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Analytical Method Development and Validation for Simultaneous Estimation of Acebrophylline and Montelukast Sodium in their Pharmaceutical Dosage Form

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ABSTRACT:

A novel combination of Montelukast sodium (MONT) and Acebrophylline (ACB) is used in the treatment of allergic rhinitis, bronchial asthma and chronic obstructive pulmonary disease. A simple, accurate and reproducible HPTLC method has been developed and validated for the simultaneous estimation of Montelukast sodium (MONT) and Acebrophylline (ACB) in their combined dosage form. Merck HPTLC aluminum plates of silica gel G60 F254, (10 × 10 cm) was used for separation of combined drug with 250 μm thickness using Chloroform : Ethyl acetate : Methanol : Triethylamine (6 : 4.5 : 2.5 : 0.8, v/v/v/v) as mobile phase. HPTLC separation of the both drugs were carried out and followed by densitometric measurement was performed in the absorbance mode at 272 nm. The drugs were resolved satisfactorily with R_f values of 0.23 ± 0.01 and 0.83 ± 0.01 for ACB and MONT, respectively. The method was validated statistically as per ICH guidelines. The method showed good reproducibility and recovery with % RSD less than 2. So, the proposed method was found to be simple, specific, precise, accuracy, linear, and robust. Hence it can be applied for routine analysis of Montelukast sodium (MONT) and Acebrophylline (ACB) in pharmaceutical formulations.

KEYWORDS: Montelukast; Acebrophylline; HPTLC; Simultaneous estimation..

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INTRODUCTION:

Acebrophylline is 4-[(2-amino-3,5-dibromophenyl) methylamino] cyclohexan-1-ol; 2-(1,3-dimethyl-2,6-dioxopurin-7-yl)acetic acid. Acebrophylline is the salt obtained by reaction of equimolar amounts of theophylline-7-acetic acid, a xanthine derivative which inhibits phospholipase A, and phosphatidylcholine leading to lesser production of the powerful pro-inflammatory substances like leukotrienes and tumour necrosis factor. By inhibiting the synthesis and release of these inflammatory mediators, acebrophylline reduces inflammation, a key factor in airway obstruction, specially in chronic forms.^[1-2] The chemical structure of MONT is shown in figure 1.

Montelukast sodium is described chemically as [R-(E)-1-[[[1-[3-[2-(7-chloro-2-quinolinyl) ethenyl] phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]thio]methyl]cyclopropane acetic acid, monosodium salt. Montelukast selectively antagonizes leukotriene D₄ (LTD₄) at the cysteinyl leukotriene receptor, CysLT₁, in the human airway. Montelukast inhibits the actions of LTD₄ at the CysLT₁ receptor, preventing airway edema, smooth muscle contraction, and enhanced

secretion of thick, viscous mucus. This reduces the bronchoconstriction otherwise caused by the leukotriene and results in less inflammation^[3-4]. The chemical structure of MONT is shown in figure 2.

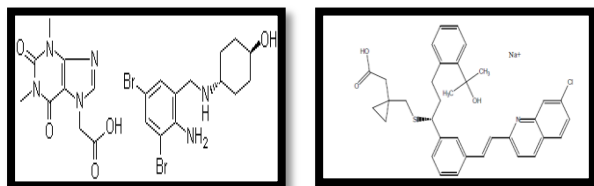


Figure 1. Structural of Acebrophylline **Figure 2.** Structure of Montelukast sodium

The review of literature revealed that there are several methods are available for the estimation of acebrophylline and montelukast sodium individually and combination with other drugs. Reported method for estimation Acebrophylline in dosage form are spectroscopic method^[5], RP-HPLC^[6-7] and HPTLC^[8] and similarly for estimation of Montelukast sodium in dosage form are spectrophotometry^[9-10], spectrofluorometry^[11], RP-HPLC^[12-14] and HPTLC^[15] But, there is no any analytical method has been reported yet for combination of these drugs. There for the present research work aims to develop a simple, sensitive, accurate and reproducible HPTLC method for simultaneous estimation of Acebrophylline and Montelukast sodium in combined dosage form.

MATERIALS AND METHODS

Acebrophylline was obtained as a gift sample from Ami life science (Baroda) and Montelukast Sodium was obtained as a gift sample from Zyduscadila, (Ahmedabad). All the reagent used in this research work are of analytical grade and were purchased from Merck Lab. and Qualigens Fine Chemicals Pvt. Ltd., India. The HPTLC instrument used was Camag HPTLC with Linomat-5 injection and camag TLC scanner-3. All the apparatus and instruments used were calibrated and validated.

METHODOLOGY

Instrumentation and chromatographic conditions

The HPTLC plates were prewashed with methanol and activated at 110 °C for 5 min prior to chromatography. The samples were spotted in the form of bands 6 mm width with a Camag 100 microlitre sample syringe (Hamilton, Bonaduz, Switzerland) on silica gel precoated

HPTLC aluminum plate 60 F254, [(10 × 10 cm) with 250 μm thickness; E. Merck, Darmstadt, Germany, supplied by Anchrom Technologies, Mumbai] using a CamagLinomat 5 applicator (Switzerland). A constant application rate of 0.1 μL/sec was used and the space between two bands was 7 mm. Linear ascending development was carried out in 20 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The mobile phase consisted Chloroform : Ethyl acetate : Methanol : Triethylamine (6 : 4.5 : 2.5 : 0.8, v/v/v/v). The optimized chamber saturation time with mobile phase was 30 min using saturation pads at room temperature (25 °C ± 2). The length of chromatogram run was 80 mm and run time was 20 min. Densitometric scanning was performed using a Camag TLC scanner III in the reflectance-absorbance mode and operated by winCATS software (V1.1.4, Camag). The slit dimension was kept at 5mm × 0.45 mm and the scanning speed was 10 mm/sec. The source of radiation used was a deuterium lamp emitting a continuous UV spectrum between 200 and 400 nm. All determinations were performed at ambient temperature with a detection wavelength of 272 nm. Concentrations of the compound chromatographed were determined from the intensity of the diffused light. Evaluation was by peak areas with linear regression

Preparation of Standard solution

Mixed stock standard solution containing 20000 μg/mL of ACB and 1000 μg/mL of MONT was prepared in methanol by dissolving 200 mg of ACB and 10 mg of MONT in 10 mL methanol. Mixed stock standard solution was further diluted with methanol to obtain working standard solutions in a concentration range of 12000 – 20000 ng/spot for ACB and 600-1000 ng/spot for MONT. Each concentration was applied six times on the HPTLC plate. The plate was then developed using the previously described mobile phase. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs as shown in figure 3 & 4.

Sample preparation

Twenty Tablets were weighed and finely powdered. The powder equivalent to 200 mg (ACB) of tablet formulation were accurately weighed and transferred to volumetric flask of 10 mL capacity. 5mL of methanol transferred to volumetric flask and sonicated it for 5 mins. The flask was shaken and volume was made up to the mark with

methanol. The above solution was carefully centrifuged at 4000 rpm for 15 min. It was filtered through vacuum filter using Whatman filter paper (No.41). The aliquot (5 mL) was transferred into 10 mL volumetric flask and volume was made up to the mark with methanol to give a solution containing 1000 µg/mL of MONT and 20000 µg/mL of ACB. The plate was developed in the previously described chromatographic conditions. The peak area of the spots were measured at 272 nm for MONT and ACB, respectively and the concentrations in the samples were determined using multilevel calibration developed on the same plate under the same conditions using linear regression equation as shown in figure 6.

VALIDATION PARAMETER

Linearity

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range. The range of analytical method is the interval between the upper and lower levels of analyte that have been demonstrated to be determined within a suitable level of precision, accuracy and linearity. Standard mixture solution of MONT and ACB having concentration of 600 to 1000 ng/spot for MONT and 12000 to 20000 ng/spot for ACB were spotted and developed as described in proposed method. Developed plates were subjected to densitometric measurements in absorbance mode at wavelength 272.0 nm using Camag TLC Scanner 3 and 3D image of linearity overlain were obtained as shown in figure 5.

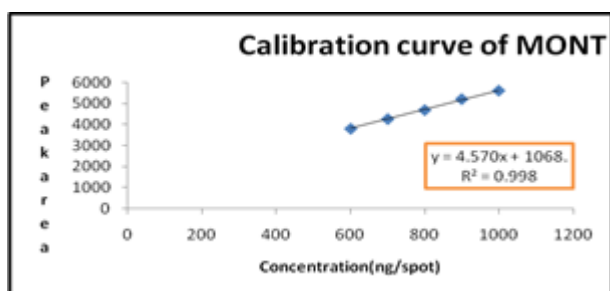


Figure 3. Calibration curve of Montelukast Sodium

Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. It provides an

indication of random error results and was expressed as coefficient of variation (CV).

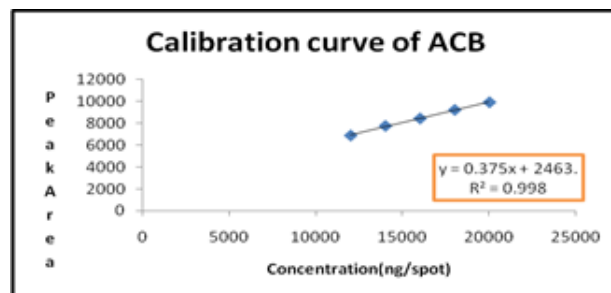


Figure 4. Calibration curve of Acebrophylline

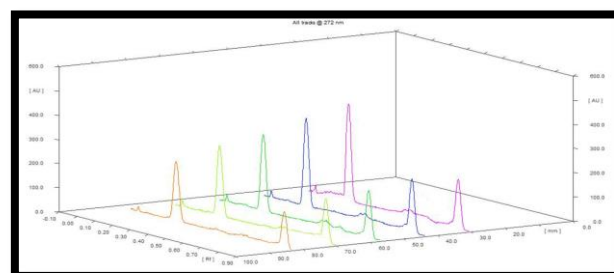


Figure 5. Three Dimension Chromatogram of calibration curve.

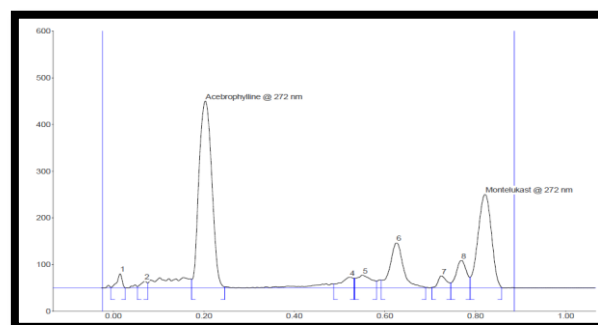


Figure 6. Chromatogram of Sample mixture in mobile phase

Intra and inter day precision

Intraday and interday precision was determined in terms of % RSD. Intraday precision was determined by analyzing in combined solution their respective calibration range for five times in the same day. Interday precision was determined by analyzing MONT and ACB in for five days.

Procedure for Intraday Precision: From combined standard solution of MONT and ACB 600+12000ng/spot, 800+16000 ng/spot, 1000+20000 ng/spot were spotted 5 times on same day and developed as described in

proposed method. Developed plates were subjected to densitometric measurements in absorbance mode at wavelength 272.0 nm using Camag TLC Scanner 3 and % RSD was calculated.

Procedure for Interday Precision: From combined standard solution of MONT and ACB 600+12000ng/spot, 800+16000 ng/spot, 1000+20000 ng/spot were spotted 5 times on different days and developed as described in proposed method. Developed plates were subjected to densitometric measurements in absorbance mode at wavelength 272.0 nm using Camag TLC Scanner 3 and % RSD was calculated result were shown in table no.1.

Accuracy

Accuracy may often be expressed as percentage recovery. It was determined by calculating the recovery of MONT and ACB by application of the analytical method to mixtures of the drug product contents to which known amount of analyte have been added within the range of the method.

Limit of Detection (LOD)

The L.O.D. was estimated from the set of 5 calibration curves.

$$LOD = 3.3 \times (S.D./Slope)$$

Where,

S.D. = Standard deviation of the Y- intercepts of the 5 calibration curves.

Slope = Mean slope of the 5 calibration curves.

LOD of MONT and ACB were described in table 1

Limit of Quantification (LOQ)

The L.O.Q. was estimated from the set of 5 calibration curves.

$$LOQ = 10 \times (S.D./Slope)$$

Where,

S.D. = Standard deviation of the Y- intercepts of the 5 calibration curves.

Slope = the mean slope of the 5 calibration curves.

LOQ of MONT and ACB were described in table 1

Specificity

Specificity of the method was determined by means of complete separation of pure drugs in the presence of other excipients normally present in the formulation. The

specificity of the method was ascertained by peak purity profiling studies. Peak purity of ACB and MONT was assessed by comparing their respective spectrum at peak start (S), peak apex (M) and peak end (E) position of the spots. The peak purity was determined on winCATS software using statistical equation.

Selectivity

Selectivity is the procedure to detect qualitatively the analyte in presence of components that may expected to be present in the sample matrix. Commonly used excipients present in selected tablet formulation were spiked into a pre-weighed quantity of drugs. The absorbance was measured, and calculations determined the quantity of the drugs.

Robustness

The robustness of the method was established by introducing small changes in mobile phase composition and chromatograms were run. The amount of mobile phase, chamber saturation time, time from spotting to chromatography and from chromatography to scanning (± 10 min) and development distance from spot application was varied in the range of $\pm 5\%$. The robustness of the method was determined at three different concentration levels of MONT and ACB.

RESULT AND DISCUSSION

The TLC procedure was optimized with a view to develop an assay method for the simultaneous estimation of ACB and MONT. The method was found to be linear concentration range of 600 - 1000 ng/spot and 12000 - 20000 ng/spot for MONT and ACB respectively. The standard solutions of both the drugs were spotted on the TLC plates and run in different solvent systems. The mobile phase consisting of Chloroform : Ethyl acetate : Methanol : Triethylamine (6 : 4.5 : 2.5 : 0.8, v/v/v/v) give sharp and symmetrical peaks with the R_f values of 0.23 ± 0.01 and 0.83 ± 0.01 for ACB and MONT respectively. Well defined spots were obtained when the chamber was saturated with mobile phase for 20 min at room temperature ($27 \pm 3^{\circ}\text{C}$). The proposed method was validated as per ICH guideline in terms of accuracy, precision, robustness and specificity.

Table 1: Summary of validation parameters for the proposed method

Parameters	Result	
	MONT	ACB
Linearity	600 - 1000	12000 – 20000
Range(ng/spot)	$y = 4.5704x + 1068.3$	$y = 0.3751x + 2463.5$
Regression equation		
Accuracy		
(%Recovery \pm SD)	100.2072 \pm 0.2685	99.8760 \pm 0.3713
Precision (%RSD)		
Inter-day (n=3)	0.4306 – 0.9008	0.4640 – 0.5384
Intra-day (n=3)	0.2704 - 0.5061	0.2042 - 0.2760
Limit Of	104.9	252.389
Detection (ng/spot)		
Limit Of	317.88	764.815
Quantification (ng/spot)		
Robustness(%RSD)	0.6937	1.5652
%Assay \pm SD (n=6)	100.04 \pm 0.6293	99.96 \pm 0.3455

CONCLUSION

The developed HPTLC technique is precise, specific, robust and accurate method for analysis of MONT and ACB in pharmaceutical preparations. Statistical analysis proves that the method is suitable for the analysis of Montelukast sodium and Acebrophylline as bulk drug and in pharmaceutical formulation without any interference from the excipients. It may be extended to study the degradation kinetics of Montelukast sodium and Acebrophylline and also for its estimation in plasma and other biological fluids. The proposed TLC method is less expensive, simpler, rapid, and more flexible than HPLC.

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