Asian Journal of Pharmaceutical Research and Development
(An International Peer-Reviewed Journal of Pharmaceutical Research and Development)

A J P R D

Open Access Available online at http://ajprd.com Research Article

RP-HPLC Method Development and Validation for Estimation of Acebrophylline

Sharma Bhavik, Agarwal Sushil Kumar
Department of Quality Assurance, Rajasthan Pharmacy collage, Jaipur, Rajasthan (India)

ABSTRACT
Acebrophylline is an anti-inflammatory and airway mucus regulator. It had ambroxol and theophylline-7-acetic acid, the former facilitates the biosynthesis of pulmonary surfactant which raises blood levels of ambroxol, by stimulating surfactant production. Chemical structure of acebrophylline is 1, 2, 3, 6- tetrahydro-1, 3-dimethyl-2, 6-dioxo-7H-purine-7-aceticacidwithtrans-4-[(2-amino-3, 5 dibromophenyl) methyl] aminio] cyclohexanol. Survey revealed that various analytical methods like spectrophotometric, HPLC, and RP-HPLC, have been reported for the determination of Ambroxol HCl and Theophylline-7-acetic acid, individually and in combination with some other drugs. The aim of present study was to develop and validate stability indicating HPLC method for the analyses of acebrophylline. High performance liquid chromatographic method has been developed for the estimation of Acebrophylline. Reported methods also include RP-HPLC method for determination of Acebrophylline. The developed UV spectrophotometric method is simple and requires less time for the analysis. It is also rapid and economic method.

Key words- Acebrophylline, RP-HPLC, Method development, Validation

Article Info: Received: 18 Oct 2018; **Review Completed**: 126 Nov 2018; **Accepted:** 30 Nov 2018**; Available online**: 15 Dec 2018

Cite this article as:
Bhavik Sharma, Department of Quality Assurance, Rajasthan Pharmacy collage, Jaipur, Rajasthan (India),Asian Journal of Pharmaceutical research and Development.**2018;6 (6): 50-55**
DOI: http://dx.doi.org/10.22270/ajprd.v6i6.446
***Address for Correspondence**
Sharma Bhavik, Agarwal Sushil Kumar, RP-HPLC Method Development and Validation for Estimation of Acebrophylline

INTRODUCTION

Acebrophylline is an anti-inflammatory and airway mucus regulator. It contains ambroxol and theophylline-7-acetic acid that facilitates the biosynthesis of pulmonary surfactant while later raises blood levels of ambroxol, by stimulating surfactant production.[1]Acebrophylline is chemically (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, which increase the adenosine monophosphate cyclic levels, which promote the relaxation of bronchial muscles.[3]Ambroxol modifies the mucous gel phase of secretions by decreasing the viscosity and increasing the serous gel phase. It will increase the mucociliary clearance by stimulating cilia motility.[4] Acebrophylline inhibits phospholipase A, and phosphatidylcholine leading to lesser production of the powerful pro-inflammatory substances like leukotrienes and tumour necrosis factor. It inhibits the synthesis and release of these inflammatory mediators, acebrophylline reduces inflammation, a key factor in airway obstruction, especially in chronic forms.[5] Survey revealed that very few analytical methods like HPLC methods, spectrophotometric and RP-HPLC have been reported for the determination of acebrophylline, individually and in combination with some other drugs. Therefore, the aim of present study was to develop and validate stability indicating HPLC method for the analytesin combination by following ICH method validation guidelines.

High performance liquid chromatographic method has been developed for the estimation of Acebrophylline. Reported methods also include RP-HPLC method for determination of Acebrophylline. The developed UV spectrophotometric method is simple and requires less time for the analysis.[6]

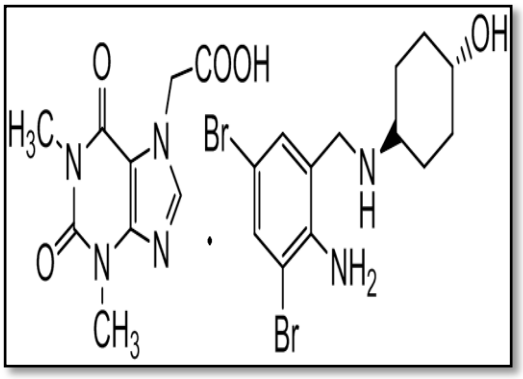

Figure 1: Structure of Acebrophylline[7]

MATERIALS AND METHODS

Reagents and Chemicals

Working standard of Acebrophylline was obtained from as gift samples from SHILPA MEDICARE LIMITED, Raichur, and Karnataka. The capsules ABPHYLLINE (containing Ambroxol and Theophylline -7- acetic acid) were procured from local market manufactured by Sun Pharmaceuticals Industries Ltd.Methanol (AR grade), ammonium acetate, acebrophylline, ambroxol hydrochloride and acefylline.

Preparation of Standard Stock Solution

Accurately weighed 10mg of Ambroxol hydrochloride was transferred into a clean dry 10 mL volumetric flask and dissolved with sufficient volume of mobile phase. The volume was made up to 10 mL with mobile phase to get a concentrationof1000 µg/mL

Selection of Detection Wavelength

The wave length for detection (UV Detector) of Acebrophylline was selected by obtaining the overlay spectra of Ambroxol hydrochloride and Acefylline in the mobile of phase of 25mM Ammonium acetate: Acetonitrile (3:7) by scanning the solutions in the range between 220nm and 360nm using UV-Visible Spectrophotometer. The overlaid spectrum of Ambroxol and Acefylline obtained are presented in Figure 2.

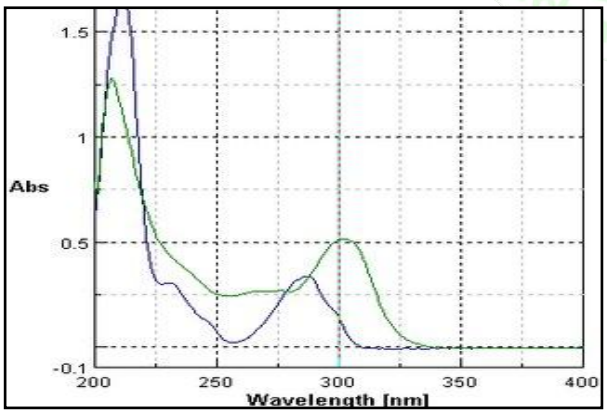

Figure 2: Absorbance Spectrum of Acebrophylline.

Method optimization

Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy may often be expressed as

The RP-HPLC was optimized for 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 0.7 mL. Mobile phase with a mixture of acetonitrile and water (3:7 v/v) pumped and detector set at 257nm gave a sharp and symmetrical peak with retention time of 3.5min.

RESULTS AND DISCUSSION

Method Validation

Specificity

Volume of 100 µ L of working placebo sample solution prepared by mixing together the commonly used excipients for capsule formulation was injected in to the chromatograph and the chromatogram was recorded and shown in Fig3.

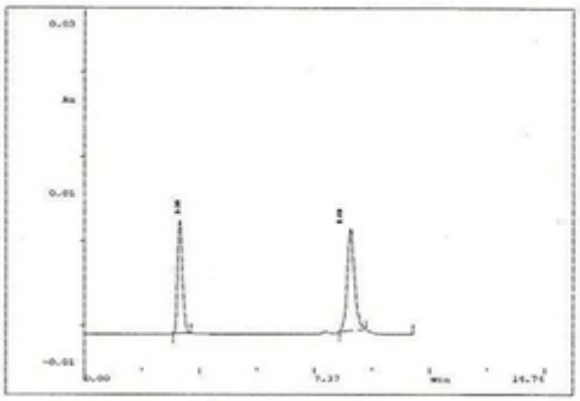

Figure. 3 Typical chromatogram for acebrophylline

Linearity

The Linearity of the developed HPLC procedure was carried out to define its ability to obtain test results which are directly proportional to concentration of analyte.100 µ L of each of these working standard solutions of Acebrophylline ranging from2- 14 µg/ mL were injected into a HPLC chromatograph at flow rate of 0.7 mL/min and UV detected at 257 nm. Retention time and peak area obtained were recorded. Standard calibration curve was plotted for Acebrophylline and linearity equation was derived.

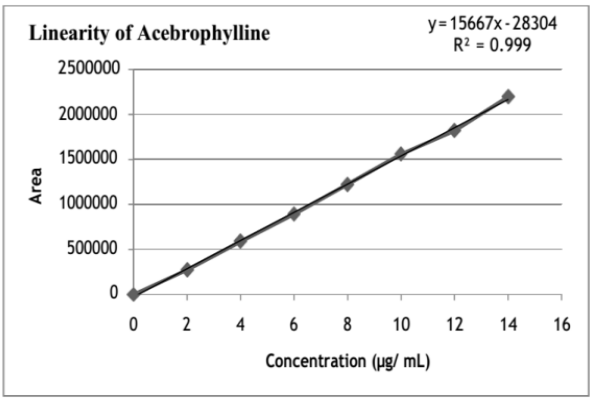

Figure. 4 Standard calibration curve of Acebrophylline

percentage recovery of the sample added to known amount of standard. It was carried out at three different levels i.e. 80%, 100% and 120%.

100 µ L solution of the mixture of sample and standard solution were injected in to a HPLC chromatograph at a flow rate of 0.7 mL/min and UV detection at 257 nm repeatedly. The chromatograms and peak areas were recorded and presented below:

Table 1: Percentage Recovery Data of Acebrophylline

Leve l	Std. Conc. (µg/mL) (a)	Sample Conc. (µg/mL) (b)	Total Conc. c = a +b	Peak area*	Total Conc. (µg/ mL) (d) From Std. graph	Amount of Std. Recovered(µg/m L) e = d - b	% Recover y (e*100/c)
80%	4	5	9	1377109	8.970473	3.970473	99.26183
100%	5	5	10	1532399	9.961656	4.961656	99.23313
120%	6	5	11	1708345	11.08469	6.084687	101.4115

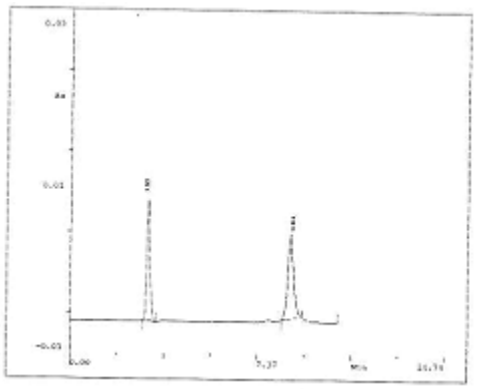

Figure. 5 Chromatogram of Acebrophylline for Recovery studies

Precision

The precision of an analytical procedure was determined to study 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.

Table 2: Precision results of Acebrophylline

Precision Parameters	% RSD	Acceptance Criteria
Method Precision	0.576728	2.0%
System Precision	0.478164	2.0%
Intra-Day Precision	0.200143	2.0%
Inter-Day Precision	0.267716	2.0%

Method sensitivity

The lowest concentration of Acebrophylline that can be detected and quantified with greatest accuracy and precision was determined by the developed HPLC method. The LOD and LOQ for Acebrophylline was determined by injecting 100 µ L of working standard solution(II) of Acebrophylline of concentration 0.6, 0.5, 0.4 and 0.3 µg/mL solutions at 0.7 mL/min flow rate and the lowest concentration that evoke peak and peak area were recorded. The LOD and LOQ values for acebrophylline were reported in the Table 3.

Table 3: LOD and LOQ results for acebrophylline

Volume of Stock Solution (mL)	Volume Made up to (mL)	Concentration (µg/ mL)	Peak Area*
0.6	10	0.6	76236
0.5	10	0.5	51946
0.4	10	0.4	5122
0.3	10	0.3	-

Robustness

The robustness of an analytical method was determined to measure its capacity to remain unaffected by small but deliberate variations in method parameters which provides an indication of its reliability during normal usage.

Table 4: Data of Acebrophylline with Change in Flow rate

Conc. (µg/ mL)	Changed low rate	RT (min)		Peak Area*	Amount Recovered (µg/ mL)	% Assay
6	0.65 mL	3.06	9.12	892770	5.879033133	97.98388555
6	**0.7 mL**	**3.04**	**8.49**	**907391**	**5.972356084**	**99.53926806**
6	0.75 mL	3.00	8.37	907026	5.970026361	99.50043935

Table 5: Data of Acebrophylline with Change in pH

Conc.(µg/ mL)	ChangedpH	RT (min)		Peak Area*	Amount Recovered (µg/ mL)	% Assay
10	7.2	3.01	9.07	1551926	10.08629549	100.8629549
10	7.3	3.05	8.49	1563092	10.15756585	101.5756585
10	7.4	3.04	8.62	1503950	9.780074168	97.80074168

Table 6: Data of Acebrophylline with Change in Composition of Mobile Phase

Conc. (µg/ mL)	Mobile Phase Ratio Buffer: ACN	RT (min)		Peak Area*	Amount Recovered (µg/ mL)	% Assay
10	25:75	2.97	9.23	1541320	10.01	100.1859949
10	**30:70**	**3.04**	**8.49**	**1563092**	**10.15**	**101.5756585**
10	35:65	3.01	9.07	1551926	10.08	100.8629549

CONCLUSION

A RP HPLC method was successfully developed for the estimation of Acebrophylline (containing Ambroxol and Acefylline) in bulk and in marketed formulation. The chromatograph used was LC 10AT Shimadzu – SPDA10A detector with 100 µ L Rheodyne injector and the column used was SGE SS Wakosil-II 5C18AR, 250 mm(length) x 4.6 mm(I.D) , 5 µm (particle size). The mobile phase comprised of 25 mM Ammonium Acetate: Acetonitrile (pH 7.3) in ratio of 3:7 v/v and a flow rate of mL/min with UV detection at 257 nm. The peaks for Acefylline and Ambroxol evoked at a retention time of 3.05 and 8.59 min respectively.

The developed method was validated by various parameters like Accuracy, Precision, Linearity, Specificity, Ruggedness and Robustness as per ICH Q2 guidelines. The results obtained were within the acceptance criteria for the respective parameters. The proposed method was applied for estimation of Acebrophylline in marketed formulations. The assay results conformed to the label claim of the formulation.

Hence the proposed method was found to be satisfactory and can be used for the routine analysis of Acebrophylline in bulk and their marketed capsule dosage formulations.

REFERENCES

1. Sravani T, Thota S, Venisetty R, Venumadhav N. RP-HPLC analysis of acebrophylline in API and capsule dosage form. Res J Pharm 2014; 5:480-6.
2. Jadhav NS, Lalitha KG. Development and validation of spectroscopic method for simultaneous estimation of acebrophylline and acetyl cysteine in capsule dosage form. Int J Pharm Phytopharma col Res 1998; 2:2249-65.
3. Tripathi KD. Essentials of Medical Pharmacology. 6thed. New Delhi: Jaypee Brothers Medica Publishers Ltd; 2010.
4. Ramanjaneyulu S, Vijay Kumar G, Chandrashekar K.B, Jyothirmai S. Int J Inv Pharm Sci 2013; 1: 404-408.
5. JithuJerin James *et al*, Estimation of Acebrophylline by HPLC, Integrated Projects Consortium Experiments, 25 Aug,2011
6. R. Aligave*et al*, Determination of Acebrophylline in bulk and pharmaceutical formulation by UV spectrophotometer, Current Pharma Research 1(3), 2011, 267-270
7. http://www.drugsupdate.com/generic/view/1114/Acebrophylline