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Research

A new analytical method development and validation for estimation of cefp odoxime, azithromycin in bulk and tablet by rp-hplc

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Abstract

Analytical Method Development and Validation for Azithromycin and Cefpodoxime in bulk and Combine Dosage Form by RP-HPLC, New method was established for simultaneous estimation of Azithromycin and Cefpodoxime by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Azithromycin and Cefpodoxime by using Symmetry ODS C18 (4.6mm×250mm, 5µm) particle size, flow rate was 1.0 ml/min, mobile phase ratio was (70:30 v/v). The system suitability parameters for Azithromycin and Cefpodoxime such as theoretical plates and tailing factor were found to be 5387, 0.97 and 5398 and 1.26, the resolution was found to be 2.97. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study n Azithromycin and Cefpodoxime was found in concentration range of 30µg-70µg and 60µg-140µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % recovery was found to be 100.14% and 100.56%, %RSD for repeatability was 0.1 and 0.5, % RSD for intermediate precision was 0.1 and 0.1 respectively. The precision study was precise, robust, and repeatable. LOD value was 0.56 and 1.2, and LOQ value was 1.7 and 3.6 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Azithromycin and Cefpodoxime in API and Pharmaceutical dosage form.

Keywords: Azithromycin and Cefpodoxime, Method Development, Validation, Accuracy.

INTRODUCTION

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure.

Chemical compounds and metallic ions are the basic building blocks of all biological structures and processes which are the basis of life. Some of these naturally occurring compounds and ions (endogenous species)

are present only in very small amounts in specific regions of the body, while others such as peptides, proteins, carbohydrates, lipids and nucleic acids are found in all parts of the body. The main object of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with certain accuracy. Analytical chemistry derives its principles from various branches of science like chemistry, physics, microbiology, nuclear science and electronics. This method provides information about the relative amount of one or more of these components.[1]

Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles relating to individual drugs and are published in the form of book called "Pharmacopoeia" (e.g. IP, USP, and BP). Quantitative chemical analysis is an important tool to assure that the raw material used and the intermediate products meet the required specifications. Every year number of drugs is introduced into the market. Also quality is important in every product or service, but it is vital in medicines as it involves life.

There is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, report of new toxicities and development of patient resistance and introduction of better drugs by the competitors. Under these conditions standard and analytical procedures for these drugs may not be available in Pharmacopoeias. In instrumental analysis, a physical property of the substance is measured to determine its chemical composition. Pharmaceutical analysis comprises those procedures necessary to determine the identity, strength, quality and purity of substances of therapeutic importance. [2]

Pharmaceutical analysis deals not only with medicaments (drugs and their formulations) but also with their precursors i.e. with the raw material on which degree of purity and quality of medicament depends. The quality of the drug is determined after establishing its authenticity by testing its purity and the quality of pure substance in the drug and its formulations.

Quality control is a concept which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production. The decision to release or reject a product is based on one or more type of control action. With the growth of pharmaceutical industry during last several years, there has been rapid progress in the field of pharmaceutical analysis involving complex instrumentation. Providing simple analytical procedure for complex formulation is a matter of most importance. So, it becomes necessary to develop new analytical methods for such drugs. In brief the reasons for the development of newer methods of drugs analysis are:

1. The drug or drug combination may not be official in any pharmacopoeias.
2. A proper analytical procedure for the drug may not be available in the literature due to Patent regulations.
3. Analytical methods for a drug in combination with other drugs may not be available.
4. Analytical methods for the quantitation of the drug in biological fluids may not be available.

The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable.

HPLC

HPLC is also called as high pressure liquid chromatography since high pressure is used to increase the flow rate and efficient separation by forcing the mobile phase through at much higher rate. The pressure is applied using a pumping system. The development of HPLC from classical column chromatography can be attributed to the development of smaller particle sizes. Smaller particle size is important since they offer more surface area over the conventional large particle sizes. The HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low.

Instrumentation of HPLC

The basic liquid chromatograph consists of six basic units. The mobile phase supply system, the pump and programmer, the sample valve, the column, the detector and finally a means of presenting and processing the results.

Mobile phase (solvent) reservoirs and solvent degassing: The mobile phase supply system consists of number of reservoirs (200 mL to 1,000 mL in capacity). They are usually constructed of glass or stainless steel materials which are chemically resistant to mobile phase**.**

Mobile phase: Mobile phases in HPLC are usually mixtures of two or more individual solvents. The usual approach is to choose what appears to be the most appropriate column, and then to design a mobile phase that will optimize the retention and selectivity of the system. The two most critical parameters for nonionic mobile phases are strength and selectivity.

Mobile phase preparation: Mobile phases must be prepared from high purity solvents, including water that must be highly purified. Mobile phases must be filtered through ≤1 μm pore size filters and be degassed before use.

MATERIALS AND METHOD

Azithromycin-Sura labs, Cefpodoxime-Sura labs, Water and Methanol for HPLC-LICHROSOLV (MERCK), Acetonitrile for HPLC-Merck, Triethylamine-Merck.

HPLC method development
Trails
Preparation of standard solution" Accurately weigh and transfer 10 mg of Azithromycin and Cefpodoxime working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.
Further pipette 0.5ml of the Azithromycin and 1ml of the Cefpodoxime stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.
Procedure: Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.
Mobile Phase Optimization: Initially the mobile phase tried was Methanol: Water and Water: Acetonitrile and Methanol: Phosphate Buffer: ACN with varying proportions. Finally, the mobile phase was optimized to Methanol: TEA Buffer (pH-3.8) in proportion 30:70 v/v respectively.
Optimization of Column: The method was performed with various columns like C18 column, Symmetry and Zodiac column. Symmetry ODS C18 (4.6mm×250mm, 5µm) particle size was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions:

Instrument used	:	Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature	:	37ºC
Column	:	Symmetry ODS C18 (4.6mm×250mm, 5µm) particle size
Mobile phase	:	Methanol: TEA Buffer (pH-3.8) (30:70v/v)
Flow rate	:	1ml/min
Wavelength	:	250 nm
Injection volume	:	20 µl
Run time	:	10 min

Method validation
Preparation of buffer and mobile phase
Preparation of Triethylamine (TEA) buffer (pH-4.2): Dissolve 1.5ml of Ttiethyl amine in 250 ml HPLC water and adjust the pH 3.8. Filter and sonicate the solution by vaccum filtration and ultra sonication.
Preparation of mobile phase: Accurately measured 360 ml (36%) of Methanol and 640 ml of buffer (64%) a were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.
Diluent Preparation: The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase	:	Methanol: TEA Buffer (pH-3.8) (30:70v/v)
Column	:	Symmetry ODS C18 (4.6mm×250mm, 5µm) particle size
Flow rate	:	1 ml/min
Wavelength	:	250 nm
Column temp	:	37ºC
Injection Volume	:	20 µl
Run time	:	10 minutes

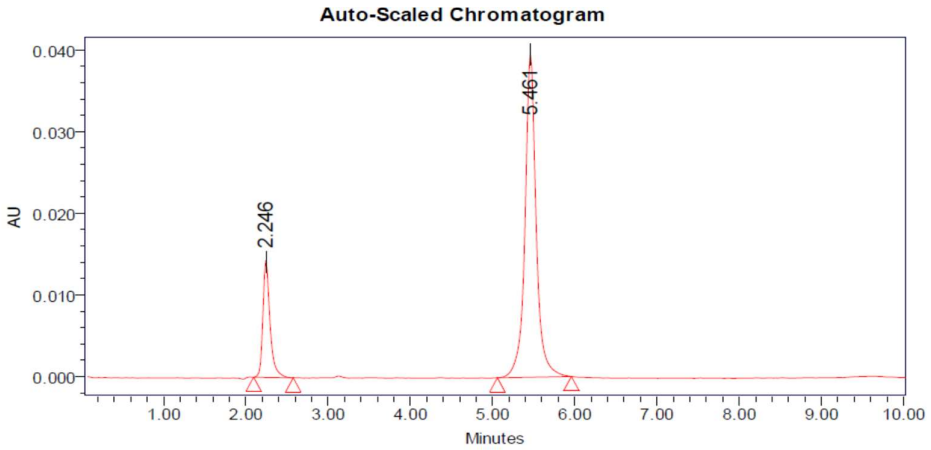

Fig 1: Optimized Chromatogram

Table 1: Peak Results for Optimized Chromatogram

S. No.	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Azithromycin	2.246	765789	69584		0.97	5587.0
2	Cefpodoxime	5.461	2532158	190049	2.97	1.26	5398.0

From the above chromatogram it was observed that the Azithromycin and Cefpodoxime peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

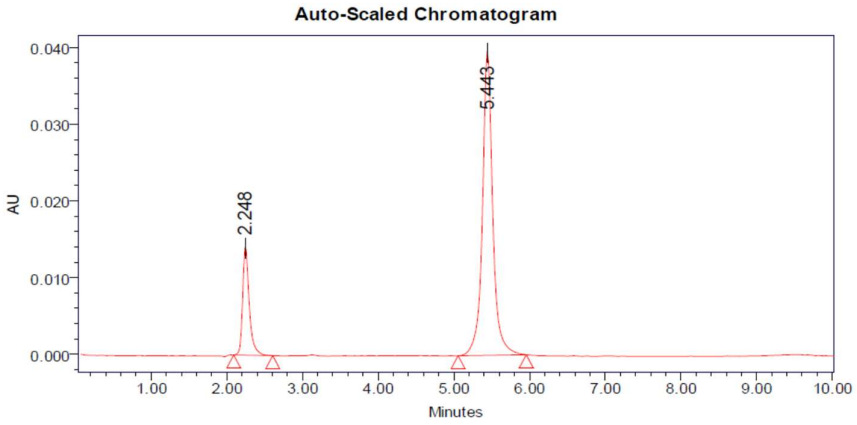

Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S. No.	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Azithromycin	2.248	775684	13124		0.99	6365.0
2	Cefpodoxime	5.443	2658478	937405	5.06	1.23	7458.0

- *Resolution between two drugs must be not less than 2.*
- *Theoretical plates must be not less than 2000.*
- *Tailing factor must be not less than 0.9 and not more than 2.*
- *It was found from above data that all the system suitability parameters for developed method were within the limit.*

System Suitability

Table 3: Results of system suitability for Azithromycin

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Azithromycin	2.247	765843	69587	5589	1.9
2	Azithromycin	2.246	766594	69854	5576	1.6
3	Azithromycin	2.248	765487	70211	5658	1.6
4	Azithromycin	2.252	765928	69213	5642	1.7
5	Azithromycin	2.248	765426	69558	5685	1.6
Mean			765855.6			
Std. Dev			466.6522			
% RSD			0.060932			

- *%RSD of five different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is suitable.*

Table 4: Results of system suitability for Cefpodoxime

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Cefpodoxime	5.452	2534658	190058	5365	1.2	2.07
2	Cefpodoxime	5.484	2536854	190052	5348	1.4	2.05
3	Cefpodoxime	5.491	2535879	190078	5389	1.5	2.0
4	Cefpodoxime	5.482	2533564	190035	5347	1.6	2.01
5	Cefpodoxime	5.491	2534214	190085	5364	1.6	2.01
Mean			2535034				
Std. Dev			1183.309				
% RSD			0.046678				

- *%RSD for sample should be NMT 2.*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Assay (Standard)

Table 5: Peak results for assay standard

Sno	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Azithromycin	2.256	759868	71255		1.7	5689	1
2	Cefpodoxime	5.427	2458754	215654	2.04	1.6	5362	1
3	Azithromycin	2.249	759458	72541		1.7	5748	2
4	Cefpodoxime	5.430	2465885	226565	2.00	1.6	5452	2
5	Azithromycin	2.248	759245	72584		1.7	5584	3
6	Cefpodoxime	5.443	2489578	221542	2.04	1.6	5456	3

Assay (sample)

Table 6: Peak results for Assay sample

Sno	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Azithromycin	2.247	756985	68958		0.98	7253	1
2	Cefpodoxime	5.452	2569856	198564	2.06	1.23	8836	1
3	Azithromycin	2.246	758745	69857		1.05	6530	2
4	Cefpodoxime	5.461	2598654	195682	2.04	0.99	7270	2
5	Azithromycin	2.243	756848	69588		1.7	7586	3
6	Cefpodoxime	5.466	2587454	192541	2.04	1.6	8371	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Azithromycin and Cefpodoxime in pharmaceutical dosage form was found to be 99.76 %.

Linearity
Chromatographic data for linearity study

Azithromycin

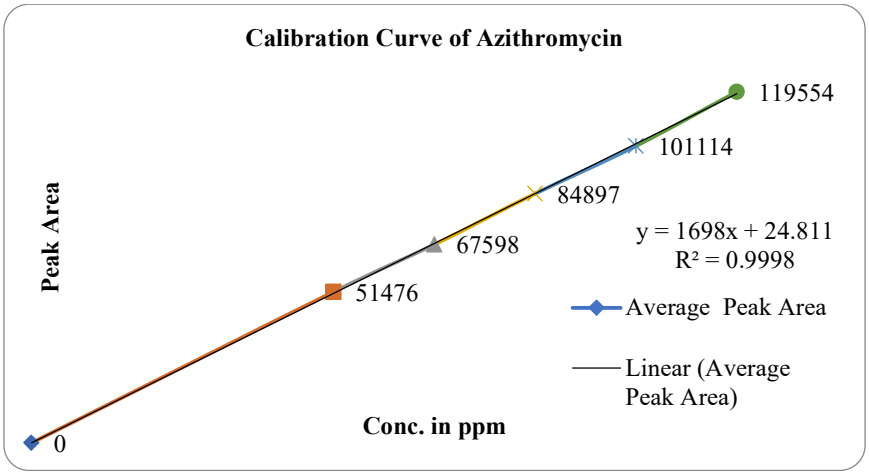

Fig 3: Calibration graph for Azithromycin

Cefpodoxime

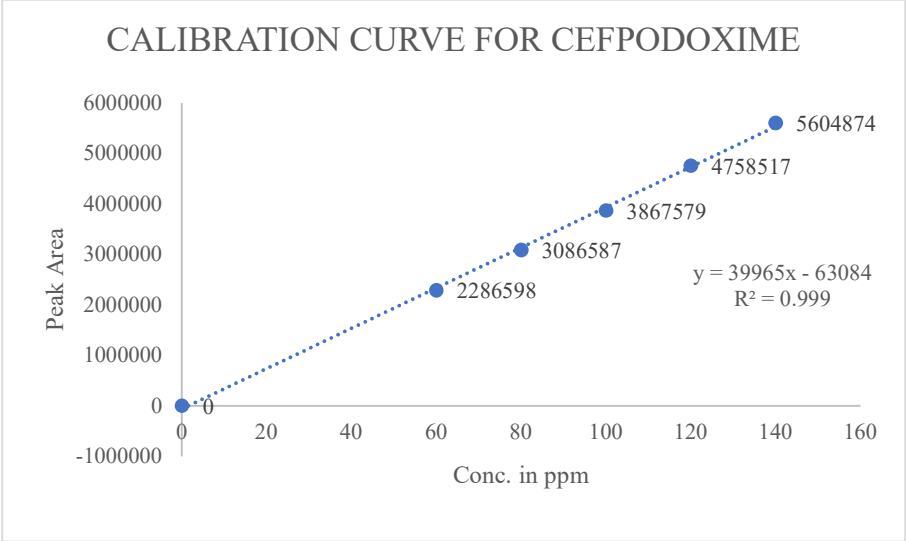

Fig 4: Calibration Graph for Cefpodoxime

Repeatability

Table 7: Results of Repeatability for Azithromycin

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Azithromycin	2.269	766854	702564	5685	1.6
2	Azithromycin	2.255	765884	698789	5584	1.4
3	Azithromycin	2.252	765842	701235	5521	1.6
4	Azithromycin	2.267	768985	700124	5525	1.9

5	Azithromycin	2.260	765845	698986	5578	1.7
Mean			766682			
Std. Dev			1357.973			
% RSD			0.177123			

- *%RSD for sample should be NMT 2.*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Table 8: Results of method precision for Cefpodoxime

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Cefpodoxime	5.274	2569865	2231111	5365	1.6
2	Cefpodoxime	5.266	2578474	2674210	5425	1.6
3	Cefpodoxime	5.265	2568985	2231261	5368	1.5
4	Cefpodoxime	5.278	2586845	2421301	5359	1.5
5	Cefpodoxime	5.305	2545898	2324710	5498	1.6
Mean			2570013			
Std. Dev			15309.45			
% RSD			0.595695			

- *%RSD for sample should be NMT 2.*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Intermediate precision
Day 1

Table 9: Results of Intermediate precision for Azithromycin

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Azithromycin	2.248	758955	68986	5785	1.6
2	Azithromycin	2.245	759869	68957	5698	1.4
3	Azithromycin	2.242	758985	68545	5689	1.6
4	Azithromycin	2.239	756894	68952	5781	1.9
5	Azithromycin	2.243	759854	68595	5785	1.7
6	Azithromycin	2.246	756985	68952	5693	1.6
Mean			758590.3			
Std. Dev			1339.793			
% RSD			0.176616			

- *%RSD of Six different sample solutions should not more than 2.*

Table 10: Results of Intermediate precision for Cefpodoxime

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Cefpodoxime	5.284	2659852	190025	5485	1.5	2.04
2	Cefpodoxime	5.293	2648574	190048	5421	1.6	2.03
3	Cefpodoxime	5.306	2659865	190054	5468	1.6	2.01
4	Cefpodoxime	5.319	2658547	190078	5487	1.6	2.05
5	Cefpodoxime	5.346	2648981	190016	5492	1.6	2.02
6	Cefpodoxime	5.352	2654652	190057	5463	1.6	2.03
Mean			2655079				
Std. Dev			5242.086				
% RSD			0.197436				

- *%RSD of Six different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is rugged.*

Day 2

Table 11: Results of Intermediate precision Day 2 for Azithromycin

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Azithromycin	2.255	766895	69858	5586	1.5
2	Azithromycin	2.260	765988	69854	5636	1.6

3	Azithromycin	2.242	766532	69824	5432	1.6
4	Azithromycin	2.245	766214	69875	5468	1.6
5	Azithromycin	2.260	765897	69854	5546	1.9
6	Azithromycin	2.255	765245	69848	5507	1.7
Mean			766128.5			
Std. Dev			567.7234			
% RSD			0.074103			

- *%RSD of Six different sample solutions should not more than 2.*

Table 12: Results of Intermediate precision for Cefpodoxime

Sno	Name	Rt	Area	Height	USP platecount	USP Tailing	USP Resolution
1	Cefpodoxime	5.266	2653254	190110	5428	1.6	7.98
2	Cefpodoxime	5.265	2648985	190058	5452	1.6	6.4
3	Cefpodoxime	5.306	2658213	190142	5498	1.6	8.9
4	Cefpodoxime	5.293	2653652	190031	5442	1.5	8.3
5	Cefpodoxime	5.265	2648978	190058	5489	1.5	7.5
6	Cefpodoxime	5.266	2658985	190047	5463	1.6	5.3
Mean			2653678				
Std. Dev			4313.355				
% RSD			0.162543				

- *%RSD of Six different sample solutions should not more than 2.*
- *The %RSD obtained is within the limit, hence the method is rugged.*

Accuracy

Table 13: The accuracy results for Azithromycin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	42594.67	25	25.070	100.280%	100.14%
100%	84867	50	49.965	99.930%	
150%	127654	75	75.164	100.218%	

Table 14: The accuracy results for Cefpodoxime

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	2079124	50	50.445	100.890%	100.56%
100%	4082412	100	100.571	100.571%	
150%	6070195	150	150.309	100.206%	

- *The percentage recovery was found to be within the limit (98-102%).*

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

ROBUSTNESS

Table 15: Results for Robustness

Azithromycin

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	765789	2.246	5387.0	0.97
Less Flow rate of 0.9 mL/min	758698	2.505	5458	0.96
More Flow rate of 1.1 mL/min	7689584	2.046	5696	0.94
Less organic phase	758412	2.505	5586	0.92
More organic phase	769852	2.046	5355	0.95

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Cefpodoxime

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	2532158	5.461	5398	1.26
Less Flow rate of 0.9 mL/min	2458692	5.599	5329	1.25
More Flow rate of 1.1 mL/min	2658642	4.576	5256	1.24

Less organic phase	2452148	5.599	5214	1.23
More organic phase	2653894	4.576	5524	1.22

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Azithromycin and Cefpodoxime in bulk drug and pharmaceutical dosage forms. Azithromycin is soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide (DMF), insoluble in acetone, chloroform & ether. Cefpodoxime was found to be freely soluble in glacial acetic acid, chloroform, slightly soluble in water, soluble in glacial acetic acid, slightly soluble or soluble in dichloromethane, slightly soluble in methanol. Methanol: TEA Buffer (pH-3.8) (30:70v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Azithromycin and Cefpodoxime in bulk drug and in Pharmaceutical dosage forms.

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