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A validated LC-method for the evaluation of advanced intermediate of montelukast

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ABSTRACT

A liquid chromatography(LC) method has been developed for the determination of montelukast advanced intermediate **(2)** and its precursors. Separation was achieved on Xterra C-18, 150×4.6 mm, 5.0 µm column using with linear gradient program comprising of mobile phase A (0.25% of triethyl amine in water, pH adjusted to 3.0 with trifluro acetic acid) and mobile phase B (acetonitrile). The analysis is carried out with flow rate of 1.0 ml/min and at 240 nm. The method is found to be accurate, linear, precise, rugged and specific. This method can be used for the analysis of montelukast advanced intermediate **(2)**.

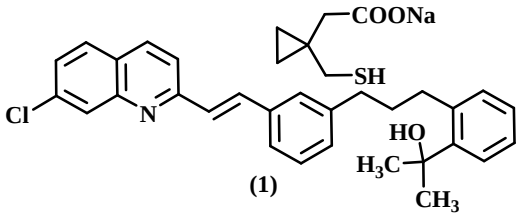
KEYWORDS

HPLC;
Montelukast;
Asthma drug;
Analysis.

INTRODUCTION

Several drugs are currently used in the treatment of asthma, including sodium cromoglycate, sodium nedocromil, theophylline, and oral or inhaled glucorticoids[1]. However, montelukast sodium **(1)** is a new anti-inflammatory drug that interferes directly with leukotriene production(5-lipoxygenase inhibitors)[2]. The leukotrienes constitute a group of locally acting hormones, produced in living systems from arachidonic acid. The major leukotrienes are leukotriene B4 (abbreviated as LTB4), LTC4, LTD4 and LTE4. The biosynthesis of these leukotrienes begins with the action of the enzyme 5-lipoxygenase on arachidonic acid to produce the epoxide known as leukotriene A4 (LTA4), which is converted to the other leukotrienes by subsequent enzymatic steps.

For the preparation of montelukast sodium **(1)**[3], involves condensation of two key intermediates, such as 2-[3(S)-[3-[2-(7-chloro-2-quinolinyl)ethenyl] phenyl]-3-hydroxypropyl]phenyl-2-propanol**(2)**and 1-(merca ptomethyl)cylcopropaneacetic acid **(3)** as shown



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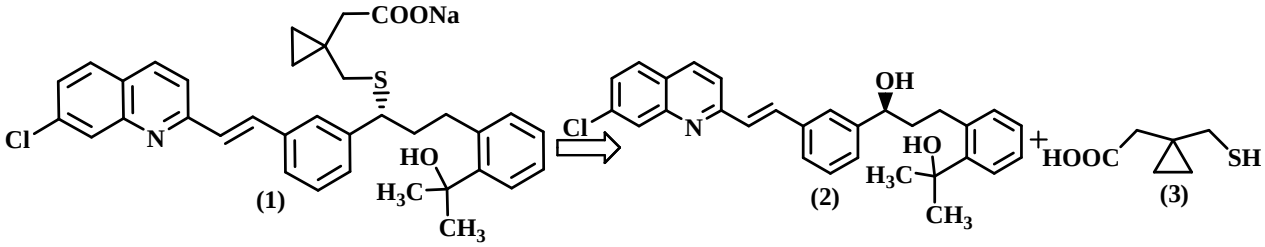

SCHEME 1

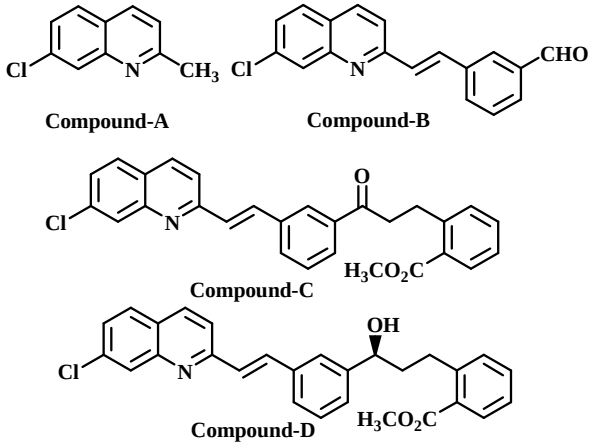

Figure 1

in the SCHEME 1.

In this publication we have developed a LC-method for the analysis of different stages in montelukast advanced intermediate **(2)** and its precursors are shown in figure 1.

EXPERIMENTAL

Material

Samples of Montelukast intermediate **(2)** and its precursors were prepared in Research and Development, Inogent laboratories (A GVK Bio Company), Hyderabad, India. Acetonitrile (HPLC grade), trifluro acetic acid, triethyl amine and methanol from Merck, Germany. High purity water was prepared by using Millipore USA.

Instrumentation

The LC system consisted of Waters 2695 separation module, connected with Waters 2996 photo diode array detector. The out put signal was monitored and integrated with Empower software.

Solutions

Mobile phase

Mobile phase A: Triethyl amine(0.25%) in water (pH adjusted to 3.0 with trifluro acetic acid), it was filtered with 0.45µm nylon membrane filter prior to use. **Mobile phase B:** Acetonitrile was filtered with 0.45µm nylon membrane filter prior to use. **Diluent:** Methanol.

Standard solutions

Solutions of montelukast intermediate **(2)** and its precursors were prepared using with diluent. The working concentration for the determination of related compounds of montelukast intermediate was 0.5 mg/ml.

4. Chromatographic conditions

The chromatographic separation was achieved on Xterra C-18, 150×4.6mm, 5.04m column using with binary gradient mixture of mobile phase A, mobile phase B. The mixture of mobile phase flow is 1.0 ml/min. linear gradient program was mentioned in TABLE 1. The column temperature was maintained at 40^0C and the wavelength was monitored at 240 nm. The injection volume was 10 µL.

Validation of method

1. Specificity

Specificity of the method to measure the analyte response in the presence of its precursors. The specificity of the developed LC method for montelukast intermediate **(2)** was carried out in the presence of its precursors namely compound-A, compound-B, compound-C and compound-D at 0.3 % with respect to 0.5 mg /ml.

2. Precision

The precision of the method was checked by injecting six individual preparations of montelukast intermediate **(2)**(0.5mg/ml) spiked with 0.15% compound-A, compound-B, compound-C and compound-D with respect to montelukast intermediate **(2)** concentration.

% RSD of area for all precursors were calculated.

3. Limit of detection (LOD) and limit of quantification

The LOD and LOQ for compound-A, compound-B, compound-C and compound-D were determined by calibration curve method, by injecting a series of dilute solutions 0.01%, 0.05%, 0.1%, 0.15%, 0.2%, 0.25% and 0.3%.

4. Linearity

Linearity of the test solutions for the related substance method was prepared by diluting stock solutions to the required concentrations. The solutions were prepared at six concentration levels from 0.01% to 0.3% (0.01%, 0.05%, 0.1%, 0.15%, 0.2%, 0.25% and 0.3%). Slope Y-intercept and correlation coefficient was calculated for each compound.

5. Accuracy

The accuracy study of all compounds were carried out in triplicate at 0.1%, 0.15%, 0.2%, 0.25% and 0.3% of the montelukast intermediate **(2)** concentration. The percentages of recoveries for compounds A-D were calculated.

6. Robustness

To determine the robustness of the developed method, experimental, theoretical plates and the resolution between compounds A-D and montelukast intermediate were recorded. The flow rate of the mobile phase was 1.0 ml/min. To study of the system suitability parameters flow was changed by 0.2 units form 0.8 ml/min to 1.2 ml/min. The effect of the column temperature on system suitability studied at 35^0C, 45^0C instead of 40^0C the effect of pH on system suitability was studied at pH 2.8 to 3.2 instead of pH 3.0 of mobile phase A.

7. Solution stability and mobile phase stability

The solution stability of Montelukast intermediate **(2)**, sample was determined at 0 hrs, 6 hrs and 12 hrs. The mobile phase stability was established at 0 hrs, 6 hrs and 12 hrs with fresh solutions.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

TABLE 1

Time	Mobile phase–A (%)	Mobile phase–B (%)	Linearity curve
0	70	30	6
0 → 10	40	60	6
10 → 20	10	90	6
20→ 25	10	90	6
25 → 27	70	30	6
27 → 30	70	30	6

TABLE 2

	Comp-A	Comp-B	Comp-C	Comp-D
Average*	26622.50	24140.00	22015.33	31509.00
Stdev	171.97	241.16	18.84	291.48
%RSD	0.65	1.00	0.09	0.93

*n= 6

TABLE 3

Linearity parameter	Comp-A	Comp-B	Comp-C	Comp-D
Correlation coefficient	0.9996	0.9995	0.9976	0.9969
Y-Intercept	-0.00701	-0.00348	-0.020168	-0.0117

The main objective of the method is to separate montelukast intermediate and its precursors, comp-A, comp-B, comp-C and comp-D were co-eluted with different mobile phase and in isocratic conditions. The separations were achieved in mentioned in gradient conditions on Xterra C-18, 150×4.6, 5.0 microns. The mobile phase consisting mobile phase-A and mobile phase -B. The flow rate of mixture of mobile phase is 1.0 ml/min and column temperature is 40°C. Gradient conditions were mentioned in TABLE 1. All compounds resolved with symmetrical peak shapes with good resolution. The retention times are 13.62, 18.89, 14.54 and 15.79 min and Montelukast intermediate 2 retention time is 3.84min. (Figure 2)

Precision

The %RSD for the area of comp-A, comp-B, comp-C and comp-D were within 1.0 %, confirming good precision of the method.

Limit of detection and limit of quantification

The limit of detection of all the compounds namely comp-A, comp-B, comp-C and comp-D is 0.011%, 0.013%, 0.026% and 0.03% with respect to montelukast intermediate **(2)** test concentration (0.5 mg/ml). The limit of quantification of all the compounds namely comp-A, comp-B, comp-C and comp-D are 0.034%, 0.038%, 0.08% and 0.092% with respect to Montelukast intermediate **(2)** test concentration (0.5 mg/ml)

Linearity

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TABLE 4

	Comp-A		Comp-B		Comp-C		Comp-D	
Spiked level	Recovery	Recovery in terms of percentage	Recovery	Recovery in terms of percentage	Recovery	Recovery in terms of percentage	Recovery	Recovery in terms of percentage
0.10%	0.105%	105.00%	0.102%	101.80%	0.102%	101.70%	0.101%	100.90%
0.15%	0.146%	97.40%	0.148%	98.50%	0.150%	100.20%	0.153%	102.20%
0.20%	0.204%	102.00%	0.201%	100.40%	0.208%	103.90%	0.203%	101.50%
0.25%	0.250%	99.90%	0.257%	102.90%	0.252%	101.00%	0.251%	100.60%
0.30%	0.298%	99.30%	0.315%	104.90%	0.300%	102.00%	0.278%	92.80%

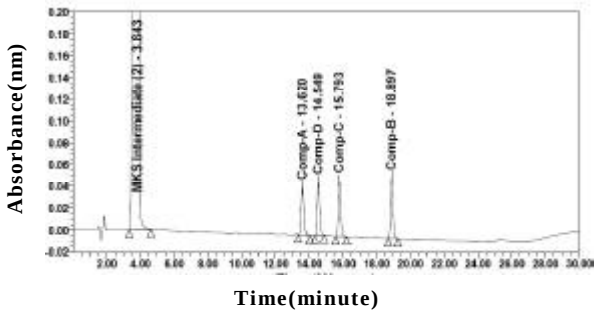

Figure 2: LC chromatogram of montelukast intermediate (2) and its precursors

The linearity calibration plot for the method was obtained over the calibration ranges tested range from 0.01% to 0.3% for comp-A, comp-B, comp-C and comp-D. The correlation coefficient obtained was grater than 0.997, the obtained results were given in TABLE 3.

The results shown that a good correlation existed between the peak area and the concentrations of comp-A, comp-B, comp-C and comp-D.

Accuracy

The percentage of all compounds carried at 0.1%, 0.15%, 0.2%, 0.25% and 0.3 levels for comp-A, comp-B, comp-C and comp-D. The percentage recovery of all compounds varied from 92.8 to 105%, the results are found satisfactory (TABLE 4).

Robustness

In all the deliberative varied chromatographic conditions (Flow rate, Column temperature and pH of Mobile phase-A) the observed resolution between of all peaks were grater than 4.0 with montelukast intermediate **(2)**.

Solution stability and mobile phase stability

The solution stability of montelukast intermediate **(2)** was established up to 12 hrs and found it sample solution is stable. The mobile phase stability was established up to 12 hrs and found to be stable.

CONCLUSION

The LC method developed for precursors of montelukast intermediate **(2)**, is precise, accurate, rugged and specific. The method was showing satisfactory data for all the method validation parameters tested. The developed method can be conveniently used by quality control department to determine the related compounds in montelukast intermediate **(2)** samples.

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