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Der Pharmacia Lettre, 2016, 8 (1):320-330
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Bioanalytical method validation of montelukast salt in human plasma using LC-MS/MS method

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ABSTARACT

Sodium salt of Montelukast is the leukotriene receptor antagonist used for the treatment of asthma and allergies. LC-MS/MS method was developed to determine Montelukast sodium in human plasma. The quality control samples were prepared between 100 and 12000 ng/mL. Plasma concentration samples were prepared in between 2.5 ng/mL and 600 ng/mL. The method was found to be linear in the range of 2.5 ng/mL and 600 ng/mL.The concentration vs. ratio of Montelukast sodium/ IS has y = 0.004x + 0.135 with R^2= 0.991.The procedure followed in this method has the least concentration i.e. LLOQ is very low than previously reported methods and is suitable for estimation of Montelukast sodium in human plasma.

Keywords: Montelukast sodium, asthma, LC MS/MS method, human plasma, analytical method

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INTRODUCTION

Montelukast is one of the leukotriene receptor antagonists [1] used for the treatment of major respiratory diseases like asthma and associated allergies [2]. It is also indicated for running nose and exercise induced bronchoconstriction, prophylactic agent for asthma and allergic rhinitis [3]. Montelukast is available in chewable tablets and can be administered to patients with one year of age and older [4]. Montelukast is also indicated for allergic rhinitis in the children of six months old.

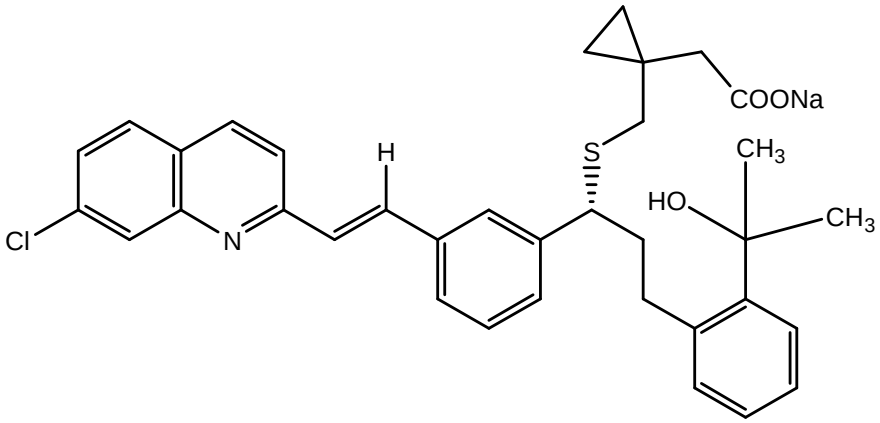

Figure 1. Montelukast Sodium

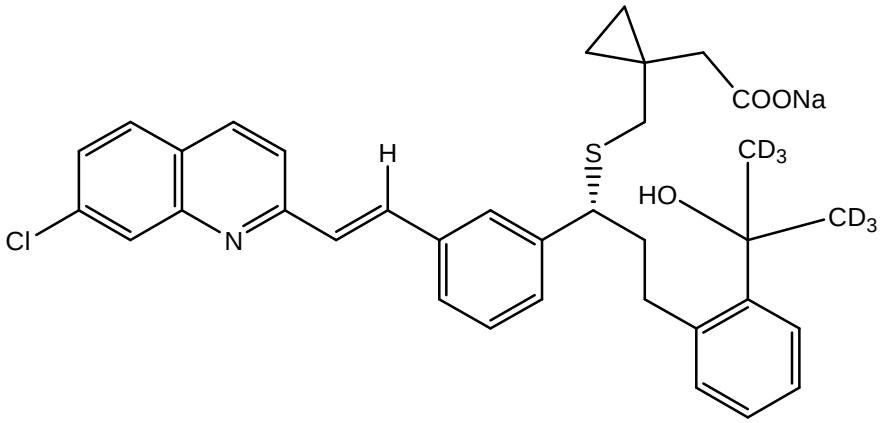

Figure 2. Montelukast Sodium D6

The molecular weight of Montelukast Sodium (2-[1-[[(1*R*)-1-[3-[2-(7-chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(2-hydroxypropan-2-yl) phenyl] propyl] sulfanyl methyl] cyclopropyl] acetic acid) is 606.192.

The products of arachidonic acid metabolism are various cysteinyl leukotrienes LTE4, LTC4 and LTD4. These are released from eosinophils and inflammatory cells. These chemical components attach to cysteinyl leukotriene (CysLT) receptors. The activation of LT receptors may lead to changes in the physiological properties of airways. Mostly intense nasal airway resistance and nasal obstruction are chief problems associated with activation of LT receptors.Montelukast is administered orally and has selective affinity for cysteinyl leukotriene I receptor and blocks the activities of LTD4 without any agonist action at the target region [5]. Montelukast oral bioavailability is 64% and reaches peak in plasma within 3 hrs. Montelukast has absolute protein binding capabilities and has volume of distribution on an average of 9.5 liters. Montelukast sodium undergoes metabolism by CYP450 3A4 [6] and 2C9 and at the steady state Montelukast is undetectable and has half-life of 2.7 to 5.5 hr [7]. The frequent side effects that can be observed are gastric upset, fever, sluggishness, lethargy and heartburn. Montelukast is affected with potential drug interactions and the AUC becomes 40% if co-administered with Phenobarbital due to hepatic metabolism.

Various analytical methods have been published related to validation of Montelukast using human plasma. Vaidya et al has reported the HPLC method using C8 column with acetonitrile: pH 3.0 ammonium acetate (35:45) [8]. Sripalakit et al reported estimation of Montelukast in human plasma using LC-MS/MS with C18 column having a 0.1M ammonium formate (pH 4.0): acetonitrile (20:80) [9]. Radhakrishnanand et al has reported validated analytical method of Montelukast sodium using HPLC method with 0.02M potassium dihydrogen phosphate: methanol (40:60) [10]. Laha et al reported estimation of Montelukast in human plasma using LC-MS/MS with monolithic column [11]. Chauhan et al has reported liquid-liquid extraction method for estimation of Montelukast using HPLC with trifluoroacetic acid: acetonitrile (45:55) [12]. Patra has reported analysis of Montelukast sodium in bulk and solid dosage forms using HPLC with acetonitrile : ethyl alcohol : water (75:25:5)[13]. Alsarra has reported Montelukast in tablets and human plasma using HPLC [14].

The main objective of the proposed study is to develop and validate suitable, sensitive and selective analytical method for the estimation of Montelukast essential for bioavailability and bioequivalence studies.

MATERIALS AND METHODS

2.1. Optimization of analytical method

Shimadzu LC Prominence with Agilent mass spectrometer having electrospray ionization. The parameters of the instrument were set to get better results with respect to sensitivity, selectivity to drug and ensuring complete extraction of analyte. The composition of ammonium acetate buffer-acetonitrile was used to prepare 200 ng/mL Montelukast determined in negative and positive ionization mode and revealed that positive ionization has shown better response. The transition was monitored 587.1 > 421.18 m/z, 592.40 > 427.6 m/z for Montelukast as well as Montelukast D6 (internal standard) respectively. Full scan mass spectra for Montelukastand internal standard were shown in Figure 2.

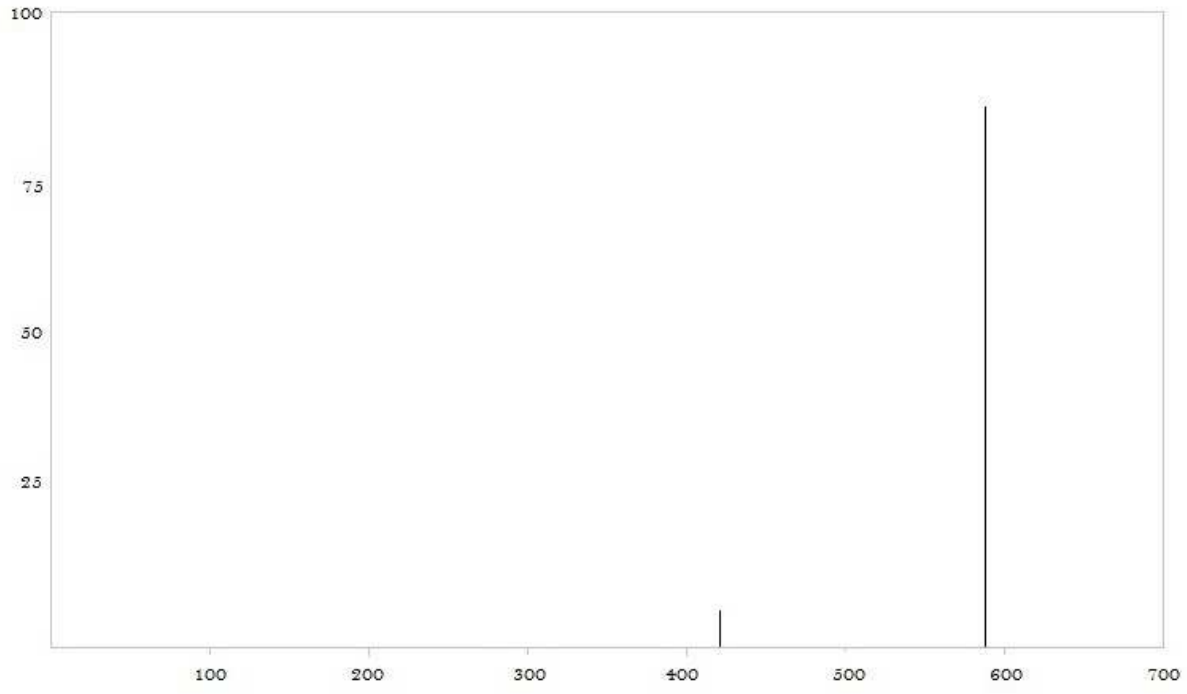

Figure 3. Mass spectrum of Montelukast sodium (587.1 > 421.18 m/z)

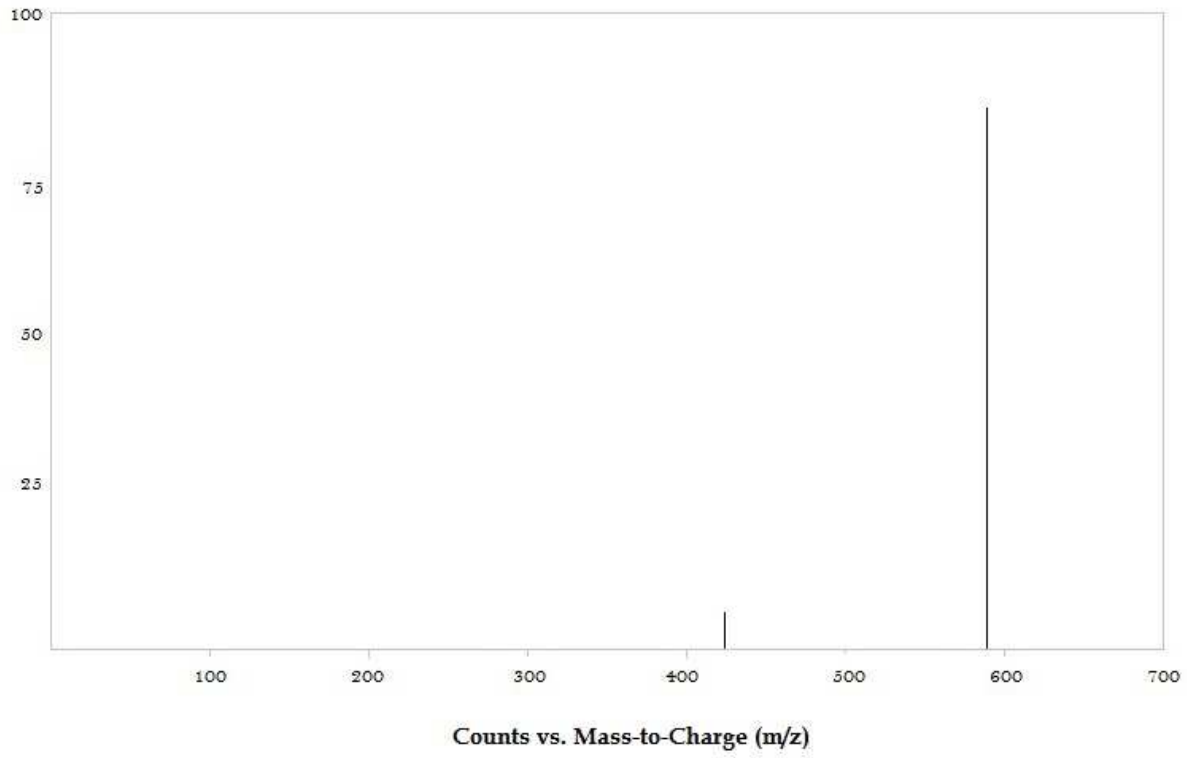

Figure 4. Mass spectrum of Montelukast sodium D6 (592.40 > 427.6 m/z)

HPLC grade acetonitrile and 0.1 M ammonium acetate (65:35) mobile phase and ODScolumn (5 µm, 250 × 4.6 mm) was selected for the study. Montelukast D6 was chosen as internal standard and the drug was extracted and injected. Few parameters were kept constant such as injection volume and flow rate etc. Solid phase precipitation technique was selected to achieve better recovery and sensitivity of Montelukast and Montelukast D6 internal standard.

2.2. Sample preparation and extraction procedure

About 225 µL of plasma with 25 µL of internal standard Montelukast were mixed in a 2 mL tube. The sample was subjected to vortex for 1 min. Furthermore, 250 µL of 1% formic acid was added and subjected to vortex for 1 min. Then the sample is injected in Orpheus sy C18, 3ml cartridges

The above sample was added with 1 mL of methanol and further 1% formic acid. Washing was performed with 1mL of 0.1M ammonium acetate and with 1 mL of distilled water. Finally eluted with 1 mL of mobile phase and filtered and further the sample was injected.

2.3. Internal Standard

To determine the reproducibility of the method,Montelukast D6 was preferred as internal standard owing to its positive ionization and good recovery profiles.

Table 1. MS Data of selected drugs

Sample	Parent Ion (m/z)	Product Ion (m/z)
Montelukast	421.18	587.1
Montelukast D6	427.6	592.4

The retention time of Montelukastand D6 was found to be 2.3 ± 0.3min with run time 5 min.

2.4. Preparation of standard stock solutions

The stock solution of Montelukast and Montelukast D6 was prepared at a concentration of 1 mg/mL and using methanol diluted subsequently to prepare calibration concentrations and quality control samples.A standard stock solution is prepared by dissolving 10 mg of drug in 100 mL of Acetonitrile and water 65: 35 % v/v.

2.5. Plasma sample preparation

The expired human blood was purchased from local blood bank and subjected to EDTA processing and further plasma was collected. The sample of plasma 500 µL and calibration concentration 500 µL were transferred to 2 mL tube and stored at –20 °C.

2.6. Mobile phase

Acetonitrile: ammonium acetate buffer 75:25, v/v was prepared and 1% formic acid was prepared and stored at –20 °C. Acetonitrile and water 65: 35 % v/v was used as solvent for dilution.

2.7. Ammonium acetate buffer, 0.1 M

The buffer was prepared by dissolving 0.77 g of ammonium acetate in 1000 mL of triple distilled water.

2.8. Calibration concentrations

Various concentrations of Montelukast beginning from 5 ng/mL to 600 ng/mL were prepared. Furthermore, samples of higher concentrations were prepared. Montelukast calibration concentrations were prepared beginning from 100 ng/mL to 12000 ng/mL using standard stock solution.

2.9. Quality Control Samples

The quality control concentrations (n = 10) as follows. 100 ng/mL as LLOQ, 1000 ng/mL as LQC, 5000 ng/mL as MQC and 10000 ng/mL as HQC.

Table 2. Preparation of various quality control samples

Respective Stock Aliquot (mL)	Diluent Added (mL)	Total Volume (mL)	Final Stock Concentration (ng/mL)
0.010	9.99	10	100
0.050	9.95	10	500
0.100	9.9	10	1000
0.250	9.75	10	2500
0.500	9.5	10	5000
0.750	9.25	10	7500
1.000	9	10	10000
1.200	8.8	10	12000

2.10. Spiking Plasma

0.250 mL of above Aqueous CC dilutions ranging from 100 to 12000 ng/mL and made up the volume to 5 mL with plasma to achieve the concentration described in the table below.

Table 3. Spiking Plasma for calibration concentrations

Stock Concentration (ng/mL)	Stock Aliquot (ng/mL)	Volume of Matrix (ng/mL)	Total Volume of Sample Matrix (ng/mL)	Final Concentration (ng/mL)
50	0.250	4.75	5	2.5
100	0.250	4.75	5	5
500	0.250	4.75	5	25
1000	0.250	4.75	5	50
2500	0.250	4.75	5	125
5000	0.250	4.75	5	250
7500	0.250	4.75	5	375
10000	0.250	4.75	5	500
12000	0.250	4.75	5	600

2.11. Spiking of Plasma for QC Samples

The stock solution ranging from 100ng/mL to 12000 ng/mL diluted to 20 mL with plasma as described in the table.

Table 4. Spiking of Plasma for QC Samples

Stock Concentration (ng/mL)	Stock Aliquot (mL)	Volume of matrix (mL)	Total Volume (mL)	Final Concentration (ng/mL)
100	1	19	20	5
1000	1	19	20	50
5000	1	19	20	250
10000	1	19	20	500

3. Method validation

The following method validation parameters were evaluated as per United States – Food and Drug Administration guidelines on bioanalytical method validation. The parameters chosen were selectivity, sensitivity, linearity, precision &accuracy, recovery and stability.

3.1. Selectivity

Montelukast and internal standard was subjected to analysis and evaluated for any interference from the both samples. We have identified that there were no such interfering components at molecular weights. The chromatograms are shown in figure 3 and 4. Furthermore, there was no interference of retention times of analyte with internal standard or with blank plasma.

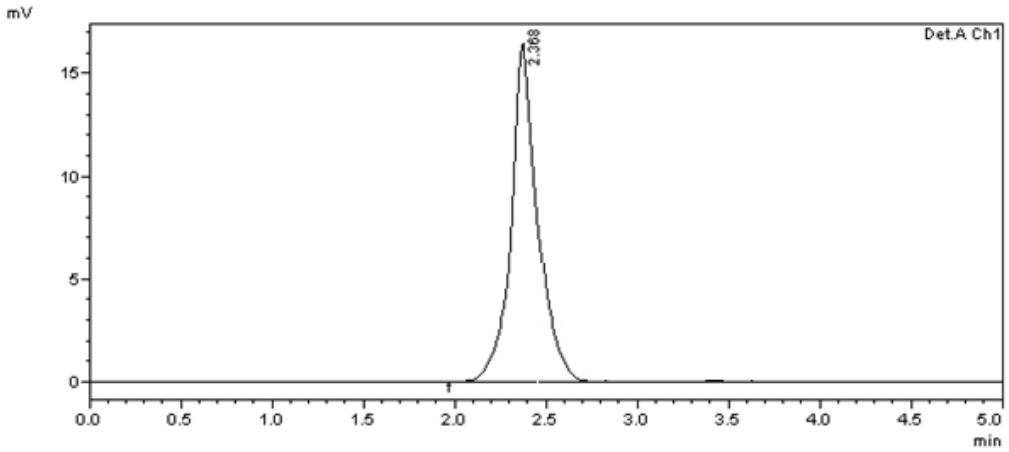

Figure 3. Chromatogram of Montelukast sodium

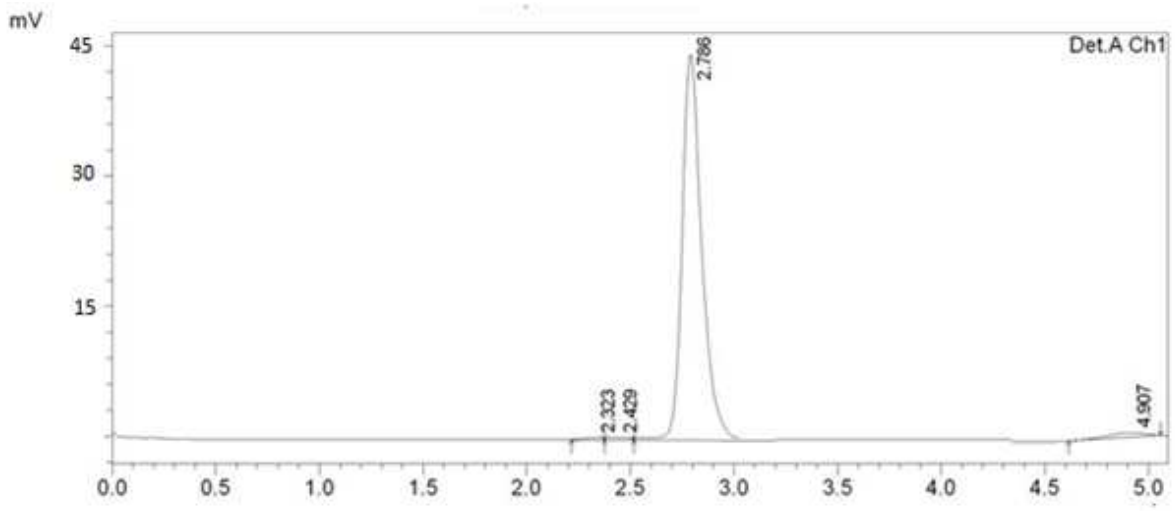

Figure 4. Chromatogram of Montelukast sodium D6

Table 5. The chromatogram result of LLOQ, Blank with IS of MS and MS D6

Conc. Parameter	Montelukast Sodium	Montelukast Sodium D6 (IS)
Area of LLOQ	11292.16 ± 335.12	678717.83 ± 21475.62
Relative Std. Dev.	2.97	3.16
Blank + IS		
Area	0	689450.31 ± 21811.16
Relative Std. Dev.	0	3.16

3.2. Sensitivity

The LLOQ of Montelukast in human plasma was 2.5 ng/mL. The precision was found to be 4.27 % and accuracy was found to be 98.43 %.

Table 6. The chromatogram result of sensitivity

Calibration Concentration (ng/mL)	2.5	5	25	50	125	250	375	500	600
Conc. Found (ng/mL)	2.46	5.06	25.38	50.56	129.27	253.47	375.78	508.15	599.52
% Estimated Value of CC	98.43	101.23	101.54	101.13	103.42	101.39	100.21	101.63	99.92

3.3. Linearity

The linear regression equation was performed with concentration 2.5 ng/mL to 600 ng/mL for Montelukast and observed the correlation coefficient.

Table 7. The chromatogram result

Calibration Concentration (ng/mL)	2.5	5	25	50	125	250	375	500	600
Conc. Found (ng/mL)	2.503	4.9565	25.105	50.575	124.425	257.1	368.363	510.5	603.96
% Estimated Value of CC	100.12	99.13	100.42	101.15	99.54	102.84	98.23	102.1	100.66

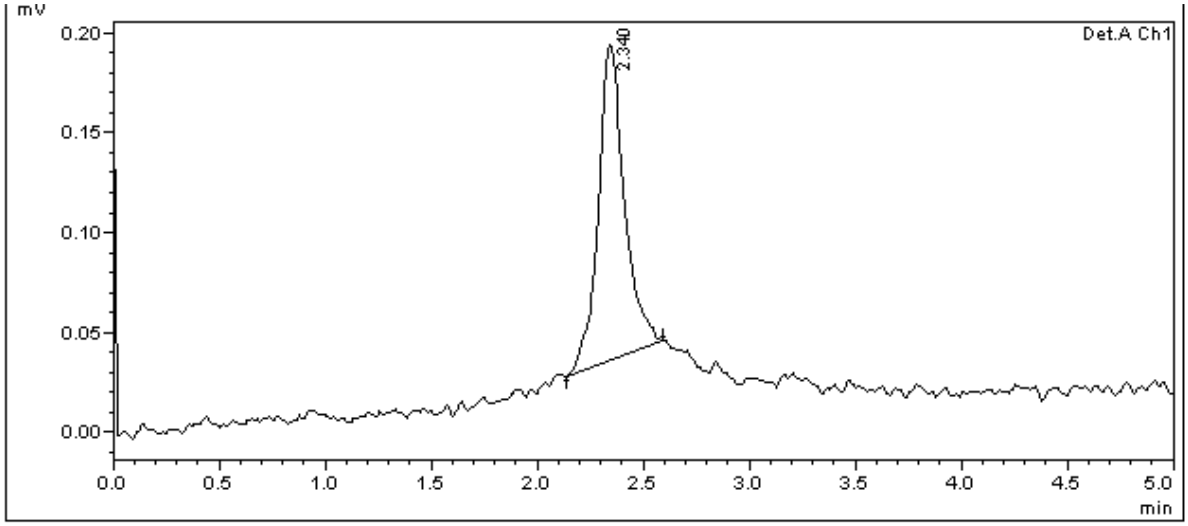

Figure 5. The chromatogram of Montelukast sodium (100 ng/mL)

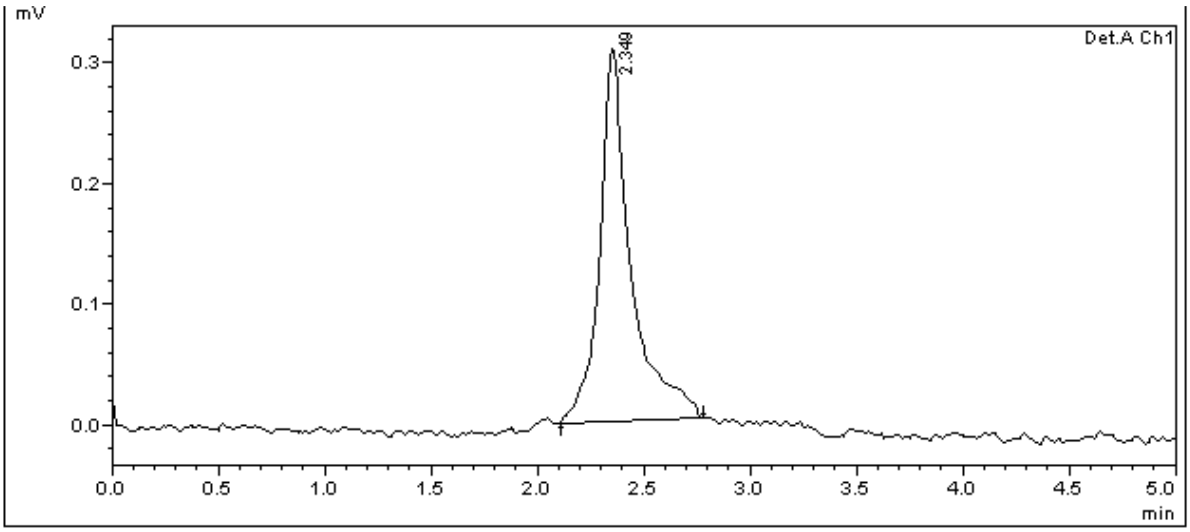

Figure 6. The chromatogram of Montelukast sodium (500 ng/mL)

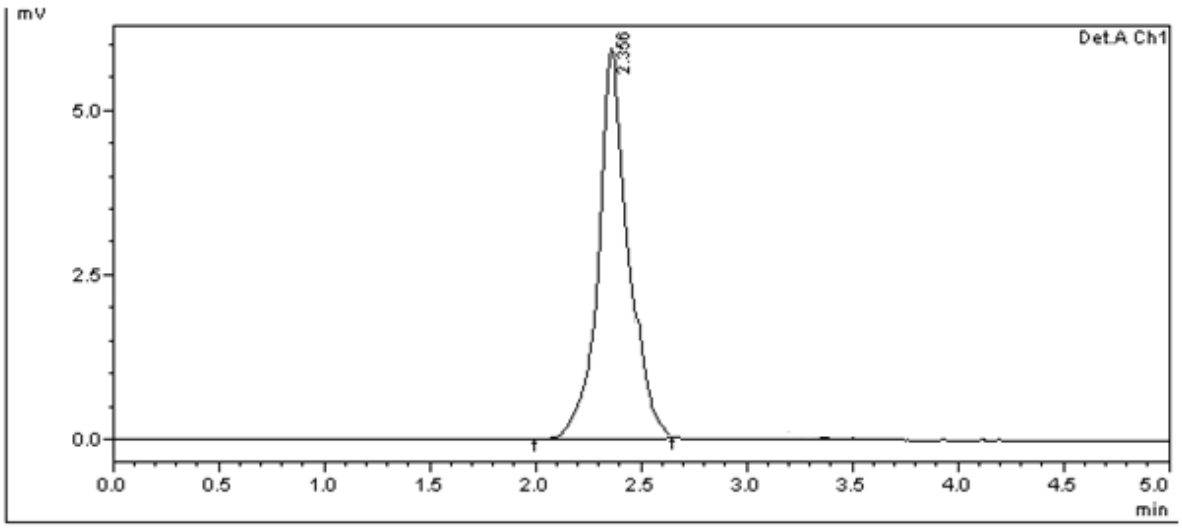

Figure 7. The chromatogram of Montelukast sodium (1000 ng/mL)

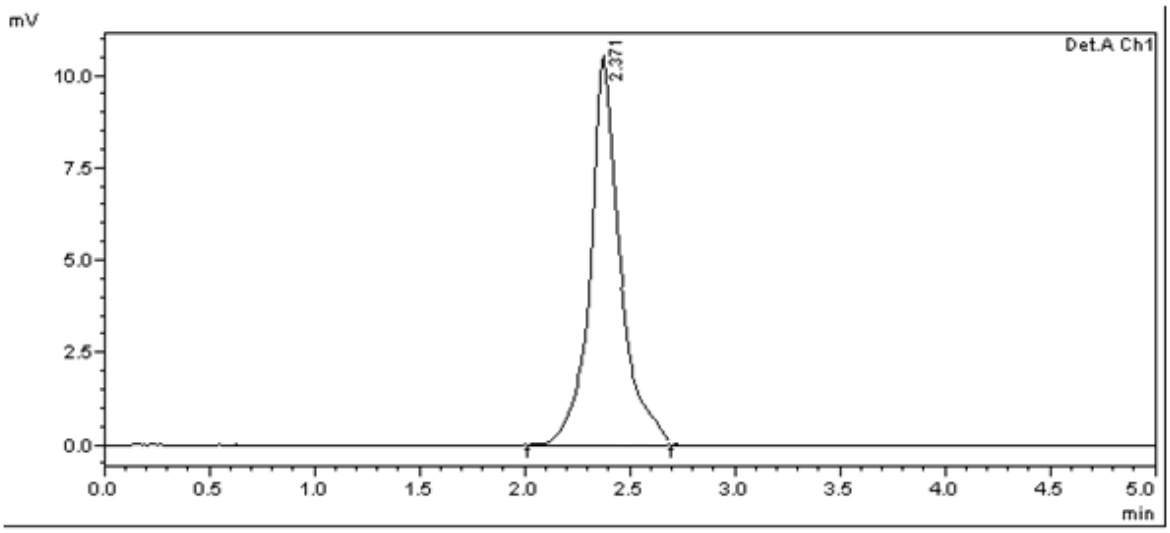

Figure 8. The chromatogram of Montelukast sodium (2500 ng/mL)

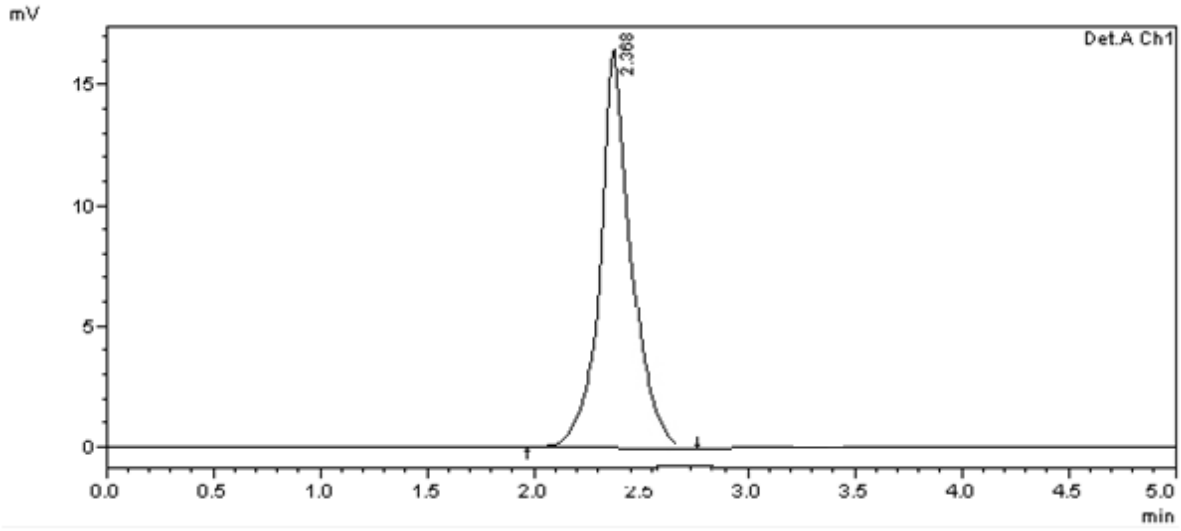

Figure 9. The chromatogram of Montelukast sodium (5000 ng/mL)

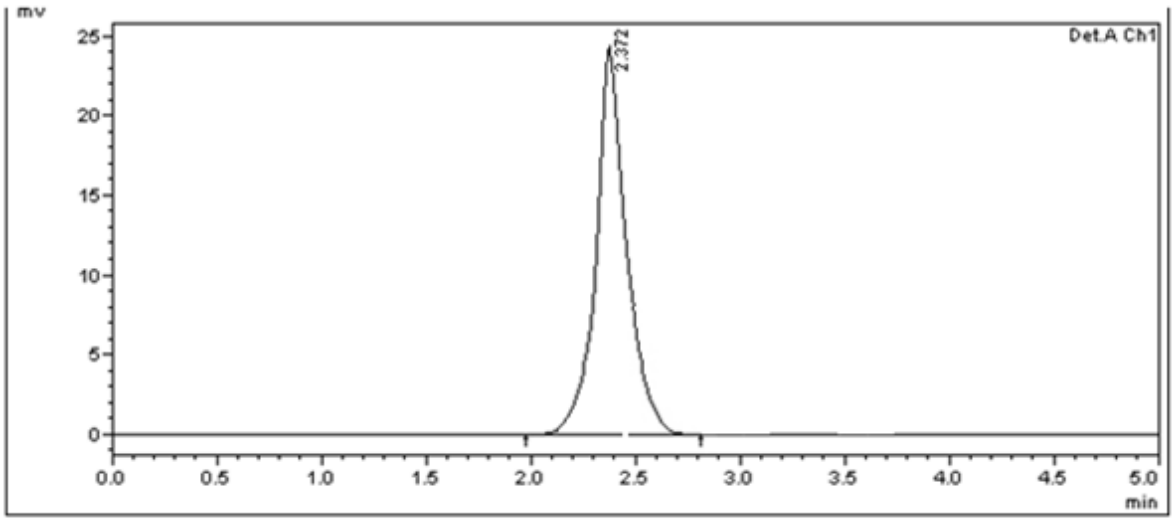

Figure 10. The chromatogram of Montelukast sodium (7500 ng/mL)

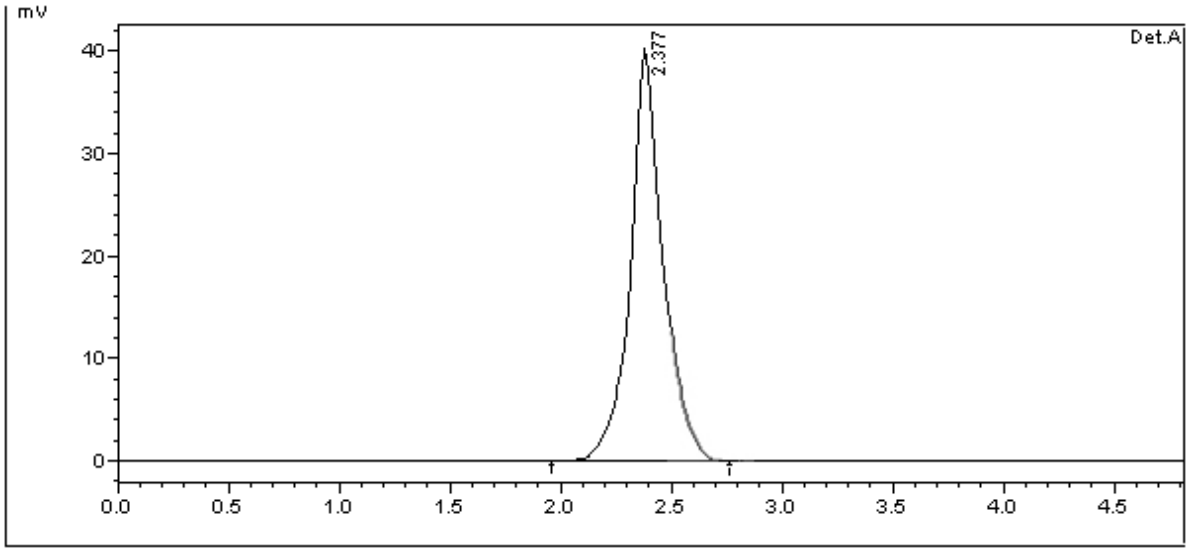

Figure 11. The chromatogram of Montelukast sodium (10000 ng/mL)

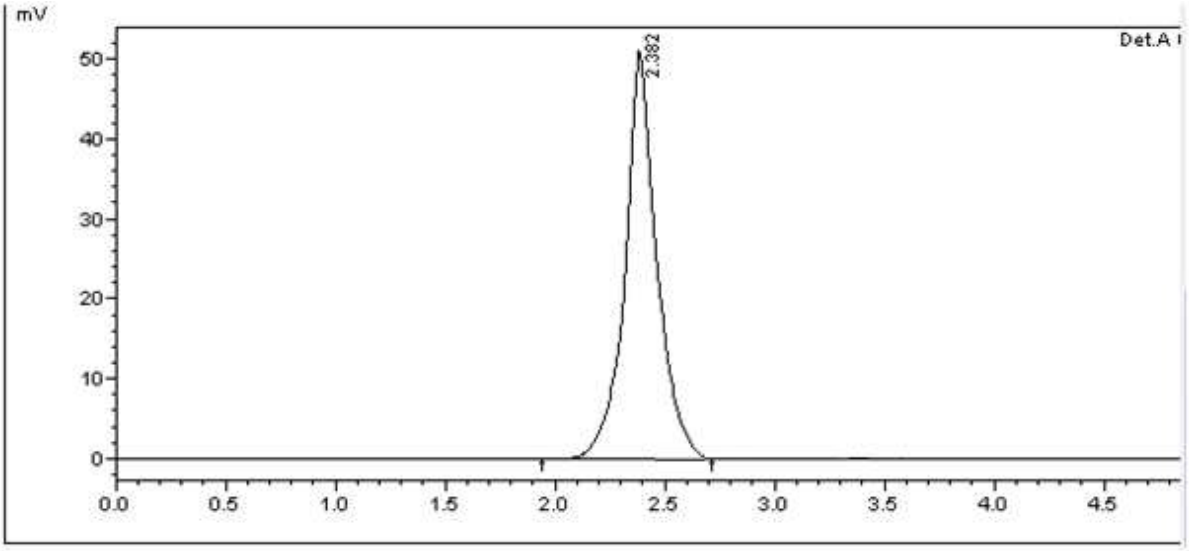

Figure 12. The chromatogram of Montelukast sodium (12000 ng/mL)

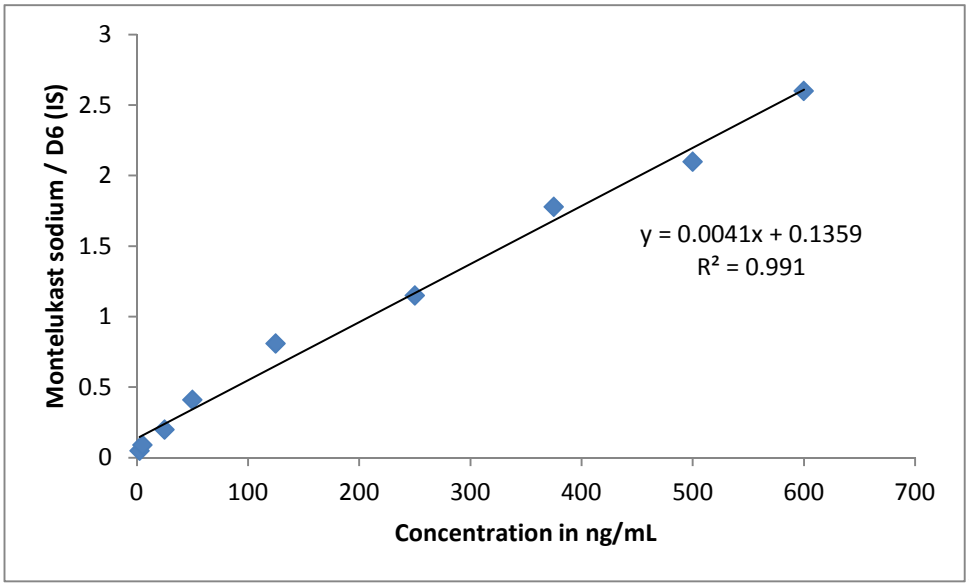

Figure 13. The linear relationship between conc. vs ratio of Montelukast sodium / D6

3.4. Precision and Accuracy

The precision of the proposed methods was performed using LLOQ, LQC, MQC and HQC. The accuracy of the proposed analytical method was calculated by taking the ratio of the average practical values of LLOQ, etc to their nominal values and expressed as %.

Sample	Conc. selected (ng/mL)	Average Conc. found (ng/mL)	S.D.	R.S.D %	% Nominal
LLOQ	**2.5**	2.44	0.26	10.65	97.6
LQC	**30**	31.82	3.21	10.08	106.06
MQC	**300**	312.67	17.65	5.64	104.22
HQC	**575**	589.43	24.65	4.18	102.50

3.5. Precision within-batch

The precision for LLOQ, LQC, MQC and HQC was found to be 10.65 %, 10.08%, 5.64% and 4.18 % respectively.

3.6. Accuracywithin-batch

The accuracy within batch was LLOQ, LQC, MQC and HQC were found to be 97.6 %, 106.06 %, 104.22 % and 102.5 % respectively.

3.7. Inter Day Precision and Accuracy

Sample	Conc. selected (ng/mL)	Average Conc. found (ng/mL)	S.D.	R.S.D %	% Nominal
LLOQ	**2.5**	2.42	0.26	10.74	96.8
LQC	**30**	29.22	3.21	10.98	97.4
MQC	**300**	309.11	17.65	5.70	103.03
HQC	**575**	586.29	24.65	4.20	101.96

Between batch precision for LLOQ, LQC, MQC and HQC was 10.74 %, 10.98 %, 5.70 % and 4.20 % respectively.

3.8. Inter Day Accuracy

Between batch accuracy for LLOQ, LQC, MQC and HQC was 96.8 %, 97.4 %, 103.03 % and 101.96 % respectively.

3.9. Recovery.

The responses of extracted internal standard samples were compared with non extracted LQC, MQC and HQC samples. The % recovery was found to be 64.70 %, 65.56 % and 61.85% respectively.

Samples		Mean	SD	CV%	% Recovery
LQC	Extracted Sample	24738	1643	6.64	64.70
	Non Extracted Sample	38231	1853	4.84	
MQC	Extracted Sample	336038	26756	7.96	65.56
	Non Extracted Sample	512534	13342	2.60	
HQC	Extracted Sample	626748	47598	7.59	61.85
	Non Extracted Sample	1013214	32518	3.20	

3.10. Stability of drug at ambient conditions

The stability of Montelukast and D_6 at 25 ± 2°C was determined. These stock solutions were exposed to this temperature for a period of 9 hours, MQC were prepared and evaluated. The precision of Montelukast at 0 hours and 9 hours was found to be between 1.13 and 2.65 % at the mentioned temperature and was found to be 101.74 %. Similarly, D6 was analyzed at 0 hours and 9 hours was 1.58 % to 2.04% and was found 101.03 %.

3.10.1. Stability of Montelukast and D_6 spiking solution at ambient temperature (Table 29, 30)

Stability of Montelukast and D_6spiking solution was conducted at ambient temperature for 9 hours equivalent to MQC. The precision of Montelukast at 0 hours and 9 hours was found to be 1.41 % to 1.87 % and stability was found to be 101.31 %. The precision of D_6 at 0 hours and 9 hours was 0.83 % to 1. 36 % respectively and percentage of stability was found to be 100.28 %.

3.10.2. Freeze-thaw Stability of plasma samples

The LQC and HQC samples of the Montelukast were subjected to four freeze thaw cycles. The precision of samples was found from 1.65 % to 1.81 % with stability of 92.15 % to 106.79 %

3.10.3. Bench Top Stability.
Bench top stability of Montelukast using LQC and HQC was determined at 12 h. The percentagewas found from 92.28 % to 101.69 % for 12 h. The precision was obtained from 0.62 % to 1.57 % for 12h.

3.10.4. Short-term Plasma Stability at -20 °C.
The stability of drugat short term storage of plasma samples at -20^{O} C for six days was carried with LQC and HQC (n=6) compared with immediately prepared calibration curve standards. The stability was obtained between 98.12 % and 102.63 % and the precision ranged from 1.54% to 1.46%.

CONCLUSION

The procedure followed in this method has the least concentration i.e. LLOQ is very low than previously reported methods. The plasma sample required is very low for the analysis. The method was found to be sensitive, simple and inexpensive than earlier methods. The method validated is suitable for quantitative determination of Montelukast sodium in human plasma.

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