

Q-ABSORBANCE RATIO METHOD FOR SIMULTANEOUS ESTIMATION OF ACETYLCYSTEINE AND ACEBROPHYLLINE

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ABSTRACT

A simple, economic, sensitive, accurate and reproducible spectroscopic method has been developed and validated for the simultaneous estimation of acebrophylline and acetylcysteine in tablet dosage form by Q-Absorbance ratio method. Acebrophylline and acetylcysteine were found to have absorbance maxima at 274 and 195 nm respectively in distilled water and iso absorptive point was found to be 210 nm. Acebrophylline was found to be linear in the concentration range of 5 to 11 µg/ ml at 274 nm and Acetylcysteine was found to be linear in the concentration range of 30 to 66 µg/ml at 195 nm. The assay of marketed tablet formulation (Pulmoclear Tablet) was found to be 103.4% and 101.58% for acebrophylline and acetylcysteine

respectively. The method was validated statistically as per ICH guidelines. The method showed good reproducibility and recovery with % RSD less than 2.

KEYWORDS: Acetylcysteine, Acebrophylline, Q-Absorbance ratio method, Validation, ICH.

INTRODUCTION

Chemically Acetylcysteine is the N-acetyl derivative of the amino acid L-cysteine and a precursor in the formation of antioxidant glutathione in the body. The thiol (sulfahydryl) group confers antioxidants effects and is able to reduce free radicals. Acetylcysteine is a (2R)-2-acetamido-3-sulfanylpropanoic acid (Figure 1). It is mucolytic drug which decreases the viscosity of secretions by splitting of disulphide bonds in mucoproteins. Intravenous and oral formulations of acetylcysteine are available for the treatment of paracetamol (acetaminophen) overdose.^[1-3] It is official in BP and USP.^[4,5]

Acebrophylline is 4-[(2-amino-3,5-dibromophenyl) methylamino] cyclohexan-1-ol-2;(1,3-dimethyl-2,6-dioxopurin-7-yl) acetic acid [Figure 1]. Acebrophylline is the salt obtained by reaction of equimolar amounts of theophylline-7-acetic acid, a xanthine derivative with specific bronchodilator activity and ambroxol, a mucolytic and expectorant. It is a novel drug with bronchodilating, anti-inflammatory and mucoregulating effect due to inhibition of phospholipase A, and phosphatidylcholine.^[1,2,6] It is not official in IP, BP, and USP.

Literature reports number of analytical procedures for the estimation of both drugs in either single dosage form, or in combination with other drugs.^[7-16] For combination of acebrophylline (ACB) and acetylcysteine (ACT), one HPLC method and one UV-spectrophotometry method have also been reported.^[17-18]

In the present work, Q-Absorbance ratio method for simultaneous estimation of acebrophylline and acetylcysteine in combined dosage form is developed and validated as per ICH Q2 (R1) guidelines.^[19]

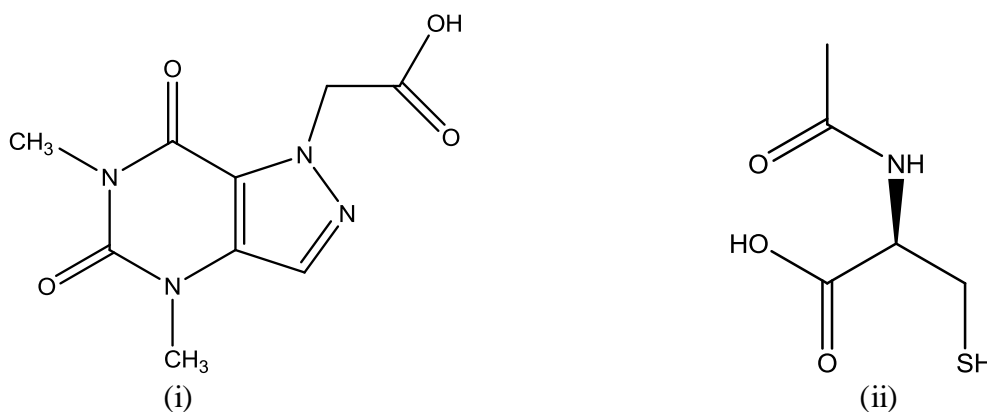


Figure 1: Chemical structure (i) Acebrophylline (ii) Acetylcysteine

MATERIALS AND METHODS

Chemicals and Reagents

Acetylcysteine and acebrophylline pure API was obtained as a gift sample from Montage Laboratory (Himatnagar) and Ami Lifescience Pvt. Ltd (Baroda) respectively. All reagent used were of AR grade. The commercial Pulmoclear (brand name) tablet formulation containing acebrophylline 100 mg and acetylcysteine 600 mg, manufactured by Fourrts (India) Laboratories Pvt. Ltd was obtained from local retail pharmacy.

Instruments

Double beam UV–visible spectrophotometer, Model: Shimadzu 1601, with a UV-Probe 2.32. version. It has 1cm quartz cell used for the spectral and absorbance measurements.

Solvent System

Distilled water.

Standard stock solution preparation

Acetylcysteine

Accurately weighed 10 mg ACT reference standard was transferred to 100 ml volumetric flask and was dissolved in minimum quantity of distilled water. The volume was made up to the mark with distilled water.

Acebrophylline

Accurately weighed 10 mg ACB reference standard was transferred to 100 ml volumetric flask and dissolved in minimum quantity of distilled water by using ultra sonication for 15 min. The volume made up to the mark with the same diluent.

Determination of λ_{max} and iso-absorptive point

The aliquots of ACT and ACB stock solution were taken and diluted with the distilled water individually to get concentration of ACT and ACB 30 $\mu\text{g/ml}$ and 5 $\mu\text{g/ml}$ respectively. The solution was scanned over the range of 190-400 nm using UV-visible Spectrophotometer and spectrum was recorded. The wavelength (λ_1 , λ_2) at which maximum absorbance was obtained, was considered as λ_{max} of the drug. Iso-absorptive point was found at 210 nm. Another wavelength used was 274 nm. These two wavelengths were used to record absorbance using method. The overlain spectra are shown in figure 2.

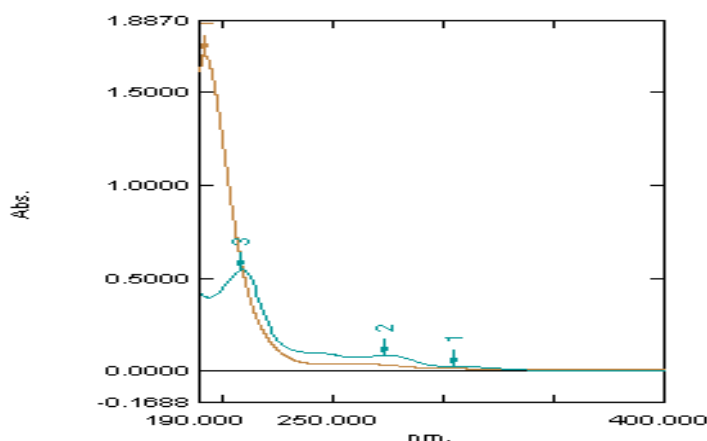


Figure 1: Overlain Spectra of ACT and ACB

Preparation of working standard solution

The aliquots (3,3.6,4.2,4.8,5.4,6 and 6.6 ml of ACT and 0.5,0.6,0.7,0.8,0.9,1 and 1.1ml of ACB) were transferred from the standard stock solution into 10 ml volumetric flask and diluted using distilled water up to the mark to get concentration of 30, 36, 42, 48, 54 ,60 and 66 µg/ml for ACT and 5,6,7,8,9,10 and 11 µg/ml for ACB.

Preparation of Sample solution

Twenty tablets of formulation (Acebrophylline 100 mg and 600 mg of Acetylcysteine) were weighed accurately and powdered. The 1143.4 mg tablet powder equivalent to 600 mg acetylcysteine and 100 mg acebrophylline was transferred into 100 ml volumetric flask and a minimum quantity of distilled water was added to dissolve the substance. It was sonicated for 15 min and diluted up to the volume with the same diluent. The content was filtered through Whatman filter paper. From the cleared solution, further dilutions were made by diluting 1 ml to 100 ml volumetric flask, further diluted 0.5 ml to 10 ml to obtain 30 µg/ml of Acetylcysteine and 5 µg/ml of Acebrophylline theoretically.

The absorbance measurements were made for the formulation at 274 nm and 210 nm. From the absorptivity values of Acetylcysteine and Acebrophylline at 274 nm, 210 nm, the amount of Acetylcysteine and Acebrophylline were determined by using Q-Absorbance ratio method.

Q- ABSORBANCE RATIO METHOD

$$C_X = \frac{Q_M - Q_Y}{Q_X - Q_Y} \frac{A_1}{a_{X1}} \quad C_Y = \frac{Q_M - Q_X}{Q_Y - Q_X} \frac{A_1}{a_{Y1}}$$

$$Q_M = A_2/A_1$$

$$Q_X = a_{X2}/a_{X1}$$

$$Q_Y = a_{Y2}/a_{Y1}$$

a_{X1} and a_{X2} = The absorptivity of ACB at 210nm and 274nm, respectively.

a_{Y1} and a_{Y2} = The absorptivity of ACT at 210nm and 274 nm, respectively.

A_1 and A_2 = The absorbance of the diluted sample at 210nm and 274nm, respectively.

Method validation

Linearity

For linearity aliquots were taken and diluted to get different concentration range for the ACT and ACB. Solutions were scanned between 200-400 nm and absorbance at 210 for ACT and

274 nm for ACB was measured. Slope, intercept and correlation coefficient (R^2) was calculated from the calibration curve.

Precision

The repeatability of the method was confirmed by the formulation analysis, repeated for five times with the same concentration. The amount of each drug present in the tablet formulation was calculated. The percentage RSD was calculated. The intermediate precision of the method was confirmed by intra-day and inter-day analysis i.e. the analysis of formulation was repeated three times in the same day and on three successive days, respectively. The amount of drugs was determined and % RSD was calculated.

Accuracy

Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of standard drug to formulation samples. The recovery was performed at three different concentrations levels (i.e. 80%, 100% and 120%). This procedure was repeated for three times for each concentration. The results of recovery studies were calculated for % RSD.

Specificity

Specificity is the ability of the method to measure the analyte in the presence of other relevant components. The evaluation of specificity of the method was determined against placebo.

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

The limit of detection (LOD) and the limit of quantitation (LOQ) of all selected combination of drugs were derived by calculating the signal to-noise ratio using the following equations as per the ICH guidelines.

$$\text{LOD} = 3.3 \times \sigma/S$$

$$\text{LOQ} = 10 \times \sigma/S$$

Where σ = standard deviation of the response and S = slope of calibration curve.

RESULTS AND DISCUSSION

Linearity

Linear relationship was found in the concentration range of range of 30 -66 $\mu\text{g/ml}$ (at 195 nm) and 5-11 $\mu\text{g/ml}$ (at 274 nm) for acetylcysteine and acebrophylline respectively. (Figure 2-3, Table 1).

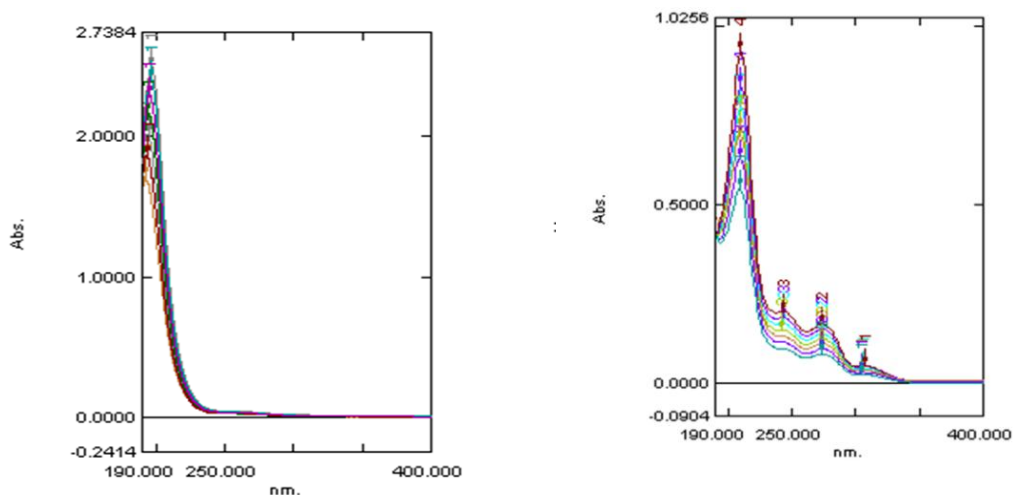


Figure 2: Spectra of ACT at 195nm and ACB at 274nm

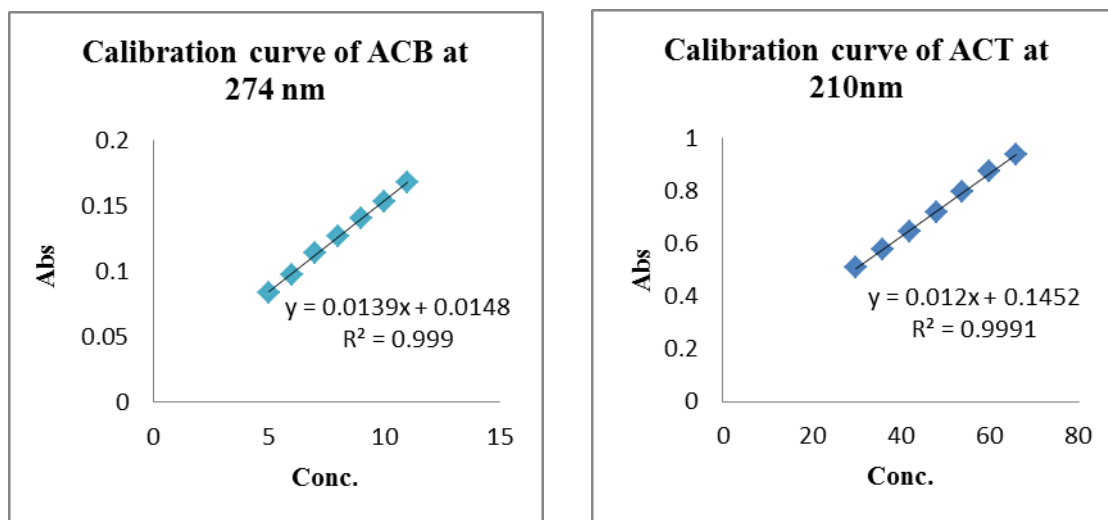


Figure 3: Calibration curves of ACT at 195 nm and ACB at 274 nm

Precision

The RSD values for Acetylcysteine and Acebrophylline were found to be 0.23554 and 0.6585 respectively (Table 2). Relative standard deviation was less than 2 %, which indicates that the proposed method is repeatable.

Accuracy

The recovery experiments were performed by the standard addition method. The mean recoveries were found to be 98 % and 98.02 % for ACT and ACB, respectively (Table 1). The low value of standard deviation indicates that the proposed method is accurate.

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

LOD and LOQ values for Acetylcysteine were found to be 0.3319 and 1.0058 µg/ml. Where, LOD and LOQ values for Acebrophylline were found to be 0.1315 and 0.3958 µg/ml, and (Table 1). These data show that method is sensitive for the determination of Acetylcysteine and Acebrophylline.

Table 1: Results of validation parameters.

Parameters		Acetylcysteine	Acebrophylline
		210nm (Iso-absorptive point)	274 nm
Linearity range (µg/ml)		30-66	5-11
Regression equation		$y = 0.012x + 0.1452$	$y = 0.0139x + 0.0148$
Correlation coefficient (R^2)		0.9991	0.999
Accuracy (%)	80%	97.89	98.02
	100%	97.94	98.02
	120%	97.77	98
Precision (% RSD)	Interday	0.01470	0.3182
	Intraday	0.03183	0.1360
Repeatability (% RSD)		0.23555	0.6585
LOD		0.3319	0.1315
LOQ		1.0058	0.3985

Assay

Analysis of sample of marketed tablet containing 600 mg acetylcysteine and 100 mg of acebrophylline was carried out and the amounts found were expressed as a percentage amount of the label claims. The percentage acetylcysteine and acebrophylline present in the formulation were found 101.58 and 103.4 respectively. Results of tablet assay are summarized in (Table 2).

Table: 2: Assay results of marketed tablet formulation

Tablet Formulation	Labeled Claim (mg/tablet)		Amount found (mg/tablet)		Potency %	
	Act	Acb	Act	Acb	Act	Acb
Pulmoclear tablet	600	100	609.48	103.4	101.58	103.4

CONCLUSION

From the results obtained by applying the suggested procedures it is obvious that the proposed method is accurate, precise, simple, sensitive, rapid and chip can be applied successfully in routine analysis for the estimation of acetylcysteine and acebrophylline in pharmaceutical formulation without interference from commonly encountered excipients and additives and with good sensitivity.

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