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Development and Validation of RP-HPLC Method for Simultaneous Estimation of Cefpodoxime Proxetil and Ambroxol Hydrochloride in Bulk and in Tablets

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Abstract

A new simple**,** precise, accurate and selective RP-HPLC method has been developed and validated for simultaneous estimation of cefpodoxime proxetil and ambroxol hydrochloride in tablet dosage form. The method was carried out on a Qualisil RP C-8 (250 mm x 4.6 mm, 5 µm) column with a mobile phase consisting of acetonitrile: 0.025 M potassium dihydrogen phosphate buffer (70:30 *v/v*) pH adjusted to 4.0 with *ortho*-phosphoric acid and flow rate of 1.0 mL/min. Detection was carried out at 248 nm. Diclofenac sodium was used as an internal standard. The retention time for cefpodoxime proxetil, ambroxol hydrochloride and diclofenac sodium was found to be 3.89, 2.69 and 5.52 min, respectively. The cefpodoxime proxetil and ambroxol hydrochloride followed linearity in the concentration range of 3- 18 µg/mL (r^2= 0.9981) and 2- 12 µg/mL (r^2= 0.9980), respectively. The amount of both these drugs estimated by proposed method was found to be in good agreement with label claim. The developed method was validated for sensitivity, accuracy, precision, ruggedness and robustness. The LOD and LOQ were found to be 0.18 and 0.55 µg for cefpodoxime proxetil and 0.09 and 0.30 µg for ambroxol hydrochloride. The proposed method can be used for routine analysis of both these drugs simultaneously in their combined dosage form.

Keywords: Cefpodoxime Proxetil; Ambroxol Hydrochloride; RP-HPLC.

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1. Introduction

Cefpodoxime proxetil, (6R,7R)-7-{[(2*Z*)-2-(2-amino-1,3-thiazol-4-yl)-2-methoxyimino-acetyl]amino}-3-(methoxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2 carboxylic acid is a broad-spectrum antibiotic implicated in the treatment of upper respiratory tract and urinary tract infections [1,2]. The drug is official in United States Pharmacopeia [3].The recommended dose of cefpodoxime proxetil is 200 to 400 mg per day [4].

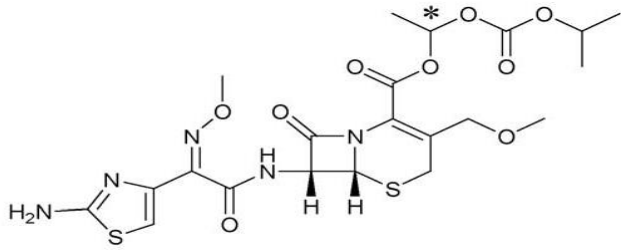
In literature, several analytical methods such as RP-HPLC [5-7] and voltammetric [8, 9] have been reported for the determination of cefpodoxime proxetil in biological fluids. Few RP–HPLC [10, 11], some hyphenated techniques such as LC/MS/MS [12], LC-MS, LC-NMR and LC-IR [13], UV-Spectrophotometric [14-16] and HPTLC [17, 18] methods have been studied for determination of cefpodoxime proxetil in bulk and in pharmaceutical formulations. One RP-HPLC method has been studied for

determination of cefpodoxime proxetil in combination with other drugs from pharmaceutical formulation [19].

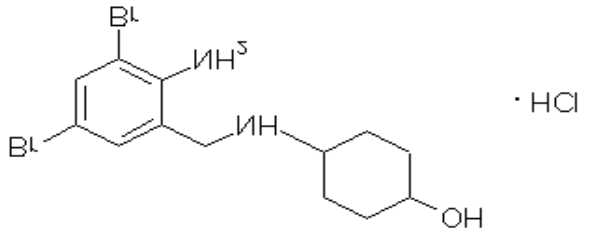
Ambroxol hydrochloride, trans- 4-[(2-amino-3, 5-dibromobenzyl) amino] cyclohexanol hydrochloride, is an active metabolite of bromohexine and used as bronchosecretolytic and expectorant [20]. The drug is official in Indian Pharmacopoeia [20], British Pharmacopoeia [21] and European Pharmacopoeia [22]. The dose of ambroxol hydrochloride is 30/60 mg per day.

In literature, HPLC–MS/ESI [23] method was found for determination of ambroxol hydrochloride in human plasma. Few RP-HPLC [24-27], Raman Spectroscopy [28], stability–indicating RP-HPLC [29], HPTLC [30] have been studied for estimation of ambroxol hydrochloride in bulk and in pharmaceutical formulations.

LC-MS/MS [31, 32] have been studied for the determination of ambroxol hydrochloride in combination with other drugs from human plasma. Many analytical techniques such as RP-HPLC [33-36], UV-Spectrophotometric [37-39], HPTLC [40] have been reported for the determination of ambroxol hydrochloride in combination with other drugs from pharmaceutical formulations. The chemical structures of both drugs are as shown in *Fig. 1*.



(a)



(b)

***Fig. 1*: Chemical structures of cefpodoxime proxetil (a) and ambroxol hydrochloride**

To our knowledge, no RP-HPLC method has been studied so far for the simultaneous determination of cefpodoxime proxetil and ambroxol hydrochloride in a combined dosage form.

Therefore, an objective of this work is to develop simple and economical RP-HPLC method for the simultaneous determination of both these drugs in their combined dosage form. The second objective is to validate the method as per the ICH guidelines [41-43].

2. Materials and Methods

2.1.Chemicals

Cefpodoxime proxetil, Ambroxol hydrochloride and Diclofenac sodium were obtained from Alkem Laboratories Ltd., Mumbai, India as a gift sample. Potassium dihydrogen ortho-phosphate (AR Grade), acetonitrile (HPLC Grade), was purchased from Merck (India) Ltd., Worli, Mumbai, India. Tablets (Finecef-AM 100) were purchased from Indian market, containing cefpodoxime proxetil 100 mg and ambroxol hydrochloride 60 mg per tablet.

2.2. Instrumentation and Chromatographic Conditions

Analysis was performed on chromatographic system of Shimadzu (Japan) liquid chromatograph comprising LC-10AT-vp solvent delivery system (pump), SPD M-10 A-vp Diode array detector, CTO-10AS- vp as a column oven and a Rheodyne injector with 20 µL loop. Class-M 10 A data station was used as a data processer. A LC-GC Qualisil C-8 column (250 mm x 4.6 mm i.d., 5-µm) was used for chromatographic separation under suitable conditions. The mobile phase consists of acetonitrile and 0.025 M Phosphate buffer (70:30 *v/v*), pH adjusted to 4.0 with *ortho*-phosphoric acid at a flow rate of 1.0 mL/min and the run time was 10 min. Before analysis both the mobile phase and sample solutions were filtered through a 0.45 µm membrane filter and degassed for 15 min in an ultrasonicator. The detection of these drugs was carried out at 248 nm. The UV- spectra of all these three drugs in methanol is shown in *Fig.2*.

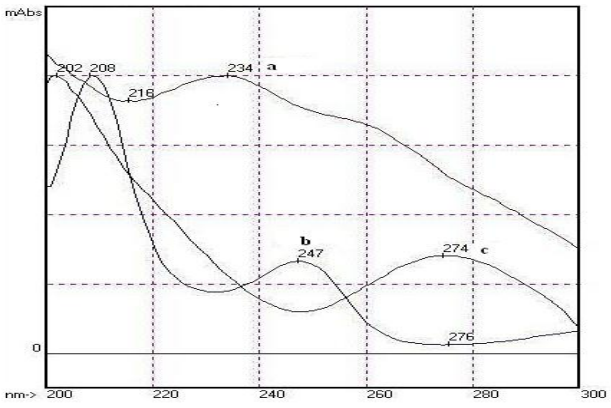


***Fig. 2*: A typical overlain spectrum of standard cefpodoxime proxetil (a), ambroxol hydrochloride (b) and diclofenac sodium (c)**

2.3 Preparation of Stock Standard Solutions and Calibration Graphs

Stock standard solutions of 1.0 mg/mL of cefpodoxime proxetil, ambroxol hydrochloride and

diclofenac sodium were prepared separately in methanol. The stock solution of cefpodoxime proxetil was diluted with the mobile phase to give working standard solutions containing 3 - 18 µg/mL concentrations, similarly the ambroxol hydrochloride stock solution was diluted with the mobile phase to give working standard solutions in the range 2 - 12 µg/mL. Diclofenac sodium, as internal standard (IS), was added at a constant level of 10 µg/mL to all the working standard solutions. These standard solutions were injected for construction of calibration curve by plotting drug-to-IS peak-area ratio (response factor) for each of the drugs against concentration.

2.4. **Analysis of marketed tablet formulation**

Twenty tablets (Finecef -AM 100) were weighed; average weight determined and crushed into fine powdered. An accurately weighed tablet powder equivalent to 50 mg of cefpodoxime proxetil and 30 mg of ambroxol hydrochloride was transferred into 50 mL volumetric flask containing 25 mL methanol, sonicated for 15 min and volume was made up to the mark with same solvent. The resulting solution was filtered using 0.45 µm filter (Mill filter, Milford, MA). From filtrate, 0.1 mL of solution was transferred into 10 mL volumetric flask, followed by addition of 0.1 mL of diclofenac sodium. Finally, the volume was made up to mark with mobile phase to obtain the concentration of 10 µg/mL of cefpodoxime proxetil and 6 µg/mL of ambroxol hydrochloride with 10 µg/mL of diclofenac sodium and was subjected to propose method and the amount of cefpodoxime proxetil and ambroxol hydrochloride were determined.

2.5. Method Validation

The HPLC method was validated in accordance with ICH guidelines [41- 43].

2.5.1.Precision

The precision of the method was studied as intra-day, inter-day and repeatability of sample injections. Intra-day precision was determined by analysis of the solutions three times on the same day. Inter-day precision was assessed by analysis of the solutions on three different days over a period of one week. Repeatability of sample injections was performed by injecting same concentration of the drugs for six times and effects on peak areas were examined.

2.5.2. **Specificity and Selectivity**

Specificity of the method was ascertained by analyzing drug standard and sample. The analytes should have no interference from other extraneous components and be well resolved from them. Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix.

The method is quite selective. There was no other interfering peak around the retention time of cefpodoxime proxetil and ambroxol hydrochloride; also, the base line did not show any significant noise.

2.5.3. Accuracy

The accuracy of the method was studied as % recovery studies. To the pre-analyzed sample solution (10 µg/mL of cefpodoxime proxetil and 6 µg/mL of ambroxol hydrochloride) a known amount of drug standard was added at three different concentration levels i.e. 80 %, 100 % and 120 % and re-analyzed by the proposed method.

2.5.4.Sensitivity

Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD)

and Limit of Quantitation (LOQ). LOD = 3.3 x ASD/S and LOQ = 10 x ASD/S, where

ASD is the average standard deviation and S is the slope of the line.

2.5.5.**Robustness**

Robustness of the method was studied by making deliberate variations in the chromatographic conditions such as variation of pH of the mobile phase; flow rate; column oven temperature and change in mobile phase composition. The robustness of the method was studied by using 10 µg/mL of cefpodoxime proxetil and 6 µg/mL solution of ambroxol hydrochloride, respectively.

2.5.6. **Ruggedness**

Ruggedness of the method was studied by two different analysts using same experimental and environmental conditions. It was performed by injecting 10 µg/mL of cefpodoxime proxetil and 6 µg/mL of ambroxol hydrochloride. Response Factor was measured for same concentration solutions for six times.

3. Results and Discussion

3.1. Selection of Chromatographic Conditions and Optimization of Mobile Phase

After trying columns containing different stationary phases, the final choice giving satisfactory resolution and run time was LC-GC Qualisil RP C-8 column (250 mm x 4.6 mm i.d., 5 µm). Mobile phase was optimized with a view to separate cefpodoxime proxetil and ambroxol hydrochloride with diclofenac sodium (IS). Initially, acetonitrile and water in the various proportions were tried as mobile phase but the splitting of the peaks for both these drugs was observed. Therefore, combination of acetonitrile and potassium dihydrogen phosphate buffer (0.025 M) in 70:30 % *v/v* was tried for resolution of both these drugs along with internal standard. Good resolution

and symmetric peaks were obtained for all three drugs when the pH of the mobile phase was adjusted to 4.0 and column oven temperature was kept at 30^0C. The flow rate of the mobile phase was 1.0 mL/min. Under optimum chromatographic conditions, the retention time for cefpodoxime proxetil, ambroxol hydrochloride and diclofenac sodium was found to be 3.89, 2.69 and 5.52 min, respectively when the detection was carried out at 248 nm. A typical chromatogram of all three drugs is shown in*Fig.3*.

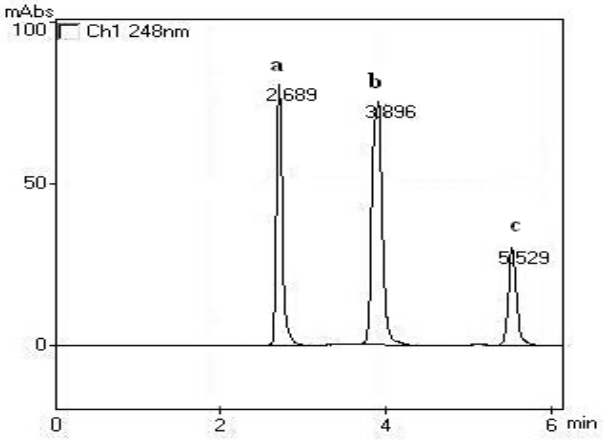


Fig. 3: Chromatogram obtained for cefpodoxime proxetil standard (b), ambroxol hydrochloride standard and diclofenac sodium (c) as IS in acetonitrile: 0.025 M potassium dihydrogen phosphate buffer (70:30 v/v), pH 4.0 as mobile phase

3.2. Linearity

The linearity was determined separately for cefpodoxime proxetil and ambroxol hydrochloride. Solutions of these drugs at six different concentrations were analyzed and calibration curves were constructed by plotting mean response factors against the respective concentrations. The method was evaluated by determining the correlation coefficient and intercept values. The cefpodoxime proxetil and ambroxol hydrochloride follow linearity in the concentration range of 3 - 18 µg/mL and 2 - 12 µg/mL; respectively. The results are shown in *Table I*.

***Table I*: Linearity Studies**

Parameter	Cefpodoxime Proxetil	Ambroxol Hydrochloride
Linearity [µg/mL]	3 – 18	2 – 12
Linearity Equation	Y= 0.1797X+0.2483	Y= 0.1997X+0.1230
Slope ± SD	0.1797 ±0.0001	0.1997 ±0.0005
Intercept ± SD	0.2483 ±0.0029	0.1230 ±0.0050
Correlation Coefficient± SD	0.9981 ±0.0001	0.9980 ±0.0007

3.3.Precision

The precision study was evaluated on the basis of % RSD value. The intra-day precision for cefpodoxime proxetil and ambroxol hydrochloride was found to be in the range 0.28 -0.75 and 0.27 - 0.92 %, respectively. And, the inter-day precision for cefpodoxime proxetil and ambroxol hydrochloride was found to be in the range 0.45 - 0.97 and 0.28 -1.43 %, respectively. The low values of % RSD indicate high precision of the method. Results of precision study are shown in *Table II*.

***Table II*: Results from Precision studies**

Drugs	conc. [µg/ml]	Intra-day		Inter-day	
		% Amount found [n =3]	% R.S.D.	% Amount found [n=3]	% R.S. D
Cefpodoxime proxetil	10	100.55	0.43	99.92	0.67
	15	100.16	0.28	100.12	0.45
	20	101.44	0.75	101.27	0.97
Ambroxol hydrochloride	6	100.52	0.27	99.88	0.28
	9	98.98	0.92	98.49	1.31
	12	100.45	0.54	100.02	1.43

3.4. Specificity and Selectivity

Specificity of the method was ascertained by comparing the chromatogram obtained from tablet and standard drug. The retention times of the standard drugs and the drugs from tablet solutions were same, so the method was specific. The method was also specific and selective because there was no interference from excipients in the tablets. The method is quite selective. There was no other interfering peak around the retention time of cefpodoxime proxetil and ambroxol hydrochloride; also, the base line did not show any significant noise.

3.5. Accuracy

The accuracy of the method studied at three different concentration levels i.e. 80 %, 100 % and 120 % showed affordable % recoveries in the range of 98.71 - 99.10 % for cefpodoxime proxetil and 98.23 - 100.38 % for ambroxol hydrochloride. The results are shown in *Table III*. The low value of % RSD indicates accuracy of the method.

***Table III*: Results of Recovery Studies**

Drugs	Label claim (mg/tablet)	Amount of standard drug added (%)	%Drug Recovered [n = 3]	% R.S.D.
Cefpodoxime Proxetil	100	80	98.71	0.66
		100	98.82	0.85
		120	99.10	0.48
Ambroxol Hydrochloride	60	80	99.26	1.08
		100	98.23	1.21
		120	100.38	0.45

3.6.Sensitivity

The LOD for cefpodoxime proxetil and ambroxol hydrochloride was found to be 0.18 and 0.09 µg, respectively. The LOQ for cefpodoxime proxetil and ambroxol hydrochloride was found to be 0.55 and 0.30 µg,

respectively. The low values of LOD and LOQ indicates adequate sensitivity of the method.

3.7.Robustness and Ruggedness study

Robustness of the method was studied by making deliberate changes in the chromatographic conditions and the effects on the results were examined. The content of the drugs was not adversely affected by these changes as evident from the low values of % relative standard deviation (less than 2 %) indicating robustness of the method.

When the method was performed by two different analysts under the same experimental and environmental conditions it was found to be rugged.

Results are reported in *Table IV*.

***Table IV*: Summary of Validation Parameter and System suitability Study**

Parameter	Cefpodoxime Proxetil	Ambroxol Hydrochloride
Linearity range [µg/mL]	3 – 18	2 – 12
Correlation coefficient	0.9981	0.9980
LOD [µg]	0.18	0.09
LOQ [µg]	0.55	0.30
% Recovery [n = 9]	98.88	99.29
Ruggedness [% R.S.D.]		
Analyst I [n = 6]	0.93	0.79
Analyst II [n =6]	0.80	0.57
Precision [%R.S.D.]		
Repeatability of Injection [n = 6]	1.21	1.15
Intra-day [n = 3]	0.28 – 0.75	0.27 – 0.92
Inter-day [n = 3]	0.45 – 0.97	0.28 – 1.43
Robustness	Robust	Robust
Specificity	Specific	Specific
Retention time [t_R]	3.896	2.689
Theoretical plates [N]	4706	5734
Capacity factor [k']	0.44	0.20
Resolution [Rs]	2.08	-
Asymmetry [T]	1.62	1.49

4. Analysis of marketed tablet formulation

Six replicates of the sample's solutions (20 µL) were injected for quantitative analysis. The amounts of cepfodoxime proxetil and ambroxol hydrochloride estimated were found to 99.43 % and 99.74 %, respectively. A good separation and resolution of these drugs indicates that there was no interference from the excipients commonly present in pharmaceutical formulations.

System Suitability Test

According to USP [3], system suitability test is integral part of liquid chromatographic methods. They are used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done. Earlier prepared solutions for chromatographic conditions were tested for system suitability testing.

The results obtained from validation of the methods and system suitability studies are summarized in *Table IV*.

5. Conclusion

The developed RP-HPLC method is simple, precise, accurate, selective and reproducible. The method has been found to be adequately rugged and robust and can be used for simultaneous determination of cefpodoxime proxetil and ambroxol hydrochloride in tablets. The method was validated as per ICH guidelines.

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