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Development of Validated RP-HPLC Method for Simultanious Estimation of Cefadroxil and Ambroxol in Pure and Pharmaceutical Dosage Form

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Authors' contributions

This work was carried out in collaboration between all authors. Authors TS and DK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AM, SA, DVKK and SP managed the analyses of the study. Authors HBS and VN managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

A precise, accurate, economical and simple HPLC (reverse phase) method was developed for the estimation of Cefadroxil and Ambroxol in bulk and pharmaceutical dosage form. Method was performed on a octadecyl silane column with dimensions 4.6 x 250 mm having particle size 5 micron. The mobile phase used in the method is methanol: phosphate buffer pH 3 (60:40v/v). The rate of flow was maintained at 1.5 ml/ min and effluent was monitored at 243 nm. Retention times were observed 2.3 min and 3.3 min for CFX and AXL respectively. The standard calibration curve

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was found linear over a range of 16–80 µg/ml and 2-10 µg/ml for CFX and AXL respectively. Similarly an average correlation coefficient was also obtained at 0.999 for CFX and AXL. LOQ limit was found at 2 µg/ml and absolute recovery was found 99.6% and 99.5% for CFX and AXL respectively. This method can be utilized for routine analysis of CFX and AXL in pure and dosage form.

Keywords: Cefadroxil; ambroxol, RP-HPLC; methanol: phosphate buffer; validation.

1. INTRODUCTION

Chemically ambroxol hydrochloride is trans-4-(2-amino-3,5-dibrombenzylamino)-cyclohexanol monohydrochloride, it is a semi synthetic derivative of vasicine obtained from the Indian shrub "Adhatoda vasica". Ambroxol hydrochloride is an N–desmethyl metabolite of bromohexine which acts by breakdown of acid mucopolysaccharide fibers in the mucous, making it thinner and less viscous and is used as a co adjuvant therapy for treating bronchitis, pulmonary emphysema, pneumonia, tuberculosis, cystic fibrosis.

Ambroxol acts as a mucolytic agent. Over production of nitric oxide (NO) causes inflammation and other disturbances of airways function. In addition NO also causes the activation of soluble guanylate cyclase and cGMP accumulation. Ambroxol inhibits nitric oxide dependent activation of soluble guanylate cyclase. Inhibition of NO-dependent activation of soluble guanylate cyclase causes suppression of excessive mucus secretion; hence, it lowers the phlegm viscosity and improves the mucociliary transport of bronchial [1,2,3].

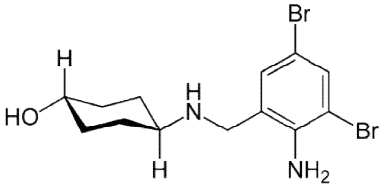

Fig. 1. Ambroxol

Cefadroxil acts on bacteria by binding to specific penicillin-binding proteins (PBPs) present inside the bacterial cell wall, it causes inhibition of bacterial cell wall synthesis. Cell lysis takes place by autolytic enzymes of bacterial cell wall such as autolysins; it is considered that cefadroxil interferes with an autolysin inhibitor. Cefadroxil monohydrate is a first generation cephalosporin antibiotic indicated for the treatment of urinary tract infections, upper respiratory tract infections, and skin and soft tissue infections in patients1. Cefadroxil is chemically (6R,7R)-7-{[(2R)-2-amino-2-(4-hydroxyphenyl)acetyl]amino}-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, and its structural formula is shown in The molecular formula is C16H17N3O5S.H2O and molecular weight is 381.40 g/mol. It is soluble in methanol. It is official drug in United States Pharmacopoeia 2004 and British Pharmacopoeia 2005. The literature review reveals that very few methods have been developed for the estimation of cefadroxil and ambroxol alone and in combination with other drugs [4,5,6]. It was observed that the previous method was slow and time consuming. So in order to improvement of method a rapid development technique is needed to be developed.

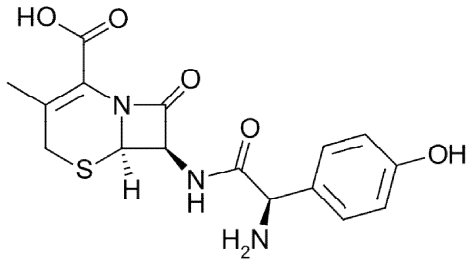

Fig. 2. Cefadroxil

2. MATERIALS AND METHODS

Cefadroxil and Ambroxol bulk drugs are supplied by NATCO Pharma. Sodium dihydrogen phosphate, 85% Orthophosphoric acid, Methanol, Acetonitrile, Dichloromethane, Hydrochloric Acid, Triethyl-Amine, Sodium Hydroxide are purchased from MERCK. HPLC used was Waters alliance 2695 HPLC system with Waters UV detector [7].

2.1 Preparation of Phosphate Buffer (PH-3)

Dissolve 0.9 g of anhydrous dihydrogen phosphate and 1.298 g of Citric acid mono

hydrate in sufficient water to produce 1000 mL. Adjust the p H 3 by using ortho phosphoric acid [8].

2.2 Preparation of Mobile Phase

Accurately measured 600 ml (60%) of Methanol and 400 ml of Phosphate buffer (40%) were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration [9,10].

2.3 Diluent Preparation

The Mobile phase was used as the diluents.

2.4 Stock Solutions and Standards

Accurately weighed and transferred 10 mg of cefadroxil and 10 mg of ambroxol working standard into a two separate 10 ml clean dry volumetric flasks and add about 10 ml of diluents, it was further sonicated to dissolve it completely, olume was made up to the mark with the same solvent.

2.5 Preparation of Sample Solution

Accurately weighed ten tablets were taken and crushed in mortar and pestle. 100 mg equivalent weight of powdered drug containing cefadroxil, and ambroxol (marketed formulation-dose of CFX is 250 mg, dose of AXL is 30mg in combination tablet) were transferred into a 100 mL volumetric flask and made the volume up to the mark with the solvent. (Stock solution). Further 1 ml pipetted out from stock solution into a 50 ml volumetric flask and diluted up to the mark with diluents [11,12].

2.6 Apparatus and Chromatographic Conditions

Quantitative HPLC was performed on Waters alliance 2695 HPLC system with UV detector. empower software is used along with a stainless steel column 4.6 x 150mm, packed with Octa decyl silane bonded to porous silica (C18) with particle size 5 micron. To develop a suitable and robust HPLC method for the determination of CFX and AXL, different mobile phases containing methanol and phosphate buffer were used in different compositions like (30:70, 40:60, 50:50, 70:30, 80:20) at different flow rates (0.5,0.75,1.0, 1.2, 1.5, ml/min). The mobile phase methanol and phosphate buffer with a flow rate of 1.5 ml/ min. It gave peaks of good resolution and were eluted at retention times around 2.3 min, 3.3 min with symmetric peak shape. The detection is performed at the wavelength 243 nm [13].

3. RESULTS AND DISCUSSION

3.1 Method Development and Optimization

The main target of the chromatographic method is to get the separation of closely eluting drugs cefadroxil and ambroxol, drugs were co-eluted by using different reverse phase column (stationary phase) like C18 and C8 with different lengths and different mobile phases containing buffers like sulphate, acetate and phosphate with varying pH (2-7) and using organic modifiers like acetonitrile, methanol and ethanol in the mobile phase. pH of the buffer has played a significant role in achieving the separation between drugs[l]. The chromatographic separation was achieved by using stainless steel column (4.6 x 250 mm) column packed with Octa decyl silane bonded to porous silica (C18) with particle size 5 micron, by using solutions methanol and phosphate buffer in the ratio of (60:40), pH adjusted to 3 using ortho phosphoric acid. The flow rate of the mobile phase was maintained at 1.5 ml/min. At 25^0 C of column temperature, the peak shape of CFX and AXL was found symmetrical with mobile phase 60:40 ratio. In the optimized conditions CFX and AXL were well separated with a good resolution and the typical retention times of CFX and AXL were about 2.3 min and 3.3 min, respectively. The system suitability results are given in Table 1 and the developed LC method was validated.

Optimized chromatogram, blank, System suitability parameters are shown in the Fig. 3.

3.2 Results of Method Validation

3.2.1 Linearity

Linear calibration plot for assay method was obtained over the calibration ranges tested, i.e. 16.6- 80 µg/ml for cefadroxil and 2 µg/ml to 10 µg/ml for Ambroxol and the correlation coefficient obtained was greater than 0.999. The results show that an excellent correlation existed between the peak area and concentration of the analyte which is given in Table 2.

3.2.2 Intermediate precision/ruggedness

The intermediate precision (also known as Ruggedness) is evaluated by performing method

Table 1. System suitability parameters

Instrument used	:	Waters HPLC with auto sampler and UV detector
Temperature	:	Ambient
Column	:	X bridge C18 (4.6×150 mm) 5 µ
Buffer	:	Phosphate buffer (pH-3)-Dissolve 0.9g of anhydrous di hydrogen phosphate and 1.298 g of Citric acid mono hydrate in sufficient water to produce 1000 ml. Adjust the pH 3 by using ortho phosphoric acid.
pH	:	3
Mobile phase	:	Methanol: Phosphate Buffer pH 3 (60:40v/v)
Flow rate	:	1.5 ml per min
Wavelength	:	243 nm
Injection volume	:	10 µl
Run time	:	6 min.

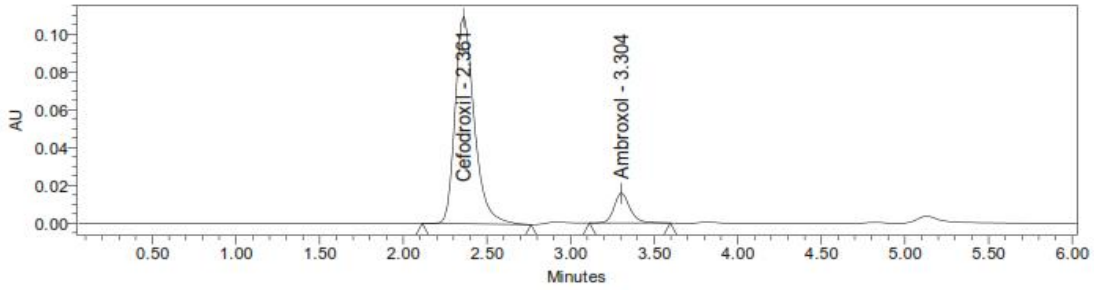

Fig. 3. Chromatogtam of standard drug of cefadroxil and ambroxol

on different day and by using different make column of same dimensions.

Table 2. Linearity results: (for cefadroxil)

S. no	Linearity level	Concentration (ppm)	Area
1	I	16.6	285532
2	II	33.3	523169
3	III	49.9	776000
4	IV	66.6	1013427
5	V	83.3	1255649
Correlation coefficient			0.999
Linearity results: (for ambroxol)			
S. no	**Linearity level**	**Concentration (ppm)**	**Area**
1	I	2	44066
2	II	4	79045
3	III	6	115348
4	IV	8	156307
5	V	10	191169
Correlation coefficient			0.999

3.2.3 Recovery and accuracy

The percentage recovery of CFX and AXL in bulk drugs samples was ranged from 99.4 - 99.6% which indicates that the method was accurate which is given in Table 4.

3.2.4 Accuracy results

The accuracy of the method was determined by preparing solutions of different concentrations of CXL and AXL that is 80%, 100% and 120% in which the amount of marketed formulation (CXL and AXL 8 mg and 1 mg respectively) was kept constant and the amount of pure drug was varied that is 6.4 mg, 12.5 mg and 15 mg for CXL and 0.8, 1 and 1.2 mg for AXL i.e. 80%, 100% and 120% respectively. The solutions were prepared in triplicates and the accuracy. Similarly was indicated by % recovery in Table 4.

3.2.5 Specificity

1 mg of CFX was spiked with 50% (0.5 mg), 100% (1 mg), and 150% (1.5 mg) of excipient mix (Magnesium Stearate), Further 01 ml is pippeted out from the all three samples and diluted to 100 ml in three separate volumetric flask, and analysed for % recovery of CFX.Similarly AXL sample were prepared and analysed.

Table 3. Results of method precession for cefadroxil and ambroxol

Results of method precession for cefadroxil							
S. no	**Name**	**Rt**	**Area**	**Height**	**USP plate count**	**USP tailing**	
1	Cefadroxil	2.375	885966	110748	2153.4	1.3	
2	Cefadroxil	2.377	886678	111931	2155.7	1.3	
3	Cefadroxil	2.377	888046	111166	2093.2	1.3	
4	Cefadroxil	2.375	888565	112210	2187.5	1.3	
5	Cefadroxil	2.376	890142	112300	2193.8	1.3	
Mean			887879.4				
Std. Dev			1637.194				
% RSD			0.1				
Results of method precession for ambroxol							
S. no	**Name**	**Rt**	**Area**	**Height**	**USP plate count**	**USP tailing**	**USP resolution**
1	Ambroxol	3.339	103737	15914	6357.6	1.2	5.0
2	Ambroxol	3.337	103946	15909	6093.6	1.2	5.0
3	Ambroxol	3.334	104197	15949	6362.9	1.2	5.0
4	Ambroxol	3.334	104210	15906	6301.1	1.2	5.0
5	Ambroxol	3.338	104406	16036	6396.0	1.2	5.0
Mean			104099.2				
Std. Dev			260.109				
% RSD			0.24				

Table 4. Results of accuracy studies for cefadroxil and ambroxol

Accuracy (recovery) data for cefadroxil					
% concentration (At specification level)	**Area**	**Amount added (mg)**	**Amount found (mg)**	**% recovery**	**Mean recovery**
80%	708330	6.4	6.35	99.2%	
100%	885413	8	7.88	98.5%	99.0%
120%	1062496	9.6	9.54	99.3%	
Accuracy (recovery) data for ambroxol					
% concentration (At specification level)	**Area**	**Amount added (mg)**	**Amount found (mg)**	**% recovery**	**Mean recovery**
80%	83492	0.8	0.79	98.7%	
100%	104365	1	0.996	99.6%	99.1%
120%	125238	1.2	1.19	99.1%	

3.2.6 LOD and LOQ

The parameter LOD for CFX and AXL was found to be 0.2 ppm and LOD for Similarly the parameter LOQ for CFX and AXL was found to be 2 ppm.

3.3 Robustness

The percent recovery of CFX and AXL was under limit in most conditions and did not show any significant change in modified critical parameters. The observed tailing factor for CFX and AXL was

Table 5. Results of specificity studies for cefadroxil and ambroxol

Speficity data for cefadroxil					
% concentration (At specification level)	**Area**	**Amount added (mg)**	**Amount found (mg)**	**% recovery**	**Mean recovery**
50%	444310	0.5	4.98	99.6%	
100%	885413	1	9.97	99.7%	99.6%
150%	1319238	1.5	14.96	99.7%	
Specificity data for ambroxol					
% concentration (At specification level)	**Area**	**Amount added (mg)**	**Amount found (mg)**	**% recovery**	**Mean recovery**
50%	50577	0.5	4.97	99.4%	
100%	104365	1	9.96	99.6%	99.5%
150%	156541	1.5	14.95	99.6%	

less than 2.0 and the components were well separated under all modifications. Considering the modifications in the system suitability parameters, as well as carrying the experiment at room temperature may conclude that the method conditions were robust.

Table 6. Results of robustness for cefadroxil and ambroxol

System suitability results for cefadroxil (Variation in flow)			
S. no	**Flow rate (ml/min)**	**System suitability results**	
		USP plate count	**USP tailing**
1	1.4	2678	1.3
2	1.5	2272	1.3
3	1.6	2534	1.3
System suitability results for ambroxol (Variation in flow)			
S. no	**Flow rate (ml/min)**	**System suitability results**	
		USP plate count	**USP tailing**
1	1.4	6685	1.2
2	1.5	6164	1.2
3	1.6	5846	1.2

4. CONCLUSION

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of cefadroxil and ambroxol was done by RP-HPLC. The proposed method is precise, simple, accurate and fast for determination of CFX and AXL in pure and dosage form. The mobile phase is easy to prepare and cheap. The sample recoveries in all formulations were in good agreement within the limit. Therefore, this method can be conveniently used for routine analysis of CFX and AXL in pure and dosage form.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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