Fast, Quantitative SPE LC-MS/MS Analysis of Montelukast in Human Plasma

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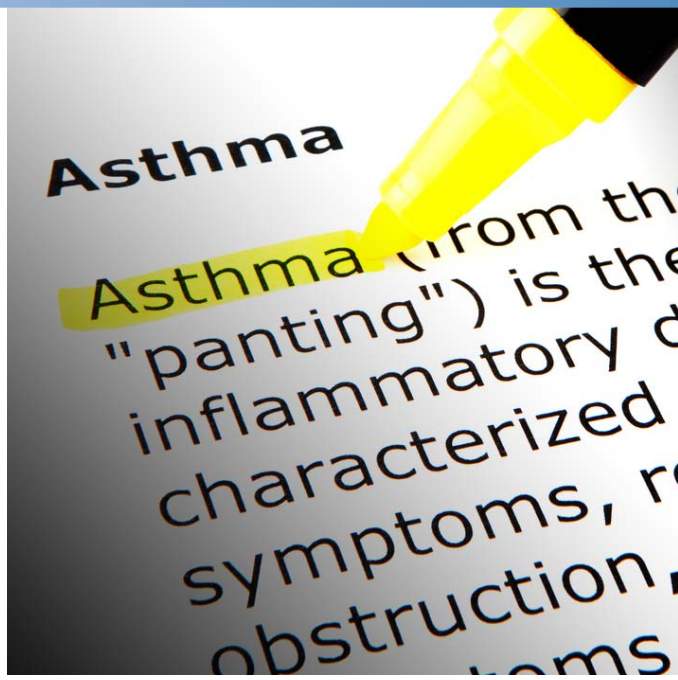
Key Words
Asthma drug, montelukast, Singulair, Montelo-10, SPE, SOLA SAX, Accucore C8

Abstract
A fast HPLC method and an efficient extraction and analysis method for montelukast in human plasma has been developed using a Thermo Scientific™ SOLA™ SAX SPE 96 well plate and a Thermo Scientific™ Accucore™ C8 HPLC column. The sample preparation was fast, reproducible, and accurate. The chromatographic method provides analysis time of less than 1.5 minutes. The dynamic range was linear between 1 and 1000 ng/mL with an R^2 of 0.9901.

Introduction

The prevalence of asthma has increased significantly since the 1970s. As of 2010, 300 million people were affected worldwide. In 2009 asthma caused 250,000 deaths globally. Causes of asthma are not well understood but it appears to be due to a combination of genetic and environmental factors. Treatment of acute symptoms is usually with an inhaled short-acting beta-2 agonist. Symptoms can be prevented by avoiding triggers, such as allergens and irritants, and by the use of inhaled corticosteroids or leukotriene antagonists. High levels of research activity continue within the pharmaceutical industry and high quality, reliable bioanalytical methods are required to support this research.

Montelukast (Figure 1, trade names Singulair® and Montelo-10) is a leukotriene inhibitor that is used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies. Leukotrienes are released when an allergen, such as pollen, is inhaled, which causes swelling in the lungs and tightening of the airway muscles. Montelukast is typically dosed at 4–10 mg, one tablet daily. A C_{max} value of 667 ng/mL has been reported following 5 mg oral dose administration for children. In this application the extraction and quantification of montelukast in human plasma are demonstrated [1–3].



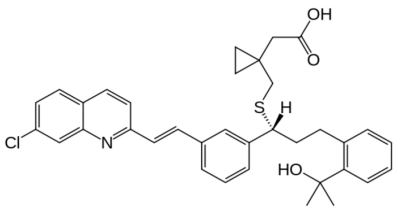


Figure 1: Montelukast [3]

To provide sample extraction and chromatographic separation of these compounds from human plasma, SOLA solid phase extraction (SPE) products and Accucore HPLC columns were utilized.

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SOLA solid phase extraction (SPE) products introduce next-generation, innovative technological advancements, which give unparalleled performance characteristics compared to conventional SPE, phospholipid, and protein precipitation products.

These include:

- Higher levels of reproducibility
- Reduced solvent requirements
- Higher levels of extract cleanliness
- Increased sensitivity

SOLA SPE plates or cartridges have significant advantages when analyzing compounds in complex matrices, particularly in high-throughput bioanalytical and clinical research laboratories where reduced failure rates, higher analysis speed, and lower solvent requirements are critical. SOLA products' superior performance gives higher confidence in analytical results and lowers cost without compromising ease-of-use or requiring complex method development.

Accucore HPLC columns use Core Enhanced Technology™ to facilitate fast and highly efficient separations. The 2.6 µm diameter particles are not totally porous, but instead have a solid core and a porous outer layer. The optimized phase bonding creates a series of high coverage, robust phases. This coverage results in a significant reduction in secondary interactions and delivers highly efficient peaks with very low tailing. The Accucore C8 HPLC column uses a shorter alkyl chain length designed to have lower hydrophobic retention than an equivalent C18 phase. The tightly controlled 2.6 µm diameter of Accucore particles results in much lower backpressures than typically seen with sub-2 µm materials.

Experimental Details

Consumables	Part Number
Fisher Scientific™ LC/MS grade water	W/0112/17
Fisher Scientific LC/MS grade methanol	M/4062/17
Fisher Scientific LC/MS grade acetonitrile	A/0638/17
Fisher Scientific formic acid	F/1900/PB08
Fisher Scientific ammonia solution	A/3295/PB05

Sample Handling Equipment	Part Number
Thermo Scientific 96 well plate vacuum manifold	60103-351
Thermo Scientific™ UltraVap™ high speed sample concentrator	CLS-229070

Sample Preparation	
Compound(s):	Montelukast, montelukast d6 (IS)
Matrix:	Human plasma
Calibration standards:	A 1.0 mg/mL stock solution of montelukast was prepared in methanol. Further dilution was carried out in methanol to prepare 20, 200, 1000, 2000, 4000, 16,000, and 20,000 ng/mL spiking solutions of montelukast. Human plasma (180 µL) was spiked with 10 µL internal standard (10 µg/mL) and 10 µL spiking standard at the appropriate level.
Quality control standards:	For QC samples, 180 µL of human plasma was spiked with 10 µL internal standard (10 µg/mL) and 10 µL spiking standard at the appropriate level. Six replicates were extracted and analyzed at 30, 300, and 600 ng/mL
Blank control standards	For blanks, 180 µL of human plasma was spiked with 20 µL of methanol.

Sample Pretreatment

To disrupt protein binding, 800 µL of methanol was added to the prepared samples and the samples were vortexed for 30 seconds. The vortexed samples were then centrifuged for 5 minutes at 14,000 rpm (radius 7 cm). The supernatant was then collected for analysis.

Solid Phase Extraction		Part Number
SPE plate:	10 mg SOLA SAX 96 well plates	60309-003
Conditioning stage:	500 µL methanol applied to SPE cartridge or well	
Equilibration stage:	500 µL water with 2% ammonia applied to SPE cartridge or well	
Load:	Crashed supernatant (under gravity) applied to SPE cartridge or well	
Wash 1:	500 µL water with 2% ammonia applied to SPE cartridge or well	
Wash 2:	500 µL methanol with 2% ammonia applied to SPE cartridge or well	
Elute:	2 × 150 µL methanol with 5% ortho-phosphoric acid solution applied sequentially to SPE cartridge or plate	
The elution stage was carried out under gravity and the vacuum was applied only to dry the bed after the last drop eluted. To adjust the polarity, 100 µL water was added to each sample followed by sonication for 10 minutes before analyzing with mass spectrometry.		

Chromatographic Conditions				Part Number
Instrumentation:	Thermo Scientific™ Dionex™ UltiMate™ 3000 Binary RSLC HPLC system			
Column:	Accucore C8, 2.6 µm, 50 × 2.1 mm			17226-052130
Guard column:	Accucore C8, 2.6 µm, 10 × 2.1 mm			17226-012105
Mobile phase A:	Water (LC-MS grade) + 0.1% formic acid			
Mobile phase B:	Acetonitrile (LC-MS grade) + 0.1% formic acid			
Gradient:	Time(min)	% A	% B	
	0.00	30	70	
	0.50	0	100	
	1.00	0	100	
	1.01	30	70	
	1.50	30	70	
Flow rate:	1.0 mL/min			
Column temperature:	60 °C			
Injection details:	10 µL			
Injection wash solvent 1:	Water / acetonitrile (80:20 v/v)			
Injection wash solvent 2:	IPA / acetonitrile / acetone (45:45:10 v/v/v)			

MS Conditions	
Instrumentation:	Thermo Scientific™ TSQ Vantage™ MS
Ionization conditions:	HESI
Polarity:	Positive
Spray voltage (V):	3500
Vaporizer temp (°C):	300
Sheath gas pressure (Arb):	50
Aux gas pressure (Arb):	30
Capillary temp (°C):	270
Collision pressure (mTorr):	1.5
Scan time (s):	0.02
Q1 (FWHM):	0.70
Q3 (FWHM):	0.70
Compound transition details are provided in Table 1.	

Compound	Montelukast	Montelukast d6 (IS)
Parent (*m/z*)	586.2	592.2
Products (*m/z*)	422.2	427.3
Collision energy (eV)	23	22
S-lens (V)	137	138

Table 1: Compound transition details

Data Processing	
Software:	Thermo Scientific™ LCQUAN™ software, version 2.6

Results

Montelukast standards, extracted from human plasma, gave a linear calibration curve over the dynamic range of 1 to 1000 ng/mL with an R^2 coefficient of 0.9901 (Table 2). QC samples were run in replicates of six at concentrations of 30, 300, and 600 ng/mL. The precision at high, medium, and low levels was 2.5%, 5.7%, and 10.6%, respectively. The calculated recovery for the high QC was 89%, for the medium QC was 84%, and for the low QC was 93%. The average recovery for all three levels was calculated to be 89% which demonstrates that SOLA SAX products give excellent recovery for montelukast (Table 3).

The chromatography of montelukast and montelukast d6 are shown in Figures 2 and 3.

Montelukast Cal. STD	Specified Conc (ng/mL)	Calculated Conc (ng/mL)	% Difference
S1	1	0.990	-0.91
S2	10	10.6	6.42
S3	50	57.5	14.9
S4	100	106	5.87
S5	200	172	-14.08
S6	800	746	-6.7
S7	1000	945	-5.49

Table 2: Accuracy of back-calculated values (montelukast)

QC Standards	Actual Amount (ng/mL)	Average Calculated Amount (n=6) (ng/mL)	Accuracy	RSD (n=6)	Recovery
QC High (600 ng/mL)	600	573	95.5%	2.5%	89%
QC Medium (300 ng/mL)	300	216	98.5%	5.7%	84%
QC Low (30 ng/mL)	30	32.3	107.6%	10.6%	93%

Table 3: Results summary

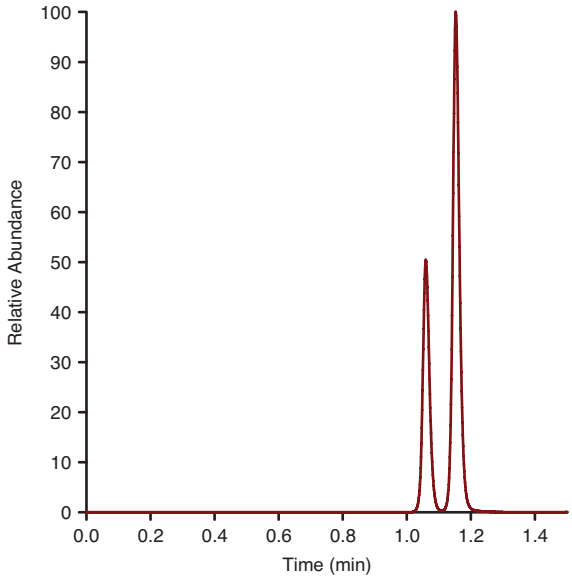

Figure 2: Chromatogram of montelukast in extracted human plasma. The peak at 1.04 minute is an isomer, which was well separated from the montelukast peak by the Accucore C8 HPLC column.

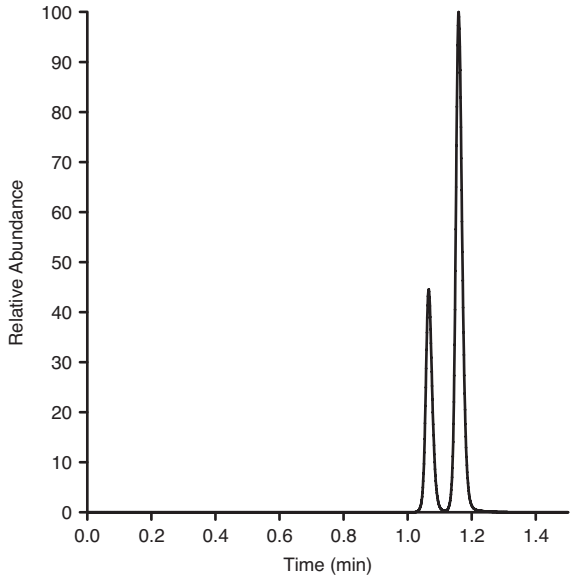

Figure 3: Chromatogram of montelukast d6 (IS). The peak at 1.04 minute is an isomer, which was separated from montelukast d6 peak by the Accucore C8 HPLC column.

Conclusion

The SOLA SAX SPE plate and Accucore C8 HPLC column can be used to extract and quantify montelukast from human plasma. In this application we demonstrated:

- Analysis and quantification of montelukast in less than 1.5 minutes using an Accucore C8 column.
- A robust UHPLC method that separates the cis/trans isomers.
- Good extraction accuracy and precision.
- High recovery of montelukast using a SOLA SAX plate.
- Excellent accuracy and reproducibility using a SOLA SAX plate.
- Low elution volume requirement for the SOLA SAX plate.
- An LOQ of 1 ng/mL for montelukast in plasma.
- A robust, simple, and highly reproducible method.

References

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