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RESEARCH ARTICLE

VALIDATED RP-HPLC METHOD DEVELOPMENT FOR THE SIMULTANEOUS ESTIMATION OF ACETYLCYSTEINE AND ACEBROFYLLINE IN CAPSULE FORMULATION

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ABSTRACT

A new simple, precise, rapid and accurate reverse phase high performance liquid chromatographic method had been developed for the simultaneous estimation of Acetylcysteine (ACST) and Acebrofylline (ACBF) in capsule dosage form. The chromatographic separation was achieved on a Hypersil BDS, C18, 100 x 4.6 mm, 5µm particle size column was used with PDA detector by using mobile phase containing mixture of 0.02M Potassium dihydrogen orthophosphate (KH_2PO_4) buffer : acetonitrile (90:10 % v/v pH 3.2) was used. . The flow rate was 0.9 ml / min and effluents were monitored at 260 nm. Chromatogram showed two main peaks corresponding to Acetylcysteine and Acebrofylline at retention times 2.365 and 5.505 min respectively. The method was liniear over the concentration range of 150-900µg/ml for Acetylcysteine and 25-150 µg/ml for Acebrofylline respectively. The developed method was validated in accordance to ICH guidelines.

Key words: Acetylcysteine, Acebrofylline, RP-HPLC, Validation, ICH, Acetonitrile

INTRODUCTION:

The present research work deals with the development and validation of a simple, specific, accurate, and precise reverse phase high performance liquid chromatographic (RP-HPLC) method for the estimation of Acetylcysteine and Acebrofylline in capsule formulations. Chemically Acetylcysteine[1] 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[1,2] IUPAC name is a (2R)-2-acetamido-3-sulfanylpropanic acid [Figure - 1], represents mucolytic drug which decreases the viscosity of secretions by splitting of disulphide bonds in mucoproteins and it also promotes the detoxification of an intermediate paracetamol metabolite which is used in the management of paracetamol overdose.

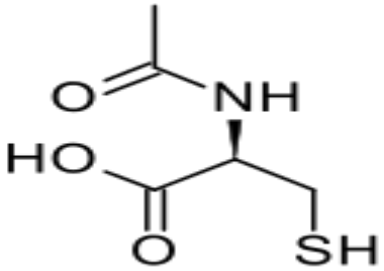

Figure 1: Structure of Acebrofylline

Acebrofylline[3] IUPAC name is 4-[(2-amino-3,5-dibromophenyl) methylamino] cyclohexan-1-ol; 2-(1,3-dimethyl-2,6-dioxopurin-7-yl)acetic acid. Acebrofylline 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 with molecular formula $C_{22}H_{28}Br_2N_6O_5$ and molecular weight 616.302 g/mol as shown in Figure 2.0. It is a novel drug with bronchodilating, anti-inflammatory and mucus regulating effect due to inhibition of phospholipase A, and phosphatidylcholine. Literature survey[4-13] reveals that some methods have been reported for the estimation of single and very few methods for the combinations, but still there is no RP-HPLC method developed for the simultaneous determination of of Acebrofylline and Acetylcysteine in capsule formulations. So the present method developed is relatively simple, rapid and highly sensitive and validated as per ICH guidelines[14] in the analysis of multicomponent of interest and it can be used for routine quality control analysis in laboratories.

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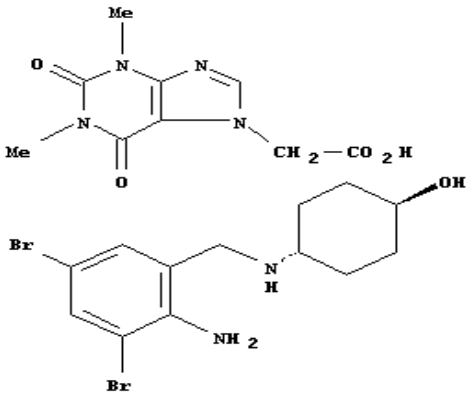

Figure 2: Structure of Acetylcysteine

Chemicals and reagents:

Acebrofylline and Acetylcysteine pure samples were obtained from SL Drugs & Pharmaceuticals, Hyderbad, India and all other chemicals were of analytical grade. The commercial capsule Acebrofylline and Acetylcysteine formulations of combined of brand Caps. Pulmoclear Manufactured by Fourrts (India) Laboratories Pvt. Ltd were obtained from local retail pharmacy.

Chromatographic conditions:

The HPLC water system was equipped with empower software for data processing. The optimize chromatographic conditioned were shown in Table No. 1.0

MATERIALS AND METHODS:

Table No. 1.0: The optimize chromatographic conditioned

Flow rate	0.9 ml/min
Column	Hypersil BDS, C18, 100 x 4.6 mm, 5µ.
Detector wave length	260 nm
Column temperature	30°C
Injection volume	5µL
Run time	8 min
Diluent	Methanol
Mobile phase	Buffer : Acetonitrile (90:10 % v/v pH 3.2)

Preparation of diluent:

The diluent was HPLC grade Methanol alone.

Preparation of buffer:

Accurately weighed 2.72gm of potassium dihydrogenorthophosphate was transferred in a 1000ml ofvolumetric flask and about 900ml of milli-Q water was added.1ml of triethylamine was added and sonicated and finally made up the volume with water. Then pH was adjusted to 3.2 with dilute ortho phosphoric acid solution.

Preparation of standard stock solution:

Accurately weighed 10mg of Acebrophylline and 12.5mg of Acetylcysteine working Standards were transferred into separate 10 ml clean and dry volumetric flasks, 7ml of diluents was added and sonicated for 30 minutes and made up to the final volume with diluents.

Preparation of sample solution:

Twenty Tablets were weighed and the average weight of each tablet was calculated. Then the weight equivalent to twenty tablets was transferred into a 100 ml volumetric flask, 50mL of diluent was added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution 0.2ml was pipetted out into a 10 ml volumetric flask and made up to 10ml with diluent.

Method validation[15]:

The developed method was validated as per the ICH guidelines with respect to system suitability, specificity, linearity, accuracy, precision, LOD and LOQ.

System suitability:

To ensure the resolution and reproducibility of the HPLC system was adequate for the analysis, a system suitability test was established. Data from six injections of 10 µL of the working standard solutions were used for the evaluation of the system suitability parameters like tailing factor, the number of theoretical plates and retention time. The system suitability results obtained for Acetylcysteine and Acebrofylline is summarized in Table No. 2.0 and Table No.3.0 respectively

Table 2, 3: The results obtained for system suitability of Acetylcysteine and Acebrofylline is summarized in Table No. 2.0 and 3.0 respectively.

Sr. No.	Retention Time	Peak Area	Theoretical plates	Tailing factor
1	2.36	686974	4492	1.09
2	2.362	685310	4513	1.1
3	2.363	686086	4502	1.1
4	2.367	683964	4290	1.1
5	2.37	686033	4376	1.09
6	2.371	688352	4430	1.09
	Mean	686120		
	Std. Dev.	1485		
	%RSD	0.2		

Table No 2.0:

Sr. No	Retention Time	Peak Area	Theoretical plates	Tailing factor
1	5.484	340354	5842	0.99
2	5.485	340140	5830	1.0
3	5.504	342289	5843	1.0
4	5.507	338474	5877	1.0
5	5.518	344216	5970	0.99
6	5.533	341513	5715	1.0
	Mean	341164		
	Std. Dev.	1982.1		
	%RSD	0.6		

Table No 3.0:

LINIARITY:

The linearity of the method was evaluated by analyzing different concentration of the drugs. According to ICH recommendations, at least six concentrations must be used. In the present study six concentrations were chosen & injected. The peak areas of the chromatograms were plotted against the concentration of drug to obtain the calibration curve and the corresponding calibration curve data and graph for ACST and ACBF shown in Table No.4.0 and Graph in Figure – 3 and Figure – 4 respectively.

Table 4: The corresponding Linearity (calibration curve) data

Sr. No	Concentration in ppm (ACST)	Peak area (ACST)	Concentration in ppm (ACBF)	Peak area (ACBF)
1	150	174619	25	88736
2	300	355507	50	175892
3	450	514460	75	257873
4	600	690463	100	345418
5	750	855800	125	431317
6	900	1039137	150	515247
SLOPE		1145	**SLOPE**	3428.7
INTERCEPT		2102.667	**INTERCEPT**	2022.333
CORRELATION COEFFICIENT		0.999	**CORRELATION COEFFICIENT**	0.999

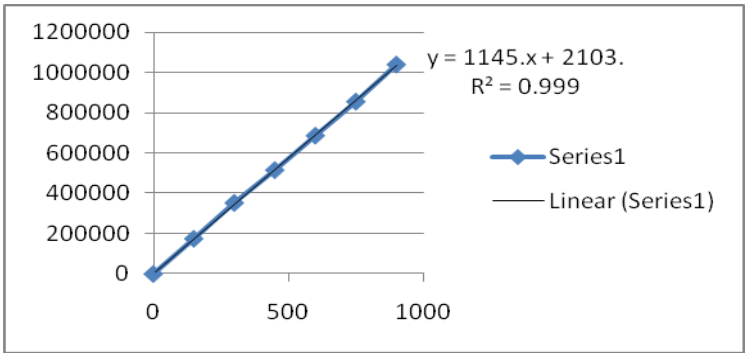

Figure 3: Calibration curve for Acetylcysteine

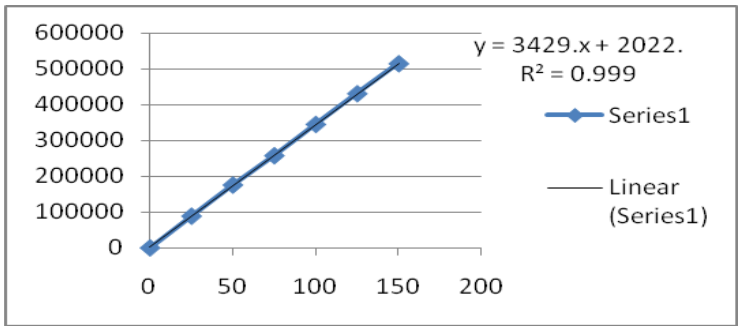

Figure 4: Calibration curve for Acebrofylline

ACCURACY:

The accuracy of the method was determined by recovery experiments. The solutions were injected in triplicate in 50%, 100% and 150% concentrations and percentage Recovery was calculated separately for ACBF and ACST and summarized in Table No. 5.0 and Table No. 6.0 Respectively.

Table 5, 6: The accuracy data (recovery study) for ACBF and ACST were summarized here

LEVEL IN %	Amount added	Amount recovered	%Recovery	% Mean	% RSD
50%	50	49.93	99.85302	100.864	0.78
50%	50	50.09	100.1831		
50%	50	50.17	100.3348		
100%	100	100.67	100.6713		
100%	100	102.15	102.1525		
100%	100	101.73	101.7314		
150%	150	152.02	101.3483		
150%	150	150.40	100.2697		
150%	150	151.9	101.2353		

Table 5.0:

LEVEL IN %	Amount added	Amount recovered	%Recovery	% Mean	% RSD
50%	300	303.0838	101.0279	100.317	0.72
50%	300	302.724	100.908		
50%	300	299.1572	99.71907		
100%	600	595.2	99.2		
100%	600	600.9755	100.1626		
100%	600	597.3328	99.55546		
150%	900	909.5546	101.0616		
150%	900	901.2856	100.1428		
150%	900	909.683	101.0759		

Table 6.0:

Precision:

Precision of the method was determined by studying intra-day and inter-day variation. In the intra-day studies, standard and sample solutions were analyzed on the same day and percentage RSD was calculated. In the inter day studies, standard and sample solutions were analyzed on consecutive days and percentage RSD were calculated and individual data for ACST and ACBF summarized in Table No 7.0

Table 7: The Precise individual data for ACST and ACBF summarized in

Assay No.	Peak Area ACBF	% Assay ACBF	Peak Area ACST	% Assay ACST
01	344629	100.6116	687668	99.82
02	341064	99.57081	686777	99.70
03	344819	100.667	690885	100.29
04	342999	100.1357	684256	99.33
05	346857	101.262	686946	99.72
06	344019	100.4335	680410	98.77
Mean		100.4468		99.61
% RSD		0.56		0.51

Specificity:

The Specificity of the method was evaluated by assessing whether excipients present in the pharmaceutical formulations interfered with the analysis. Excipients for each capsule were mixed in order to prepare a placebo, and solutions were prepared by following the procedure described in the section on sample preparation. The capsule excipients did not interfere with the method.

Limits of detection(LOD) and Limit of quantitation(LOQ):

In accordance with ICH recommendations, the method based on the standard deviation of the response and the slope of the calibration plots was used to determine detection and quantification limits. LOD and LOQ values were estimated [(standard deviation of repeatability)/ (Slope of the regression equation)] by multiplying with 3.3 and 10 respectively. And corresponding results given in Table No. 8.0

Table 8: The results and summary for the developed and validated method of Acetylcysteine(ACST) and Acebrofylline (ACBF) was given below

Sr. No.	Parameter	Acetylcysteine	Acebrophylline
1.	Peak area (%RSD)	686120(0.2)	341164(0.6)
2.	Retention Time	2.365	5.505
3.	USP Theoretical Plate	4434	5846
4.	USP Tailing	1.09	0.99
5.	Specificity	No peak	No peak
6.	Linearity (µg/ml)	150-900	25-150
7.	Slope	1145	3429
8.	Y-Intercept	2103	2022
9.	Correlation coefficient	0.999	0.999
10.	Accuracy	0.72	0.78
11.	Precision	0.51	0.6
12.	LOD	1.5042	0.1874
13.	LOQ	4.558	0.568
14.	Ruggedness	0.48	0.76
15.	Flow rate(+0.1)	695877(0.3)	345226(0.4)
16.	Flow rate(-0.1)	0.46	0.51
17.	Mobile phase (+2%)	0.51	0.56
18.	Mobile phase(-2%)	0.87	0.81
19.	Column temp(+5)	0.43	0.46
20.	Column temp(-5)	1.53	1.56

Robustness:

Robustness is a measure of capacity of analytical methods to remain unaffected by small but deliberate variation of the operating conditions. This was tested by studying the effect of changing column temperature ±5°C, the mobile phase composition by 2%, and flow rate by ±0.1ml. And corresponding results given in Table No. 8.0

RESULTS AND DISCUSSION:

The results and summary for the developed and validated method of Acetylcysteine(ACST) and Acebrofylline (ACBF) was given below Table No- 8.0

CONCLUSION:

The RP-HPLC assay method was developed and validated for simultaneous determination of Acetylcysteine (ACST) and Acebrofylline (ACBF) in capsule dosage forms. The method was found to be simple, specific, Precise and Robust and can be applied for the routine and stability analysis for commercially available formulation.

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