

On-line SPE LC/MS/MS Configurations for Effective Biological Sample Preparation

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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 96-well 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.

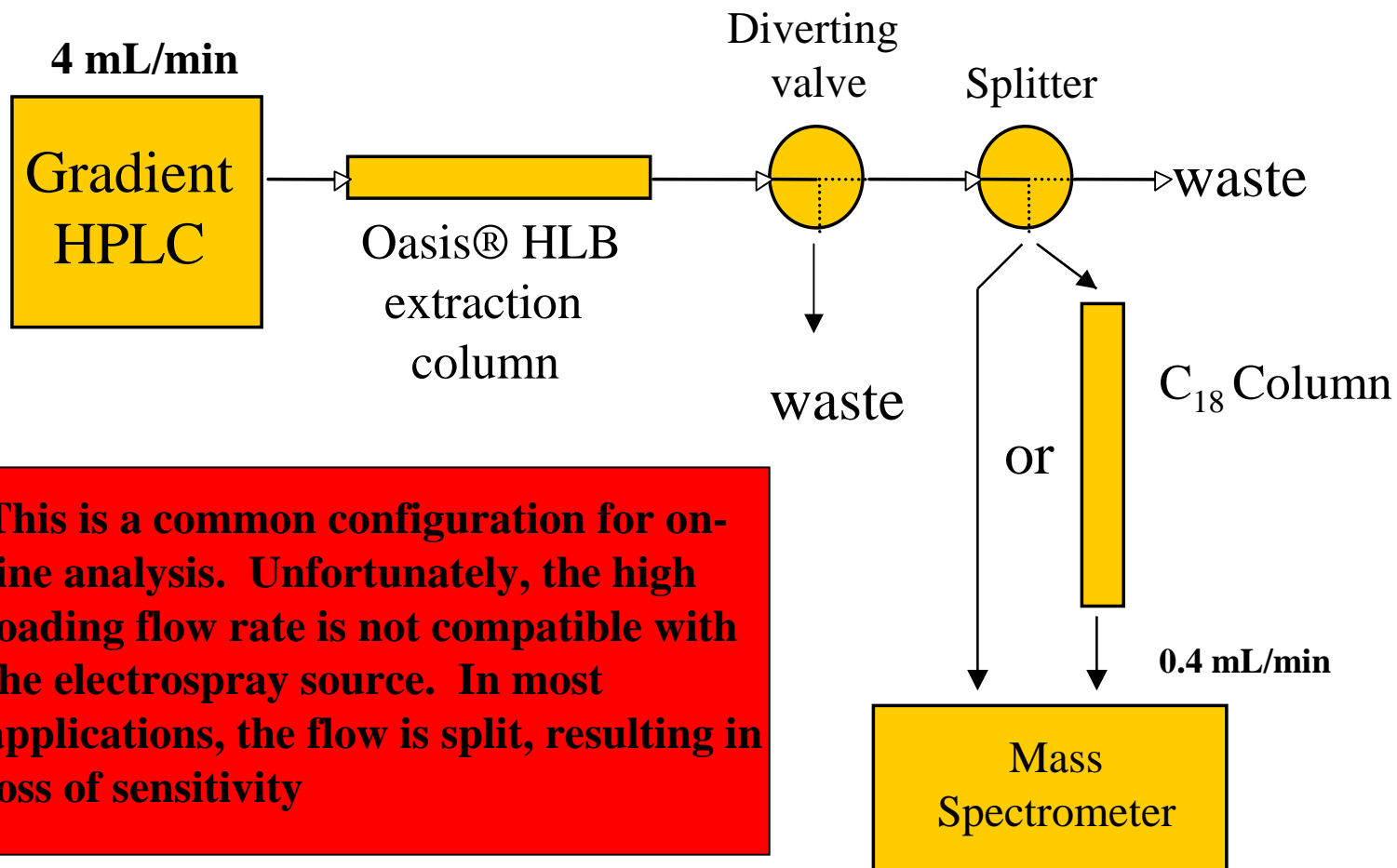
Abstract (continued)

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.

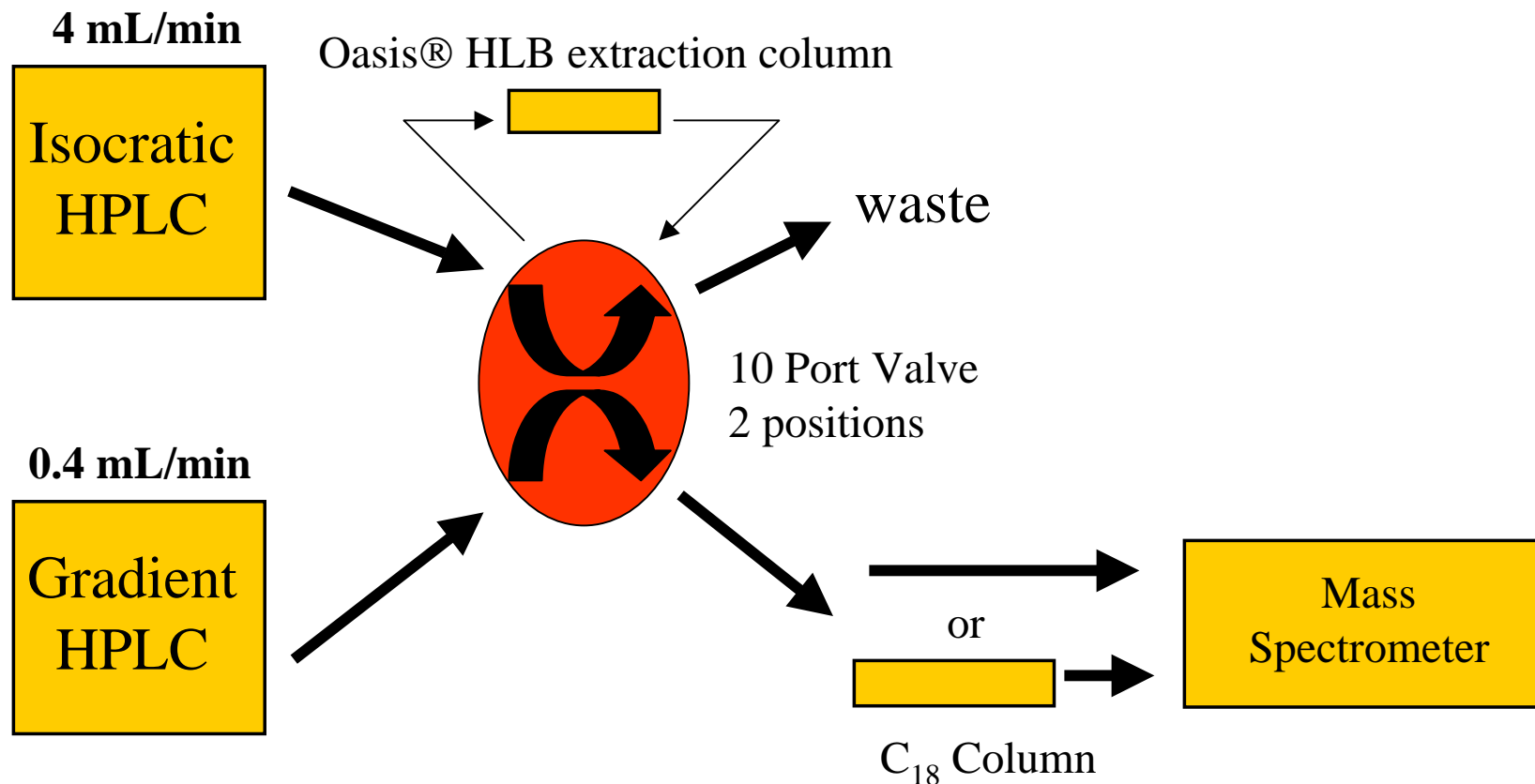
Abstract (continued)

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 (2.1x20mm, 25 μ m) using a Waters Alliance 2790™ 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 μ 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 Ultima™ 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 μ m

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 µL NH₄OH

Sample preparation: 500 µL of spiked rat plasma + 400 µL 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

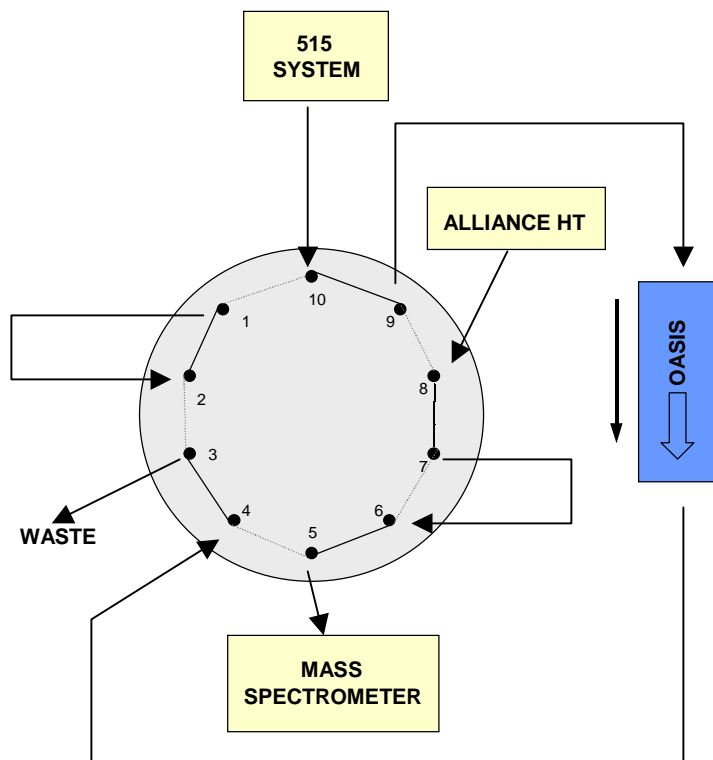
Time	HPLC gradient Flow 4 mL/min		Valve position
	A	B	Function
0.0	5	95	switch position 2 to 1
0.5			
1.0	95	5	
2.60	95	5	switch position 1 to 2
2.90			
3.0	5	95	

A - Acetonitrile + 0.5 % Formic Acid

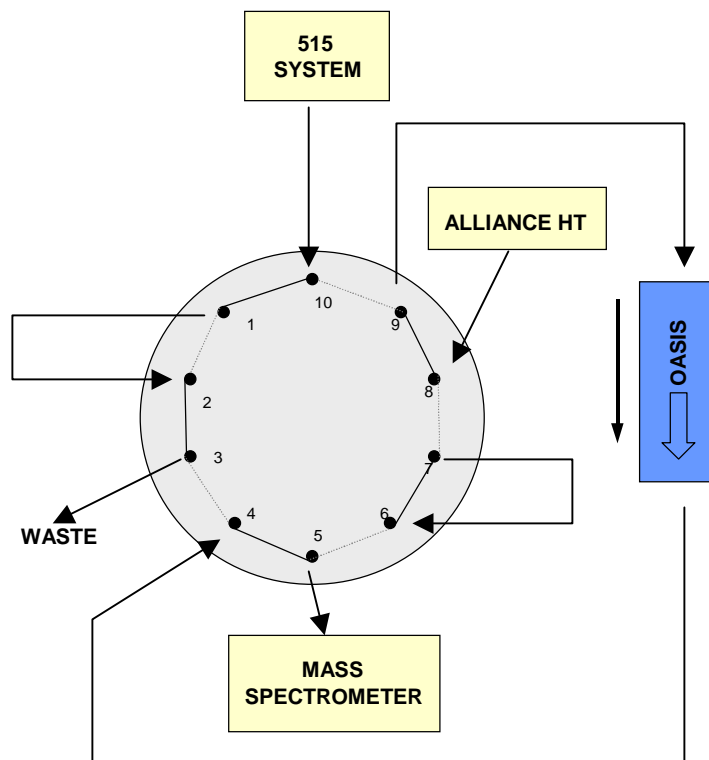
B - Water + 0.5 % Formic Acid

Oasis® HLB Column Configuration

Load position



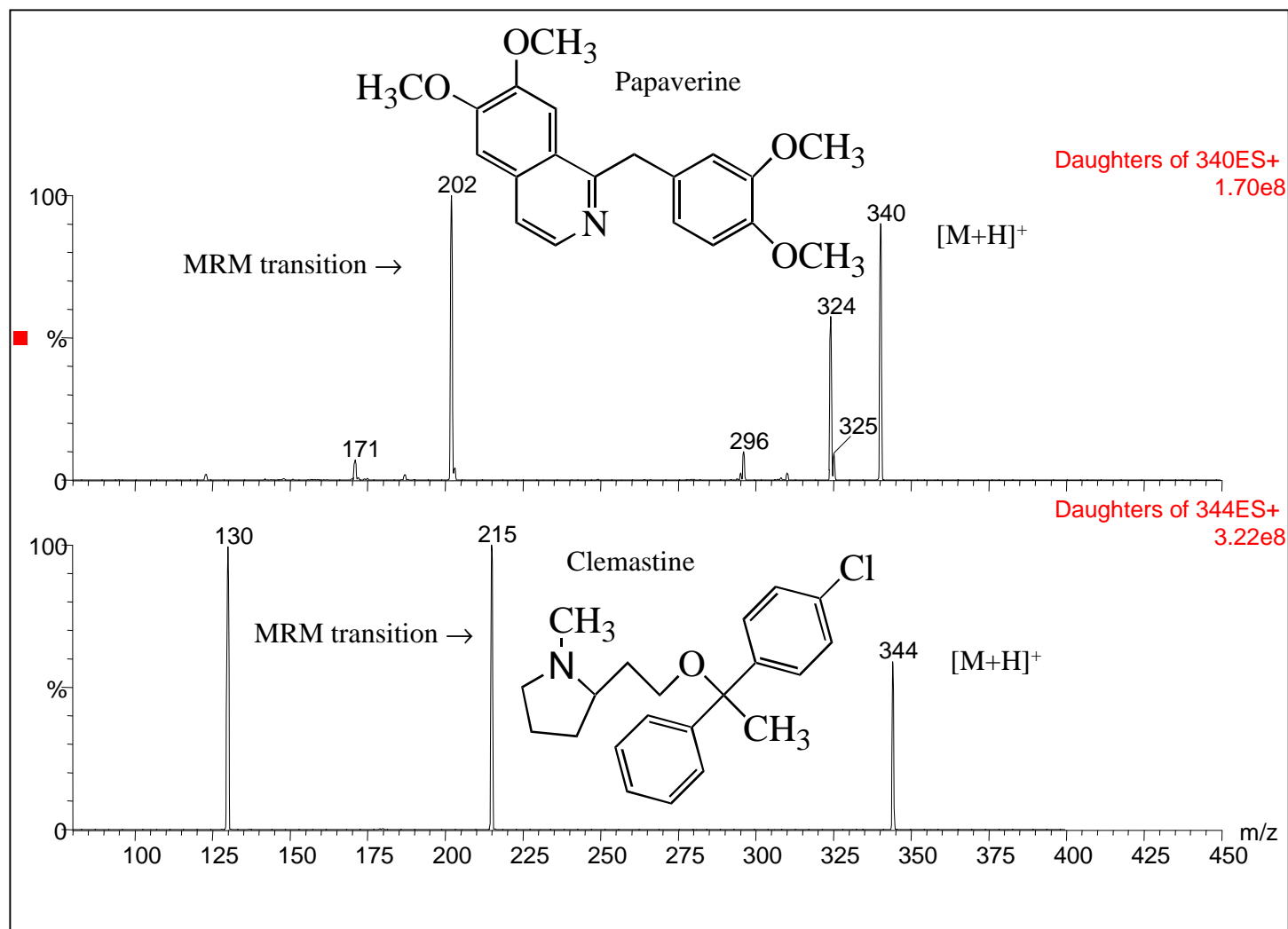
Injection position



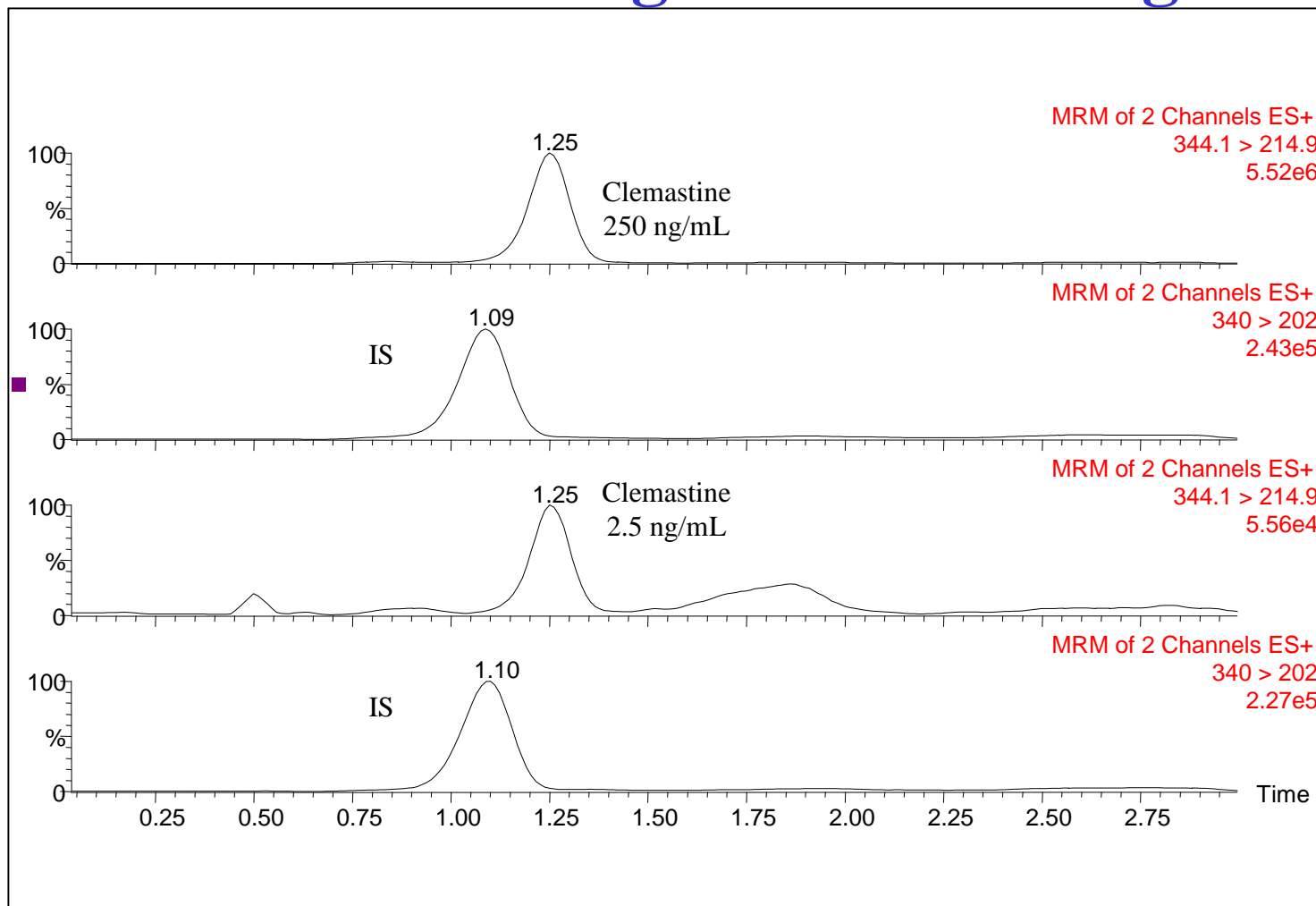
LC₁: Alliance 2790 - 0.4 mL/min
 LC₂: Waters 515 - 4.0 mL/min
 Loading mobile phase: 100 % water
 Eluting mobile phase: 1 minute gradient 5% ACN to 95% ACN
 Eluting mobile phase additive: 0.5 % Formic acid
 Extraction column temperature: 40 °C
 Switching valve: Rheodyne LabPro 10 ports, 2 position

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

Clemastine Daughter Mass Spectra

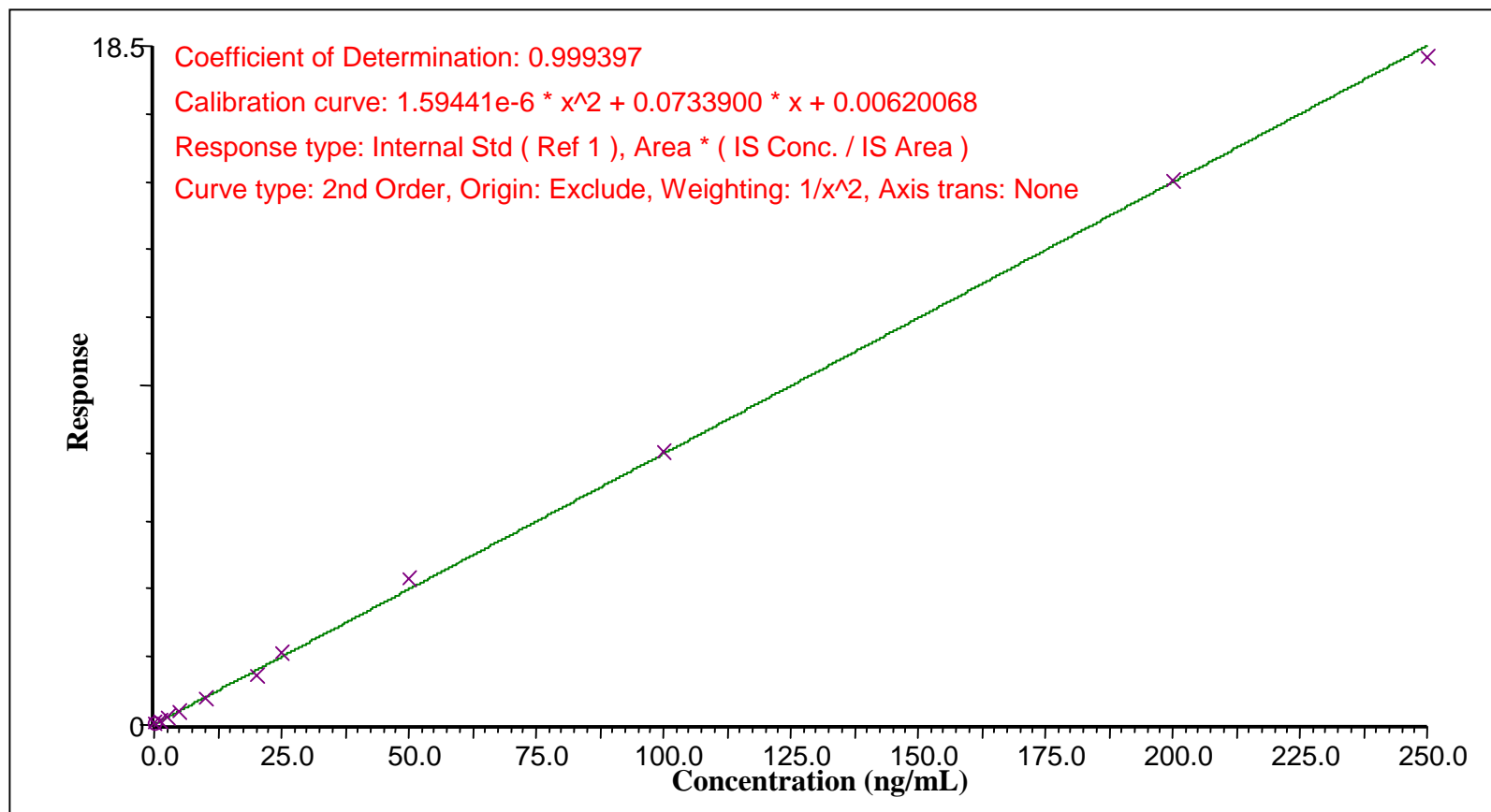


Oasis® HLB LC/MS/MS Analysis of Clemastine at 2.5 ng/mL and 250 ng/mL

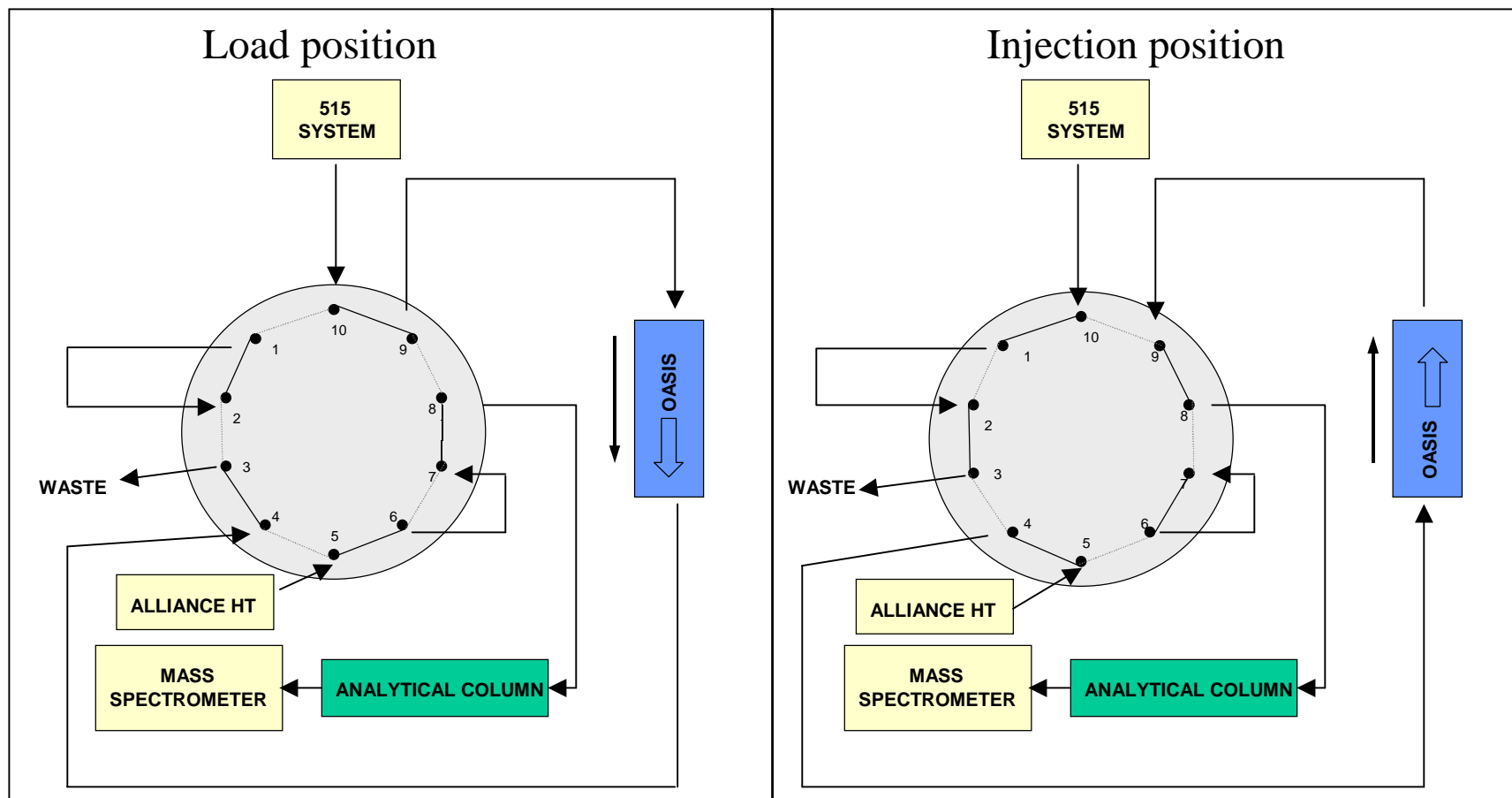


Oasis® HLB Clemastine Calibration Curve

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



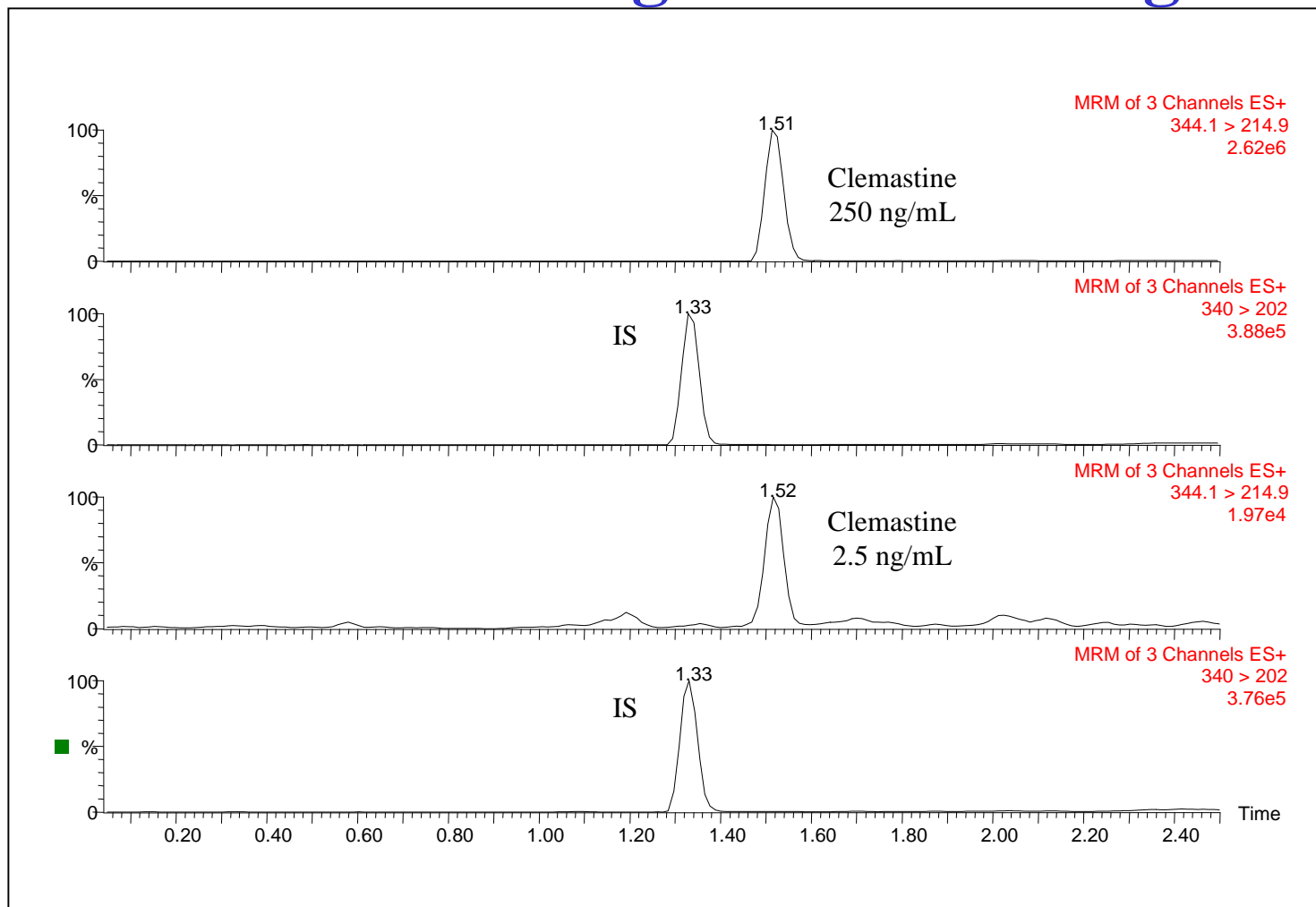
Oasis[®] HLB/XTerra[®] Columns Configuration



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

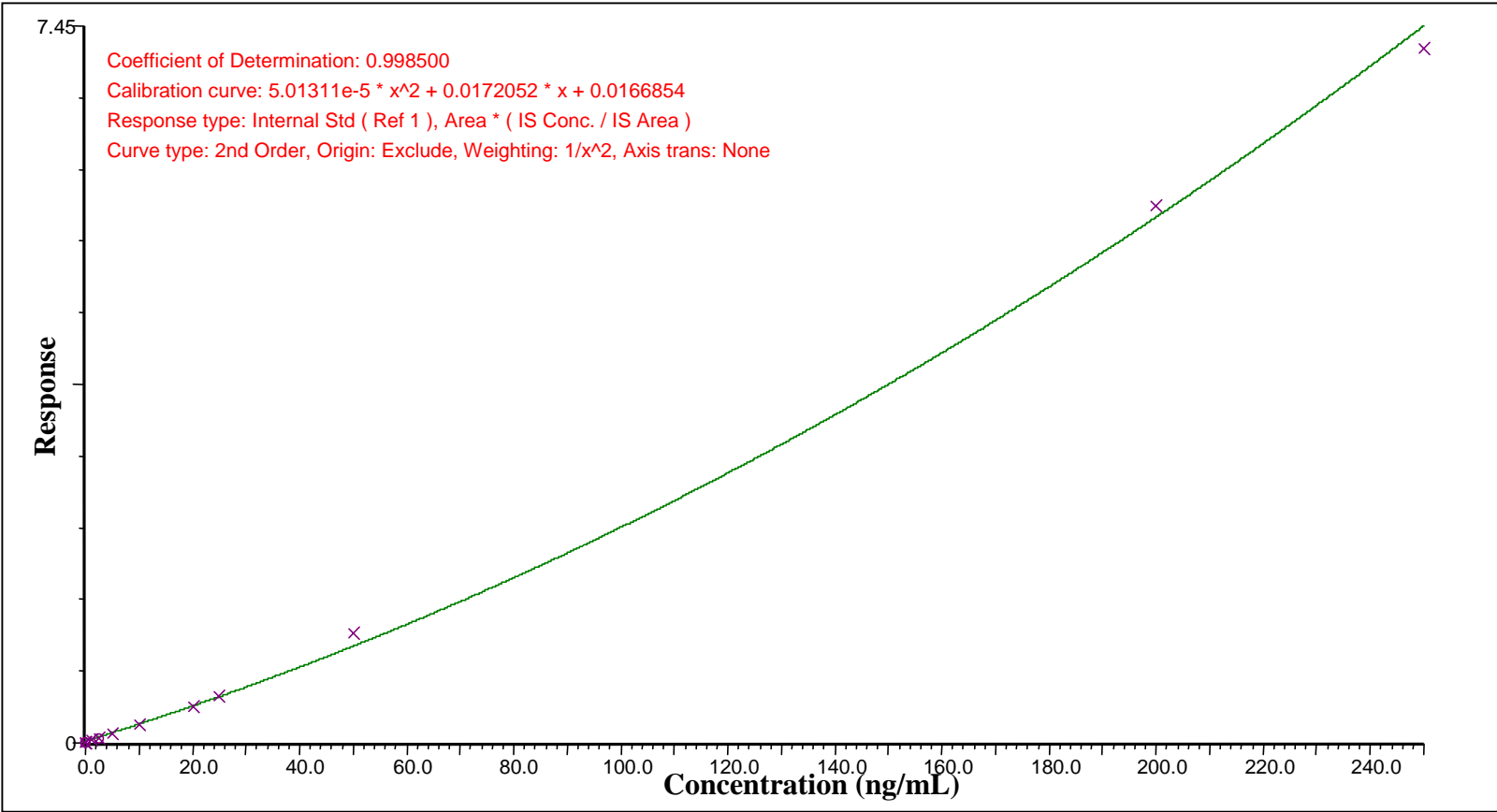
MS: Quattro Ultima Triple Quadrupole
Source: Electrospray positive
Source temperature: 150 °C
Desolvation gas: 600 L/hr
Gas cell: 1.5e-3 mbar
Cone Voltage: 20 volts
Collision energy: 20

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

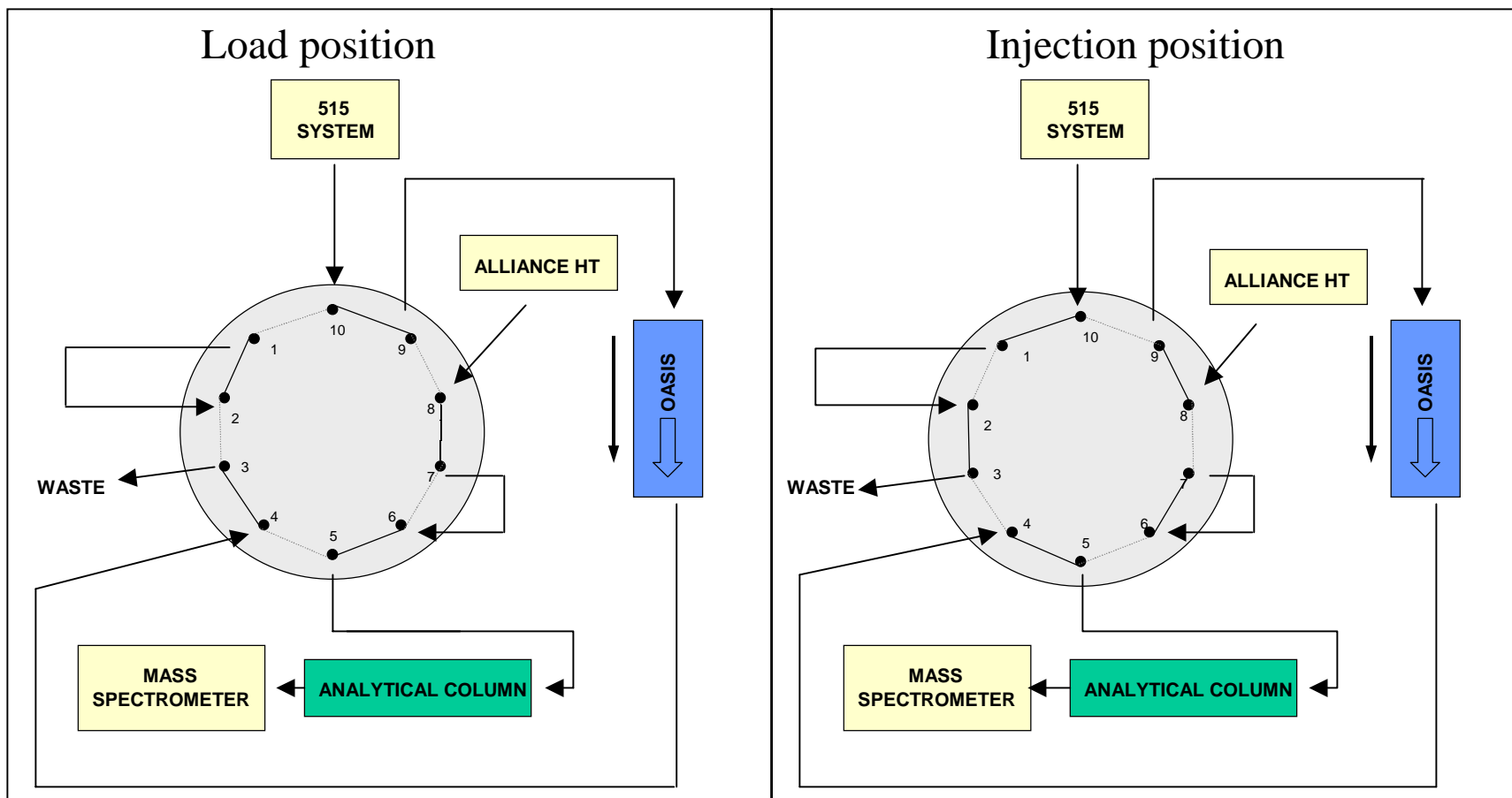


Oasis®HLB/XTerra® Clemastine Calibration Curve

Conc. ng/mL N=6	1	5	10	20	25	200	250
Average	1.01	4.8	9.7	20.3	24.3	206.9	243.1
Standard Deviation	0.01	0.1	0.2	0.9	0.9	4.2	3.1
RSD %	1.3	2.0	1.7	4.4	3.5	2.0	1.3



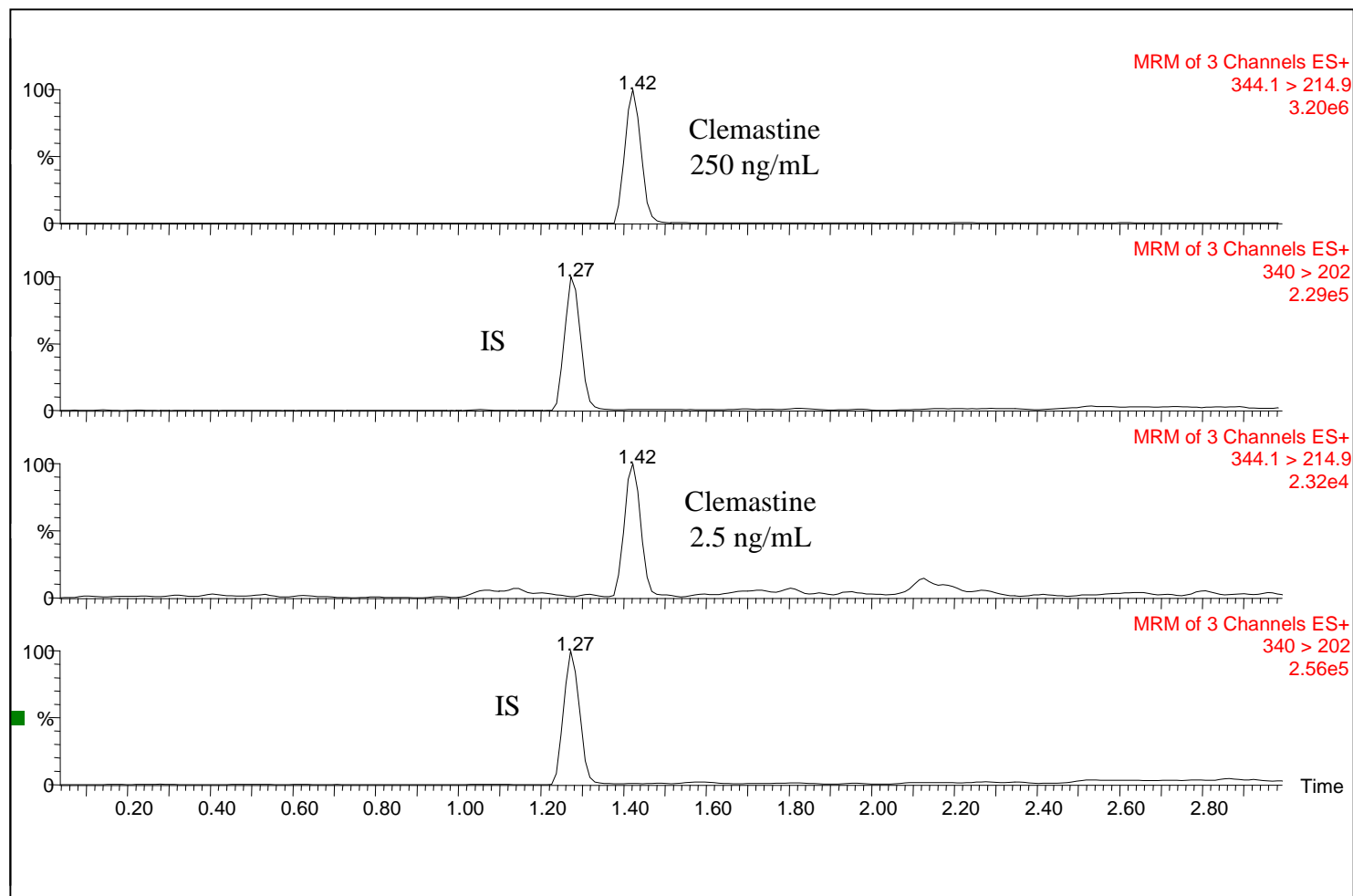
Oasis® HLB/Symmetry Columns Configuration



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

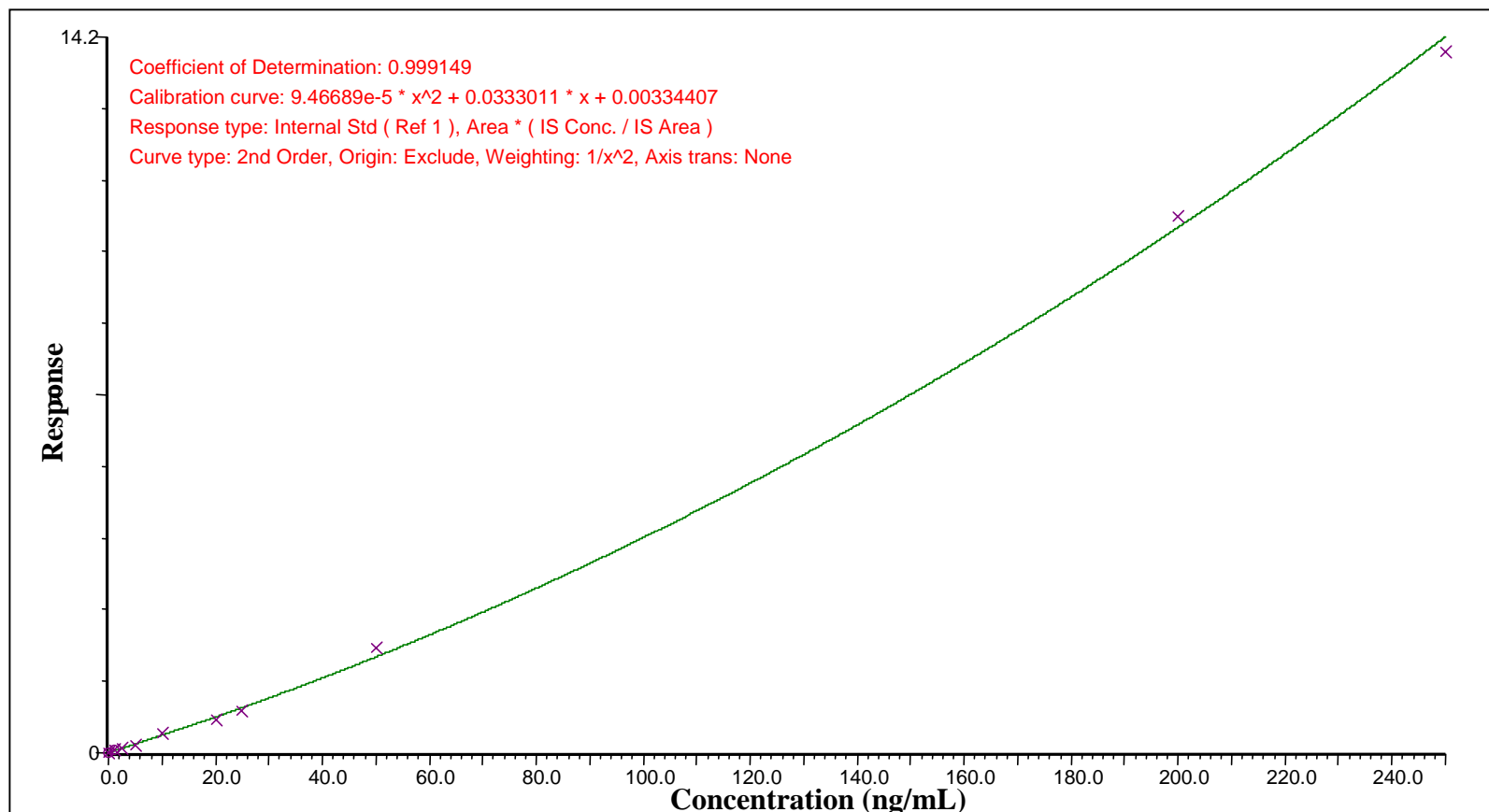
MS: Quattro Ultima Triple Quadrupole
Source: Electrospray positive
Source temperature: 150 °C
Desolvation gas: 600 L/hr
Gas cell: 1.5e-3 mbar
Cone Voltage: 20 volts
Collision energy: 20

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



HLB/Symmetry® Clemastine Calibration Curve

Conc. ng/mL N=6	1	5	10	20	25	200	250
Average	1.01	4.7	10.4	19.7	25.1	205.7	244.4
Standard Deviation	0.007	0.1	0.5	0.8	1.1	3.4	2.4
RSD %	0.7	2.8	4.3	4.0	4.2	1.7	1.0

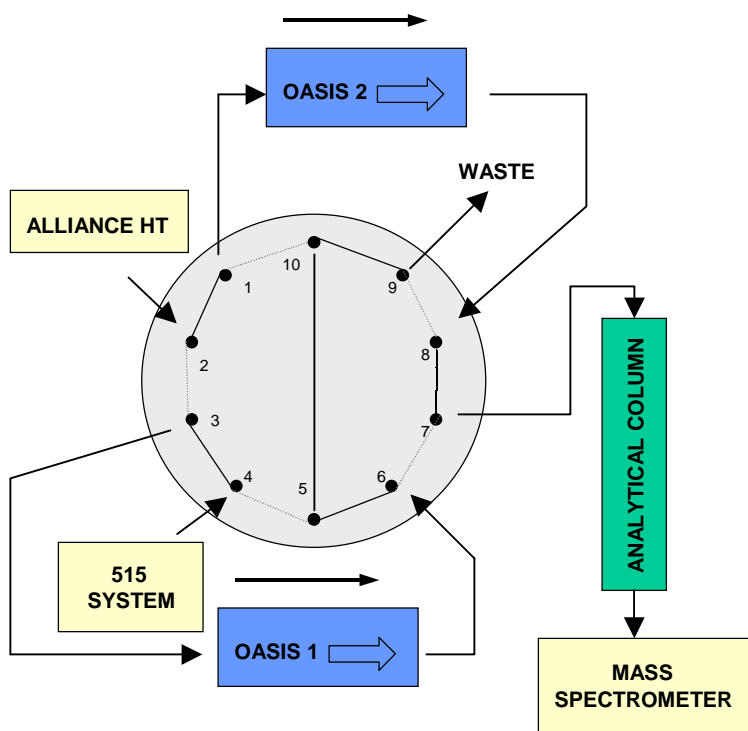


Benefits of the Oasis® HLB Extraction Column

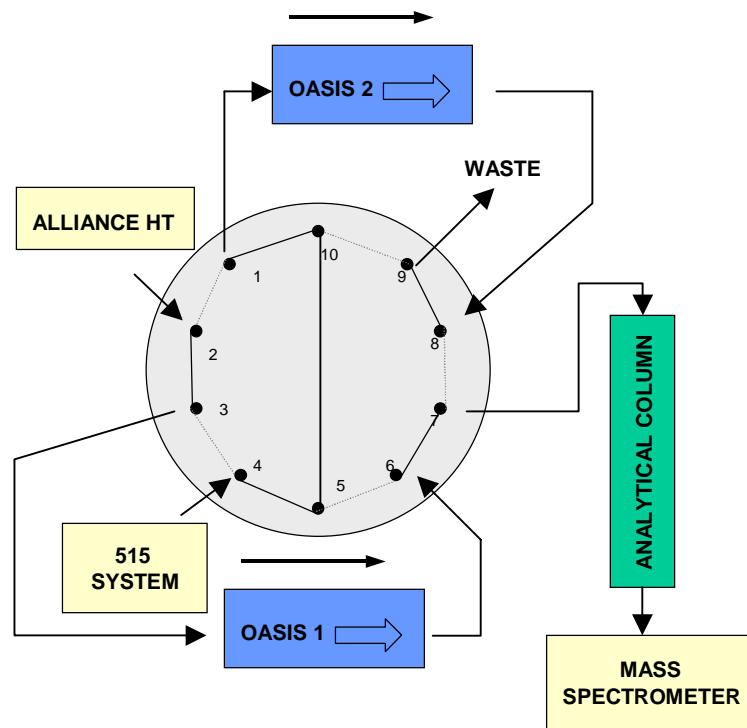
- No off-line sample preparation
- Rapid isolation of analyte from a complex sample matrix
- Rapid clean up
- Short cycle time (3.0 min)

Dual HLB/XTerra® Columns Configuration

Position 1: Load HLB 1 / Elute HLB 2



Position 2: Load HLB 2 / Elute HLB 1



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
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 Source temperature: 150 °C
 Desolvation gas: 600 L/hr
 Gas cell: 1.5e-3 mbar
 Cone Voltage: 20 volts
 Collision energy: 20