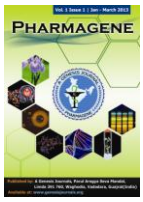
**PHARMAGENE**
**Vol:1 Issue:1**
*Research Article*
www.genesisjournals.org



# Development And Validation of Derivative Spectroscopic Method For Estimation of Acebrophylline In Bulk And Its Dosage Form & In Presence of Impurity, Ambroxol HCl

**Bhagavati Solanki[1], Harshal Trivedi[2], Ankita Khodiyar[1], Falguni Tandel[1], Lalit Lata Jha[1], Rajesh KS[1]**

**[1]Parul Institute of Pharmacy, Limda, Vadodara, India.**
**[2]Analytical Research Lab, Cadila Pharmaceutical Ltd., Vadodara, India.**

**Abstract:** A sensitive, selective and validated first derivative spectrophotometric method using methanol have been proposed for the determination of Acebrophylline which is widely used as anti asthmatic agent. The developed spectrophotometric method is simple, rapid, precise, accurate, reliable and economical. Method is specific in presence of Ambroxol which is the impurity of Acebrophyllin. The method is accurate, precise and linear over a range of 5-50µg/ml for Acebrophyllin. The limit of detection was observed as 0.178µg /ml, the limit of quantification was observed as 0.598µg/ml. The %RSD is less than 2% in methanol. Method accurately estimates Acebrophyllin in presence of Ambroxol with 99-100% recovery. The method can be applied for Acebrophylline tablet formulation with 99-100% recovery.

**Keywords:** Acebrophylline, Anti asthmatic, Methanol, UV spectroscopy.

**Received on 20-12-2012** **Modified on 07-02-2013** **Accepted on 24-02-2013**

## INTRODUCTION

Acebrophylline, 4-[(2-amino-3,5-dibromo-phenyl methylamino] cyclohexan-1-ol; 2-(1,3-dimethyl- 2,6-dioxo-purin-7-yl)acetic acid(figure 1) is a hydroxy naphthaquinone derivative used for treatment of asthama. It 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 agent. Theophylline-7-acetate, as with other xanthinic derivatives, 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 simulating cilia motility.

A few methods have been reported to estimate Acebrophylline levels in bulk and pharmaceutical formulation by UV spectrophotometer. Methods have been reported also for the determination of Acebrophylline in bulk and capsule formulation. Several other methods for bioequivalence study of Acebrophylline capsules have been reported. High performance liquid chromatographic method has been developed for the estimation of Acebrophylline. Reported methods also includes RP-HPLC-PDA 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. It involves estimation in presence of Ambroxol.

*Address for correspondence:
Falguni Tandel
Parul Institute of Pharmacy, Limda-391760
E-mail: falgunibt@gmail.com
Contact:9328990735

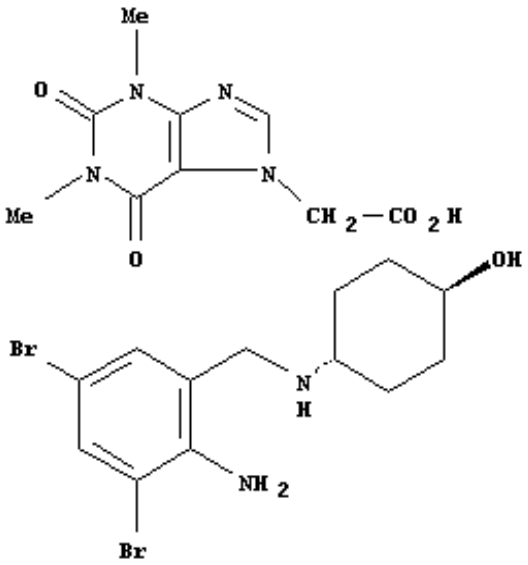



**Figure 1: - Chemical Structure of Acebrophylline**

## MATERIALS AND METHODS

**Instruments:**

UV-Visible double beam spectrophotometer (Shimadzu 1800) with 10mm matched quartz cells were used for spectrophotometric measurement. All weighing were done on electronic balance (Mettler Toledo).

**Reagents & Materials:**

The chemicals used were methanol, obtained from (Rankem Pharmaceutical Limited, Mumbai) and Acebrophylline, reference standard (Ami Life Science Pvt. Ltd., Vadodara, India) having a potency of 99.8%.

**Selection of solvent**

Methanol was selected as solvent for spectrophotometric analysis of Acebrophylline.

**Preparation of standard solution and calibration curve:**

Stock solution of drug having concentration 1000µg/ml was prepared by dissolving Acebrophylline in methanol. Aliquot of stock solutions were further diluted in methanol to prepare solution of 100µg/ml. This stock solution was further diluted in methanol to prepare solutions in the range of 5-50 µg/ml and were scanned in the wavelength range of 200–400 nm. The zero order spectra were converted to first derivative spectra. The λmax was obtained at 244.3 and λmin at 255.6 nm in methanol (figure-2). Derivative amplitude was obtained and was used for construction of calibration curve. A calibration graph was plotted at the selected wavelengths of the first derivative spectra with the aim of best linearity and maximum absorption. The Beer's law was obeyed over the concentration range 5-50 µg/ml by Acebrophylline (figure 3).

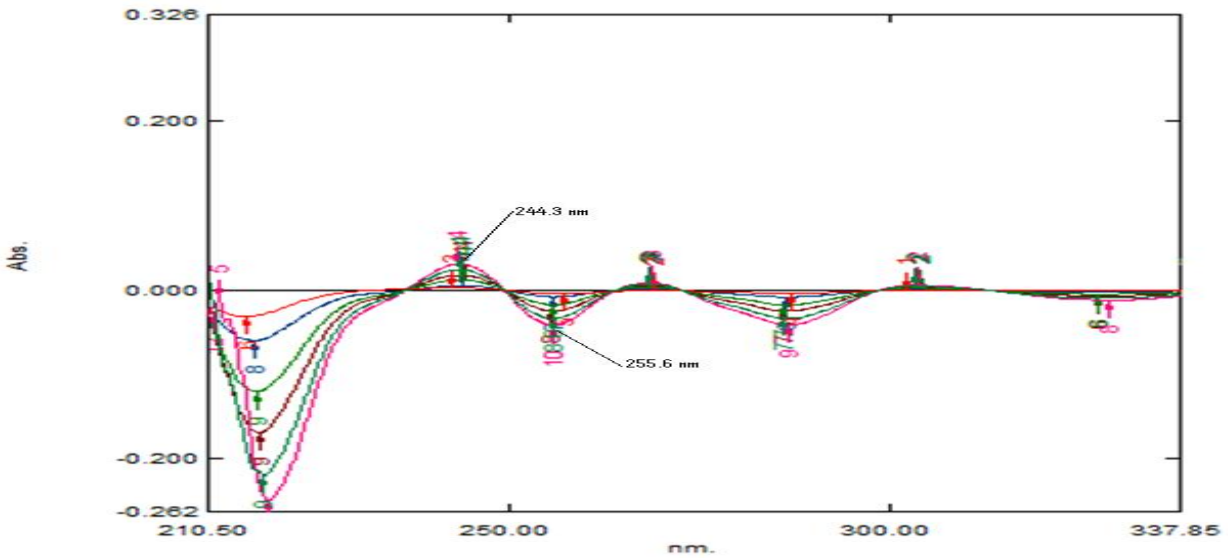



**Figure 2: First Derivative Spectra for Acebrophylline**

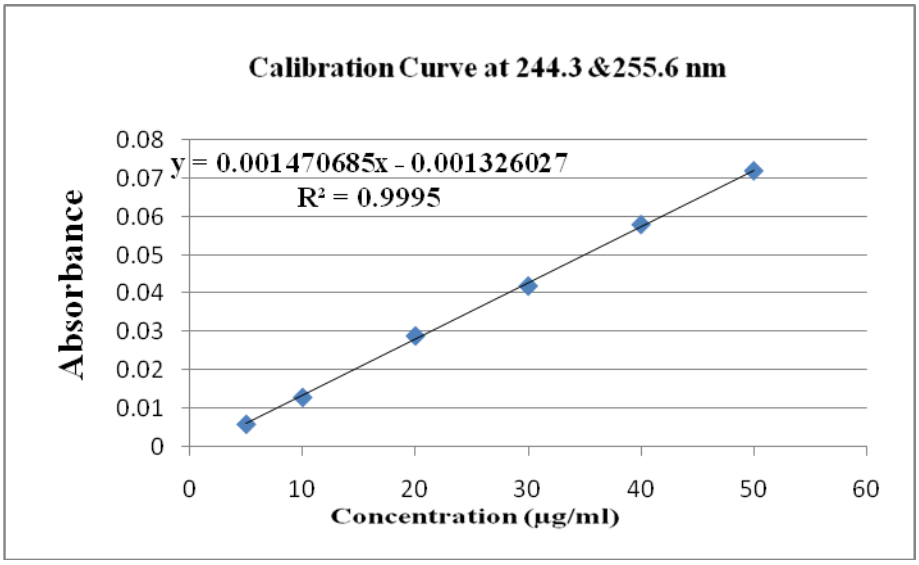



**Figure 3: Calibration curve for Acebrophylline**

**Preparation of sample solution and formulation analysis:**
Twenty capsules were weighed accurately and a quantity of capsule powder equivalent to 100mg of Acebrophylline was transferred to 100 ml volumetric flask, 60 ml of methanol was added to the same flask, sonicated for 5 min and diluted to 100 ml with methanol and filtered through whatmann filter paper no. 41. Resulting solution was further diluted with methanol to obtain solution having concentration 10µg/ml of Acebrophylline. The sample solution was scanned in the wavelength range of 200–400 nm. Derivative amplitude was obtained and a concentration of the drug was calculated by using calibration curve

**VALIDATION:**
**Specificity**
A method is said to be specific when it produces a response only for a single analyte. For specificity, drug was estimated in presence of Ambroxol which is the impurity of the drug.

**Sensitivity**

**Limit of Detection (LOD) and Limit of Quantitation (LOQ)**
The LOD and LOQ were estimated from the set of 5 calibration curves used to determine method linearity.
**LOD= 3.3*σ/S and LOQ= 10*σ/S**
Where, σ = the standard deviation of y- intercepts of regression lines
S = the slope of the calibration curve
**Linearity and range**
Linearity of the concentrations was determined in the range of 5-50µg/ml and $R^2$ was calculated from the calibration curve.

**Accuracy**
Accuracy of proposed method from excipients was determined by recovery experiments. Recovery experiments were carried out at three levels of concentration. The amounts of standard recovered were calculated in the terms of mean recovery.

**Precision**
It is expressed as the percentage coefficient of variation (%CV)/%RSD which is calculated as per the following expression:
%CV= (standard deviation /mean)*100

**Intraday precision**
It was determined by calculating the %coefficient of variation (%CV) of the results of three determinations obtained in the same day.
**Inter day precision** It was determined by calculating the percentage coefficient of variation (%CV) of the results obtained over three days.

**RESULTS & DISCUSSION**

**Specificity**
The method was found to be specific in presence of Ambroxol which is the impurity of the drug.

**Sensitivity**
The limit of detection value for Acebrophylline was obtained as 0.178 µg/ml. The limit of quantification was determined as 0.598 µg/ml (table 1).

**Linearity and Range**
The linearity was determined over the range of 5-50µg/ml. The $R^2$ value was obtained as 0.9995 for first derivative. The results are depicted in table 1 and in figure 3.

| **PARAMETERS** | **RESULTS** |
|---|---|
| Linearity and range | 5-50 µg/ml |
| Equation | Y = 0.001x - 0.001 |
| $R^2$ | 0. 9995 |
| LOD | 0.178 |
| LOQ | 0.598 |

**Table:1 Liniarity range and Sensitivity**

**Precision**
By the precision studies the relative standard deviation values were obtained as less than 2%. The values were given in table 2.

| **PARAMETER** | **FIRST DERIVATIVE** | | | | | |
|---|---|---|---|---|---|---|
| | **Conc. (µg/ml)** | | | | | |
| | 5 | 10 | 20 | 30 | 40 | 50 |
| **Mean Absorbance** | 0.0062 | 0.0133 | 0.0306 | 0.0421 | 0.0583 | 0.0716 |
| **SD** | 0.00054 | 0.0000070 | 0.002687 | 0.000707 | 0.000473 | 0.0000042 |
| **% RSD** | 0.586% | 0.053% | 0.462% | 1.682% | 0.812% | 0.0591% |

**Table 2: Precision studies for Acebrophylline.**

**Accuracy**
The mean absolute recovery of Acebrophylline was found to be 99-100%. The values are given in table 3.

| | **CONCENTRATION TAKEN (µg/ml)** | **CONCENTRATION ADDED (µg/ml)** | **TOTAL (µg/ml)** | **OBSERVED TOTAL (µg/ml)** | **% RECOVERY** |
|---|---|---|---|---|---|
| **Level 1** | 14 | 10 | 24 | 24.99 | 99.92 |
| **Level 2** | 20 | 10 | 30 | 30.80 | 99.12 |
| **Level 3** | 26 | 10 | 36 | 36.99 | 99.96 |

**Table 3: % Recovery for Acebrophylline**

**Estimation of Acebrophylline in presence of Ambroxol**
It was determined by calculating the % recovery of Acebrophylline from tablet formulation by standard addition method in presence of Ambroxol HCl impurity. 5 ml of 10 ppm solution of Ambroxol HCl impurity was added in each prepared solution. Each solution was scanned between 200nm to 400nm against methanol as a blank. The first derivative spectrum of each was obtained. The amount of Acebrophylline was calculated at each level and % recoveries were computed in presence of Ambroxol HCl. The data is given in table 4.

| | **Concentration taken (µg/ml)** | **Concentration added (µg/ml)** | **Total** | **Observed Total** | **%Recovery** |
|---|---|---|---|---|---|
| **Level 1** | 14 | 10 | 24 | 24.98 | 99.85 |
| **Level 2** | 20 | 10 | 30 | 30.98 | 99.99 |
| **Level 3** | 26 | 10 | 36 | 37.10 | 100.38 |

**Table 4: Estimation in Presence of Ambroxol**

## CONCLUSION

Finally with the above results it is concluded that the developed method is specific, simple, rapid and accurate which can be applied to the estimation of Acebrophylline in capsule formulations and for estimation in presence of Ambroxol with minimum errors.

## ACKNOWLEDGEMENT

The authors are thankful to Ami Life Science Pvt. Ltd. for providing gift samples of Acebrophylline. The authors are thankful to Management of Parul Institute of Pharmacy, Vadodara for providing necessary facility for execution of the work.

## REFERENCES

1. R. Aligave *et al*, Determination of Acebrophylline in bulk and pharmaceutical formulation by UV spectrophotometer, Current Pharma Research ISSN: 2230-7842 CPR 1(3), 2011, 267-270.
2. S. R. Dhaneshwar *et al*, Development and Validation of Stability Indicating RP-HPLC-PDA Method for Determination of Acebrophylline and Its Application for formulation Analysis and Dissolution Study ISSN-2090-4304 Journal of Basic.Appl.Sci.Res.,1(11)1884-1890,2011.
3. D. Saraswathi *et al*, Spectrophotometric Estimation of Acebrophylline in Bulk and Capsule Formulation, Research Journal of Pharmacy and Technology, Volume 03, Issue 04, October-December 2010.
4. A. Singhai *et al*, Development and Validation of Acebrophylline by Reverse phase High Performance Liquid Chromatographic method in Tablet, Journal of Pharmaceutical Science, 2011.
5. Jithu Jerin James *et al* , Estimation of Acebrophylline by HPLC, Integrated Projects Consortium Experiments, 25 Aug,2011
6. R.K Smith, Hand book of Environmental Analysis Genium, New York 1999.
7. Y.R. Lee, Bioequivalence Study of Acebrophylline Capsules in Healthy Korean Male Volunteers, AAPS Pharmaceutics, 2005.
8. K.S. Sumanth *et al*, Development and validation of simple spectrophotometric, first and second derivative spectroscopy for estimation of Atovaquone in bulk and pharmaceutical formulations, Int. J. Res. Pharm. Sci., 2(2), 2011, 261-265.
9. Drug Profile of Acebrophylline , P. Arjun http/imakmj.com
10. ICH, Q2B (1993). Validation of Analytical Procedure: Methodology, International Conference on Harmonization, Geneva,