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SIMULTANEOUS ESTIMATION OF ACEBROPHYLLINE AND ACETYLCYSTEINE IN TABLET DOSAGE FORM BY RP –HPLC METHOD

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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 and N-Acetylcysteine in tablet dosage form. An enable Hypersil BDS, C18, 100 x 4.6 mm, 5 µ particle size column was used as stationary phase. The mobile phase consisting of a mixture of buffer solution and acetonitrile (90:10) was pumped isocratically at a flow rate of 1 ml/min with detection at 260nm. The retention time of Acebrophylline and N-Acetylcysteine were found to be 5.5 min and 2.3 min respectively. The calibration curves were linear over a concentration range of 25-150 µg/ml with coefficient regression (r^2) = 0.9995 for Acebrophylline and (r^2) = 0.9996 for N-Acetylcysteine. The limits of detections were 0.18 µg/ml and 1.50 µg/ml for Acebrophylline & N-Acetylcysteine respectively. The selectivity, specificity, system suitability, ruggedness and robustness were performed as per ICH guidelines. In quantitative and recovery studies was 100.37% and 100.8% for Acebrophylline and N-Acetylcysteine respectively. The percentage RSD was found to be less than 2. Due to the simplicity, rapidity and accuracy of the method with believe that the method will be useful for routine quality control analysis of Acebrophylline and N-Acetylcysteine in pharmaceutical formulation.

Key words: Acebrophylline-ACB, N-Acetylcysteine-NAC, Accuracy, RP-HPLC and Validation.

INTRODUCTION

Acebrophylline is an anti-inflammatory and airway mucus regulator. It contains ambroxol and theophylline-7-acetic acid are 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]. 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].

Acetylcysteine (NAC), ((2*R*)-2-(acetylamino)-3-sulfanylpropanoic acid) is a mucolytic agent used in bronchitis, pulmonary diseases and respiratory disorders associated with active cough. It depolymerises mucopolysaccharides, reduces the viscosity of pulmonary secretions. Besides mucolytic effect it also has anti-oxidant and antiinflammatory effects and it is used as an antidote in Paracetamol poisoning [3]. More recently, animal and

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human studies of NAC have shown it to be a powerful antioxidant and a potential therapeutic agent in the treatment of cancer, heart disease, HIV infection, heavy metal toxicity, and other diseases characterized by free radical and oxidant damage [3].

The literature survey reveals that there was no analytical method for the simultaneous estimation of Acebrophylline and Acetylcysteine in tablet dosage form. Sravani Takkarusu et al [1] had developed a precise and accurate RP-HPLC method for the determination of Acebrophylline in bulk and capsule dosage form. G. Lalitha et al [2] had developed a UV-visible spectroscopical estimation for Acebrophylline and N-Acetylcysteine in bulk and capsule dosage form. So their is a need to develop a simple, less time consuming and economical method for the estimation of Acebrophylline and N-Acetylcysteine in tablet dosage form. Hence an attempt was made to develop and validate a simple, precise and accurate RP-HPLC method as per ICH guidelines [9, 10].

MATERIALS AND METHODS

Reagents

All the reagents and chemicals used were of HPLC grade. Acebrophylline and N-Acetylcysteine working standards were obtained from Sun Pharma, Mumbai and Torrent Pharmaceuticals, Ahmadabad respectively. The Pulmoclear Tablets were obtained from Fourrts (India) Laboratories Pvt. Ltd. HPLC grade Acetonitrile was procured from SDFCL Mumbai, India. All other chemical reagents were of analytical grade.

Instrumentation

A Waters Alliance 2695 with Empower 2 software HPLC system provided with PDA detector of model 2998 and an automatic injector were used. The chromatographic analysis was performed on Hypersil BDS, C18, 100 x 4.6 mm, 5µ particle size column.

Preparation and selection of mobile phase

Buffer: (0.02M KH2PO4)

Accurately weighed and transferred 2.72gm of Potassium dihydrogen orthophosphate in a 1000ml of volumetric flask, add about 900ml of milli-Q water and 1ml of triethylamine then sonicated to degasses the solution, finally the volume make up with water. The pH adjusted to 3.2 with dil. ortho phosphoric acid solution.

Mobile phase

Buffer and Acetonitrile taken in the ratio 90:10.

Preparation of Standard solution

Accurately Weighed and transferred 10mg of Acebrofylline and 12.5mg of N-Acetylsteine working standards into a 10 ml clean dry volumetric flask, add 7ml of diluents, sonicated for 30 minutes and make up to the volume with diluents (methanol).

Preparation of Sample solution

20 tablets were weighed and powdered. Calculated the average weight of each tablet and weighed equivalent to 50 mg was transferred into a 500 ml volumetric flask. 50ml of diluent was added and sonicated for 30 min; further the volume was made up to with diluent and filtered through 0.45 µ nylon filters. From the above filtrate 1ml was pipette out into a 10 ml volumetric flask and made upto 10ml with diluent. The desired concentrations for the drug were obtained by accurate dilution, and the analysis was followed up as in the general analytical procedure.

RP-HPLC Method Development and optimization of chromatographic conditions

The method development was conducted on a Hypersil BDS, C18, 100 x 4.6 mm, 5µ particle size column used for separation. The chromatographic conditions were optimised with respect to specificity, resolution and time of analysis. The optimal experimental conditions fixed with a mixture of mobile phase consist of buffer and acetonitrile in ratio of 90:10 and a flow rate of 0.9ml/min with detection at 260nm. The ACB and NAC depicted a well defined chromatographic separation within a run time of 8 min. The retention times of ACB and NAC were 5.5min ± 0.32 and 2.3 min ± 0.35, respectively. Fig 2 shows chromatograms of standard and sample of Acebrophylline and Acetylcysteine.

RP-HPLC Method Validation

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

Specificity of the method

It is an ability to measure the accurate concentration of analyte in the presence of all other excipient. The 10.0mg of placebo was transfer into a 100 ml volumetric flask and added sufficient amount of diluents. The sample solution was sonicated to dissolve and make up the volume with diluents. Further dilute 1 ml of the above solution to 10 ml with diluents. The final solution of placebo was injected in to a chromatographic system and chromatogram was observed.

Accuracy 50%, 100% and 150%

Accurately weighed 10mg of acebrophylline and 12.5mg of acetylcysteine were transferred into a10 ml volumetric flask. The compounds were first dissolved in 7 ml of diluent and it was sonicated for 5 minutes then the volume was made up to the mark with diluents and mixed well. Further 0.5 ml, 1ml and 1.5ml were diluted to 10 ml in different volumetric flasks with diluents to get 50%, 100% and 150% solutions respectively and mixed well, filtered through 0.45µ nylon filter. The accuracy of the

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method was computed by the determination of recovery for three concentrations [5]. The amount of ACB and NAC was recovered and then percentage of drug content was calculated.

Precision

Precision was studied to find out system precision, method precision and interday variation. In method precision, a homogenous sample of a single batch was analyzed six times. The % RSD of the assay value for six determinations was calculated. The system precision was checked by measuring six successive injections of Acebrophylline and Acetylcysteine standard to ensure that the analytical system was precise [7]. The percentage RSD was calculated for ACB and NAC shown in table 4.

Ruggedness

The standard stock solution and sample stock solution were prepared by different analysts on different days and the resulting solutions were injected and chromatograms are recorded.

Precision was studied to find out system precision, method precision and interday variation. The percentage RSD was calculated for ACB and NAC, Table (4) shows the results of the test obtained by running the samples for two days by various analysts.

Linearity

Linearity was determined by the regression analysis and was studied by preparing standard solution at different concentration levels (25-150%). Acebrophylline and Acetylcysteine standard solutions are prepared across the range of the analytical method with a minimum of five concentrations that are within the specified range that 25%, 50%, 75%, 100%, 125%, 150% of Acebrophylline and Acetylcysteine. The calibration curves were plotted between certain concentrations [5] and average peak areas. The results were shown in fig 5.

Robustness

Robustness study with effect of flow rate

Robustness was carried by small deliberate changes in chromatographic conditions at three different levels and retention time was noted. The factors selected were flow rate and pH. To carry out the test method with variation of flow rate (± 0.2 ml/minute of set value, i.e. 0.8 and 1.2 ml/minute) and P^H (± 0.2 of set value). Prepare standard preparation and perform analysis as per test method and evaluate the system suitability parameters [8].

System suitability test

To establish the chromatographic conditions, a system suitability test during the development and optimization of the method was performed. The test was performed by injecting standard mixture to the chromatographic system and the various parameters, retention time, tailing factor and theoretical plates were computed as reported by the International conference harmonized guidelines [4, 6].

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

The limit of quantification (LOQ) and limit of detection (LOD) were calculated using the signal-to-noise-ratio of 10 and 3 the results were tabulated III.

Table 1. Data showing assay of tablet formulation

Component	Label claim	% Amount Mean	S.D	%RSD
Acebrophylline	100mg	100.09	0.56	0.566
Acetylcysteine	600mg	99.75	0.51	0.512

Table 2. Results of Accuracy

S.No	Spike level	Mg added(ppm)		Mg Recovered(ppm)		%Recovery	
		ACB	NAC	ACB	NAC	ACB	NAC
1	50%	50	300	49.93	303.0	99.8	101.02
2	50%	50	300	50.09	302.7	100.1	100.9
3	50%	50	300	50.17	299.1	100.3	99.7
4	100%	100	600	100.6	595.2	100.6	99.2
5	100%	100	600	102.1	600.9	102.1	100.1
6	100%	100	600	101.7	597.3	101.7	99.5
7	150%	150	900	152.02	909.5	101.3	100.06
8	150%	150	900	150.4	901.2	100.2	100.1
9	150%	150	900	151.9	909.6	101.2	100.07

Table 3. Results of intermediate precision

Sample	Assay%	
	Acebrohylline	Acetylcysteine
1	100.6116	99.82471
2	99.57081	99.69537

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3	100.667	100.2917
4	100.1357	99.32941
5	101.262	99.71991
6	100.4335	98.77111
AVG	100.4468	99.60537
SD	0.566493	0.512353
%RSD	0.56	0.51

Table 4. Linearity Results, Limit of Detection (LOD) and Limit of Quantification (LOQ)

Parameters	Acebrophylline	Acetylcysteine
Number of samples	5	5
Correlation range (µg /mL)	25-150 (µg/mL)	25-150 (µg/mL)
Regression coefficient	0.9995	0.9996
Limit of Quantification (µg/mL)	0.568100676	4.558335662
Limit of Detection (µg/mL)	0.187473223	1.504250768

Table 5. Summaries of Validation Parameters

S.No.	Validation Parameters	Requirement	Results		Acceptance Criteria
			Acebrophylline	Acetylcysteine	
1.	Specificity	No Interference	Pass	Pass	No Interference
2.	Linearity	Correlation Coefficient	0.9999	0.9998	NLT 0.999
3.	Accuracy	%Recovery	100.8%	100.37%	100 ± 2%
4.	Method Precision	%RSD	0.51	0.51	NMT 2%
	System Precision		0.6	0.2	NMT 2%
	Ruggedness		0.78	0.72	NMT 1%
5.	Robustness	%RSD	0.97	0.57	NMT 1%
6.	System Suitability	RT	5.564	2.401	-
		Tailing Factor	0.99	1.09	NMT 2
		Plate Count	5945	4448	NLT 4000
		Assay Value	100.44%	99.6%	100 ± 2%
7.	LOQ	Concentration of analyte	0.56	4.55	-
8.	LOD	Lowest concentration	0.18	1.5	-

Fig 1-2. chromatogram of Acebrophylline and N-Acetylcysteine sample and standard

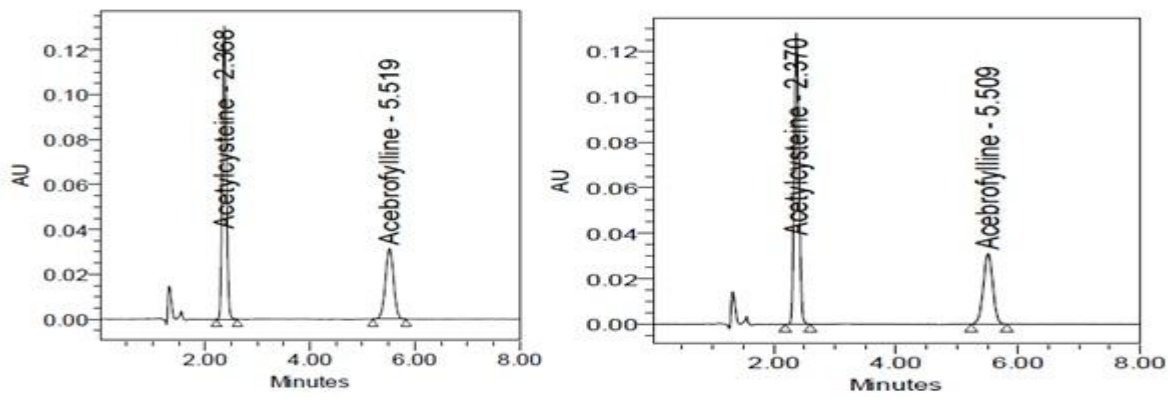

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Fig 3. Chromatogram for system suitability

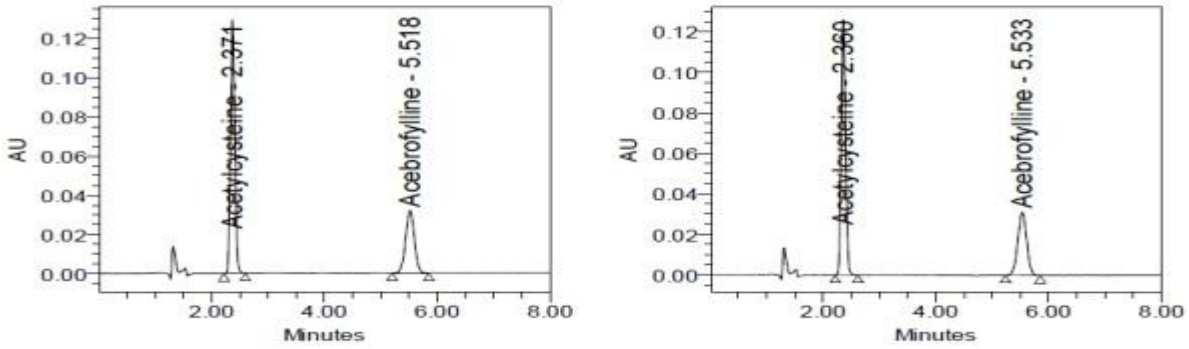

Fig 4. Linearity plot of Acebrophylline and Acetylcysteine

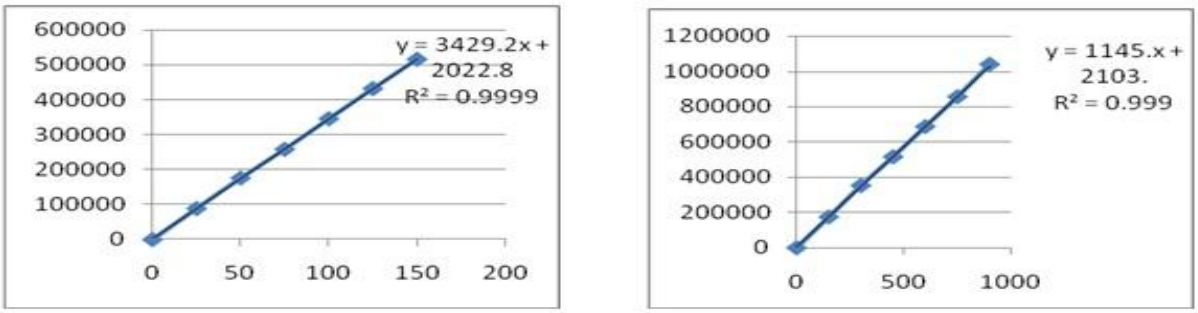

Fig 5. Chromatogram for linearity concentration at 25%, 50%, 75%, 100%, 125%, 150%

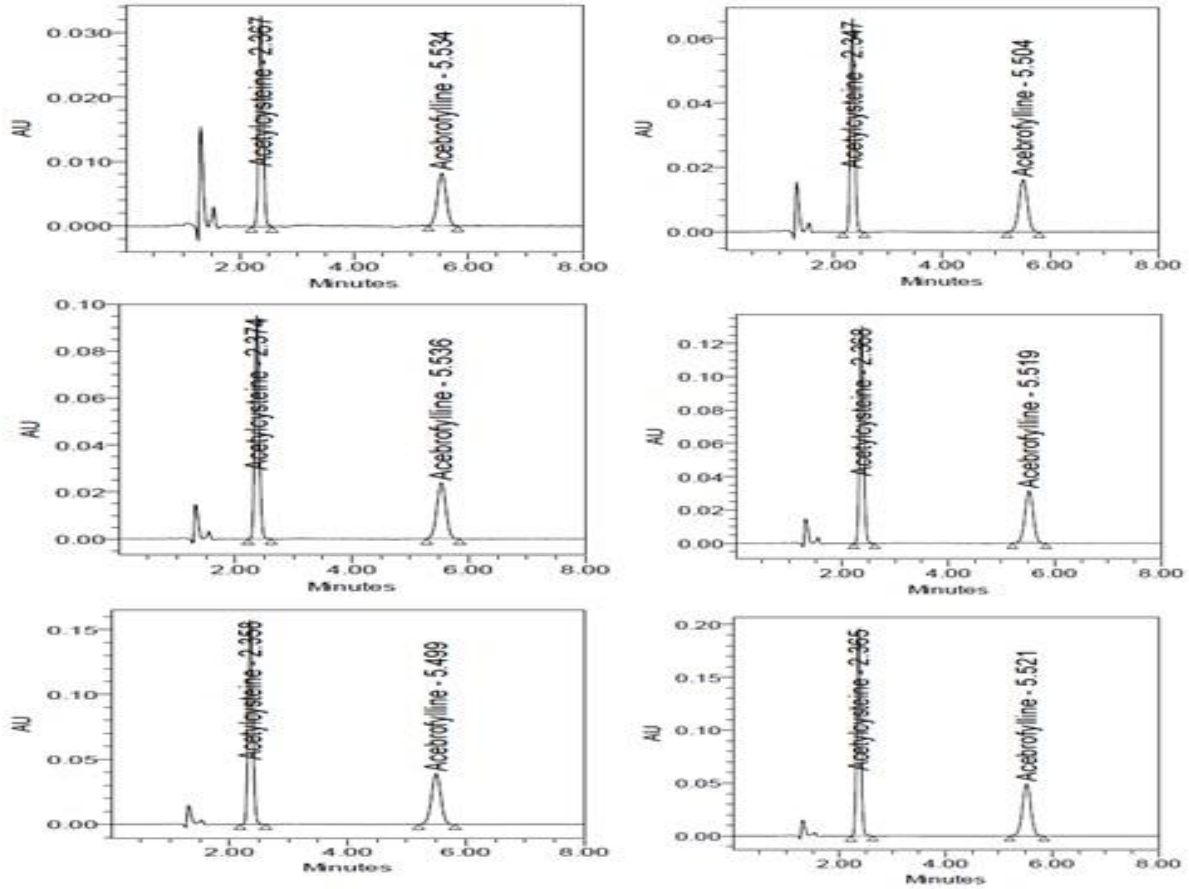

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Fig 6. Accuracy 50% level

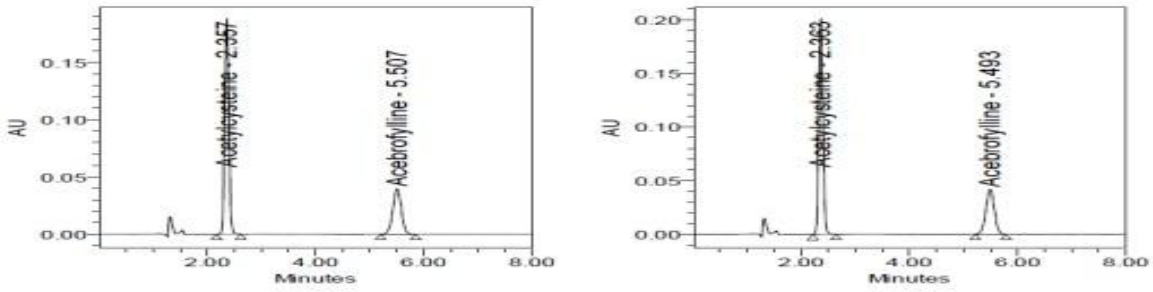

Fig 7. Accuracy 100% level

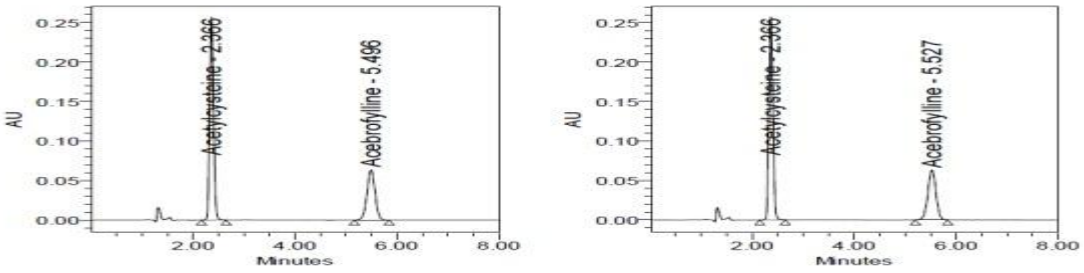

Fig 8. Accuracy 150% level

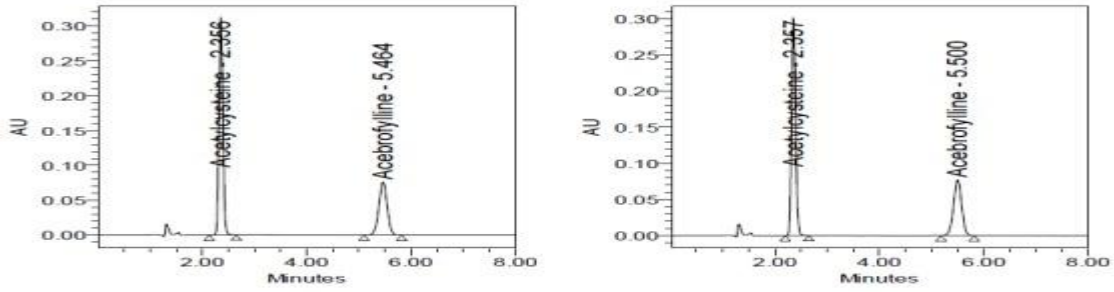

Fig 9. Method Precision chromatogram

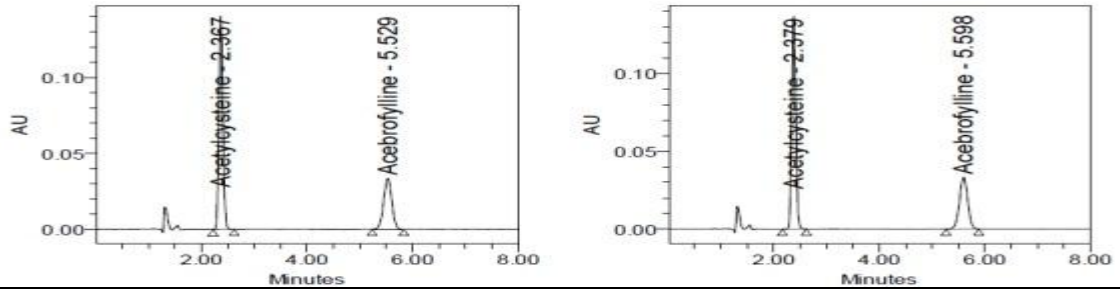

Fig 10. Ruggedness chromatogram for day1and day2

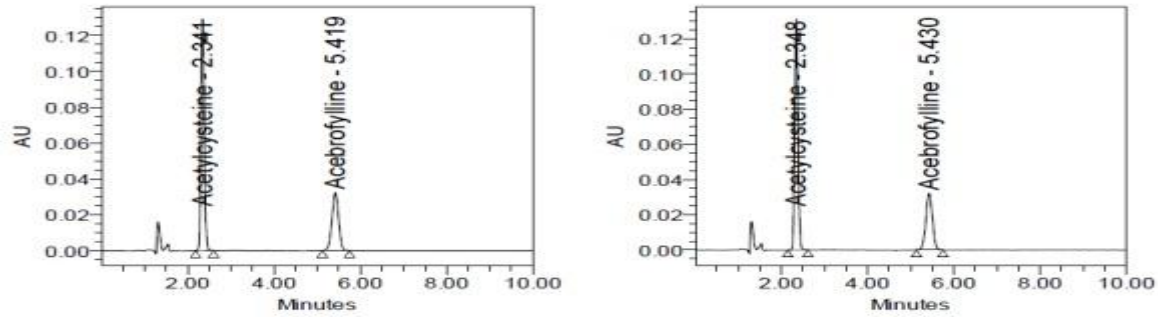

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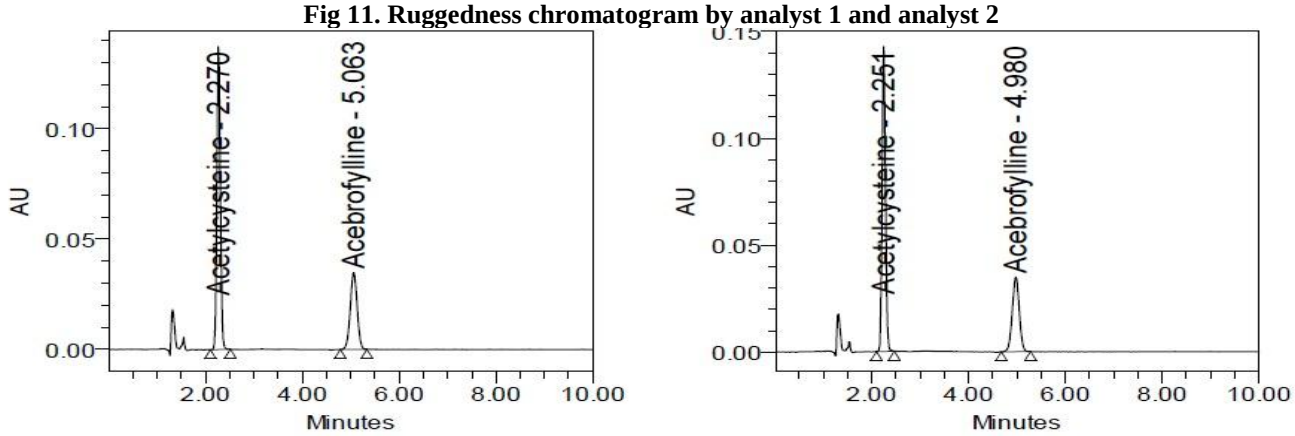
Fig 11. Ruggedness chromatogram by analyst 1 and analyst 2



RESULT AND DISCUSSION

The chromatogram of sample and standard are shown in the figure 1 and 2 indicating retention time for ACB was 5.504 and 5.509 respectively. Similarly the retention time for NAC was 2.370 and 2.370 respectively which is not so different it can be conclude that the developed RP-HPLC method is accurate and precise without any interference and overlapping (Fig 3). The assay of acebrophylline and acetylcysteine was calculated and mentioned in Table I. This data of both the drugs complies with the standard specifications of 50%, 100% and 150% recovery data for acebrophylline and acetylcysteine are shown in Table II .The % recovery of sample was observed between 99 to 101% and relative standard deviation was 0.5 and 0.4 for NAC, ACB respectively. Hence the data shown under accuracy parameters were found within the prescribed limits, indicating that the developed method was accurate and précised (Fig 6-9). The linearity parameter and corresponding regression data indicated excellent linear relationship (R^2 =0.9999) for ACB and (R^2 =0.9998) for NAC (table 3). Fig.4 indicates ACB and NAC were obeying Beer Lambert's law limit. The LOD and LOQ for the ACB were found 0.18 (µg/mL) and 0.56 (µg/mL), while for NAC was 1.50 (µg/mL) and 4.55 (µg/mL) respectively. This specificity test of the proposed method demonstrated that the excipients of tablet formulation do not interfere in the drug peak (Fig 2&3). The ruggedness study of standard solution (Fig 10-11) indicated that the retention times for both the drugs were not so different and relative standard deviations of drugs are less than 2. This might be due to proper system suitability (Table 4). Table 5 Indicated that system suitability parameters for drugs Acebrophylline and Acetylcysteine. The resolution for both the drugs was found to be more than 2. Hence it can be concluded that the RP-HPLC method shown the reproducibility for the method systems.

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 and Acetycysteine in tablet 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 and Acetylcysteine in tablet dosage form.

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