Application Note: ANCCSCETRETZAF

Zafirlukast in Human Plasma Using Retain AX and Accucore RP-MS Column

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Key Words

- Asthma drug
- Zafirlukast
- Accolate
- Retain AX
- Bioanalysis
- Glybenclamide

Abstract

A fast HPLC method and an extraction method for zafirlukast from human plasma has been developed using a Thermo Scientific Accucore RP-MS column and a Retain AX SPE well plate. The sample preparation was fast, reproducible and accurate. The chromatographic method provide a run time of less than 3 minutes.

Introduction

Accucore™ HPLC columns use Core Enhanced Technology™ to facilitate fast and high efficiency separations. The 2.6µm diameter particles are not totally porous, but rather have a solid core and a porous outer layer. The optimised phase bonding creates a series of high coverage, robust phases. Accucore RP-MS uses an optimized alkyl chain length for more effective coverage of the silica surface. This coverage results in a significant reduction in secondary interactions and thus highly efficient peaks with very low tailing. The tightly controlled 2.6µm diameter of Accucore particles results in much lower backpressures than typically seen with sub-2µm materials.

HyperSep Retain AX is a high capacity polymeric anion exchange SPE material with high purity, highly porous polystyrene DVB material partially modified with quaternary amine functional group. Retain AX provides exceptionally high and consistent recoveries and is stable at pH 0 to 14.

Zafirlukast (trade name Accolate) is a leukotriene inhibitor and is used for chronic treatment of asthma. Leukotriene is a chemical released in an asthmatic's body when an allergen such as pollen is inhaled, which causes a swelling in the lungs and tightening of the muscle of airways. Zafirlukast is typically dosed at 10-20 mg tablet daily. A C_{max} value of 326 ng/mL has been reported following 20 mg oral dose administration to an adult male volunteer [1, 2, 3]. In this application the extraction and quantification of zafirlukast in human plasma are demonstrated.

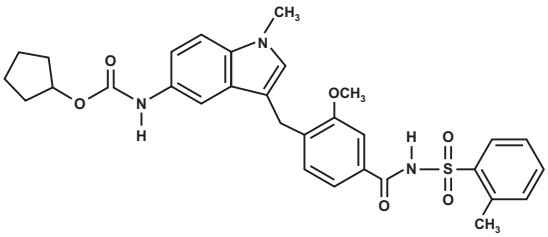

Figure 1: Structure of zafirlukast [3]

Experimental Details

Chemicals and Reagents	Part Number
Fisher Scientific HPLC grade Water	W/0106/17
Fisher Scientific LC-MS grade Water	W/0112/17
Fisher Scientific HPLC grade Methanol	M/4056/17
Fisher Scientific LC-MS grade Methanol	M/4062/17
Fisher Scientific LC-MS grade Acetonitrile	A/0638/17
Zafirlukast (Sigma Aldrich)	Z4152
Glybenclamide (Sigma Aldrich)	G2539
Fisher Scientific Formic Acid	F/1900/PB08

Sample Handling Equipment	Part Number
Fisher Scientific Finn pipettes	9402151, 02-707-408, 02-707-423
96-well manifold	60103-351
30 mg Retain AX 96-well plates	60303-407
Thermo Fisher Scientific Ultra Vap	CLS-229070

Sample and Calibration Preparation

Compound(s):	Zafirlukast and glybenclamide (IS)
Matrix:	Human Plasma (SeraLab, part PLHI-123-H)
Stock Solution:	1000 µg/mL stock solutions of zafirlukast were prepared in LC-MS grade acetonitrile. From 1000 µg/ml glybenclamide solution made in LC-MS grade acetonitrile, a concentration of 6.0 µg/mL internal standard stock solution was prepared by diluting 6 µL with 994 µL LC-MS grade acetonitrile + 5% ammonia solution. Calibration standards: S1-S6 calibration standards were prepared (Table 1). 180 µL of plasma was spiked with 10 µL internal standards and 10 µl of the appropriate zafirlukast spiking solution to provide the calibration standards.

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Standard	Zafirlukast Concentration in plasma (ng/mL)	Spiking solution conc (ng/mL)	Take from spike solution	Amount to Spike (µL)	Acetonitrile + 5% Ammonia solution added (µL)
S6	500	10000	Stock (1 mg/mL)	50.00	4950.00
S5	500	10000	Stock	50.00	4950.00
S4	400	8000	S6	800.00	200.00
S3	200	4000	S6	400.00	600.00
S2	100	2000	S6	200.00	800.00
S1	50	1000	6 µg/mL	200.00	1000.00

Table 1: Preparation of spiking solution

Single level accuracy check: 5 replicates were extracted and analyzed at 200 ng/mL, to indicate accuracy and precision at this level.

Solid Phase Extraction		**Part Number**
SPE product:	30 mg Retain AX 96-well Plates	60303-407
Conditioning stage:	500 µL acetonitrile	
Equilibration stage:	500 µL water	
Load:	200 µL plasma (spiked with zafirlukast and IS)	
Wash 1:	500 µL water	
Wash 2:	500 µL acetonitrile	
Elute:	500 µL acetonitrile with 5% formic acid solution	

Extracts were dried using an UltraVap™ and reconstituted in 200 µL LC-MS grade acetonitrile. The reconstituted solutions were sonicated for 30 minutes and analyzed with mass spectrometry.

Chromatographic Conditions			**Part Number**
Instrumentation:	Thermo Scientific Accela 600 with Open Auto Sampler		
Column:	Accucore RP-MS 2.6 µm, 50 x 2.1 mm		17626-052130
Guard column:	Accucore RP-MS 2.6 µm, 10 x2.1 mm		17626-012105
Guard holder:			852-00
Mobile phase:	A: water (LC-MS grade) + 0.1% formic acid		
	B: methanol (LC-MS grade) + 0.1% formic acid		
	T/min	% A	%B
	0.00	35.00	65.00
	2.00	0.00	100.0
	2.02	35.00	65.00
	3.00	35.00	65.00
Flow rate:	0.60 mL/min		
Column temperature:	25 °C		
Injection details:	5 µL		
Injection wash solvent:	water/methanol (10:90 v/v)		

MS Conditions

Instrumentation:	Thermo Scientific TSQ Vantage

Ionization conditions	HESI
Polarity	Positive
Spray voltage (V)	3500
Vaporizer temp (°C)	450
Sheath gas pressure (Arb)	40
Aux gas pressure (Arb)	30
Capillary temp (°C)	270
Collision pressure (mTorr)	1.5
Scan time (s)	0.500
Q1 (FWHM)	0.70
Q3 (FWHM)	0.70

Table 2: TSQ Vantage™ conditions

Compound	Zafirlukast	Glybenclamide
Parent (m/z)	574.32	492.24
Products (m/z)	462.24	367.147
Collision energy (V)	32	30
S-lens	178	153

Table 3: Compound transition details

Data Processing

Software:	Thermo Scientific LC QUAN

Results

The dynamic range was shown to be linear between 50 and 500 ng/mL with a r^2 (goodness of fit) of 0.9903 (Figure 2). QC samples were run in replicate (n= 5) at a mid range concentration of 200 ng/mL and the precision for these calculated to be 4.2% (Table 2). The calculated recovery was 85%.

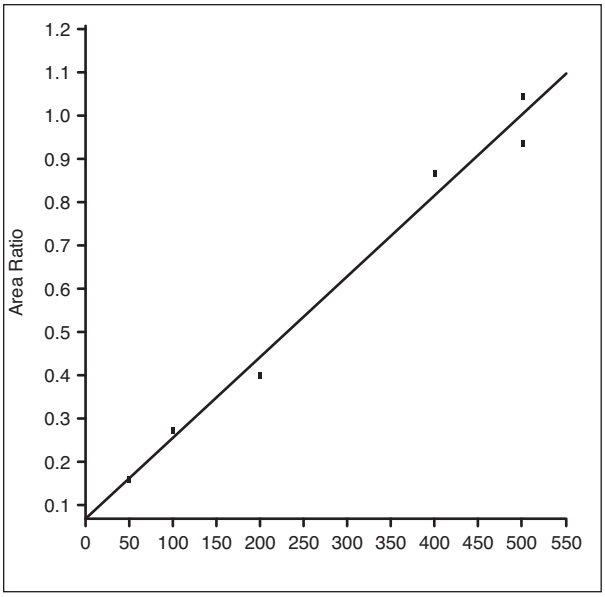

Figure 2: Extracted calibration line

Single level Standards	Actual Amount /ng.ml^{-1}	Calculated Amount /ng.ml^{-1}	Accuracy
1	200	223.37	111.68%
2	200	216.21	108.10%
3	200	218.76	109.38%
4	200	210.32	105.16%
5	200	200.16	100.08%
Mean	200	213.76	106.02%
RSD		4.19%	

Table 4: Determination of zafirlukast in spiked plasma samples using an internal standard

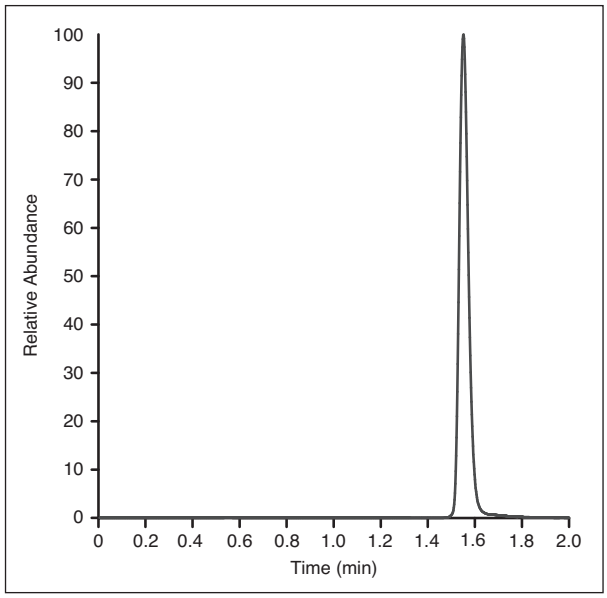

Figure 3: Chromatogram of zafirlukast in extracted human plasma

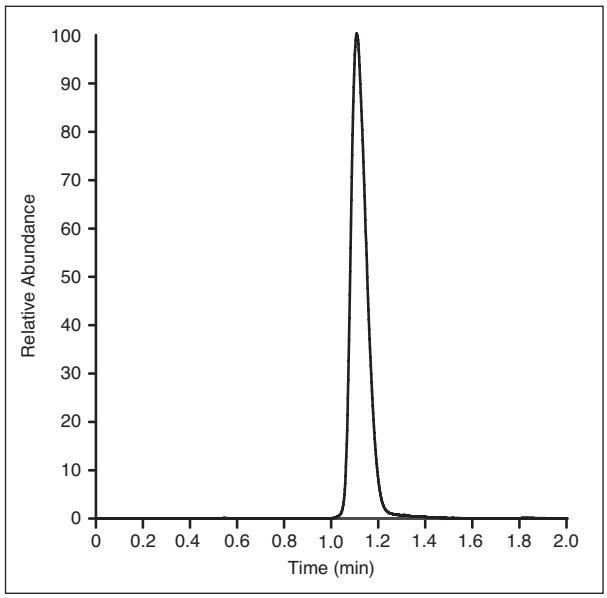

Figure 4: Chromatogram of glybenclamide (IS)

Conclusions

Retain AX SPE cartridges and Accucore RP-MS HPLC column can be used to extract and quantify zafirlukast from human plasma. The extracts were clean and showed no carry over. In this application we demonstrated that:

- Accucore RP-MS column can be used to analyze and quantify zafirlukast in less than 2 minutes
- Good method accuracy and precision was achieved
- Hypersep Retain AX gives good recovery of Zafirlukast
- Hypersep Retain AX is effective in removing endogenous interferences from a plasma matrix

References

[1] Drug Information, http://www.drugs.com/mtm/zafirlukast.html, Dated 24/11/2011

[2] The Internet Drug Index, http://www.rxlist.com/accolate-drug.htm, Dated 24/11/2011

[3] FDA, US Food and Drug Administration http://www.fda.gov/Safety/MedWatch/SafetyInformation/SafetyAlertsforHumanMedicalProducts/ucm166246.htm, Dated 29/11/2011

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