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METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF AMOXYCILLIN AND POTASSIUM CLAVULANATE

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ABSTRACT: A rapid, sensitive and specific RP-HPLC method involving UV detection was developed [1-15] and validated [16-21] for determination and quantification of Amoxycillin and Potassium Clavulunate. Chromatography was carried out on a pre-packed Hypersil C18 (5µm, 250x4.6mm) column using filtered and degassed mixture of Phosphate buffer:Methanol (95:05) as mobile phase at a flow rate of 1.0ml/min and effluent was monitored at 220nm. The method was validated [16-21] in terms of linearity, precision, accuracy, and specificity, limit of quantification and limit of detection. The assay was linear over the concentration range of Amoxycillin and Potassium Clavulunate was 25mcg-200mcg/ml and 5mcg to 40mcg/ml respectively. Accuracy of the method was determined through recovery studies by adding known quantities of standard drug to the pre analyzed test solution and was found to be 97.70%-103.00% and 96.80%-102.01% within precision RSD of 0.432 and 0.988 for Amoxycillin and Potassium Clavulunate respectively. The system suitability parameters such as theoretical plates and tailing factor were found to be 3189.33, 1.225 and 7852.83, 1.08 respectively for Amoxycillin and Potassium Clavulunate. The method does require only 15 minutes as run time for analysis which prove the adoptability of the method for the routine quality control of the drug.
Key words: Amoxycillin, Potassium Clavulunate, Method development, Validation.

INTRODUCTION

Amoxycillin is chemically 7-[2-amino-2-(4-hydroxy phenyl)-acetyl] amino-3,3 dimethyl-6-oxo-2-thia-5-azabicyclo[3.2.0] heptanes-4-carboxylic acid and used as an anti-bacterial and anti infective. Potassium clavulanate is chemically (2R,5R,Z)-3-(2hydroxy ethylidene)-7-oxo-4-oxa-1-aza-bicyclo[3.2.0] heptanes-2-carboxyllic acid and used as beta lactamase inhibitors. There is a plethora of analysis of such formulations without prior separation. For the estimation of multi-component formulation, the instrumental techniques, which are commonly employed, are spectrophotometry, GLC, high performance thin layer chromatography (HPTLC), HPLC etc. These methods are based upon the measurement of specific and nonspecific physical properties of the substances. The literature survey reveals that there is no HPLC methods have been reported. The present study is to develop [1-15] an accurate and reliable HPLC method for simultaneous estimation of Amoxycillin and Potassium Clavulunate in solid dosage form.

OBJECTIVE: In this paper we describe a simple, inexpensive, sensitive and validated [16-21] HPLC method for the simultaneous determination of amoxicillin and potassium clavulanate in pharmaceutical formulation.

EXPERIMENTAL

Working standards of amoxicillin and potassium clavulanate were obtained from well reputed research laboratories. HPLC grade Methanol, Merck grade KH_2PO_4 and Milli-Q water were procured from the market. The separation was carried out on isocratic HPLC system (YOUNGLIN ACME-900) with pre-packed (Hypersil-C18(5µm,250x4.6mm)) column using filtered and degassed mixture of Buffer: Methanol(95:05) as mobile phase.

Standard preparation: About 100mg of amoxicillin and 100mg of potassium clavulanate were accurately weighed and transferred to a 100ml volumetric flask

individually and dissolved in the water by sonication to give standard stock solution.

Chromatographic conditions: Flow rate 1.0ml/min; detection wavelength 220nm; injection volume 20µl; column used Hypersil C18 (5µm, 250x4.6mm); column temperature: 25°C; mobile phase: Buffer: Methanol (95:05).

Method development[1-15]: Working standard of various concentrations was prepared by taking aliquots of standard solution and diluted to get required concentration for calibration plot and which was injected.

Assay preparation for commercial formulation: Twenty capsules were taken; average weight was determined and mixed well fine powder. Powder equivalent to 100mg of amoxicillin and potassium clavulanate was transferred into 100ml volumetric flask and dissolved in sufficient amount of diluent and sonicated to dissolve. Solution was filtered through 0.45µ membrane filter and then the filtrate was further diluted to get the required concentrations.

Procedure: 20µl of the standard preparation and assay preparation were separately injected and chromatographed.

Method validation[16-21]:

Linearity: Linearity was demonstrated by analysing six different concentrations of active compound. Peak areas were recorded for all the peaks and calibration plot was constructed by plotting peak area vs concentrations of amoxicillin and potassium clavulanate which were found to be linear in the range of 25mcg-200mcg/ml and 5mcg to 40mcg/ml respectively. Coefficient of correlation was 0.9998 and 0.9997(Fig-1).

Accuracy: accuracy was done by recovery study using standard addition method, known amount of standard amoxicillin and potassium clavulanate in to pre-analysed samples and subjected to proposed HPLC method. The results of recovery studies are shown in Table-1.

RP-HPLC estimation of amoxicillin and potassium clavulanate

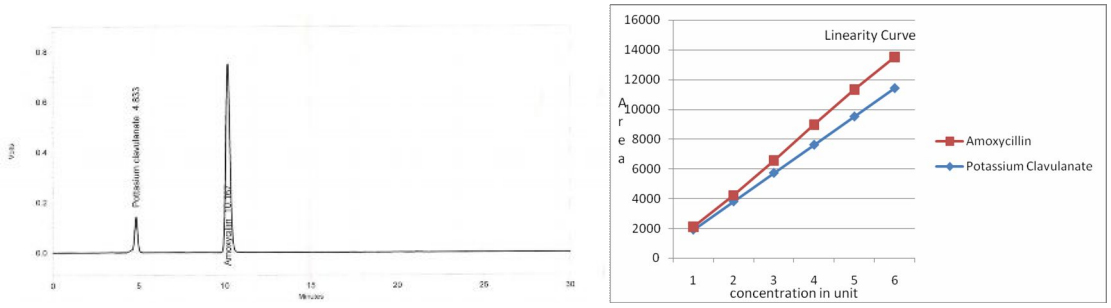

Table-1: Analysis of capsule containing amoxicillin and potassium clavulanate

Formulation	Drug	Injected sample (mcg/ml)	Amount found (mcg/ml)	Found (%)	Amount std. added	Amount recovered (mcg/ml)	Recovery (%)
Capsule	Amoxicillin	40	39.76	99.4	40	39.78	99.45
	Potassium clavulanate	20	20.26	101.3	20	20.09	100.45

Precision: To demonstrate agreement among results, a series of measurements are done with amoxicillin and potassium clavulanate six replicate injections of the specific standard at various time intervals on the same day were injected into the chromatograph and the value of %RSD was found to be 0.710 and 1.000 for amoxicillin and potassium clavulanate respectively. In inter-day precision same standard was injected on different days and the found %RSD were 0.432 and 0.988 for amoxicillin and potassium clavulanate respectively.

RESULTS AND DISCUSSION

The regression value was found to be 0.9998 and 0.9997 for amoxicillin and potassium clavulanate respectively, which shows the response, is linear from 25mcg-200mcg/ml and 5mcg to 40mcg/ml respectively. Coefficient of correlation was 0.9998 and 0.9997. Selectivity experiment showed that there is no interference or overlapping of the peaks either due to excipients or diluents with the main peak of amoxicillin and potassium clavulanate. The percentage RSD for precision is <2 which confirms that method is sufficiently precise and the total run time required for the method is only 15mins for eluting both amoxicillin and potassium clavulanate. The proposed method is simple, fast, accurate, and precise and can be used for routine analysis in quality control of amoxicillin and potassium clavulanate.

Amount found on	Intra-day		Inter-day	
	Mean %	RSD (%)	Mean %	RSD (%)
Amoxicillin	98.686	0.710	99.712	0.432
Potassium clavulanate	97.506	1.000	100.240	0.988

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