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METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF AMBROXOLAND CEFPODOXIME BY RP-HPLC METHOD IN BULK AND PHARMACEUTICAL DOSAGE FORM

M Navaneetha[*1], Paul Richards M[2], V Kiran Kumar [3], P Govardhan Reddy [4]

Department of Pharmaceutical Analysis and QA, Unity College of Pharmacy, Raigir (V), Bhongir (M), Yadadri Bhongir (Dt), India,

***Corresponding Author:** Email: richie2626@gmail.com*

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ABSTRACT

The Present work was to develop a simple, fast, accurate, precise, reproducible, reverse phase high performance liquid chromatographic method for simultaneous estimation of ambroxol and cefpodoxime in pharmaceutical tablet dosage form marketed as doxinate. Chromatographic separation was done using Inertsil ODS RP C18 column having dimension of 4.6×250mm having particle size of 5µm, with mobile phase consisting of phosphate buffer pH 3 ±0.02 pH adjusted with ortho phosphoric acid and acetonitril (50:50 %v/v), flow rate was adjusted to 1.0 ml/min and detection wavelength at 263nm. The retention times of ambroxol and cefpodoxime was found to be 2.35 and 4.80min. The Proposed method has been validated for accuracy, precision, linearity, range, and robustness were within the acceptance limit according to ICH guidelines. Linearity for ambroxol and cefpodoxime was found in range of 25 µg-150 µg and correlation coefficient was found to be 0.999 and 0.999, %RSD for method precision was found to be 0.76, 0.82 and for system precision was 0.80 and 0.71 respectively, % mean recovery for ambroxol and cefpodoxime was found to be 99.18% to 99.48%. The method was found to be robust even by change in the mobile phase ±5% and in less flow condition. The developed method can be successfully employed for the routine analysis of ambroxol and cefpodoxime in API and Pharmaceutical dosage forms.

Keywords *– Ambroxol, Cefpodoxime, RP-HPLC, Method development, Method validation.*

1. INTRODUCTION

Ambroxol Hcl, trans-4-[(2-amino-3, 5 dibromobenzyl) amino] cyclohexanol hydrochloride, Bronchosecretolytic and Expectorant, used to treat asthma. It is a mucolytic expectorant that inhibits the release of arachidonic acid cell membrane phospholipids it blocks nitric oxide stimulated activation of guanylate cyclise**.** Cefpodoxime Proxitile IUPAC name is 6R,7R)-7-[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-methoxyimino) acetamido]-3-(methoxymethyl)-8-oxo-5-thia-1- azabicyclo 4.2.0 oct-2-ene-2-carboxylic acid belongs to oral third generation antibiotic. It is active against most gram positive and negative bacteria. It is commonly used to treat acute otitis media, pharyngitis, and sinusitis and its prodrug which is absorbed and de-esterified by the intestinal mucosa and belongs to Anti bacterial with mild to moderate infections and inactivated by certain extended spectrum beta-lactamases [1-5].

2. MATERIALS AND METHODS

Equipment HPLC equipped with auto sampler and PDA detector and Empower 2 (Waters) Symmetry C18 (4.6 x 150 mm, 5µm, Make: Waters) Lab India 3000 – double beam UV-Visible spectrophotometer. All the reagents are HPLC grade.

2.1 Preparation of Phosphate buffer (pH-3.5), mobile phase and diluents

Weigh 7 grams of K Di H Ortho Phosphate and make up the volume to 1000 ml to get Ph 3.5with ortho Phosphoric acid from this take 250 ml of Buffer and add 750 ml of ACN and degas for 5 min and filter through 0.45 µ filter under vacuum filtration and use this mobile phase as diluent .

2.2 Preparation of the Ambroxol HCl and Cefpodoxime Proxitil Standard and Sample Solution

Standard and Sample Solution Preparation

Accurately weigh 12 mg and 10 mg of Ambroxol HCl and cefpodoxime Proxitil (Std) and 7 ml of the diluents and make up to 10 ml from this pipette out 0.3ml and 0.6 ml and make up the solution to 10 ml. Weigh accurately 256.9 mg of sample solution of both add 70 ml of diluent and make up the solution to 100 ml from this pipette out 0.6 ml of both and make up the solution to 10 ml .

2.3 Analytical Method Development

Six trails were injected into HPLC equipped with Auto Sampler and PDA detector like methanol and water then phosphate buffer pH 3.5 is adjusted to methanol and Acetonitrile with the final optimization trial is 30:70 and no impurities was detected and sharp peak was observed with less retention time.

2.4 Method Validation

2.4.1 System suitability studies

Weigh accurately 12 mg and 10 mg of both the drugs and make up the volume to 10 ml pipette out 0.3ml and 0.6 ml and make up the solution with diluent prepared as above and is given below.

2.4.2 Specificity

Weigh accurately 12 mg and 10 mg of both drugs and make up the volume to 10 ml with diluents. Pipette out 0.1 ,0.2 ,0.3 ,0.4 and 0.5 ml for ambroxol and 0.2,0.4,0.6,0.8 and 1.0 ml for Cefpodoxime for five different concentrations and inject the drug into instrument and noted the peak area value and listed in Table-2

2.4.3 Precision

Weigh accurately 12 mg and 10 mg of both the standard drugs and pipette out 0.3 ml of Ambroxol and 0.6 ml of Cefpodoxime and inject to HPLC instrument for six times and note down the peak area values and listed in Table-3.

2.4.4 Accuracy

Accurately weighed 10 mg and 12 mg of Ambroxol and Cefpodoxime transferred to two separately 10 ml and volumetric flasks, 3/4th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution (1000 µg/ml of Belinostat)

2.4.5 Robustness

Standard solution 36 ppm of Ambroxol HCl and 60 ppm of Cefpodoxime Proxitil was prepared and analysed using the varied flow rates along with method flow rate.

The Organic composition in the Mobile phase was varied from 70 % to 80 %. Standard solution 36 µg/ml of Ambroxol HCl and 60 µg/ml of Cefpodoxime Proxitil was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

2.4.6 Limit of Detection and Quantitation

Weigh accurately 12 mg and 10 mg of ambroxol and cefpodoxime and make up the solution with diluents to 10ml from this pipette out 0.3 ml and 0.6 ml and make up the volume to 10 ml again both 1 ml pipette out and make the solution to 10 ml gives 0.03 and 0.05 micro g/ml for limit of detection and quantitation and all the limits of S/N ratio are within the range.

3. RESULTS AND DISCUSSION

The selectivity of the method was revealed by the repeated injection of mobile phase and no interference was found. The recovery studies were carried out by preparing three individual samples with same procedure from the formulation and injecting. The standard drug solution of varying concentration ranging from 12-60µg / ml for Ambroxol Hcl and 20-100 µg / ml for Cefpodoxime proxitile. Both peaks are eluted clearly, more plate count and more resolution was observed.

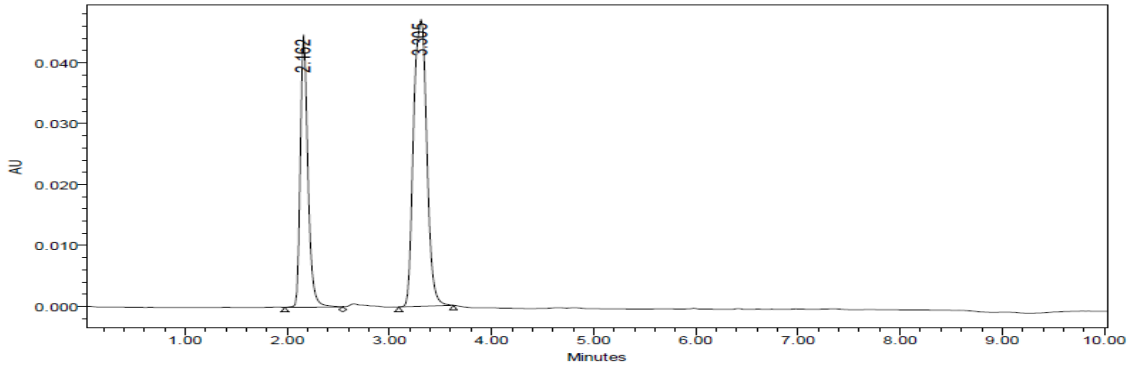

Fig 1: Representative chromatogram of standard

Table 1: System Suitability Parameters of AMB and CEF.

Sl. No	Parameters	Cefpodoxime	Ambroxol
1	System Suitability	6.3	6.3
2	Tailing factor	1.2	1.2
3	No of theoretical plates	3744	4750
4	Retention time	3.304	2.162

Table 2: Linearity Parameters of AMB and CEF

Sl. No	Linearity level	Concentration	Peak area AMB	Peak area CEF
1	I	12ppm	374052	545062
2	II	24ppm	682802	1052605
3	III	36ppm	1012619	1515308
4	IV	48ppm	1324938	2050501
5	V	60ppm	1708316	2635141

Table 3: Precision Parameters of AMB and CEF.

Injection	AMB	CEF
Injection-1	1015722	1496209
Injection-2	1016087	1507963
Injection-3	1018135	1521163
Injection-4	1019549	1522810
Injection-5	1032335	1528916
Injection-6	1020365	1515412

Table 4: Accuracy Parameters of AMB and CEF

Concentration	Area		Amount added		Amount Found		% Recovery		Mean Recovery
	AMB	CEF	AMB	CEF	AMB	CEF	AMB	CEF	
50%	605652.5	774787.7	6	5	5.8	5	98.1	101	99.5
100%	1246314	1537580	12	10	12.1	10.0	101.0	100	100
150%	1869868	2285575	18	15	18.1	14.9	101.0	99.4	99.5

Table 5: Robustness Parameters of AMB and CEF

Sl. no	Flow rate (ml/min)	Ambroxol		Cefpodoxime	
		USP Plate count	Tailing	USP Plate count	Tailing
1	0.8	4479	1.3	3086	1.1
2	1.0	4750	1.2	3744	1.2
3	1.2	4099	1.2	3072	1.1

4. CONCLUSION

Where the RP-HPLC method in which determination of Cefpodoxime proxitile and Ambroxol HCl was carried out on a Symmetry C18 (4.6 x 150mm, 5µm, Make: Waters) using a mobile phase consisting of pH 3.5 phosphate buffer : Acetonitrile (30:70). The mobile phase was pumped at a rate of 1.0 ml/min and the detection was carried out at 254nm. The retention time of Cefpodoxime proxitile and Ambroxol HCl was found to be 2.162 and 3.305 min.

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