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NEW ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ACEBROPHYLLINE (BRONCHODILATOR) DRUG

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ABSTRACT

A simple, economic, precise, and accurate reverse phase high performance liquid chromatographical method has been developed for the simultaneous estimation of acebrophylline in tablet dosage form. An enable x-bridge^{TM}, $C_{18,}$ 4.6×250mm, 5µm particle size column was used as stationary phase. The mobile phase consisting of a mixture of buffer (ammonium acetate P_H 4.7) solution and acetonitrile (70:30) was pumped isocraticcally at a flow rate of 1ml/min with detection of 273nm. The retention time of acebrophylline were found to be 2.260 min. The calibration curves were linear over a concentration range of 10-60 µg/ml. with coefficient regration (r^2) =0.9945 for acebrophylline. The limit of detection and limit of quantification were 0.329µg/ml 1.0975 µg/ml respectively for acebrophylline. The selectivity, specificity, system suitability, ruggedness and robustness were performed as per ICH guidelines. The percentage RSD was found to be less than 2. Due to simplicity, rapidity, and accuracy of the method with believe that the method will be useful for routine quality control analysis of acebrophylline in pharmaceutical dosage form.

KEY WORDS: Acebrophylline, Accuracy, LOD, LOQ, RP-HPLC and validation.

INTRODUCTION

Acebrophylline, a xanthine derivative, is prescribed as a bronchodilator; for the treatment of chronic bronchitis bronchial asthma and COPD in adults. It is a novel drug with bronchodilating, anti-inflammatory and Mucoregulating effect. Chemically it is 4-[(2-amino-3,5-dibromophenyl) methylamino] cyclohexan-1-ol;2-(1,3-dimethyl-2,6-dioxopurin-7-yl)

acetic acid. Acebrophylline is the salt obtained by reaction of equimolar amounts of theophylline-7-acetic acid, a xanthine derivative which inhibits phospholipase A, and phosphatidylcholine leading to lesser production of the powerful pro -inflammatory substances like leukotrienes and tumor necrosis factor. Acebrophylline alter mucus gel secretion phase by lowering viscosity and increasing the serous gel phase. By augmenting ciliary motility, acebrophylline increases the mucociliary clearance. It is available as the white crystalline powder which is slightly soluble in water, slightly soluble in ethanol, ether, chloroform, slightly soluble in alcohol, more soluble in hot water, soluble in alkaline solutions. Acebrophylline comes under the drugs affecting the respiratory system category.[39-43]

Literature survey revealed that very few analytical methods like HPLC methods spectrophotometric and HPTLC 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. Therefore, the objective of the present work was to develop an accurate, specific and reproducible method for the estimation of acebrophylline in pharmaceutical oral dosage forms. Parameters are established for standardization of the methods including statistical analysis of data.

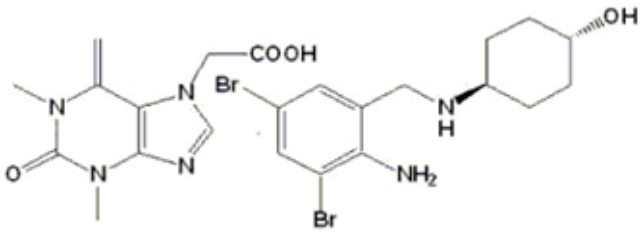

Figure 1- Chemical structure of Acebrophylline.

MATERIALS AND METHODS

Reagents and chemicals

Ab- Phylline (Sun Pharma) is a tablet dosage form each contains 200mg of Acebrophylline. HPLC grade Acetonitrile (Merck) and Analytical grade Ammonium Acetate was used as the solvents throughout the experiment. Pharmaceutical formulation Ab-Phylline tablet (label claim contains (200mg) was used in HPLC analysis. HPLC grade water obtained by using Direct-Q water purification system (Millipore, Milford, USA) was used in HPLC study and all the apparatus and instruments used were calibrated and validated. The optimized chamber

saturation time with mobile phase was 30 min using saturation pads at room temperature (25 ˚C ± 2). Evaluation was by peak areas with linear regression.

Instrumentation

The Agilent 1120 Compact LC HPLC system consisting of gradient pump (LC-10AT vp pump) (4MPa or 40barr), rheodyne injector, UV variable wavelength detector, Standard cell and Agilent syringe was used. The separations were achieved on a X- Bridge^{TM} C_{18} column (5µm 4.6x250mm), column length is 15 cm with UV detection at 273nm. Analytical weighing balance. (Shimadzu AUX 220) was used for weighing, sonicator (EQUITRON230VAC, 50Hz), vacuum pump (SUPER FIT), filtration kit (TARSONS) and Nylon membrane filter (Merck Millipore) for solvents and sample filtration were used throughout the experiment. Double beam UV Visible spectrophotometer (SHIMADZU-UV 1700) was used for wavelength detection. The EZ Chrome Elite software-single channel was used for acquisition, evaluation and storage of chromatographic data.

Chromatographic condition

After several trials with the different combination and ratio of solvents, the mobile phase Ammonium acetate (buffer): Acetonitrile (70:30v/v) at P^{H}-4.7. Retention time (R_t) 2.260 min for Acebrophylline. Wavelength was selected by scanning the standard drug over a wide range of wavelength 200 nm to 400 nm. The component shows reasonably good response and maximum peak at 273nm.

Preparation of standard solution for the HPLC estimation of Acebrophylline

A tablet is powdered which contain 200 mg of active ingredient. Equivalent weight of 10mg of drug is transferred into 10 ml of volumetric flask and is dissolved in mixture of acetonitrile and the buffer (30:70) volume were made up to the mark with same solvent. This gave the concentration of 1000 µg ml^{-1} of Acebrophylline (Stock-1). From stock solution 1, 6 dilutions were prepared between 10-60 µg ml^{-1} which is working concentration.

RP-HPLC Method Development and optimization of chromatographic conditions

The method development was conducted on an enable x-bridge^{TM}, $C_{18,}$ 4.6×250mm, 5µm particle size column used for separation. The chromatographic conditions were optimized with respect to specificity, resolution and time of analysis. 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 ammonium acetate (30:70 v/v) ratio is used. The

flow rate was 1 mL/min and flow rate with 1 mL/min was found to be optimum. Mobile phase with a mixture of acetonitrile and ammonium acetate (30:70 v/v) pumped at a flow rate of 1 mL/min and detector set at 273nm gave a sharp and symmetrical peak with retention time of 2.263min.

RESULTS AND DISCUSSIONS

Method development

The developed method was validated according to ICH guidelines [4] with respect to specificity, accuracy, precision, linearity, limit of detection (LOD), limit of quantification (LOQ) ruggedness, robustness and system suitability.

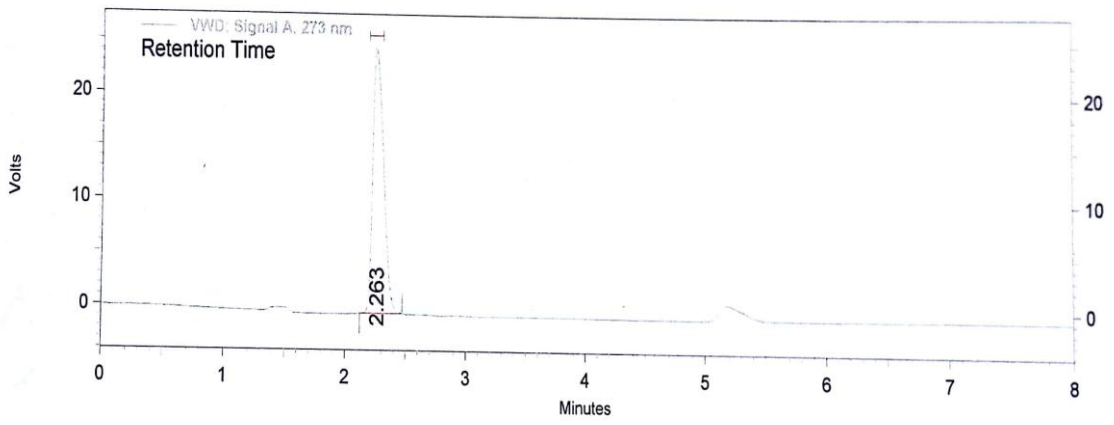

Figure 2- A typical chromatogram for acebrophylline.

Linearity and range

By using the working standard, a liquots of 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, 60µg/ml, were prepared with acetonitrile. Six dilutions of each of the above-mentioned concentrations were prepared separately and from these six dilutions, 20µl of each concentration were injected into the HPLC system. Then their chromatogram was recorded. Peak areas were recorded for all the peaks and a standard calibration curve of peak area against concentration was plotted.

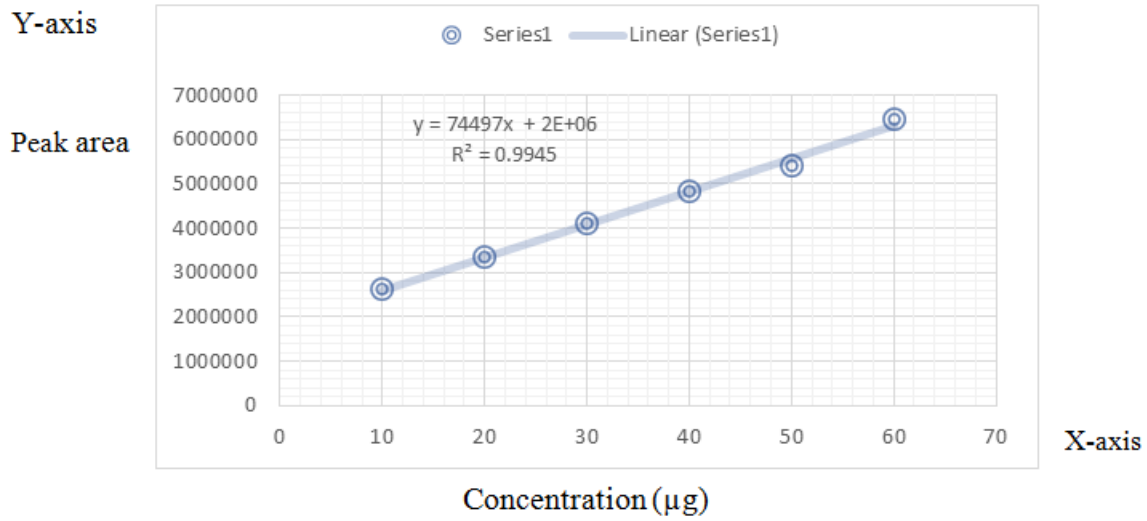

Figure 3- linearity graph of acebrophylline

In Y-axis peak area should be there while in X- axis concentration (µg) should be there. (peak area vs concentration)

linearity (µg)	area
10	2622770
20	3344289
30	4105348
40	4823074
50	5395679
60	6463187

Table 1- linearity data for acebrophylline.

Precision

The precision of the assay was determined in terms of intra and inter day variation in the peak area for a set of drug solution 30µg/ml, assayed six times on the same day and on different 2 days.

The intra and inter day variation in the peak ratio of the drug solution was calculated in terms of co-efficient of variation (CV) and obtained by multiplying the ratio of the standard deviation to the mean with

100(CV=SD/MEAN × 100) shown in the graph.

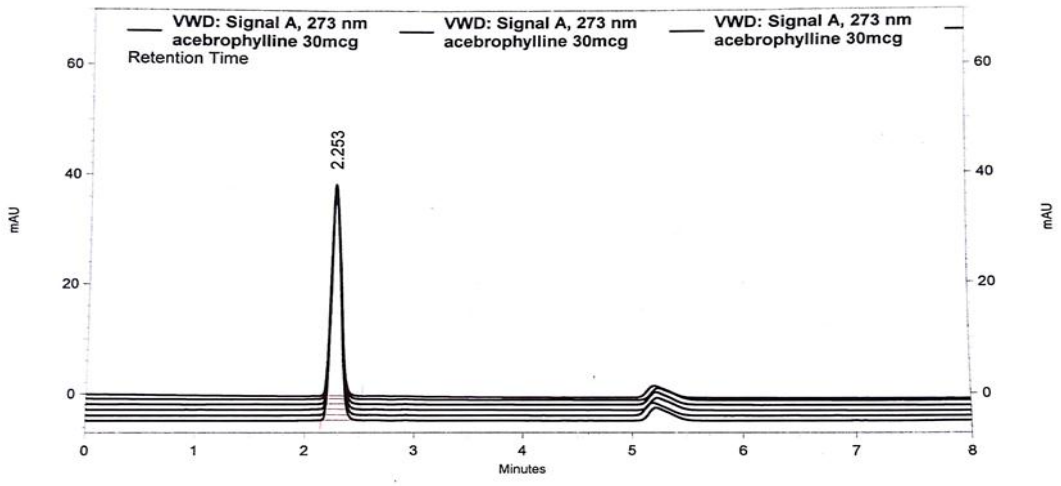

Figure 4 - Chromatogram showing intraday precision –morning.

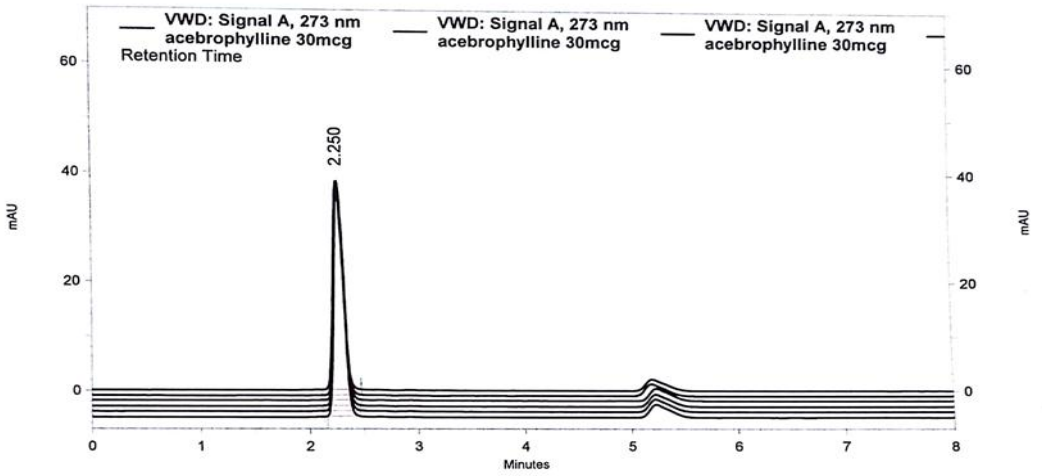

Figure 5 - Chromatogram showing intraday precision –afternoon.

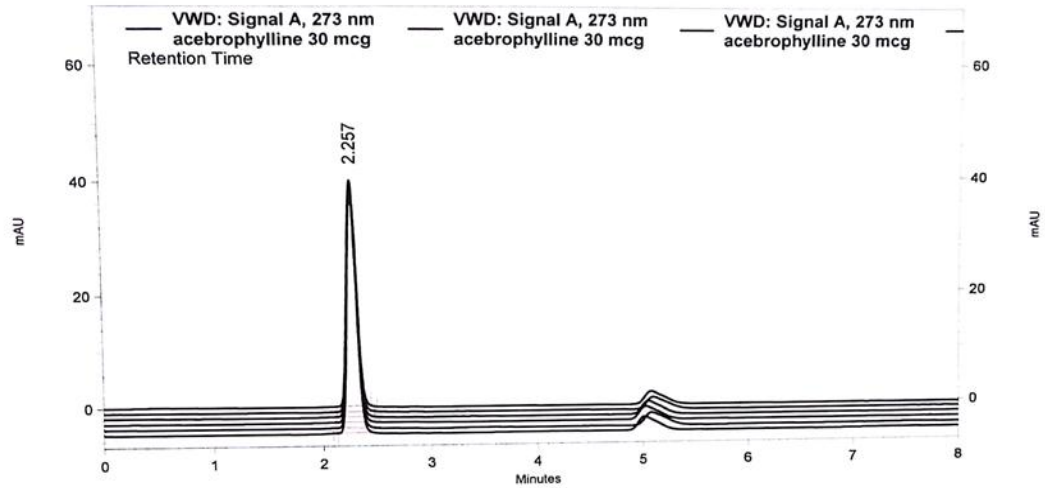

Figure 6 - Chromatogram showing inter day precision (Day-1).

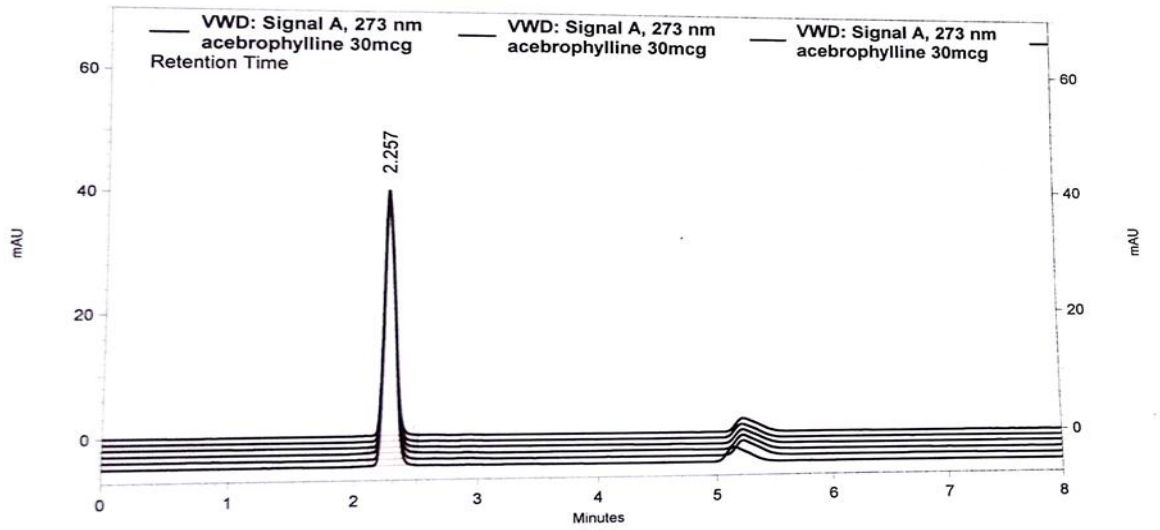

Figure 7 - Chromatogram showing inter day precision (Day-2).

Accuracy

The procedure for the preparation of the solutions for Accuracy determination at 80%, 100% and 120% level were prepared in the acetonitrile.

For 80% Accuracy for Acebrophylline:

10 mg of the formulation was added to 8mg of pure drug

For 100% Accuracy for Acebrophylline:

10mg of the formulation is added to 10mg of pure drug

For 120%Accuracy for Acebrophylline

10 mg of the formulation is added to 12 mg of pure drug

Table 2 - Accuracy data for estimation of Acebrophylline.

Level of percentage recovery	Amount of Drug present in Sample	Amount of Standard drug added	Area	Mean	Standard Deviation	%RSD
80%	10mg	8mg	4153523	4153757	3553.6	0.08
			4157431			
			4150336			
100%	10mg	10mg	4806780	4811013	4377.15	0.09
			4815521			
			4810737			
120%	10mg	12mg	5301128	5354872	46554.91	0.86
			5882767			
			5380721			

Robustness

As defined by the ICH, the robustness of an analytical procedures describes to its capability to remain unaffected by small and deliberate variation in the chromatographic conditions and found to be unaffected by small variation ±0.1ml/min in flow rate of mobile phase, wavelength ±5nm results are shown.

Table 3 - Robustness of acebrophylline.

Sl.no.	Parameter	Optimized	Used	Retention time(mins)
1	Flow rate	1 ml/min	0.9 ml/min	2.517
			1.1ml/min	2.060
2	Detection wavelength	273 nm	278nm	2.263
			263 nm	2.260

CONCLUSION

From the above results, method was found to be accurate, precise, linear, specific, system suitable, robust proved to be sensitive, convenient and cost effective for the estimation of Acebrophylline in oral solid dosage form. The proposed method has a run time of 8mintues, which makes the method simple, cost effective and suitable for the routine analysis of Acebrophylline in oral solid tablet dosage form.

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