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Performance of an ultra low elution volume 96-well plate

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Today's Challenges Faced by Analytical Chemists in Methods Development

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

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Method Development

Sample Preparation → **Chromatography** → **Mass Spectrometry**

Raw sample:
- CACO2, microsomes, P450, hepatocytes … etc
- tissue, CSF, plasma, serum urine, tears … etc
- water, sediment, food … etc

Extracted sample For LC/MS/MS

Ideally, the final sample should be as clean as a non extracted standard.

Polarity:
Silica- C_{18}, C_8, C_4, C_2
Hybrid- C_{18}, C_8, C_4, C_2
Polymer- C_{18}, C_8, C_4, C_2
Embedded polar group
Cyano, Phenyl

Particle size:
2.5, 3.5, 5 or 7 µm

Internal diameter:
4.6, 3.9, 2.1, 1.0,
0.32 mm and 75 µm

Length:
150, 100, 50, 30, 20 mm

Source:
ESI
APcI
Nano-ESI

Mass analyzers:
magnetic sectors
electric sectors
time of flight
quadrupole
ion trap
FT-ICR

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Extraction Techniques

"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

Extraction Methods in Organic Analysis – A.J. Handley 1998

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Drug Development Overview

DRUG DISCOVERY → **Target ID, Lead Generation, Lead Optimization**

Matrix: CaCO2, microsomes P450, hepatocytes, rat plasma
Sample volume: 50 – 100 μL
Linearity range: 5 – 250 ng/mL

DRUG DEVELOPMENT → **Pre-Clinical, Clinical Trials, Pilot Plant**

Matrix: plasma/serum from rat, rabbit, dog, monkey, human
Sample volume: 50 μL up to 1 mL
Linearity range: 0.1 ng/mL – 500 ng/mL

DRUG MANUFACTURE → **QA/QC, Patent Protection**

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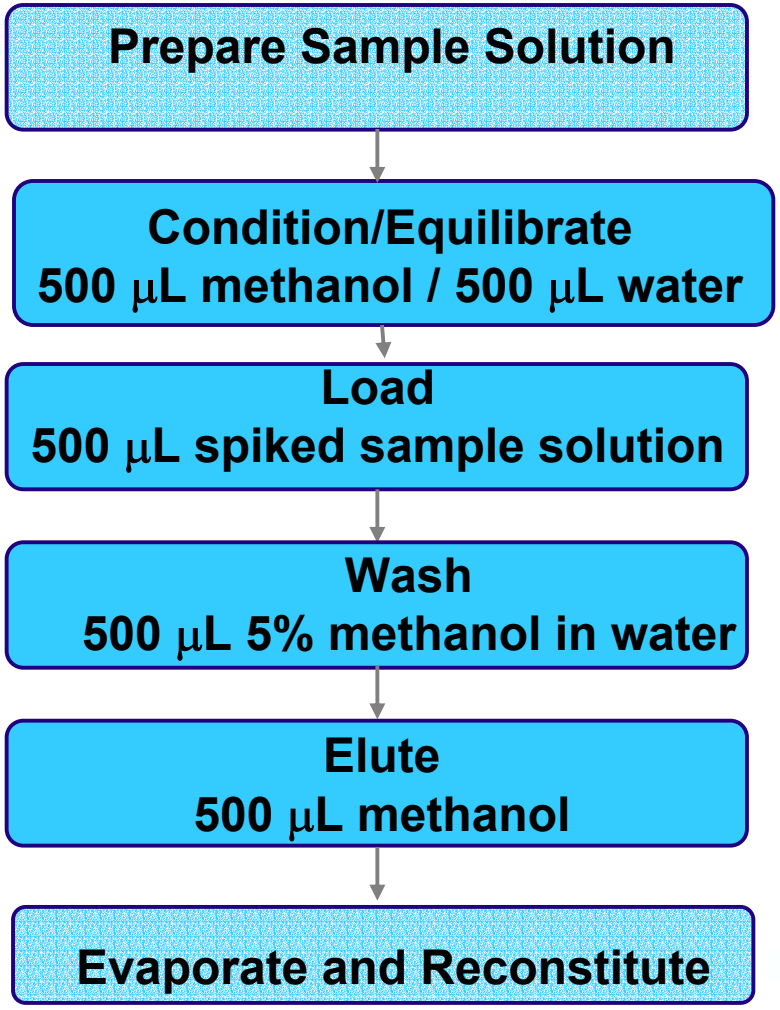
96-Well Plates and Cartridges

96-well plate

barrel

Generic reversed phase SPE Method



Typical values for a 10 mg bed packing

Pre concentration step

Note: For larger bed packing (e.g. 30 or 60 mg), increase the condition, load, wash and elution volumes

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Choice of Sorbent Weight Based on Sample Size

Sorbent per Well	Maximum Mass Capacity	Typical Sample Volume	Typical Elution Volume
5 mg	0.15 to 1 mg	10 to 100 µL	≤ 150 µL
10 mg	0.35 to 2 mg	50 to 400 µL	≤ 250 µL
30 mg	1 to 5 mg	100 µL to 1 mL	> 400 µL
60 mg	2 to 10 mg	200 µL to 2 mL	> 800 µL

With decreasing limit of quantification (LOQ's), it will be necessary to include an evaporation and reconstitution step, which is extremely time consuming.

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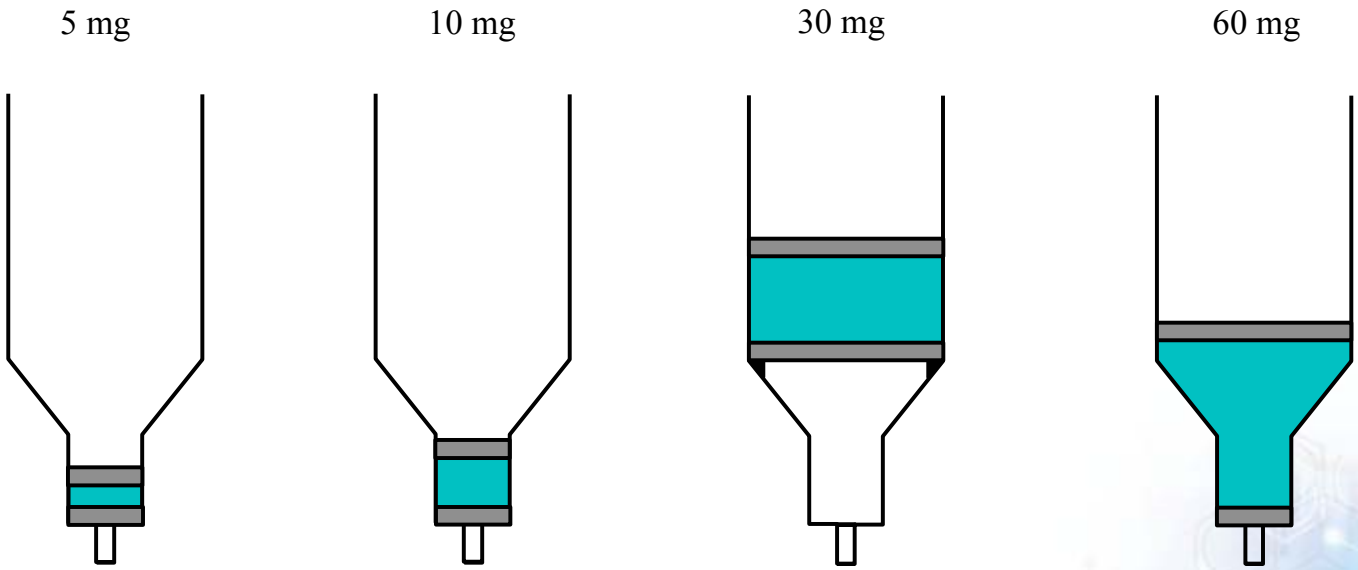
Waters two-Stage Well Design in 96-Well Extraction Plate

Enabling technology for 96-well plates - Oasis® sorbent in the amount required to meet your capacity and elution volume needs.

Designed for Optimal Flow Property



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Oasis® µElution 96-well plate

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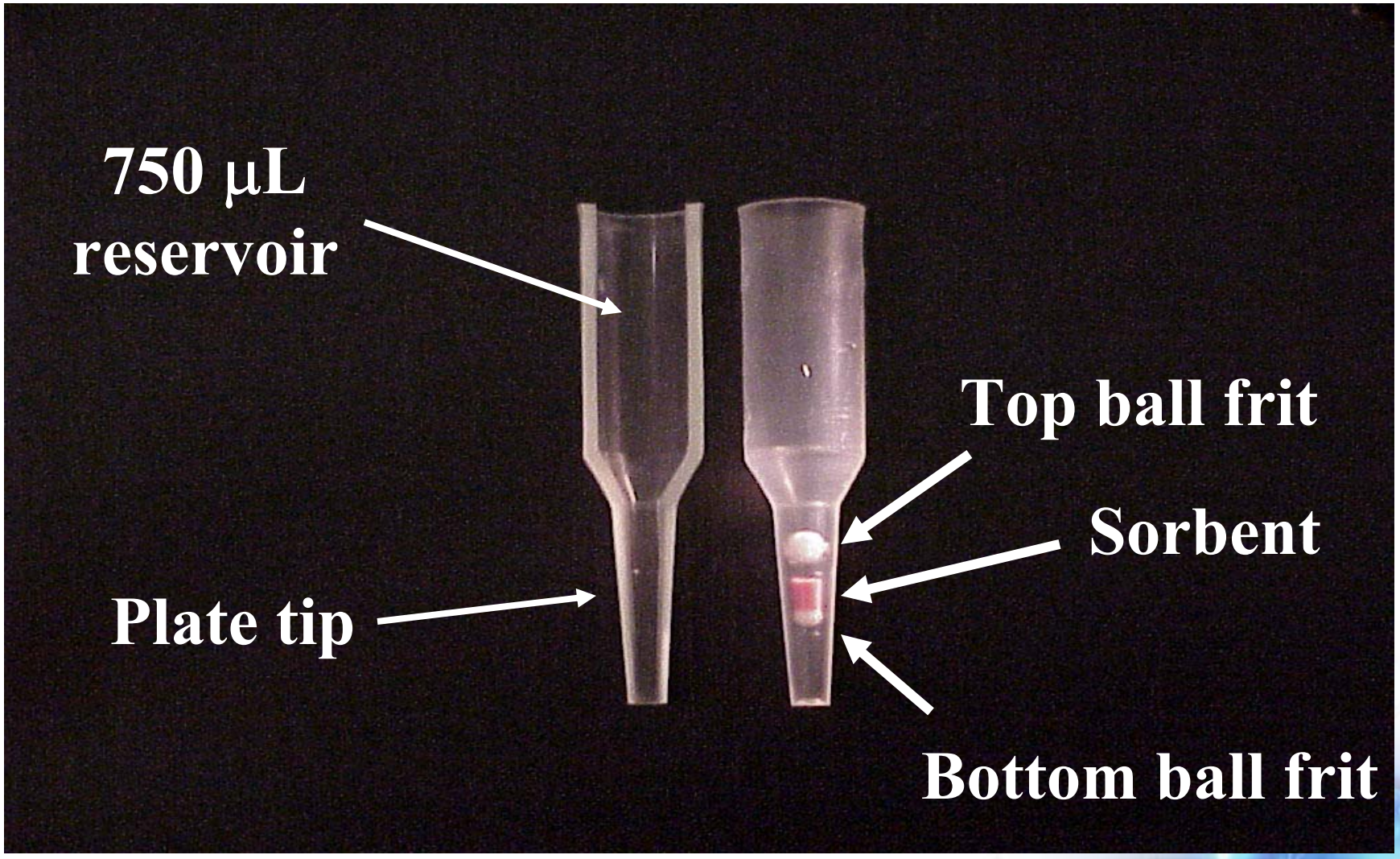
µElution tip



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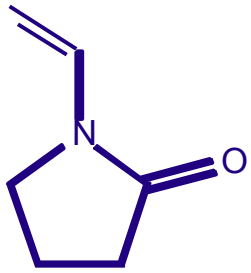
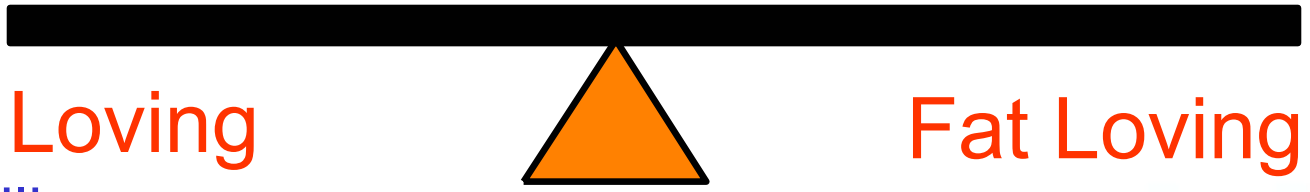
Oasis® HLB Sorbent

Hydrophilic-Lipophilic Balanced copolymer



N-Vinyl-Pyrrolidone

Di-Vinyl-Benzene



Water Loving

Hydrophilic monomer

- Provides wetting properties
- No impact of sorbent drying

Fat Loving

Lipophilic monomer

-Provides reversed phase properties for analyte retention

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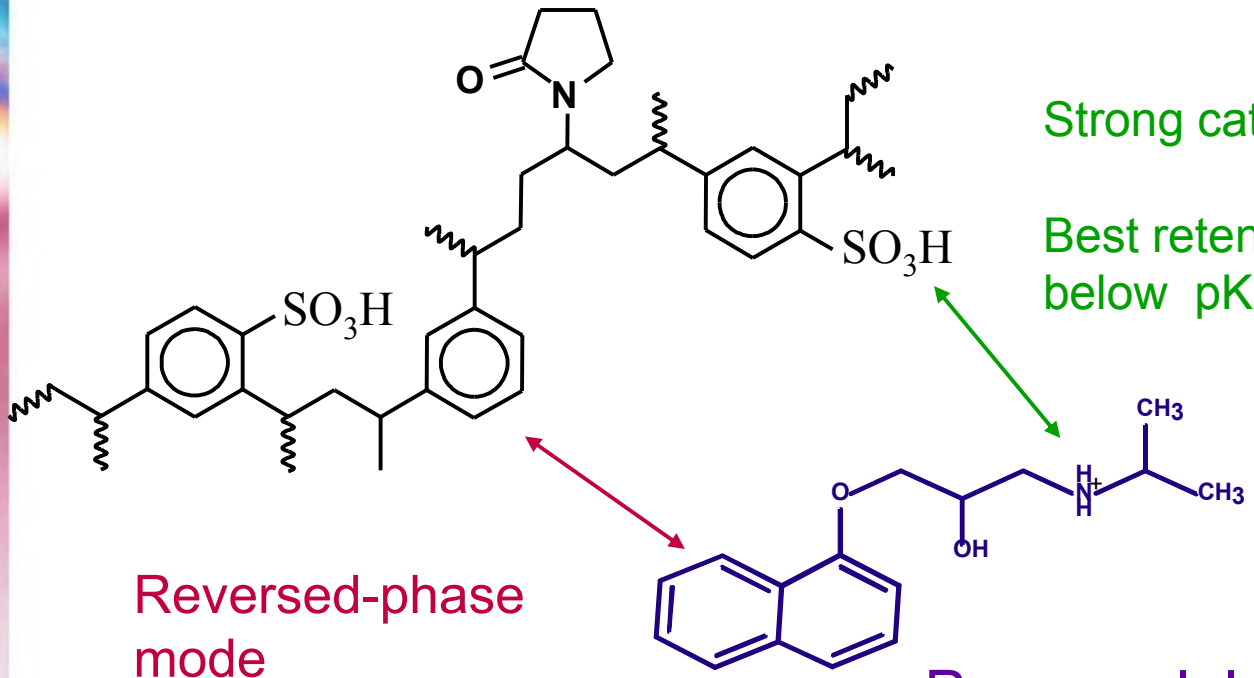
Oasis® MCX Sorbent



Strong cation-exchange mode

Best retention at least 2 pH units below pKa

Reversed-phase mode

Propranolol

Basic drug

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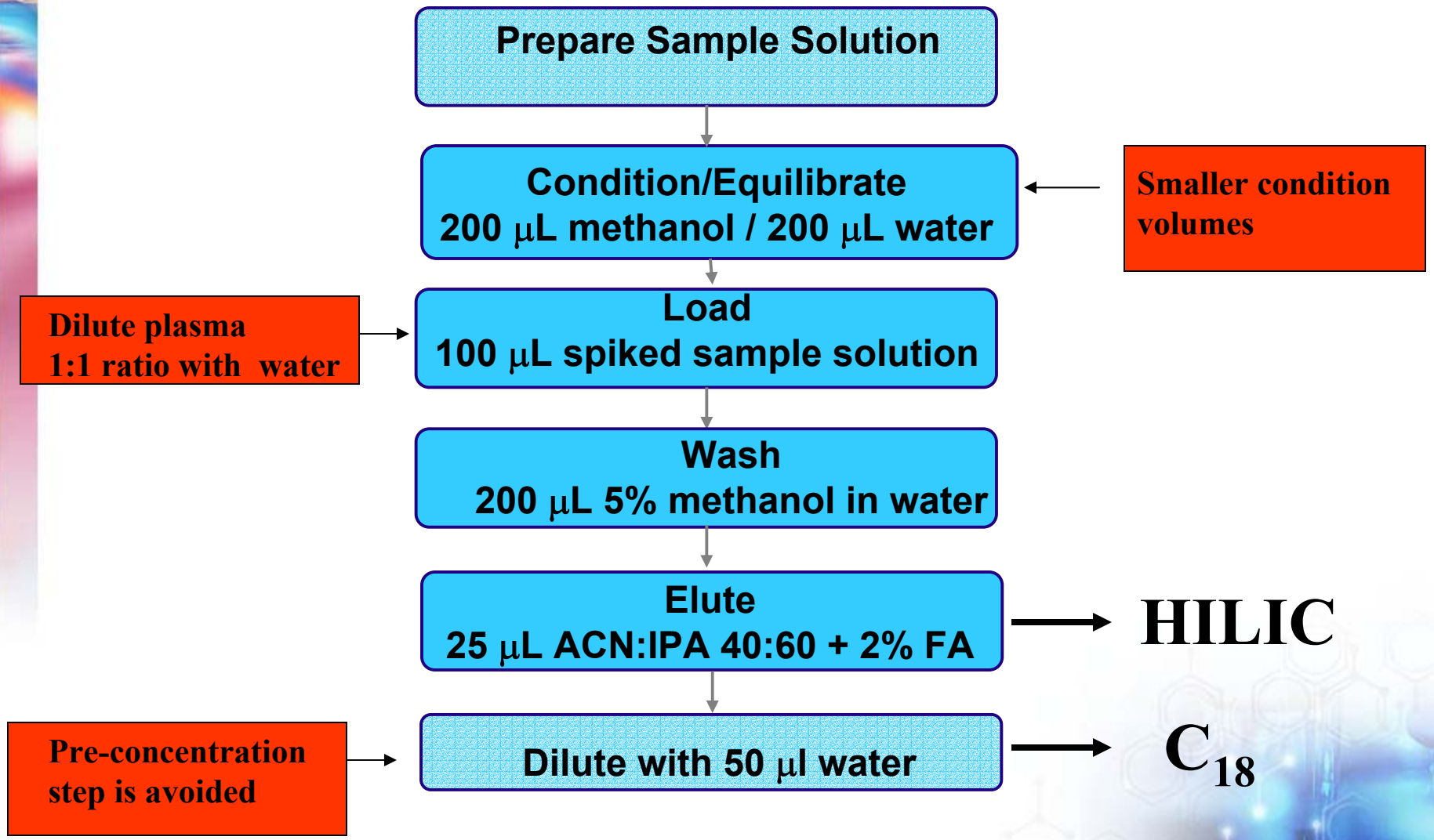
Generic micro-elution reversed phase SPE Method



Generic micro-elution mixed mode SPE Method

Prepare Sample Solution

Condition/Equilibrate
200 µL methanol / 200 µL water

Smaller condition volume

Dilute plasma
1:1 ratio with water

Load
100 µL spiked sample solution

Wash 1
200 µL Water + 2 % FA

Locks basic drug on ion exchanger

Removes polar interferences

Wash 2
200 µL MeOH

Elute
25 µL ACN:IPA 40:60 + 2% NH_4OH

Pre-concentration step is avoided

Dilute with 50 µl water

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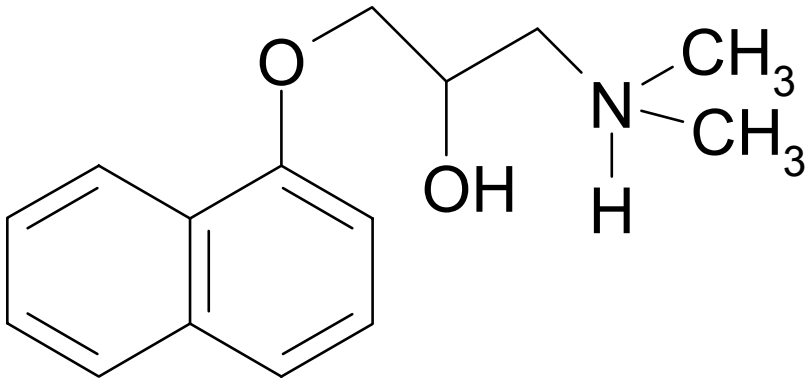
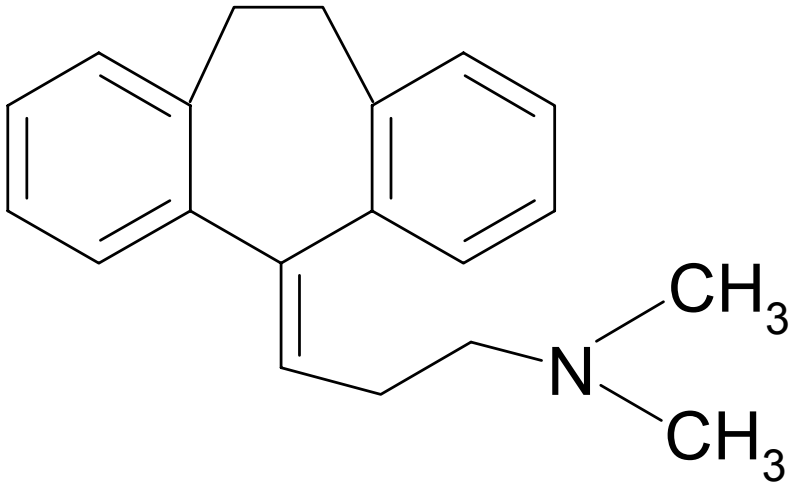
Discovery Application



Propranolol
Antihypertensive



Amitriptyline
Antidepressant

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LC/MS/MS conditions

MS: Micromass Quattro Ultima
Ion source: ESI (+)
Source temperature: 150 °C
Gas cell: 2.0 e-3 bar Argon
Desolvation temperature: 350 °C
Capillary voltage: 3.5 kV
Drying gas flow: 500 L/hr
Cone gas flow: 50 L/hr
Cone voltage: 35 volts

LC: Waters Alliance® 2795
Flow rate: 0.2 mL/min
Mobile phase A: Water + 0.5 % NH_4OH
Mobile phase B: ACN + 0.5 % NH_4OH
Column: XTerra MS C18
2.1 x 30 mm, 3.5 µm
LC conditions: 5% to 95% in 1 min.
Column temperature: ambient

MRM transition: Metoclopramide (IS) m/z 299.8 → 226.7
Propranolol m/z 259.9 → 154.9
Amitriptyline m/z 278.1 → 232.9

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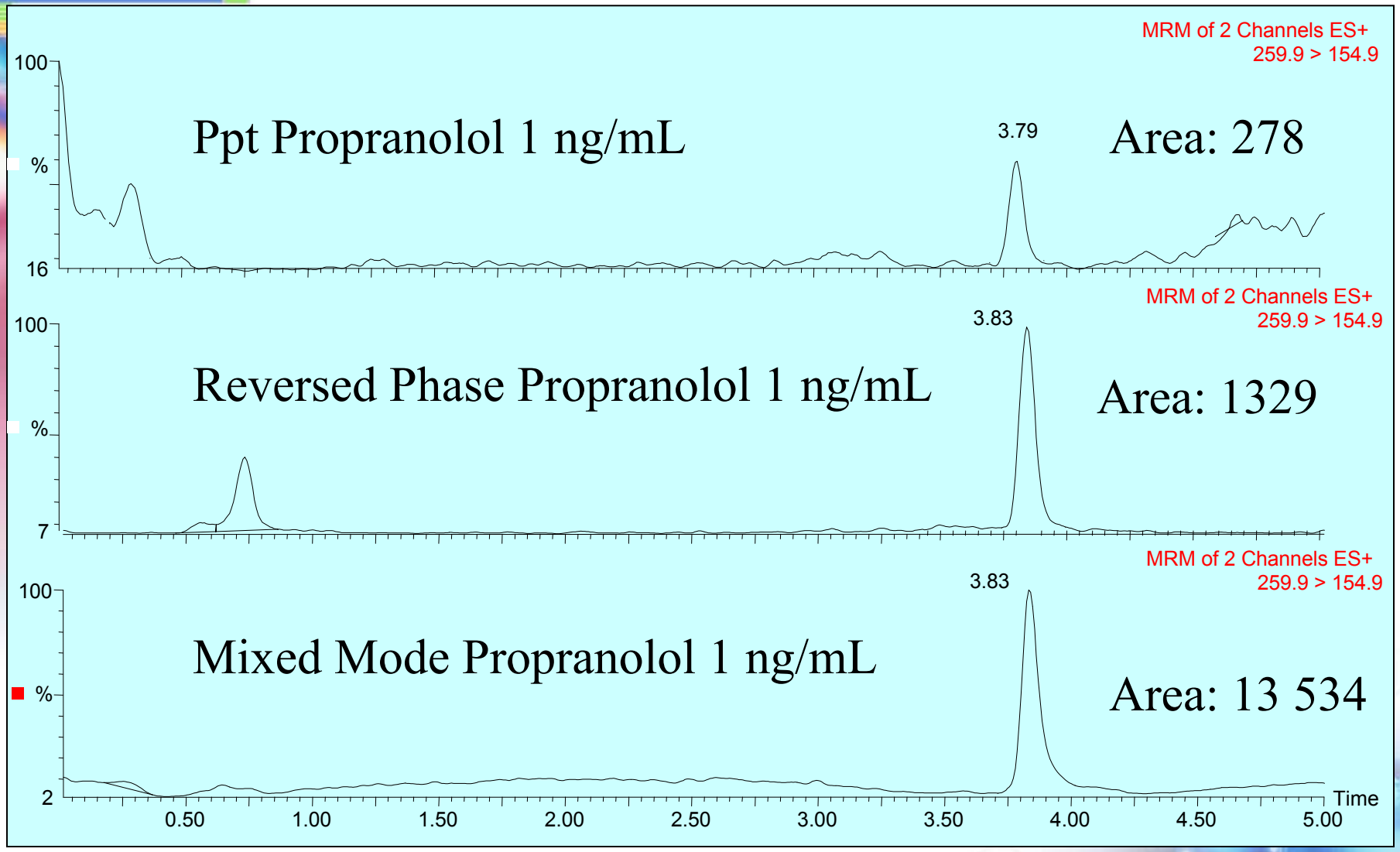
Comparison of Ppt vs HLB vs MCX



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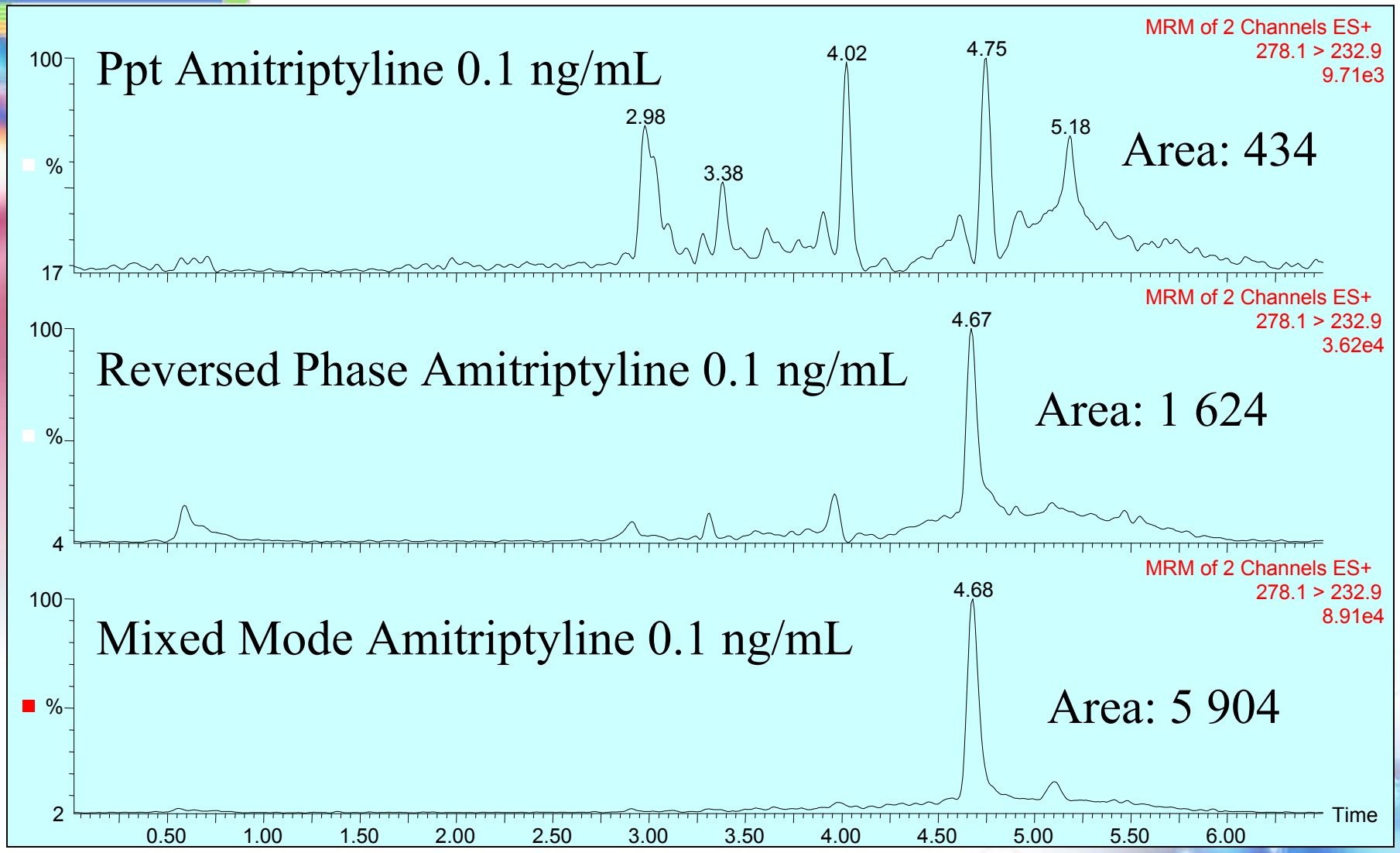
Comparison of Ppt vs HLB vs MCX



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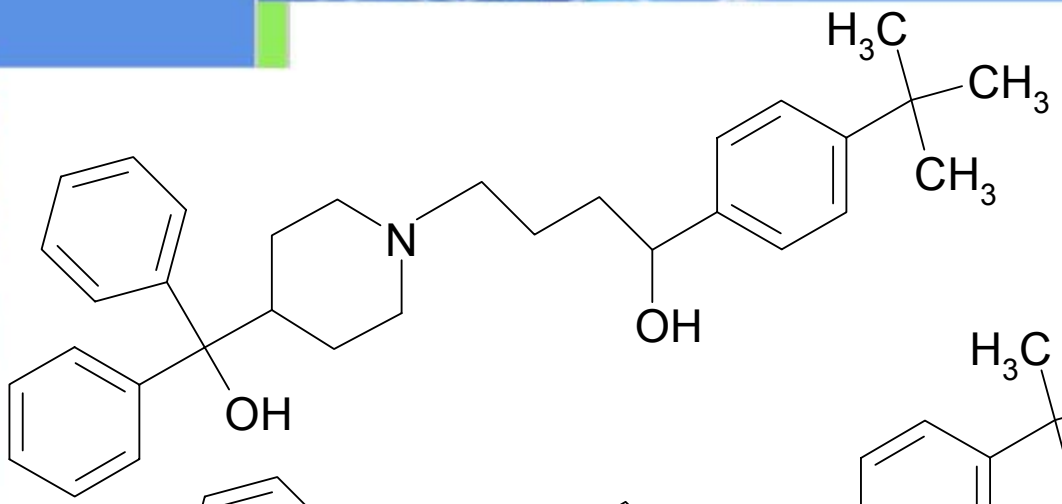
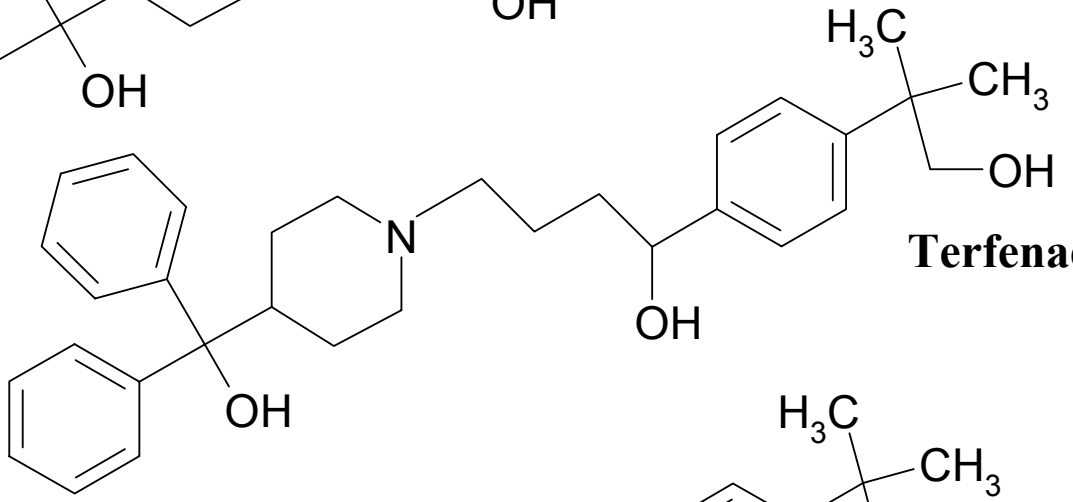
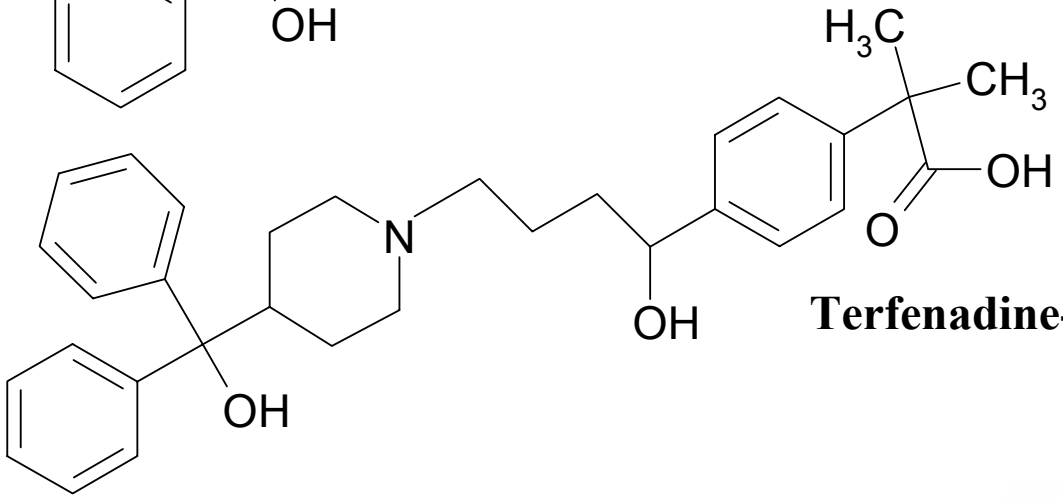
Development Application



Terfenadine
Antihistaminic



Terfenadine-alcohol metabolite



Terfenadine-carboxylate metabolite

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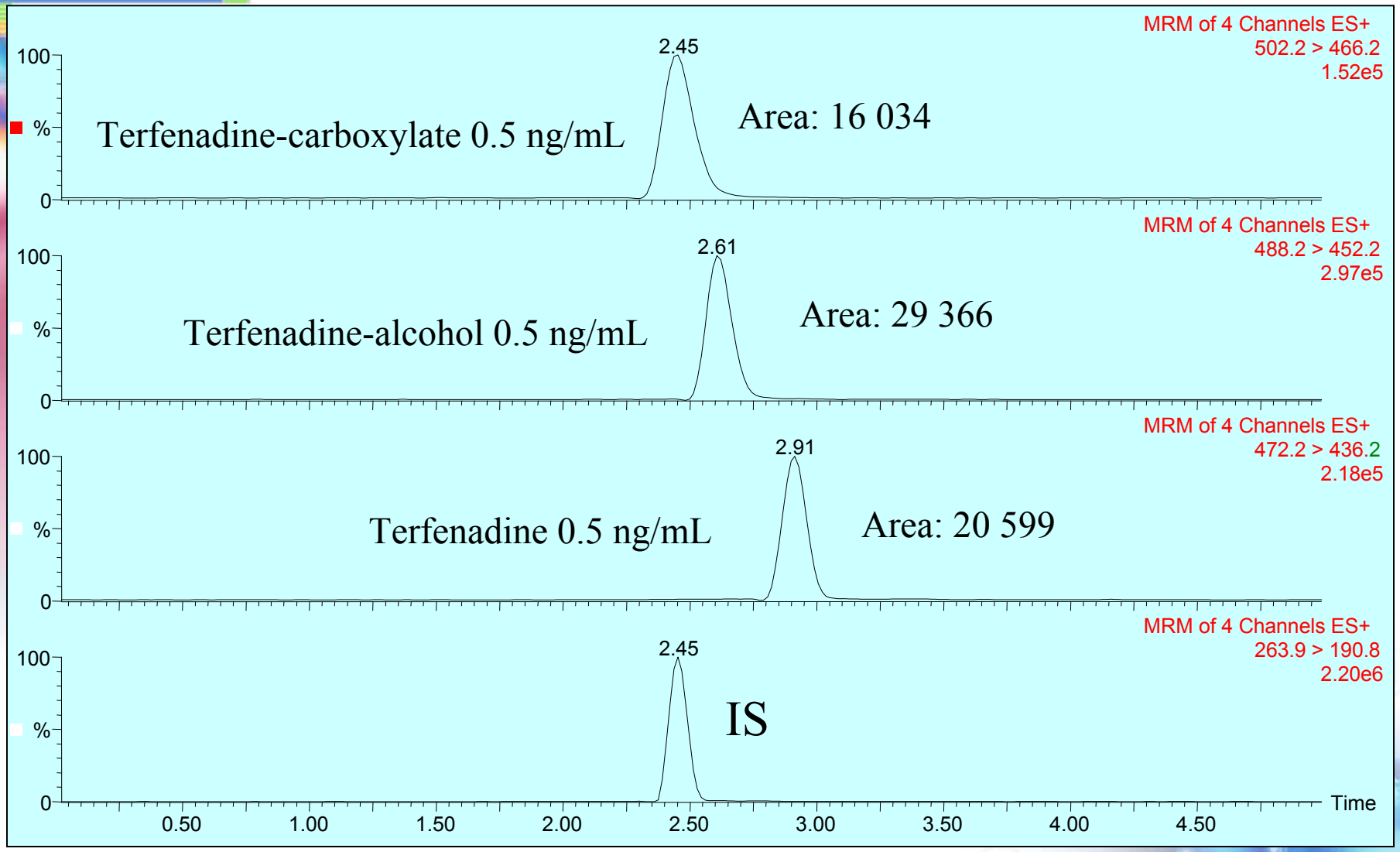
LC/MS/MS analysis of Terfenadine and Metabolites on mixed mode



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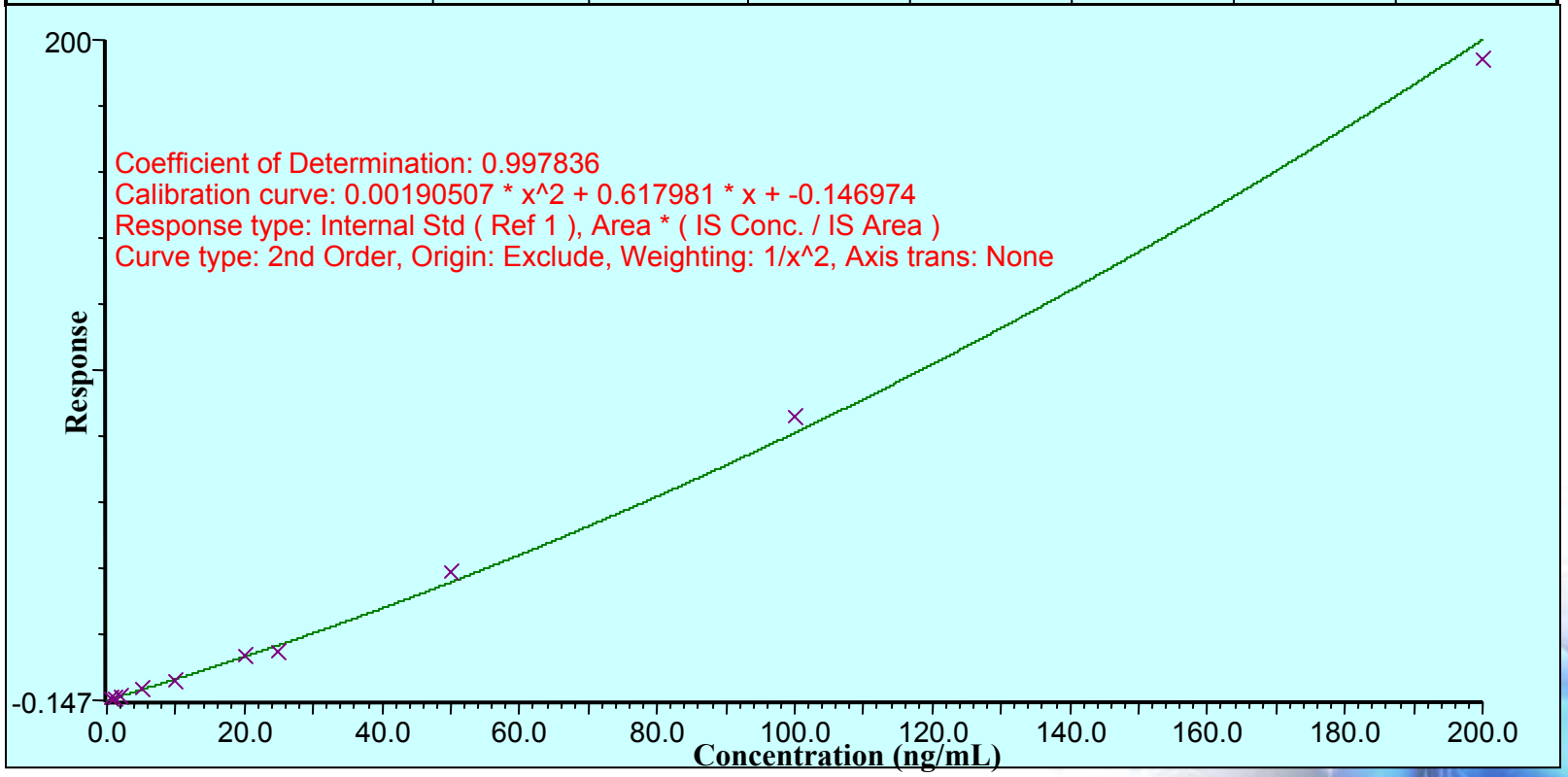
Calibration curve of Terfenadine on mixed mode

Conc. ng/mL N=6	0.5	1.0	5.0	10.0	20.0	100.0	200.0
Average	0.50	0.96	4.99	10.11	19.26	103.25	197.18
Standard Deviation	0.008	0.048	0.25	0.51	0.38	3.68	1.92
RSD %	2	5	5	5	2	4	1



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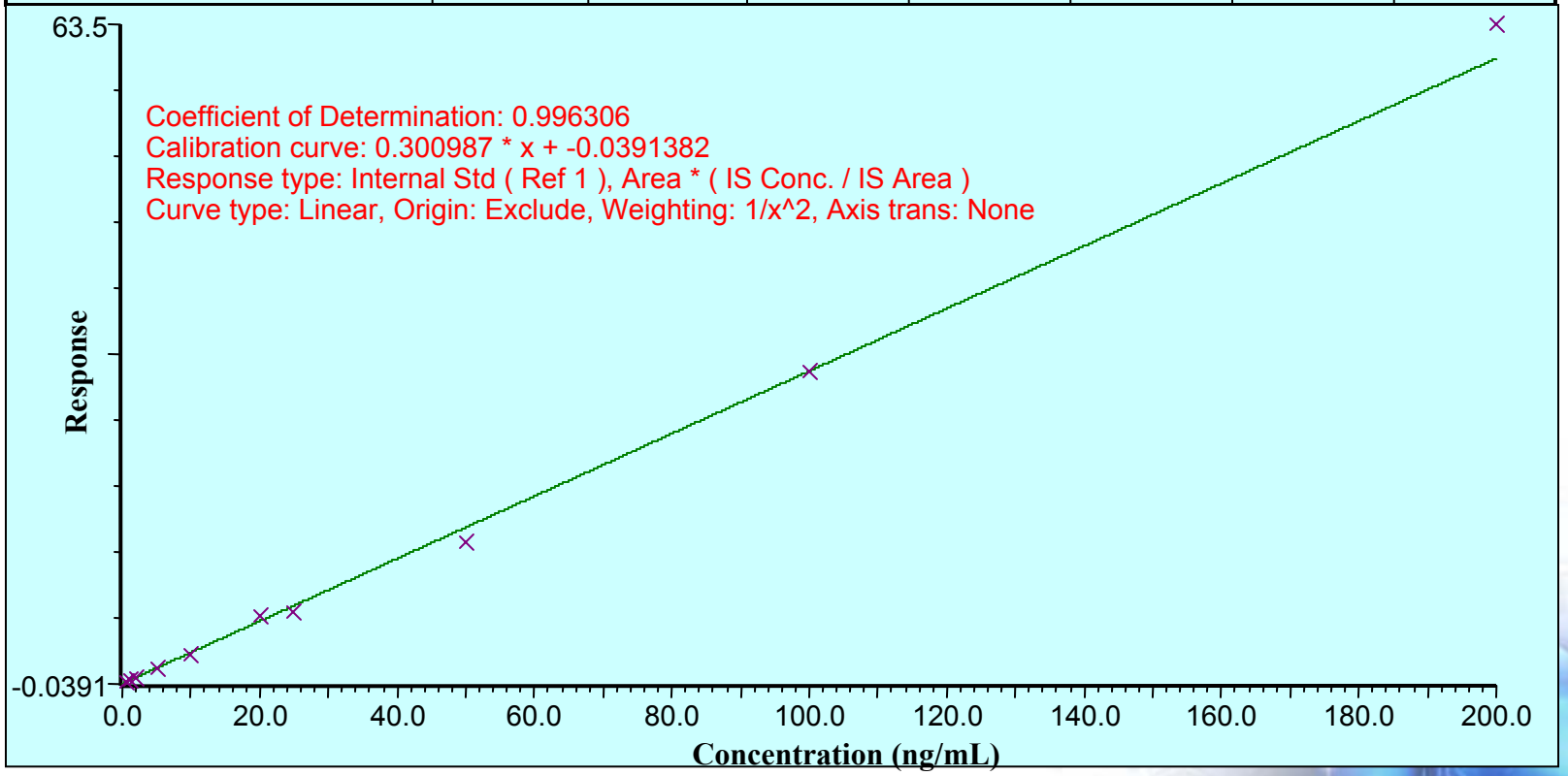
Calibration curve of Terfenadine-Carboxylate on mixed mode

Conc. ng/mL N=6	0.5	1.0	5.0	10.0	20.0	100.0	200.0
Average	0.49	1.02	5.05	10.12	19.71	101.69	196.39
Standard Deviation	0.013	0.052	0.26	0.49	0.46	3.25	4.41
RSD %	3	5	5	5	2	3	2



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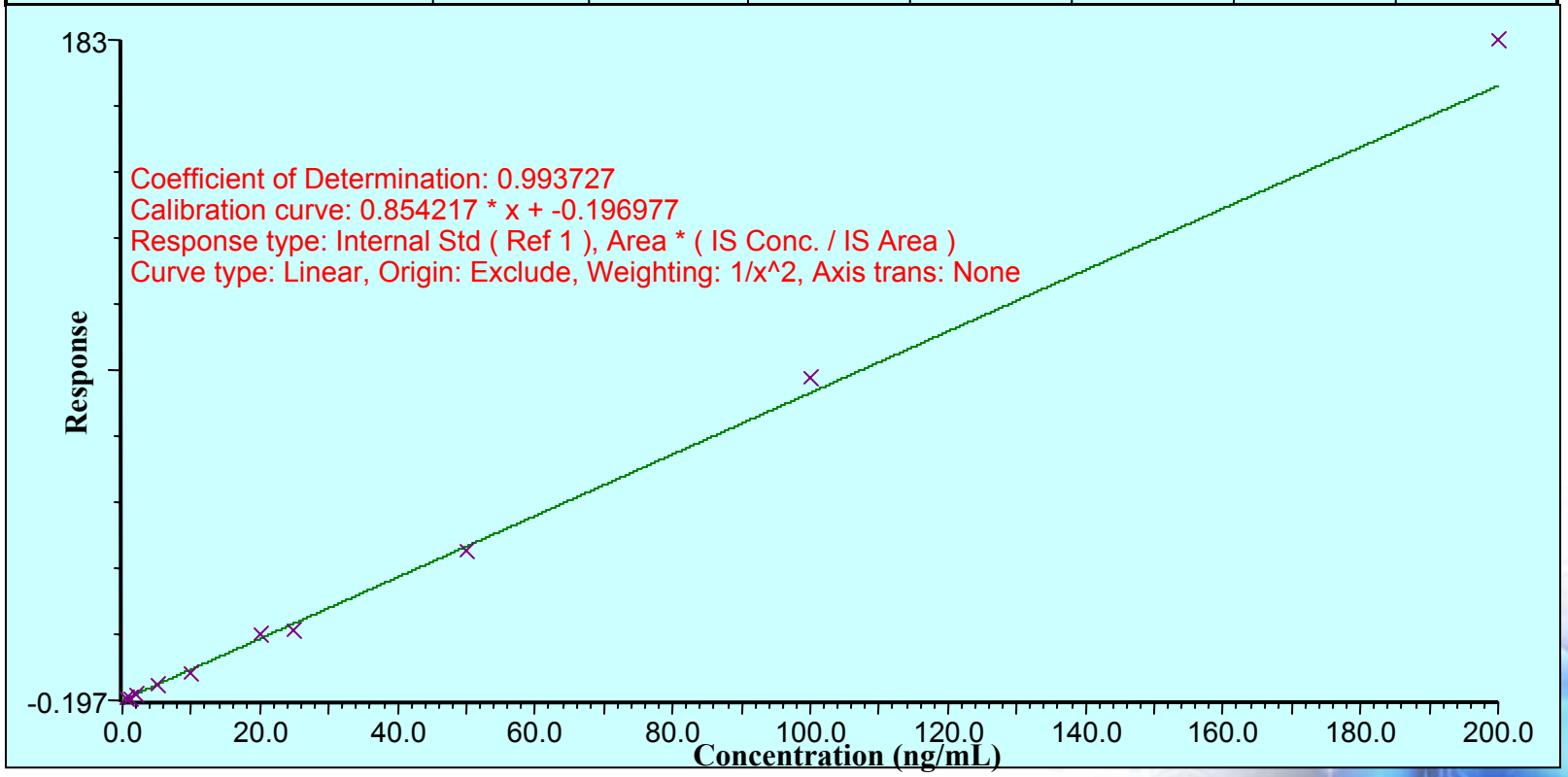
Calibration curve of Terfenadine-Alcohol on mixed mode

Conc. ng/mL N=6	0.5	1.0	5.0	10.0	20.0	100.0	200.0
Average	0.51	0.97	5.19	10.24	19.80	102.05	196.55
Standard Deviation	0.008	0.041	0.27	0.44	0.77	3.15	7.92
RSD %	2	4	5	4	4	3	4



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Long term stability of Terfenadine and Metabolite on mixed mode

Conc. (ng/mL)	0.5	1.0	5	10	20	100	200
Terfenadine							
Day 1 (N=6) (C.V.)	0.50 (3)	0.97 (8)	4.96 (4)	9.93 (2)	19.89 (4)	105.29 (4)	195.87 (2)
Day 2 (N=6)	0.51 (2)	0.92 (2)	5.29 (2)	10.51 (4)	19.41 (3)	100.56 (4)	200.70 (1)
Day 3 (N=6)	0.50 (3)	0.98 (5)	5.09 (6)	9.64 (5)	19.22 (3)	106.38 (3)	194.47 (2)
Day 4 (N=6)	0.51 (2)	0.89 (1)	5.21 (3)	9.94 (5)	19.20 (3)	107.04 (2)	197.1 (1)
Terfenadine-carboxylate							
Day 1 (N=6) (C.V.)	0.50 (2)	0.96 (5)	4.88 (6)	10.45 (5)	20.74 (6)	na	na
Day 2 (N=6)	0.50 (3)	1.01 (6)	4.79 (1)	9.68 (4)	21.11 (3)	97.12 (5)	204.06 (4)
Day 3 (N=6)	0.45 (3)	0.99 (5)	5.21 (4)	10.08 (5)	20.79 (4)	98.36 (4)	194.85 (4)
Day 4 (N=6)	0.50 (2)	1.00 (4)	5.10 (6)	10.35 (3)	20.34 (3)	96.02 (5)	192.70 (3)
Terfenadine-alcohol							
Day 1 (N=6) (C.V.)	0.52 (1)	0.92 (2)	4.99 (2)	10.30 (4)	20.53 (2)	96.74 (4)	195.23 (5)
Day 2 (N=6)	0.51 (1)	0.94 (2)	5.01 (4)	10.03 (4)	20.55 (3)	100.83 (3)	191.65 (3)
Day 3 (N=6)	0.50 (2)	0.97 (5)	4.99 (5)	9.88 (2)	20.27 (5)	99.34 (5)	204.10 (3)
Day 4 (N=6)	0.50 (2)	1.00 (5)	5.03 (4)	10.36 (4)	20.23 (6)	97.43 (7)	196.04 (3)

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Conclusions

Wide volume range for loading (50 to 800 µL)

Low elution volume (25 – 50 µL)

Generic protocols for reversed phase and mixed mode

Pre-concentration factor up to 10x

Sub ng/mL quantitation limits

96-well plate format for high throughput

No evaporation and reconstitution step needed