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RP-HPLC Analysis of Acebrophylline in API and Capsule Dosage Form.

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ABSTRACT

A simple, precise and accurate RP-HPLC method has been developed for the determination of acebrophylline in bulk andcapsule dosage form. Theλ_{max} of acebrophyllline was found to be 274 nm.An enable C_{18} (250x4.6mm, 5µm) column was used as a stationary phase. Mobile phase consisting of a mixture of acetonitrile and double distilled water (70:30) pumped isocratically at a flow rate of 1mL/min at ambient temperature. The retention time of acebrophylline was found to be 1.75 min. The calibration curve was linear over a concentration rangeof 5 to 50µg/mL with coefficient regression (r^2) =0.9986. Percentage relative standard deviation value is below 2.0 for intraday (n=3) and interday (n=3) precision. The percentage recovery value (average 98.47 %) indicated the accuracy of the method. The proposed method has a short retention time of 1.75 min, which makes the method suitable for the routine analysis of acebrophylline in bulk and capsule dosage form.

Keywords: Acebrophylline; RP-HPLC;Method development; Validation

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INTRODUCTION

Acebrophylline is an anti-inflammatory and airway mucus regulator. It contains ambroxol and theophylline-7-acetic acid, the former facilitates the biosynthesis of pulmonary surfactant while later raises blood levels of ambroxol, by stimulating surfactant production[1]. Chemically acebrophylline (Fig 1) is (1, 3- dimethyl-2, 6- dioxo-1, 2, 3, 6-tetrahydro-7H-purine-7yl) acetic acid-4[((2-amino-3, 5-dibromophenyl) methyl) amino]cyclohexanol.It is a salt obtained by reaction of equimolar amounts of theophylline-7-acetic acid and ambroxol [2].

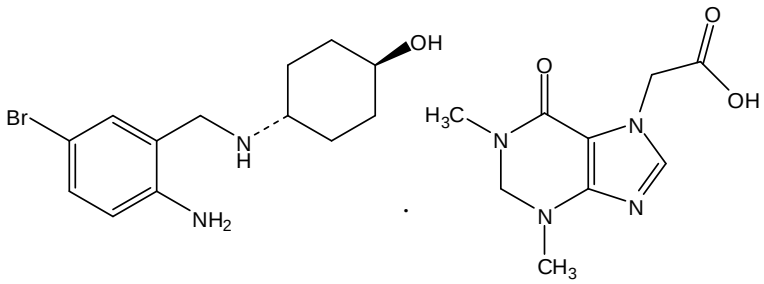

Fig.1.Chemical structure of acebrophylline

Theophylline-7-acetate has a bronchodilator effect due to inhibition of the intracellular phosphodiesterases, followed by an increase of adenosine monophosphate cyclic levels, which promote the relaxation of bronchial muscles. Ambroxol modifies the mucous gel phase of secretions by decreasing the viscosity and increasing the serous gel phase. It increases the mucociliary clearance by stimulating cilia motility. Acebrophylline inhibits phospholipase A, and phosphatidylcholine leading to lesser production of the powerful pro-inflammatory substances like leukotrienes and tumour necrosis factor. By inhibiting the synthesis and release of these inflammatory mediators, acebrophylline reduces inflammation, a key factor in airway obstruction, especially in chronic forms [2].

Literature survey revealed that very few analytical methods like HPLC methods [1-5], spectrophotometric[6-8] and HPTLC [9] have been reported for the determination of acebrophylline, individually and in combination with some other drugs. All these methods are expensive, time consuming, complex in nature. In these methods, mobile phases used were mostly buffers, which are very much hazardous for the column life and efficiency. Consequently, there was still a need to develop a simple, less time consuming and economical method for the determination of acebrophylline in API and dosage form. Therefore, we attempted to develop a fast and reproducible RP-HPLC method for the estimation acebrophyllinein bulk and capsule dosage form by following ICH method validation guidelines.

EXPERIMENTAL

Materials and Reagents

Acetonitrile and methanol HPLC grade were procured from SD fine chemicals. Acebrophylline, API sample was gifted by Synokem Pharmaceuticals Ltd, Haridwar, India.

Capsule formulation AB-Phylline, manufactured by Sun Pharma Ltd. Sikkim, India containing acebrophylline 100 mg per capsule was used.

HPLC Instrumentation and Conditions

Chromatographic analysis was performed on a Shimadzu HPLC system consisting of UV-visible detector (SPD-20A), rheodyne port with 20 µL loop volume and windows based LC-10 solution software. An enable C_{18}G column (250 × 4.6mm, 5µm) was used for analysis. The elution was carried out isocratically at a flow rate of 1 mL/min using acetonitrile:water (70:30v/v) mobile phase.

Preparation of stock solutions:

The primary stock solution (1mg/mL) was prepared by dissolving 100 mg of acebrophylline bulk drug in a small amount of methanol and the volume was made up to 100 mL with methanol.The secondary stock solution (100µg/mL) was prepared with methanol.

Preparation of working solutions:

A series of dilutions were prepared from secondary stock solution (100 µg/mL) using methanol as a solvent. The concentrations prepared are in the range of 1-50 µg/mL.

METHOD OPTIMIZATION

The RP-HPLC procedure was optimized with a view to develop an accurate and precise analytical method. Mobile phase consisting of a mixture of acetonitrile and water is used in different ratios. The flow rate was also varied from 0.6 mL to 1 mL/min and flow rate with 1 mL/min was found to be optimum. Mobile phase with a mixture of acetonitrile and water (70:30 v/v) pumped at a flow rate of 1 mL/min and detector set at 274nm gave a sharp and symmetrical peak with retention time of 1.749min.

RESULTS AND DISCUSSION

Method Validation

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. The determination of method specificity can be achieved by comparing the chromatogram of blank with that of standard acebrophylline peak. Typical chromatogram for acebrophylline was shown in Fig.2.

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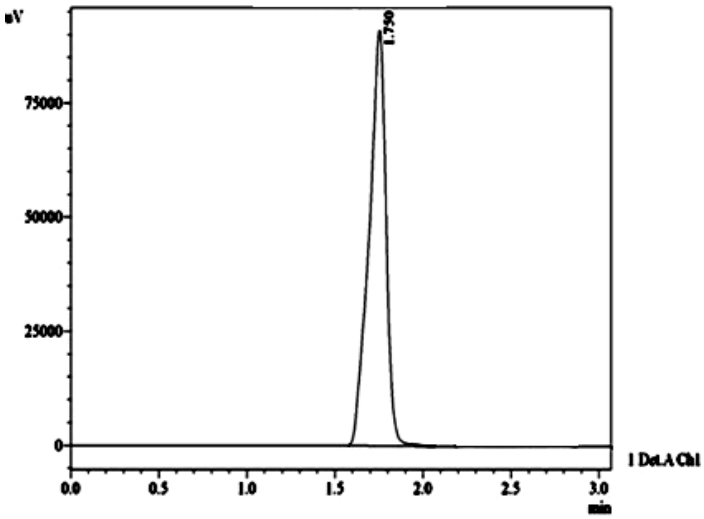

Fig.2 Typical chromatogram for acebrophylline

Linearity

Serial dilutions of acebrophylline (1-50 µg/mL) were injected into the column and detected at a wavelength set at 274nm. The calibration curve was obtained by plotting the concentration vs. peak area Fig.3. Concentration range 5-50 µg/mL was found to be linear with correlation coefficient r^2=0.9986

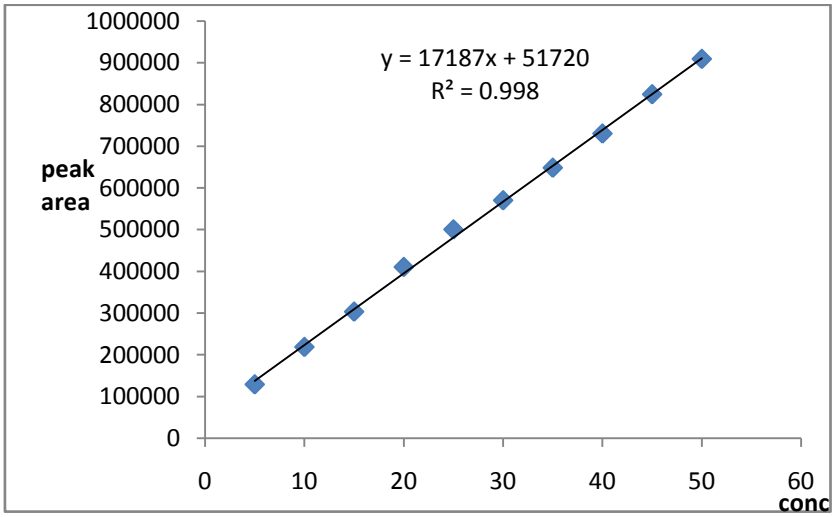

Fig.3 Linearity curve of acebrophylline (5-50 µg/mL)

Accuracy

The accuracy of a measurement is defined as the closeness of the measured value to the true value.Accuracy was calculated by recovery studies at three levels by standard addition method. The percentage recovery was found to be 98.47%-99.2%.The values accuracy results are shown in Table 1.

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Table.1 Accuracy results of acebrophylline

Drug name	Method	Concentration	Amt found	% Assay
Acebrophylline	Standard addition method	25 µg	24.8 µg	99.2 %
		30 µg	29.68 µg	98.93 %
		35 µg	34.5 µg	98.47 %

Precision

Precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.Intra-day studies were performed by injecting three repeated injections within a day. Peak area and %RSD were calculated and reported in Table.2. Inter-day precision studies, was done by injecting three (3) repeated injections for three consecutive days. Peak area and %RSD were calculated and reported in Table.2.

Table.2. Precision results of acebrophylline

ACEBROPHYLLINE	DAY-1 (%RSD)	DAY-2 (%RSD)	Day-3 (%RSD)	INTER-DAY (%RSD)
5 µg/mL	1.2	1	1.6	0.7
25 µg/mL	1.3	1.2	0.6	1
50µg/mL	1.2	1.4	0.4	0.4

Method sensitivity

The sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ).

The LOD and LOQ values for acebrophylline were reported in the Table 3.

Table.3 LOD and LOQ results for acebrophylline

Sample	LOD	LOQ
Acebrophylline	1.56 µg/mL	4.74 µg/mL

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. %RSD was calculated from peak areas. Results of robustness are summarized in Table 4-6. Percentage RSD of peak areas of acebrophylline was found be less than 2.0 % for variation in flow rate, composition of mobile phase and wavelength. Hence the developed method was found to be robust

Assay: The validated method was applied to the determination of acebrophylline in commercially available capsules. Twenty were accurately weighed and sample equivalent to 100 mg of was transferred to a 100 mL volumetric flask, dissolved in 60 mL methanol and sonicated for 10 min and the volume was made upto the mark with methanol. The solution was then filtered through whatmann filter paper No.41. Five mL of the above solution was diluted to 50 mL with methanol. From this solution, different dilutions were made and injected into the column. The percentage assay was found to be 98.47%. The results of assay indicate that the developed method is selective without interference from excipients of tablet.

Table.4 Data for variation in flow rate

Flow rate (mL/min)	Retention time (min)	Peak area	% RSD
0.9	1.77	584170	1.2 %
1.0	1.75	583184	
1.1	1.68	580450	

Table.5 Data for variation in wavelength

Wavelength (nm)	Retention time (min)	Peak area	% RSD
272	1.744	574322	1.0 %
274	1.750	564570	
276	1.747	563415	

Table.6 Data for variation in mobile phase composition

Mobile Phase Ratio	Retention time (min)	Peak area	% RSD
68:32	1.758	568723	0.7 %
70:30	1.750	563890	
72:28	1.744	574201	

CONCLUSION

The proposed RP-HPLC method for the estimation of acebrophylline in bulk and pharmaceutical formulation was found to be simple, rapid, precise, accurate and robustic. The intra-run andinter-run variability and accuracy results were found in acceptable limits. Simplicity of the method, shorter run time, and economical nature makes the method superior to the other reported HPLC methods. The proposed method has a short retention time of 1.75 min, which makes the method suitable for the routine analysis of acebrophylline in bulk and capsule dosage form.

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