WA20263

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Performance of an Ultra Low Elution Volume 96-Well Plate

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Introduction

Achieving both high throughput and low detection limits (LOQ's) is now the challenge of pharmaceutical analysis. Since the mid 90's, additional emphasis has been directed on the sample preparation at sub ng/mL levels and extraction speed. Classical extraction techniques, like liquid-liquid extraction (LLE) or protein precipitation (ppt), are not suitable formats for high throughput for good clean-up. For example, LLE gives better recovery and clean-up requires extensive manual

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Introduction (continued)

labor and is not easily automated. Ppt, on the other hand, is fast and can be automated, but the extract is not as clean as LLE or SPE techniques (potential loss of signal due to ion suppression). As more drug candidates shows increased levels of potency, less dosage is required, hence the need for lower LOQ's in order to define the pharmacokinetic profile. During pre-clinical trials of a drug candidate, sample volume is relatively low, most common in the 50 µL range. Clearly, using solid phase extraction (SPE) in a 96-well plate format

Introduction (continued)

(1,2) can keep up with these new demands for low sensitivity using less sample volume. With the assistance of robotic liquid handler, a 96-well plate can be prepared in less than a minute per sample (3). The performance of a new 96-well ultra low elution volume plate for LC/MS/MS applications will be summarized. Each well in the plate contains 2 mg of a high capacity solid phase extraction polymer (Oasis® HLB and MCX) (4) that has the capacity of extracting analytes from several hundred microliters of plasma. A novel tip design permits quantitative

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Introduction (continued)

quantitative recovery of the extracted analytes with as little as 25 microliters of elution solvent. Therefore, the plate provides as much as 5-fold concentration of the analyte from plasma. The plate allows for simple dilution of the elution solution with water prior to LC/MS/MS analysis, thus avoiding an evaporation and reconstitution step.

1- Zweigenbaum, T. Heing, K., Steinborner, S. Wachst, T., Henion, J., Anal. Chem., 1999, **71**, 2294

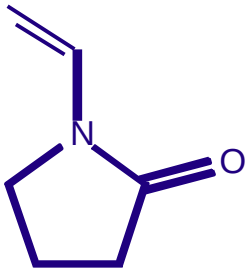
2- Show, W.Z., Jiang, X., Beato, B.D., Naidong, W., Rapid Commun. Mass Spectrom., 2001, **15**, 466

3- Jemal, M., Huang, M., Mao, Y., Whigan, D., Powell, M., Rapid Commun. Mass Spectrom, 2001, **15**, 994

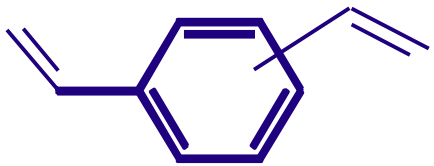
4- Cheng, Y.F., Neue, W., Bean, L., J. Chromatogr. A, 1998, **828**, 273

Oasis® HLB Sorbent

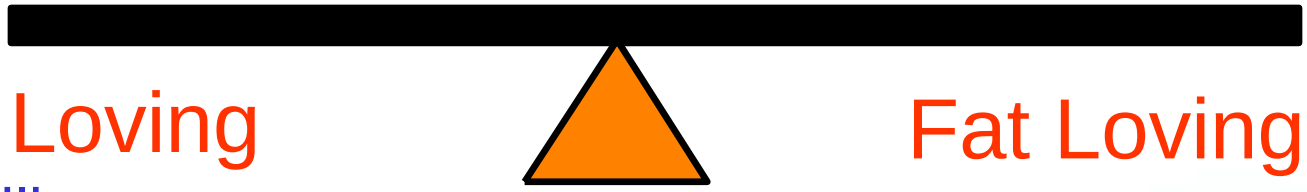
Hydrophilic-Lipophilic Balanced copolymer



N-Vinyl-Pyrrolidone



Di-Vinyl-Benzene



Water Loving

Hydrophilic monomer

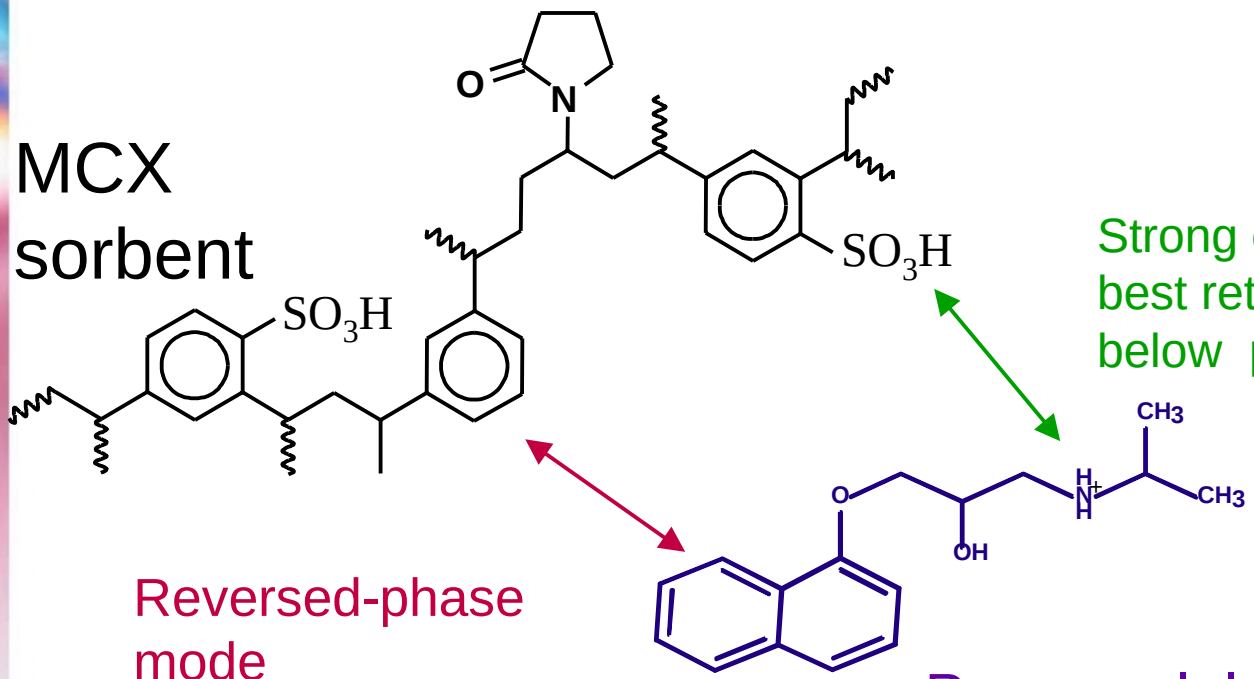
- Provide wetting properties
- No impact of sorbent drying

Fat Loving

Lipophilic monomer

-Provide reverse phase property for analyte retention

Oasis® MCX Sorbent



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Oasis® HLB micro elution 96-well plate

Instrumentation

Alliance® HT 2795

Micromass® Ultima
Triple quadrupole

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
Drying gas flow: 500 L/hr
Cone gas flow: 50 L/hr
Cone voltage: 25 volts
Capillary voltage: 3.5 Kv

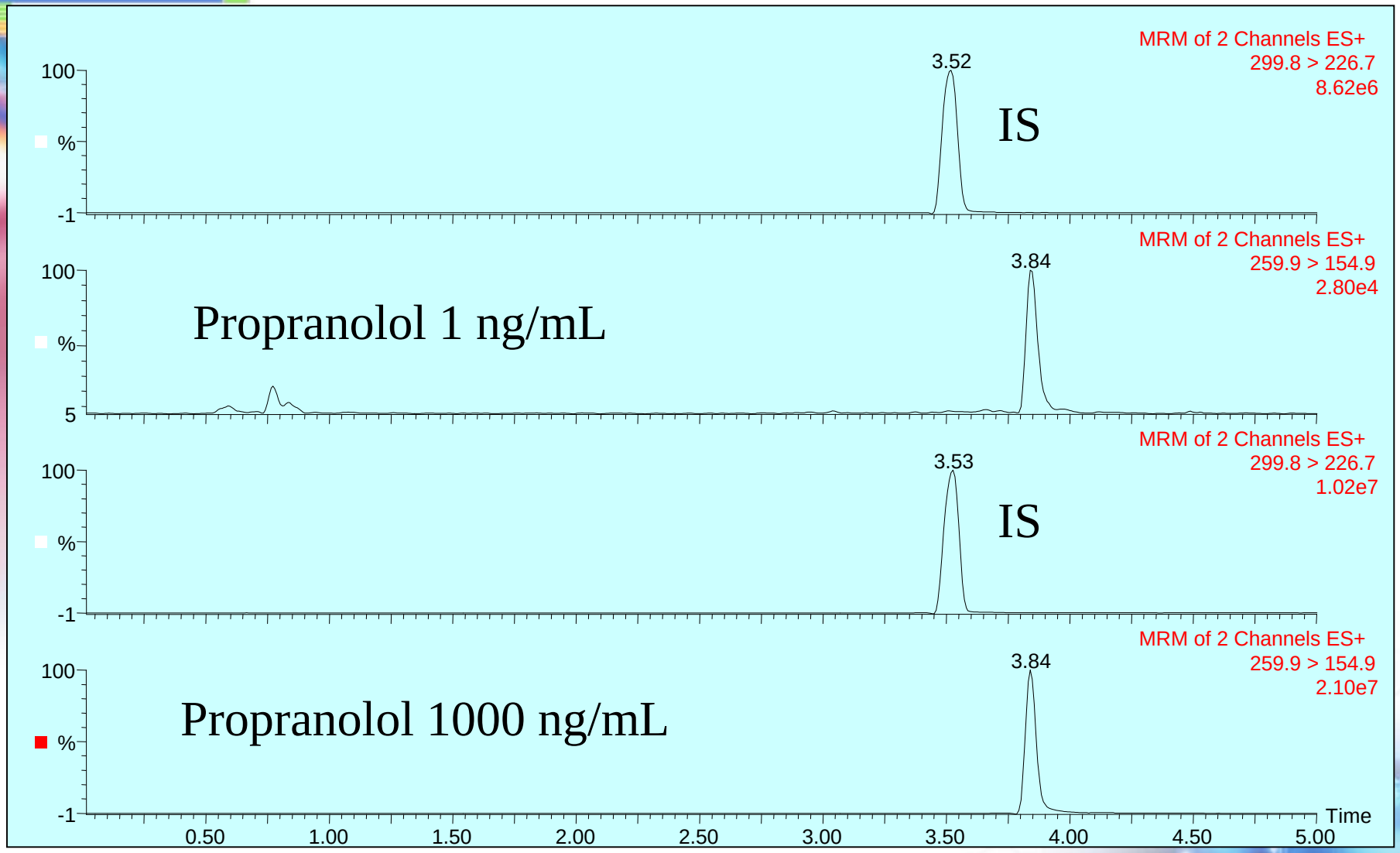
LC: Waters Alliance® HT 2795
Flow rate: 0.2 mL/min
Mobile phase A: Water + 0.5 % NH_4OH
Mobile phase B: ACN + 0.5 % NH_4OH
Column: XTerra® MS C18
2.1 x 30 mm, 3.5 µm
LC conditions: 5% to 95% in 1 min.
Column temperature: ambient

MRM transition: Metoclopramide (IS) m/z 299.8 → 226.7
Propranolol m/z 259.9 → 154.9
Amitriptyline m/z 278.1 → 232.9
Nortriptyline m/z 263.9 → 190.8

Oasis® HLB 1D Generic Extraction Protocol

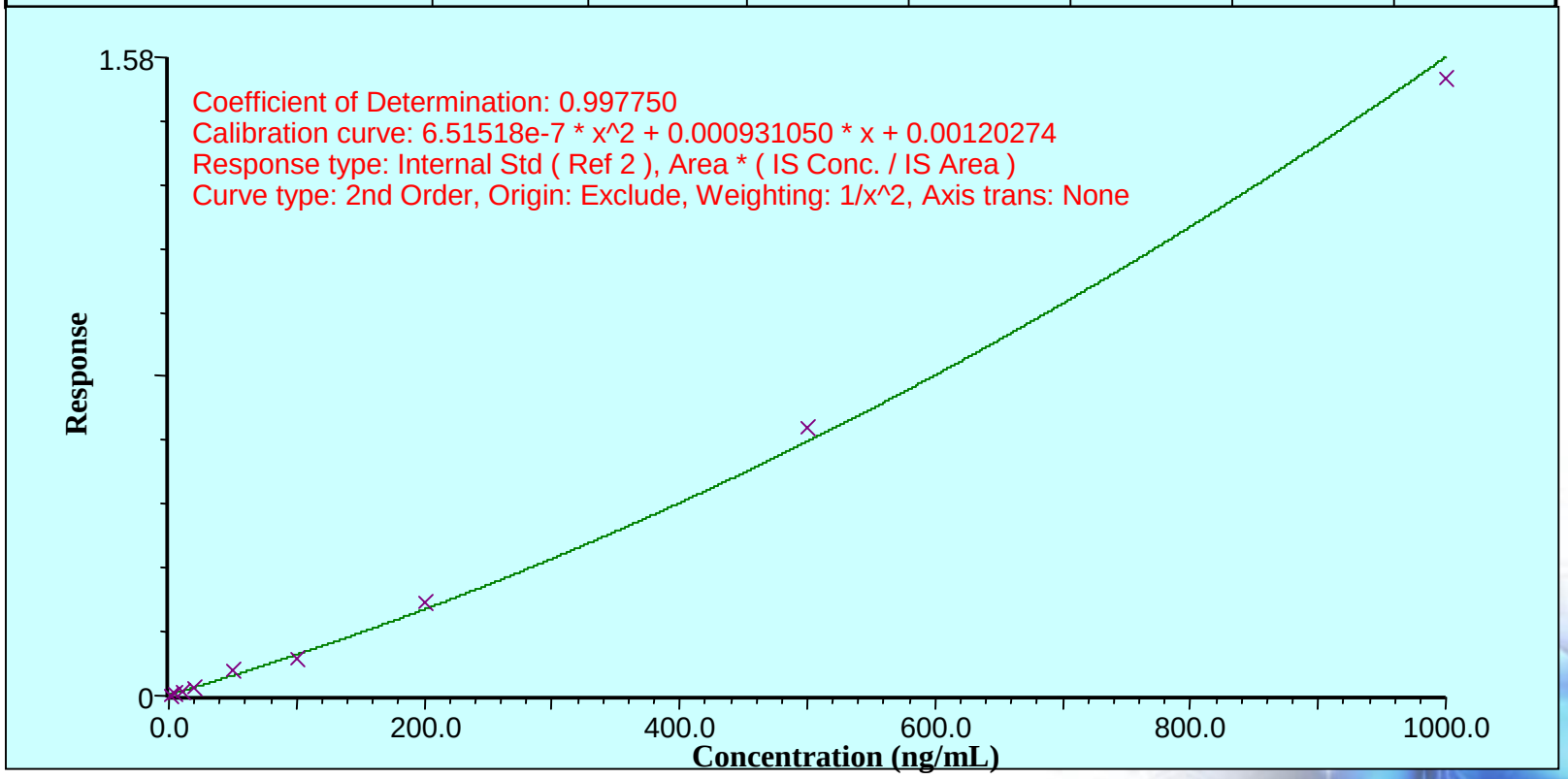
Condition	200 µL MeOH
Equilibrate	200 µL Water
Load	50 µL spiked rat plasma
Load	50 µL IS (10 pg/µL) in water
Wash	200 µL 5% MeOH in water
Elute	25 µL ACN:IPA 40:60 + 2% FA
Dilute	50 µL Water
Inject	20 µL

LC/MS/MS analysis of Propranolol on Oasis® HLB



Calibration curve of Propranolol on Oasis® HLB

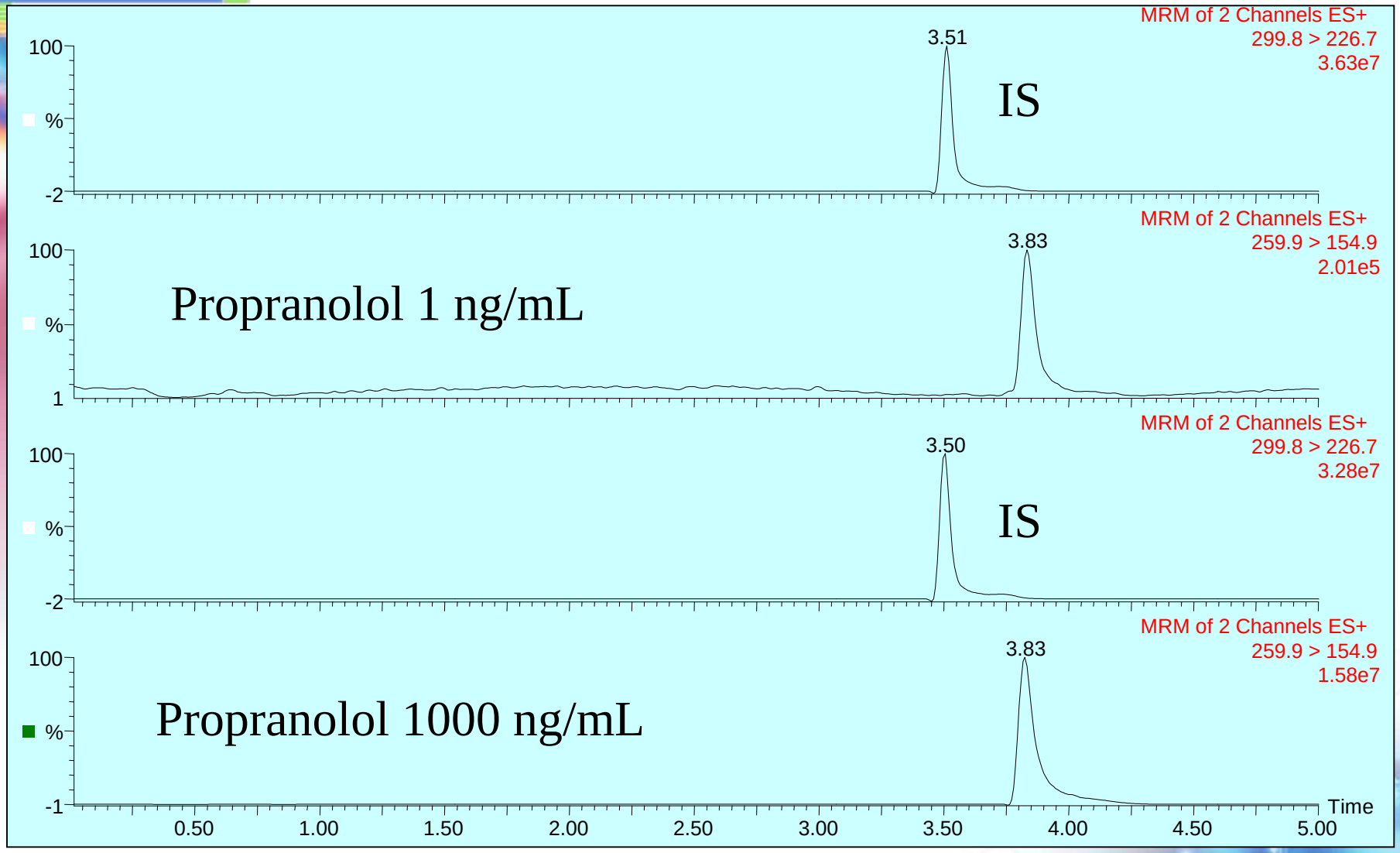
Conc. ng/mL N=6	2.0	5.0	10.0	20.0	50.0	500.0	1000.0
Average	1.95	5.25	9.98	19.01	50.98	508.06	994.60
Standard Deviation	0.056	0.24	0.51	0.89	2.21	23.97	14.27
RSD %	2.8	4.7	5.1	4.7	4.3	4.7	1.4



Oasis® MCX Generic Extraction Protocol

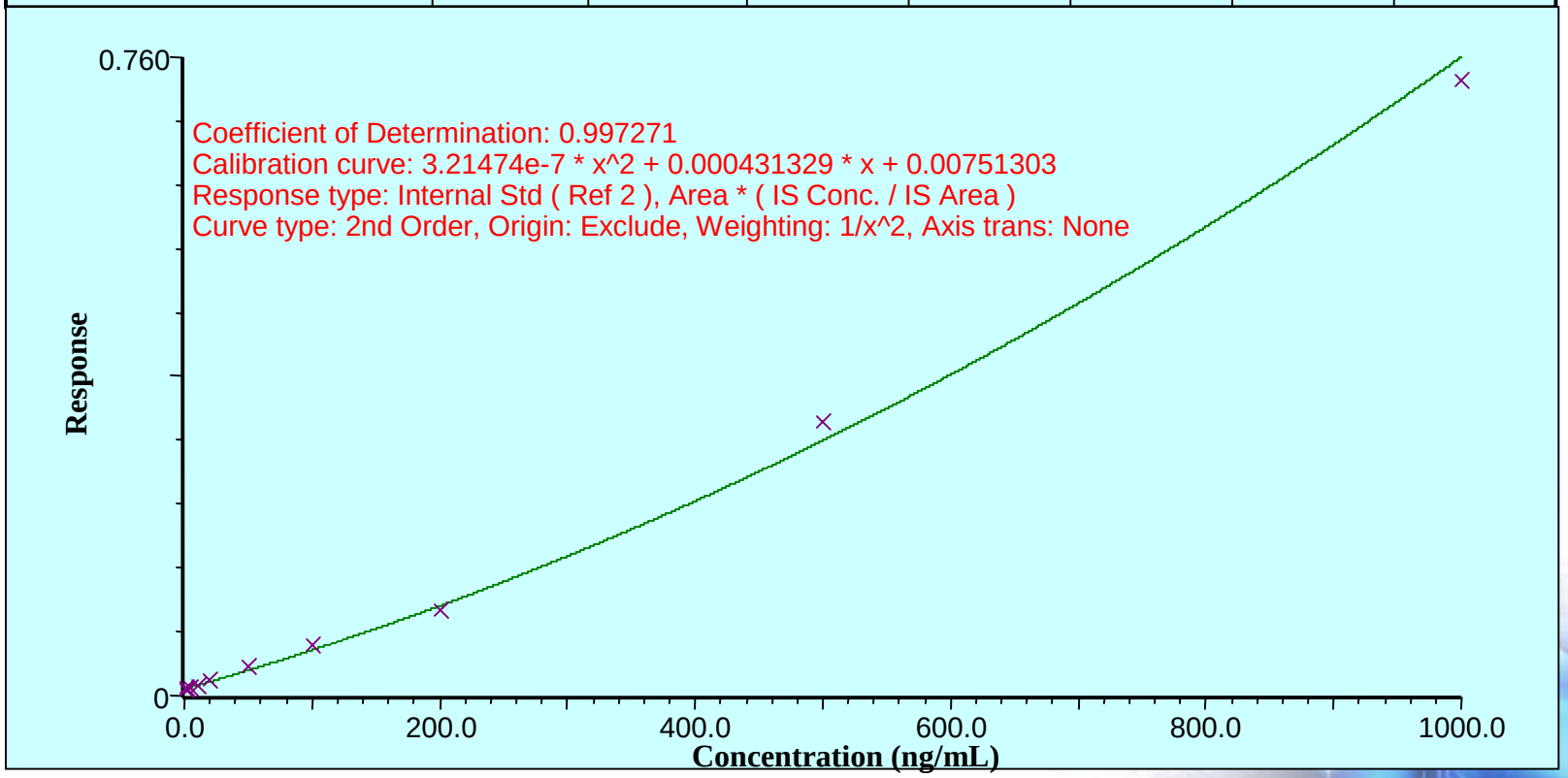
Condition	200 µL MeOH
Equilibrate	200 µL Water
Load	50 µL spiked rat plasma
Load	50 µL IS (10 pg/µL) in water
Wash 1	200 µL Water + 2% FA
Wash 2	200 µL MeOH
Elute	25 µL ACN:IPA 40:60 + 2% NH_4OH
Dilute	50 µL water
Inject	20 µL

LC/MS/MS analysis of Propranolol on Oasis® MCX

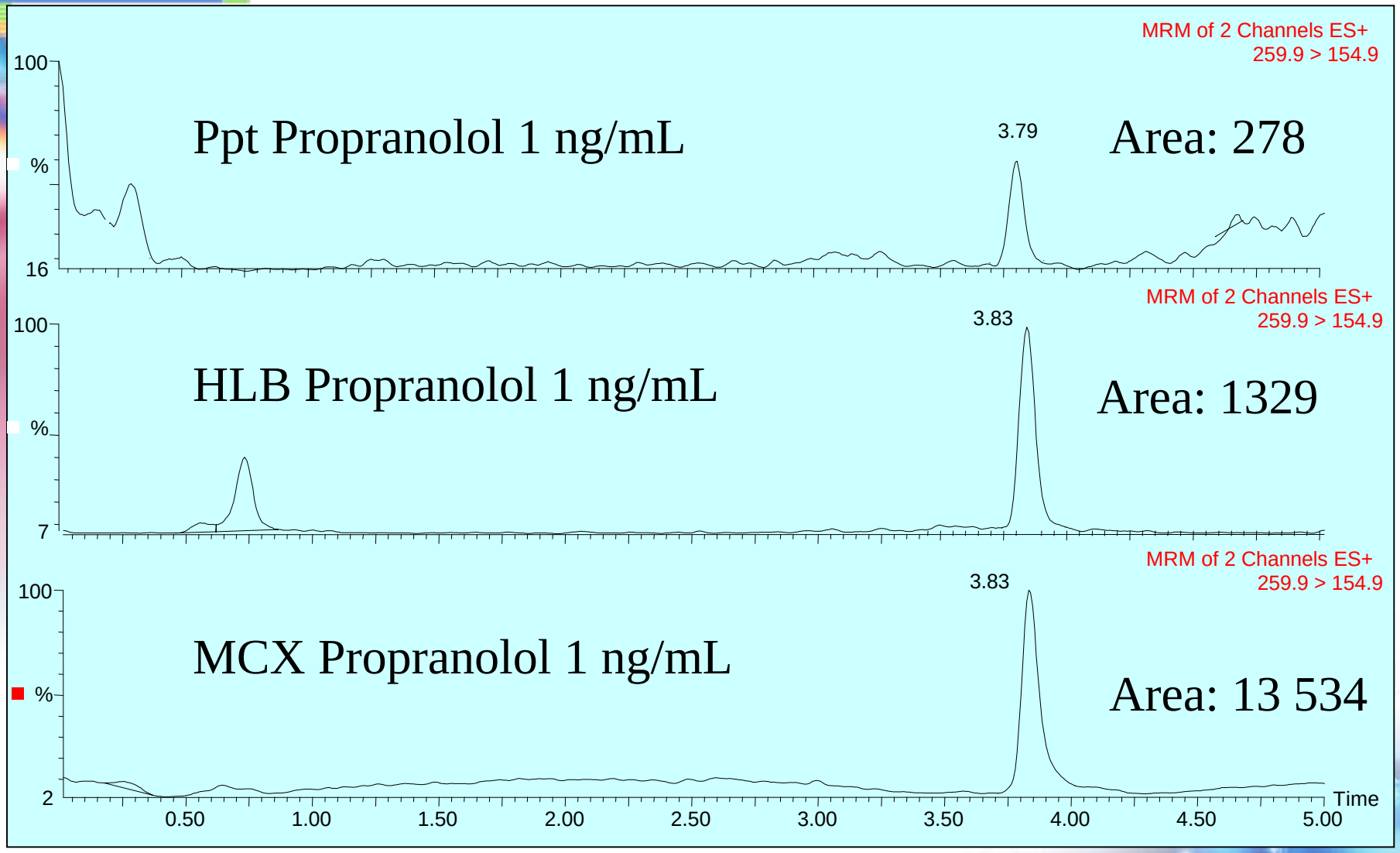


Calibration curve of Propranolol on Oasis® MCX

Conc. ng/mL N=6	2.0	20	50.0	100.0	200.0	500.0	1000.0
Average	2.02	19.10	50.77	103.78	198.72	534.95	968.75
Standard Deviation	0.056	1.17	2.54	5.84	13.14	25.48	16.37
RSD %	2.8	6.1	5.0	5.6	6.6	4.7	1.6

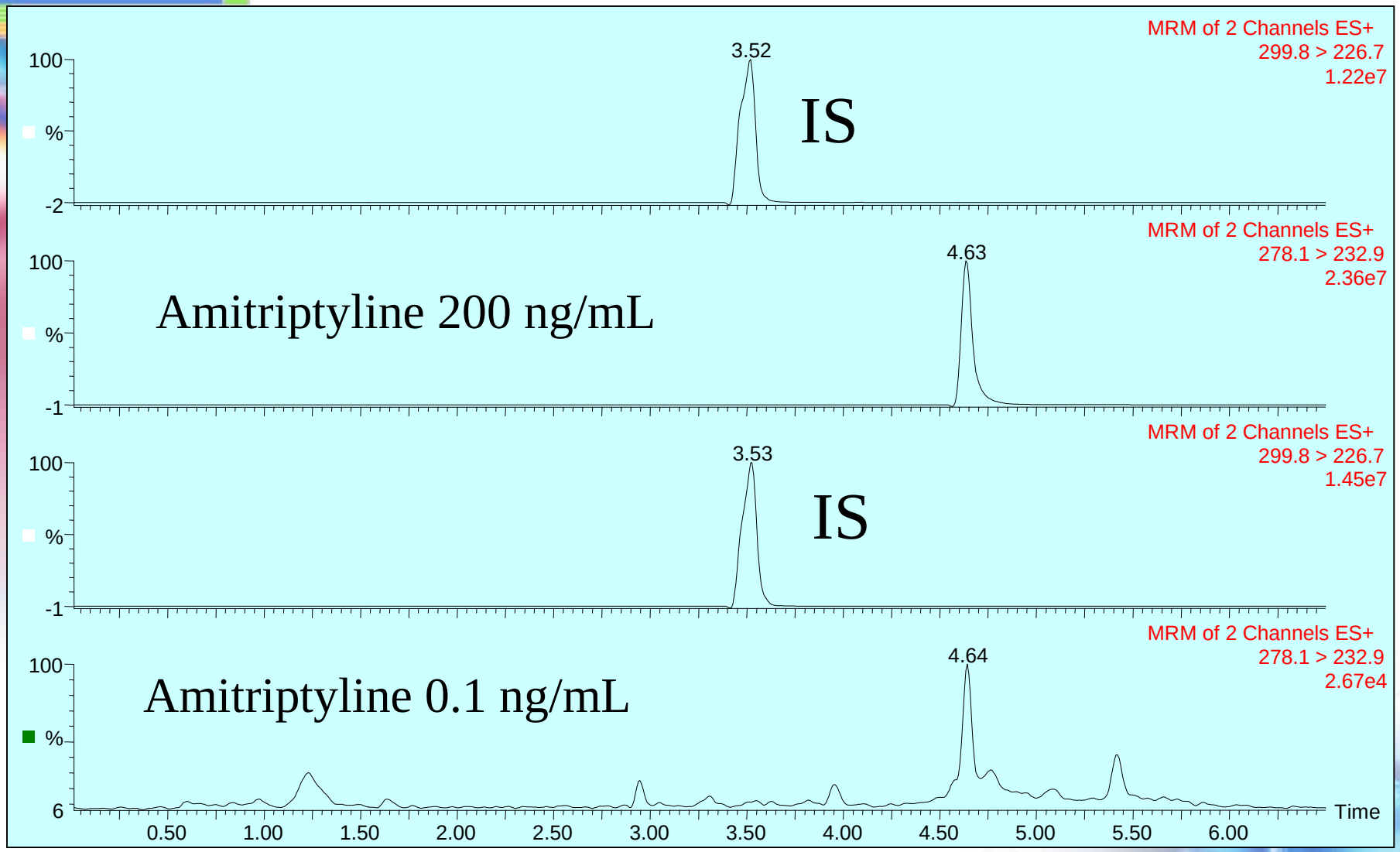


Comparison Ppt vs HLB vs MCX



LC/MS/MS analysis of Amitriptyline on Oasis® HLB

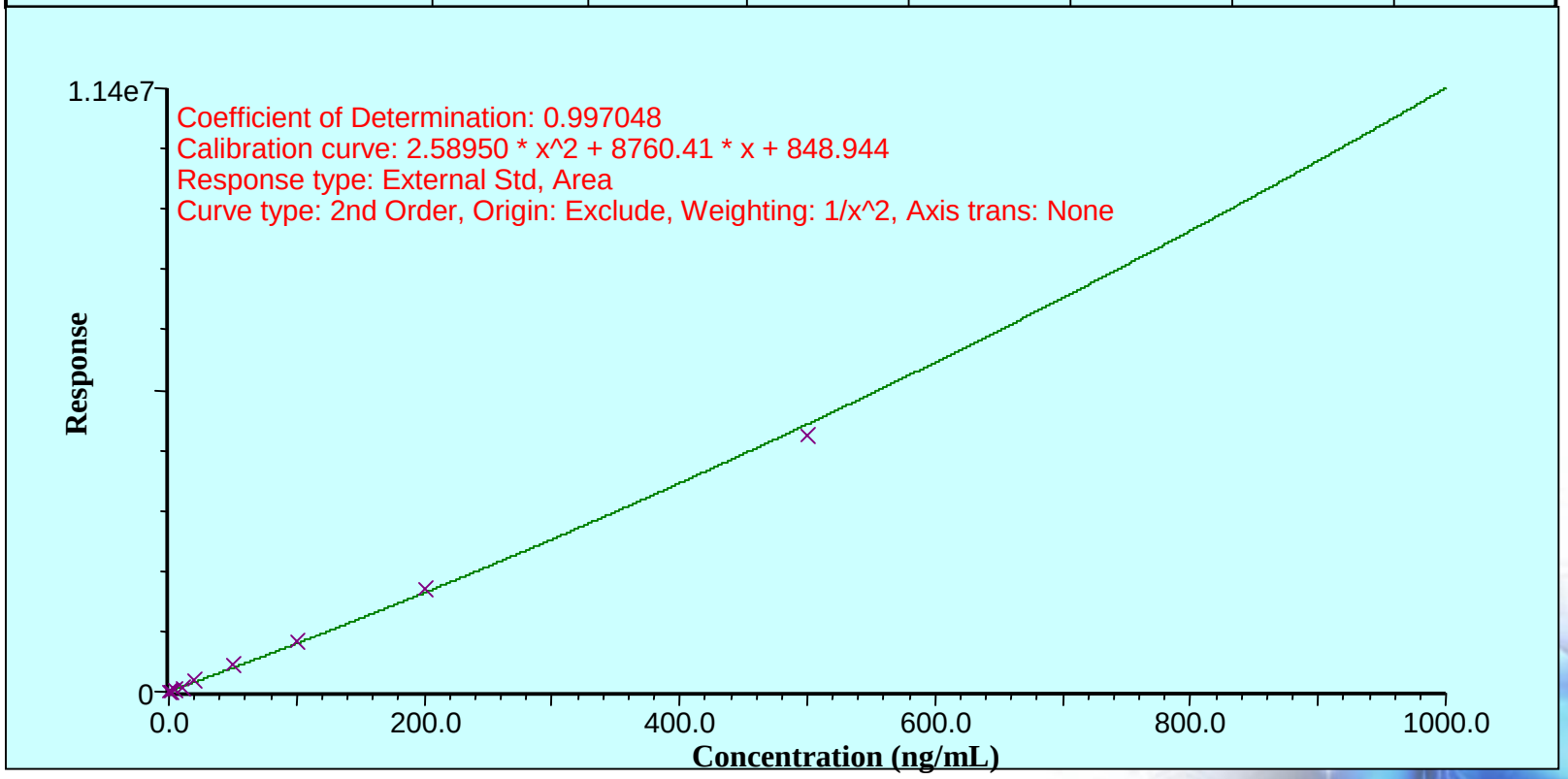
* Elution with 40:60 ACN:IPA + 2% FA



Calibration curve of Amitriptyline on Oasis® HLB

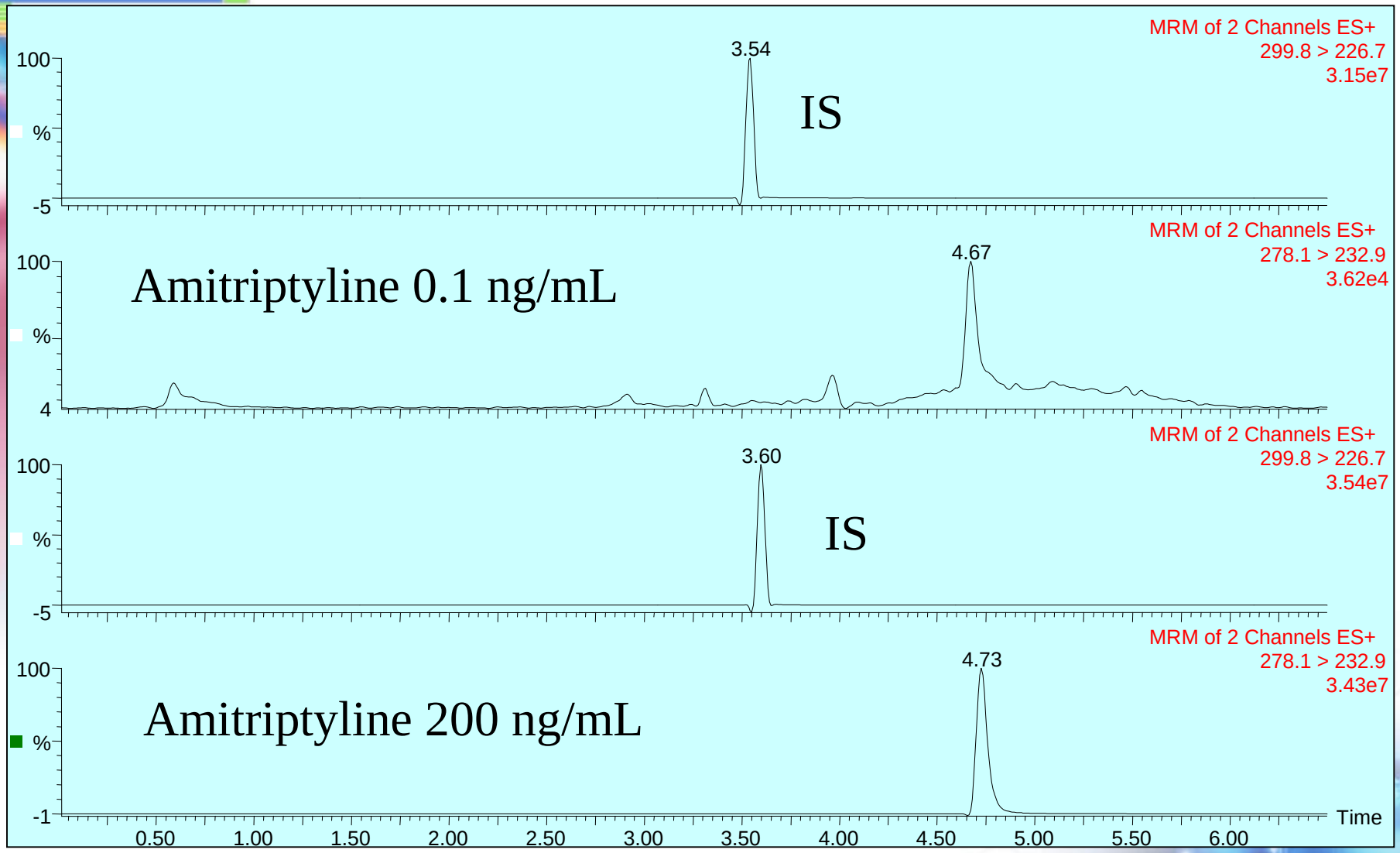
* Elution with 40:60 ACN:IPA + 2 % FA

Conc. ng/mL N=6	0.1	0.2	0.5	5.0	10.0	20.0	50
Average	0.1	0.24	0.49	5.02	10.12	21.0	51.74
Standard Deviation	0.005	0.005	0.017	0.34	0.63	1.27	1.86
RSD %	5.0	2.1	3.6	6.9	6.2	6.0	3.6



LC/MS/MS analysis of Amitriptyline on Oasis® HLB

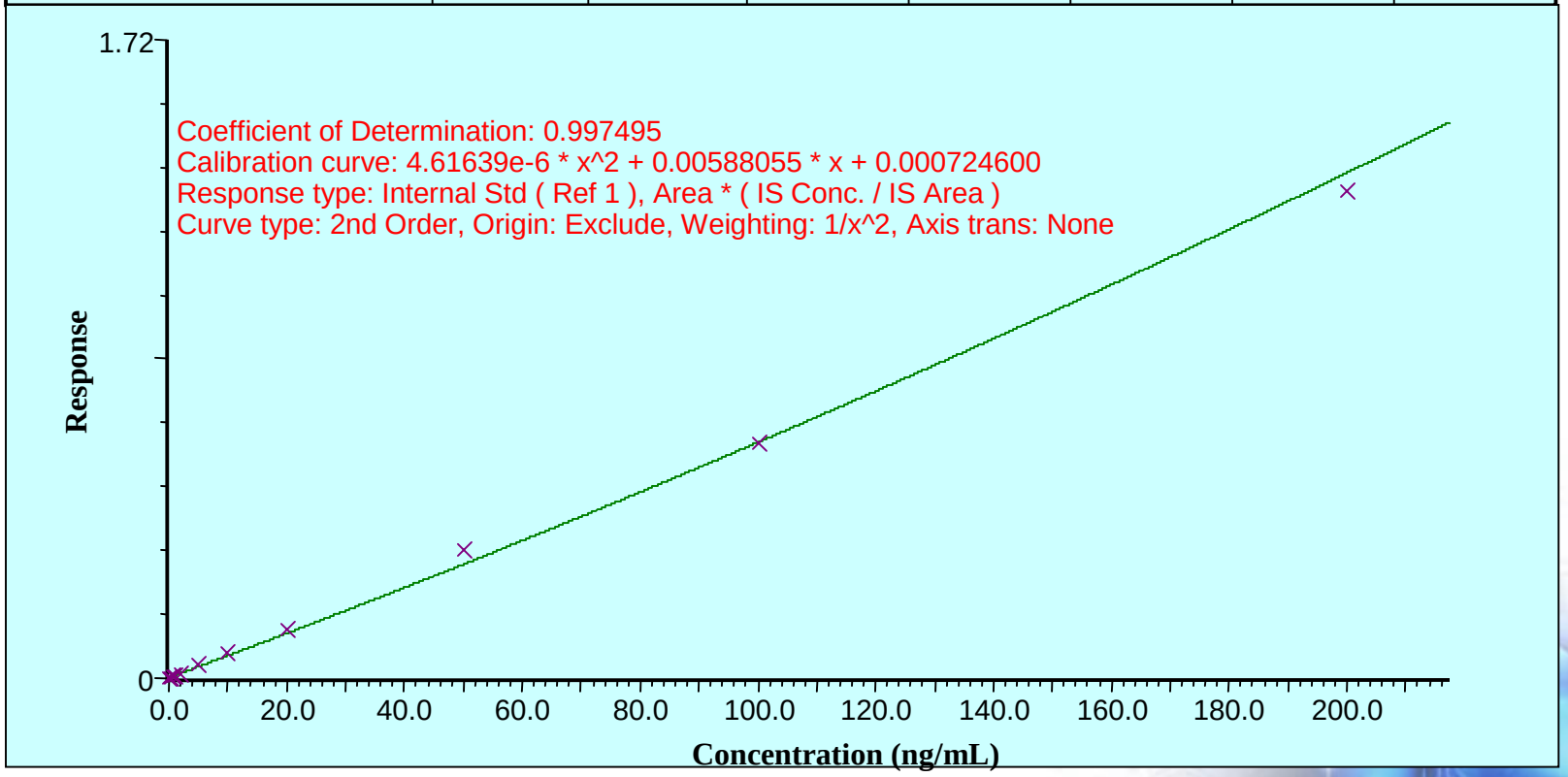
* Elution with 40:60 ACN:IPA + 2 % NH_4OH



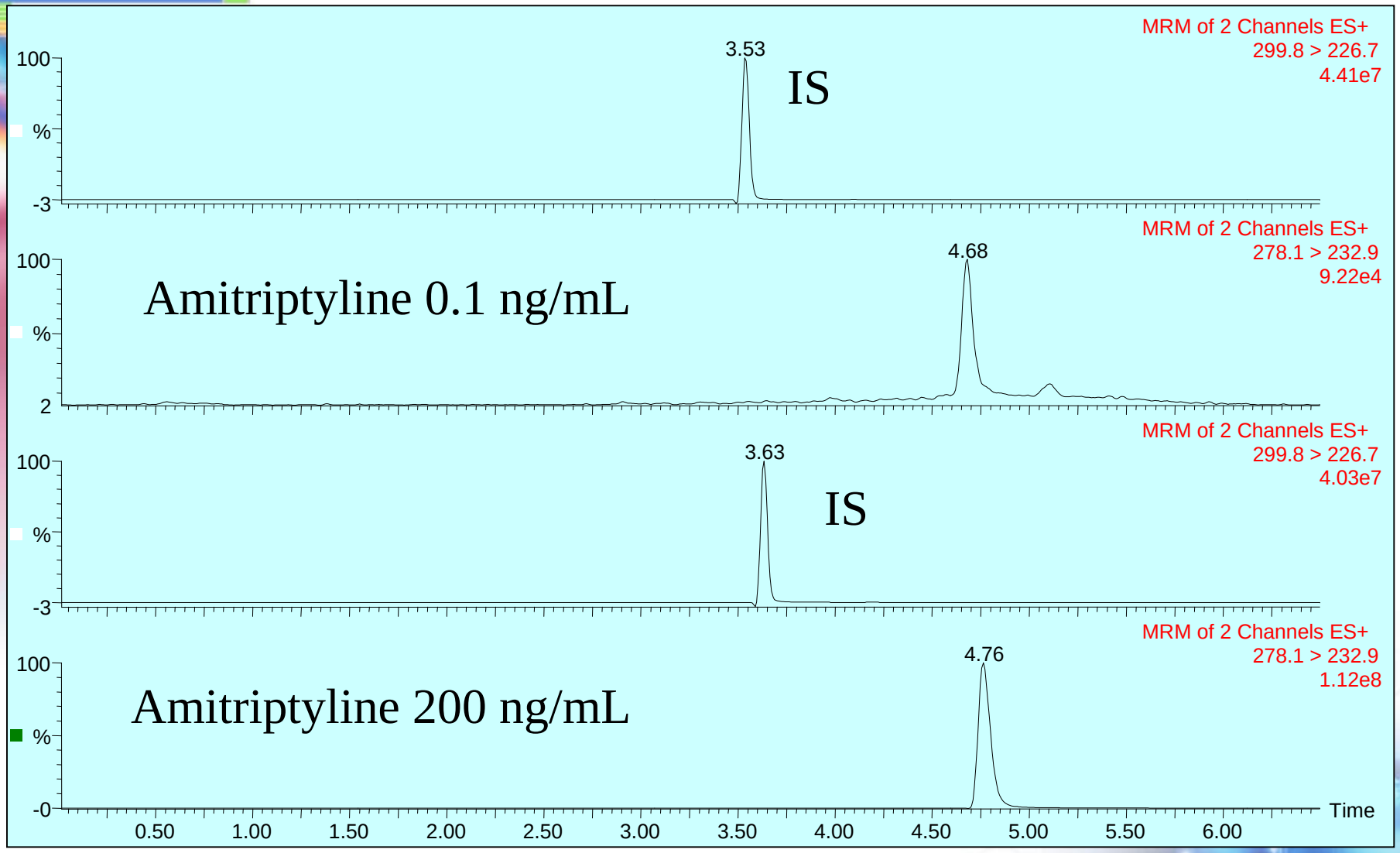
Calibration curve of Amitriptyline on Oasis® HLB

Elution with 40:60 ACN:IPA + 2 % NH_4OH

Conc. ng/mL N=6	0.1	0.5	1.0	5.0	10.0	50.0	100.0
Average	0.101	0.47	0.975	5.05	9.89	53.47	96.58
Standard Deviation	0.003	0.012	0.07	0.35	0.52	1.19	1.33
RSD %	3.6	2.5	7.2	7.0	5.2	2.2	1.3

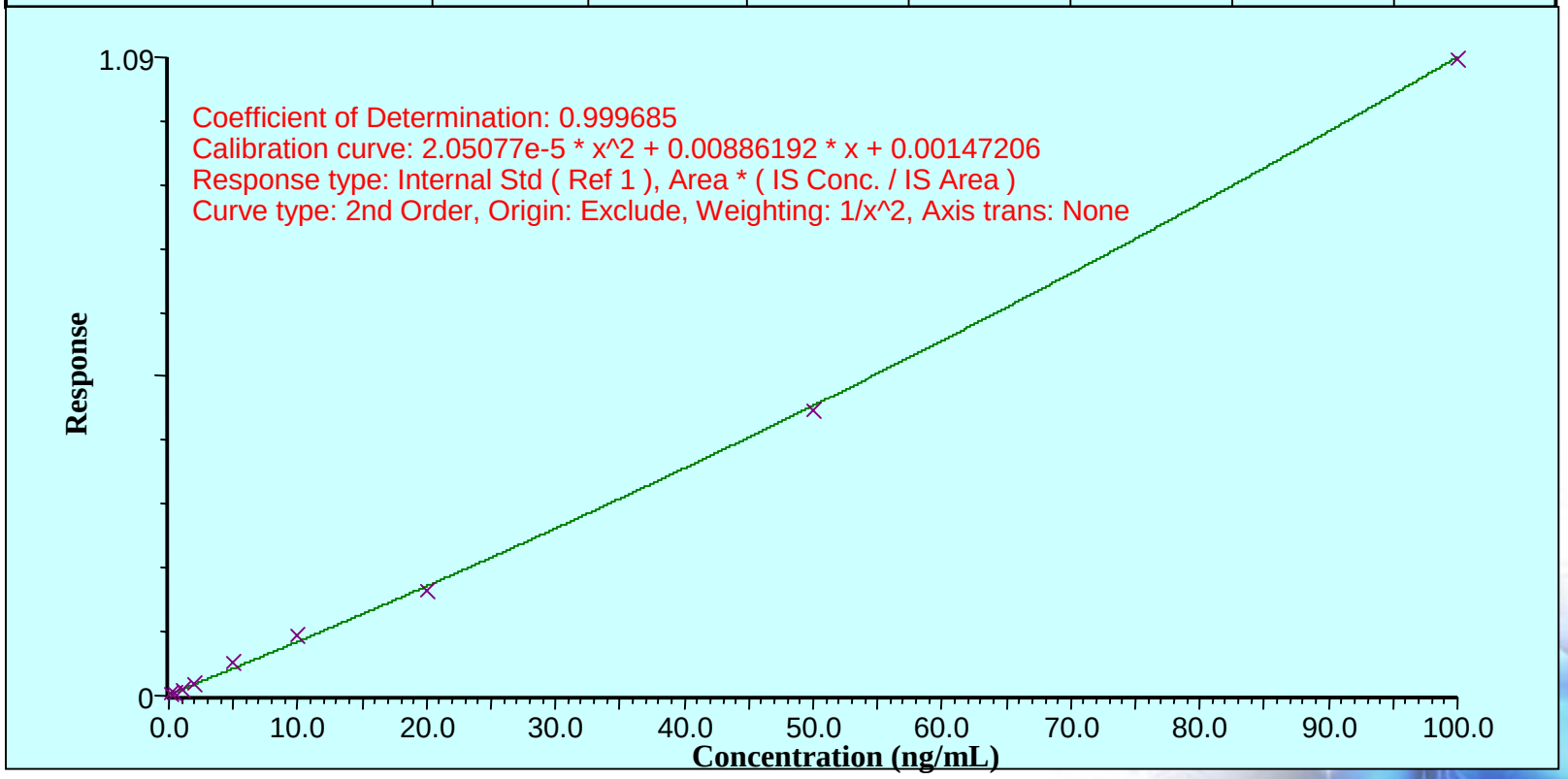


LC/MS/MS analysis of Amitriptyline on Oasis® MCX

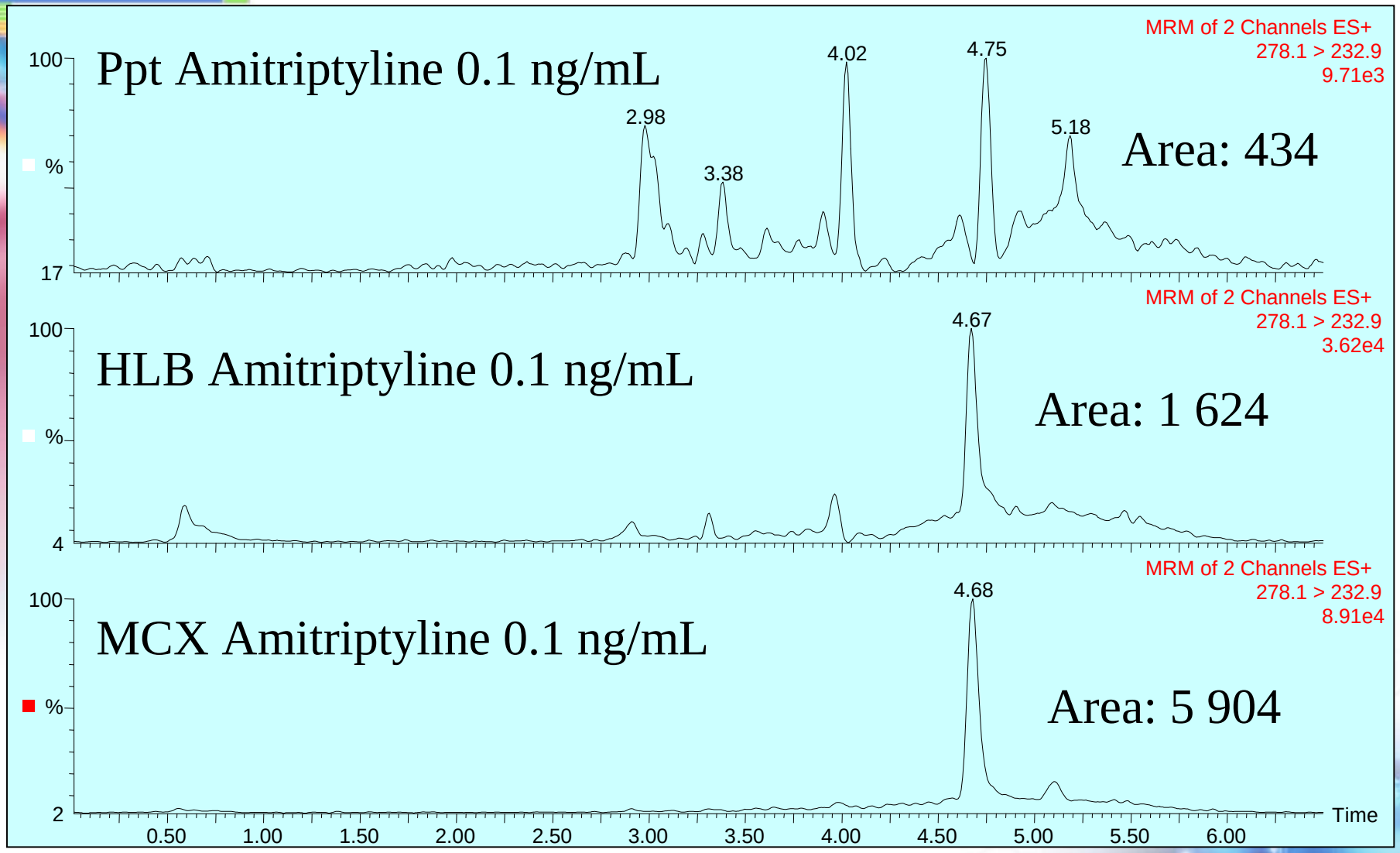


Calibration curve of Amitriptyline on Oasis® MCX

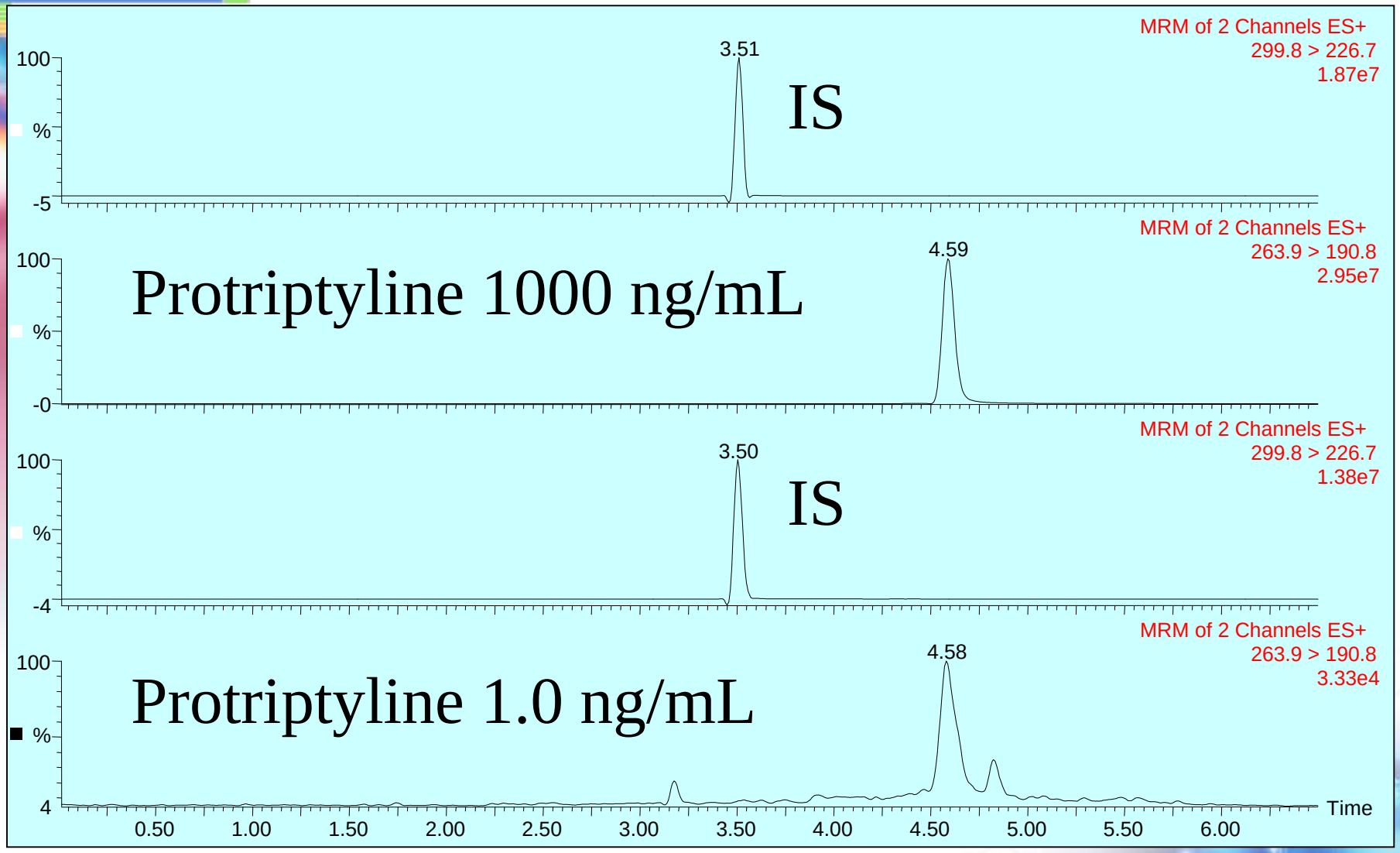
Conc. ng/mL N=6	0.1	0.2	0.5	1.0	5.0	10.0	50.0
Average	0.105	0.188	0.481	1.023	5.104	10.59	52.0
Standard Deviation	0.005	0.009	0.027	0.019	0.23	0.15	2.92
RSD %	4.7	5.2	5.8	1.9	4.6	1.4	5.6



Comparison of Ppt vs HLB vs MCX



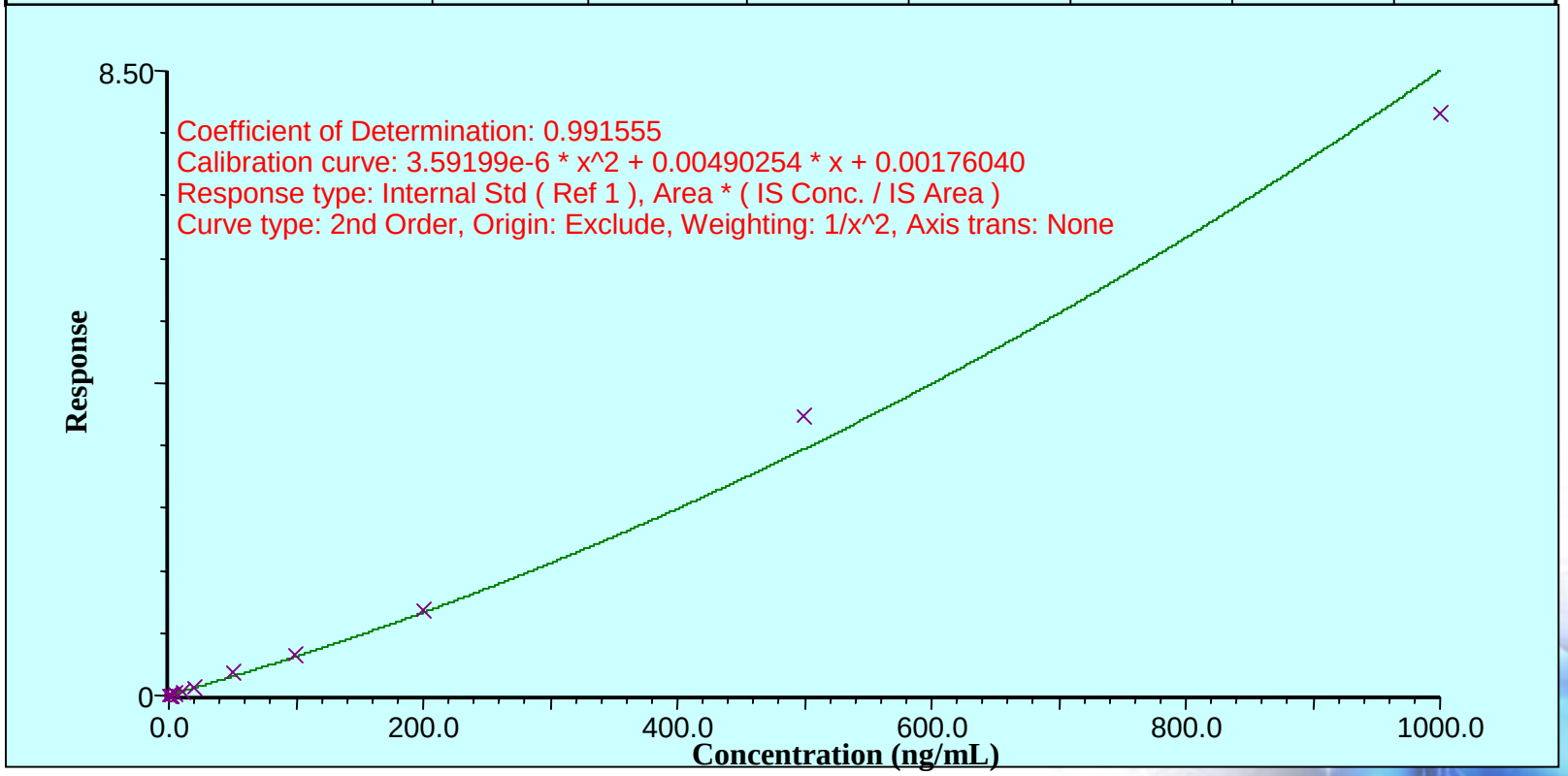
LC/MS/MS analysis of Protriptyline on Oasis® HLB



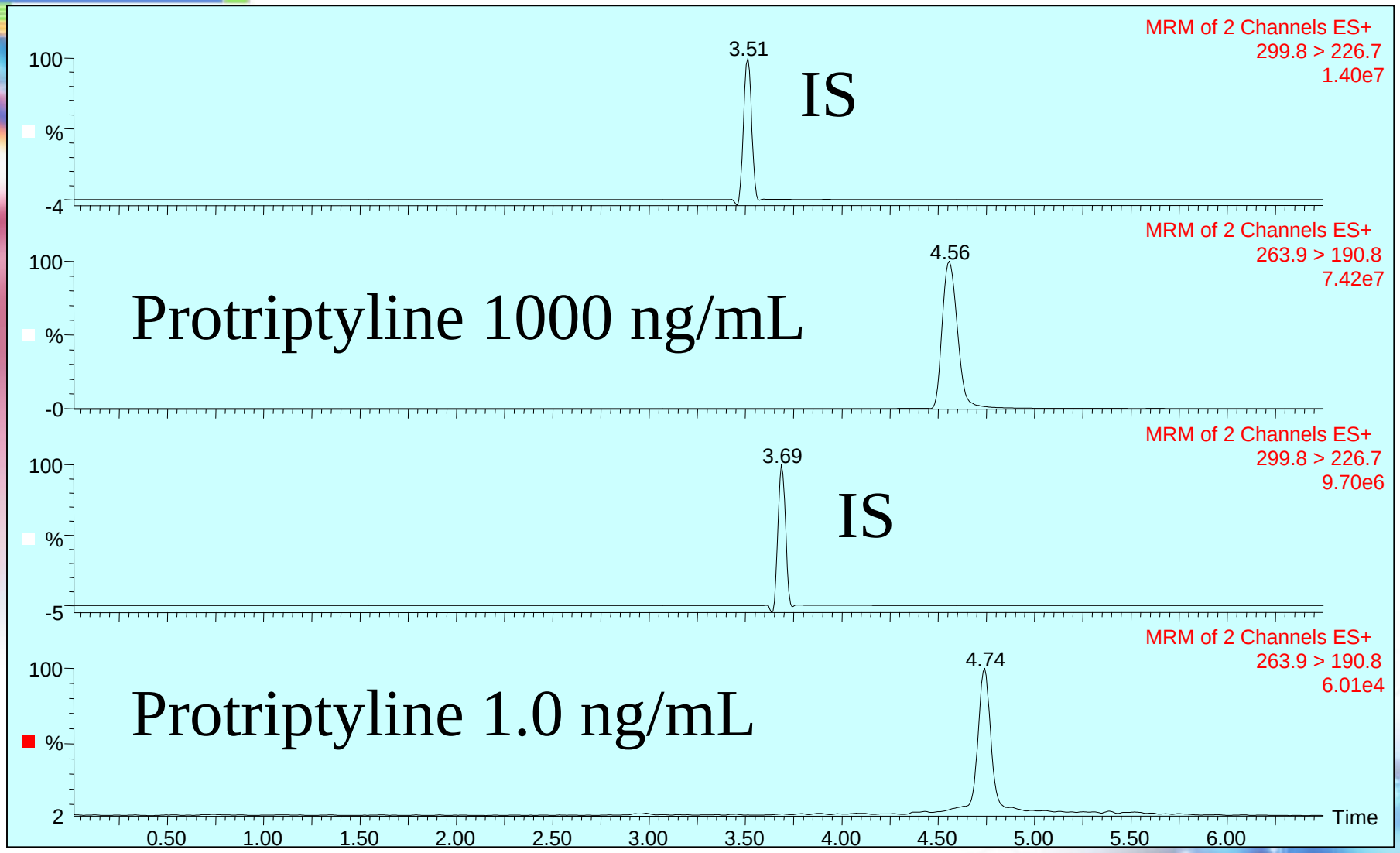
Calibration curve of Protriptyline on Oasis® HLB

Elution with 40:60 ACN:IPA + 2 % NH_4OH

Conc. ng/mL N=6	5.0	10.0	20.0	100.0	200.0	500.0	1000.0
Average	4.8	9.8	20.0	104.3	204.0	538.7	988.3
Standard Deviation	0.28	0.48	1.19	3.11	10.13	1.34	1.33
RSD %	5.8	4.9	5.9	2.9	4.9	0.2	1.3



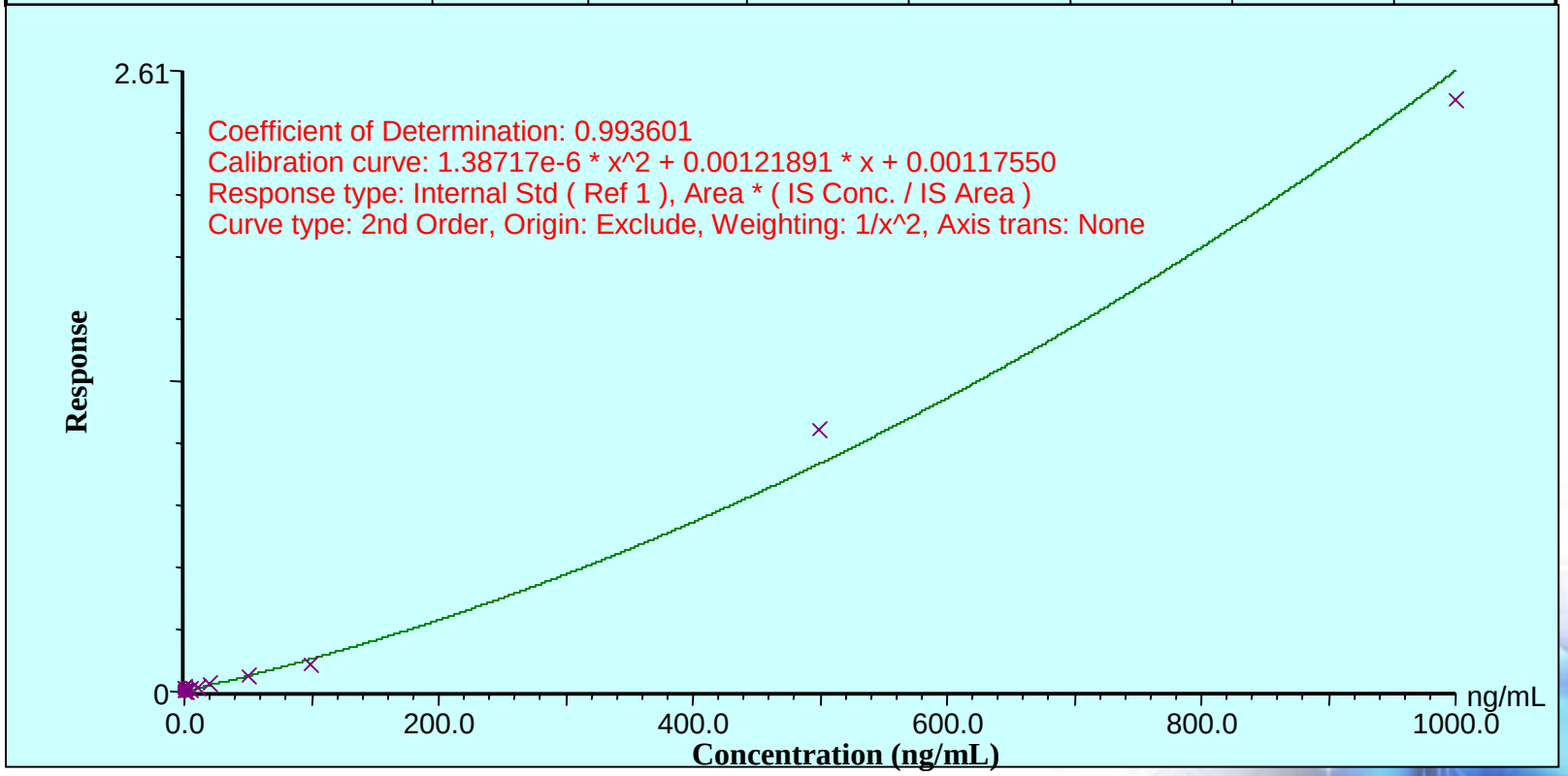
LC/MS/MS analysis of Protriptyline on Oasis® MCX



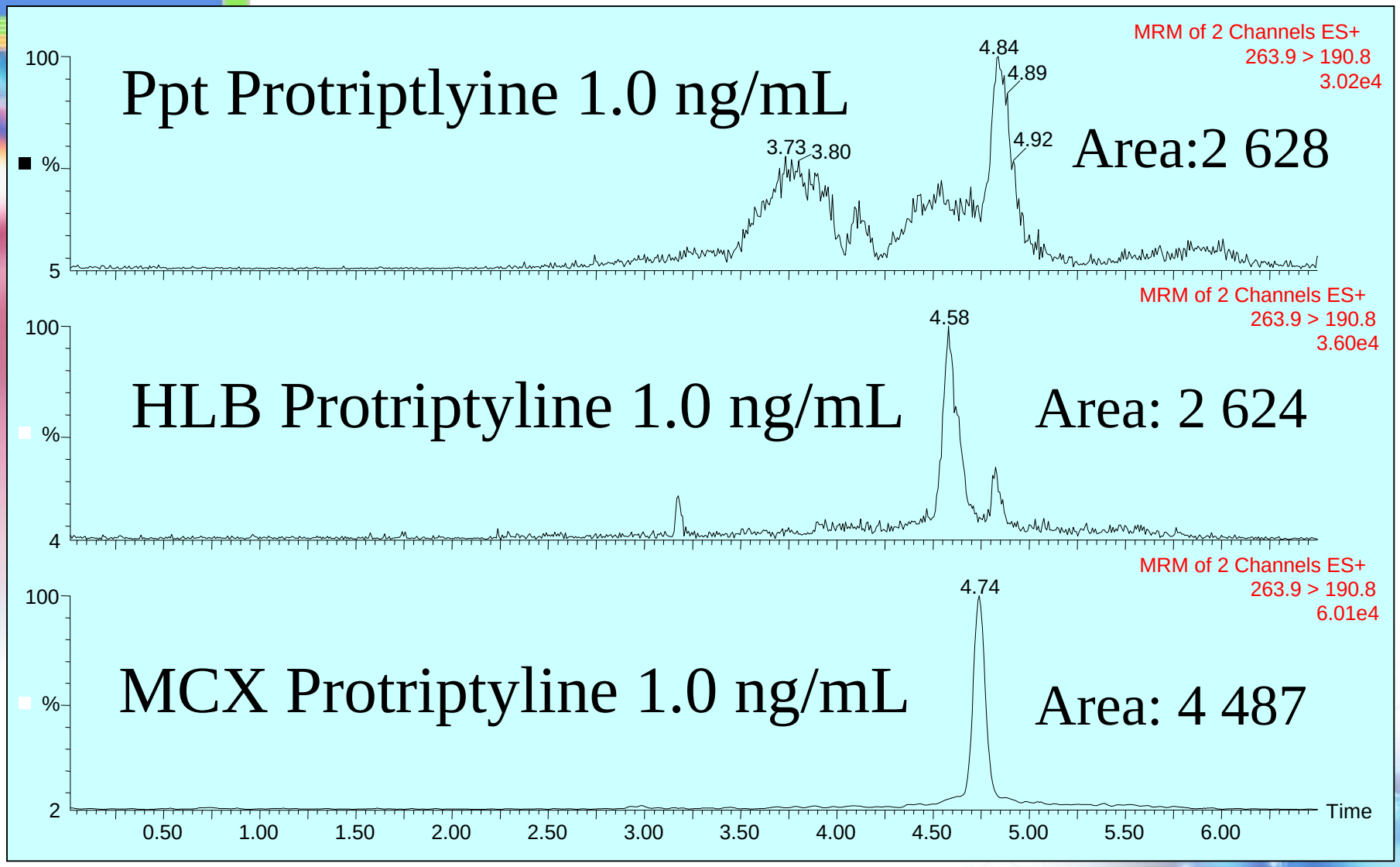
Calibration curve of Protriptyline on Oasis® MCX

Elution with 40:60 ACN:IPA + 2 % NH_4OH

Conc. ng/mL N=6	1.0	5.0	10.0	20.0	50.0	500.0	1000.0
Average	1.01	4.9	9.6	19.4	51.7	515.9	989.8
Standard Deviation	0.01	0.3	0.55	1.06	1.62	22.21	20.34
RSD %	1.5	6.1	5.7	5.4	3.1	4.3	2.0



Comparison of Ppt vs HLB vs MCX



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Conclusions

Wide volume range for loading (50 to 800 µL)

Low elution volume (25 – 50 µL)

Generic protocols for HLB and MCX

Pre-concentration factor up to 10x

Sub ng/mL quantitation limits

96-well plate format for high throughput