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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF CEFADROXIL AND AMBROXOL IN THE BULK AND SYNTHETIC MIXTURE

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ABSTRACT

A simple, rapid, accurate, and precise stability-indicating RP-HPLC method was developed to simultaneously estimate Cefadroxil and Ambroxol HCl in the bulk and synthetic mixture. This method was developed using the Nuclosil C18 column (150mm x 4.6mm) with a mobile phase consisting of 0.01N sodium dihydrogen phosphate-acetonitrile (60:40v/v). The isosbestic point of Cefadroxil and Ambroxol HCl was identified at 244 nm. Linearity was observed in the concentration range of 62.5-375 µg/ml for Cefadroxil and 7.5-45.0 µg/ml for Ambroxol HCl, with R^2 values of 0.998 and 0.999, respectively. The method was validated statistically and by recovery studies. The mean % recovery was found to be 98.74 % and 99.25 % for Cefadroxil and Ambroxol HCl respectively. Stability testing of Cefadroxil and Ambroxol HCl was carried out according to ICH guideline Q1A (R2). Cefadroxil and Ambroxol HCl were subjected to various stress conditions including acidic, alkaline, oxidation, thermal, and UV decomposition. Significant degradation was observed under acidic, alkaline, oxidative, thermal, and UV conditions. Among the degradation pathways, oxidative degradation occurred at a faster rate compared to other degradation processes.

Keywords: RP-HPLC, Cefadroxil, Ambroxol HCl, Nuclosil C_{18} column, 0.01N Sodium dihydrogen phosphate, Acetonitrile, Stability testing, Synthetic mixture

INTRODUCTION

Cefadroxil is a cephalosporin antibiotic **[1]** used to treat bacterial infections in various parts of the body. Ambroxol, on the other hand, is a medication that helps to dissolve phlegm and is commonly used in the treatment of respiratory diseases **[2-3]** associated with excessive mucus. It is often included as an active ingredient in cough syrups. Chemical name of cefadroxil is 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 **(Figure 1)**. Ambroxol's chemical nameis trans-4-(2-Amino-3,5-dibrombenzylamino)-cyclohexanol **(Figure 2)**.

Through an extensive literature review, it was found that a few RP- HPLC **[4-6]** methods have been reported for the simultaneous estimation of cefadroxil and ambroxol in bulk and pharmaceutical dosage forms. Additionally, various methods such as HPTLC **[7]**, HPLC **[8-10]**, UV **[11-12]** have been reported individually for cefadroxil, while one review literature focused on analytical methods for the quantitative estimation of ambroxol HCl **[13-15]** in pharmaceutical preparations. However, no method has been found thus far for the analysis of a synthetic mixture of cefadroxil and ambroxol.

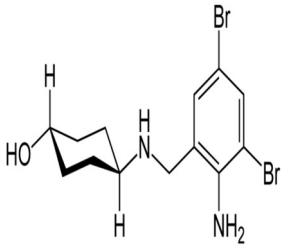


Figure 1: Chemical Structure of Ambroxol

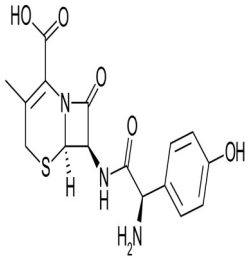


Figure 2: Chemical Structure of Cefadroxil

MATERIALS AND METHODS

Active Pharmaceutical Ingredients: The active pharmaceutical ingredients such as Cefadroxil and Ambroxol HCl were obtained as gift samples from Richer Pharmaceutics, Hyderabad.

The remaining raw materials and diluents, including HPLC grade water, acetonitrile, methanol, sodium dihydrogen orthophosphate, orthophosphoric acid, and hydrogen peroxide, were purchased from Yarrow Pharmaceutical Private Limited, Mumbai.

Instrumentation: Waters HPLC (e-2685), Empower software (2.00062), UV visible double beam visible spectrophotometer

(Shimadzu), Analytical balance (Sortorious), pH meter (Polmon microprocessor analyser, LP– 139S), Sonicator (QC1-014 Biotechnic India).

Mobile phase solution: A combination of 0.01N Sodium dihydrogen orthophosphate buffer and acetonitrile in a ratio of 60:40% v/v was chosen after conducting multiple trials. The 0.01N Sodium dihydrogen orthophosphate buffer was adjusted to a pH of 3.0 using dilute orthophosphoric acid. The prepared mobile phase solution was then filtered through a 0.45 µm membrane filter and subjected to sonication to remove any dissolved gases and air bubbles. This ensured the mobile phase was degassed and ready for use in the analysis.

Standard stock solution for Cefadroxil and Ambroxol: Weighed and transferred 250 mg of Cefadroxil standard and 30 mg of Ambroxol standard into 100 ml volumetric flask, add 25 ml of diluents (Mobile phase) and sonicated to dissolve. Once the standards are dissolved, dilute the solutions to the mark (100 ml) with the diluent.

Working Standard solution for Cefadroxil and Ambroxol: Transferred 10 ml of the standard stock solution into a 100 ml volumetric flask using a pipette and dilute to volume with diluent to get concentrations of 250µg/ml of Cefadroxil and 30µg/ml of Ambroxol.

Composition of synthetic mixture of Cefadroxil and Ambroxol (400mg): Synthetic mixture preparation consisting Cefadroxil-250mg, Ambroxol -30mg, Starch- 59mg, Magnesium stearate-15mg, Aerosil-10mg, Talc -6mg, Poly vinyl pyrrolydine- 30mg.

Sample stock Solution: Finely grind pre-weighed 20 tablets of the synthetic mixture and transferred ground sample quantitatively equivalent to 250 mg of Cefadroxil and 30 mg of Ambroxol into 100 ml volumetric flask add 25 ml of mobile phase and sonicate it for 10 minutes and dilute to volume with the mobile phase. Further, filter the solution through 0.45µ filter paper. Diluted 10 ml of the filtrate to 100 ml with mobile phase to get concentrations of 250µg/ml of Cefadroxil and 30µg/ml of Ambroxol.

RESULTS AND DISCUSSIONS

Selection of wavelength: Isosbestic point was determined by using 10µg/ml of Cefadroxil and 10µg/ml of Ambroxol HCl prepared and scanned in between 200-400nm wavelength. Isosbestic point was found to be 244 nm for Cefadroxil and Ambroxol **(Table 1, Figure 3)**.

Method Validation:

Linearity: The Linearity of detector response for Cefadroxil and Ambroxol is demonstrated by Concentration versus area over the range of 25 to 150% level of the target. The linearity was performed by using a standard stock solution by transferring 2.5mL -15mL into a series of 10mL volumetric flask and making up with mobile phase to get a concentration of 62.5-375.0 µg/ml of Cefadroxil and 7.5-45 µg/ml of Ambroxol. From these 20 µl of the solution was injected into the HPLC column **(Table 2, 3, Figure 4, 5)**.

Method Precision: (Table 4)

Accuracy:

The accuracy of the method developed was determined by the percentage of the recovery. To assess accuracy, the sample solutions were spiked with a known amount of the standard at three concentration levels, typically at 80%, 100%, and 120% of the expected concentration **(Table 5)**.

Robustness: The robustness of the test method was demonstrated by carrying out method variations like flow rate (0.6ml/min and 0.75 ml/min) and Column oven temperature variations ($\pm 5^0$C) **(Table 6)**.

Limit of detection (LOD) and Limit of quantification (LOQ): (Table 7)

The LOD and LOQ were calculated by the following equations.

$$\mathbf{LOD} = \frac{\mathbf{3.3 \times Standard\ Deviation}}{\mathbf{Slope}}$$

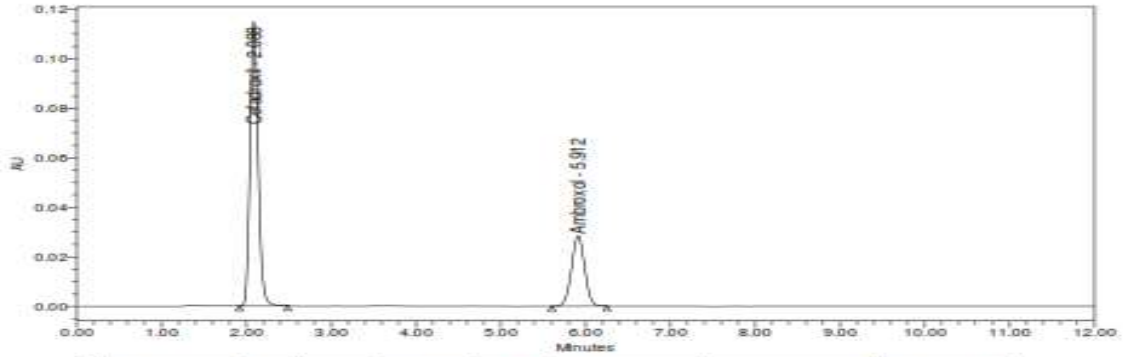
$$\mathbf{LOQ} = \frac{\mathbf{10 \times Standard\ Deviation}}{\mathbf{Slope}}$$

Assay: (Table 8, 9)

Stability studies: Stability studies were conducted to evaluate the behavior of Cefadroxil and Ambroxol under various degradation conditions such as acidic, alkali, oxidative, thermal and photolytic studies **(Table 10, Figure 6-10)**.

Table 1: Optimized Chromatographic Conditions

Mobile Phase	**0.01N sodium dihydrogen phosphate: Acetonitrile [60 :40]**
Column	**Nucleosil C_{18} 150 × 4.6 ×5**
Flow rate	**0.7 ml/min**
Detection	**244 nm by UV**
Injection volume	**20 µL**
Elution	**Gradient**
Column Temperature	**Ambient**



	Peak Name	RT	Area	Height	% Area	USP Resolution	USP Plate Count	USP Tailing
1	Cefadroxil	2.088	760842	114877	71.34		2314.44	1.14
2	Ambroxol	5.912	305635	28463	28.66	16.47	6920.89	1.01

Figure 3: Chromatogram of Optimized method

Table 2: Linearity results for Cefadroxil

S. No.	Concentration (µg/ml)	Peak area
1	62.50	226260
2	125.00	468032
3	187.00	703503
4	250.00	932387
5	312.50	1179448
6	375.00	1399110

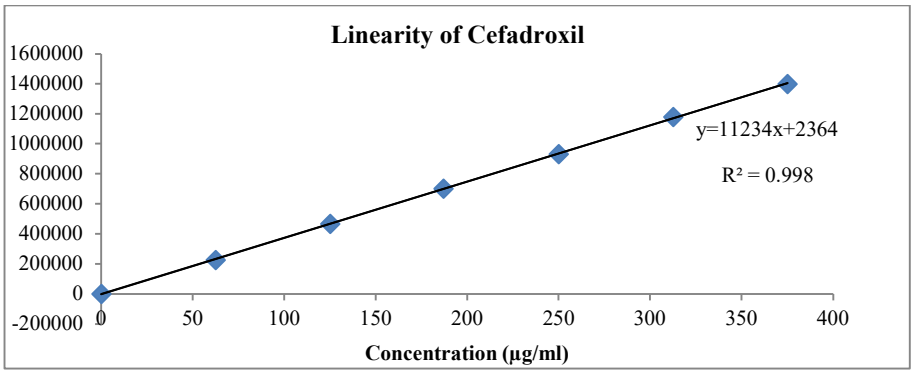


Figure 4: Standard curve of Cefadroxil for Linearity

Table 3: Linearity results for Ambroxol

S. No.	Concentration (µg/ml)	Peak area
1	7.5	90998
2	15.0	189180
3	22.5	284113
4	30.0	376650
5	37.5	476779
6	45.0	568398

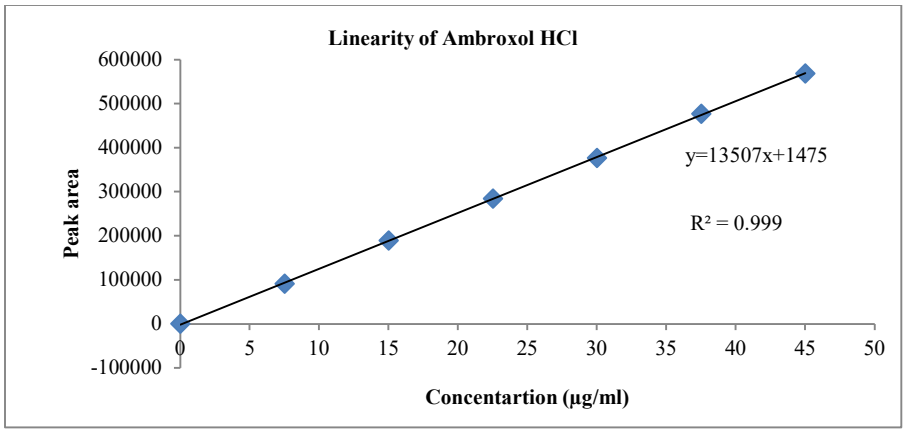


Figure 5: Standard curve of Ambroxol HCl for Linearity

Table 4: Method precision results for Cefadroxil and Ambroxol

S. No.	Name	Cefadroxil		Ambroxol	
		RT	Area	RT	Area
1	Injection-1	2.075	761542	5.923	312514
2	Injection-2	2.071	759454	5.928	311545
3	Injection-3	2.074	760214	5.931	312335
4	Injection-4	2.068	759227	5.941	312457
5	Injection-5	2.069	766666	5.965	313854
6	Injection-6	2.061	758947	5.975	311876
Average		2.070	761008	5.944	312430
Standard Deviation		0.0050	2924.5	0.021	791.80
% RSD		0.244	0.384	0.359	0.253

Table 5: Accuracy results for Cefadroxil and Ambroxol

Injections	80% Concentration		100% Concentration		120% Concentration	
	Cefadroxil	Ambroxol	Cefadroxil	Ambroxol	Cefadroxil	Ambroxol
	Peak area	Peak area	Peak area	Peak area	Peak area	Peak area
Injection-1	754789	295647	921394	375468	1093264	452157
Injection-2	748659	300245	919356	368954	1111152	459547
Injection-3	712447	301146	916246	371647	1102694	425765
Average	738632	299013	918999	372023	1102370	445823
Amount Recovered	79.64	79.85	98.78	99.25	118.85	119.06
% Recovery	99.54	99.82	98.78	99.25	99.04	99.22

Table 6: Robustness results for Cefadroxil and Ambroxol HCl

S. No.	Parameter	Cefadroxil		Ambroxol	
		RT (minutes)	Area	RT (minutes)	Area
1	At flow rate 0.6ml/min	2.492	1140487	7.146	458967
2	At fiow rate 0.8 ml/min	1.801	810499	5.11	326020
3	Column oven temp at 25°C	2.102	931017	5.899	376547
4	Column oven temp at 35°C	2.098	933154	5.902	376754

Table 7: LOD and LOQ results for Cefadroxil and Ambroxol HCl

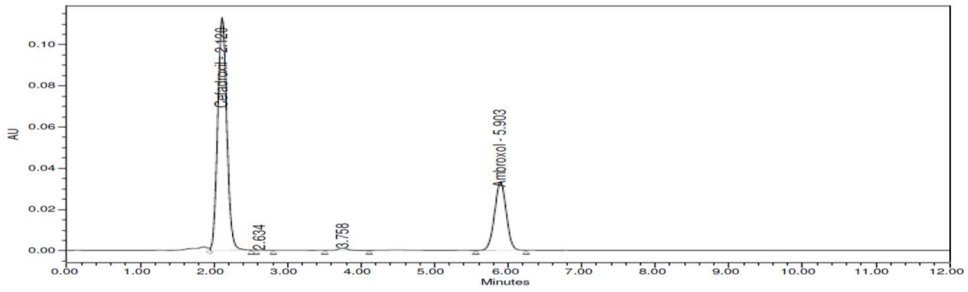
Drug name	LOD	LOQ
Cefadroxil	0.69 µg/ml	2.10 µg/ml
Ambroxol HCl	0. 26 µg/ml	0.79 µg/ml

Table 8: Assay results for Cefadroxil and Ambroxol HCl

S No	Name	Cefadroxil		Ambroxol	
		RT	Area	RT	Area
1	Standard-1	2.069	756845	5.926	320457
2	Standard-2	2.076	758697	5.931	319954
Average		2.073	757771	5.929	320206
3	Sample-1	2.024	757215	5.968	319002
4	Sample-2	2.068	755684	5.949	318959
Average		2.046	756450	5.959	318981

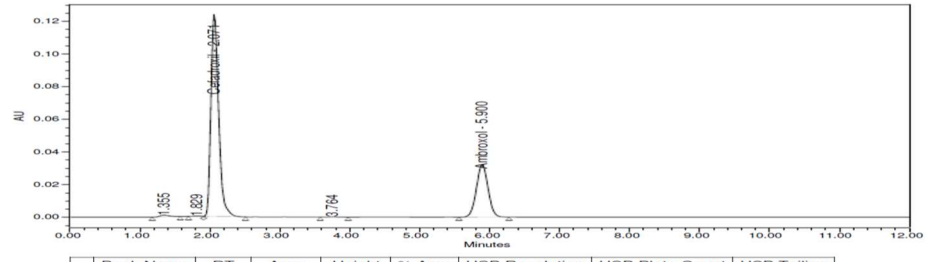
Table 9: Percentage purity for Cefadroxil and Ambroxol HCl

S. No	Drug name	Peak Area		% Purity
		Standard	Sample	
1	Cefadroxil	757771	756450	99.57
2	Ambroxol HCl	320206	318981	99.66



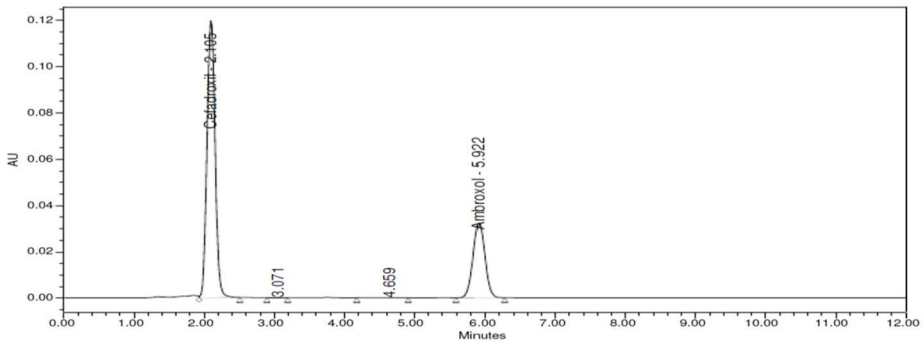
	Peak Name	RT	Area	Height	% Area	USP Resolution	USP Plate Count	USP Tailing
1	Cefadroxil	2.120	707105	113020	71.98		1405.27	1.08
2		2.634	193	28	0.01	2.66	4644.42	2.40
3		3.758	8566	1033	0.64	5.94	4704.23	1.16
4	Ambroxol	5.903	295805	33350	27.36	8.24	6536.24	0.99

Figure 6: Chromatogram of Cefadroxil and Ambroxol HCl for Acid degradation



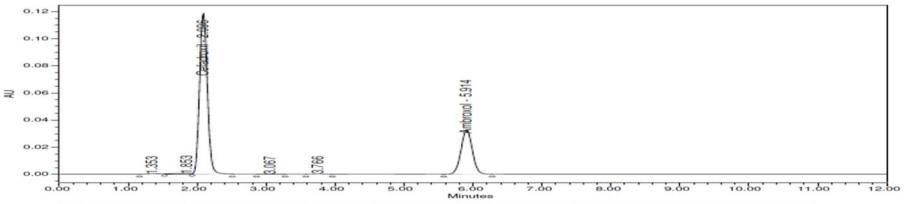
	Peak Name	RT	Area	Height	% Area	USP Resolution	USP Plate Count	USP Tailing
1		1.355	9694	1060	0.73		489.12	1.13
2		1.829	2716	342	0.20		824.89	
3	Cefadroxil	2.071	697057	123706	71.05		1722.91	1.32
4		3.764	1274	153	0.10	7.86	4592.51	1.16
5	Ambroxol	5.900	285057	32207	27.92	7.94	5981.88	1.02

Figure 7: Chromatogram of Cefadroxil and Ambroxol HCl for alkali degradation



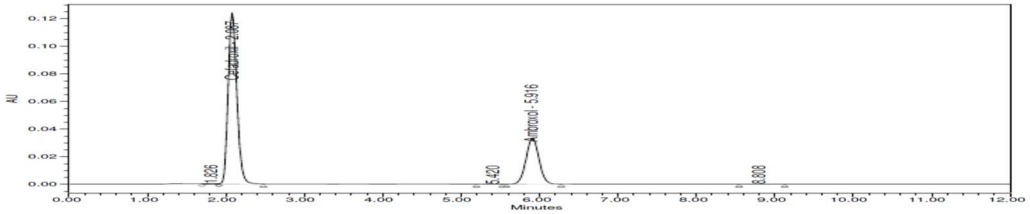
	Peak Name	RT	Area	Height	% Area	USP Resolution	USP Plate Count	USP Tailing
1	Cefadroxil	2.105	657027	119858	72.18		1589.18	1.06
2		3.071	753	106	0.06	4.71	3993.18	1.03
3		4.659	2495	88	0.19	2.70	597.13	0.76
4	Ambroxol	5.922	265023	32348	27.57	1.97	6243.48	1.02

Figure 8: Chromatogram of Cefadroxil and Ambroxol HCl for Oxidative degradation



	Peak Name	RT	Area	Height	% Area	USP Resolution	USP Plate Count	USP Tailing
1		1.353	5885	459	0.45			
2		1.853	12648	855	0.96			
3	Cefadroxil	2.096	710547	118695	70.29		1656.01	1.10
4		3.067	689	101	0.05	5.19	5478.89	0.89
5		3.766	2004	240	0.15	3.55	4553.60	1.13
6	Ambroxol	5.914	296002	32829	28.10	8.08	6279.37	1.01

Figure 9: Chromatogram of Cefadroxil and Ambroxol HCl for Thermal Degradation



	Peak Name	RT	Area	Height	% Area	USP Resolution	USP Plate Count	USP Tailing
1		1.826	4764	509	0.36			
2	Cefadroxil	2.087	697125	124032	71.00		1706.52	1.18
3		5.420	661	75	0.05	14.72	7838.70	0.82
4	Ambroxol	5.916	284507	33446	28.58	1.79	6134.10	1.02
5		8.808	168	10	0.01	5.80	7571.31	1.37

Figure 10: Chromatogram of Cefadroxil and Ambroxol HCl for UV Degradation

Table 10: Results for degradation studies

Condition	Hours	% Degradation	
		Cefadroxil	Ambroxol
Acidic (0.1 N Hcl)	24	6.61	7.27
Alkaline (0.1N NaOH)	24	7.94	10.64
Oxidative (3% v/v H_2O_2)	2	13.25	16.92
Thermal (300^0 C)	3	4.81	7.20
Photolytic (at 254 nm)	5	7.93	10.81

CONCLUSION

Based on the observed results, a new stability-indicating RP-HPLC method was developed and validated for the simultaneous estimation of Cefadroxil and Ambroxol HCl. The method was designed to meet the criteria outlined in the International Council for Harmonisation (ICH) guidelines.

The developed method is considered simple, cost-effective, accurate, and precise. It is suitable for routine analysis in quality control laboratories for the estimation of Cefadroxil and Ambroxol HCl in pharmaceutical formulations.

The method demonstrated good reproducibility, with a percentage relative standard deviation (RSD) of less than 2%. This indicates consistent and reliable results when analyzing multiple samples using the proposed method.

Stability studies revealed that the drugs undergo significant degradation under various conditions, including acidic, basic, oxidative, thermal, and UV light conditions. Among these, the presence of hydrogen peroxide (H_2O_2) accelerated the degradation process, while thermal conditions showed relatively lower degradation rates.

These findings highlight the importance of stability testing and confirm that the developed RP-HPLC method is capable of identifying and quantifying degradation products, making it suitable for stability studies and quality control analysis of Cefadroxil and Ambroxol HCl formulations.

Conflict of Interest: There was no conflict of interest relevant to this work.

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