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Direct analysis of basic drugs in cell culture lysate using on-line extraction LC/MS/MS

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Today's Challenges Faced by Analytical Chemists in Methods Development

- Sample Preparation
  - Faster methods development
  - Generic and simple extraction protocol
  - Increased sensitivity and selectivity
- Instrumentation
  - Faster analysis (1000-analysis-per-day barrier)
  - On-line analysis (automation)

Sample Preparation

"Many extractions practices are based on classical methodologies of liquid-liquid or liquid-solid extraction using practices which have not changed for the last one hundred years"

Classical

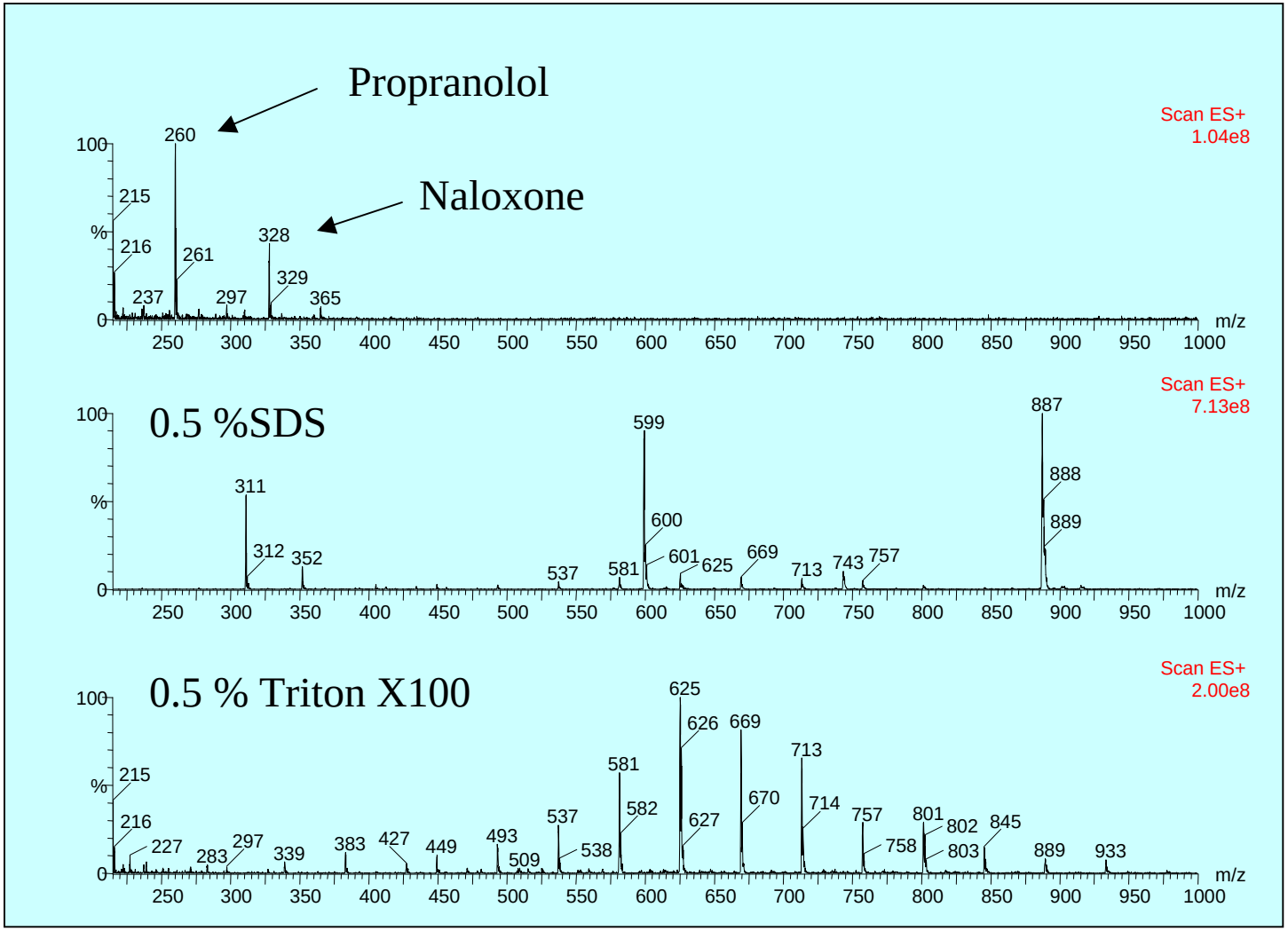
Protein precipitation
Liquid-liquid extraction
Membrane extraction
Soxhlet

New technologies

Solid phase extraction
Solid phase micro extraction
Accelerated fluid extraction
Supercritical fluid extraction
Microwave-assisted extraction

What happen is the sample is not in aqueous base ? Or contains surfactants?

Ion Suppression of Surfactants



Cell Lysing Protocols

10^6 Jurkat cells were grown in RPMI 1640 culture medium

Cells were centrifuged at 1500 g for 5 min

Cells were washed twice with cold PBS

Cells were centrifuged at 1500 g and supernatant was removed

Cell pellets were spiked at various level

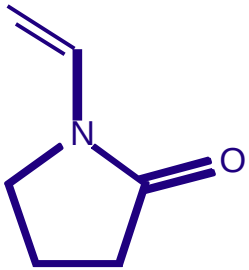
Lyse with 1 mL 50/50 MeOH/ACN → Dilute with 4 mL H_2O Inject 400 µL

Lyse with 1 mL 1 % SDS → Dilute with 4 mL H_20 Inject 400 µL

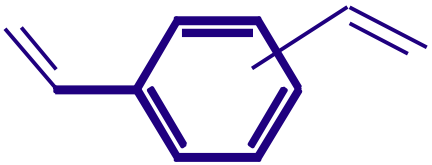
Lyse with 1 mL 1 % Triton X100 → Dilute 4 mL H_20 Inject 400 µL

Oasis® HLB sorbent

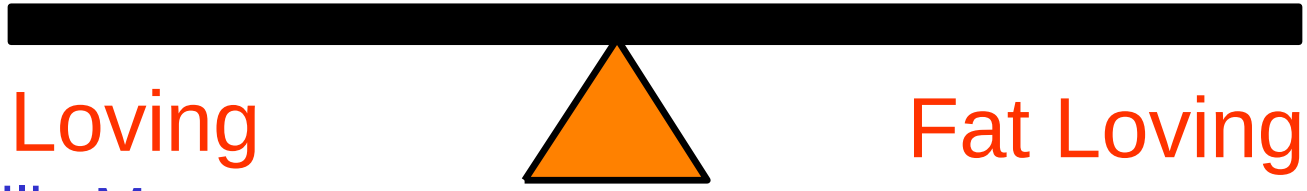
Hydrophilic-Lipophilic Balanced copolymer



N-Vinyl-Pyrrolidone



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 Oasis® HLB and MCX 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

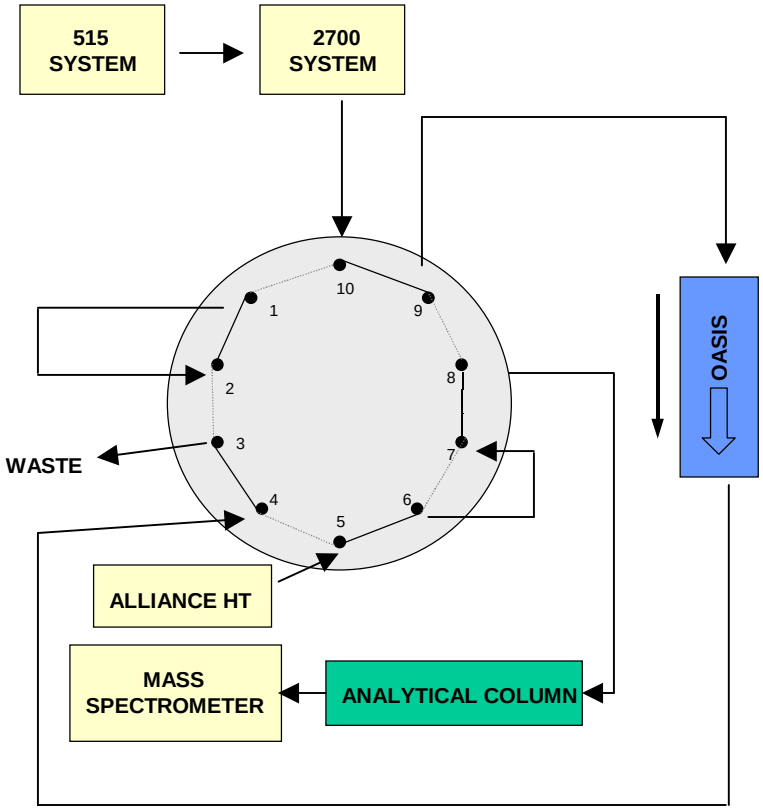
2 Positions Switching Valves

10 ports

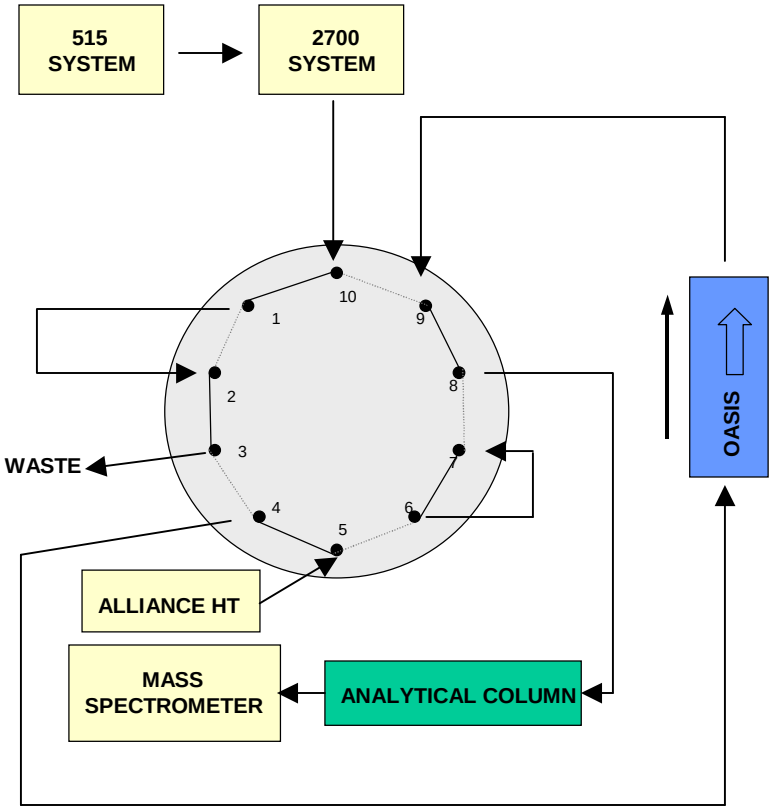
6 ports

Oasis® HLB/XTerra® Columns Configuration

Load position (backflush configuration)



Injection position (backflush configuration)



LC_1: Alliance 2690 - 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: room temperature
Switching valve: Rheodyne LabPro 10 ports, 2 position

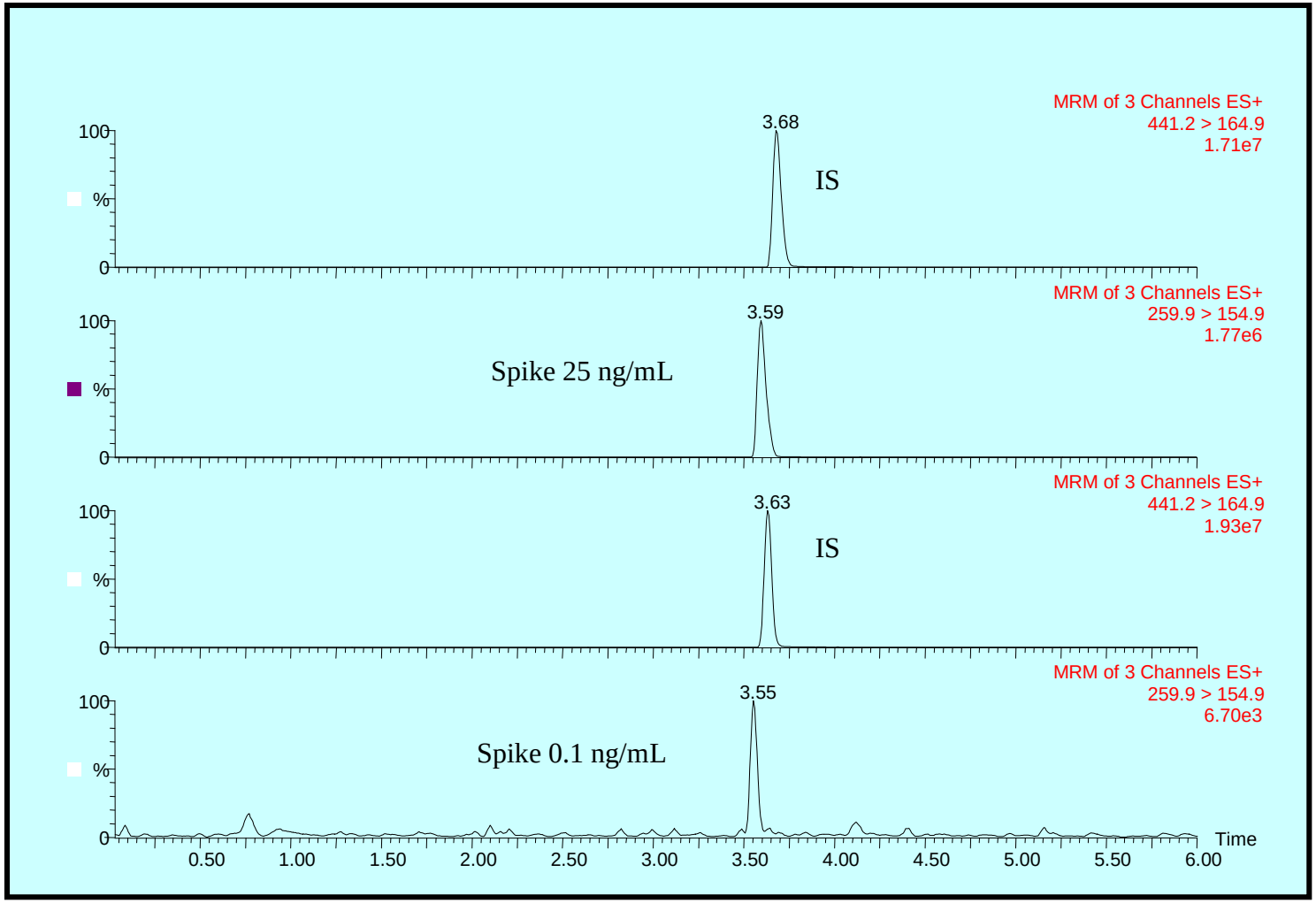
MS: Quattro Ultima Triple Quadrupole
Source: Electrospray positive
Source temperature: 150 °C
Desolvation gas: 600 L/hr
Gas cell: 2.0e-3 mbar
Cone Voltage: 20 volts
Collision energy: 20

HPLC Gradient and Wash Conditions

Time	HPLC gradient Flow 0.4 mL/min		Valve position	Function
	A	B		
0.0	5	95		
0.5	5	95	position 1	Loading with 100 % H_2O
1.5	95	5	position 2	Elution with 1 min gradient
4.40	95	5		
4.50	5	95	position 1	Return to loading position
6.0	5	95		

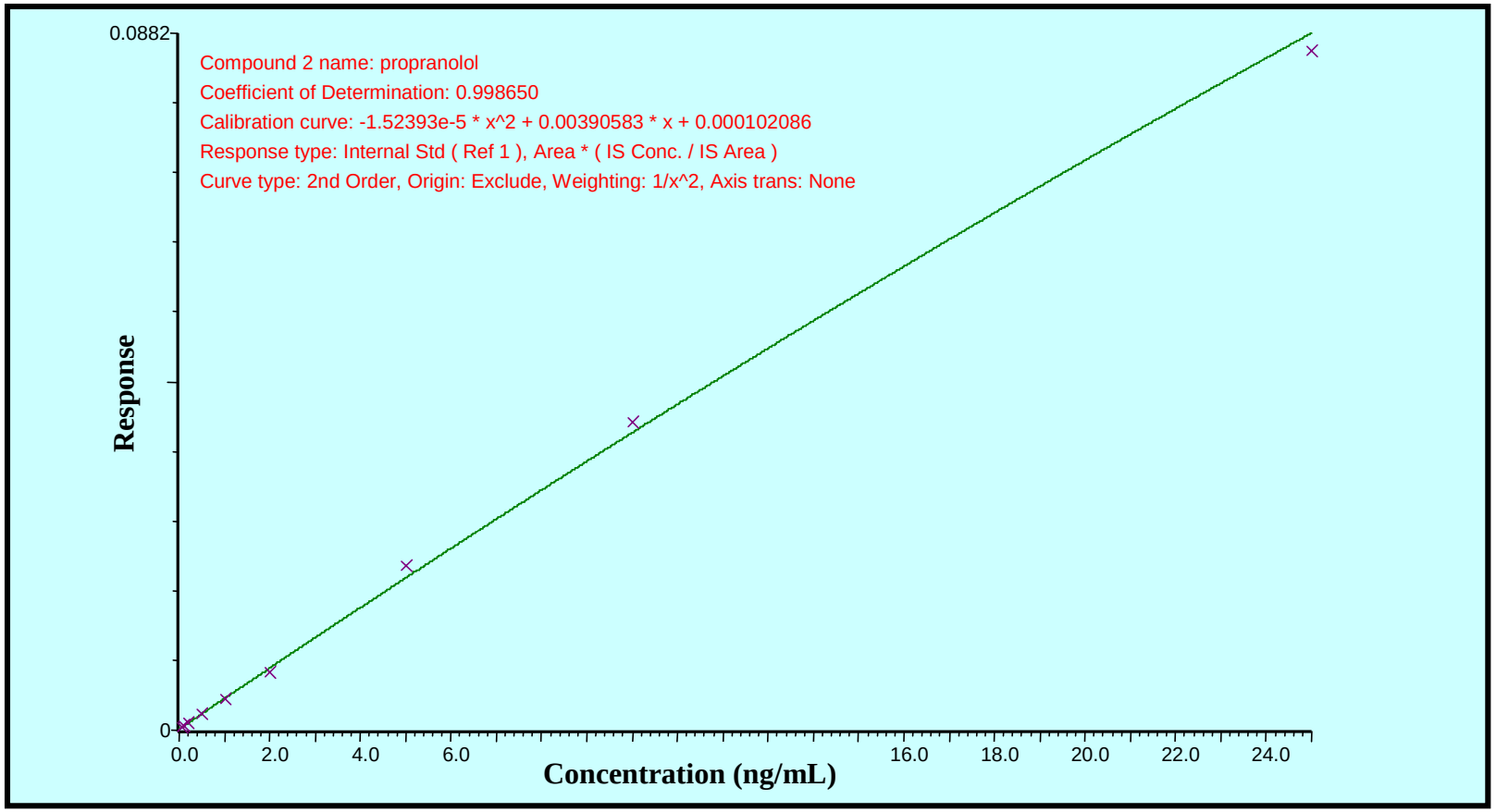
A - Acetonitrile + 0.5 % Formic Acid
B - Water + 0.5 % Formic Acid

50/50 MeOH/ACN Cell Lysing Propranolol at 0.1 ng/mL and 25 ng/mL

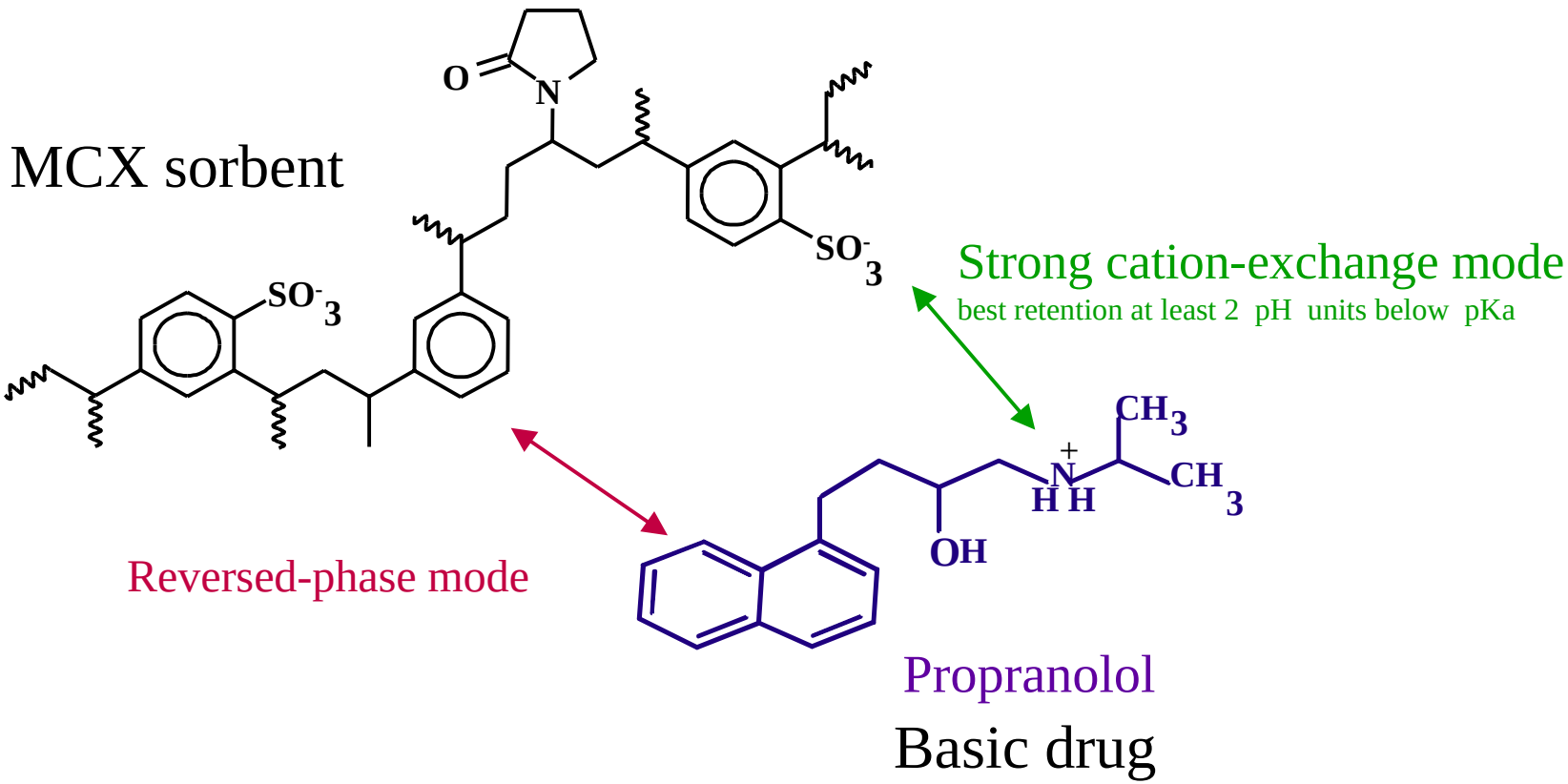


Propranolol Calibration Curve

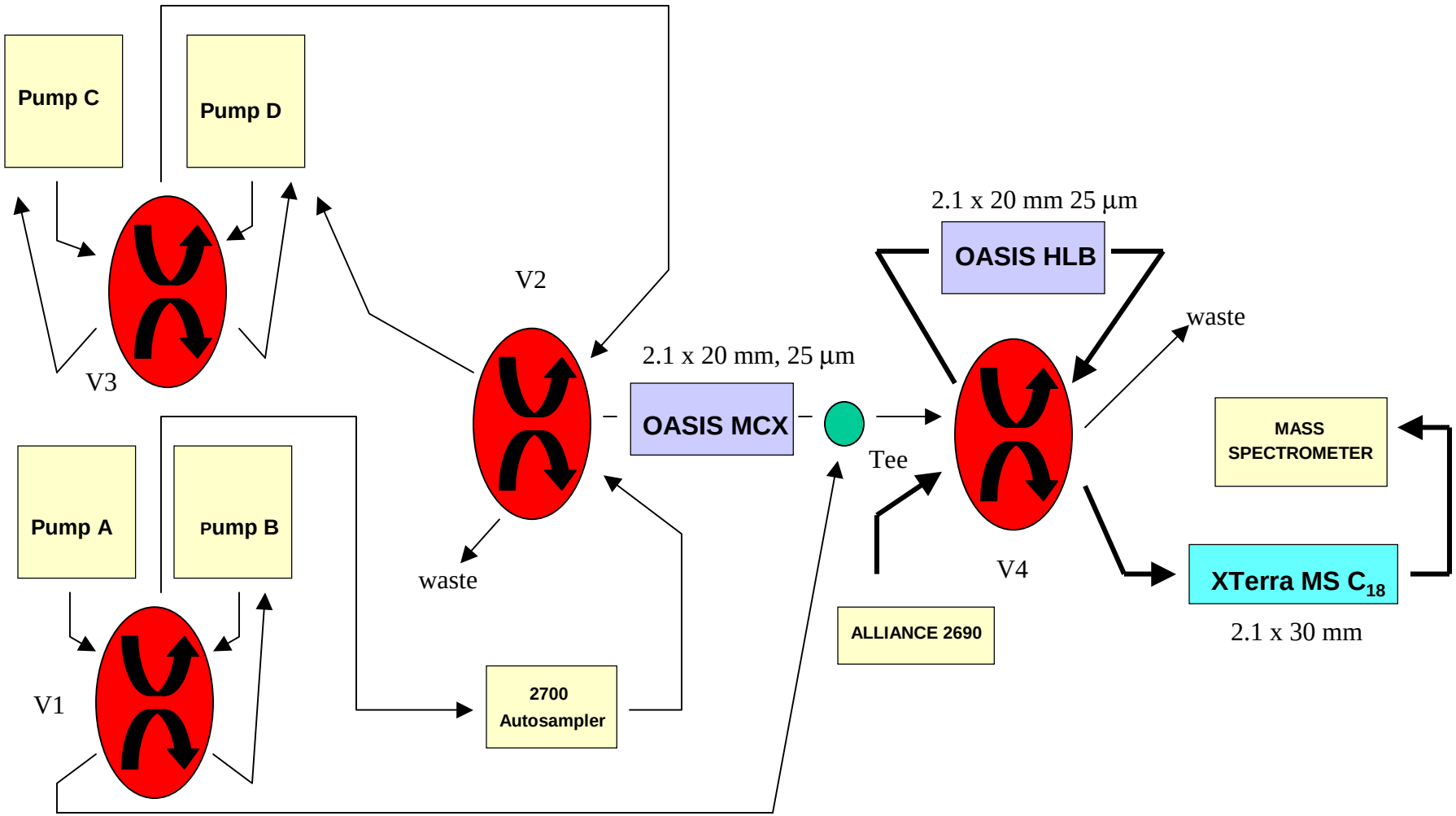
Conc. ng/mL N=6	0.1	0.5	1.0	5.0	10	20	25
Average	0.09	0.49	1.03	4.93	9.76	20.21	25.21
Standard Deviation	0.05	0.03	0.04	0.26	0.34	0.89	0.69
RSD %	4.8	5.9	3.9	5.3	3.5	4.4	2.7



Drug - Sorbent Interactions on Oasis® MCX Products



Oasis® HLB / MCX / XTerra® Configuration



Pump A: 100 % water + 2 % NH_4OH
Pump C: 100 % Water + 4 % Formic Acid

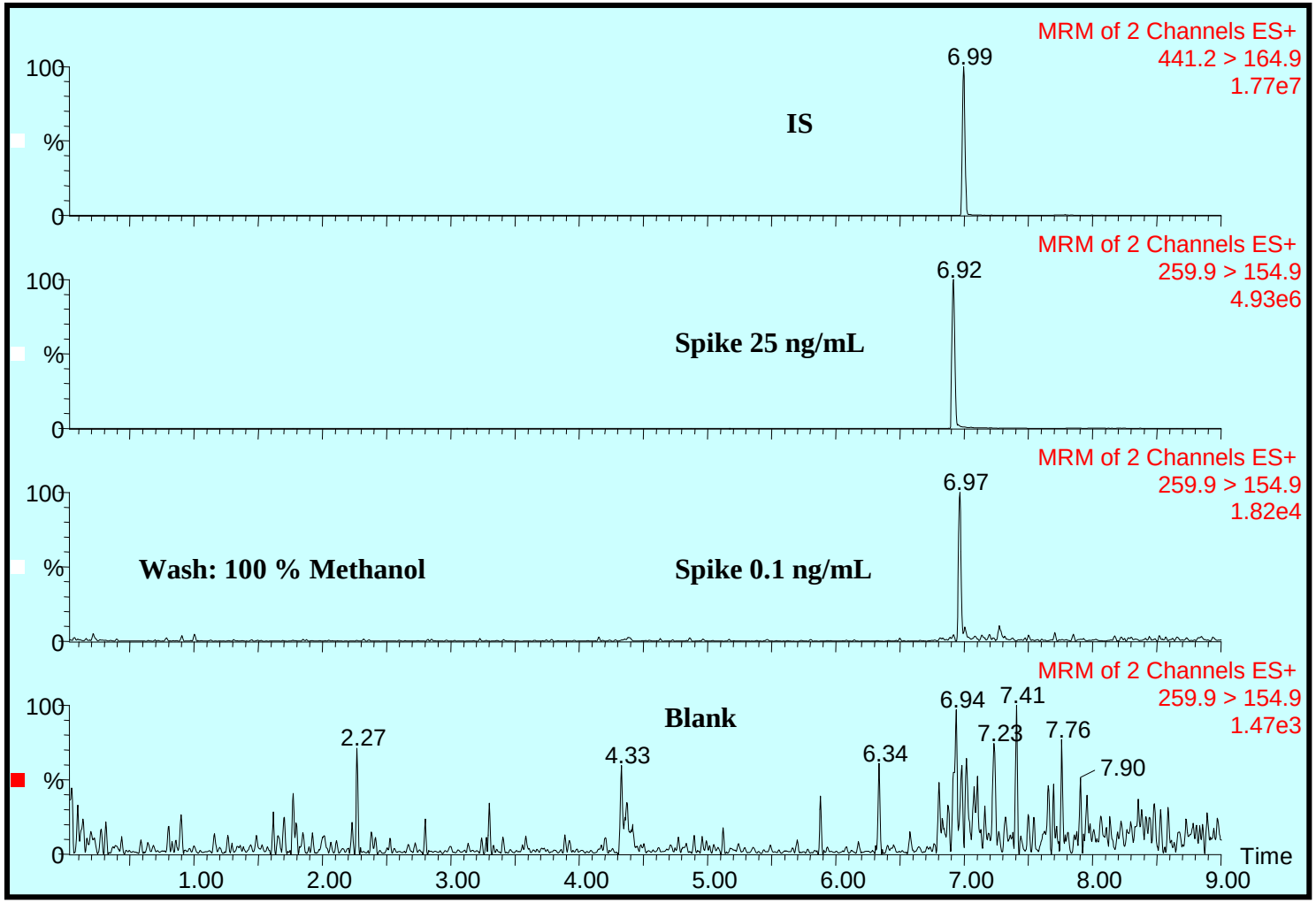
Pump B: 100 % Methanol
Pump D: 100 % Methanol + 2 % NH_4OH

HPLC Gradient and Wash Conditions

Time (min)	HPLC gradient Flow 0.4 mL/min		Valve Position V1, V2, V3, V4	Function
	A	B		
0.0	5	95	2 – 2 – 2 – 2	Load 100 % H_2O pH 11
1.0	5	95	1 – 1 – 2 – 2	Wash 100 % H_2O pH 2
2.0	5	95	1 – 2 – 2 – 2	Wash (see chromatograms)
3.0	5	95	1 – 1 – 1 – 2	Elution of MCX onto HLB (pH 11)
4.0	5	95	1 – 1 – 1 – 1	Elution of HLB onto Xterra (pH 3)
5.0	95	5		
7.0	95	5		
7.5	5	95		
8.0	5	95	2 – 2 – 2 – 2	Reset to starting position
9.0	5	95		

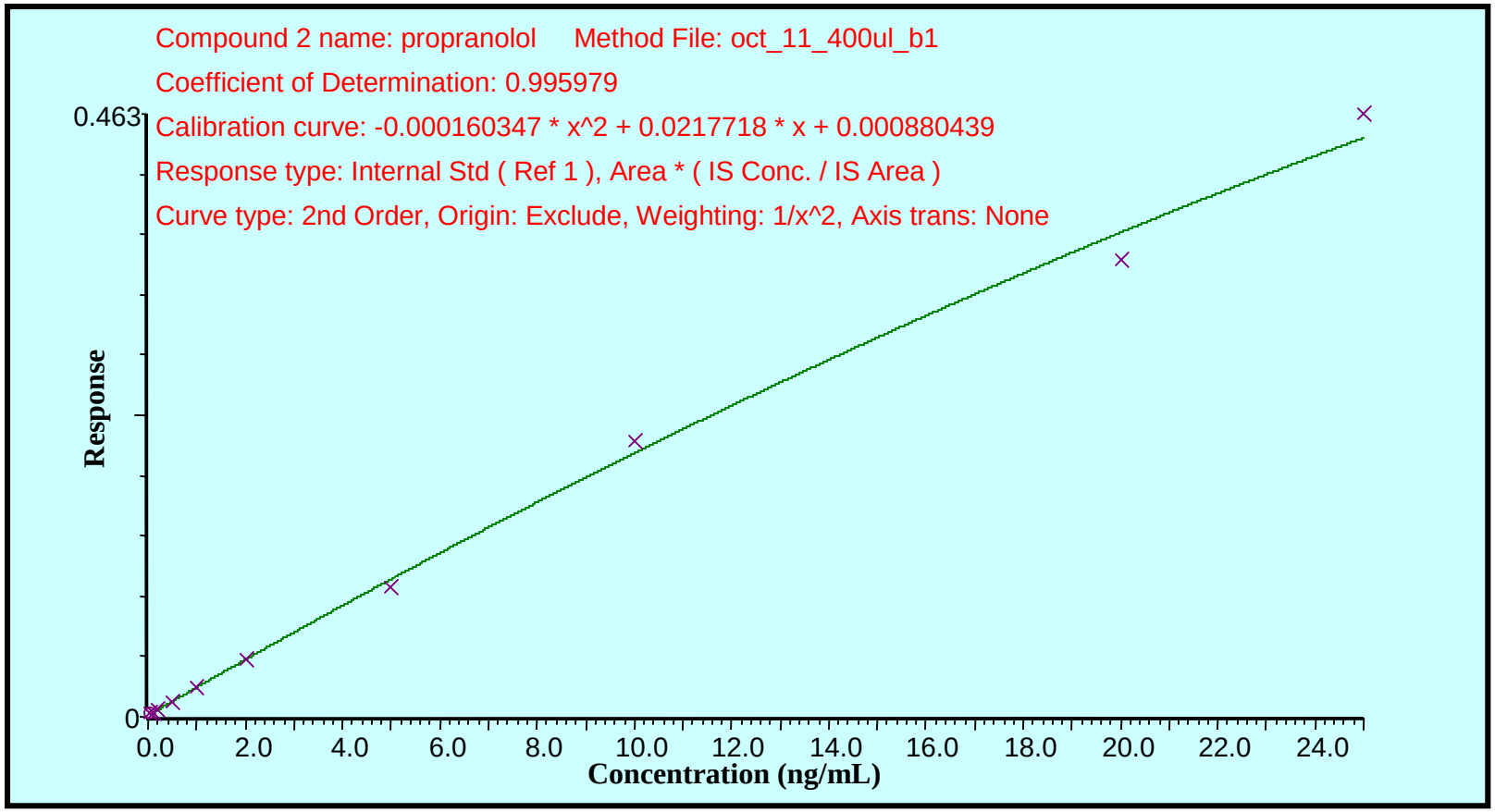
A - Acetonitrile + 0.5 % Formic Acid
B - Water + 0.5 % Formic Acid

1 % Triton X100 Cell Lysing Propranolol at 0.1 ng/mL and 25 ng/mL

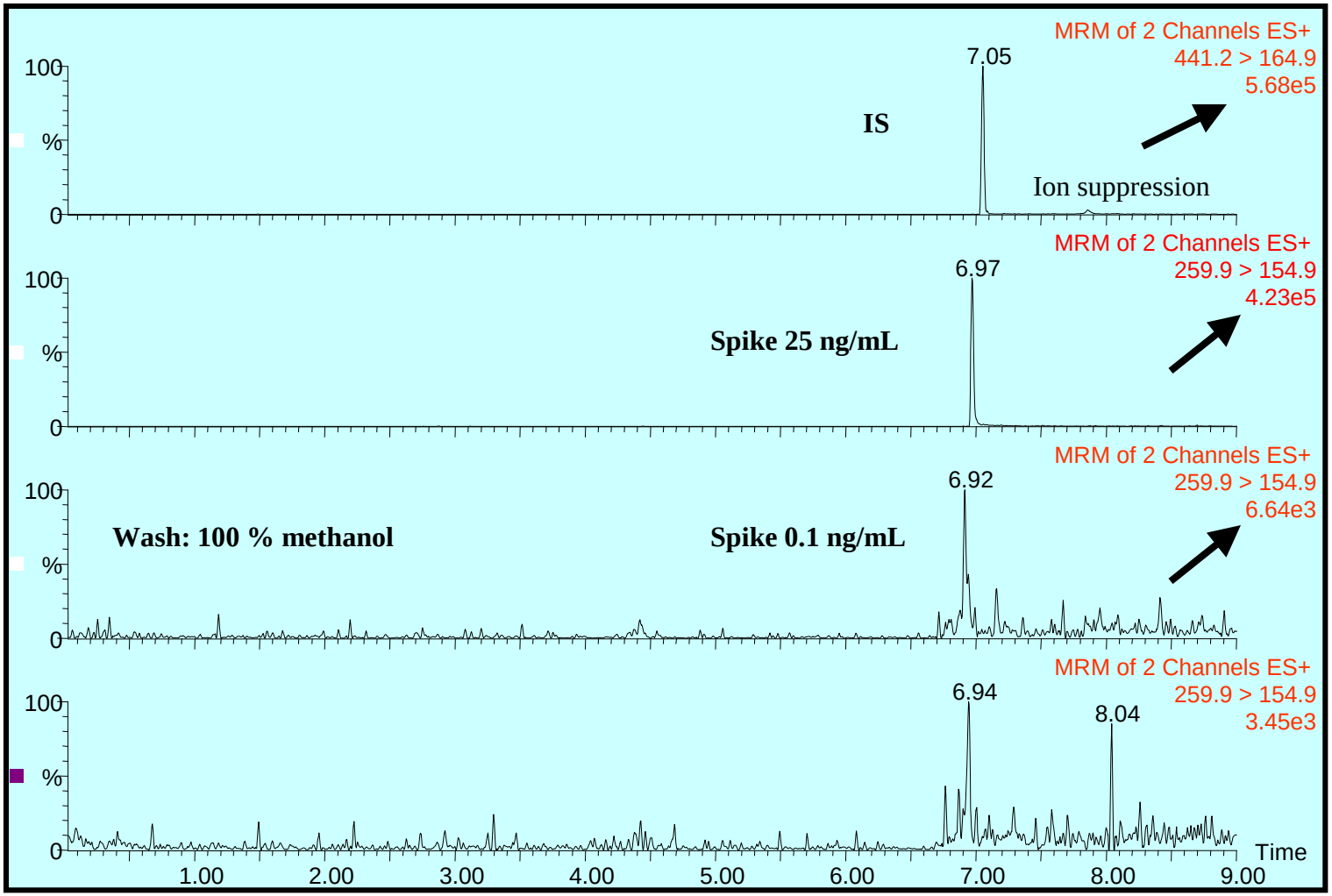


Propranolol Triton Calibration Curve

Conc. ng/mL N=6	0.1	0.2	1.0	2.0	10.0	20.0	25.0
Average	0.10	0.19	0.99	1.99	9.94	19.56	25.63
Standard Deviation	0.001	0.008	0.02	0.06	0.3	0.5	0.5
RSD %	1.7	4.2	2.4	3.2	3.9	2.6	2.0



1 % SDS Cell Lysing
Propranolol at 0.1 ng/mL and 25 ng/mL



1 % SDS Cell Lysing
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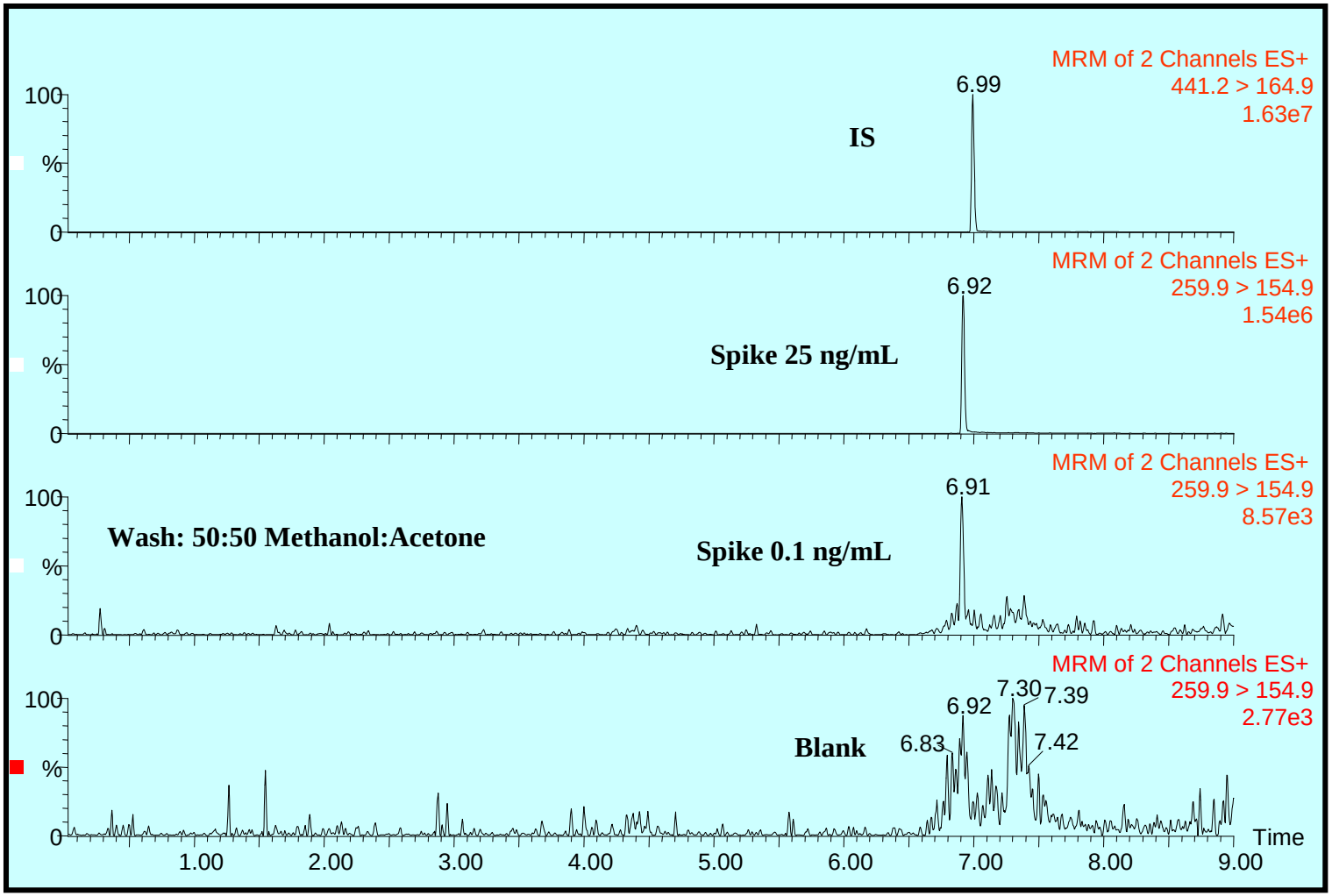
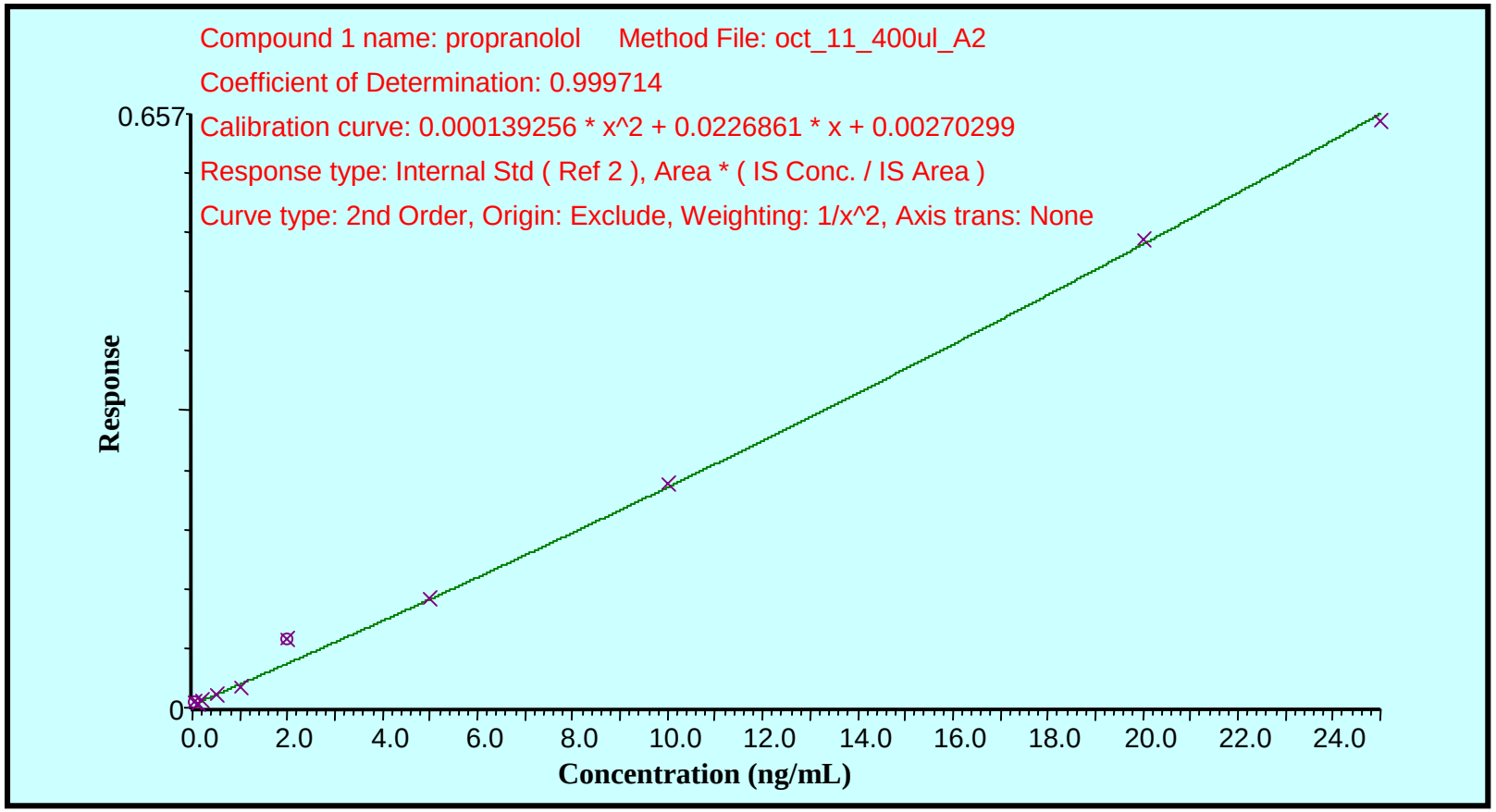


Propranolol SDS Calibration Curve

Conc. ng/mL N=6	0.1	0.5	1.0	2.0	10.0	20.0	25.0
Average	0.10	0.5	1.0	1.8	10.2	19.0	25.5
Standard Deviation	0.001	0.001	0.05	0.1	0.4	0.7	0.7
RSD %	1.4	5.2	5.1	5.6	4.5	4.1	2.9



Benefits of the Oasis® HLB/MCX on-line extraction column

- Simple off-line sample preparation
- Rapid isolation of analyte from a complex sample matrix
- High injection volume
- Lower detection limits