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RP- HPLC Method Development and Validation for Simultaneous Estimation of Ambroxol Hydrochloride and Cefpodoxime Proxetile in Pharmaceutical Dosage form

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ABSTRACT

A Reversed-Phase High Performance liquid chromatographic (RP-HPLC) method was developed for the simultaneous determination of Ambroxol Hydrochloride and Cefpodoxime Proxetile in combined tablet dosage form. The analysis was carried out using Phenomenex Luna C – 18, pre-packed column. Mobile phase, containing Acetonitrile: 0.05 M Potassium Dihydrogen Ortho Phosphate Buffer (70:30) pH adjusted to 6.7 with Tri ethyl Amine was pumped at a flow rate of 1.0 mL/min with UV-detection at 245 nm. Retention time was 3.34 ± 0.01 min and 4.77 ± 0.01 min for Ambroxol Hydrochloride and Cefpodoxime Proxetile, respectively. The method was validated for linearity, accuracy, precision, and specificity. The method showed good linearity in the range of 30 - 60 µg/ml for Ambroxol Hydrochloride and 50 - 100 µg/ml for Cefpodoxime Proxetile. The detection limit of the proposed method was 4.56 and 12.51 µg/ml and the quantification limit was 13.82 and 37.92 µg/ml for Ambroxol Hydrochloride and Cefpodoxime Proxetile, respectively. The % recovery was within the range between 99.57% and 100.27% for Ambroxol Hydrochloride and % recovery was within the range between 99.89% and 100.86% for Cefpodoxime Proxetile. The % R.S.D for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Ambroxol Hydrochloride and Cefpodoxime Proxetile in combined tablet dosage form.

Keywords Ambroxol Hydrochloride, Cefpodoxime Proxetile, High Performance Liquid Chromatography Method, Validation

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INTRODUCTION

Ambroxol Hydrochloride (AMB) is chemically trans–4–(2–Amino-3, 5–dibromobenzylamino)-cyclohexanol[1] is a secretolytic agent used in the treatment of tracheobronchitis, emphysema with bronchitis pneumoconiosis, chronic inflammatory pulmonary conditions, bronchiectasis, bronchitis with bronchospasm asthma[2]. It is official in Indian Pharmacopoeia (IP) and British Pharmacopoeia (BP). IP[1] describes High Performance Liquid Chromatography (HPLC) method and BP[3] describes HPLC, Spectrophotometric and Thin Layer Chromatography (TLC) method. Literature survey also reveals Spectrophotometric[4-5], HPLC[6-7], Ultra Performance Liquid Chromatography (UPLC) [8] and HPTLC[9] methods for determination of AMB with other drugs.

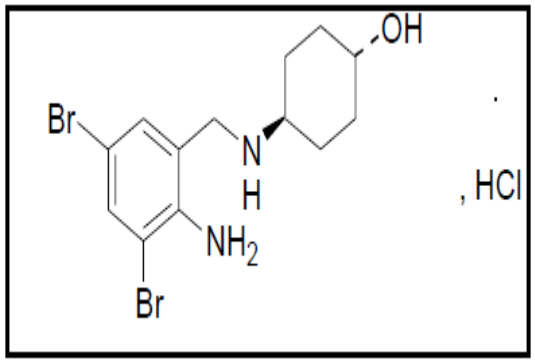

Figure 1: Structure of Ambroxol hydrochloride

Cefpodoxime Proxetile (CEFPO) is chemically 1-(isopropoxy carbonyloxy) ethyl (6R, 7R)-7-[2-(2-amino-4-thiazolyl)-(z)-2-(methoxyimino) acetamido]-3-methoxymethyl-3-cephem-4-carboxylate [10], is a third generation cephalosporin antibiotic. It is used for infections of the respiratory tract, urinary tract and skin and soft tissues. It has greater activity against staphylococcus aureus [11]. Cefpodoxime Proxetile is official in IP and USP. IP[12] and USP[13] describe liquid chromatography method for its estimation. Literature survey reveals Spectrophotometric[14-15], RP-HPLC [16] and HPTLC [17] methods for determination of CEFPO with other drugs.

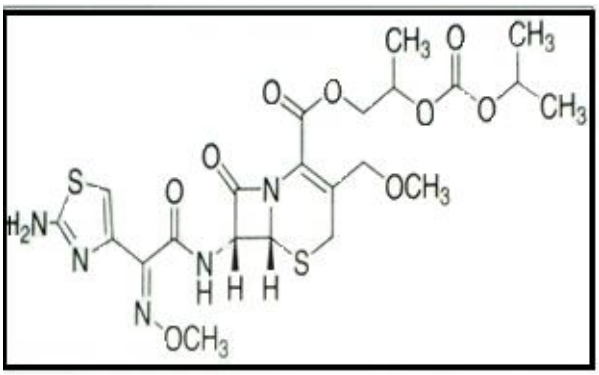

Figure 2: Structure of Cefpodoxime Proxetile

The combined dosage forms of AMB and CEFPO are available in the market (FINECEF – AM) for the treatment of lower Respiratory Tract Infection in adults. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of AMB and CEFPO in their combined dosage forms. Literature survey does not reveal any simple HPLC method for simultaneous estimation of AMB and CEFPO in combined dosage forms. The present communication describes simple, specific, rapid, accurate and precise chromatographic method based on High Performance Liquid Chromatographic method for simultaneous estimation of both drugs in their combined tablet dosage forms.

MATERIALS AND METHODS

Reagents and Materials

AMB and CEFPO bulk powder was kindly gifted by Cadila Pharmaceuticals Ltd. Ahmedabad, Gujarat, India and Baroque Pharmaceutical Ltd., Khambhat, Anand, Gujarat, India respectively. The commercial fixed dose combination product FINECEF- AM (AMB – 60 mg, CEFPO – 100 mg) was procured from the local market which is manufactured by Abott Healthcare Private Limited (AHPL). HPLC grade acetonitrile and water were obtained from Finar Chemicals Limited, Ahmedabad, Gujarat. Potassium di hydrogen orthophosphate AR and Tri Ethyl Amine AR grade were procured from local sources unless specified.

Instrumentation

Shimadzu 20A high performance liquid chromatographic system was used for this experiment. Shimadzu 20A system equipped with SPD - 20A UV-VIS detector, LC – 20AT Liquid Chromatograph double reciprocating plunger design. Spin chrome software was applied for data collecting and processing. A Chemiline pH-meter was used for pH measurements.

Chromatographic Conditions

Table 1: Chromatographic condition is given in following table.

Parameters	Condition
Column	Phenomenex Luna C18 (250 x 4.6 mm, 5 µm)
Detector	245 nm
Injection Volume	20 µl
Flow Rate	1.0 ml/min
Temperature	30°C
Run Time	10 minute
Mobile Phase	Acetonitrile : 0.05 M Potassium Di hydrogen Ortho Phosphate Buffer (70 : 30) (pH adjusted to 6.7 with Tri ethyl Amine)

Preparation of 0.05 M Potassium Di hydrogen Ortho Phosphate (KH_2PO_4) buffer (pH 6.7).

Accurately measured Potassium Dihydrogen Ortho Phosphate buffer 3.4 gm was dissolved in 500ml of HPLC grade water pH was adjusted to 6.7 with the help of Triethylamine.

Preparation of mobile phase

A mixture of 70 ml Acetonitrile and 30 ml of 0.05 M Potassium Di – hydrogen Ortho Phosphate buffer (pH 6.7) filtered through 0.45 µm filter paper, sonicated for 10 minutes to degas the mixture and used as mobile phase..

Preparation of Standard Stock Solution

A 60 mg of standard AMB and 100 mg of standard CEFPO was accurately weighed and transferred to a 100 ml volumetric flask and dissolved in 50 ml mobile phase. The flask was sonicated for 10 min. The flask was shaken and volume was made up to the mark with mobile phase to give a solution containing 600µg/ml AMB and 1000µg/ml CEFPO. From this solution 25 ml was transfer to 100 ml volumetric flask. The volume was adjusted to the mark with the mobile phase to give a solution containing 150µg/ml AMB and 250µg/ml.

VALIDATION OF THE PROPOSED METHOD

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines[18].

Linearity and range

The calibration curves were plotted over a concentration range of 30-60 µg/ml for AMB and 50-100 µg/ml for CEFPO. Appropriate volume of aliquot from standard stock solution AMB (120 µg/ml) and CEFPO (250 µg/ml) was transferred to different volumetric flasks of 25 ml capacity. The volume was adjusted to the mark with the mobile phase to obtain concentration of 30, 36, 42, 48, 54 and 60 µg/ml AMB and 50, 60, 70, 80, 90 and 100µg/ml. The responses were measured as peak area. The peak area was plotted against concentration and regression equation was obtained.

Precision

The precision of the method was verified by repeatability and intermediate precision studies.

Repeatability

Repeatability studies were performed by analysis of all concentrations (30, 36, 42, 48, 54, and 60 µg/ml for AMB and 50, 60, 70, 80, 90 and 100 µg/ml for CEFPO) of the drug in six times on the same day.

Intermediate precision

The intermediate precision of the method was checked by intraday and inter day study. The intraday and inter day precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of AMB and CEFPO (36, 42, 48 µg/ml for AMB and 60, 70, 80 µg/ml for CEFPO). The result was reported in terms of relative standard deviation (% R.S.D).

Specificity

The specificity of the method was determined by analyzing standard drug and test samples. The peak for AMB and CEFPO in the samples was confirmed by comparing the retention time and chromatogram peak with that of a standard. .

Accuracy

Accuracy of the method was carried out by applying the method to drug sample (AMB and CEFPO combination tablet) to which know amount of AMB and CEFPO standard powder corresponding to 50, 100 and 150% of label claim had been added (standard addition method), mixed and analyzed by running chromatogram in optimized mobile phase.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were separately determined on the basis of standard calibration curve. The residual standard deviation of the regression line or the standard deviation of y-intercepts of regression lines was used to calculate LOD and LOQ. Following formulae were used; LOD= 3.3×D/S and LOQ= 10×D/S, where, D is the standard deviation of the y-intercepts of regression line and S is the slope of the calibration curve.

System Suitability Parameters

For system suitability parameters, six replicate injections of mixed standard solution were injected and parameters such as the resolution, capacity factor, tailing factor, theoretical plate, and asymmetry factor of the peaks were calculated.

Analysis of AMB and CEFPO In Combined Tablet Dosage Form

Twenty Tablets were weighed and powdered. The powder equivalent to 60 mg of AMB and 100 mg of CEFPO was transferred to a 100 ml volumetric flask. 50 ml mobile phase was added to it and sonicated for 20 min. The solution was filtered through Whatman filter paper (0.45µ) and the volume was adjusted up to the mark with mobile phase. This solution is expected to contain 600 µg/ml of AMB and 1000 µg/ml of CEFPO. From this solution 25 ml was transfer to 100 ml volumetric flask. The volume was adjusted to the mark with the mobile phase to give a solution containing 150µg/ml AMB and 250µg/ml. Further 4.5 ml of this solution was transferred to

volumetric flask of 25 ml capacity. Volume was made up to the mark with the mobile phase to give a solution containing 45µg/ml AMB and 75µg/ml CEFPO. This solution was used for the estimation of AMB and CEFPO. The prepared sample solution was chromatographed for 10 minutes using mobile phase at a flow rate of 1.0 ml/min. From the peak area obtained in the chromatogram, the amounts of both the drugs were calculated.

RESULTS AND DISCUSSION

The results of validation studies on simultaneous estimation method developed for AMB and CEFPO in the current study involving Acetonitrile: 0.05 M potassium di hydrogen orthophosphate buffer (70: 30 v/v) pH adjusted to 6.7 with Tri Ethyl Amine as the mobile phase for HPLC are given below.

The proposed method was found to be simple, specific, accurate, and precise for the routine simultaneous estimation of two drugs. The optimum mobile phase containing Acetonitrile: 0.05M potassium dihydrogen orthophosphate buffer 70: 30 (v/v) pH adjusted to 6.7 was selected because it could resolve the peaks of AMB (RT = 3.34 ± 0.01) and CEFPO (RT = 4.77 ± 0.01) with a resolution factor of 6.58. The linearity range for AMB and CEFPO were found to be 30 – 60 µg/ml and 50 - 100 µg/ml respectively. Regression analysis data and summary of all validation parameters is given in Table 2. Precision was calculated as repeatability (% R.S.D) and intra and inter day variation (% R.S.D) for both the drugs. Accuracy was determined by calculating the recovery and the mean was determined. The LOD and LOQ were found to be 4.56 and 12.51 µg/ml respectively for AMB and 13.82 and 37.92 µg/ml respectively for CEFPO indicates sensitivity of the proposed method.

Analysis of marketed tablets (FINECEF – AM) was carried out using optimized mobile phase. The % drug content of tablets obtained by the proposed method was found to be between 99.51% and 100.30%, which showed that the estimation of dosage forms were accurate within the acceptance level of 95% to 105%. The method was successfully used to determine the amounts of AMB and CEFPO present in tablets. The results obtained are in good agreement with the corresponding labelled amount. By observing the validation parameters, the method was found to be specific, accurate and precise. Hence the method can be employed for the routine analysis of these drugs in combinations.

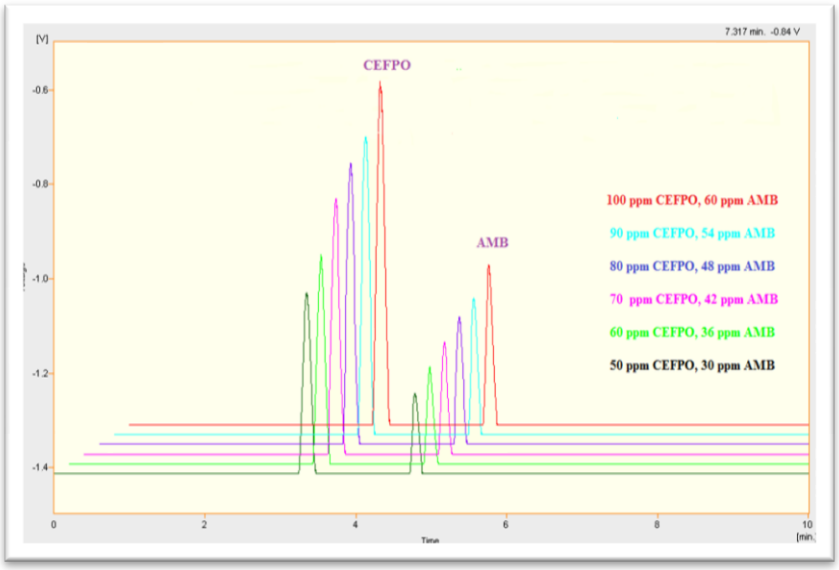

Figure 3: Overlay Chromatogram of AMB (30 – 60 µg/ml) and CEFPO (50 – 100µg/ml)

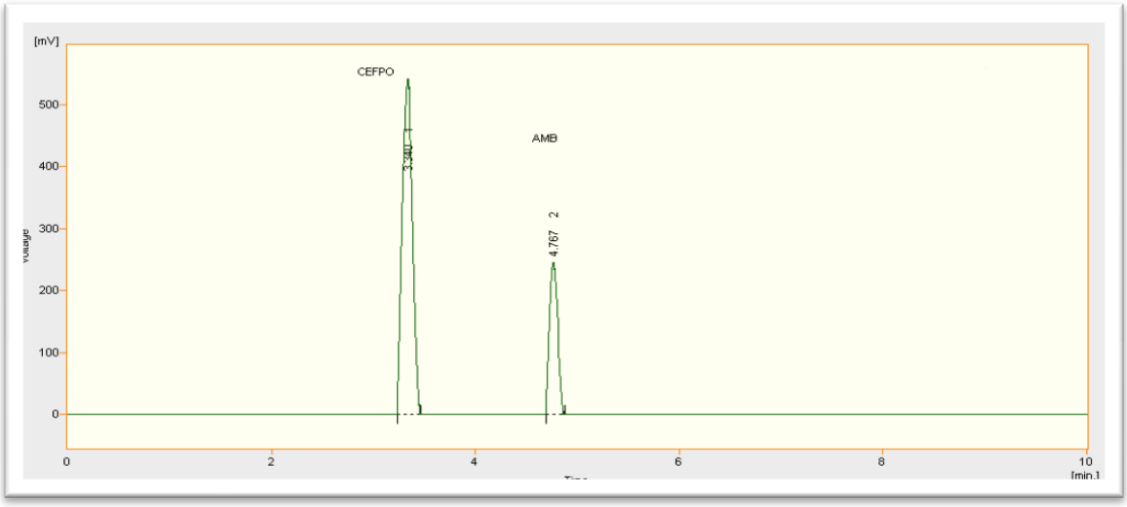

Figure 4: Chromatogram of AMB (45µg/ml) and CEFPO (75µg/ml) marketed formulation

Table 2: Regression analysis data and summary of validation parameters for the proposed method

Parameters	HPLC method	
	AMB	**CEFPO**
Concentration Range (µg/ml)	30 – 60	50 – 100
Slope (m)	33.8936	48.1118
Intercept (c)	-79.5924	308.7933
Correlation Coefficient (r^2)	0.99642	0.99037
Accuracy (% recovery) (n = 3)	99.57 – 100.27 %	99.89 – 100.86%
Repeatability (%RSD) (n = 6)	0.32 %	0.17 %
Intraday (n = 3) (%RSD)	0.18 – 0.39 %	0.09 – 0.15 %
Interday(n = 3) (%RSD)	0.29 – 0.61 %	0.13 – 0.36 %
LOD (µg/ml)	4.56	12.51
LOQ (µg/ml)	13.82	37.92

Table 3: System Suitability Parameters:

Parameters	High Performance Liquid Chromatography method	
	AMB	CEFPO
Retention Time	4.77	3.34
Resolution	7.29	-----
Theoretical Plate	9875	4462.24
Asymmetry factor	1.25	1.03

Table 4: Recovery data of proposed method

Drug	Level	Amount taken (µg/ml)	Amount added (µg/ml)	Amount Recovered (µg/ml) (n=3)	% Recovery (n=3)
AMB	0 %	30	0	30.04	100.12 ± 0.50
	50 %	30	15	44.81	99.57 ± 1.15
	100 %	30	30	60.16	100.27 ± 0.81
	150 %	30	45	74.97	99.96 ± 0.75
CEFPO	0 %	50	0	50.01	100.02 ± 0.26
	50 %	50	25	75.64	100.86 ± 0.65
	100 %	50	50	99.89	99.89 ± 0.33
	150 %	50	75	125.19	100.15 ± 0.50

Table 5: Analysis of AMB and CEFPO by proposed method

	Label claim (mg)		Amount taken (µg/ml)		Amount Recovered (µg/ml) (n=3)		% Label claim	
Tablet	AMB	CEFPO	AMB	CEFPO	AMB	CEFPO	AMB	CEFPO
FINECEF – AM	60	100	45	75	44.78	75.23	99.51%	100.30%

CONCLUSION

Introducing HPLC into pharmaceutical analysis represents a major step in terms of quality assurance. Pharmacopoeias are replacing the chemical methods and Spectrophotometric methods by more sensitive and accurate HPLC methods for the analysis of drugs as well as their formulations. Today HPLC is rapidly becoming a routine analytical technique due to its advantages of qualitative and quantitative analysis. The developed HPLC technique is precise, specific and accurate. Statistical analysis proves that the method is suitable for the analysis of AMB and CEFPO in pharmaceutical formulation without any interference from the excipients. The common excipients and other additives are usually present in the tablet dosage form do not interfere in the analysis of AMB and CEFPO in method, hence it can be conveniently adopted for routine quality control.

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