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Research Article

Development and Validation of Stability Indicating HPTLC Method for Estimation of Acebrophylline in Their Dosage Form

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ABSTRACT
A rapid, accurate and precise stability indicating HPTLC method has been developedandvalidatedfor the estimation of Acebrophylline in pharmaceutical formulation. In this method, standard andsample solutions of Acebrophylline were applied on pre-coated 6 x 10 silica gel 60F254 TLC plate, and developed using Toluene: Methanol:Acetone (8: 2: 2v/v) as mobile phase. Acebrophylline was well resolved at Rf 0.49 ±0.03. A Camag HPTLC system comprising of Camag Linomat-5-applicator, Camagtwin trough chamber, Camag TLC-4 scanner operated with WINCATS software, was used for theanalysis. The drugs on the plate were scanned at 247 nm. The response for the drug was found to be linear in the concentration range 600-2200 ng/band for Acebrophylline with correlation coefficient of 0.9992.The LOD and LOQ were found to be 1.133ng/band and 3.434ng/band respectively by standard equation method.Acebrophyllinewas exposed to acid & base hydrolysis, oxidation and heat. Under all these stress conditions, degraded productswerewell separated. The method was validated according to the ICH guidelines with respect toaccuracy, precision, linearity, specificity, limits of detection and limits of quantitation. As the proposed method could effectivelyseparate the drug from its degradation products, it can be employed as stability indicating method.

Key words: Stability indicating HPTLC method, Acebrophylline.

INTRODUCTION

Acebrophylline is an anti-inflammatory and airway mucus regulator. It contains ambroxol and theophylline-7-acetic acid, the former facilitates the biosynthesis of pulmonary surfactant while later raises bloodlevels of ambroxol, by stimulating surfactant production [1]. Chemically 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 [2, 3, 10]. From the literature survey it was found that only three methods UV [4, 5], HPLC [6-8] and HPTLC [9] have been reported very recently for the determination of Acebrophylline. But many methods have been reported for estimation of Ambroxol or Theophylline individually or in combination with other drugs.There is no stability indicating HPTLC method reported yet.Hence in the present projectwork, attempts will be made to develop rapid, accurate, precise and economic stability indicating HPTLC method for estimation of Acebrophylline in bulk and marketed formulation.

MATERIALS AND METHODS

Chemicals and Equipment

Acebrophylline Active Pharmaceutical Ingredient (API) was supplied by Sava Medica ltd. (Surendranagar, Gujarat, India) & Tablet formulation (ABPhylline®200 mg by Sun Pharma) was procured from local market. Reagents used are toluene, methanol, acetone, Conc. HCL, Sodium Hydroxide, Hydrogen peroxide. All the chemicals and reagents used were of analytical grade.Chromatographic separation of drugs was performed on Merck TLC plates pre-coated with silica gel 60 F254 (20 cm ×10 cm with 250 mm layer thickness).

Preparation of Standard Solutions

Accurately weighed Acebrophylline standard (100 mg) was transferred into a 100 ml volumetric flask and dissolved in and diluted to the mark with methanol to obtain the standard stock solution (1000 µg/ml). An aliquot (1 ml) was transferred into a 10 ml volumetric flask and diluted to the mark with methanol to obtain the Acebrophylline standard solution (100µg/ml).Standard solutions having concentration ranging from 600-2200ng/spot of Acebrophylline were applied on TLC plates.

Preparation ofsample solution

Twenty tablets of Acebrophylline were powdered. Powder equivalent to 100 mg of Acebrophylline was weighed and transferred into a 100 ml of volumetric flask and 60 ml of methanol was added. It was sonicated for 20 min and diluted up to mark with methanol.The solution was filtered through a 0.45 µm HVLV Millipore filter. An aliquot (1 ml) was transferred into a 10 ml volumetric flask and diluted to the mark with methanol to obtain the Acebrophylline sample solution (100µg/ml).

Development and Optimization of Method[11]

Selection of Wavelength

The sensitivity of the method depends upon the proper selection of wavelength. An ideal wavelength is the one that gives good response for the drug that is to be detected. The standard solution of Acebrophylline (10 µg/ml) was scanned between 200-400 nm wavelengths. Acebrophylline shows higher absorbance at 247nm so it was selected as a detection wavelength.

Selection of chromatographic condition

Various solvents like Toluene, Methanol, Chloroform, Acetone, Acetic acid, Ammonia were tried in different

proportions and different combinations for the separation of Acebrophylline and its degradation product.

Analysis of Market Formulation (Tablet)

Sample solution of (1400 ng/band) was applied on the TLC plate five times. Plate was developed, scanned in densitometer and peak area is measured and % assay was calculated against the peak area of band of standard solution applied in same concentration.

Forced Degradation Studies[13]

The degradation samples were prepared by transferring 100 mg of Acebrophylline into 100 ml volumetric flask and diluted to the mark with 0.1 N HCl, 0.1 N NaOH and 3% H_2O_2 for acidic, basic and oxidative degradation studies respectively. Hydrolytic reactions were carried out in 0.1 N HCl, 0.1 N NaOH and 3% H_2O_2 under refluxing for 4 hrs at80 ºC. Powder solid drug (in 1 mm thick layer petridish) was exposed to dry heat at 80 ºC in an oven for 48 hrs. Samples were withdrawn and applied by HPTLC after suitable dilution.

METHOD VALIDATION[12]

This method was validated for the parameters listed below as per ICH guidelines.

Linearity

Different concentrations of Acebrophylline (600 ng to 2200 ng/ band) were applied on TLC plate and peak area were measured in densitometer. The calibration curve was constructed by plotting the peak area versus concentration.

Precision

Interday and Intraday precision were evaluated by analyzing three concentrations for Acebrophylline (1200, 1400 and 1600 ng/band) three times on same day and on three different days and % RSD value obtained was calculated to determine any Intraday and Interday variation.

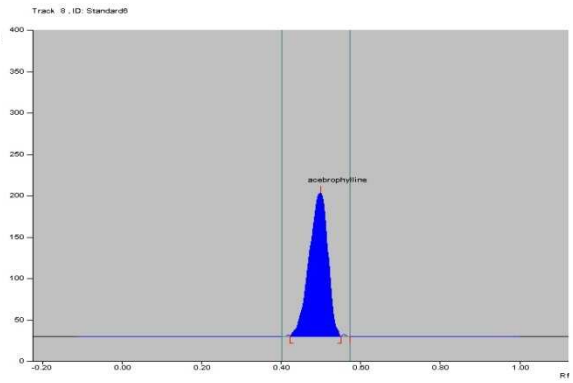

Fig 1 Representative HPTLC chromatogram of Acebrophylline Standard (1400 ng/band)

Accuracy

Accuracy was determined by calculating the % recovery of Acebrophylline by standard addition method. Known amount of standard solution of Acebrophylline (400, 800 and 1200 ng/band) were added in pre analyzed sample solution of Acebrophylline (800 ng/band). Each solution was applied in triplicates and recovery was calculated by measuring the peak area and fitting themselves into the regression equation.

Limit of Detection and limit of quantitation

The limit of Detection (LOD) and limit of quantitation (LOQ) of the drug were calculated using following equations as per ICH guideline.

$$LOD=3.3(\sigma/S),$$

$$LOQ=10(\sigma/S)$$

Where σ is Standard deviation of the intercepts and S is slope of the calibration curve.

Specificity

Specificity was performed by scanning the band of sample solution of Acebrophylline at upper, middle and bottom position in spectrum mode. If spectrum obtained from each position are nearly same indicates the no interference of any placebo, solvent or any degradation product which may expected to be present.

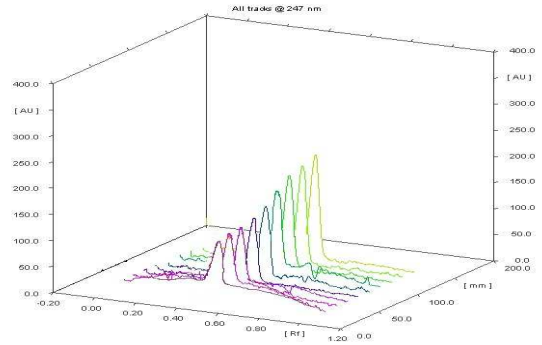

Fig 2 A 3D HPTLC chromatogram of Acebrophylline (600-2200 ng/band)

RESULT AND DISCUSSION

Development for optimum Mobile Phase

For the selectionof appropriate mobile phase for Acebrophylline, several runs wereexercised using mobile phases containing solvents of varyingpolarity, at different concentration levels. Among the differentmobile-phase combinations employed, the mobile phaseconsisting of Toluene: Methanol: Acetone (8: 2: 2 v/v)gave a sharp and well-defined peak at *R*fvalue of 0.49±0.03 (Fig.1).Well distinct bands were found when thechamber was saturated with the mobile phase for 30 min atroom temperature.

Chromatographic Conditions

Mixture of Toluene: Methanol: Acetone in the ratio of 8:2:2 v/v/v % was optimized for thin layer chromatography plate development. The samples were applied onto the plates using Camag 100 µl sample syringe (Hamilton, Switzerland) with an applicator (Camaglinomat 5). Linear ascending development was carried out in a twin trough glass chamber (for 20 x 10 cm).The chamber was saturated with the mobile phase at room temperature for 60 min. A run distance was kept about 90 mm and 10 ml of the mobile phase was used for single development. The dosing speed of nitrogen applicator was kept 150nl/sec with a predosage volume of 5 µl. Samples were applied as bands of 6 mm width with the gaps of 10 mm in between keeping 20 mm and 20 mm distance from X-axis and Y-axis respectively. Developed plates were dried at room temperature for 5 min. Densitometric scanning was performed using Camag TLC scanner4 and operated by WINCATS software. Detection was done at 247 nm using deuterium lamp in absorption-re-emission mode. The slit dimension of detection was kept 0.4 mm x 0.02 mm. The Rf of Acebrophylline was found to

be 0.49 ± 0.03. HPTLC is carried out for the separation Acebrophylline and its degradation product.

VALIDATION OF METHOD[12]

Calibration curve

The linear regression data for the calibration curve showed good linear relationship over the concentration range 600-2200ng/band. Linear regression equation was found to bey = 2.3033x - 121.91, r^2 = 0.9992 (Figure 2)

Precision

The precision of the developed methodwas represented in terms of % relative standard deviation(%RSD) of the peak area. The results depicted indicated high precision of the method are presented in Table 1.

Table 1 Intraday and Interday precision for Acebrophylline

Sr. No.	Conc. (ng/band)	Intraday		Interday	
		Peak area* ± SD	%RSD	Peak area* ± SD	%RSD
1	1200	2659.2± 4.56	0.172	2658.9 ± 4.4	0.166
2	1400	3118.7± 3.24	0.104	3120.2 ± 4.7	0.149
3	1600	3571.6 ± 5.39	0.151	3572.4 ± 5.3	0.147

*Average of three determinations.

Recovery studies

The recovery studies were executed out at 50%, 100%, and 150% of the test concentration as per ICH guidelines. The % recovery of Acebrophylline at all the three levels was found to be satisfactory. The amounts of drug added and determined and the % recovery are listed in Table 2.

Table 2 Accuracy data of Acebrophylline

Amt. of sample (ng/band)	Amt. of drug added (ng/band)	Mean peak area*±SD	Amt. recovered Peak area*	% Recovery ± % CV
800	400	2639.67 ±2.52	1198.88	99.93 ± 0.095
800	800	3561.00 ±1.00	1598.96	99.92 ± 0.0281
800	1200	4440.00 ±5.00	1982.55	99.00 ± 0.113

*Average of three determinations

Summary of validation parameters for Acebrophylline

Linearity	600-2200 ng/band
Regression equation	y = 2.3033x - 121.91
Correlation coefficient	0.9992
Precision	
(a) Intraday (n=3)	0.10-0.17 % RSD
(b) Interday (n=3)	0.14-0.16 % RSD
% Recovery	99.00 - 99.93 %
LOD	1.133 ng/band
LOQ	3.434 ng/band
Specificity	No interference
Rf (Acebrophylline)	0.49 ± 0.03

Applicability of the method for analysis of marketed formulation

Acebrophylline powder content of Tablet FormulationABPhylline®SR(200mg) tablet was found to be 99.12 % with a % RSD of 0.28.

DEGRADATION STUDIES[13]

Forced degradation studies of Acebrophylline were carried out under various stress conditions and resultant chromatograms are depicted in Figure (3-6). Percentage degradation was calculated and recorded. Acebrophylline undergoes 34.5 %, 33.8 %, 46.34%, 36.72% decomposition under acidic, alkaline, oxidative and thermal conditions with more than one degradation product. Acebrophylline was moderately degradable in acidic and oxidative conditions.

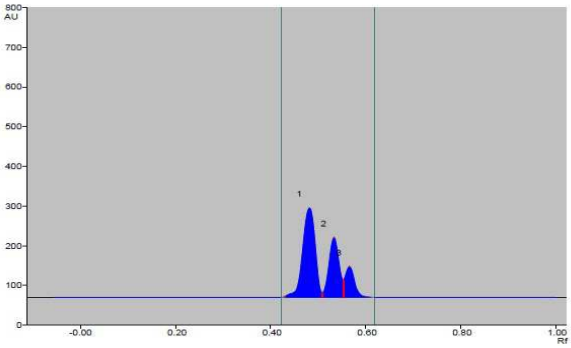

Fig 3 Degradation of Acebrophylline in 0.1 N HCl [Rf (1) std-0.48 (2) d1-0.53 (3) d2-0.57]

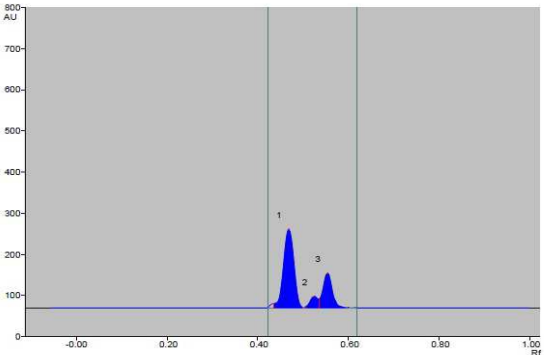

Fig 4 Degradation of Acebrophylline in 0.1 N NaOH [Rf (1) std-0.47(2) d1-0.53(3) d2-0.56]

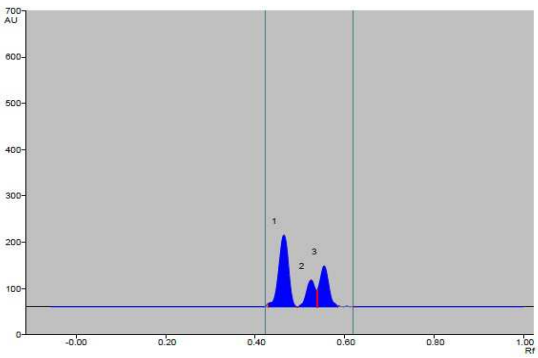

Fig 5 Degradation of Acebrophylline in 3% H2O2 [Rf (1) std-0.47 (2) d1-0.53(3) d2-0.57]

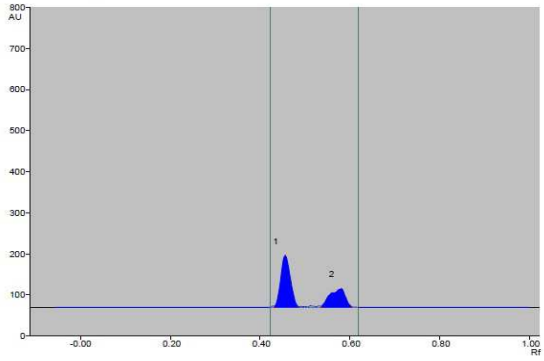

Fig. 6 Degradation of Acebrophylline in Thermal condition [Rf (1) std-0.48 (2) d1-0.58]

CONCLUSION

The proposed Stability indicating assay method for determination of Acebrophylline and its degradation product in tablet dosage form is found to be simple, precise, accurate and sensitive. The excipients and degradant did not interfere in the analysis of Acebrophylline, which proved the specificity of the method for this formulation. This method can be used for routine quality control of Acebrophylline even in presence of degradation product in its formulation.

REFERENCES

1. CIMS-106 [Update-3] pp: 13 (July-2009).
2. http://www.medlineindia.com/respiratory/acebrophylline.html,accessed Sept.10, 2012.
3. AC1L427N–Compound summary (cid176595) http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=176595&loc=ec_rcs [19/11/2009] accessed Sept. 10, 2012.
4. D Saraswathi, Priyadharsini, A Aruna and A J Suresh,"Spectrophotometricestimation of Acebrophylline in bulk and capsule formulation,"*Res. J Pharm.Tech.* 3 (4), 1222-1225 (2010).
5. A R Aligave, H S Dhamne, S S Gaikwad, M. S. Kondawar, "Determination of Acebrophylline in bulk and pharmaceutical formulation by UV-spectrophotometer,"*CPR*1(3),267-270 (2011).
6. D Saraswathi, G P Gigi, V Niraimathi, AJSuresh, "Estimation of Acebrophylline in Pharmaceutical Oral Solid Dosage formby RP-HPLC,"*J. Pharm. Res.*, 9 (3), (2010).
7. S R Dhaneshwar, V N Jagtap, "Development and Validation of Stability Indicating RP-HPLC-PDAMethod for Determination of Acebrophylline and Its Application for Formulation Analysis and Dissolution Study," *J. Basic. Appl. Sci. Res.*, 1(11), 1884-1890 (2011).
8. M D Bauskar, P Sonawane, S Y Nandedkar, R D Wagh, T Shaikh and V Jagtap,"Development and Validation of Reverse Phase Liquid Chromatographic Methods for the Determination of Acebrophylline in Capsule Form," *Res. J pharm. & Techno*, 4(10), 1542-1546 (2011).
9. W D Sam Solomon, M Manu, R Sivakumar, PRVijaiAnand and RVenkatanarayanan,**"**Application of TLC - Densitometry method for estimation of acebrophyllineinpharmaceutical dosage forms," *J. pharm. Res*, 3(11), 2561-2563 (2010).
10. Sean C Sweetman. Martindale "The Complete Drug Reference". Pharmaceutical press London,Chicago, 36th ed. (1), 2009, pp. 1108-1115.
11. P D Sethi, High Performance Thin Layer Chromatography(Quantitative Analysis of Pharmaceutical Formulations), CBS Publishers, New Delhi, India, (1996).
12. International conference on Harmonization ICH/CPMP guidelines Q2 (R1), Validation of Analytical Procedures: Text and Methodology, ICH, Geneva, Switzerland, 2005, accessed Nov.13, 2013.
13. International Conference on Harmonization Q1A, Stability Testing of New Drug Substances and Products, ICH, Geneva,Switzerland, 1993,accessed Nov. 13, 2012.