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STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF AMBROXOL AND CEFADROXIL IN BULK AND ITS TABLETS

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Abstract

A simple, sensitive, accurate, precise and selective stability indicating reverse phase high performance liquid chromatographic method was developed and validated for the determination of ambroxol and cefadroxil in bulk drug and pharmaceutical dosage form. Separation and quantification were achieved on a Phenomenex C18, 5µm, 150 x 4.6 mm i. d. column using PDA detector. The mobile phase was 0.1% OPA: methanol (50:50 v/v), at a flow rate of 1 ml/min and injection volume was 10µL. Detection was carried out at a wavelength of 249 nm. The method was validated for precision, accuracy and robustness. Ambroxol and cefadroxil were exposed to acidic, basic, oxidative, neutral, thermal and photolytic stress conditions and the stressed samples were analyzed by the proposed method. Good linear relationship in the concentration range of 12-36µg/ml for ambroxol with correlation coefficient of 0.999 and 60-180 µg/ml for cefadroxil with correlation coefficient of 0.999. The LOD and LOQ for Ambroxol were found to be 0.0680 mg/ml and 0.2268 mg/ml, respectively. The LOD and LOQ for cefadroxil were found to be 0.244mg/ml and 0.813mg/ml, respectively. The precision was less than 2% RSD for both analytes. The stressed sample chromatograms demonstrate the specificity of the proposed method for the determination of target analytes in presence of their degradants.

Keywords: *RP-HPLC, Ambroxol, Cefadroxil, Analysis,Stability Indicating.*

Introduction

Ambroxol [1-3] is a secretolytic agent used in the treatment of respiratory diseases associated with viscid or excessive mucus. It is the active ingredient of mucosolvan, lasolvan or mucoangin. The substance is a mucoactive drug with several properties including secretolytic and secretomotoric actions that restore the physiological clearance mechanisms of the respiratory tract which play an important role in the body's natural defense mechanisms. It stimulates synthesis and release of surfactant by type II pneumocytes. Surfactants act as an anti-glue factor by reducing the adhesion of mucus to the bronchial wall, in improving its transport and in providing protection against infection and irritating agents. The chemical structure of ambroxol is given in Figure 1.

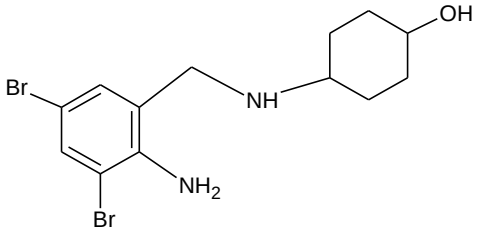

Figure1. Chemical structure of ambroxol

Cefadroxil [4-6] is a first-generation cephalosporin antibacterial drug that is the para-hydroxy derivative of cefalexin, and is used similarly in the treatment of mild to moderate susceptible infections such as the bacterium *Streptococcus pyogenes*, causing the disease popularly called strep throat or streptococcal tonsillitis, urinary tract infection, reproductive tract infection, and skin infections. The chemical structure of cefadroxil is given in Figure 2.

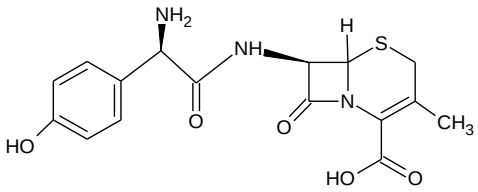

Figure 2. Chemical structure of cefadroxil

The literature survey has revealed few analytical techniques for the simultaneous quantification of ambroxol and cefadroxil in bulk, pharmaceutical formulation and biological samples. They include UV spectrophotometry [7], HPLC [7,8] and HPTLC [8,9]. In the present manuscript, we report a new simple, accurate and precise stability indicating RP-HPLC method for simul-

taneous estimation of ambroxol and cefadroxil in bulk and in its pharmaceutical formulations. The developed method was validated according to ICH guidelines.

2. Materials and Method

2.1. Instrumentation

The HPLC separation was carried out on HPLC system with Waters 2695 alliance with binary HPLC pump. Waters 2998 PDA detector is used for detection. Waters Empower2 software is used to access the data. The separation was done with Phenomenex C18 (150mm × 4.6 ; 5µm) analytical column.

2.2. Chemicals and reagents

Ambroxol and cefadroxil was a gift sample by Lara drugs pvt Ltd., Hyderabad. Methanol of HPLC grade was purchased from Merck (India) Ltd., Mumbai. Ortho phosphoric acid (OPA) of AR grade was obtained from S.D. Fine Chemicals Ltd., Mumbai. mille Q water was used throughout the process.

2.3. HPLC conditions

The mobile phase consisting of 0.1% OPA: methanol was degassed and was pumped from the solvent reservoir in the ratio of 50:50 *v/v* was pumped into the column at a flow rate of 1.0 ml/min. The column temperature was 30°C. The detection was monitored at 249 nm and the run time was 7 min. The volume of injection loop was 10 µl. Prior to injection of the drug solution the column was equilibrated for at least 15 min. with the mobile phase.

2.4. Preparation of standard solution

30 mg of ambroxol and 150 mg of cefadroxil was weighed accurately and transferred into 100 ml volumetric flask. It was dissolved in 10 ml of mobile phase, sonicated for 10 minutes and diluted upto the mark with mobile phase. The solution was filtered through the 0.45 µm filter paper. Four ml of the above prepared standard stock standard stock was transferred into 50 ml volumetric flask dilute to volume with mobile phase

2.5. Preparation of sample solution

Accurately weighed 438.10 mg of sample powder was transferred into 100 ml of volumetric flask containing 10 ml mobile phase and dissolved with the aid of sonication for 20 min. The volume was made up to the mark with mobile phase and filtered through the 0.45 µm filter paper. Transfer 4 ml of above solution into 50 ml volumetric flask and made up the volume with mobile phase.

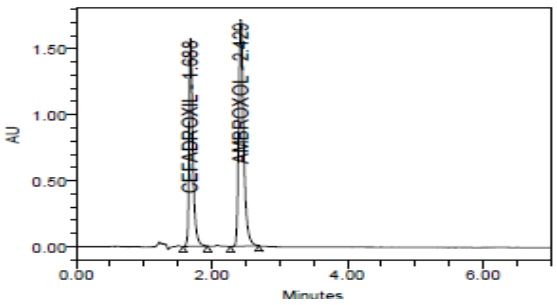

Figure 3. Standard chromatogram for ambroxol and cefadroxil

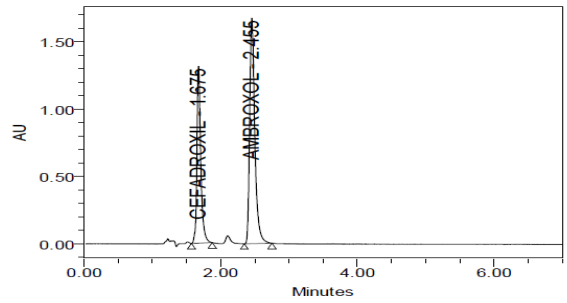

Figure 4. Formulation chromatogram for ambroxol and cefadroxil

2.6. Method validation

The method was validated according to ICH guidelines [10]

2.6.1. System Suitability Studies

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions (Table I). The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ± 1% standard deviation range during routine performance of the method.

Table 1. System Suitability Parameters

Parameter	Cefadroxil	Ambroxol
Retention time	1.688	2.429
Theoretical plates	5055	5716
Tailing factor	1.22	1.36
% RSD	0.8	0.6

2.6.2. Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may expect to be present. Typically these might include impurities, degradants, matrix, etc.

2.6.3. Forced degradation studies (Specificity)

Forced degradation of the drug product was carried out as per the ICH guideline. The forced degradation study of Ambroxol and Cefadroxil was performed in acidic, alkaline & peroxide media and exposing the drug to heat and sunlight conditions. The results are shown in Table II. The respective chromatograms are shown in Figures 5-9.

Table 2. Forced degradation studies

S.No	Ambroxol Area	Cefadroxil area	%Assay of Ambroxol	% Assay of Cefadroxil
Acid	8405199	5813384	96	94
Base	8411445	5857347	96	95
Peroxide	8341445	5887347	95	95
Heat	8327254	5898878	95	96
Sunlight	8431758	5832035	96	95

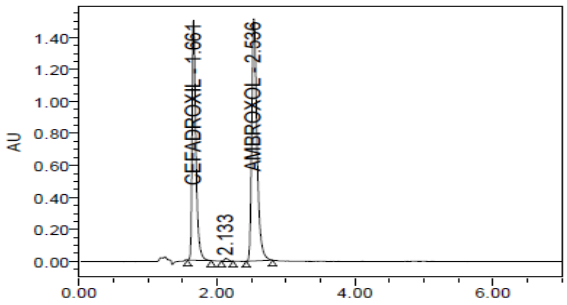

Figure 5. Chromatogram of sample after acid hydrolysis

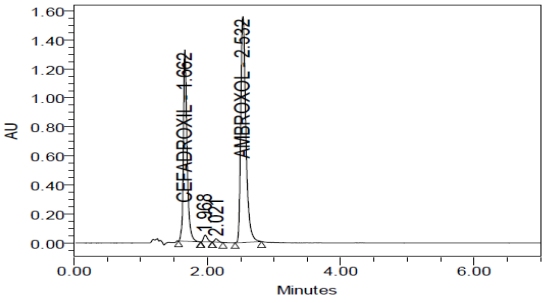

Figure 6. Chromatogram of sample after base hydrolysis

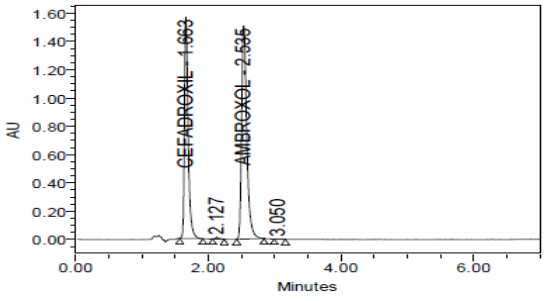

Figure 7. Chromatogram of sample after peroxide hydrolysis

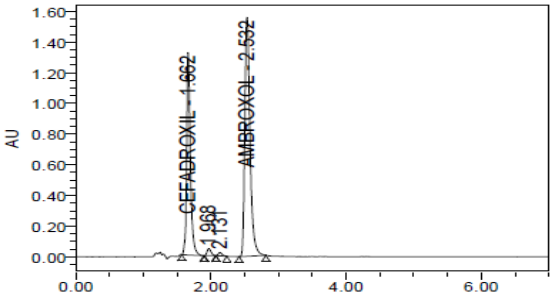

Figure 8. Chromatogram of sample after heat degradation

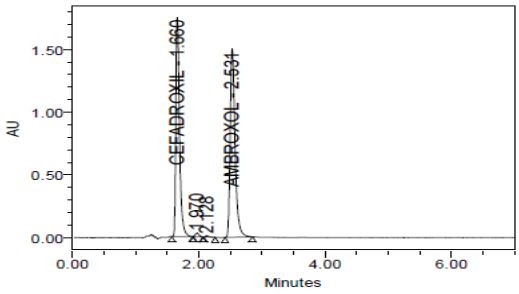

Figure 9. Chromatogram of sample after sunlight degradation

2.6.4. Accuracy and precision

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out thrice. The percentage recovery and its standard deviation were calculated. From the data obtained, added recoveries of standard drugs were found to be accurate (Table III).

Table 3. Accuracy of the method

Accuracy level	Sample weight	µg/ml added	µg/ml found	% Recovery	% Mean
Ambroxol					
50%	219.05	12.00	11.96	99	100
	219.05	12.00	11.96	100	
	219.05	12.00	11.99	100	
100%	438.10	24.00	23.96	100	100
	438.10	24.00	23.92	100	
	438.10	24.00	24.00	100	
150%	657.15	36.00	36.09	100	100
	657.15	36.00	35.72	99	
	657.15	36.00	36.04	100	
Cefadroxil					
50%	219.05	60.000	59.71	100	100
	219.05	60.000	59.91	100	
	219.05	60.000	59.85	100	
100%	438.10	120.000	119.93	100	100
	438.10	120.000	119.75	100	
	438.10	120.000	119.55	100	
150%	657.15	180.000	178.84	99	99
	657.15	180.000	178.88	99	
	657.15	180.000	178.37	99	

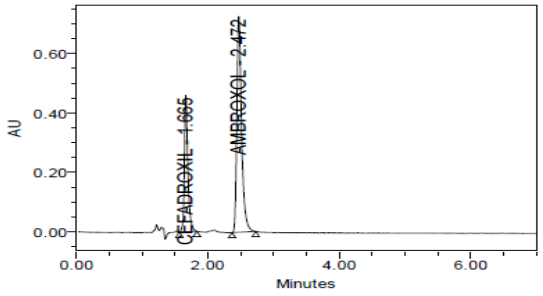

Figure 10. Chromatograms of ambroxol and cefadroxil at 50% level

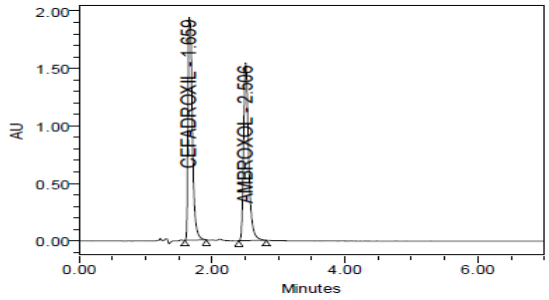

Figure 11. Chromatograms of ambroxol and cefadroxil at 100% level

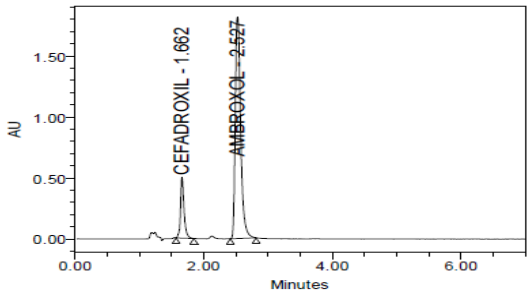

Figure 12. Chromatograms of ambroxol and cefadroxil at 150% level

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intraday studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage RSD were calculated. In the inter-day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drugs peaks and percentage RSD were calculated. From the data obtained, the developed RP-HPLC method was found to be precise (Table IV).

Table 4: Precision studies

S.No.	Ambroxol		Cefadroxil	
	Area	% Assay	Area	% Assay
1	8747787	100	6194395	100
2	8745763	100	6186949	100
3	8751078	100	6146044	100
4	8741012	100	6166045	100
5	8742140	100	6191852	100
6	8730473	100	6176679	100

2.6.5. Linearity and Range

The developed method was tested for linearity by plotting peak area against concentration of solutions. The plot of peak area versus the respective concentrations of ambroxol and cefadroxil were found to be linear in the concentration range of 12-36 µg/ml and 60-180 µg/ml respectively. The regression equation for ambroxol is y = 87738x with a coefficient of correlation (R^2) of 0.999. The regression equation for cefadroxil is y = 43363x with a coefficient of correlation (R^2) of 0.999. The results shows that an excellent correlation exists between area and concentration of drug within the concentration range indicated above. The results for calibration curves are given in Figures 13 &14.

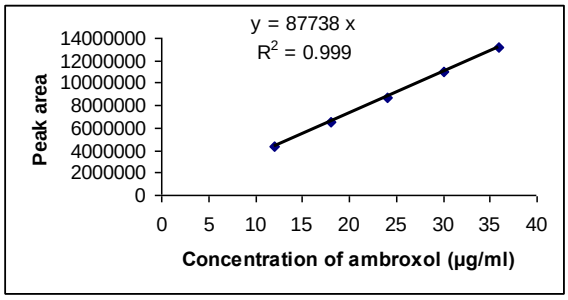

Figure 13. Linearity curve for ambroxol

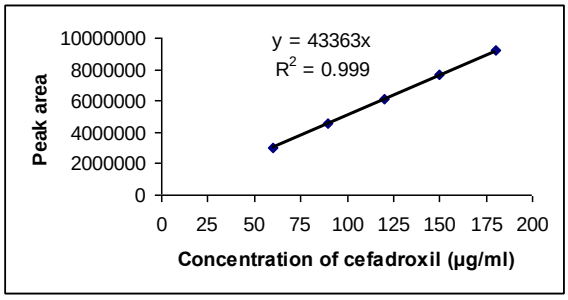

Figure 14. Linearity curve for cefadroxil

2.6.7. Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms which demonstrated that the RP-HPLC method developed is are robust (Table V).

Table 5. Robustness of the method

S. No	Sample Name	RT	Area	Theoretical plates	USP Tailing
Ambroxol					
1	Temp-1	2.536	8182384	5926	1.22

2	Temp-2	2.533	8241244	5771	1.35
3	Flow-1	3.050	9871135	6259	1.38
4	Flow-2	2.539	8174485	5926	1.28
Cefadroxil					
1	Temp-1	1.660	5798685	5097	1.34
2	Temp-2	1.658	5792846	5071	1.34
3	Flow-1	2.000	6968834	5401	1.20
4	Flow-2	1.663	5804282	4988	1.38

2.6.7. Limit of detection (LOD) & Limit of quantification (LOQ)

Limit of quantification and detection were predicted by plotting linearity curve for different nominal concentrations of ambroxol and cefadroxil. The LOQ and LOD values were calculated as follows:

LOQ = 10 σ / S

LOD = 3.3 σ / S

Where σ = residual standard deviation of response

S = slope of the calibration curve.

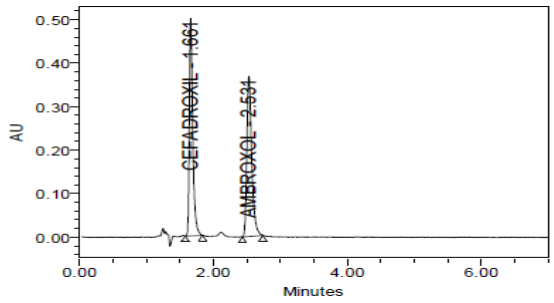

Figure 15. Chromatograms for LOD

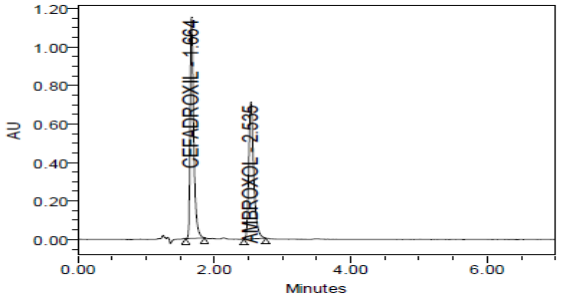

Figure 16. Chromatograms for LOQ

Table 6: LOD and LOQ for Ambroxol and Cefadroxil

S No.	Sample Type	Sample Name	RT	Area
1	LOD	Ambroxol	2.531	1853278
2	LOQ	Ambroxol	2.532	3561813
1	LOD	Cefadroxil	1.661	1759404
2	LOQ	Cefadroxil	1.664	4134428

3. Results and Discussion

A new stability indicating RP-HPLC method was developed for the estimation of simultaneous ambroxol and cefadroxil in bulk and its pharmaceutical formulations. The developed method was validated according to ICH guidelines. The results of validation indicated that the developed method was sensitive, linear, specific, precise and accurate for the simultaneous estimation of ambroxol and cefadroxil. The developed method was successfully applied to pharmaceutical formulation. The excipients commonly present in the formulations did not interfere in the assay. Therefore, the proposed method can easily and conveniently adopt for rountine quality control analysis of simultaneous determination of ambroxol and cefadroxil in pure and in its pharmaceutical dosage forms.

4. Conclusion

The proposed method can easily and conveniently adopt for routine quality control analysis of ambroxol and cefadroxil in pure and its pharmaceutical dosage forms.

5. Acknowledgement

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