Direct analysis of basic and acidic drugs in rat plasma using on-line extraction LC/MS/MS

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Abstract

During the last ten years, pharmaceutical companies have constantly pushed for shorter analysis time in order to breach the one-thousand-analyses-per-day barrier. With this demand for high speed analysis, new techniques, such as 96well plates, fast gradients or ultra-high-flow chromatography, are showing promising results.

Recently, we have focused our attention toward on-line extraction techniques for high-throughput analysis. We are injecting a plasma sample, without pretreatment, onto an extraction column at high flow rate (i.e. 4mL/min) (1-3) to remove macromolecular compounds such as proteins, but trap smaller analytes on the head of the column.

Abstract (continued)

Several configurations for direct injection are possible. In the simplest configuration, the extraction column is connected directly to the MS/MS system. Other versions are configured with a single or a dual extraction column coupled to an analytical column. It is often necessary to split the flow. However, in cases where sensitivity is low, this option is not recommended. For efficient high speed analysis, the use of a second pump and a 10 port valve is also a good choice. One line (high flow rate) can be dedicated to the extraction column, while the other (low flow rate) drives the analytical column and the mass spectrometer.

Abstract (continued)

Three different configurations were studied for the analysis of 2 basic drugs in rat plasma. The on-line analysis was performed on an Oasis® HLB extraction column $(2.1 \times 20 \text{ mm}, 25 \text{ um})$ using a Waters Alliance 2790^{TM} in the gradient mode and a 515 stand-alone pump in the isocratic mode. In the dual column configuration, the analytes were backward flushed onto an XTerra column ($2.1x30, 3.5 \mu m$) or forward flushed onto a Symmetry column (2.1x30, 3.5) μ m). These columns were added to provide additional separation power. The drugs were quantified using a MicroMass UltimaTM triple quadrupole mass spectrometer equipped with an electrospray source and operated in the multiple reaction monitoring mode (MRM).

Common Setup for On-line SPE-LC/MS/MS



10 Port Valve Setup for On-line SPE-LC/MS/MS



On-line Oasis® HLB Extraction Column



Oasis is a trademark of Waters Corporation

• Features

- Direct plasma injection
- >100 injections per column
- Compatible with rapid
 HPLC gradients
- Fast cycle time for MS detection
- Description:
 - Sorbent: Oasis® HLB
 - 2.1 mm I.D. x 20 mm 25 µm

Oasis[®] HLB sorbent Hydrophilic-Lipophilic Balanced copolymer





Di-Vinyl-Benzene

Water Loving

- Hydrophilic monomer
- Provide wetting properties
- No impact of sorbent drying

Fat Loving Lipophilic monomer

-Provide reverse phase property for analyte retention

On-line Protocol for Clemastine in Rat Plasma Oasis[®] HLB Extraction Column

Goals : High Loading Flow Rate and Low Elution Flow Rate

Oasis[®]HLB Extraction Column 2.1x20 mm, 25 μm

Sample preparation: centrifuge rat plasma

Sample preparation: Spike 5 mL of rat plasma + 100 μ L NH₄OH

Sample preparation: 500 μ L of spiked rat plasma + 400 μ L IS in water

Loading: 200 µL at 4 mL/min in 100 % water

Elution: 0.4 mL/min gradient 5% ACN to 95 % ACN in 1 minute

HPLC Gradient and Wash Conditions

Time	HPLC gradient Flow 0.4 mL/min		Valve position		
	Α	В	Function		
0.0 0.5	5	95	valve position 2 to 1		
1.0	95	5	Ĩ		
2.60	95	5			
2.90		. .	valve position 1 to 2		
3.0	5	95			

A - Acetonitrile + 0.5 % Formic Acid

B - Water + 0.5 % Formic Acid

Oasis® HLB Column Configuration



Injection position



 LC_1 : Alliance 2790 - 0.4 mL/min LC_2 : Waters 515 - 4.0 mL/min Loading mobile phase: 100 % water Eluting mobile phase: 1 minute gradient 5% ACN to 95% ACN Eluting mobile phase additive: 0.5 % Formic acid Extraction column temperature: 40 °C Switching valve: Rheodyne LabPro 10 ports, 2 position MS: Quattro Ultima Triple Quadrupole Source: Electrospray positive Source temperature: 150 °C Desolvation gas: 600 L/hr Gas cell: 1.5e-3 mbar Cone Voltage: 20 volts Collision energy: 20

Clemastine Daughter Mass Spectra



Oasis[®] HLB LC/MS/MS Analysis of Clemastine at 2.5 ng/mL and 250 ng/mL



Oasis® HLB Clemastine Calibration Curve

Conc. ng/mL N=6	1.0	2.5	5.0	10.0	100	200	250
Average	0.98	2.56	5.25	9.5	101.43	201.14	247.1
Standard Deviation	0.02	0.11	0.12	0.25	2.69	3.2	1.58
RSD %	2.1	4.4	2.3	2.7	2.6	1.6	0.6
18.5 Coefficient of Determination: 0.999397 Calibration curve: 1.59441e-6 * x^2 + 0.0733900 * x + 0.00620068 Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area) Curve type: 2nd Order, Origin: Exclude, Weighting: 1/x^2, Axis trans: None 0							

Oasis[®] HLB/XTerra[®] Columns Configuration



Injection position (backflush configuration



LC₁: Alliance 2790 - 0.4 mL/min LC₂: Waters 515 - 4.0 mL/min Loading mobile phase: 100 % water Eluting mobile phase: 1 minute gradient 5% ACN to 95% ACN Eluting mobile phase additive: 0.5 % Formic acid Extraction column temperature: 40 °C Switching valve: Rheodyne LabPro 10 ports, 2 position MS: Quattro Ultima Triple Quadrupole Source: Electrospray positive Source temperature: 150 °C Desolvation gas: 600 L/hr Gas cell: 1.5e-3 mbar Cone Voltage: 20 volts Collision energy: 20

Oasis[®]HLB/XTerra[®] LC/MS/MS Analysis of Clemastine at 2.5 ng/mL and 250 ng/mL



Dual Oasis[®]/XTerraTM Configuration



 $\label{eq:LC1} \begin{array}{l} \text{LC}_1\text{: Alliance 2790 - 0.4 mL/min} \\ \text{LC}_2\text{: Waters 515 - 4.0 mL/min} \\ \text{Loading mobile phase: 100 \% water} \\ \text{Eluting mobile phase: 1 minute gradient 5% ACN to 95% ACN} \\ \text{Eluting mobile phase additive: 0.5 \% Formic acid} \\ \text{Extraction column temperature: 40 °C} \\ \text{Switching valve: Rheodyne LabPro 10 ports, 2 position} \end{array}$

MS: Quattro Ultima Triple Quadrupole Source: Electrospray positive Source temperature: 150 °C Desolvation gas: 600 L/hr Gas cell: 1.5e-3 mbar Cone Voltage: 20 volts Collision energy: 20

HPLC Gradient and Valve Positions

Time	HPLC Flow	C gradient 0.4 mL/min	Valve position			
	Α	В	Function			
0.0	5	95				
0.5	5	95	valve 2 - position 1 to 2			
			valve 1 - position 2 to 1			
			valve 3 - position 2 to 1			
1.5	95	5				
2.50	95	5				
3.0	5	95	valve 2 - position 2 to 1			
			valve 3 - position 1 to 2			

A - Acetonitrile + 0.5 % Formic Acid or 0.5 % NH_4OH B - Water + 0.5 % Formic Acid or 0.5 % NH_4OH

Dual HLB[®]/XTerraTM LC/MS/MS Analysis of Clemastine at 2.5 ng/mL and 250 ng/mL



Sequential versus Parallel



HPLC Gradient and Valve Positions

Time	HPLC gra Flow 0.4 1	adient mL/min	Valve position		
0.0 (elution)	A 5	B 95	Function valve 2 – position 1 to 2		
0.5 (loading)			valve 3 - position 2 to 1		
1.0 1.7	95 95	5			
2.0	5	95	valve 2 - position 2 to 1 valve 3 - position 1 to 2 valve 1 - position 1 to 2		

A - Acetonitrile + 0.5 % Formic Acid or 0.5 % NH_4OH

B - Water + 0.5 % Formic Acid or 0.5 % NH_4OH

Dual Oasis[®]HLB LC/MS/MS Analysis of Clemastine at 2.5 ng/mL and 200 ng/mL



Clemastine Calibration Curve

Conc. ng/mL N=6	1.0	2.5	5.0	10.0	100	200	250	
Average	1.0	2.5	4.9	9.9	106.7	196.1	246.9	
Standard Deviation	0.02	0.1	0.2	0.3	3.4	3.1	3.4	
RSD %	1.6	5.0	3.2	3.3	3.1	1.6	1.3	
2.00 Compound 2 name: clemasti Coefficient of Determination: Calibration curve: -6.71807e- Response type: Internal Std Curve type: 2nd Order, Origin	KSD % 1.6 3.0 3.2 3.3 3.1 1.6 1.3 2.00 Compound 2 name: clemastine Coefficient of Determination: 0.999859 Calibration curve: 6.71807e-6* x*2 ± 0.00964430 * x ± 0.00444024 Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area) Curve type: 2nd Order, Origin: Exclude, Weighting: 1/x*2, Axis trans: None Image: Compound 2 name: clemastine (IS Conc. / IS Area) Image: Compound 2 name: clemastine (IS Conc. / IS Area) Image: Compound 2 name: clemastine (IS Conc. / IS Area) Image: Compound 2 name: clemastine (IS Conc. / IS Area) Image: Compound 2 name: clemastine (IS Conc. / IS Area) Image: Compound 2 name: clemastine (IS Conc. / IS Area) Image: Compound 2 name: clemastine (IS Conc. / IS Area) Image: Compound 2 name: clemastine (IS Conc. / IS Area) Image: Compound 2 name: clemastine (IS Conc. / IS Area) Image: Compound 2 name: clemastine (IS Conc. / IS Area) Image: Compound 2 name: clemastine (IS Conc. / IS Area) Image: Compound 2 name: clemastine (IS Conc. / IS Area) Image: Compound 2 name: clemastine (IS Conc. / IS Area) Image: Compound 2 name: clemastine (IS Conc. / IS Area) Image: Compound 2 name: clemastine (IS Conc. / IS Area) Image: Clemastine (IS Conc. / IS Area) Image: Clemastine (IS Conc. / IS Area) Image: Clemastine (IS Area)							

Benefits of the Oasis® HLB on-line extraction column

- simple off-line sample preparation
- Rapid isolation of analyte from a complex sample matrix
- Rapid clean up
- Short cycle time (3.0 min)