Pharmaceuticals and Personal Care Products Analysis in Bottled, Tap and Surface Water Using Two-Dimensional Liquid Chromatography Mass Spectrometry (2D LC/MS/MS)

C.R. Mallet
Waters Corporation

NEMC 2014
Washington DC

Time De-Coupled Chromatography
This is why…

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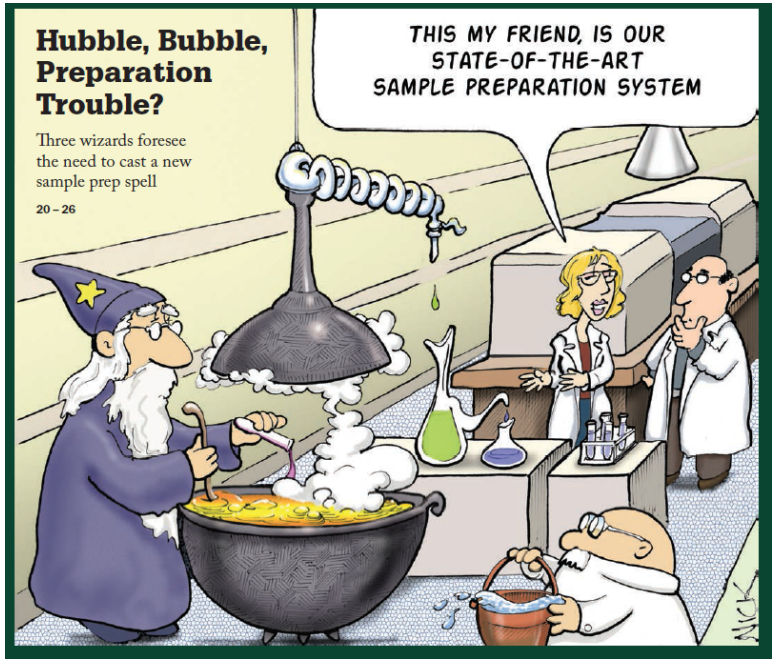
JULY 2014 # 18

the Analytical Scientist



managers underestimate the importance of good sample prep: "Want to buy a €350,000 GCxGC QToF system? You have it, no problem. Want to spend €20,000 for sample preparation? No. That's too much." What are your thoughts?

a little strange, indeed unacceptable, that papers are still being published that show amazing data concerning sensitivity but rely on sample preparation methods based on liquid-liquid extraction of a 1L water sample with 100 mL dichloromethane, followed by evaporative concentration to 100 µL and injection of 1 µL.

Traditional Extraction Protocol
Class A (Circa 1990)

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Traditional

0 min — Condition 5 mL ACN/Dichloromethane (1:1)

Condition 5 mL Methanol

6 min — Condition 5 mL Water

Load Sample – 1000 mL @ 10 mL/min

120 min

Milli Q:	2-3 hrs
Tap Water:	5-6 hrs
Surface Water:	12-15 hrs

Wash 5 mL Water

Air Dry – 10 min

Elute 5 mL ACN

60 min — Evaporate to dryness

Reconstitute 1 mL ACN/Water (1:9)

3.0 hrs

Inject 10 μL into LC/MS/MS

1000:1 enrichment from sample protocol
1% of final extract use for analysis

Traditional Extraction Protocol
Class A (Circa 2013)

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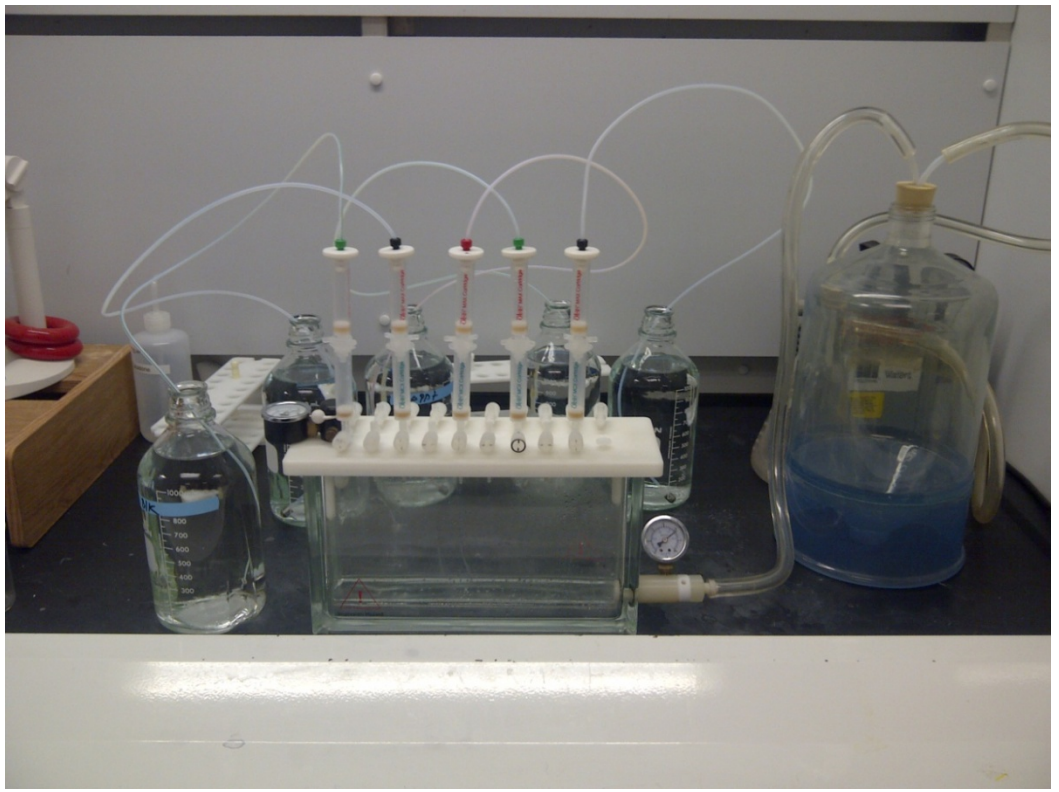
Traditional

0 min	Condition 5 mL ACN/Dichloromethane (1:1)
	Condition 5 mL Methanol
6 min	Condition 5 mL Water
120 min	Load Sample – 1000 mL @ 10 mL/min Milli Q: 2-3 hrs Tap Water: 5-6 hrs Surface Water: 12-15 hrs
	Wash 5 mL Water
	Air Dry – 10 min
	Elute 5 mL ACN
60 min	Evaporate to dryness
	Reconstitute 1 mL ACN/Water (1:9)



3.0 hrs

→ Inject 10 μL into LC/MS/MS

1000:1 enrichment from sample protocol
1% of final extract use for analysis

NEMC 2007 – Cambridge MA
Drinking Water Analyzer (2-1 configuration)

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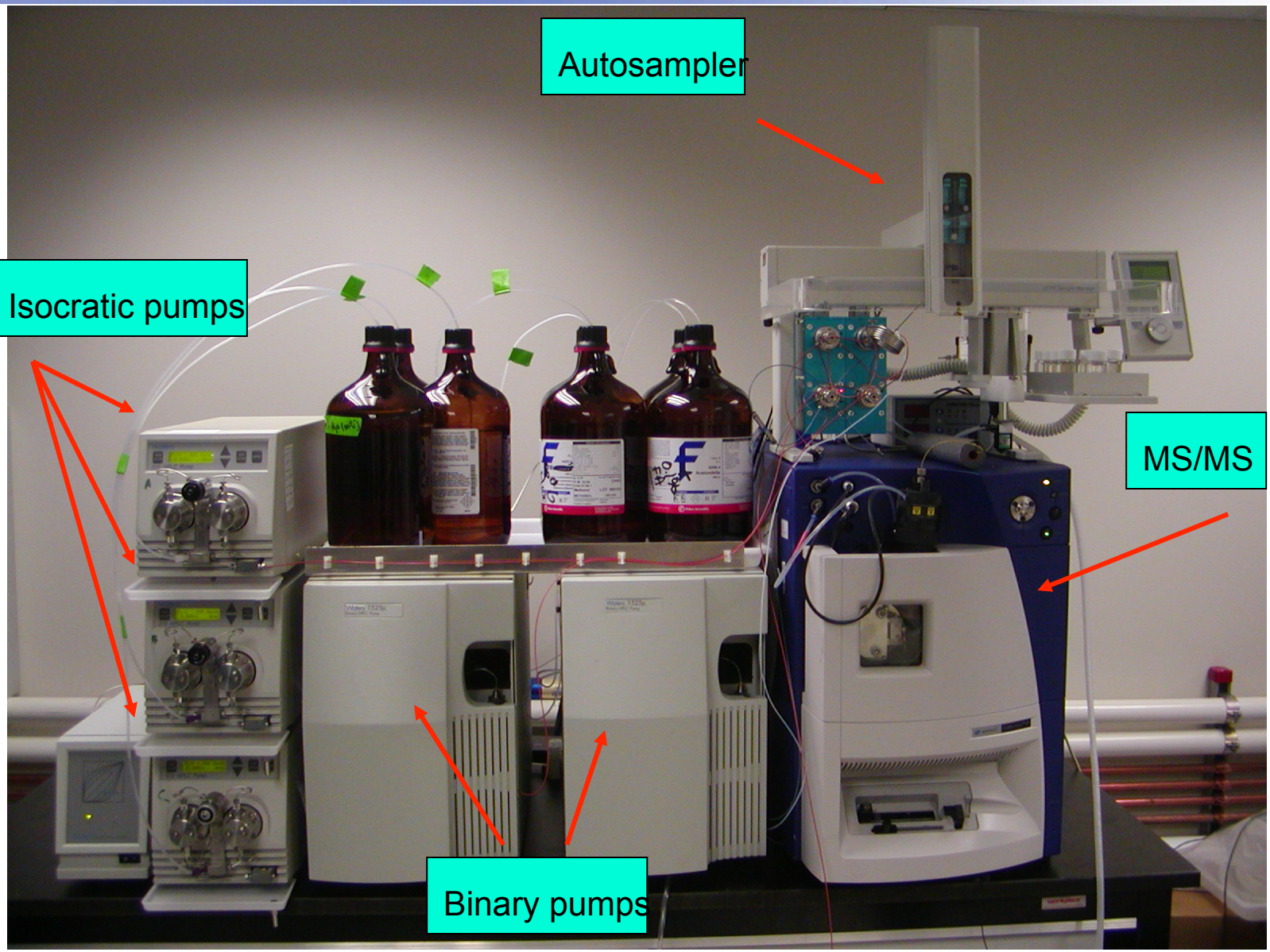

NEMC 2007 – Cambridge MA
OFF-line Vs ON-line

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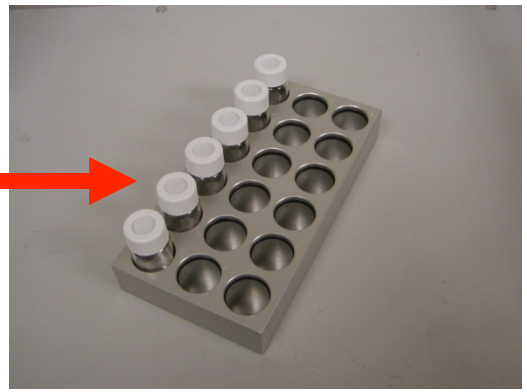
1000 mL Container

20 mL vial



Autosampler rack

NEMC 2007 – Cambridge MA
Triazine in drinking water

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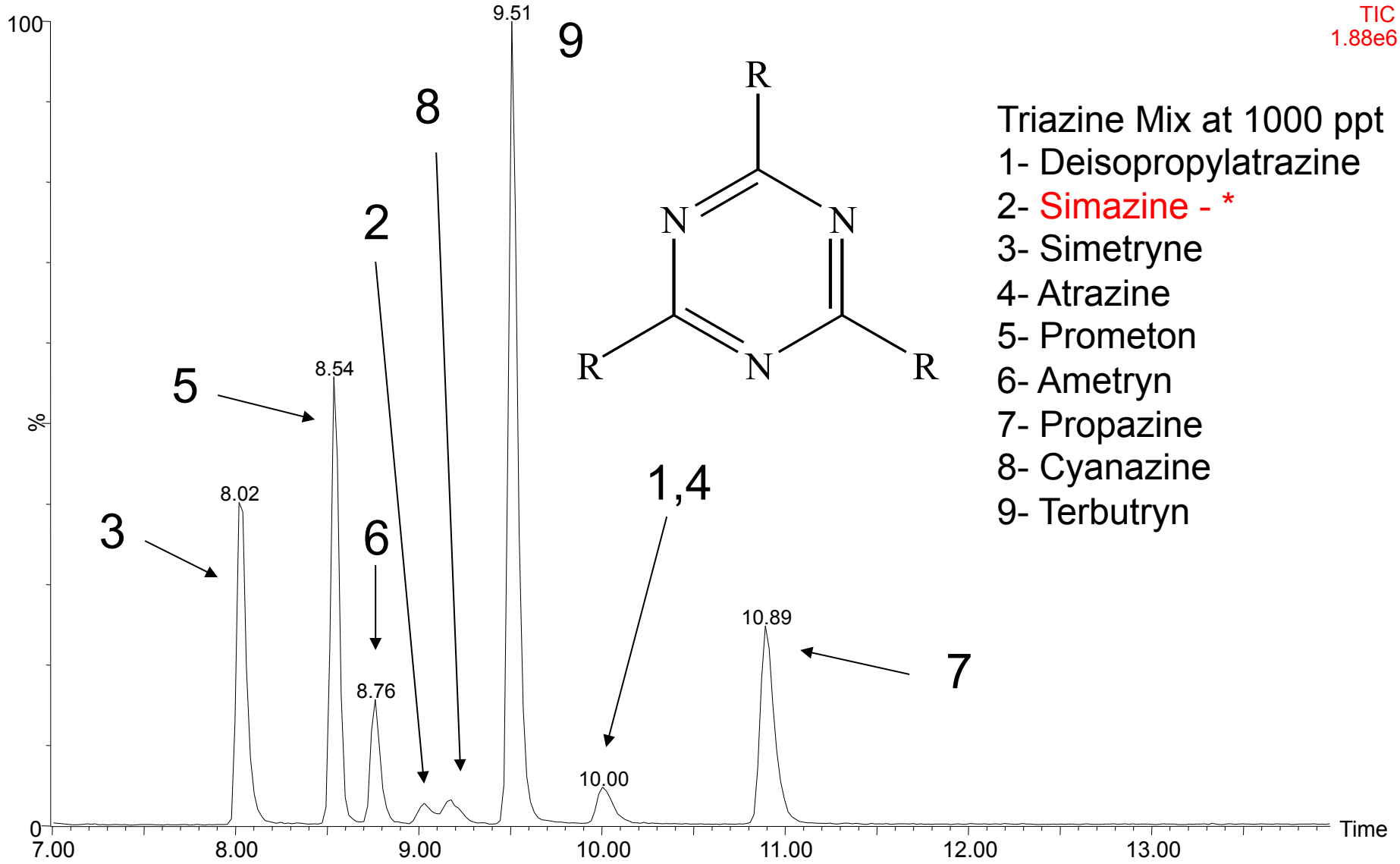

Triazine Mix at 1000 ppt
1- Deisopropylatrazine
2- Simazine - *
3- Simetryne
4- Atrazine
5- Prometon
6- Ametryn
7- Propazine
8- Cyanazine
9- Terbutryn

NEMC 2008- Washington DC
Drinking Water Analyzer (2-1 configuration)

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NEMC 2008- Washington DC
15 mL well water (10 ppt)

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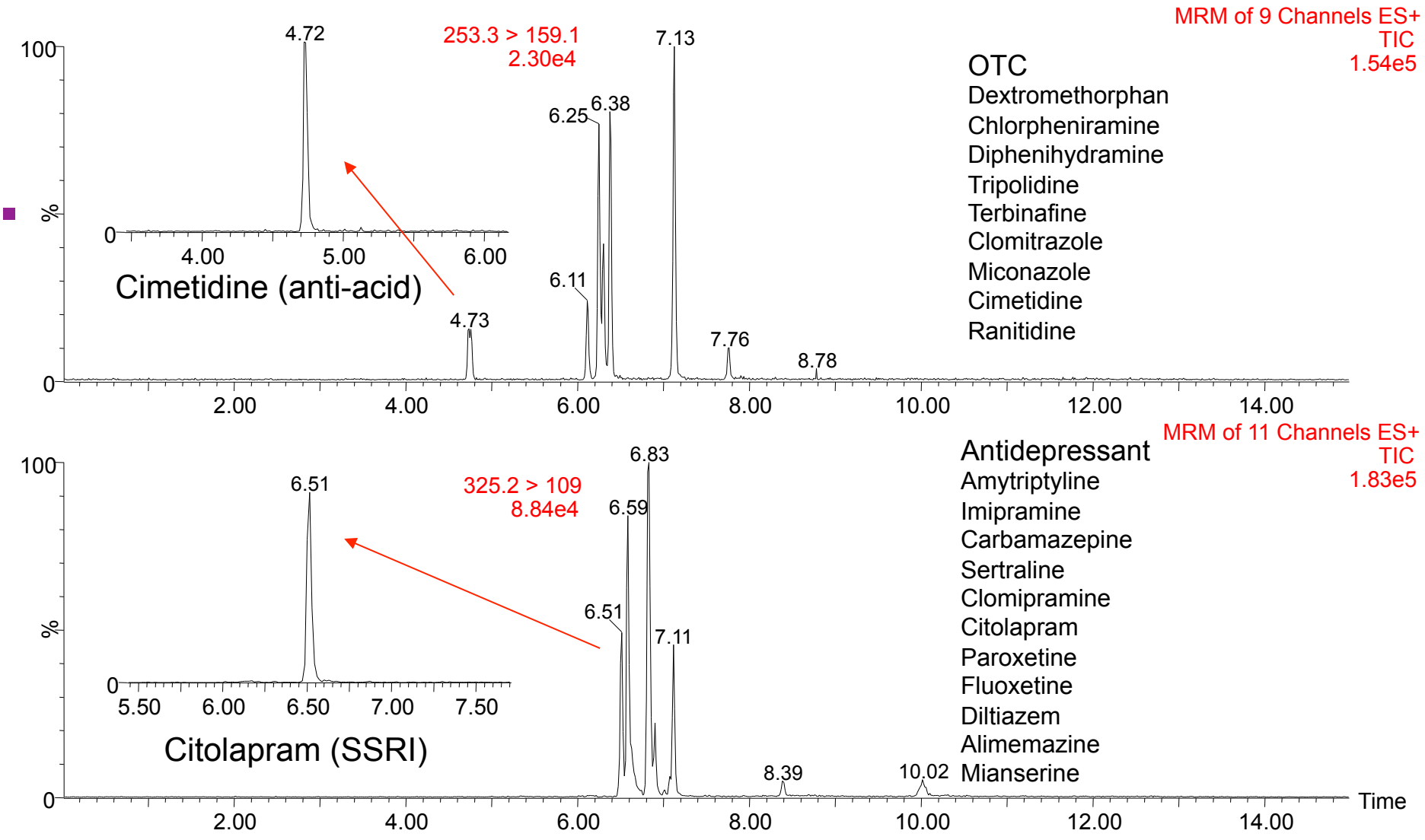

NEMC 2009- San Antonio, TX
15 mL surface water 10 ppt

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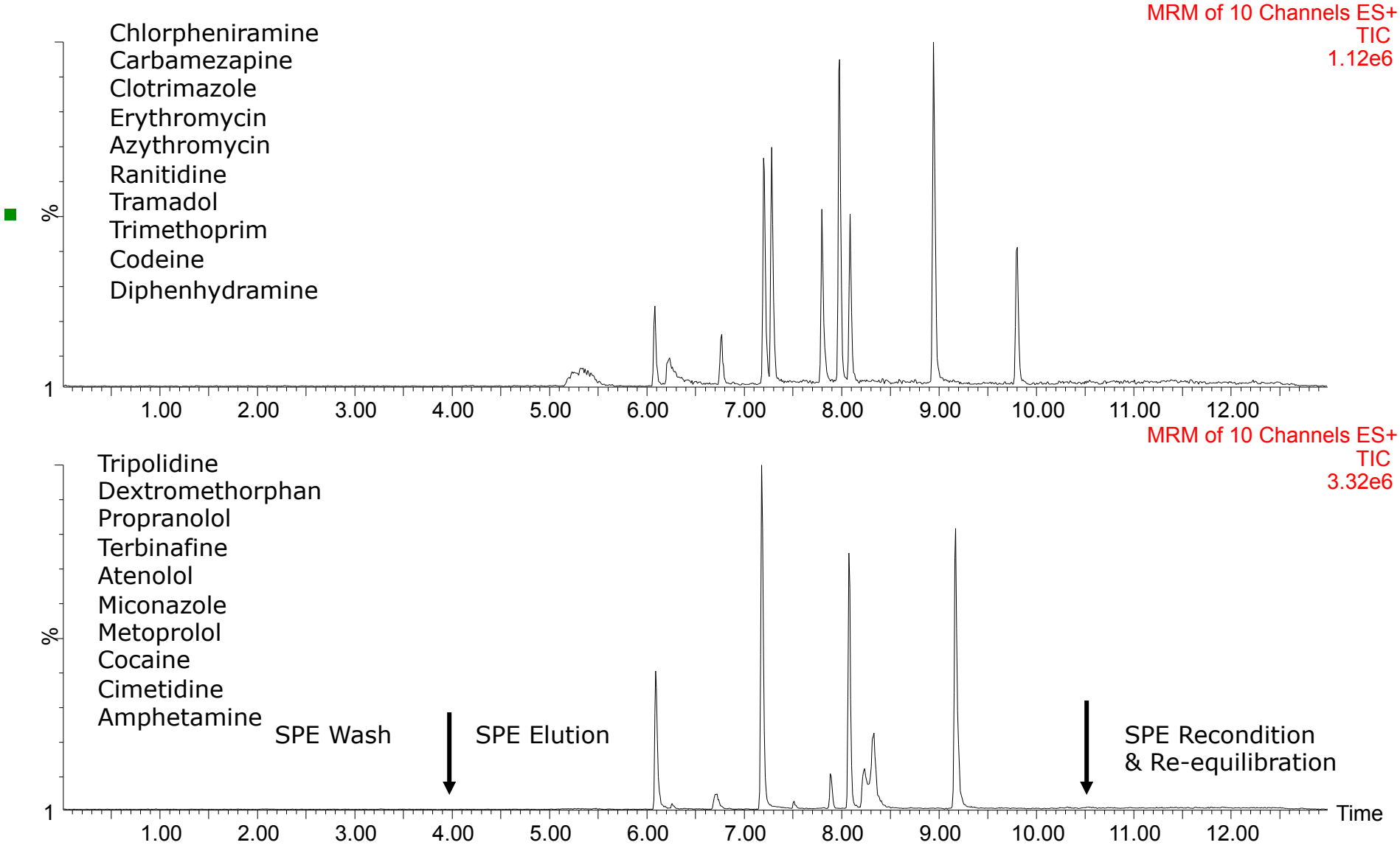

NEMC 2010- Washington DC
Open Architecture 2D & Acquity 2D

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Acquity 2D

Open Architecture 2D

NEMC 2010
Corticosteroids in reactor effluents

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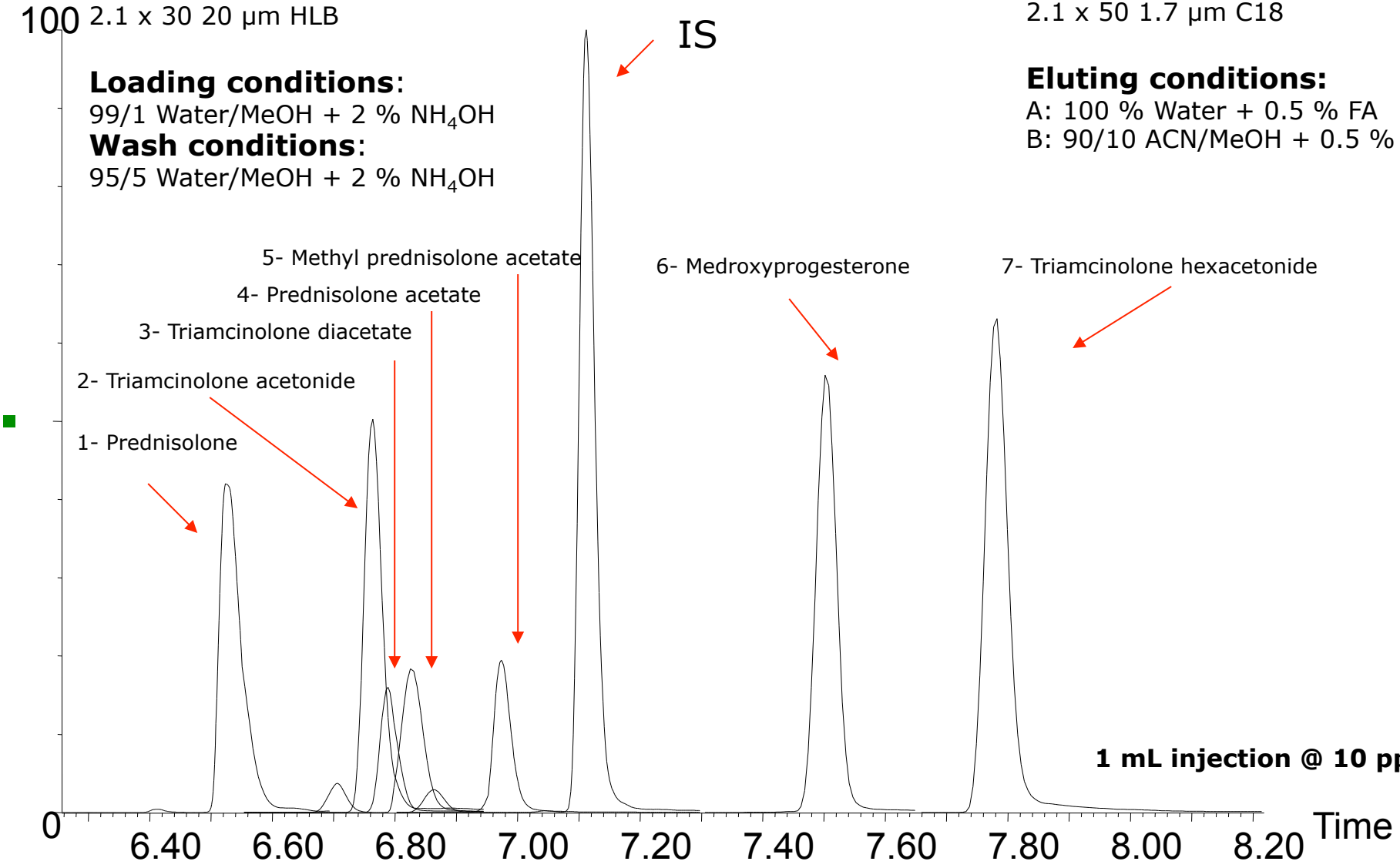
SPE:
2.1 x 30 20 µm HLB

Loading conditions:
99/1 Water/MeOH + 2 % NH_4OH

Wash conditions:
95/5 Water/MeOH + 2 % NH_4OH

UPLC:
2.1 x 50 1.7 µm C18

Eluting conditions:
A: 100 % Water + 0.5 % FA
B: 90/10 ACN/MeOH + 0.5 % FA



1 mL injection @ 10 ppb

NEMC 2014- Washington DC
Time De-Coupled Chromatography (2D/3D)

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2D “Trap & elution” configuration

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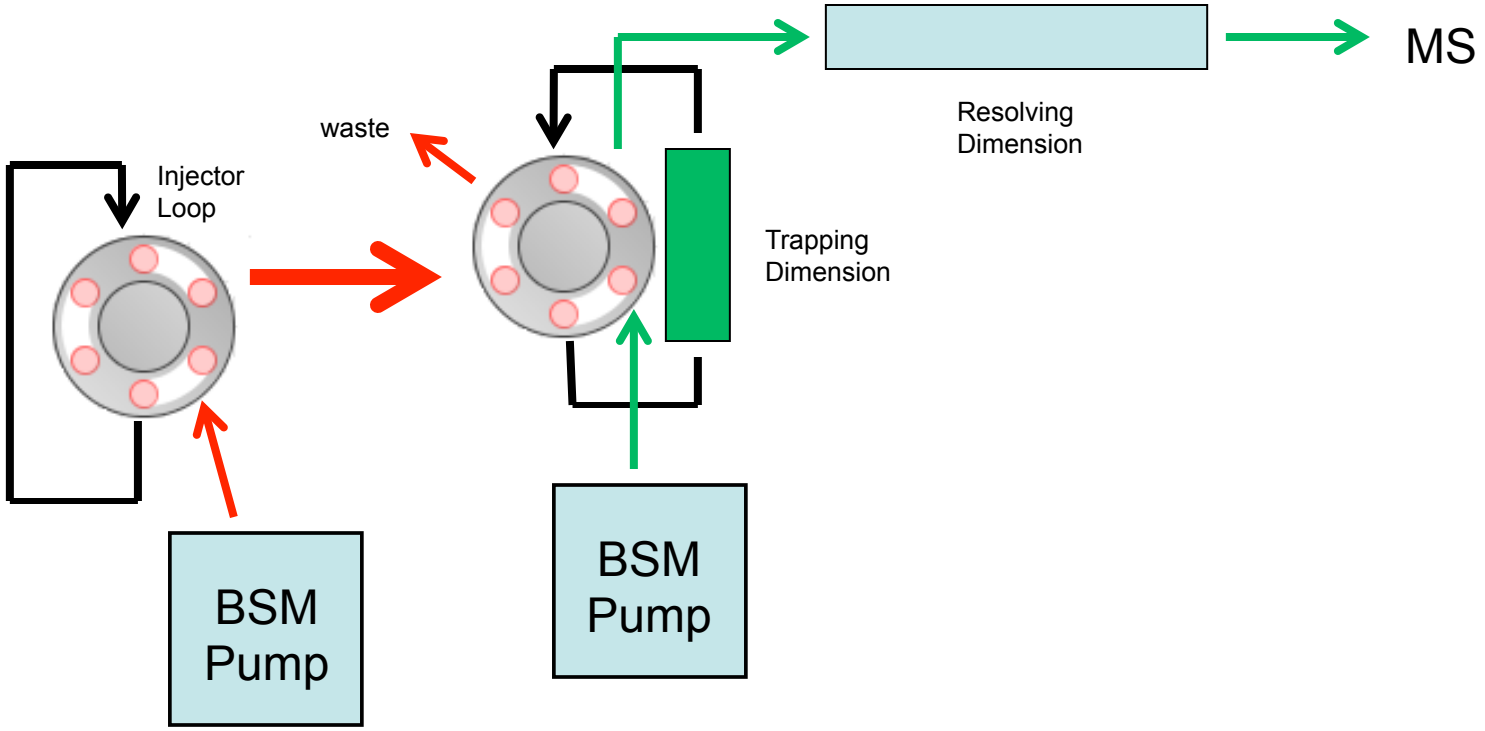

2D “Trap & elution” configuration
Aqueous Extract

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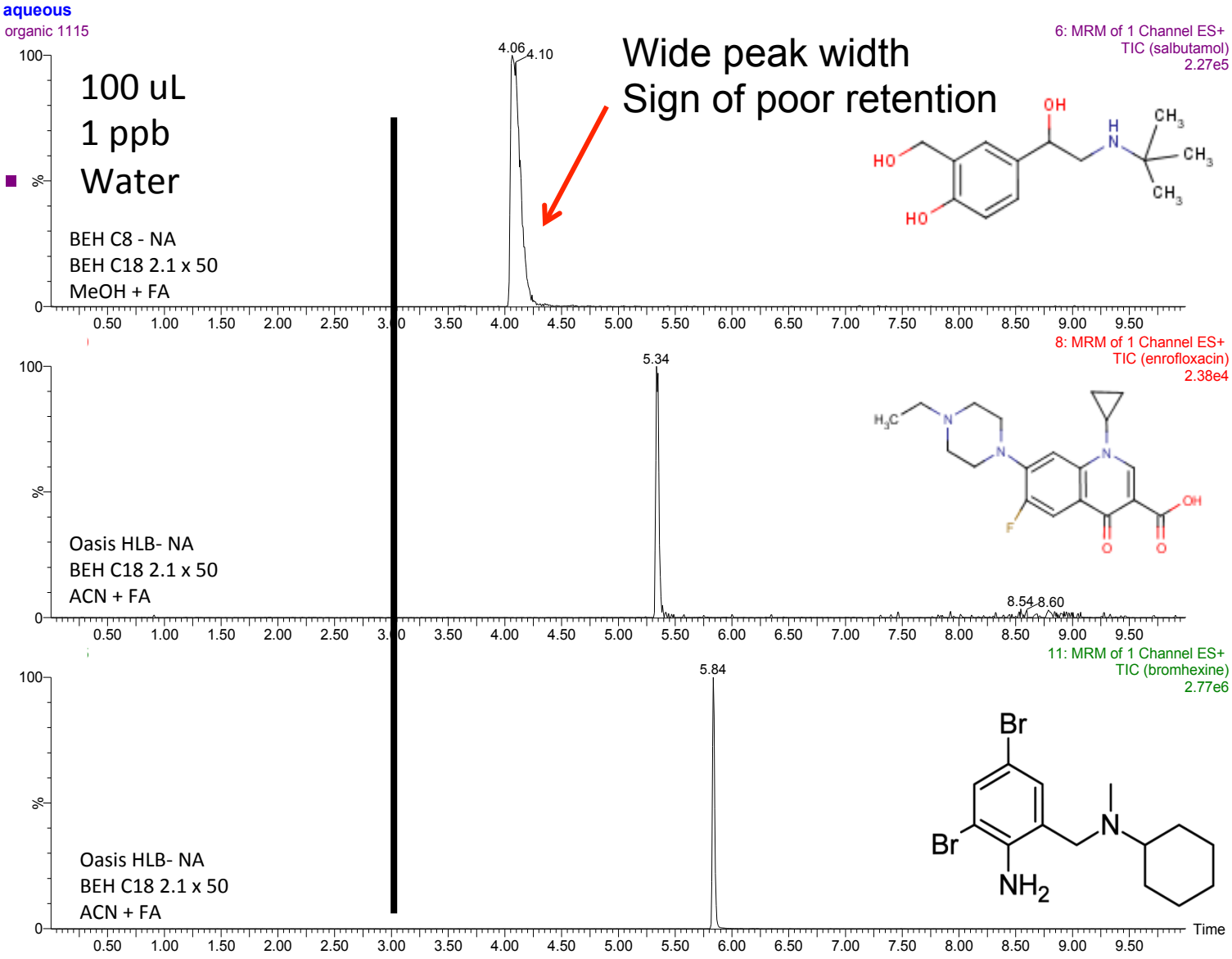

2D “Trap & elution” configuration
MeOH Extract

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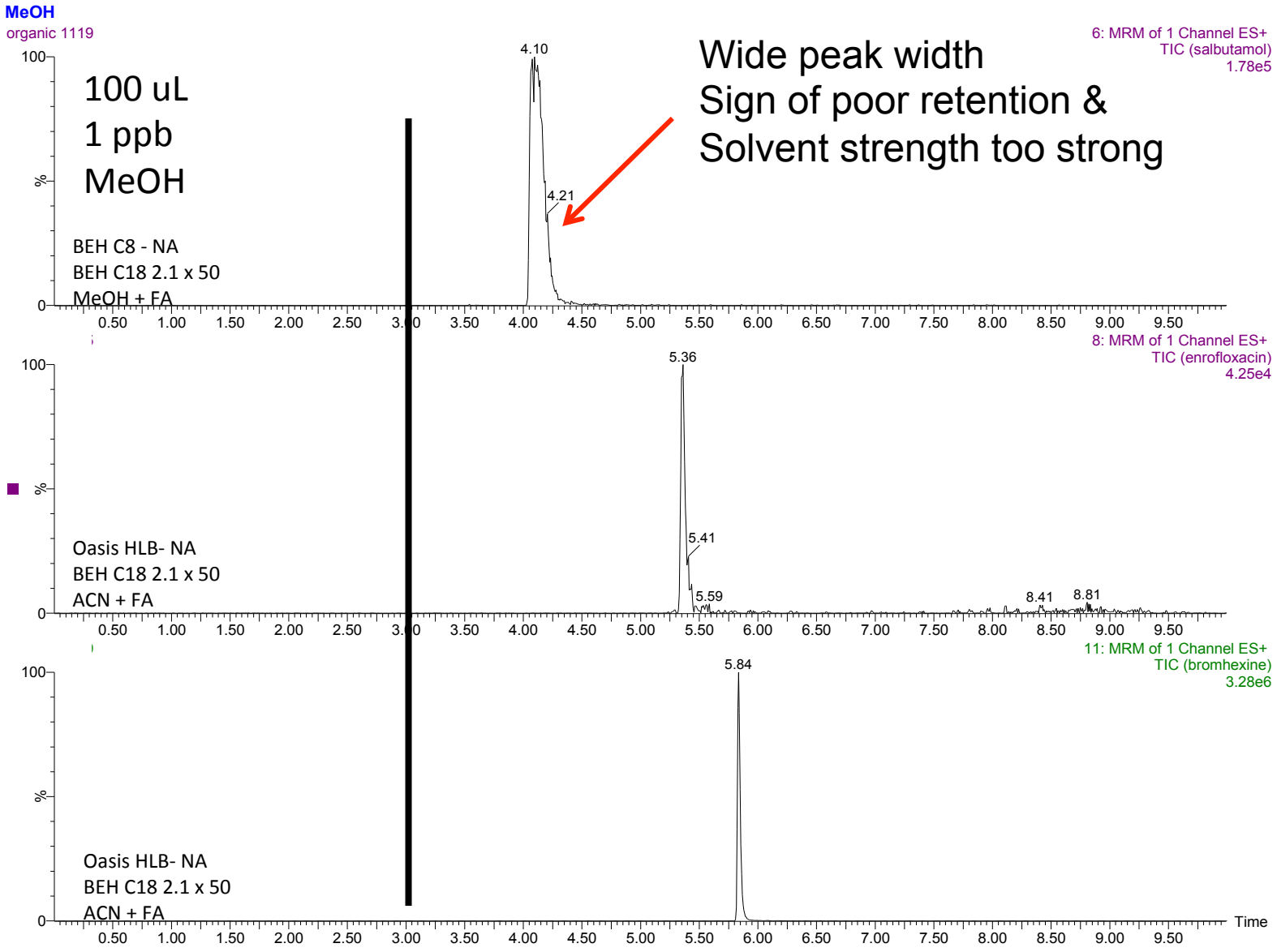

2D “Trap & elution” configuration
ACN Extract

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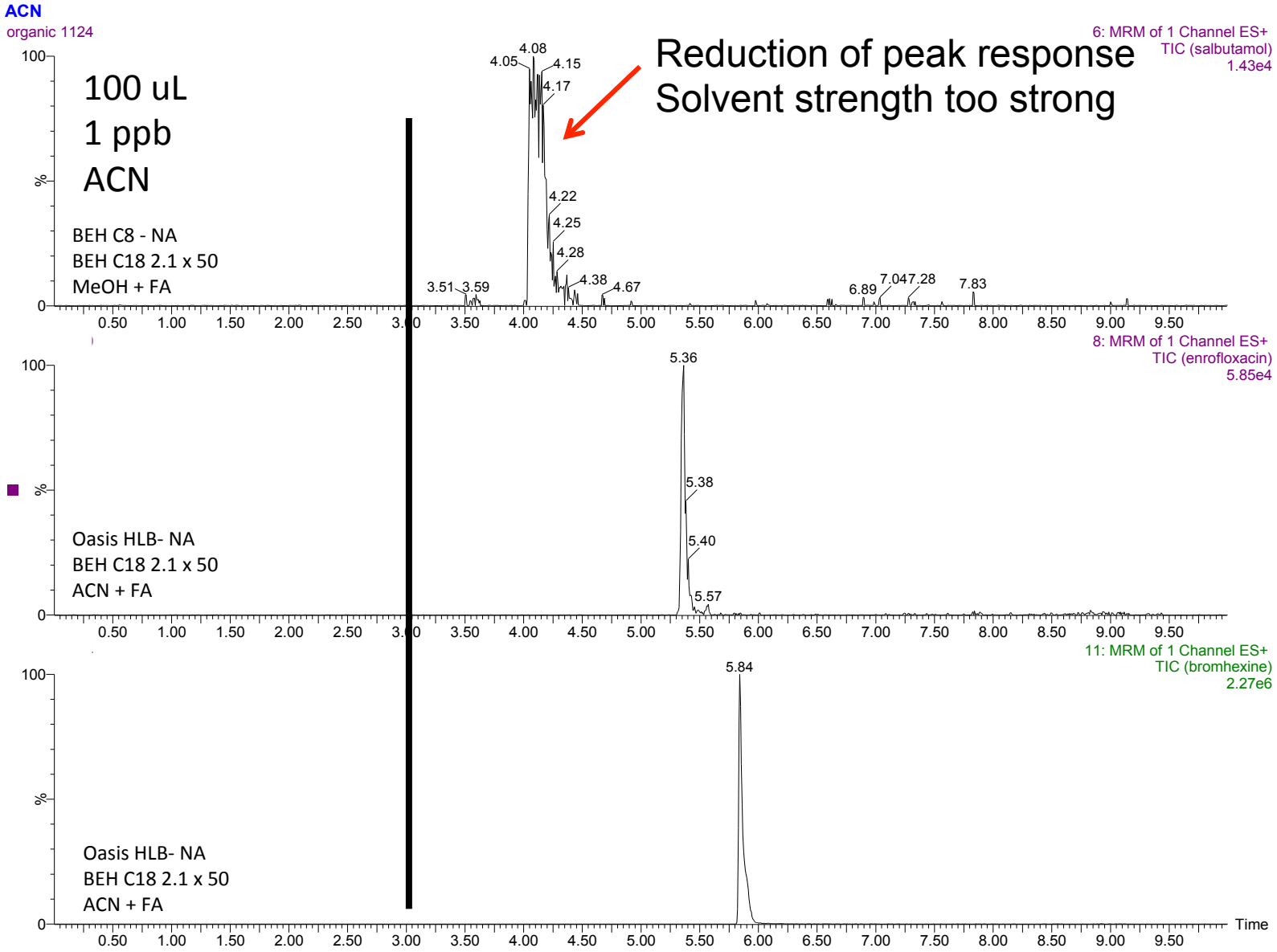

2D “Trap & elution” configuration
Acetone Extract

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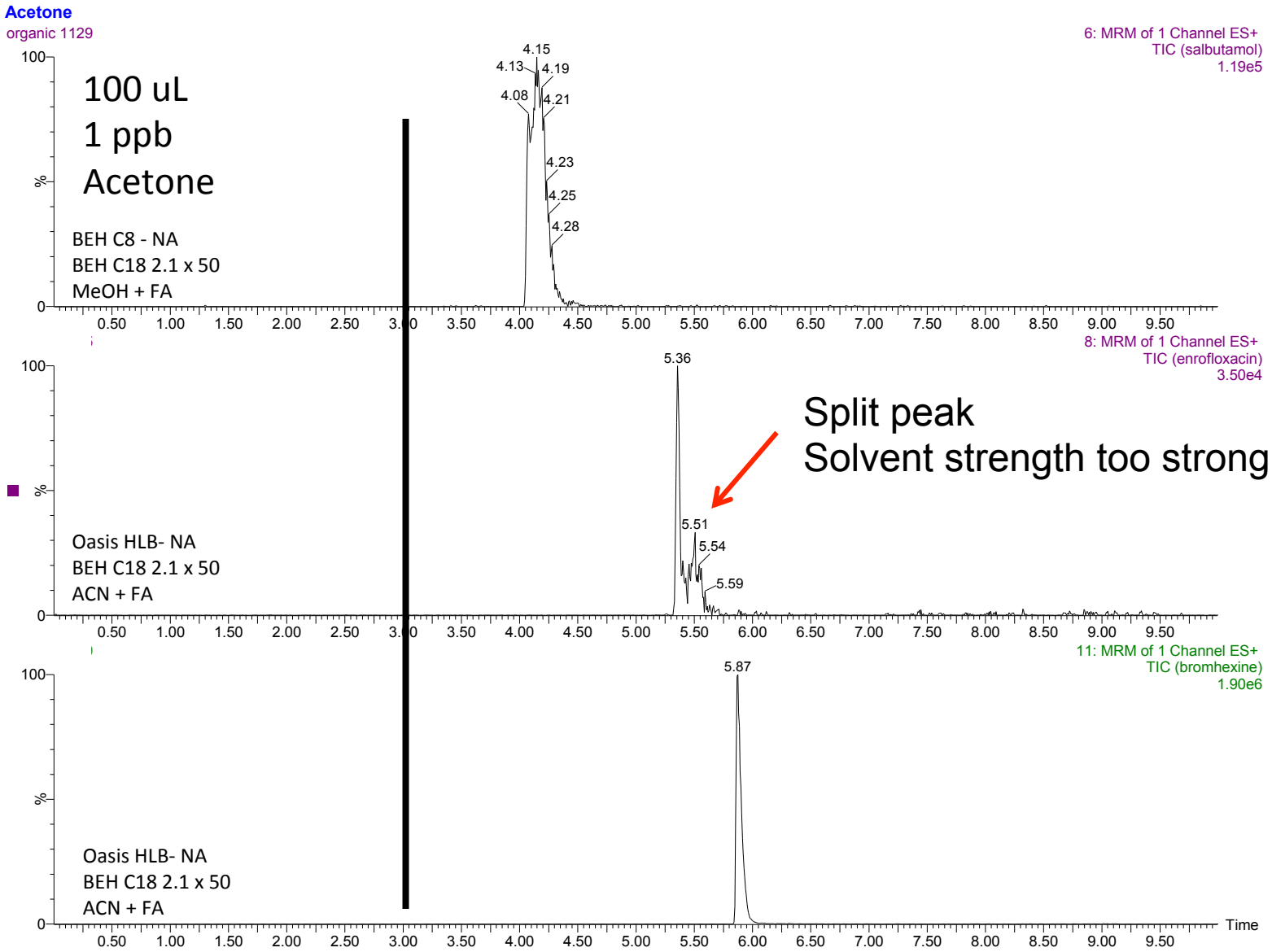

2D “AT-column dilution” configuration

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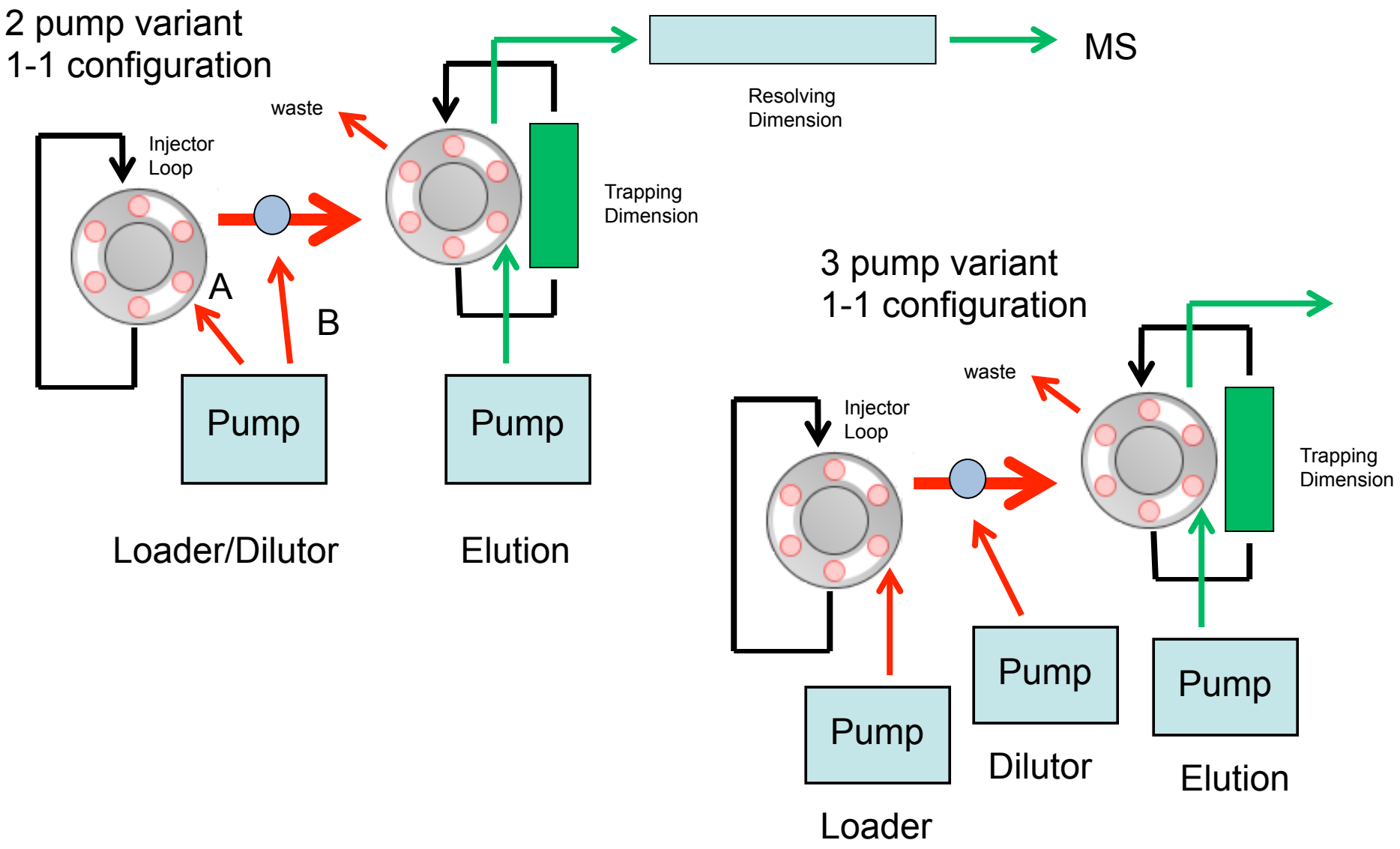

2D "AT-column dilution" configuration
Aqueous Extract

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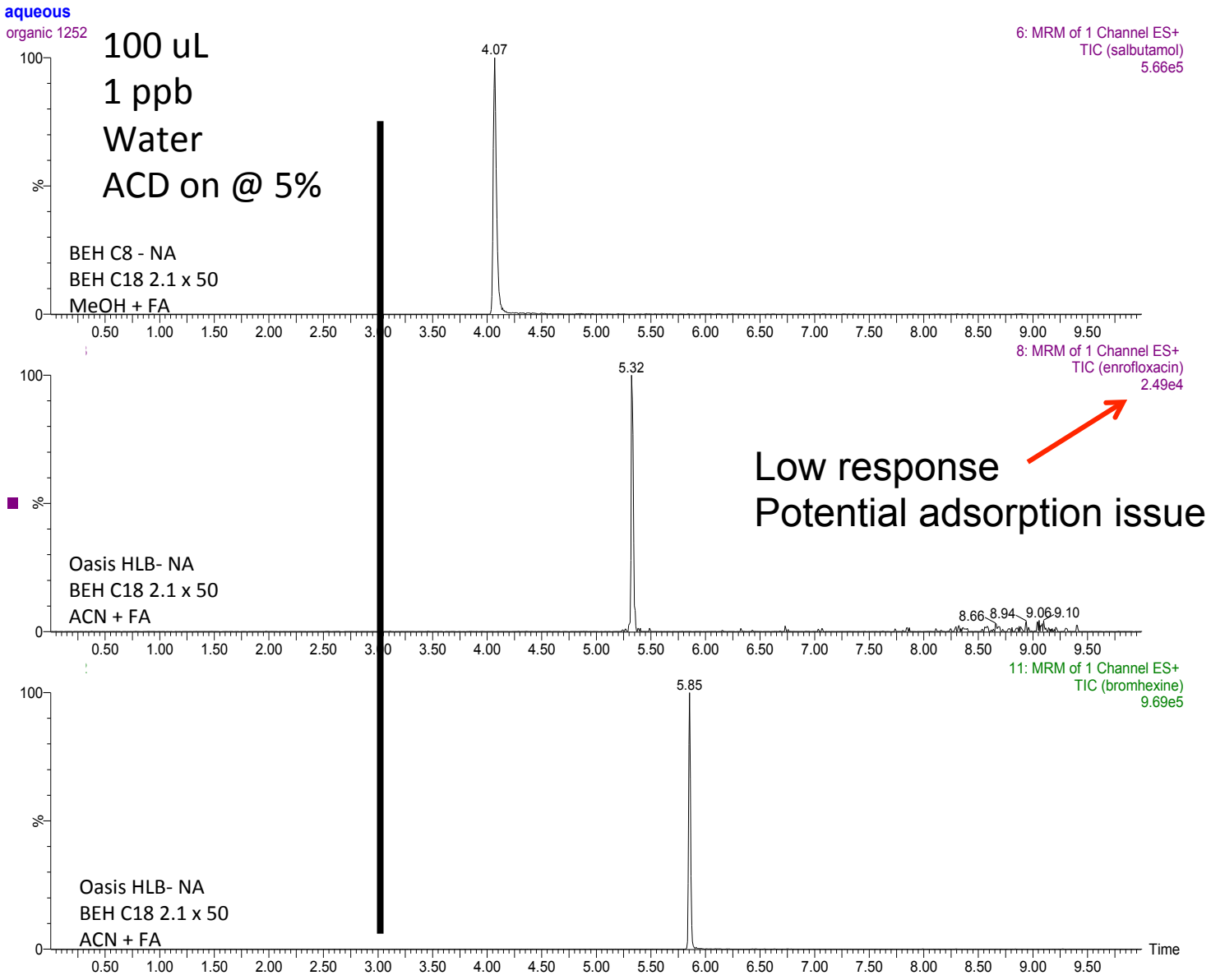

2D “AT-column dilution” configuration
MeOH Extract

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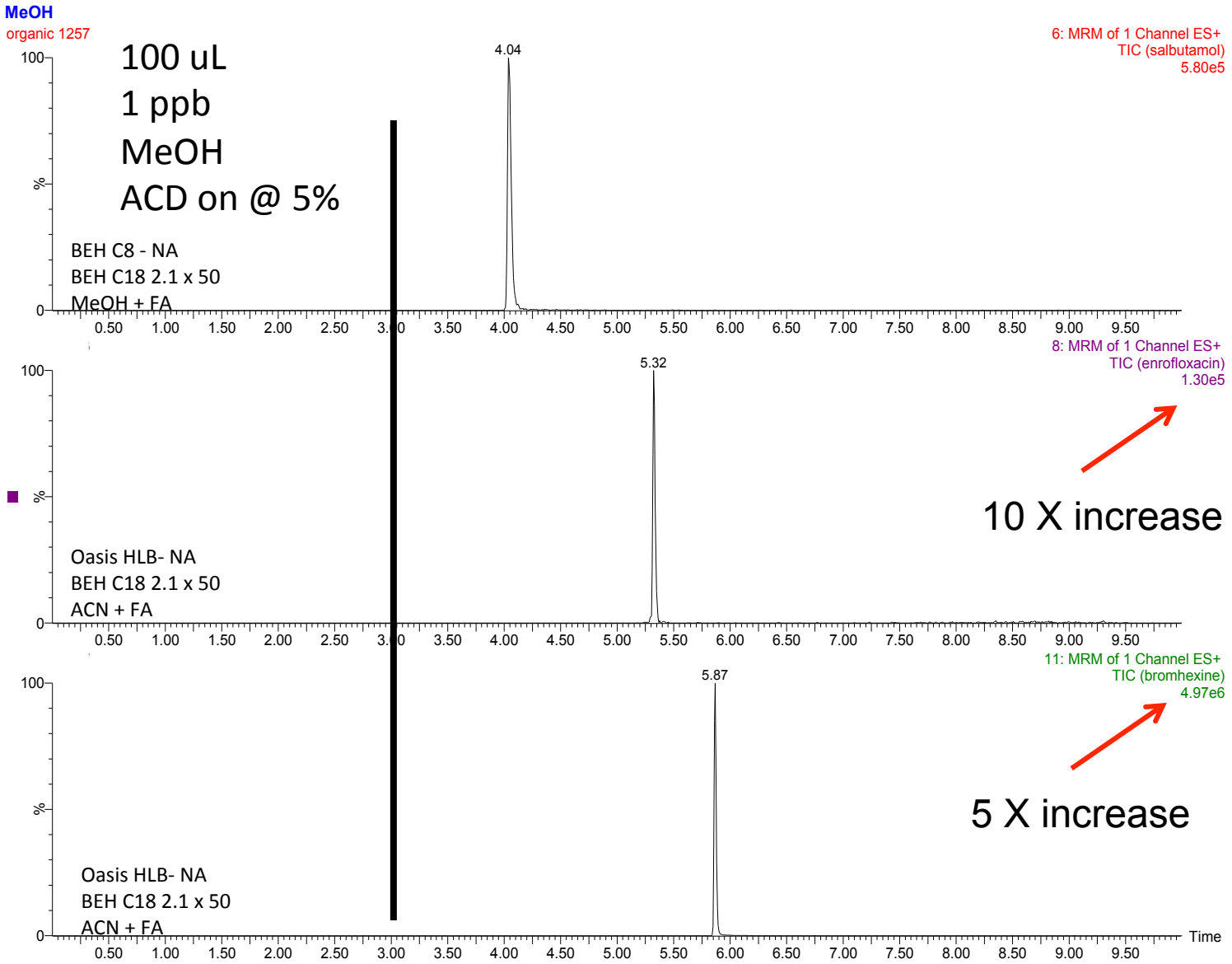

2D “AT-column dilution” configuration
ACN Extract

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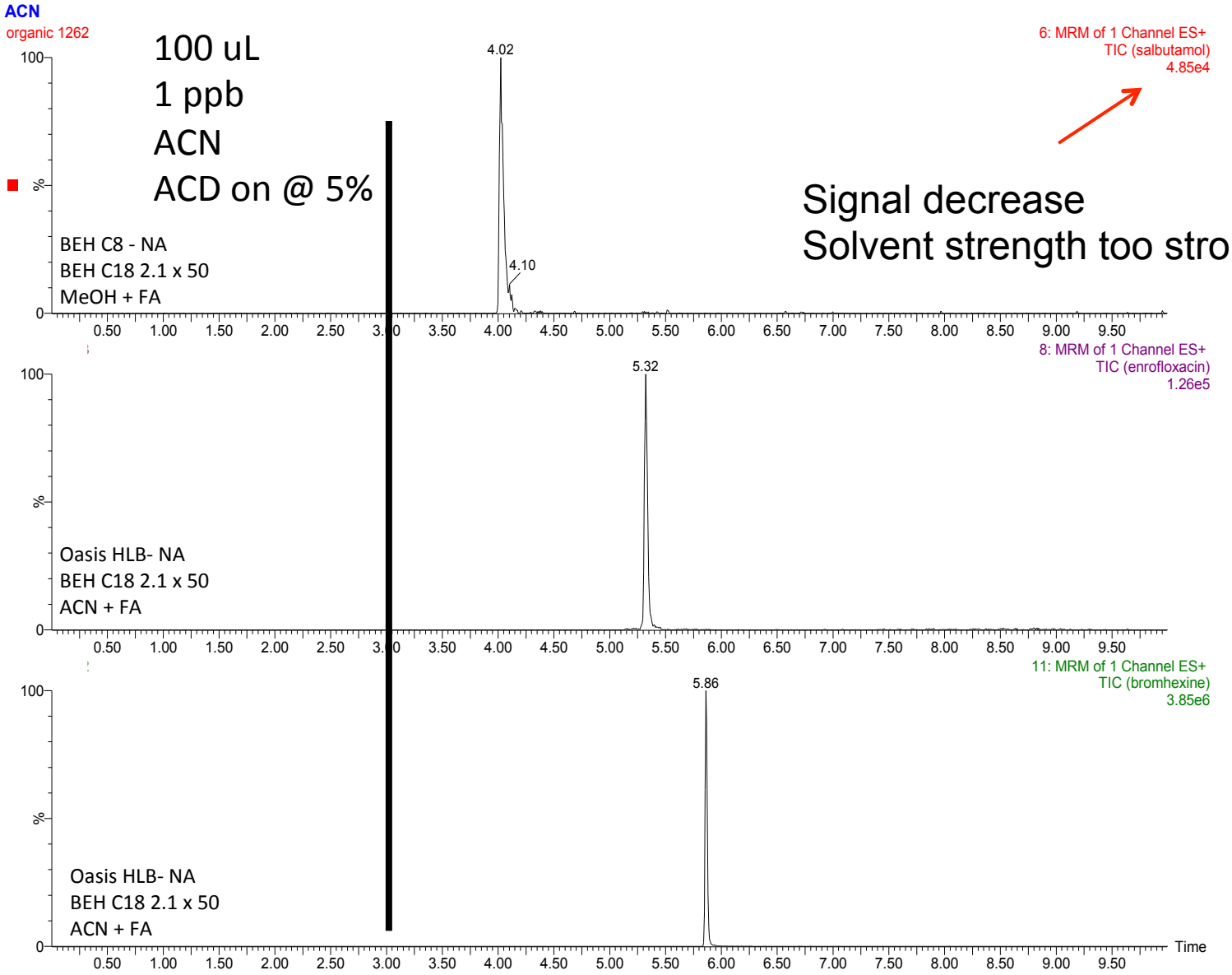

2D “AT-column dilution” configuration
Acetone Extract

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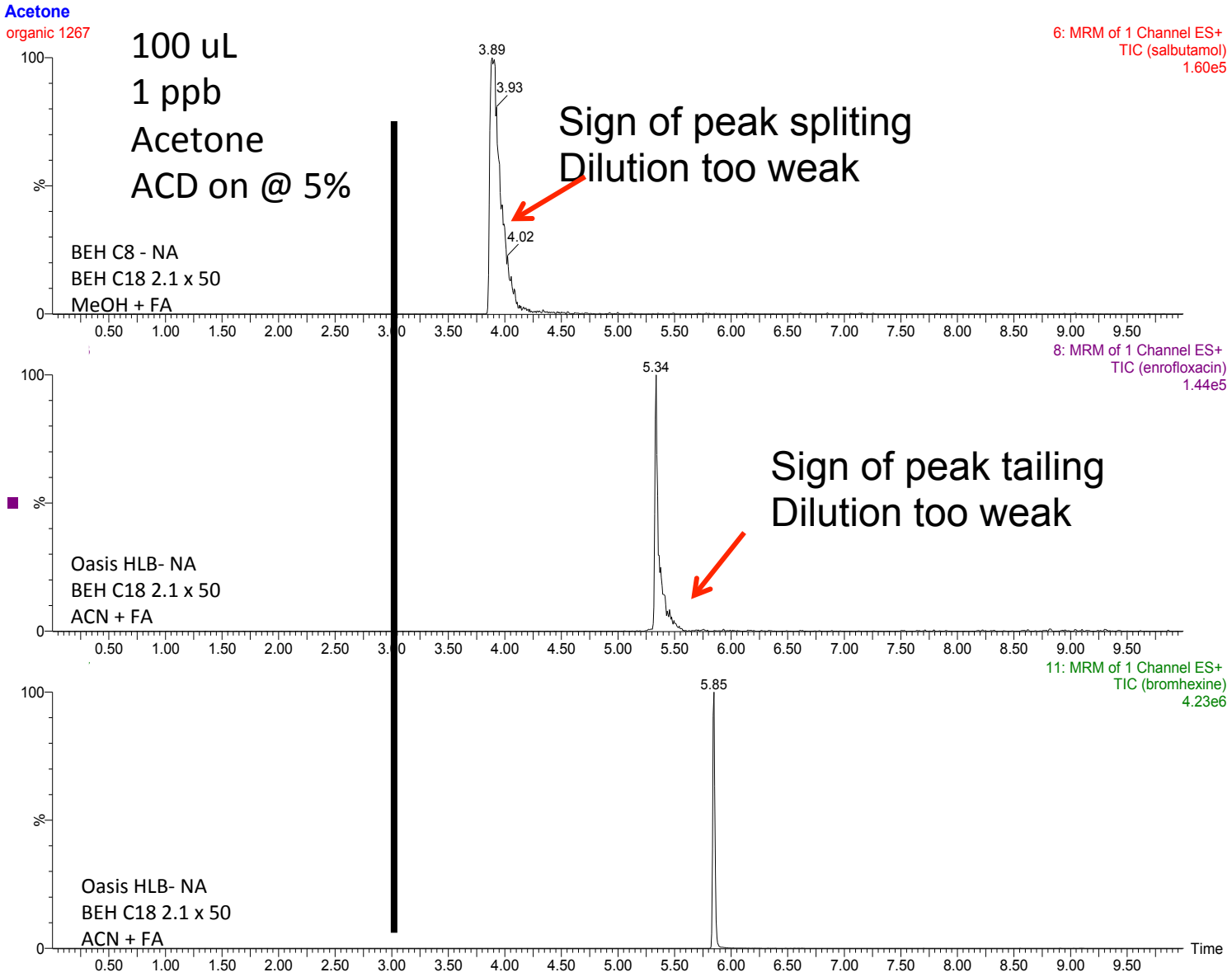

2D “AT-column dilution” configuration
Acetone Extract @ 2 %

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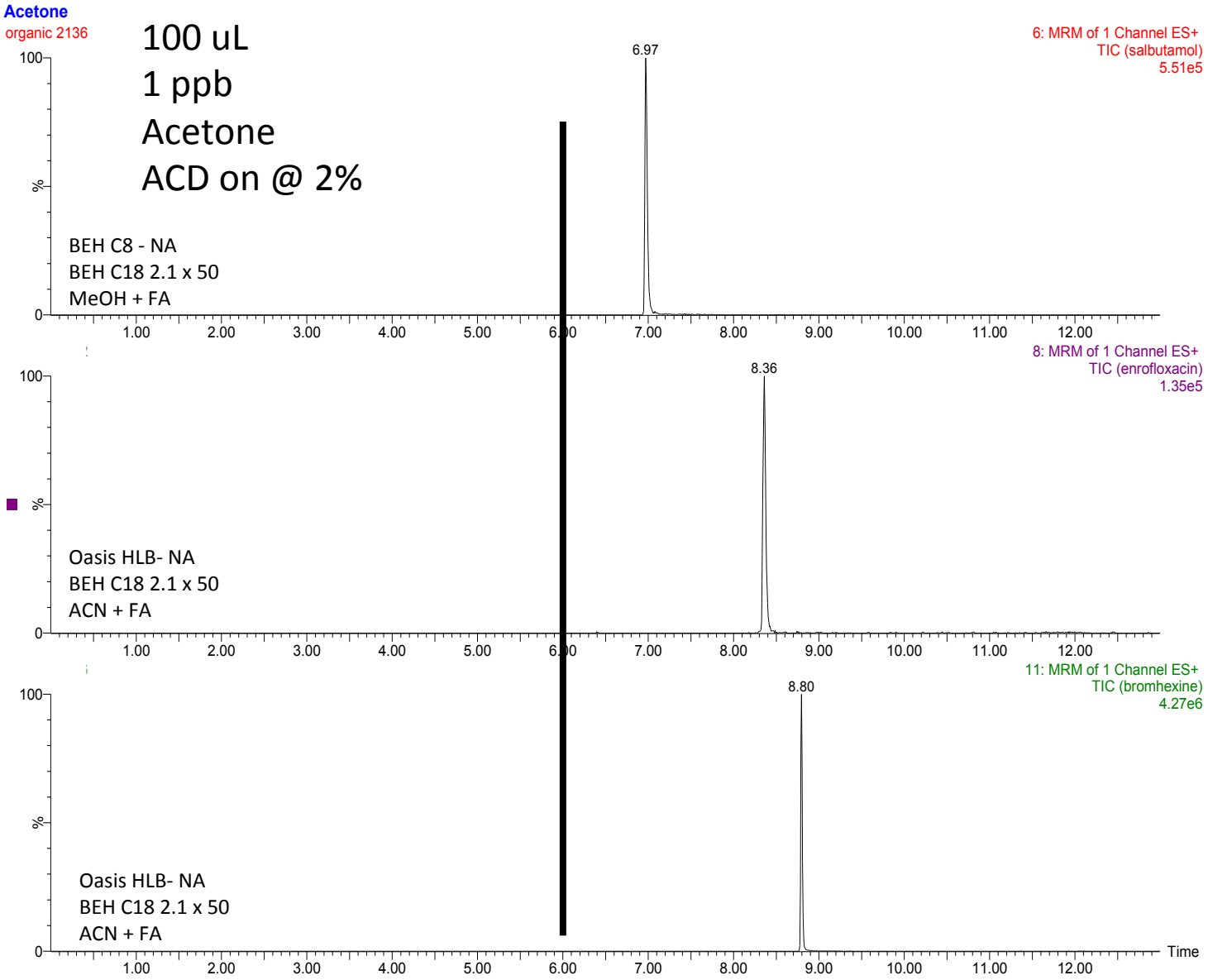

Macro vs Micro Extraction Protocol Class A

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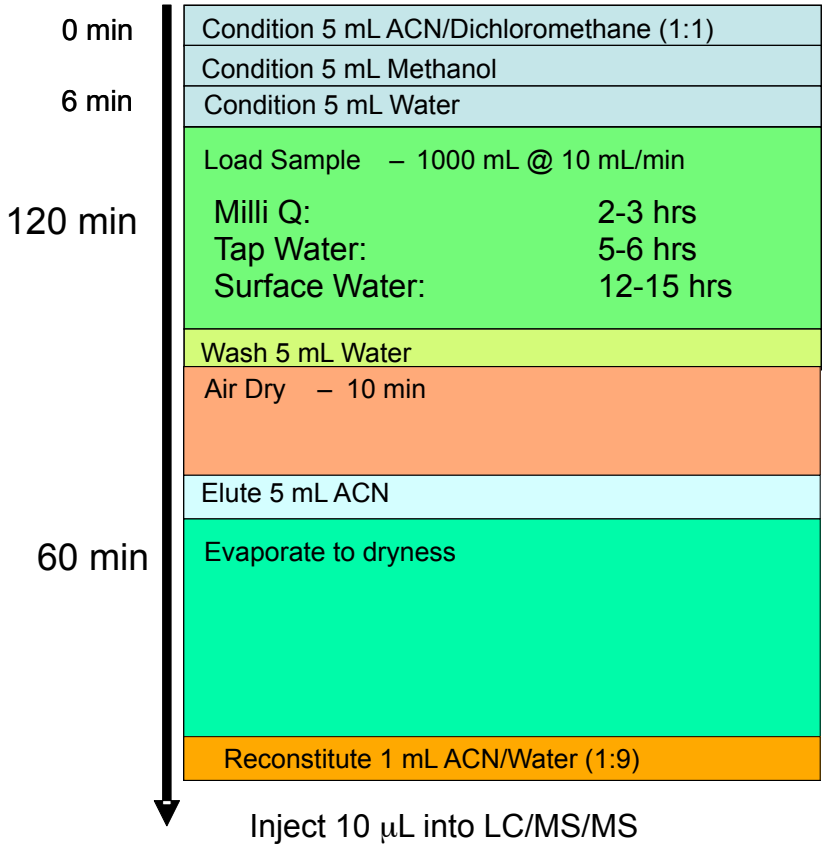
Macro Extraction Protocol

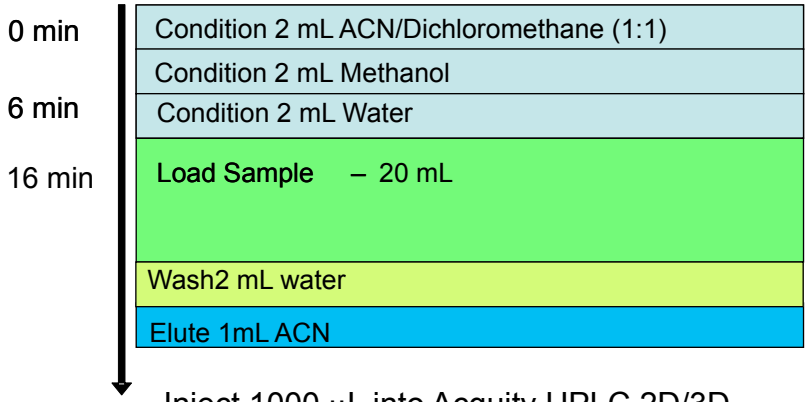


Inject 10 µL into LC/MS/MS

3.0 hrs

1000:1 enrichment from sample protocol
1% of final extract use for analysis

Micro Extraction Protocol



Inject 1000 µL into Acquity UPLC 2D/3D

0.3 hrs

20:1 enrichment from sample protocol
100:1 enrichment from injection volume
2000:1 final enrichment
100% of final extract use for analysis

In-sight analysis during method development – targeting interferences

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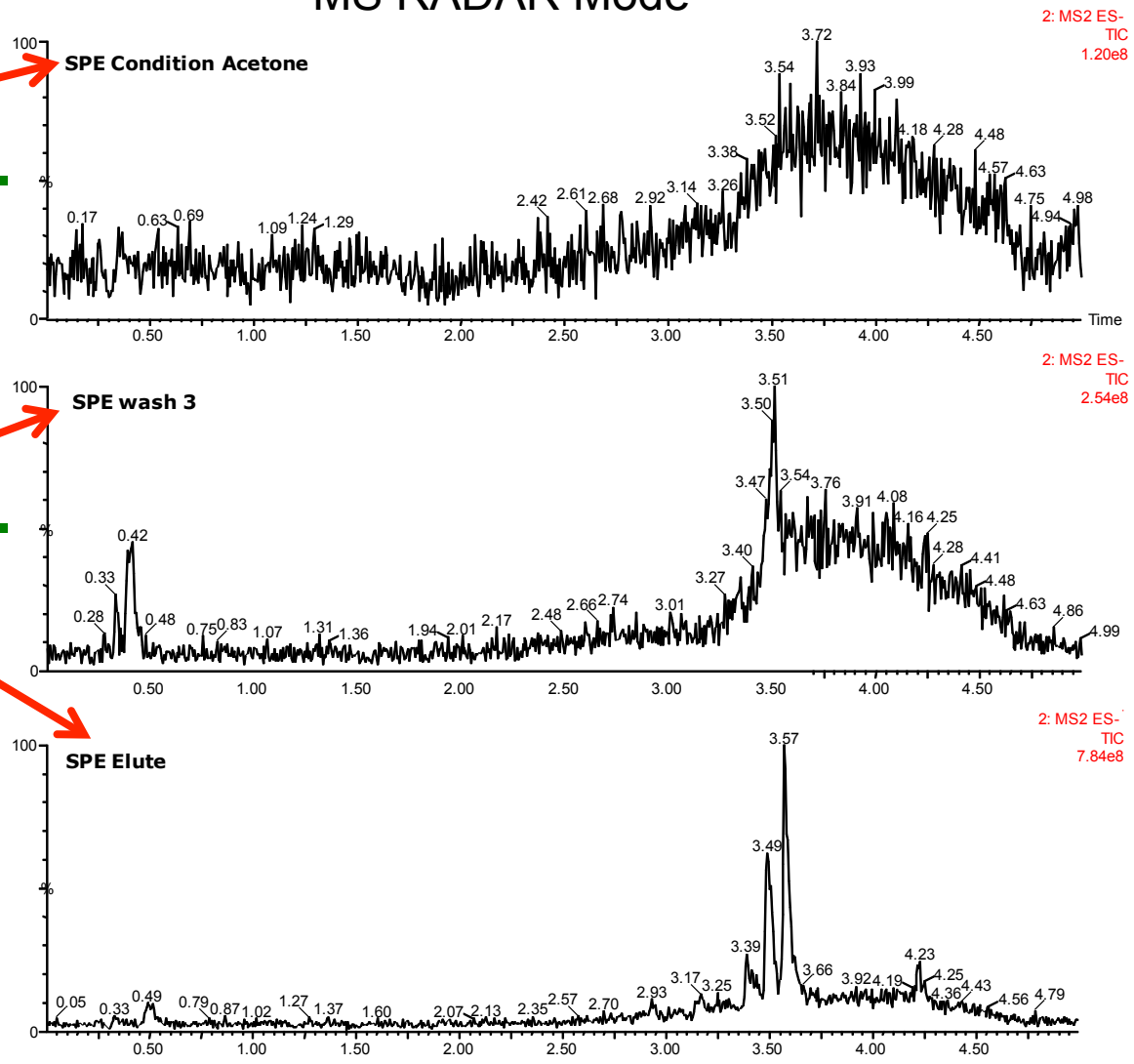
Condition 2 mL Acetone
Condition 2 mL Methanol
Condition 2 mL Water
Load Sample – 20 mL @ 10 mL/min
Wash # 1: 5% MeOH + 2 %FA
Wash # 2: 5% MeOH + 2 % NH_4OH
Wash # 3: X/X MeOH/H_2O + 2 % NH_4OH
Air Dry – 10 min
Elute 1 mL ACN

MS RADAR Mode



Rapid Method Optimization

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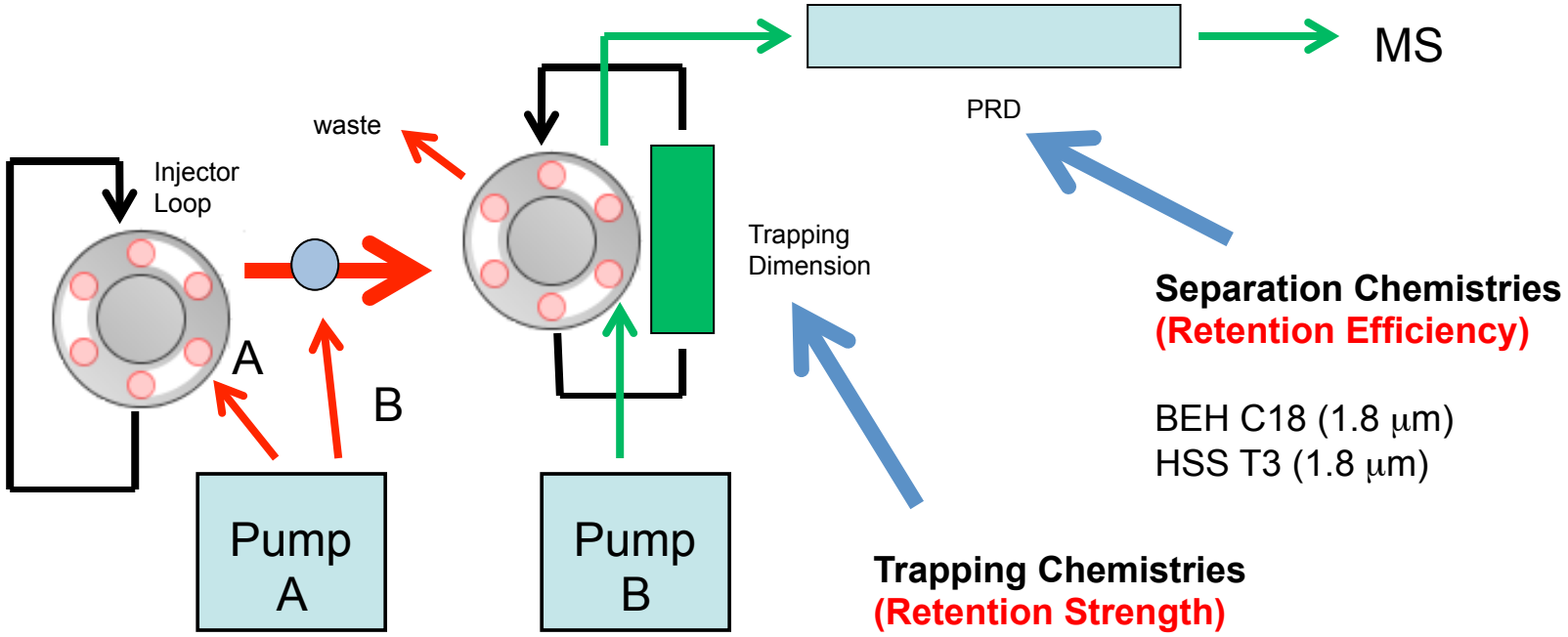

Separation Chemistries
(Retention Efficiency)

BEH C18 (1.8 μm)
HSS T3 (1.8 μm)

Trapping Chemistries
(Retention Strength)

Oasis HLB (20 μm)
XBridge C18 (10 μm)
XBridge C8 (10 μm)

Loading Conditions
(Trapping Efficiency)

Low pH (5% Formic acid)
High pH (5% NH_4OH)
Neutral pH

Eluting Conditions
(Elution Strength)

Low pH (0.5% Formic acid) with MeOH
Low pH (0.5% Formic acid) with ACN

36 Methods
16 hours
(over-night automated analysis)

Rapid Method Optimization
Loading Efficiency vs Retention Strength

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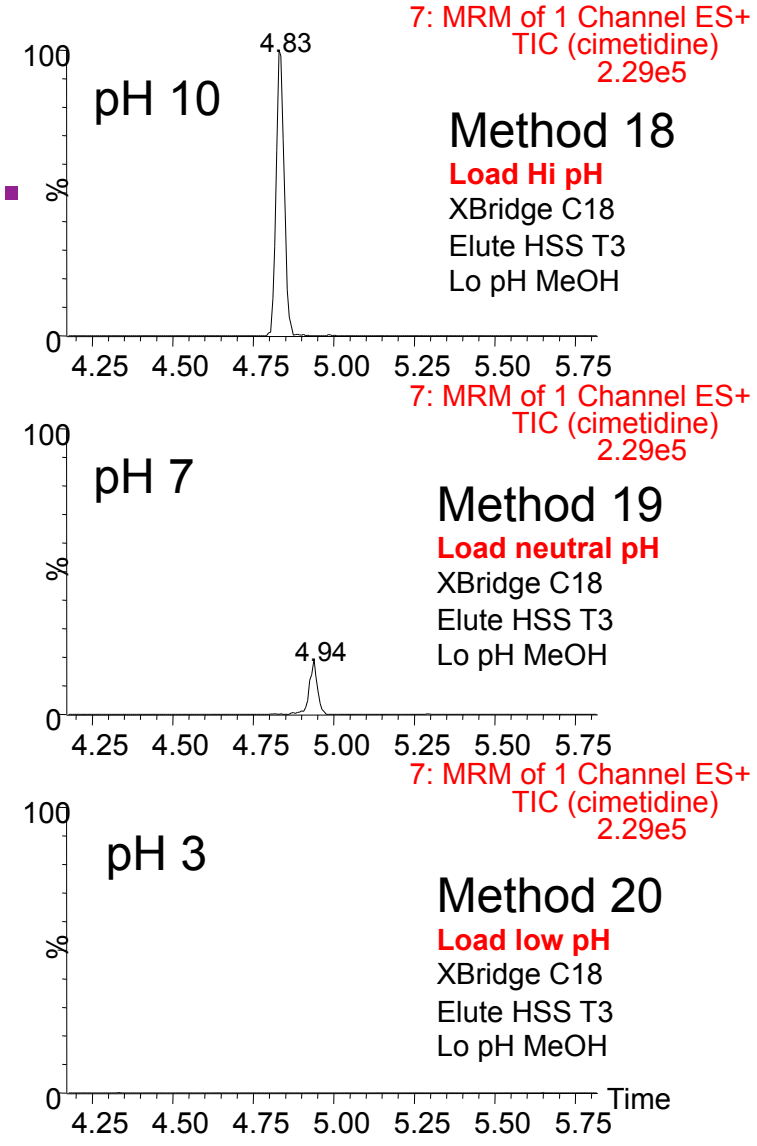

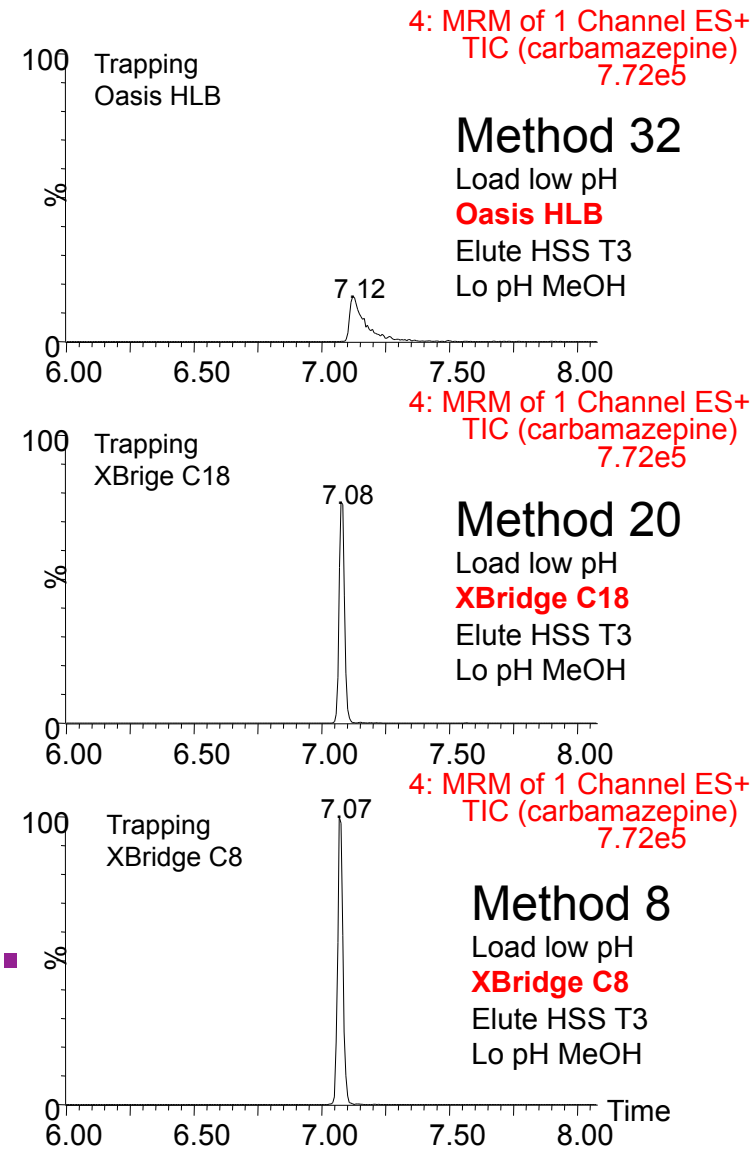

Rapid Method Optimization
Target Extraction

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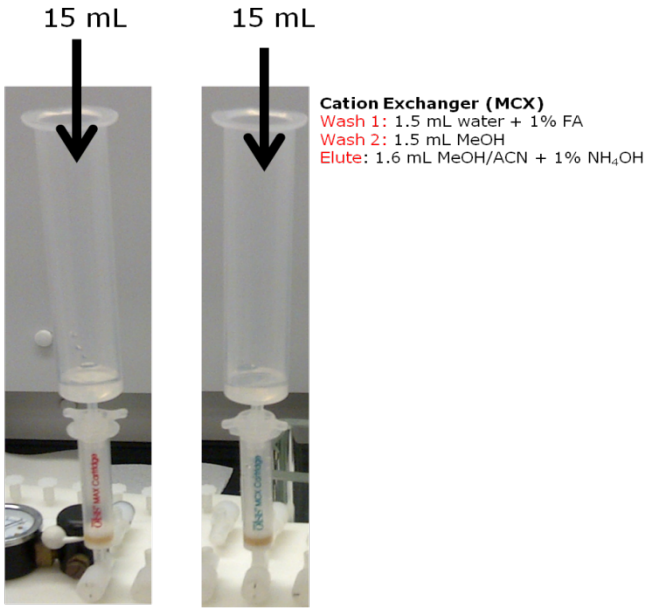
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
MAX Wash	1	1	4	0	2	1	1	0	2	3	3	4	0	3	3	0	2
MAX Elute (RP)	49	20	7	90	7	1	7	1	5	13	11	12	0	11	7	1	8
MAX Elute (IE)	5	1	1	12	2	0	0	99	110	3	2	5	84	104	99	2	5
MCX Wash	0	2	10	0	4	0	1	0	0	3	2	5	0	2	4	3	8
MCX Elute (RP)	7	2	4	0	6	0	2	0	0	3	7	12	1	4	4	7	7
MCX Elute (IE)	1	80	26	0	15	0	8	0	8	93	94	87	1	8	0	0	6
MCX Wash	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MCX Elute (RP)	1	0	0	106	0	0	0	11	2	0	1	0	9	1	0	0	0
MCX Elute (IE)	53	112	97	10	83	99	106	0	1	88	102	80	1	37	27	92	106
MAX Wash	4	1	10	1	1	1	6	0	3	6	12	1	1	7	10	4	2
MAX Elute (RP)	3	3	2	3	2	1	1	0	21	6	2	12	0	11	11	1	3
MAX Elute (IE)	1	4	0	1	1	0	0	1	1	0	0	2	1	22	7	1	5

A- benzocaine,**B**- diethylcabarmazine,**C** levamisole,**D**- carbamezapine,**E**- procaine,**F**- salbutamol,**G**- cimetidine,**H**- sulfamethoxaxole,**I**- sulfamerazine
J- tripolidine,**K**- trimethoprim,**L**- buflomedil,**M**- sulfamethoxine,**N**- enrofloxacine,**O**-bromhexine,**P**- diltiazem,**Q**- miconazole

Acidic PPCP

Sulfamethoxazole
Sulfamerazine
Sulfadimethoxine
Enrofloxacine
Bromhexine

15 min extraction time
10:1 enrichment



Basic PPCP

Benzocaine
Diethylcarbamazine
Levamisole
Procaine
Salbutamol
Cimetidine
Tripolidine
Trimethoprim
Buflomedil
Diltiazem
Miconazole

Rapid Method Optimization
Surface water spike @ 1 ppt

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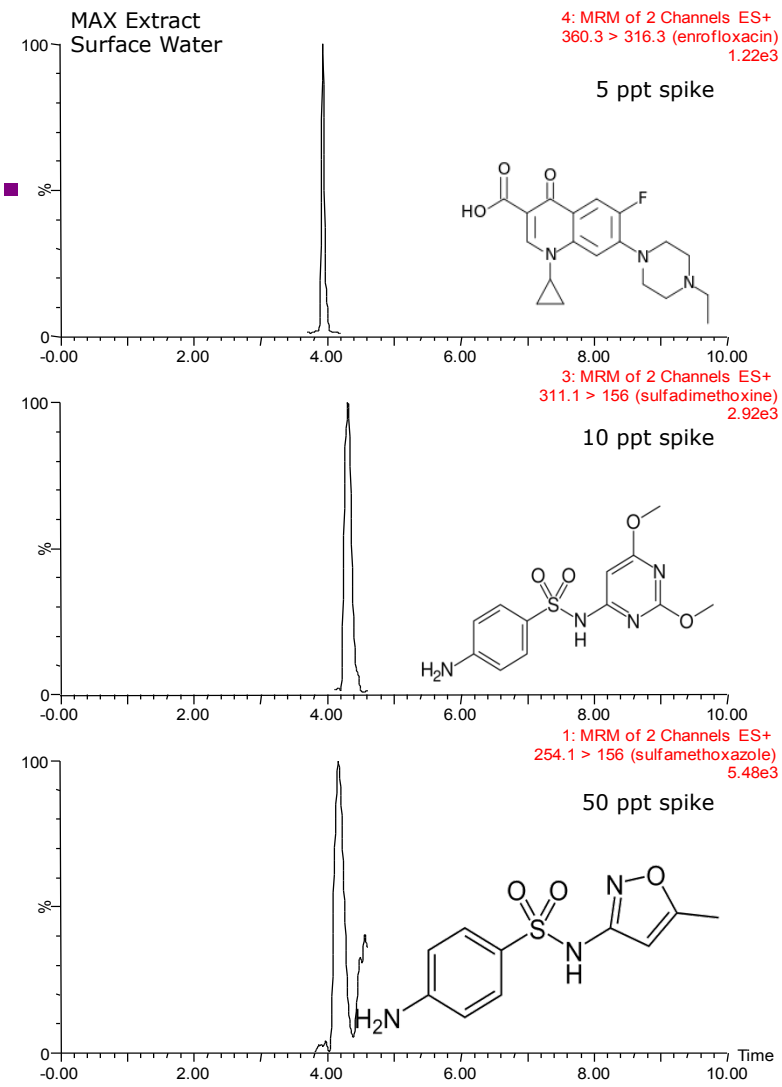

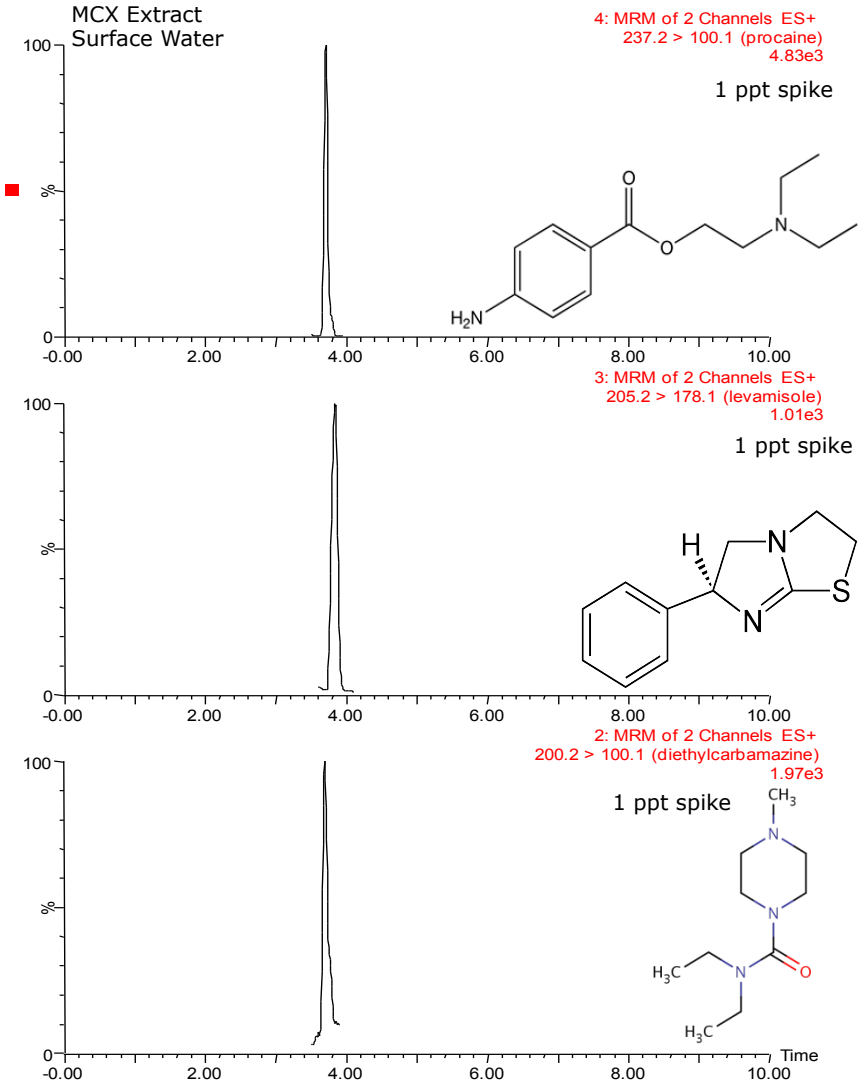

Recoveries, LOD and Linearity
Bottled, Tap & Surface Water @ 10ppt

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Pharmaceuticals MAX	**LOD ppt**	**Linearity (r^2)**	**Bottled**	**Tap**	**Surface**
Recoveries @ 10 ppt					
Sulfamethoxazole	50	0.991	na	na	na
Sulfamerazine	5	0.993	73.7	70.3	112.4
Sulfadimethoxine	10	0.994	80.5	79.2	75.6
Enrofloxacine	10	0.993	92.7	85.0	84.5
Bromhexine	1	0.991	101.0	87.4	80.4
Pharmaceuticals MCX					
Recoveries @ 10 ppt					
Benzocaine	2	0.991	88.5	90.9	92.6
Diethylcarbamazine	1	0.997	90.1	99.7	105.1
Levamisole	1	0.997	102.6	104.9	114.2
Procaine	1	0.990	84.7	87.2	95.8
Salbutamol	10	0.990	88.9	89.1	81.3
Cimetidine	1	0.992	90.6	81.0	102.4
Tripolidine	1	0.994	103.0	88.4	113.9
Trimethoprim	1	0.993	94.3	99.8	97.6
Buflomedil	1	0.992	97.5	95.0	101.6
Diltiazem	1	0.994	96.2	103.2	91.5
Miconazole	1	0.998	99.1	89.9	79.3

Conclusions

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- Trace level detection (ppt)
- Minimum sample preparation
- High reduction of manual labor (80%)
- Wide pH range SPE & LC sorbents
- Rapid method development
- High level of reproducibility and robustness
- Good precision & accuracy (< 15 %)
- Reduce field sample volume by 50 times