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Simultaneous Estimation of Montelukast Sodium and Rupatadine Fumarate in Tablet Formulation by HPTLC Method.

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Abstract

A new, rapid, accurate, precise High-performance thin layer chromatographic (HPTLC) method was developed for the simultaneous estimation of Montelukast sodium and Rupatidine fumarate in pharmaceutical dosage forms. Estimation was performed on TLC aluminum plates precoated with silica gel 60F-254 as stationary phase. Linear ascending development was carried out in twin trough glass chamber saturated with mobile phase consisting of Toluene: Ethyl acetate: methanol (5:3: 2v/v) at room temperature (25 ± 2 0C). After development of the plate, Camag TLC scanner 4 (scanning speed 20mm sec-1 and data resolution 100µm/step) was used for spectrodensitometric scanning with win CATS software (slit-micro, 6 x 0.30 mm). Analysis of the plate in absorbance mode at 280 nm was carried out. The system was found to give compact spots for Montelukast sodium and Rupatidine fumarate with Rf. (Retardation factor) value of 0.61± 0.02 and 0.45± 0.03 respectively. The data for calibration plots showed good linear relationship with correlation coefficient of 0.99875 and 0.99796 in the concentration range of 0.2-1.4 and 1.4–9.8 µg/spot for Montelukast sodium and Rupatidine fumarate respectively. The present method was validated according to the ICH guidelines.

Key Words

Montilukast Sodium, Rupatadine fumarate, HPTLC, Method validation.

Introduction

Rupanex M tablets (Dr. Reddy's Laboratories Ltd), which contain Montelukast Sodium and Rupatidine Fumarate, are one of the most commonly used formulations for treatment of asthmatic condition when one medicine (monotherapy) is not sufficiently effective. Montelukast, sodium (2-[1-[[(1*R*)-1-[3-[2-(7 chloroquinolin-2-yl) ethenyl] phenyl]-3-[2- (2 hydroxypropan-2-yl) phenyl] propyl] sulfanylmethyl] cyclopropyl] acetic acid; Fig. 1) is a. Montelukast sodium is Anti-Asthmatic Agents, Antiarrhythmic Agents, Leukotriene Antagonists. 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

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muscle contraction, and enhanced secretion of thick, viscous mucus.

Several analytical methods, including spectrophotometry [1,3] and HPLC [1,4,9,11,12,13] have already been reported for its determination, either alone or in combination with other drugs. Rupatidinefumarate (8-chloro-6, 11-dihydro-11-[1-[(5-methyl3-pyridinyl) methyl]-piperidinylidene]-5H-benzo [5, 6] cyclohepta [1,2b] pyridine, Fig. 2) is an Antihistaminic. The literature contains very few methods for analysis of Rupatidine fumarate; those reported include HPLC detection with sability indicating [10,17]. HPLC and ratio derivative spectrophotometric methods have been used for simultaneous determination of the two compounds [1] Fig. 1 Chemical structure of Montelukast sodium, Fig. 2 Chemical structure of Rupatidine fumarate. In this paper we describe a simple, inexpensive, sensitive, and validated HPTLC method with for simultaneous determination of Montelukast sodium and Rupatidine fumarate in pharmceutical formulations. The method has been successfully used for quality-control analysis of the drugs and for other analytical purposes. To the best of our knowledge, this is the first time report of a quantitative determination method for Montelukast sodium and Rupatidine fumarate in combination from an Tablet formulation using HPTLC. The objective of the present study is to develop a method for quantification of Montelukast sodium and Rupatidine fumarate from a Tablet formulation using HPTLC.

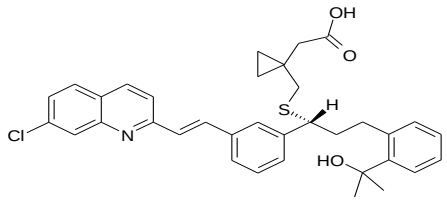

Fig.1: Chemical structure of Montelukast sodium

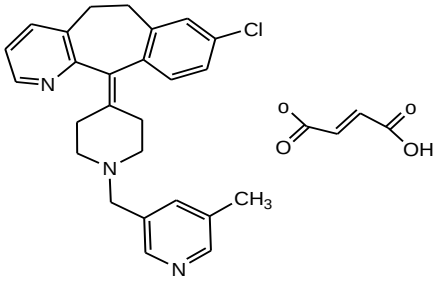

Fig.2: Chemical structure of Rupatidine fumarate

Experimental

Materials and Reagents

Montelukast sodium and Rupatdine fumarate standards were procured from INDOCO REMEDIES LTD. Mumbai, Hetero Health Care Ltd. Mumbai, India, respectively. Silica gel 60 F_{254} TLC plates (20 x 10 cm, layer thickness 0.2 mm, E. Merck, Germany) were used as a stationary phase. All chemicals and reagents were of analytical grade and obtained from Qualigens Fine chemicals. Prepared formulation was used for analysis.

Standard solutions

It was used stock solutions each of Montelukast sodium were 10mg-in 10 ml methanol. Rupatidine fumarate (2 µL) were 10mg-in 5 ml methanol. The binary mixture containing 10and 10 mg of Montelukast sodium and Rupatidine fumarate. Montelukast sodium (0.2 µL) was prepared by transferring 1 ml from respective

stock solutions to a 5mL volumetric flask and make up with methanol.

Chromatographic Condition

Chromatographic separation was performed on Merck TLC plates precoated with silica gel 60 F_{254}. The samples were applied onto the plates as a band with 8 mm width using Camag 100 microlitre sample syringe (Hamilton, Switzerland) with a Camag Linomat 5 applicator (Camag, Switzerland). Linear ascending development was carried out in a twin trough glass chamber (20 x 10 cm) with the mobile phase Toluene: Ethyl acetate: methanol (5:3: 2v/v). Mobile phase was developed by trial and error method. Scanning was performed using Camag TLC scanner 4 at 280 nm and operated by winCATS software (V 1.4.6 Camag). Ultrasonicator was used for extraction of the drugs from the Tablet.

Preparation of Standard and Sample Solution

10 mg of each Montelukast sodium and Rupatidine fumarate were weighed separately and transferred in two different 10 ml volumetric flasks. These drugs were dissolved in 5 ml of methanol solvent by vigorous shaking and then volume was made up to mark with methanol to obtained final concentration of 1 mg/ml of each component. Out of that 1 ml was pipette out and transferred to 10 ml volumetric flask and volume was made up to mark with methanol to get 100 µg/ml solutions. Combination of standards was prepared by taking in 1:1 proportion from both solutions. 276.4 mg Tablet were weighed and transferred into another volumetric flask containing 50 ml of methanol and kept in ultrasonicator for 20 minutes for extraction of drugs from Tablet. From resulting solution 1 ml of solution was withdrawn and transferred in 10 ml volumetric flask and then volume was made up to the mark with methanol to obtained final concentration of 0.2 mg/ml.

Method Validation

Method was validated and carried out as per the ICH guidelines [21]. The parameters checked were precision, reproducibility, limit of detection, limit of quantification and recovery.

Calibration Curves of Montelukast sodium and Rupatidine fumarate

A stock solution of Montelukast sodium and Rupatidine fumarate (0.2 µg/ml& 2µg/ml) was prepared in methanol. Out of that 1 ml was pipette out and transferred to 10 ml volumetric flask and volume was made up to mark with methanol to get 100 µg/ml solution. Different volumes of stock solution 1,2,3,4,5,6,7µl for Montelukast sodium & 0.7,1.4,2.1,2.8,3.5,4.2,4.9 µl for Rupatidine fumarate were spotted in duplicate on TLC plate to obtain concentrations of 0.2,0.4,0.6,0.8,1.0, 1.2 and 1.4 µg & 1.4,2.8,4.2,5.6,7.0,8.4and 9.8 µg per spot of for Montelukast sodium and Rupatidine fumarate. The data of peak area versus drug concentration were treated by linear least-square regression.

Precision

Repeatability of sample application and measurement of peak area were carried out using 6 replicates of the same spot i.e. 1 µg per spot of Montelukast sodium and Rupatidine

fumarate and was expressed in terms of percent relative standard deviation (%R.S.D.) and standard error (S.E.). The intra- and inter-day variation for the determination of Montelukast sodium and Rupatidine fumarate were carried out at concentration levels of 1 µg per spot.

Reproducibility

As per ICH guideline reproducibility of sample application and measurement of peak area were carried out using 6 replicates of the same spot i.e. 1 µg per spot of Montelukast sodium and Rupatidine fumarate.

Limit of Detection and Limit of Quantification

The detection limit (LOD) of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. LOD was calculated using the following formula.

LOD = (3.3 x Standard deviation of the Y-intercept) / slope of calibration curve.

The quantification limit (LOQ) of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. LOQ was calculated using the following formula.

LOQ = (10 x Standard deviation of the Y-intercept) / slope of calibration curve

Recovery Studies

The accuracy of proposed method was evaluated by addition of standard drug solution to pre-analyzed tablet sample solution at three different concentration levels i.e. 80,100, and 120% of linearity of both the drug.

Tablet Analysis

Tablet were weighed accurately and ground to fine powder and dissolved in 50 ml of methanol. The solution was sonicated for 20 min. The extracts were filtered through Whatman filter paper No. 41 and transferred to 10 ml volumetric flask and volume was made up to 10 ml with methanol. Required dilutions were made to get desired concentrations of Montelukast sodium and Rupatidine fumarate.

Results and Discussion

Development of the Optimum Mobile Phase

The TLC procedure was optimized with a view to quantify Montelukast sodium and Rupatidine fumarate in a tablet formulation. The mobile phase Toluene: Ethyl acetate: methanol (5:3: 2 v/v) gave good resolution with Rf = 0.45 ± 0.03 for Rupatidine fumarate and Rf = 0.65 ± 0.02 for Montelukast sodium. Under the chromatographic condition employed, standard compounds Montelukast sodium and Rupatidine fumarate and the formulation have shown sharp peaks and good separation (Figure 1, 2 and 3).

Calibration Curves

The developed HPTLC method for estimation of Rupatidine fumarate showed a good correlation coefficient (r^2 = *0.99796*) in concentration range of 1.4 -9.8 µg per spot with respect to the peak area and for estimation of Montelukast sodium showed a good correlation coefficient (r^2 = *0.99875*) in concentration range of 0.2 -1.4 µg per spot with respect to the peak area (Table 1). The mean value (±S.D.) of slope and intercept were 278 and

569.240 respectively for Montelukast sodium. The mean value (±S.D.) of slope and intercept were 283 and 103.088 respectively for Rupatidine. No significant difference was observed in the slopes of standard curves (ANOVA, P > 0.05).

Method Validation

The measurement of the peak area showed low values of S.E. and % R.S.D. (<1%) for inter- and intra-day variation, which suggested an excellent precision of the method (Table 2). Limits of detection (LOD) and limits of quantitation (LOQ) are described as shown in table 4. The calibration curve for Montelukast sodium and Rupatidine fumarate in this study was plotted between amount of analyte versus peak area and the regression equation was obtained (Y = 569.240+ 3.999 X) and (Y = 103.088 + 617.257X) with a regression coefficient of 0.99875 and 0.99796 respectively (Table 2). The formulation was analyzed and found to contain 4.36µg of montelukast sodium and 0.57µg of Rupatidine in a tablet (Table 3). In recovery studies the analyzed samples were spiked with extra 80, 100, 120% of the standard montelukast sodium and Rupatidine fumarate and the mixtures were reanalyzed by the proposed method. The experiment was conducted in triplicate. This was done to check for the recovery of the drug at different levels in the formulation. The proposed method when used for extraction and subsequent estimation of curcumin and gallic acid from the formulation afforded recovery of 98.8%, 99.19%, 102.6% and 89.69%, 89.70%, 88.2% respectively as listed in (Table 6). The peak purity of montelukast sodium and Rupatidine fumarate was assessed by comparing the spectra at peak start, peak apex and peak end positions of the spot as shown in graph 1, 2 and 3. Good correlation was obtained between the standard and the sample overlain spectra of montelukast sodium (Table 7).

Analysis of the Formulation

Montelukast sodium and rupatudine fumarate from tablet formulation showed single spots at *R*f = 0.61 ± 0.02 and 0.45 ± 0.03 respectively (Figure 3). The % of Montelukast sodium and rupatudine fumarate from tablet was found to be 96.66 and 114% and was well within the limits. By considering Rf values of standard Montelukast sodium and rupatudine fumarate and spots observed of samples, fingerprint analysis, presence of these active chemical marker compounds was detected.

Conclusion

The developed HPTLC technique is a rapid, simple, precise, specific, accurate and robust for the simultaneous determination of titled ingredients. Statistical analysis proves that the method is reproducible and selective for the analysis of title ingredients. Since, the proposed mobile phase effectively resolves titled ingredients and the method can be used for qualitative as well as quantitative analysis of Montelukast sodium and Rupatidine fumarate in commercial formulations as well as in laboratory prepared mixtures.

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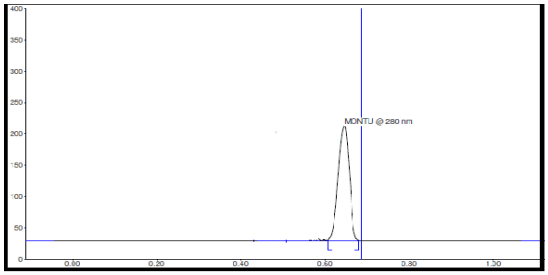

Fig. 1: HPTLC chromatogram of standard Montelukast sodium.

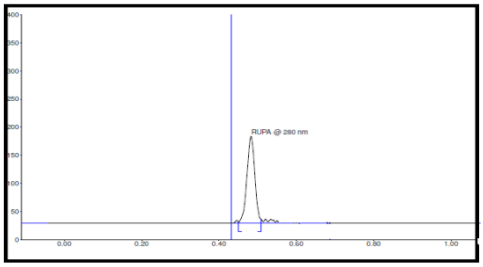

Fig. 2: HPTLC chromatogram of standard Rupatidine fumarate.

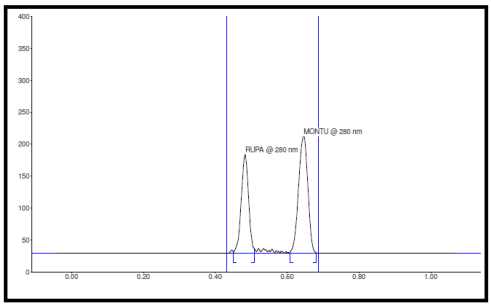

Fig. 3: HPTLC chromatogram of formulation showing peaks ofMontelukast sodium and Rupatidine fumarate.

Table 1: Result for calibration curve.

Sr. No.	**Conc.** µg/ml	**Peak area for Montelukast**	**Sr.No.**	**Conc.** µg/ml	**Peak area for Rupatidine**
1	0.2	235.2	1	1.4	753
2	0.4	1001.3	2	2.8	1729.3
3	0.6	1798.3	3	4.2	2762.7
4	0.8	2707.9	4	5.6	3648.9
5	1.0	3483.8	5	7.0	4452.4
6	1.2	4157.6	6	8.4	5205.3

Table 2: Intra and Inter-day precision of HPTLC method (n = 6).

Component	**Intraday Precision**					**Interday precision**				
	Mean area	% found	S.D	% RSD	S. E	Mean area	% found	S.D	% RSD	S. E
Montelukast sodium	1895.670	97.38	2.78	0.014	1.13	1895.670	98.70	2.78	0.014	1.13
Rupatidine fumarate	2406.882	97.69	2.83	0.001	1.15	2406.882	99.24	2.83	0.001	1.15

Table 3: Precision of HPTLC method.

Sr. No.	Conc. µg/ml	Peak Area MONT	Conc µg/ml	Peak Area RUPA
1	3.0	1897.0	2.1	2508.3
2	3.0	1951.1	2.1	2397.9
3	3.0	1837.0	2.1	2357.3
4	3.0	1910.5	2.1	2404.6
5	3.0	1877.3	2.1	2409.7
6	3.0	1900.9	2.1	2363.6
	Avg.	1895.66	Avg.	2406.8

Table 4: Method validation parameters for the estimation of Montelukast sodium and Rupatidine fumarate by HPTLC.

Parameter	Montelukast sodium	Rupatidine fumarate
Linearity range (µg/spot)	0.2-1.4	1.4-9.8
Slope	569.240	103.088
Intercept	3.999	617.275
Coefficient of correlation	0.99875	0.99796
Limit of Detection (LOD)	0.016 µg/spot	0.09 µg/spot
Limit of Quantitation (LOQ)	0.04 µ/spot	0.27 µg/spot
Reproducibility	97.38%	97.69%

Table 5: Analysis of Tablet by HPTLC.

Drugs	Rf	Amount found	% drug found
Montelukast sodium	0.61	4.36 µg	91.25
Rupatidine fumarate	0.45	0.57 µg	89.34

Table 6: Recovery studies of Montelukast sodium and Rupatidine fumarate.

Level of % Recovery	Amount of standard added (µg)		Total amount recovered (µg)		% Recovery	
	Montelukast	rupatidine	Montelukast	rupatidine	Montelukast	rupatidine
80	1.6	1.6	1.58	1.55	98.7	96.87
100	2	2	1.96	1.98	98.0	99.00
120	2.4	2.4	2.37	2.39	98.75	99.5

Table 7: Statistical validation.

Component	Mean	Standard Deviation	Coefficient of Variation	Standard Error
Montelukast sodium	100.19%	2.78	2.254	1.13
Rupatidine fumarate	89.1%	2.83	1.988	1.15

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