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METHOD DEVELOPMENT AND VALIDATION OF MONTELUKAST BY USING LC-MS MS

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ABSTRACT

High performance liquid chromatography mass spectrometric method in positive ion mode for the estimation of Montelukast in human plasma was developed and validated using Montelukast D_6 as internal standard (ISTD). Sample preparation was accomplished by solid phase extraction technique. The eluted samples were chromatographed on ZORBAX Eclipse XDB phenyl 4.6*75 mm, 3.5 µm column (Agilent Technologies) using a mobile phase consisting of acetonitrile: 5 mM ammonium acetate (85:15 v/v). The method was validated over a concentration range of 5.032 ng/mL to 602.362 ng/mL for Montelukast. This validation report provides the results of selectivity, matrix effect. Sensitivity determinations, calibration standards and quality control samples data, precision and accuracy data, and dilution integrity along with all pertinent supporting documentation.

KEYWORDS: Montelukast, Reverse phase HPLC, Solid phase extraction technique, Method development, Method Validation.

INTRODUCTION

Montelukast (trade name **Singulair**) is a leukotriene receptor antagonist[1] (LTRA) used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies.[2][3] Montelukast comes as a tablet, a chewable tablet, flash tablet and granules to take by mouth.[4] Montelukast is usually taken once a day with or without food.

The mechanism of action of Montelukast

The cysteinyl leukotrienes (LTC4, LTD4, and LTE4) are potent inflammatory eicosaloids released from mast cells and eosinophils. These important pro-asthmatic mediators bind to cysteinyl leokutriene receptors. Montelukast is an orally active compound that binds with high affinity and selectivity to the CYSLT1 receptor.[5] It inhibits physiologic actions of LTD4 at the receptor.

Structure of Montelukast

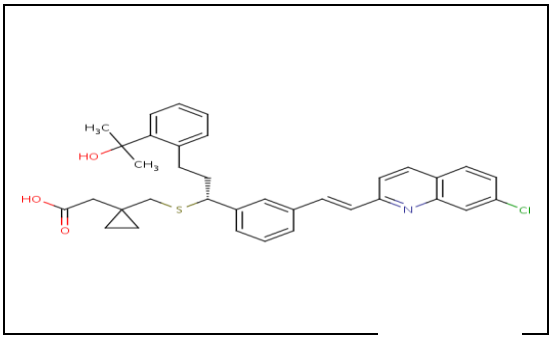


Fig: 1. Molecular Structure of Montelukast

MATERIALS AND METHODS

Table 1: List of chemicals

Reagents/Materials	Manufacturer/Supplier
Methanol (HPLC grade)	JT Baker
Ammonium acetate (AR grade)	Merck
Acetonitrile (HPLC grade)	JT Baker
HPLC grade water	Rankem
Milli-Q water	In house

Table 2: List of equipment

S.No.	Name of equipment	Make	Model
1	Analytical Balance	Sartorius	CPA2250
2	Micro Balance	Sartorius	SE-Z
3	Ph Meter	Drjon	3 STAR
4	Reciprocating Shaker	Orbitek	SCIGENICS
5	Refrigerate Centrifuge	Heraens	MEGAFUSE20 R
6	Turbo Evaporator	Zymark	BE-TE-01
7	Positive processor Pressure	Orochen	SZYPRESS 48
8	HPLC MS-MS	Shimadzu	API 4000

Chromatographic and Mass Spectrometric Conditions

Column: ZORBAX Eclipse XDB Phenyl, 4.6 x 75 mm, 3.5 µm (Make: Agilent)
Mobile phase: HPLC grade Acetonitrile: 5mM Ammonium acetate buffer (85:15, v/v)
Rinsing solution: HPLC grade Acetonitrile: Milli-Q water (60:40, v/v)
Flow rate: 0.600 mL/minute (direct)
Sample Cooler Temperature: 5°C
Injection volume: 20 µL
Needle Rinsing Volume: 700 µL
Retention time: Montelukast 1.70 ± 0.3 minutes
Montelukast D_6 1.70± 0.3 minutes
Run Time: 3.00mns

Montelukast Stock Solution

Weighed accurately, about 5 mg of Montelukast sodium working standard and transferred to a 5 mL clean glass volumetric flask, dissolved in HPLC grade Methanol and made up the volume with the same to produce a solution of 1000000.0000ng/mL. Corrected the above concentration of Montelukast solution accounting for its potency and the actual amount weighed for free compound weight. A batch number was provided and the 'Stock Weighing and Solution Preparation' form was completed. The stock solution was stored in refrigerator at 2-8°C and used for maximum of four days.

The stock solutions were diluted to suitable concentrations using diluent for spiking into plasma to obtain calibration curve (CC) standards, quality control (QC) samples and DIQC samples. All other final dilutions (system suitability dilutions, aqueous mixture, etc.) were prepared in mobile phase.

Montelukast D_6 Stock Solution (Internal Standard)

Weighed accurately, about 5.0000 mg of Montelukast D_6 sodium transferred to a 5 mL volumetric flask, dissolved in HPLC grade Methanol and made up the volume with the same to produce a solution of 1000000.0000 ng/mL. Corrected the above concentration of Montelukast D_6 accounting for its potency and the actual amount weighed. A batch number was provided and the 'Stock Weighing and Solution Preparation' form was completed. The stock solution was stored in refrigerator at 2-8°C and used for maximum of four days. The stock solution was diluted to suitable concentration using diluent for internal standard dilution.

Biological Matrix

Eight lots of K_2EDTA human plasma including one hemolytic and one lipemic plasma were screened All eight human plasma lots including hemolytic and lipemic plasmas were found free of any significant interference for Montelukast and Montelukast D_6. Six plasma lots are used to prepare calibration standards, quality control samples. Selectivity and matrix effect tests were performed After bulk spiking, aliquots of 600µL for CCs and 600µL for QCs of spiked plasma samples were pipetted out into a prelabelled polypropylene micro centrifuge tubes and then all the bulk spiked samples were stored to deep freezer at –70°C ± 10°C.

Sample Preparation

The thawed samples[9] were vortexed to ensure complete mixing of the contents. 500µL of the plasma sample was pipetted into a RIA vial, 50µL of Montelukast D_6 dilution (1020.554ng/mL) was added to it, as an internal standard whereas, in blank plasma, 50µL of Diluent solution was added and vortexed. 500µL of 1% formic acid in water was added to it and vortexed.

RESULTS AND DISCUSSION

Selectivity

Selectivity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants. There was no significant interference from endogenous components observed at the mass transitions of Montelukast and internal standard. Refer figure no: 02 and 03 for representative chromatograms.[6] For analyte selectivity, there was no significant interference observed.

Table 3: Selectivity Data for Montelukast (LLOQ Area)

M-2142011 SEL-LLOQ	Montelukast Area	IS Area
1	21031	721742
2	20606	725139
3	19879	705262
4	18904	692428
5	19164	698075
6	19149	669661
Mean	**19788.8**	**702051.2**
SD	**871.96**	**20438.32**
% CV	**4.41**	**2.91**

Table 4: Selectivity Data for Montelukast (Blank+IS Area)

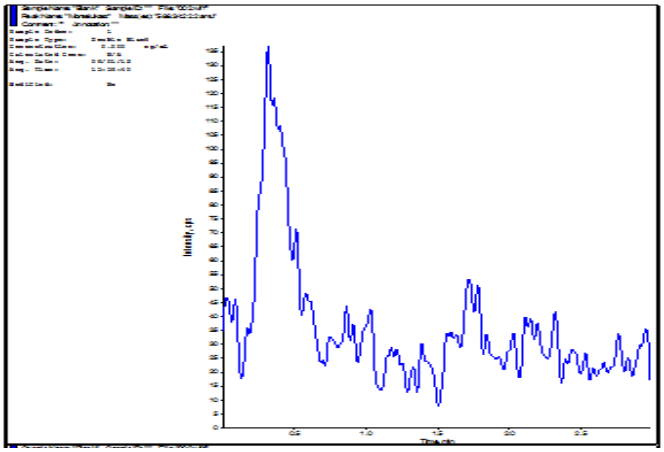
M-2142011 Sample ID	Montelukast Area	IS Area
Blank+IS (Montelukast D6) -1	0	673185
Blank+IS (Montelukast D6) -2	0	683800
Blank+IS (Montelukast D6) -3	0	665731
Blank+IS (Montelukast D6) -4	0	687144
Blank+IS (Montelukast D6) -5	0	666870
Blank+IS (Montelukast D6) -6	0	671618
Mean Response of Montelukast in presence of Montelukast D6	0.0	
Mean Response of Montelukast in SEL- LLOQ	19788.8	
% Interference at RT of Montelukast in Presence of Montelukast D6	0.0	

Table 5: Selectivity Data for Montelukast (ULOQ Area)

M-2142011 Sample ID	Montelukas Area	IS Area
ULOQ (Montelukast)-1	1774600	1016
ULOQ (Montelukast)-2	1767794	2162
ULOQ (Montelukast)-3	1743268	2541
ULOQ (Montelukast)-4	1744443	2324
ULOQ (Montelukast)-5	1796231	1195
Mean Response of Montelukast D6 in presence of Montelukast		1912.3
Mean Response of Montelukast D6 in SEL-LLOQ		702051.2
% Interference at RT of Montelukast in Presence of Montelukast D6		0.27

Calibration[7] Curve Standards and Quality Control Samples

Calibration curve standard consisting of a set of nine non-zero concentrations ranging from 5.032ng/mL to 602.362 ng/mL of Montelukast was prepared. Prepared quality control samples consisted of concentrations of 5.036 ng/mL (LLOQ QC), 15.076 ng/mL (LQC), 90.278 ng/mL (MQC-1), 300.927ng/mL (MQC-2) and 501.545 ng/mL (HQC) for Montelukast. These samples were stored at –70°C ± 10 °C until use.



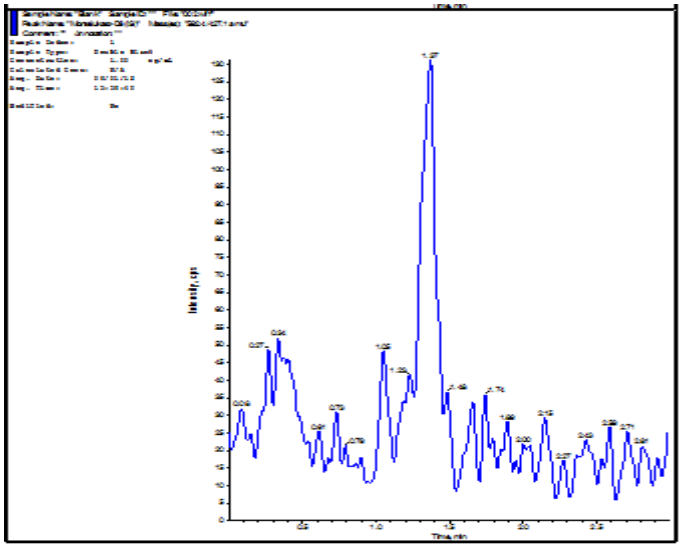


Figure2: A Representative Chromatogram of an Aqueous Standard and Internal Standard Mixture of Montelukast

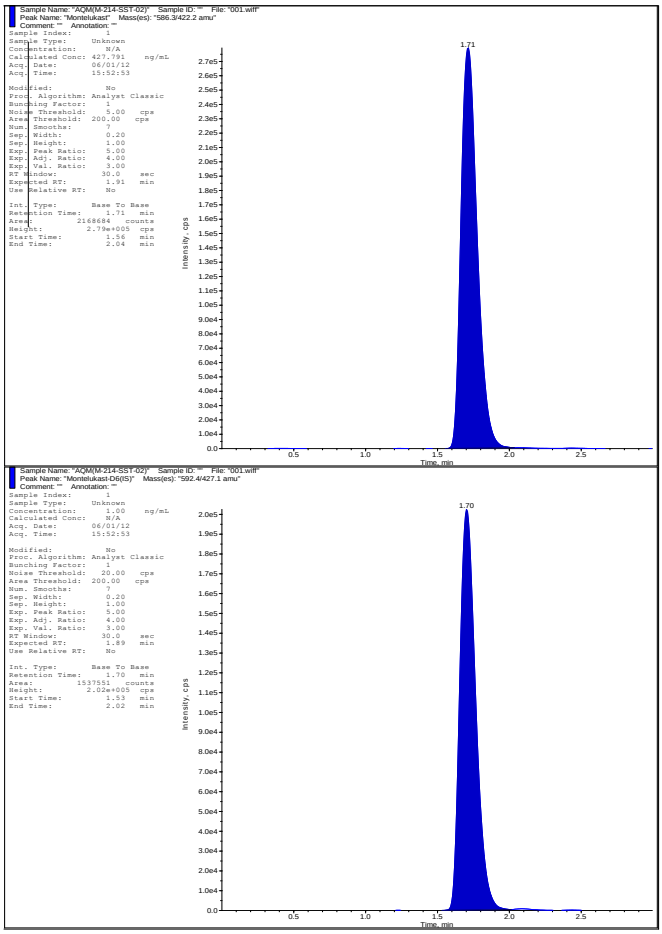


Figure 3: Representative Chromatogram of Blank Plasma Sample of Montelukast.

Linearity

The linearity of an analytical procedure is its ability within a given range to obtain test results which are directly proportional to the concentration of analyte[8] in the sample. A regression equation with a weighting factor of 1/ (concentration ratio) ^{2} of drug to ISTD concentration was judged to produce the best fit for the concentration-detector response relationship for Montelukast in human plasma. The representative calibration curve for regression analysis is illustrated in Figure 9. Correlation coefficient (r^2) was greater than 0.98 in the concentration range of 5.032 ng/mL to 602.362 ng/mL for Montelukast.

Table: 6: Concentration-response Linearity Data of Montelukast for Ruggedness

	Concentration (ng/mL)								
	STD-A	**STD-B**	**STD-C**	**STD-D**	**STD-E**	**STD-F**	**STD-G**	**STD-H**	**STD-I**
Upper Limit	6.038	11.574	34.653	69.306	138.613	277.225	415.630	554.172	692.716
Lower Limit	4.026	8.554	25.613	51.226	102.453	204.905	307.204	409.606	512.008
CC#	5.032	10.064	30.133	60.266	120.533	241.065	361.417	481.889	602.362
3	5.172	9.285	31.283	63.883	123.255	237.336	323.255	485.843	627.532
% Nominal	102.77	92.26	103.81	106.00	102.26	98.45	89.44	100.82	104.18
M-2142011		Slope			Intercept		r^2		
CC# 3		0.0019			-0.0002		0.9960		

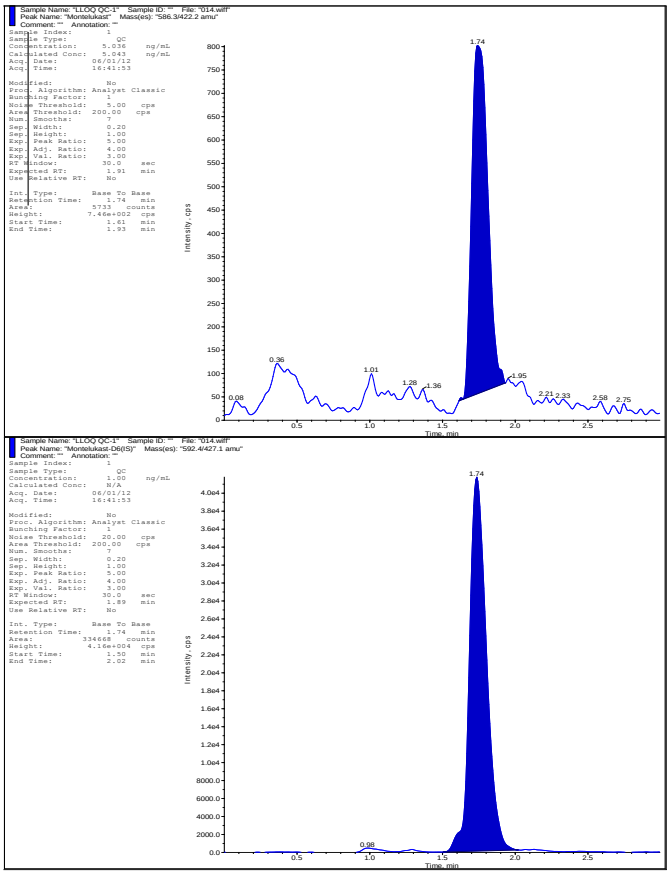


Figure: 4 A Representative Chromatogram of Blank Plasma with Internal Standard Sample of Montelukast

Robustness and Ruggedness

The Ruggedness[10] of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Table: 7 Within Batch Precision and Accuracy of Montelukast for Ruggedness

M-2142011	**Concentration (ng/mL)**				
	LLOQ QC	**LQC**	**MQC1**	**MQC2**	**HQC**
Upper Limit	6.043	17.337	103.820	346.066	576.777
Lower Limit	4.029	12.815	76.736	255.788	426.313
QC#	5.036	15.076	90.278	300.927	501.545
13	5.753	16.131	97.014	330.868	531.492
14	5.063	16.464	95.839	332.958	531.994
15	5.734	16.343	96.975	331.162	523.269
16	5.268	16.194	94.628	325.034	513.333
17	5.205	16.271	93.557	318.946	515.638
18	5.212	16.604	91.471	320.926	509.996
Mean	5.3725	16.3345	94.9140	326.6490	520.9537

S.D.	0.29529	0.17587	2.15828	5.87653	9.43088
C.V.%	5.50	1.08	2.27	1.80	1.81
% Nominal	106.68	108.35	105.14	108.55	103.87
N	6	6	6	6	6
6		2623833		2568896	
Mean		2614977.8		2560690.7	
SD		19012.52		14407.26	
CV%		0.73		0.56	
N		6		6	
% Stability		**101.96**			

Sensitivity

The lowest limit of reliable quantification for Montelukast in human plasma was set at the concentration of the LLOQ 5.032 ng/mL. The precision and accuracy[11] for Montelukast at this concentration was found to be 3.10% and 97.04.

Table: 8 Concentration-response Linearity Data for Sensitivity of Montelukast

M-2142011	**Concentration (ng/mL)**								
	STD-A	**STD-B**	**STD-C**	**STD-D**	**STD-E**	**STD-F**	**STD-G**	**STD-H**	**STD-I**
Upper Limit	6.038	11.574	34.653	69.306	138.613	277.225	415.630	554.172	692.716
Lower Limit	4.026	8.554	25.613	51.226	102.453	204.905	307.204	409.606	512.008
CC#	5.032	10.064	30.133	60.266	120.533	241.065	361.417	481.889	602.362
1	4.893	10.480	30.959	61.758	125.181	245.090	319.380	484.142	596.639
% Nominal	97.24	104.13	102.74	102.48	103.86	101.67	88.37	100.47	99.05

M-2142011	Slope	Intercept	r^2
CC# 1	0.0033	0.0005	0.9985

Table9: within Batch Precision and Accuracy for Sensitivity of Montelukast

M-214-SEN LLOQ	**Concentration (ng/mL)**	
		LLOQ
	Upper Limit	**6.038**
	Lower Limit	**4.026**
	5.032	
1	4.811	
2	4.958	
3	5.001	
4	4.795	
5	4.664	
6	5.068	
Mean	4.8828	
SD	0.15144	
CV%	3.10	
% NOMINAL	97.04	
N	6	

CONCLUSION

A high performance liquid chromatography mass spectrometric method for the estimation of Montelukast in human plasma in positive ion mode was developed and validated using Montelukast D6 as internal standard (IS). Sample preparation was accomplished by Solid phase extraction technique. The reconstituted samples were chromatographed, using a mobile phase consisting of HPLC grade acetonitrile: 5mM ammonium acetate (80:20, v/v). The method was validated over a concentration range of Montelukast 5.032 ng/mL to 602.36. ng/mL with the detection of Montelukast m/z - 586.30 (parent) and 422.20 (product) and internal standard Montelukast D_6 m/z - 592.40 (parent) and 427.10 (product) in positive ion model.

This validation report provides the results of selectivity, and sensitivity determinations, calibration standards and quality control samples data, precision and accuracy data, the results of recovery, various stabilities and dilution along with all pertinent supporting documentation.

REFERENCES

1. http://en.wikipedia.org/wiki/Montelukast

2. Bharathi DV, Hotha KK, Jagadeesh B, Mullangi R, Naidu A.
3. Sripalakit P, Kongthong B, Saraphancho.
4. Determination of montelukast (MK-0476) and its S-enantiomer in human plasma by stereoselective high-performance liquid chromatography with column-switching Article *in* "*Journal of Pharmaceutical and Biomedical Analysis* ", March, 1997; 15(5): 631-8.
5. Hartmann c, Smeyers-Verbeke, Massart DL, MC Dowel RD. Validation of Bioanalytical chromatographic methods. *Journal of Pharmaceutical and Biomedical Analysis*, 1998; 17(2): 193–218.
6. Roger Cousin, Validation of chromatographic methods in biomedical analysis view point and discussion, "*Journal of Chromatography"*, 1997; 175-180.
7. Bressolle, F, Bromet-pitit, M. & Audran, M, Validation of Liquid Chromatography and Gas Chromatographic Methods Application to Pharmacokinetics, "*Journal of Chromatography".* 1996; 3-10.
8. Howard Hill., "*High throughput Bio analytical Sample Preparation Methods and Automation Strategies", 1st Ed.*, 2009; 1(1): 116-209.
9. Jurgen J.G, John Willey & Sons, Chichester, "*Mass Spectrometry Instrumentation Interpretation and application"*, 1st Ed, 2009; 216-389.
10. Stooge DA, Holler FJ, Crouch, "*Principles of Instrumental Analysis", 5th Ed*, 1998; 550-55.
11. Bressolle, F, Bromet-pitit, M. & Audran, M, Validation of Liquid Chromatography and Gas Chromatographic Methods Application to Pharmacokinetics, "*Journal of Chromatography",* 1996; 3-10.
12. Bioanalysis by LC–MS/MS, "*J. Pharm. Biomed. Anal"*, 2007; 44(2): 342-355.
13. Howard Hill., "*High throughput Bio analytical Sample Preparation Methods and Automation Strategies", 1st Ed.*, 2009; 1(1): 116-209.
14. Jurgen J.G, John Willey & Sons, Chichester, "*Mass Spectrometry Instrumentation Interpretation and application"*, 1st Ed, 2009; 216-389.
15. Stooge DA, Holler FJ, Crouch, "*Principles of Instrumental Analysis", 5th Ed*, 1998; 550-55.