulanic Acid in Injection. *AJAC* 2010; 12: 95-101
8. Jadhav SS, Salunkhe VR. Development and validation of HPLC method for simultaneous estimation of Amoxycillin trihydrate and Potassium clavulanate in pure and marketed tablet dosage form. *Curr Pharma Res* 2013; 3: 994-998.
9. Mulla S, Mohommadisak. Development and validation of Analytical method for estimation of Amoxicillin Trihydrate from bulk and pharmaceutical dosage form. *IJPRD* 2013; 5: 14-19.