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Research Article

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Development and Validation of HPTLC Method for Simultaneous Estimation of Montelukast and Theophylline in Bulk and Pharmaceutical Dosage Form

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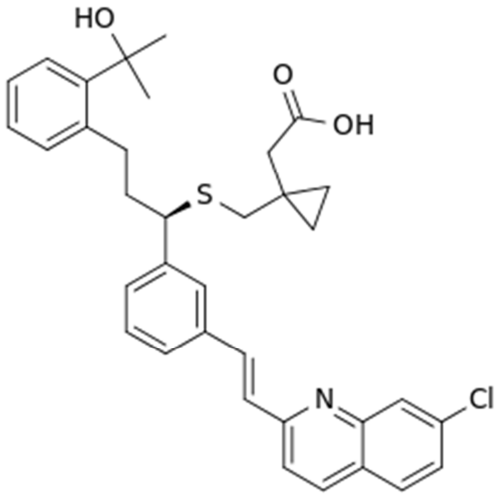
ABSTRACT

A simple, precise, specific and accurate high performance thin layer chromatographic method has been developed for the simultaneous estimation of montelukast(MONT) and theophylline(THEO) in pharmaceutical dosage form. The separation was carried out on Merck HPTLC aluminum plates of silica gel G6O F254 (20 X 10 CM) with 250 µm thickness using Ethylacetate: Chloroform: Ethanol: Ammonia(6:4:3:1v/v/v/v) as mobile phase. HPTLC separation of the two drugs followed by densitometric measurement was carried out in the absorbance mode at 254 nm. The drugs were resolved satisfactorily with R_f values of 0.32 ± 0.01 and 0.52 ±0.01for MONT and T HEO respectively. The linear regression analysis data for the calibration plots showed good linear relationship with R^2 =0.9999 and 0.9999 for MONT and THEO respectively at the concentration range of 100-500ng/spot for MONT and 4000 to 8000ng/ spot for THEO. The method was validated for accuracy, precision, specificity and robustness. The limit of detection and quantitation were 131.01 and 597.82 ng/spot and 399.54 and 181.15ng/spot for MONT and THEO respectively. The proposed developed HPTLC method can be applied for identification and quantitative determination of MONT and THEO in bulk and drug formulation.

Keywords: Montelucast, Theophylline, HPTLC, Validation.

INTRODUCTION

Montelukast is a leukotriene receptor antagonist (LTRA) used for the treatment of asthma and to relieve symptoms of seasonal allergies. It is usually administered orally. Montelukast is a $CysLT_1$ antagonist and it blocks the action of Leukotriene D_4 (and secondary ligands LTC_4 and LTE_4) on the cysteinyl leukotriene receptor $CysLT_1$ in the lungs and bronchial tubes by binding to it. This reduces the bronchoconstriction otherwise caused by the leukotriene and inflammation reduces swelling that narrows airways. Montelukast also relaxes bronchial tube walls. It is also used to treat symptoms of hay fever and allergic rhinitis. Montelukast is chemically[R–(E)]–1–[[[1-[3-[2-(7-chloro-2-quindinyl)ethenyl]phenyl]-3-[2-(1-hydroxy-1– methyl ethyl)phenyl]propyl]thio]methyl] cyclopropane acetic acid (Fig. 1).Theophylline (THEO) has maintained an important role as a potent bronchodilator and used to treat asthma . Chemical name is1,3-dimethyl-2,6-dione as shown in (Fig. 2). THEO is determined alone or in combination with other drugs by HPTLC. The chemical structures of montelukast and theophylline are shown in the Figure 1 and 2.



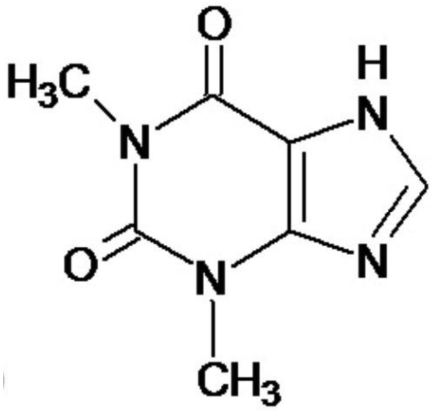


Figure 1. Structural formula of Montelucast **Figure 2.Structural formula of Theophylline**

The review of literature revealed that several methods are available for the determination of montelukast and theophylline individually. Reported method for estimation of montelukast in dosage form are spectrophotometry[6], spectroflourometry[7],RP HPLC[8-9],voltammetry[10]. And similarly for estimation of theophylline in dosage form are spectrophotometry [11-14],RP-HPLC[15-20],HPTLC[21].The present research work aims to develop a simple, sensitive, accurate and reproducible method for simultaneous estimation of montelukast and theophylline in combined dosage form by HPTLC method.

EXPERIMENTAL SECTION

Working standards of pharmaceutical grade MONT(99.8% w/w) and THEO(99.9% w/w) were obtained as gift samples from panacea biotech. All the chemicals and reagents of analytical grade were purchased from Merck chemicals, Mumbai India. Formulation ZOMONT – THEO tablet (10 mg of Montelukast and 200 mg of Theophylline) was procured from local market. The HPTLC instrument used was CAMAG TLC scanner – 3. All the apparatus and instruments used were calibrated and validated.

Selection of analytical wavelength

Stock solutions of drugs were prepared in methanol separately; UV spectrum of 100 µg /ml of individual drug was taken. Further, insitu HPTLC spectral overlain of MONT AND THEO was taken.

Instrumentation and chromatographic conditions

The HPTLC plates were prewashed with methanol and activated at 110º 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 gelprewated HPTLC aluminium plate 60 F 254, [(20 X 10 cm) with 250 µm thickness, E.Merck, Darmstadt, Germany, supplied by Anchrom Technologies, Mumbai] using a Camaglionmat 5 applicator (Switzerland). A constant application rate of 0.1 µl / sec was used and the space between two bands was 7mm. Linear ascending development was carried out in 20 cm X 10 cm twin trough glass chamber (camag, Muttenz, Switzerland) saturated with the mobile phase. The mobile phase consisted of Ethylacetate: Chloroform: Ethanol: Ammonia (6:4:3:1/v/v/v) and 20 mL was used per chromatography run. The optimized chamber saturation time with mobile phase was 30 min using saturation pads at room temperature (25ºC ± 2). The lengths of chromatogram run was 80mm and runtime was 10 min. Densitometric scanning was performed using a Camag TLC scanner 111 in the reflectance absorbance mode and operated by win CATS software (V1.1.4 camag). The slit dimension was kept at 5mm X 0.45mm and the scanning speed was 20 mm / sec. The source of radiation used was a deuterium lamp emitting continuous UV spectrum between 200 and 400 nm. All determinations were performed at ambient temperature with a detection wave length of 254 nm.

Preparation of calibration curve

Mixed stock standard solutions were prepared by dissolving 2 mg of montelukast and 40 mg THEOPHYLLINE in methanol and final volume was adjusted with same solvent in 10 ml volumetric flask to get strength of 200mg/ml of MONT and 40000 mg/ml of THEO. Each concentration were spotted five times on the HPTLC plate. The plate was

then developed using mobile phase as described above. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.Linear calibration curves were generated and shown in Figure 3 and 4.

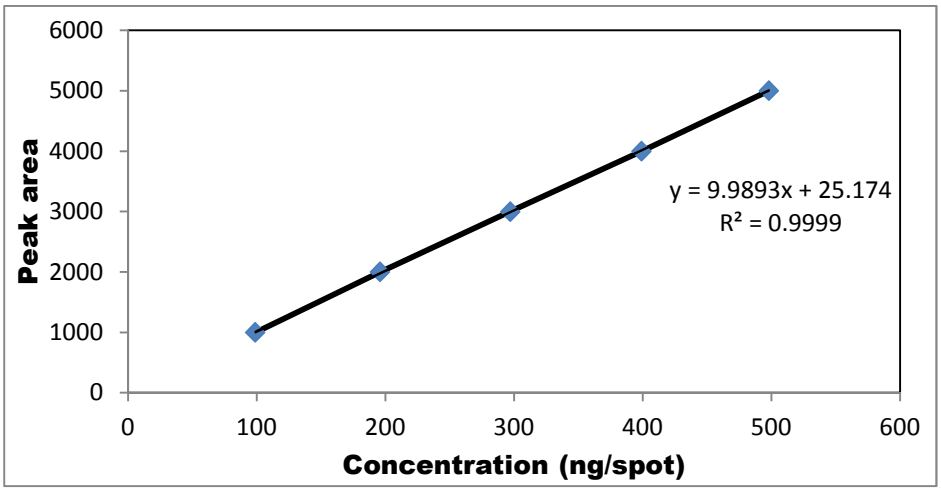


Figure 3 Calibration curve of Montelucast

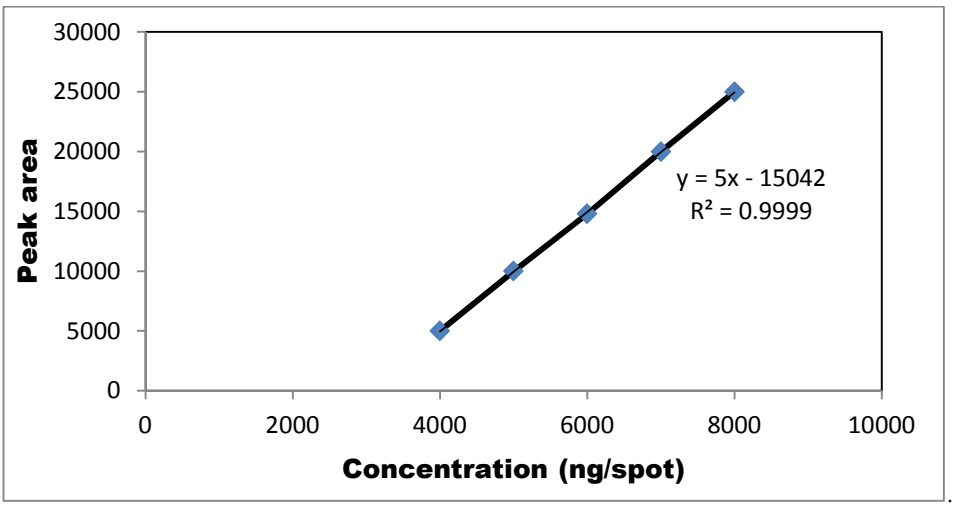


Figure 4 Calibration curve of Theophylline

Sample Preparation

Twenty tablets were weighed and finely powdered. The powder equivalent to 200mg(THEO) of tablet formulation were accurately weighed and transferred to volumetric flask of 50 ml capacity. 15 ml of methanol is 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 40000 rpm for 15 min. It was filtered through vacuum filter using whatman filter paper(no.41). The aliquot (1.0ml) was transferred into 10 ml volumetric flask and volume was made up to the mark with methanol to give a solution containing 100 µg/ml of MONT and 4000 µg/ml of THEO. The plate was developed in the previously described chromatographic conditions. The peak area of the spots were measured at 254 nm for MONT and THEO respectively and the concentrations in the samples were determined using multilevel calibration developed on the same plate under the same condition using linear regression equation.

Validation parameter of the developed methods [4]

The method was validated in accordance with ICH guidelines. The parameters assessed were linearity, Accuracy, Limit of Detection (LOD), Limit of Quantification (LOQ), Precision, Reproducibility and Robustness.

Linearity

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyze in sample within a given range. The range of analytical method is the interval between the upper and lower levels of analyze that have been demonstrated to be determined within a suitable level of precision, accuracy and linearity. Standard mixture solution of MONT and THEO having concentration of 100 to 500 ng/spot of MONT and 4000 to 8000ng / spot of THEO were spotted and developed as described in proposed method. Developed plates were subjected to densitometric measurement in absorbance mode at wavelength 254 nm using Camag TLC scanner 39 (Figure5).

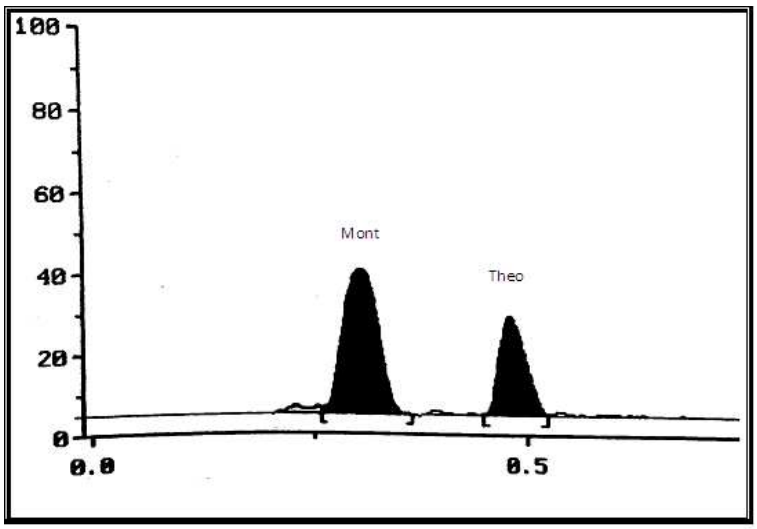


Figure5. Typical chromatogram of MONT and THEO in Pharmaceutical dosage form

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.

Intra and Interday 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 THEO in for five days and results were shown in Table 2

Accuracy

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

Tablet Formulation Solution

Weigh accurately 200mg equivalent weight of tablet powder and dissolved in 50ml of methanol to get concentrations of 10 mg/ml of MONT and 400 mg/ml of THEO and this solution applies on TLC plate as a 100ng/SPOT and 4000ng/SPOT, results were obtained as shown in Table 1.

Table 1. Analysis of marketed formulations

Tablet	Concentration of formulation (ng/spot)	Concentration found (ng spot)		% Mean Recovery	
ZOMONT -THEO	MONT :THEO	MONT	THEO	MONT	THEO
	10 + 200	100.13±0.9160	1998.20±0.7241	101.33	99.91

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 MONT and THEO was assessed by comparing their respective spectrum at peak starts(S), peak apex(M) and peak end (E) position of the spots. The peak purity was determined on win CATS software using statistical equation.

Selectivity

Selectivity is the procedure to detect qualitatively the analyte in presence of components that may expect 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 Table 2.

Table 2. Specificity and Selectivity Study

Parameter	MONT	THEO
Specificity	99.06%	99.32%
Peak Purity	r(s,m)=0.9985 r(m,E) = 0.9999	r(s,m)=0.9997 r(m,E) = 0.9998
Selectivity	Selective	Selective

Limit of Detection(LOD)

The LOD was estimated from the set of 5 calibration curves

LOD = 3.3 X (SD / Slope)

Where,

SD = Standard deviation of the Y – intercepts of the 5 calibration curves .

Slope = Mean slope of the 5 calibration curves.

LOD of MONT and THEO were described in Table 3.

Limit of Quantification(LOQ)

The LOQ was estimated from the set of 5 calibration curves

LOQ = 10 X (SD / Slope)

Where,

SD = Standard deviation of the Y – intercepts of the 5 calibration curves .

Slope = Mean slope of the 5 calibration curves.

LOQ of MONT and THEO were described inTable3.

Table 3. Summary of validation parameters for the proposed method

PARAMETERS	RESULT	
	MONT	THEO
Linearity range (ng / spot)	100-500	2000-10000
Accuracy % Recovery ±SD	101.3351 ± 0.4544	100.0015 ± 0.2340
Precision (% RSD) Inter-day (n = 3) Intra – day (n= 3)	0.3124 – 1.101 0.2347 – 0.3560	0.7345 – 1.1364 0.2451 – 0.5641
Limit of Detection (ng/spot)	131.04	5978.2
Limit of Quantification (ng/spot)	0.399	1811.57
Robustness(% RSD)	0.7042	0.8206

Robustness

The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions. The effect on the results was examined. The amount of mobile phase was varied over the range of ± 5%. The time from spotting to chromatography and from chromatography to scanning was varied by + 10 min. The robustness of the method was determined at three different concentration levels of 100,200,300 ng / Spot for MONT and 2000,4000, 6000 ng/spot for THEO respectively.

RESULTS AND DISCUSSION

A new HPTLC method has been developed for simultaneous estimation of montelukst and theophylline in tablet formulation. It was shown that method was found to be linear, accurate, precise, reproducible and robust proving reliability of method. One can easily identify and estimate drugs on TLC plate within a shorter period of time and both drugs are linear over concentration range 100-500ng/spot for MONT and 4000-8000ng/spot for THEO which obey's the beer's law having correlation coefficient 0.9999 and 0.9999 for MONT and THEO respectively. The

proposed method was also evaluated by the assay of commercially available tablet containing MONT and THEO. The percentage assay was found to be 101.33% for MONT and 99.91% for THEO.

CONCLUSION

The proposed HPTLC method involving the simultaneous estimation of both drugs in pharmaceutical formulation which provides simple, accurate, fast and reproducible quantitative analysis for simultaneous determination of MONT and THEO in tablets. It can be successfully applied for simultaneous estimation of MONT and THEO in tablet dosage form without prior separation and interference in quality control.

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