Asian Journal of Advances in Medical Science

3(1): 416-425, 2021

A STUDY ON DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF CEFPODOXIME IN BULK AND A PHARMACEUTICAL DOSAGE FORMS

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author SKSB did the conception and design. Authors NBJ, RT, RS and GS did the provision of study material or patients: All authors. Authors SKSB, NBJ, RT, RS and GS did the collection and assembly of data. Authors SKSB, NBJ, RT, RS and GS did the data analysis and interpretation. All authors did the manuscript writing, read and approved the final manuscript.

Received: 04 September 2021
Accepted: 08 November 2021
Published: 19 November 2021

Original Research Article

ABSTRACT

A simple, rapid, precise, sensitive and, reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of Cefpodoxime in a pharmaceutical dosage form. Chromatographic separation of Cefpodoxime was achieved on Waters Alliance -2695, by using Luna Pheny Hexyl (250mm x 4.6mm, 5µm) column and the mobile phase containing 0.1% TEA adj pH-2.5 with OPA & ACN in the ratio of 75:25% v/v. The flow rate was 1.0 ml/min, detection was carried out by absorption at 222nm using a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factor for Cefpodoxime were NLT 2000 and should not be more than 2 respectively. The linearity of the method was excellent over the concentration range 7-105 µg/ml for Cefpodoxime respectively. The correlation coefficient was 0.999. % Relative standard deviation of peak areas of all measurements always less than 2.0. The proposed method was validated according to ICH guidelines. The method was found to be a simple, economical, suitable, precise, accurate & robust method for quantitative analysis of Cefpodoxime and study of its stability.

Keywords: HPLC; Cefpodoxime; stability; pharmaceutical dosage.

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1. INTRODUCTION

1.1 Drug

A drug may be defined as a substance meant for diagnosis, cure mitigation, prevention or treatment of diseases in human beings or animals or for altering any structure or any function of the body.

The development of human civilization plays a vital role in finding cures for diseases. Today the majority of drug users is of synthetic origin. These are produced in the bulk and used for their therapeutic effects in pharmaceutical formulations [1-2]. There are biologically active chemical substances generally formulated into convenient dosage forms such as tablets, capsules, ointments and, injectables, these formulations deliver the drug substance in stable, non-toxic and acceptable form, ensuring its bioavailability and, therapeutic activity [3-4].

1.2 Methodology

Purpose and objectives of the study

In order to develop a simple, reliable and, accurate method for the assay of Cefpodoxime tablet dosage form by reverse-phase HPLC and validate the method for its repeatability and reproducibility [5-6].

The plan work includes:

- ✔ Procurement of raw materials.
- ✔ Establishment of system suitability parameters.
- ✔ Trials for the method development for Cefpodoxime.
- ✔ Setting the optimized method.
- ✔ Validation of optimized methods for Cefpodoxime.

Validation parameters like:

- ❖ System suitability and system precision.
- ❖ Specificity
- ❖ Linearity and range.
- ❖ Accuracy.
- ❖ Precision.
  - ● Method precision
  - ● System precision
  - ● Intermediate precision.
- ❖ Robustness
- ❖ Force degradation

2. MATERIALS AND EQUIPMENTS

1. Chemicals and Reagents:

Table 1. Chemicals and reagents

S. NO	NAME of REAGENT	MAKE	GRADE
1	Water	Rankem	HPLC Grade
2	Triethyl amine	Merck	HPLC Grade
2	Ortho phospharic Acid	Merck	HPLC Grade
3	Acetonitrile	Merck	HPLC Grade
4	Hydrochloric acid	Merck	Pure
5	Sodium hydroxide	Merck	Pure
6	Hydrogen peroxide	Merck	Pure
7	0.45 µm nylon filter	Zodiac life sciences	# 100428036

2. Standards and samples:

Table 2. Standards and Samples

S. NO	NAME of the DRUG	MANUFACTURER or SUPPLIER
1	Cefpodoxime working standard	Cipla pvt .Ltd
2	Cefpodoxime drug substance	Cipla pvt .Ltd
3	Cefpodoxime tablets 200 mg	Cipla pvt .Ltd

3. Instruments or equipments details:

Table 3. Instruments or equipment's

S. NO	INSTRUMENT NAME	MAKE and MODEL
1	HPLC	Waters(alliance 2695)
2	Ultra-sonicator	Unichrome
3	pH meter	GT Sonic
4	Electronic balance	Ohaus
5	HPLC column	Luna Pheny Hexyl, 250mm x 4.6mm, 5µm.
6	Centrifuge	Remi
7	Refrigerator	Whirlpool

2.1 Experimental Details

Analytical method development:

In the proposed project a successful attempt has been made to develop a simple, accurate for analysis of Cefpodoxime tablets (200mg) by RP-HPLC.

Method development parameters:

Selection of following parameters is very important in method development.

- Mode of chromatography.
- Wave length.
- Column.
- Mobile phase composition and buffer P^H.
- Column temperature.
- Solvent delivery system.
- Flow rate.
- Injection volume.

Selection of mode of chromatography:

Selected mode of chromatography: Reverse phase

Basis of selection: polarity of the molecule

Reason for selection: As Cefpodoxime is a polar molecule it elutes at faster rates along with the mobile phase.

Selection of detector wavelength:

Selection of detector wavelength is a critical step in finalization of the analytical method. To determine exact wave length standard API is prepared and injected into a chromatographic system with a PDA detector and the wave length which gives higher response for the compound [7-9].

3. RESULTS AND DISCUSSION

PDA Spectrum

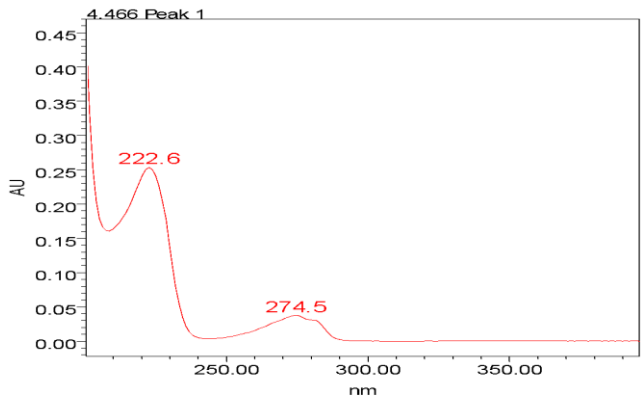

Fig. 1. PDA spectrum

S. NO	WAVE LENGTH
1.	222 nm

ANALYTICAL METHOD DEVELOPMENT OF FENOFIBRATE BY RP-HPLC METHOD TRAIL CHROMATOGRAMS

TRAIL -1

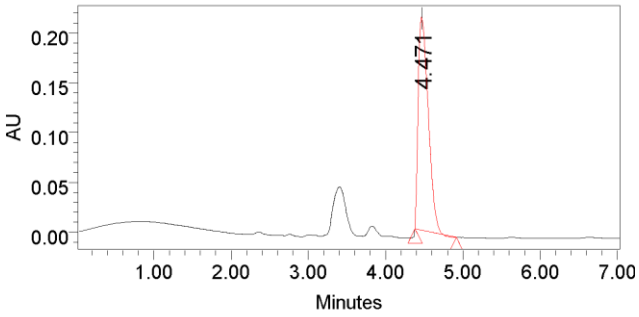

Fig. 2. Typical chromatogram trail 1

Name	Retention Time	Area	% Area	USP Tailing	USP Plate Count
1	4.471	1937340	100		

TRAIL - 2

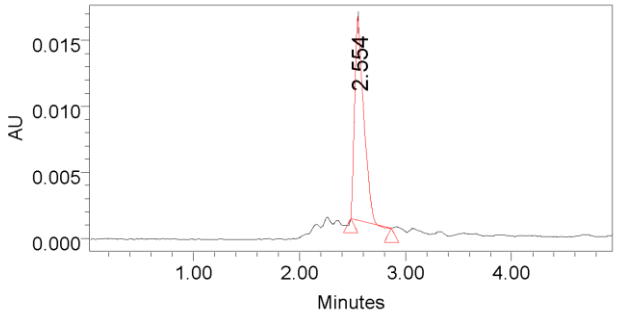

Fig. 3. Typical chromatogram trail 2

Name	Retention Time	Area	% Area	USP Tailing	USP Plate Count
1	2.554	92149	100		

TRAIL – 3

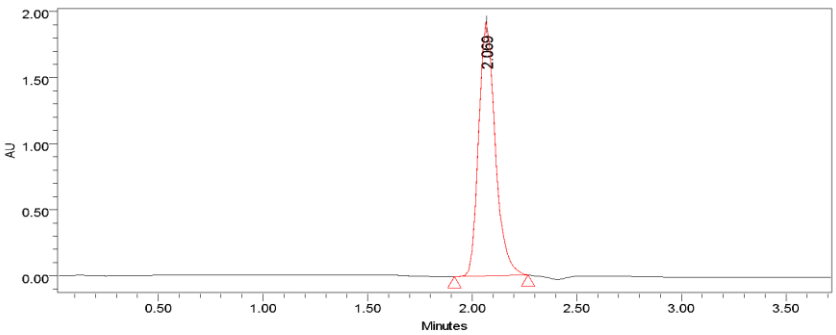

Fig. 4. Typical chromatogram trail 3

Name	Retention Time	Area	% Area	USP Resolution	USP Tailing	USP Plate Count
	2.088	9551426	100.12		1.14	3345

TRAIL – 4

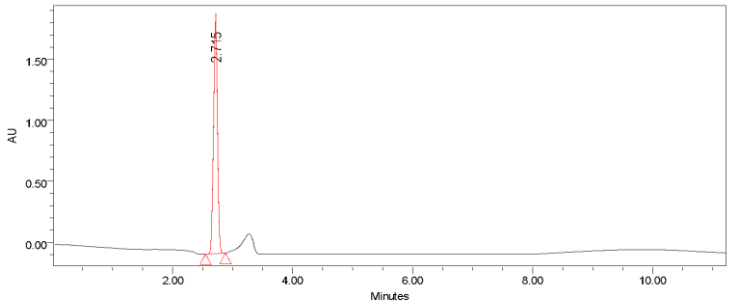

Fig. 5. Typical chromatogram trail 4

Name	Retention Time	Area	% Area	USP Tailing	USP Plate Count
1	2.715	8456322	100.48	1.14	2563

TRAIL – 5

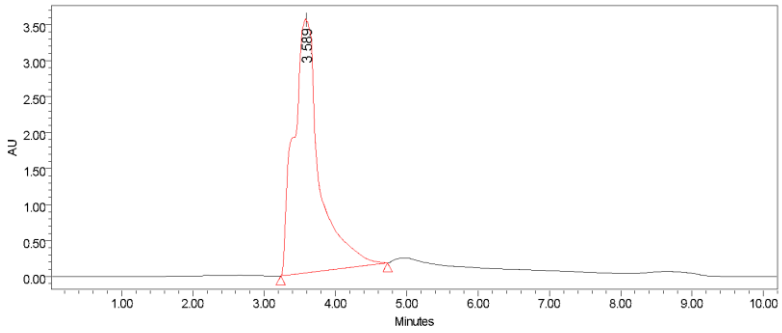

Fig. 6. Typical chromatogram trail 6

Name	Retention Time	Area	% Area	USP Tailing	USP Plate Count
1	3.589	18586274	100.82	3.56	1858

TRAIL – 6

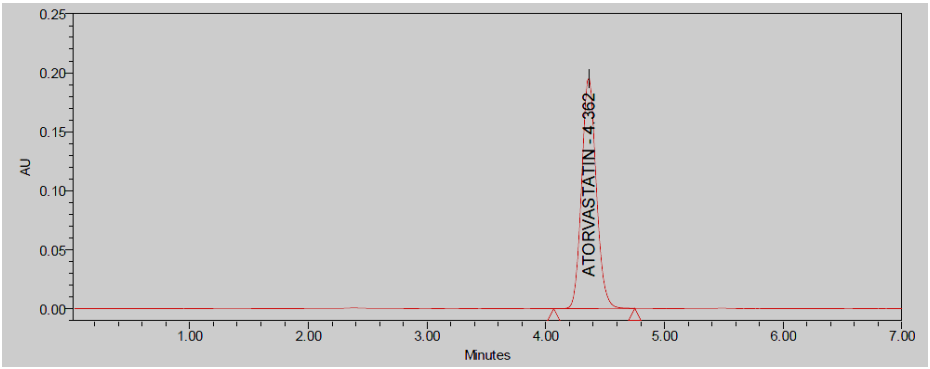

Fig. 7. Typical chromatogram trail 6

	Name	Retention Time	Area	% Area	USP Tailing	USP Plate Count
1	Fenofibrate	4.357	2062896	100	1.20	5666

ANALYTICAL METHOD VALIDATION OF FENOFIBRATE:-

System suitability:

Results for system suitability of Cefpodoxime**:**

Acceptance Criteria:

- The % RSD for the retention times of Cefpodoxime peaks from 6 replicate injections of each standard solution should be not more than 2.0 %**.**
- The % RSD for the peak area responses of Cefpodoxime peaks from 6 replicate injections of each standard solution should be not more than 2.0%.
- The number of theoretical plates (N) for the Cefpodoxime peaks is not less than 2000.
- The Tailing factor (T) for the Cefpodoxime peaks is not more than 2.0.

Table 4. Results for system suitability of cefpodoxime

Injection	Retention time (min)	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	3.179	1613079	5059	1.21
2	3.187	1608749	5035	1.21
3	3.211	1606510	4913	1.23
4	3.208	1610506	5135	1.21
5	3.222	1614302	5033	1.21
6	3.255	1608089	52250	1.20
Mean	3.210	1610206	5070	1.21
SD		3012.83	--	--
%RSD		0.1871	--	--

Specificity:

BLANK

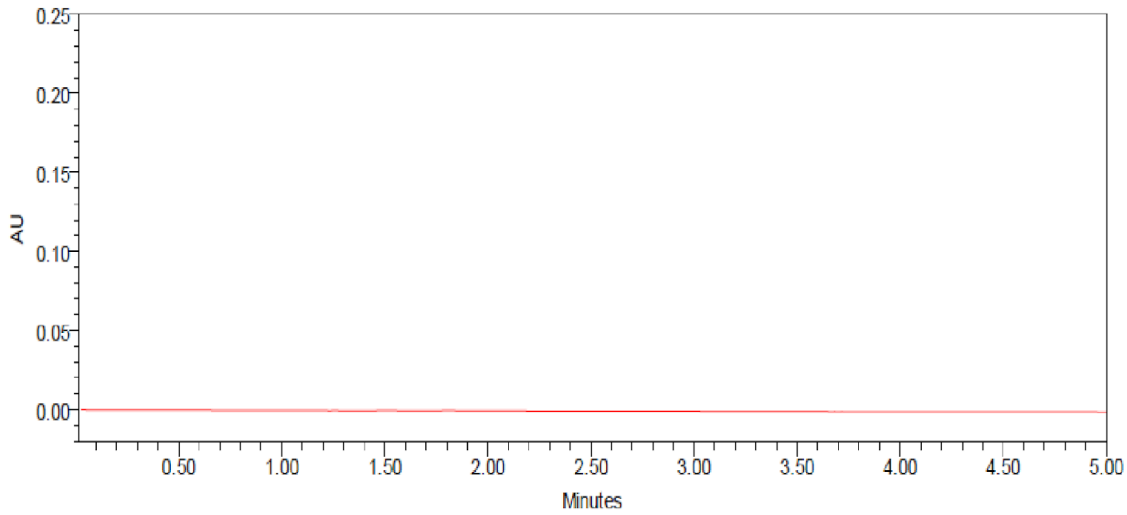

Fig. 8. Typical chromatogram of blank

S. No.	Name	Rt (min)	Peak Area	Efficiency
1	Blank	-	-	-

Acceptance Criteria:-

Chromatograms of blank should not show any peak at the retention time of analytic peak.

SAMPLE

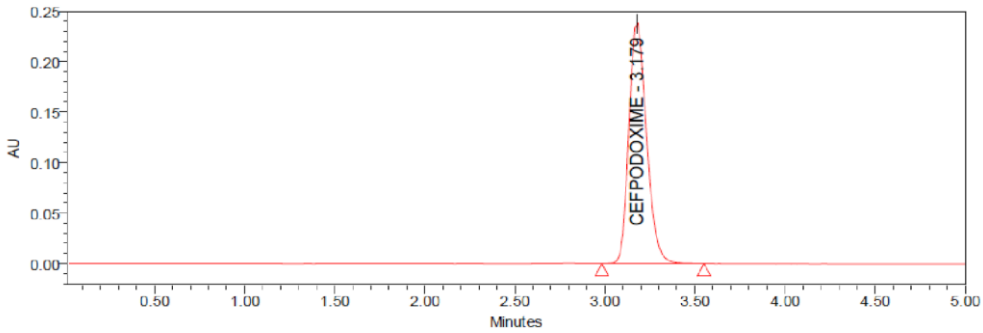

Fig. 9. Typical chromatogram of sample

S. No.	Name	Rt (min)	Peak Area
1	Cefpodoxime	3.179	1608089

STANDARD

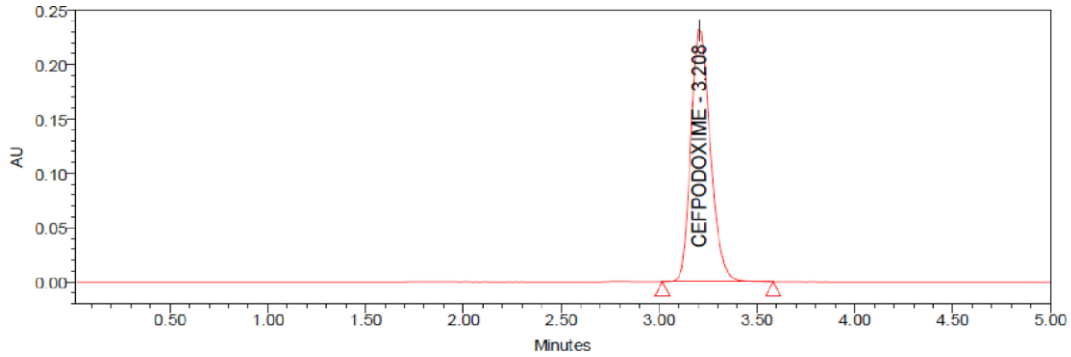

Fig. 10. Typical chromatogram of standard

S.No.	Name	Rt (min)	Peak Area
1	Cefpodoxime	3.208	1606510

Linearity:-

Linearity of detector response for Cefpodoxime:

Table 5. Linearity of detector response for atrovastatin

S.No.	Conc.(µg/ml) of Cefpodoxime	Area	Acceptance criteria
		Cefpodoxime	
1	7.0 µg/ml	214633	Squared correlation coefficient should be not less than 0.999.
2	17.5 µg/ml	524156	
3	35 µg/ml	1032584	
4	52.5 µg/ml	1526321	
5	70 µg/ml	2062896	
6	87.5 µg/ml	2524821	
7	105 µg/ml	3025963	

Linearity graphs of Atrovastatin:

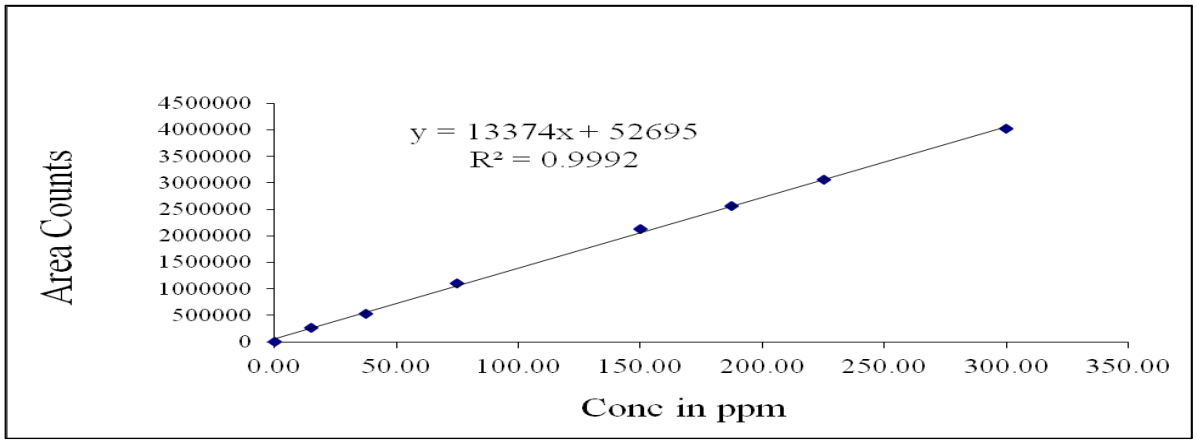

Fig. 11. linearity of detector response graphs for Atrovastatin

Table 6. linearity of detector response graphs for Atrovastatin

S. No	Linearity Parameters	Atrovastatin
1.	Linearity range	7.0-105 µg/ml
2.	Correlation coefficient	0.999
3.	Y intercept	13374x + 52695

Acceptance criteria:

1. Individual % recovery and mean % recovery of both drugs should be between 98.0 and 102.0 .
2. For replicate preparations the %RSD should not more than 2.0.
3. For linearity of test method, the squared correlation coefficient derived from least square fit of the data should not be less than 0.999.

Chromatographs of linearity:

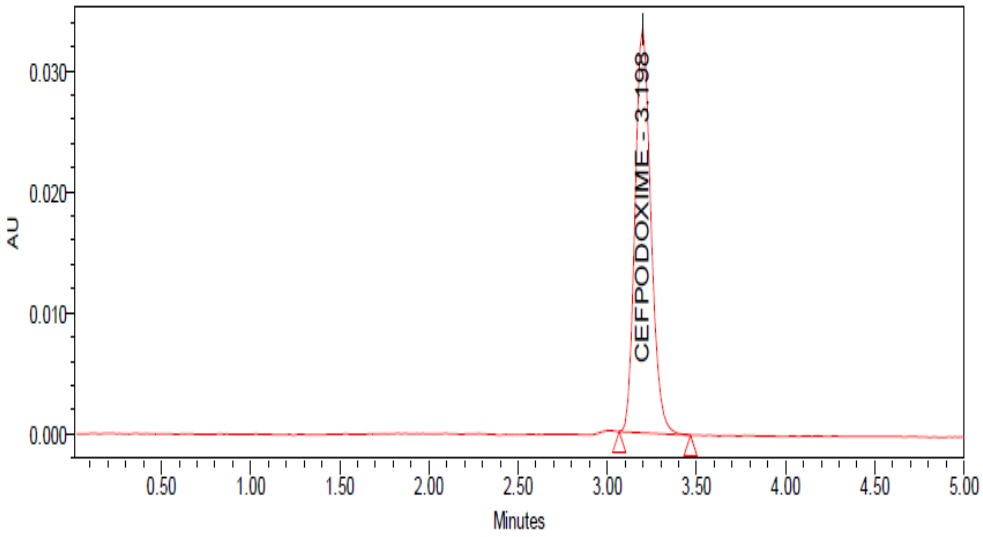

Fig. 12. Typical chromatogram of linearity 7.0 µg/ml

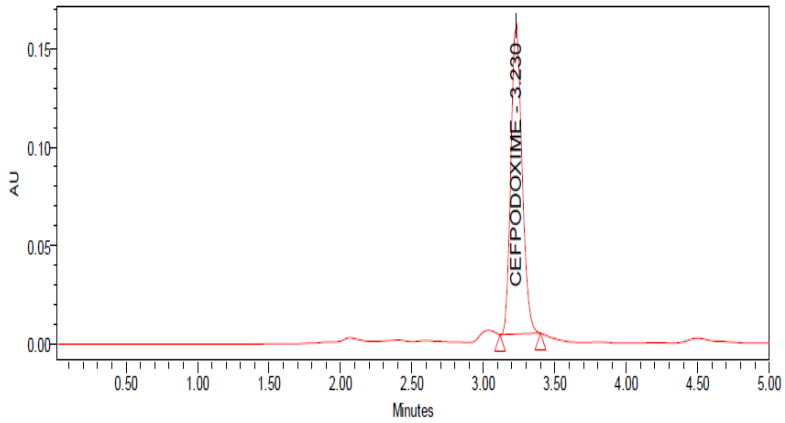

Fig. 13. Typical chromatogram of linearity 17.5 µg/ml

Summary of Results:

S. No	Parameter	Result
1.	System suitability	Relative standard deviation for area of Cefpodoxime peak for six replicate injections of standard solution 0.82 Tailing factor is 1.12 Theoretical plates are 3525
2.	Specificity Blank interferences	No peaks are observed in the blank chromatogram at the retention time of the Cefpodoxime peaks.
3	linearity	The square of correlation coefficient value of Cefpodoxime is 0.999.
4	accuracy	Individual % recovery for Cefpodoxime is found to be in the range of 95.0 to 105%. Mean % recovery for Cefpodoxime is found to be in the range of 95.0 to 105%. %RSD for of Cefpodoxime is found to be in the range of 0.10 to 1.52. For the linearity of the test method, the squared correlation coefficient derived from the least square fit of the data for Cefpodoxime is 0.99.
5.	Precision Method precision System precision Intermediate precision	The % assay of each individual preparation is found to be in range of 95.0 to 105%. The % RSD for assay of six replicate preparations of Cefpodoxime is 0.32 and 0.34. The %RSD of individual preparation of Cefpodoxime should be in the range of less than 2. i.e. 1.21
6.	Robustness From rate variation organic phase variation	All the parameters are found to be within the acceptable limits for all the robustness parameters.

4. CONCLUSION

Development and validation of RP-HPLC method for the estimation of Cefpodoxime in bulk and Pharmaceutical dosage forms with the facilities and the results are incorporated in this paper. In conclusion a validated RP-HPLC method has been developed for the determination of Cefpodoxime the bulk and tablet dosage forms. The results show that the method was found to be specific, simple, accurate, precise and sensitive. The method was successfully applied for the determination of the Cefpodoxime tablet dosage form.

Several analytical procedures have been proposed for the quantitative estimation of Cefpodoxime separately and in combination with other drugs.

So the attempt was taken to develop and validate a reversed-phase high performance liquid chromatographic method for the quality control of Cefpodoxime in pharmaceutical preparations with lower solvent consumption along with the short analytical run time that leads to an environmentally friendly chromatographic procedure and will allow the analysis of a large number of samples in a short period of time.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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