RESEARCH ARTICLE

Development and validation of RP-HPLC method for the estimation of Cefadroxil Monohydrate in bulk and its tablet dosage form

K. Govinda Rao, B. Uma Shankar[1], B. Phanindra[2], B. Mahesh Naik*

Department of Pharmaceutical Analysis & Quality Assurance
[1]Aditya Institute of Pharmaceutical Sciences & Research, Surampalem
[2]Bapatla College of Pharmacy, Bapatla

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ABSTRACT

A new simple and precise reverse phase high performance liquid chromatographic method has been developed and subsequently validated for the estimation of cefadroxil monohydrate in bulk and its pharmaceutical dosage form. The chromatographic separation was performed by using mobile phase consisting of KH_2PO_4: acetonitrile in the ratio of 65:35 % v/v and the pH 3.5 adjusted with 0.2% orthophosphoric acid. The column used was Hypersil ODS C_{18} (250 × 4.6 mm, 5µ) with flow rate of 1 ml/min using PDA detection at 220 nm. The described method was found to be linear over the range of 20-80µg/ml and correlation coefficient was found to be 1 with a retention time of 3.257min. The assay of cefadroxil was found to be 99.98 %. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise, reliable, accurate and economical which is useful for the routine determination of cefadroxil monohydrate in bulk and its pharmaceutical dosage form.

Keywords: cefadroxil monohydrate, method validation, ICH guidelines

INTRODUCTION

Cefadroxil hydrochloride is a beta lactam antibiotic. It binds to specific penicillin-binding proteins (PBPs) located inside the bacterial cell wall, causing the inhibition of the third and last stage of bacterial cell wall synthesis. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins; it is possible that cefadroxil interferes with an autolysin inhibitor.

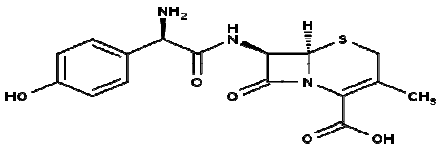

Fig.1. Structure of cefadroxil hydrochloride

(6*R*, 7R)-7-{[(2*R*)-2-amino-2-(4-hydroxyphenyl) acetyl] amino}-3-methyl-8-oxo-5-thia azabicyclo -[4.2.0] oct-2-ene carboxylic acid[9]. Literature survey reveals the availability of three analytical methods for the analysis of cefadroxil hydrochloride by RP-HPLC.[1,2,3] and two bio analytical methods are available for estimation of cefadroxil in bulk and pharmaceutical dosage form.[4,5] , two HPTLC methods are also available for this cefadroxil formulation. [6, 7] The reported RP-HPLC methods are not economical in terms of mobile phase composition, flow rates and less efficient. Hence there is a need to develop an RP-HPLC method for the estimation of cefadroxil in the tablet formulations. The aim of the present analytical research is to develop simple, precise, accurate, rapid and economical RP-HPLC method for the assay of cefadroxil hydrochloride in tablet formulation.

Address for correspondence

B. Mahesh Naik,
Department of Pharmaceutical Analysis and Quality Assurance, Surampalem
Email: maheshbhukya89@gmail.com

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MATERIALS

Cefadroxil monohydrate gift sample was provided by Sipra labs, Hyderabad. A commercial tablets containing cefadroxil monohydrate 100 mg was purchased from local market, Hyderabad. All other chemicals used were of HPLC grade.

METHODS

Selection of wavelength of detection

Cefadroxil standard solution of 100 ppm was scanned at 200-400 nm and UV Spectrum was recorded. By observing the spectrum of standard solution, λmax of

220 nm was taken for trails to develop the proposed method.

Instrumentation and Chromatographic Conditions

High performance liquid chromatography waters 2690 series equipped with PDA detector and Hypersil ODS C_{18} (250 mm × 4.6 mm) ODS column containing 5 µm particle size column was used. Mobile phase comprising of Phosphate buffer: acetonitrile in a ratio 65:35 % v/v at p^H 3.5 adjusted with 0.2 % orthophosphoric acid at a flow rate of 1 ml/min and the effluent was detected at 220 nm. The Column temperature was maintained at ambient and the volume of injection is 10 µL.

Preparation of buffer

Phosphate buffer was prepared by dissolving 7 gm. of potassium dihydrogen orthophosphate in 1000 mL of double distilled water. P^H was adjusted to 3.5 with 0.2% ortho phosphoric acid and solution was filtered through 0.45 µ Millipore Nylon filter.

Mobile phase preparation

Degassed buffer and acetonitrile were taken in the ratio of 65:35%, sonicated for 15 minutes and filtered through 0.45µ Millipore Nylon filter under Vacuum filtration. The prepared solution was used as Mobile phase.

Preparation of solutions

Standard stock solution

10mg of Cefadroxil RS drug was weighed and dissolved in 10ml of Mobile phase and taken in 10ml of volumetric flask and sonicated for 20 minutes to get 1000ppm and 1 ml was taken from this and diluted to 10 ml with mobile phase.

Working Standard solution

The stock solution equivalent to 25ppm to 150ppm were prepared, sonicated and filtered through 0.45µ membrane.

Sample stock solution

Twenty tablets containing Cefadroxil of each marketed formulation were taken and powdered. The powder equivalent to 500 mg of the active ingredient was accurately weighed and taken in a 100ml volumetric flask containing 50 ml mobile phase and sonicated for 15 minutes and the solution was made up to volume with mobile phase and filtered through 0.45micron membrane.

Method validation

The method was validated in terms of the following parameters; linearity, specificity, accuracy, precision, and system suitability parameters as per the ICH guidelines[8].

Specificity

To determine specificity, a volume of 10µl of working standard, sample and blank solution were injected separately and the chromatograms were recorded and are shown in fig. 2 and 3.

Linearity

A series of working standard solutions of cefadroxil monohydrate were prepared in the concentration range from 20-80(µg/mL) and injected into the chromatographic system. A calibration graph is plotted between concentration (µg/mL) and chromatographic peak area (mV). The results are tabulated in Table no.1 and linearity graph was shown in Fig 4.

Accuracy studies

A known amount of working standard at three different levels i.e. 50%, 100%, and 150% were added to pre analysed sample solution of 100% concentration and injected each three times in to the chromatographic system. From this % recovery was calculated. Results of the recovery studies are shown in Table no.2

Precision Studies

System precision:

The system precision was established by injecting six replicate injections of working standard solution into the chromatographic system and the results are shown in table no.3

Method precision:

The method precision was established by injecting six freshly prepared sample solutions into the

chromatographic and the results are shown in table no.4

RESULTS AND DISCUSSION

The proposed method was developed and validated as per the ICH guidelines. Linearity was observed over a concentration range of 20 to 80 µg/ml. System suitability parameters were satisfactory and the theoretical plates were obtained above 2000. Tailing factor was found below 2. %RSD also found below 2%. The assay of cefadroxil hydrochloride was found to be 99.98% and the low % RSD value confirms the robustness of the method.

Table 1: Data of linearity study

Sl. No.	Concentration (µg/mL)	Peak Area	Statistical analysis
1	20	1710658	Slope=85508 Intercept=188.2 Correlation coefficient 1
2	30	2565406	
3	40	3420581	
4	50	4275443	
5	60	5130645	
6	70	5985694	
7	80	6840877	

Table 2: Data of Accuracy (Recovery studies)

Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% injection 1	19.99	20	99.95	**MEAN**	100.02
50% injection 2	20.01	20	100.05	**%RSD**	0.057
50% injection 3	20.01	20	100.05		
100 % injection 1	39.99	40	99.97	**MEAN**	99.98
100 % injection 2	40.00	40	100.00	**%RSD**	0.017
100% injection 3	39.99	40	99.97		
150% injection 1	59.99	60	99.98	**MEAN**	99.99
150% injection 2	60.00	60	100.00	**%RSD**	0.011
150% injection 3	60.00	60	100.00		

Table 3: Data of system precision

	Injection	Peak Areas	%Assay
Concentration 40ppm	1	3420032	99.98
	2	3420106	99.99
	3	3420087	99.98
	4	3420825	100.01
	5	3420540	100.00
Statistical Analysis	**Mean**	3420318	99.99
	SD	348.724	0.013
	% RSD	0.0101	0.013

Table 4: Data of method precision

	Injection	Peak Areas	%Assay
Concentration 40ppm	1	3420653	100.00
	2	3420106	99.98
	3	3420885	100.01
	4	3420825	100.01
	5	3420438	99.99
	6	3420349	99.99
Statistical Analysis	**Mean**	3420543	99.99
	SD	299.4052	0.0121
	% RSD	0.0087	0.0121

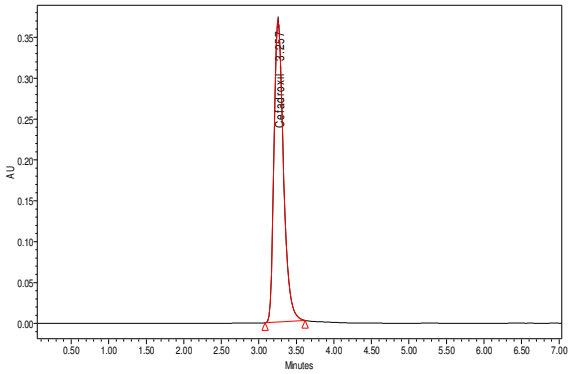

Fig. 2: Chromatogram of cefadroxil hydrochloride in standard solution

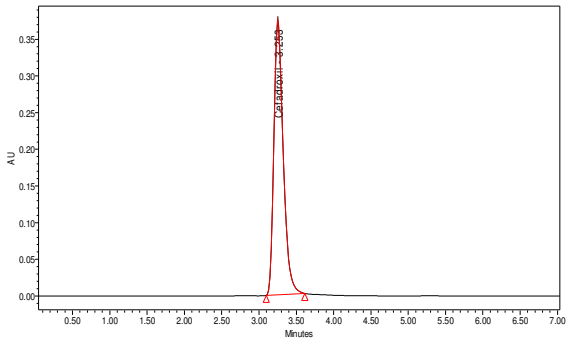

Fig.3: Chromatogram of cefadroxil hydrochloride in sample solution

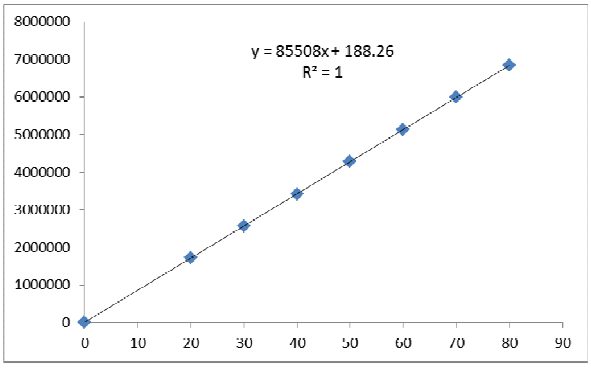

Fig.4: Calibration curve of cefadroxil hydrochloride (20-80 µg/ml)

Table 5: Summary of Validation Parameters

S. No	Parameter	Results
1.	Linearity Range	20-80 µg/ml
2.	Regression equation Slope Intercept Correlation coefficient(r^2)	Y=85508x + 188.2 85508 188.2 1
3.	Accuracy	99.98-100.02
4.	Precision System precision Method precision	 0.013 0.012
5.	LOD µg/ml	0.0085
6.	LOQ µg/ml	0.26

CONCLUSION

The proposed RP-HPLC method was found to be simple, accurate, precise, linear, robust and specific for quantitative estimation of Cefadroxil Monohydrate in bulk and its formulation. The proposed RP-HPLC method was cost effective and less time consuming. The values for system suitability parameters showed feasibility of this method for routine pharmaceutical application. Hence, the present RP-HPLC method is suitable for routine assay of cefadroxil monohydrate in bulk and tablet dosage form in the quality control laboratories.

ACKNOWLEDGMENTS

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REFERENCES

1. Arshad Anjum, Development and Validation of Rp-Hplc Method for the Quantitative Estimation of Cefadroxil Monohydrate in Bulk and Pharmaceutical Dosage Forms; Int. J. Chem. Sci.: 10(1), 2012, 150-158.
2. Sheetal Vikas patil; Reverse phase HPLC Method for the analysis of cefadroxil in pharmaceutical dosage form, International Journal of Pharmaceutical Research and Development; 2011; vol 3(6): August 2011(155-160).
3. Roopam Devaliya and U. k. Jain Novel estimation of cefadroxil in tablet dosage form by Rp-Hplc, Oriental journal of chemistry; 2009; vol.25 (4), 1053-1058.
4. Prashant U Tompe , Nandan V Shinde, Development and Validation of Bio analytical method for the determination of Ambroxol Hydrochloride and Cefadroxil in Human Plasma, International Journal of Research in Pharmaceutical and Biomedical Sciences; Vol. 4 (4) Oct – Dec 2013.
5. Janaki Sankarachari Krishnan Nagarjuna, Simultaneous pharmacokinetic assessment of cefadroxil and clavulanic acid in human plasma by LC–MS and its application to bioequivalence studies; Journal of Pharmaceutical Analysis 2013; 3(4):285–291.
6. Vasudha B.Pisal et.al, High performance thin layer chromatographic determination of Potassium clavulanate and cefadroxil in combined Tablet dosage form, Der Pharmacia Sinica, 2011, 2 (2): 79-85.
7. Madhura V Dhoka*, Shakuntala S. Chopade, Method Development & Comparative Statistical Evaluation of HPLC & HPTLC Method for Simultaneous Estimation of Cefadroxil Monohydrate & Ambroxol Hydrochloride, Indo Global Journal of Pharmaceutical Sciences, 2012; 2(2): 203-212.
8. ICH, Validation of Analytical Procedures: Methodology, ICH – Q2B, International Conference on Harmonization, 1996, 1-3.
9. Indian Pharmacopoeia, 2010, vol-1, vol-2, 167, 2103-2105.

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