



## Application of TLC - Densitometry method for estimation of acebrophylline in pharmaceutical dosage forms

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### ABSTRACT

A rapid, accurate and precise HPTLC method has been developed for the estimation of Acebrophylline in Pharmaceutical formulation. In this method standard and sample solutions of Acebrophylline were applied on pre-coated 6 x 10 silica gel 60F<sub>254</sub> TLC plate, and developed using chloroform: isopropanol: toluene (8: 1: 1 v/v), as mobile phase. A Camag HPTLC system comprising of Camag Linomat -5-applicator, Camag twin trough chamber, Camag TLC-3 scanner was used for the analysis. The drugs on the plate were scanned at 254 nm. The dynamic linearity range was 1µl, 2µl, 3µl, 4µl & 5µl (1000-5000ng/spot) for Acebrophylline. The method was validated for precision, accuracy and recovery.

**Key words:** Estimation, HPTLC, Acebrophylline.

### INTRODUCTION

Acebrophylline is an anti-inflammatory and airway mucus regulator. It contains ambroxol and theophylline-7-acetic acid, the former facilitates the bio-synthesis of pulmonary surfactant while later raises blood levels of ambroxol, there by stimulating surfactant production<sup>1</sup>. Chemically acebrophylline is 1, 2, 3, 6-tetrahydro-1, 3-dimethyl-2, 6-dioxo-7H purine-7-acetic acid with trans- 4-[(2-amino-3, 5-dibromophenyl) methyl] amino] cyclohexanol. Literature survey revealed that various analytical methods like spectrophotometric<sup>2-6</sup>, HPLC<sup>5-8</sup>, and HPTLC<sup>9-10</sup> methods, have been reported for the determination of Ambroxol HCl and Theophylline -7- acetic acid, individually and combination with some other drugs. No HPTLC method for estimation of Acebrophylline in single dosage form has so far been reported. The review of literature prompted us to develop a rapid, accurate and precise HPTLC method for the estimation of Acebrophylline in pharmaceutical dosage forms.

### MATERIALS AND METHODS

#### Chemicals and Equipment

BESTOPHYLLINE-A, capsules used for the formulation analysis contains Acebrophylline manufactured by Bestochem, Vikasmarg, Delhi. Pure samples were procured from Sun Pharmaceutical Ltd., Jammu & Kashmir. All the chemicals and reagents used were of analytical grade. A Camag HPTLC system comprising of Camag Linomat -5-applicator, 500µl Hamilton syringe, Camag twin trough chamber, Camag TLC-3 scanner, and stationary phase pre coated with Silica gel 60F<sub>254</sub> were used.

#### Preparation of Standard Solutions

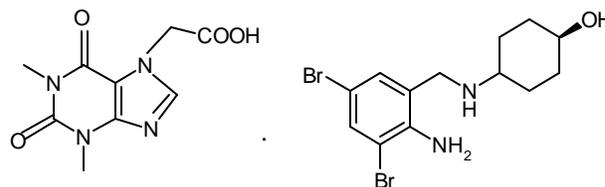
The given standard Acebrophylline 10mg was dissolved in Water and made-up to 10ml in a volumetric flask, this solution was used as working standard solution (1µg/1µl) for the analysis. Standard solutions having concentration ranging from 1000-5000ng/spot of Acebrophylline were applied on TLC plates.

#### Analysis of Formulation

Twenty number of BESTOPHYLLINE-A capsules were powdered using Pestle & Mortar to fine powder. From this, 100mg equivalent of powdered sample was extracted and dissolved in Methanol: Water(1:1), centrifuged and the supernatant liquid was made-up to 25 ml in a volumetric flask with Water and filtered through Whatman filter paper no 41. This solution contains 4µg drug sample in 1µl Water, used as test solution for quantitative analysis of Acebrophylline from BESTOPHYLLINE -A capsules. 2.5 µl of the test solution was applied on the pre-coated silica gel 60F<sub>254</sub> plate and from the peak area obtained; the amount of Acebrophylline in formulation was simultaneously calculated using the respective calibration graph. The amount obtained per capsule and percentage label claim are shown in Table 1.

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Structure of Acebrophylline

#### Development of Chromatograms

The TLC plates were washed with methanol and activated by keeping at 115° for about 30 min. The samples were spotted in the form of bands of width 5mm with 500µl Hamilton syringe on the pre-coated silica gel 60F<sub>254</sub> plate (6x10cm) and the slit dimension was kept at 15 min respectively. The mobile phase used was chloroform: isopropanol: toluene (8: 1: 1 v/v) in chamber and the plate saturation time was 15 min, migration distance was allowed up to 80 mm, linear ascending development was carried out in (20x10cm) twin trough glass chamber. Subsequent to the development, TLC plates were dried in current of air and kept in photo documentation chamber. The images of developed plate were captured at white light, UV 254 nm using Camag - Reprostar -3 instrument. The plate was scanned at 254nm using Camag-TLC- scanner-3 instrument.

#### Validation Parameters

The method was validated for linearity, accuracy, limit of detection, limit of quantification, inter-day and intra-day assay precision, repeatability of measurement and repeatability of sample application. Samples applied on the plate were developed with the mobile phase and the peak areas were noted. The mobile phase, chloroform: isopropanol: toluene (8: 1: 1 v/v) gave R<sub>f</sub> value of 0.22 ± 0.02 for Acebrophylline (Fig.1).

#### Linearity and Regression

A good linear relationship was obtained over the concentration range 1000 - 5000ng/spot of Acebrophylline. The linear regression data showed a regression coefficient of 0.9987 for Acebrophylline.

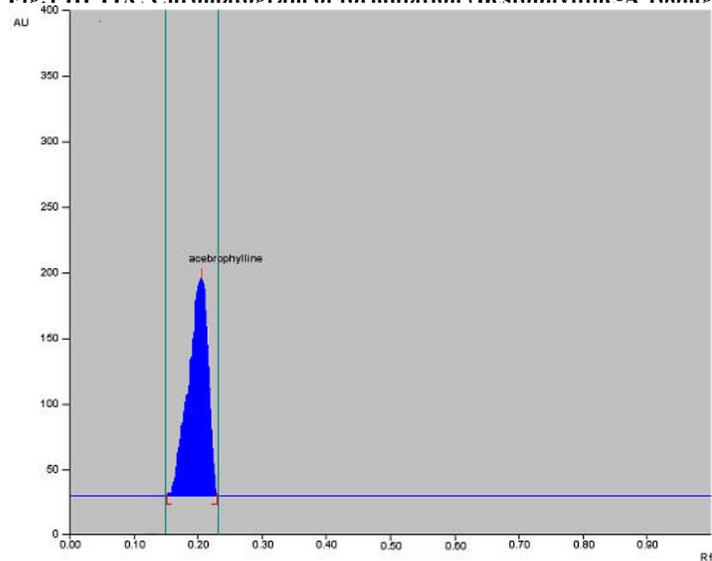
#### LOD and LOQ

The LOD with signal/ noise ratio were found to be 250ng/spot for Acebrophylline. The LOQ with signal/ noise ratio was found to be 1000ng / spot for Acebrophylline.

#### Precision

Intra-day assay precision was found by analysis of standard drug at three times on the same day. Inter-day assay precision was carried out using at three different days, and percentage relative standard deviation (%RSD) was calculated. The RSD was found to be less than 2 for both intra-day and inter-day precision. Repeatability of sample application was assessed by spotting 1 µl of

Fig.1 HPTLC Chromatogram of formulation (Bestonhville-A 100mg)



Formulation Solution Containing Acebrophylline 10µg/ml showing Rf Value = 0.22.

Fig.2. 3D Display of Acebrophylline calibration samples and formulation

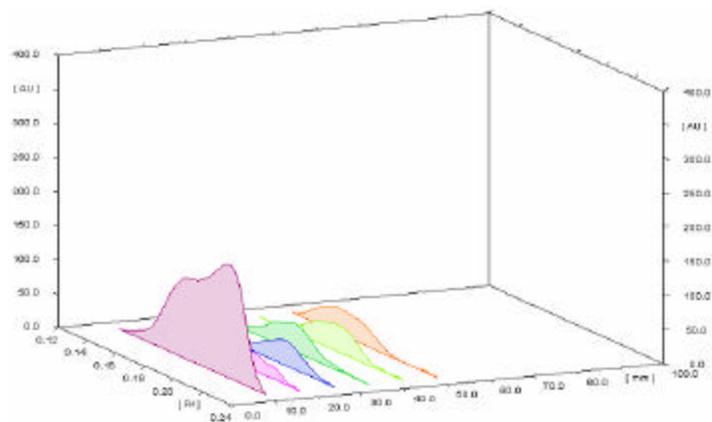


Fig.3 Spectral display of calibration samples and formulation

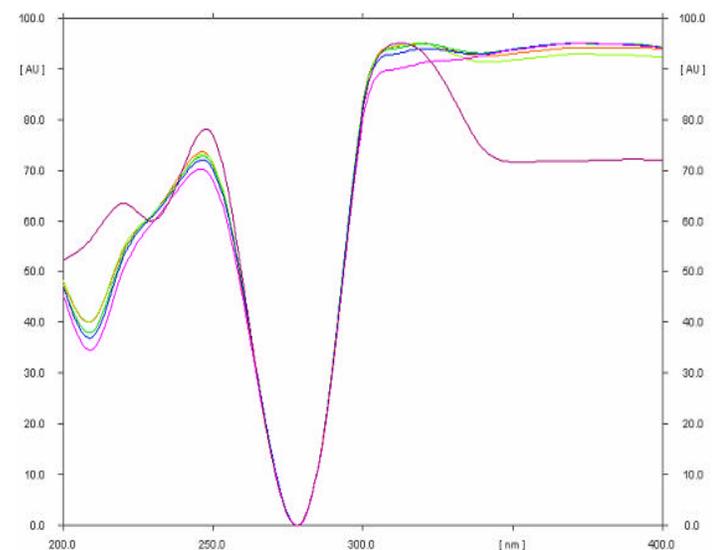


Table.1.Result of analysis of formulation

Drug	Amount (mg/Capsule) Labeled	Amount (mg/Capsule) Found <sup>a</sup>	% label claim <sup>a</sup>	S.D <sup>a</sup>
Acebrophylline	100	99.32	99.32	0.58

<sup>a</sup>An average value ± relative standard deviation of 5 observations.

Table.2.Validation Parameters

Parameters	Value
R <sub>f</sub>	0.22 ± 0.03
Linearity (µg/ml)	1000-5000ng
Correlation co efficient	0.9987
LOD (ng/spot)	250ng
LOQ (ng/spot)	1000ng
<b>Precision (% RSD)</b>	
Inter-day	0.86
Intra-day	0.62
Repeatability (% RSD)	0.57

R<sub>f</sub> - resolution factor, RSD- relative standard deviation, LOD – limit of detection, LOQ – limit of quantification.

Table.3.Recovery data

Level	Amount added (mg)	Amount found (mg) <sup>a</sup>	% Recovery <sup>a</sup>	% RSD <sup>a</sup>
80%	80	81.23	101.53	1.19
100%	100	100.46	100.46	0.79
120%	120	119.76	99.79	0.83

<sup>a</sup>An average value ± relative standard deviation of 5 observations.

drug solution, six times. From the peak areas, the percentage RSD was determined. The complete validation parameters are shown in Table 2.

### Recovery Studies

The recovery study was carried out at three levels, 80%, 100 % and 120%. To the powdered formulation, the standard drug of Acebrophylline were added at 80%, 100% and 120% levels, dilutions were made and analyzed by the method. The % recovery and % RSD were calculated and found to be within the limit, as listed in Table 2.

### RESULTS AND DISCUSSION

During the stage of method development different mobile phases were tried and the mobile phase comprising of chloroform: isopropanol: toluene in the proportion of (8:1:1) was confirmed. The R<sub>f</sub> value was found to be 0.22 Acebrophylline. Linearity of the drug was determined by the calibration curve and the linearity based on the peak area was in the range of 1000-5000ng (table.2). The regression coefficient value for Acebrophylline was 0.9987. The limit of quantification was determined by injecting minimum concentration of the drugs. The limit of quantification (LOQ) was found as 1000ng/spot. The recovery was less than 101.53 % for 80,100 and120% Acebrophylline samples(table.3) and the repeatability showed excellent % RSD less than 0.57(table.2). The method passes all the validation parameter limits and proves to be selective, sensitive and precise. Hence the proposed method can be used for the routine assay of Acebrophylline using HPTLC.

### CONCLUSION

Since, the developed HPTLC method is rapid, precise and accurate; the statistical analysis proved that the method is repeatable and selective for the analysis of Acebrophylline in bulk drugs and in pharmaceutical dosage forms without any interference from the excipients.

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