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Original Article

Development and Validation of HPLC Method for Simultaneous Estimation of Amoxycillin Trihydrate and Potassium Clavulanate in Pure and Marketed Tablet Dosage Form.

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Abstract

A rapid, sensitive and specific RP-HPLC method involving UV detection was developed and validated for determination and quantification of Amoxycillin Trihydrate and Potassium Clavulanate. Chromatography was carried out on pre-packed Inertsil C_{18} (5µm, 250×4.6mm) column using filtered and degassed mixture of Methanol: Aqueous disodium hydrogen phosphate pH 5 as mobile phase at flow rate of 1.0 ml/min and effluent was monitored at 249 nm. The method was validated in terms of Linearity, precision, accuracy, specificity, limit of detection, limit of quantification as per the ICH guidelines.The assay was linear over the concentration range of amoxycillin trihydrate and potassium clavulanaterespectively.The accuracy and precision were found to be within the specified limits. The method does require only nine minutes as run time for analysis which proves its adoptability.

Keywords: *Amoxycillin Trihydrate, Potassium Clavulanate, Method development, validation.*

1. Introduction

Amoxycillin Trihydrate (AT) is chemically 7-{2-amino-2-(4-hydroxyphenyl)-acetyl} amino-3, 3 dimethyl-6-oxo-2-thia-5azabicyclo [3.2.0] heptanes-4-carboxylic acid and used as an anti bacterial and anti infective. Potassium Clavulanate (PC) is chemically (2R,5R,Z)-3-(2-hydroxyetylidene)-7-oxo-4-oxa-1-aza-bicyclo[3.2.0]heptanes-2-carboxylicacid and used as beta lactamase inhibitors.[1] For the estimation of multicomponent formulation, the instrumental techniques, which are commonly employed are Spectrophotometry, Gas Liquid Chromatography, High performance liquid chromatography, High performance thin layer liquid chromatography etc.[2] These methods are based upon the measurement of specific and non specific physical properties of the substances.[3] The literature survey reveals that there several HPLC methods have been reported for the simultaneous estimation of the above drugs.Acetonitrile, methanol, aqueous disodium hydrogen phosphate were used as solvents for mobile phase. Use of costly solvents and other optimizing conditions are in fictitious or unaffordable. It was our intention to develop an accurate, economic and reliable method for the simultaneous estimation of amoxycillin trihydrate and potassium clavulanate in combined tablet dosage form.

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2. Method development and Validation

HPLC method development is a part of good quality control process which includes information on samples, need for experimental procedures, choosing detectors and liquid chromatographic methods, optimizing separation conditions, checking problems, calibration and validation of instruments. Validation provides high degree assurance that specific developed method produces products consistently meeting its prescribed limits.Robustness is measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability in normal usage whereas Ruggedness is measure of reproducibility of test results under normal and expected operational conditions from laboratory to laboratory and from analyst to analyst

2.1. Material and Methods

Working standards of amoxicillin trihydrate and potassium clavulanate were obtained from Cipla pharmaceuticals, Goa. HPLC grade methanol was procured from merk pvt.ltd. The separation was carried out on isocratic HPLC system (AGILLENT 1100) with pre-packed Inertsil C18 (5µm, 250×4.6mm) column using filtered and degassed mixture of methanol: aqueous disodium hydrogen phosphate (10:90) pH 5 as mobile phase [.4,5,12]

2.1.1. Method development

Chromatographic conditions

The analysis was carried out on HPLC system using the column Inertsil C18 (5µm, 250×4.6mm); with UV detection of 249 nm, column temperature was

maintained upto 37^0 C. The injection volume used was 20µl with a flow rate of 1.0ml/min. [6,7]

2.1.2. Selection of Wavelength

The bands of peaks are showing the maximum detection level for Amoxycillin trihydrate in the range of 272 nm and Potassium clavulanate in the range of 262 nm. A single wavelength has been selected for estimation of amoxicillin trihydrate and potassium clavulanate at 249 nm as both the peaks have the significant response [8]. Overlaid spectrum of amoxicillin trihydrate and potassium clavulanate is given in fig. 1. below

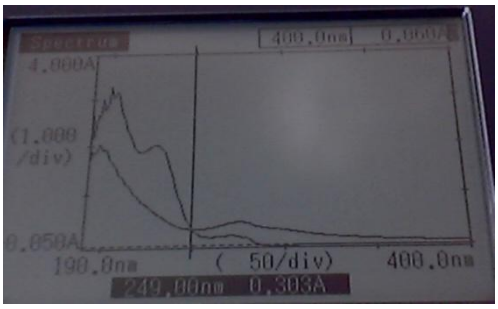


Fig.1. Overlain spectra of Amoxycillin Trihydrate and Potassium Clavulanate.

2.1.3. Selection of Mobile phase

A degassed mixture of methanol: aqueous disodium hydrogen phosphate (10:90) pH 5 was selected as a mobile phase. Buffer of required concentration was prepared by dissolving 7.8 gm of sodium dihydrogen phosphate monohydrate to 1000 ml distilled water and the pH was adjusted by using dilute orthophosphoric acid solution.

2.1.4. Calibration

Six different concentrations (20-120 µg/ml) of amoxycillin trihydrate and (10-60µg/ml) of potassium clavulanate were prepared for linearity studies. The responses were measured as peak areas and plotted against concentration [11]. Calibration curves are given in fig.2 and 3.

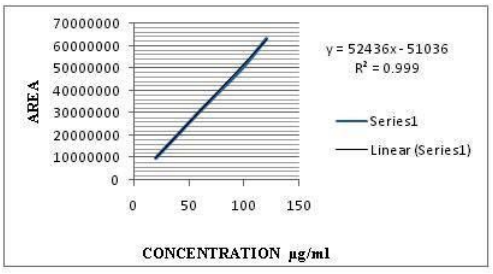


Fig. 2. Calibration Curve for Amoxycillin Trihydrate.

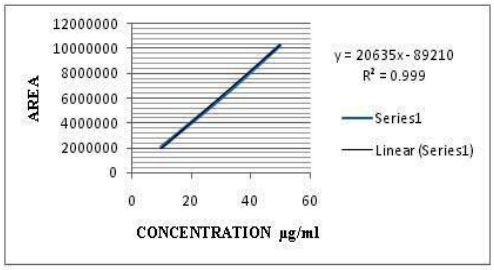
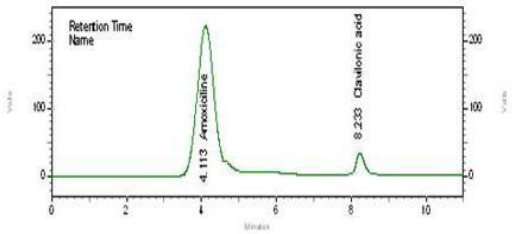


Fig.3. Calibration Curve for Potassium Clavulanate.

2.1.5. Standard preparation

About 100mg of amoxicillin trihydrate and 100mg of potassium clavulanate were accurately weighed and transferred to a 100ml volumetric flask individually and dissolved in the aqueous disodium hydrogen phosphate pH 5 by sonication to give standard stock solution. Furthur 1 ml of the above standard stock solution was withdrawn and volume was made upto the mark by phosphate buffer pH 5 solution to get 100µg/ml concentration. Then 4 ml of solution was withdrawn from the above 100µg/ml concentration of amoxycillin trihydrate to get the final concentration of 40 µg/ml amoxycillin trihydrate solution and 2ml of solution was withdrawn from the above 100µg/ml concentration of potassium clavulanate to get final concentration of 20 µg/ml potassium clavulanate solution [9,10]. A typical chromatogram is given in fig.4.



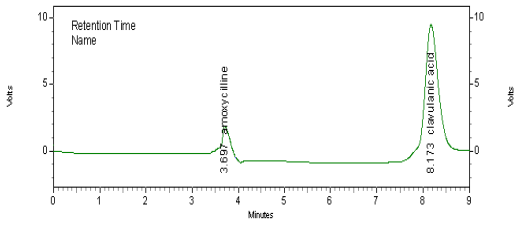
Name	RT	Area	TP (USP)	R USP)	Asymmetry
AT	4.113	11325123	3922	0.10000	0.70000
PC	8.233	7903651	6884	2.94914	1.23984

Fig.4. Chromatogram of Amoxycillin Trihydrate and Potassium Clavulanate (Standard drug).

2.1.6. Estimation of Amoxycillin Trihydrate and Potassium Clavulanate from marketed dosage form

Twenty tablets were taken and quantity of powder equivalent to 250 mg of amoxycillin trihydrate and 125 mg of potassium clavulanate was transferred to 100 mL volumetric flask and dissolved in phosphate buffer pH 5 solution and final volume was made upto the mark. The sample solution was then filtered through Whatman filter paper no.41. From the above solution 1 mL of solution was taken and diluted to 50 mL with phosphate buffer pH 5 solution to get final concentration containing 250 µg/mL of amoxycillin trihydrate and 125 µg/mL of potassium clavulanate [12]. 20µl of the standard preparation and marketed preparation were separately injected

and chromatographed. A typical chromatogram is given in fig. 5.



Name	RT	Area	TP (USP)	R (USP)	Asymmetry
AT	3.697	734751	2008	0.00000	0.97132
PC	8.173	2305970	3701	2.94914	1.29242

Fig.5. Chromatogram of Amoxycillin Trihydrate and Potassium Clavulanate (marketed drug).

3. Method validation

3.1. Linearity

Linearity was demonstrated by analyzing 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 Amoxycillin Trihydrate and Potassium Clavulanate which were found to be linear in the range of 20-120µg/ml and 10-60µg/ml foramoxycillin trihydrate and potassium clavulanate respectively. Coefficient of correlation was found to be 0.999 for both the drugs [13]. Result of validation parameters Table no.1. below.

3.2. Precision

To demonstrate agreement among results, a series of measurements are done with amoxycillin trihydrate 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 were calculated for amoxycillin trihydrate and potassium clavulanate respectively [14,15] as shown in Table no.2 .

3.3. Accuracy

It was done with the help of recovery study by using standard addition method, known amount of standard amoxycillin trihydrate and potassium clavulanate into pre-analyzed samples and subjected to proposed HPLC method. The results of the recovery studies are shown in Table no. 3.

3.4. Limit of detection

The detection limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

$$DL = \frac{3.3\ \sigma}{S}$$

Where σ = the standard deviation of the response
S = the slope of the calibration curve

3.5. Limit of quantitation

The quantitation limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

$$QL = \frac{10\ \sigma}{S}$$

Where σ = the standard deviation of the response
S = the slope of the calibration curve
The result of analysis of marketed tablet is given in Table no.4

3.6. Ruggedness and Robustness

Ruggedness study was carried out by two different analysts at similar chromatographic conditions viz. pH 5, flow rate 1ml/min, (10:90) methanol: phosphate buffer and the results shown by both the drugs are within the limits shown in Table no. 5. Robustness study was carried out by changing the mobile phase concentrations as 80:20,11:89 v/v. pH was changed from 5 to 4.5,5.5 and flow rate was varied from 1 to 0.8, 0.9 respectively.

Table 1.
Result of validation parameters.

Parameters	Amoxycillin Trihydrate	Potassium Clavulanate
Detection wavelength	249 nm	249 nm
Linearity range	20-120 µg/mL	10-60 µg/mL
Slope	5242	1857
Intercept	5163	-3860
Correlation coefficient	0.9995	0.9998
Regression equation (y =mx+c)	Y=52436x-51036	Y=20635x-89210
Limit of detection	1.19µg/mL	0.42µg/mL
Limit of quantitation	3.61µg/mL	1.3µg/mL

Table 2.
Result of Interday and Intraday precision.

	Interday precision		Intraday precision	
	%Amount found±SD*	% RSD	%Amount found±SD*	% RSD
AmoxycillinTrihydrate	49.6±0.57	0.28	49.9±0.1	0.28
Potassium Clavulanate	24.5±0.51	0.12	24.7±0.1	0.12

Table 3.
Result of Recovery studies.

Concentration of the drug added to the combination	Amoxycillin Trihydrate % Recovery ± SD*	%RSD	Potassium Clavulanate % Recovery ± SD*	%RSD
80%	39.9±0.5	0.29	24.7±0.57	0.11
100%	47.6±1.52	0.72	28±1	0.28
120%	58.3±0.57	0.33	33.6±0.57	0.19

Table 4.
Result of analysis of marketed tablet.

Marketed Tablet	Drug	Label Claim	Amount found ± S.D*	% label claim ±S.D*
	Amoxycillin Trihydrate	250 mg	247±0.565	99±0.12
	Potassium Clavulanate	125 mg	122.2±0.2	90±1.2

Table 5.
Result of Ruggedness studies.

Marketed	Drug	Label Claim	Amount found ± S.D*	% label claim ±S.D*
Analyst 1	Amoxycillin Trihydrate	250mg	248±1.16	100.7±0.287
	Potassium Clavulanate	125 mg	124±0.04	85.6±0.331
Analyst 2	Amoxycillin Trihydrate	250 mg	250.8±2.46	100±0.617
	Potassium Clavulanate	125 mg	125.12±0.036	85.16±0.276

Table 6.
Result of Robustness study.

Chromatographic Condition	Ret. Time*		Asymmetry*	
	AT	PC	AT	PC
	A] Flow Rate			
0.8	3.311	7.548	0.000	1.204
0.9	4.059	8.505	0.048	1.093
1.0	4.113	8.233	0.700	1.239
Avg.	3.827	8.095	0.249	1.178
SD	0.448	0.493	0.391	0.076
%RSD	0.017	0.039	0.009	0.008
	B] Mobile Phase of Buffer : Methanol (v/v)			
9:91	3.224	7.488	0.026	0.305
10:90	4.113	8.233	0.700	1.239
11:89	5.337	5.547	1.005	3.198
Avg.	4.224	7.08	0.577	1.580
SD	1.060	1.386	0.500	1.476
%RSD	0.040	0.09	0.02	0.02
	D] PH effect			
4.5	3.342	5.35	0.073	1.186
5	4.113	8.233	0.700	1.239
5.5	6.288	9.533	1.012	3.217
Avg.	4.581	7.705	0.595	1.880
SD	1.527	2.140	0.478	1.157
%RSD	0.06	0.16	0.002	0.02

Results and Discussion

The proposed method is simple, fast, accurate and precise and can be used for routine analysis in quality control of amoxycillin trihydrate and potassium clavulanate as it has shown the retention time 4.113 and 8.233 for amoxycillin trihydrate and potassium clavulanate the total run time required for the method is 9 mins for eluting both amoxycillin trihydrate and potassium clavulanate. Flow rate was maintained at 1ml/min for the separation of the peaks of both the standard and marketed preparations. The areas of peaks for standard amoxycillin trihydrate and potassium clavulanate were found to be 11325123 and 7903651 respectively. The thereotical plates were found to be 3922 and 6884 for amoxycillin trihydrate and potassium clavulanate respectively. The resolution was found to be 0.10000 and 2.94914 for amoxycillin trihydrate and potassium clavulanate respectively. The asymmetries of the peaks were found to be 0.70000 and 1.23984 respectively. The regression value was found to be 0.999 and 0.999 for amoxycillin trihydrate and potassium clavulanate respectively, which shows the response is linear from 20-120µg/ml and 10-60µg/ml for amoxycillin trihydrate and potassium clavulanate respectively. No overlapping of the peaks were seen with the main peak of amoxicillin trihydrate and potassium clavulanate. The precision was found to be 49.6 ± 0.57 and 24.5 ± 0.51 for amoxycillin trihydrate and potassium clavulanate respectively. The limit of detection was found to be 1.19 and 0.427 for amoxycillin trihydrate and potassium clavulanate respectively. The Limit of Quantitation was found to be 3.61 and 1.3 for amoxycillin trihydrate and potassium clavulanate respectively. The recovery was found to be 47.6 ± 1.52 and 28 ± 1 for amoxycillin trihydrate and potassium clavulanate respectively. The robustness was found to be 248 ± 1.16 and 124 ± 0.04 for amoxycillin trihydrate and potassium clavulanate respectively. The percentage relative standard deviation for precision is < 2. The evaluation of robustness is considered during the development phase and depends on the type of procedure under study. It is the reliability of an analysis with respect to deliberate variations in method parameters. If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure. One consequence of the evaluation of robustness should be that a series of system suitability parameters (e.g., resolution test) is established to ensure that the validity of the analytical procedure is maintained whenever used [16,17].

Conclusion

In present work a simple and rapid RP- High performance liquid chromatographic method was developed for the separation and determination of Amoxycillin Trihydrate and Potassium Clavulanate from bulk and combined tablet dosage form.

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References

[1] Florey, Analytical profiles of drug substance, 27, 265.
[2] Willard H.H, Merritt, Jr. L.L, Dean, J.A Settle, Jr. F.A, Introduction to Instrumental Methods of Analysis, Delhi, 2001.
[3] Lloyd R Snyder, Joseph J Kirkland, Joseph L. Glajch, Practical HPLC Method Development, New York, 21, 235-264.
[4] Robert A Nash, Alfred H Watcher, Pharmaceutical Process Validation, 129, 159 - 507.
[5] Kasturi A V, Mahadik V R, Principle of spectroscopy In Pharmaceutical analysis, 2003.
[6] Kalsi P.S, Ultraviolet and Visible spectroscopy; Spectroscopy of organic compounds, New Delhi, 2001.
[7] D.M.R Tippa, N. Singh. J.American Analytical Chemistry, 1 (2010) 95-101.
[8] Kemp W, Ultra and Visible Spectroscopy, Organic spectroscopy, Hampshire, 1996.
[9] Becket A K, Stenlase J B, spectroscopy in Practical pharmaceutical chemistry, New Delhi, 4, 275-299.
[10] Silverstein R M, Morril T Y C, Bassler G C, Ultraviolet spectrophotometer, New York, 1997.
[11] Davidson A.G, The basis of spectrophotometry, New Delhi, 1997.
[12] AGILLENT- 1100, Instrumental Manual, High Performance Liquid Chromatogram.
[13] Department of Trade and Industry, Valid analytical measurement programe, the manager's guide to VAM, U.K.
[14] Guideline for Industry ANDA, Impurities in drug substance, 2002.
[15] ICH Harmonised Tripartite Guideline, Code Q2A- Text on Validation of analytical Procedures Step-3 Consensus Guidelines, 1994.
[16] ICH Harmonised Tripartite Guideline, Code Q2B- Validation of Analytical Procedures-Methodology step- 4 Consesus Guidelines, 1994.
[17] Validation of analytical procedures, Centre for Veternary Medicine Guidance Document 63, 1999.