



Drugs of Abuse Screen by UHPLC-MS/MS (I)

ACE Excel 1.7 C18 1.7 μ m, 100 x 2.1mm

Gradient analysis

A: 5mM NH₄OAc in water

B: 5mM NH₄OAc in MeOH

Time (mins)	%B	Time (mins)	%B
0	10	13.4	10
10	90	15.5	10
11.9	90		

Flow rate: 0.3ml/min

Column temperature: 40°C

Injection volume: 10 μ l

Sample: 5pg/ μ l

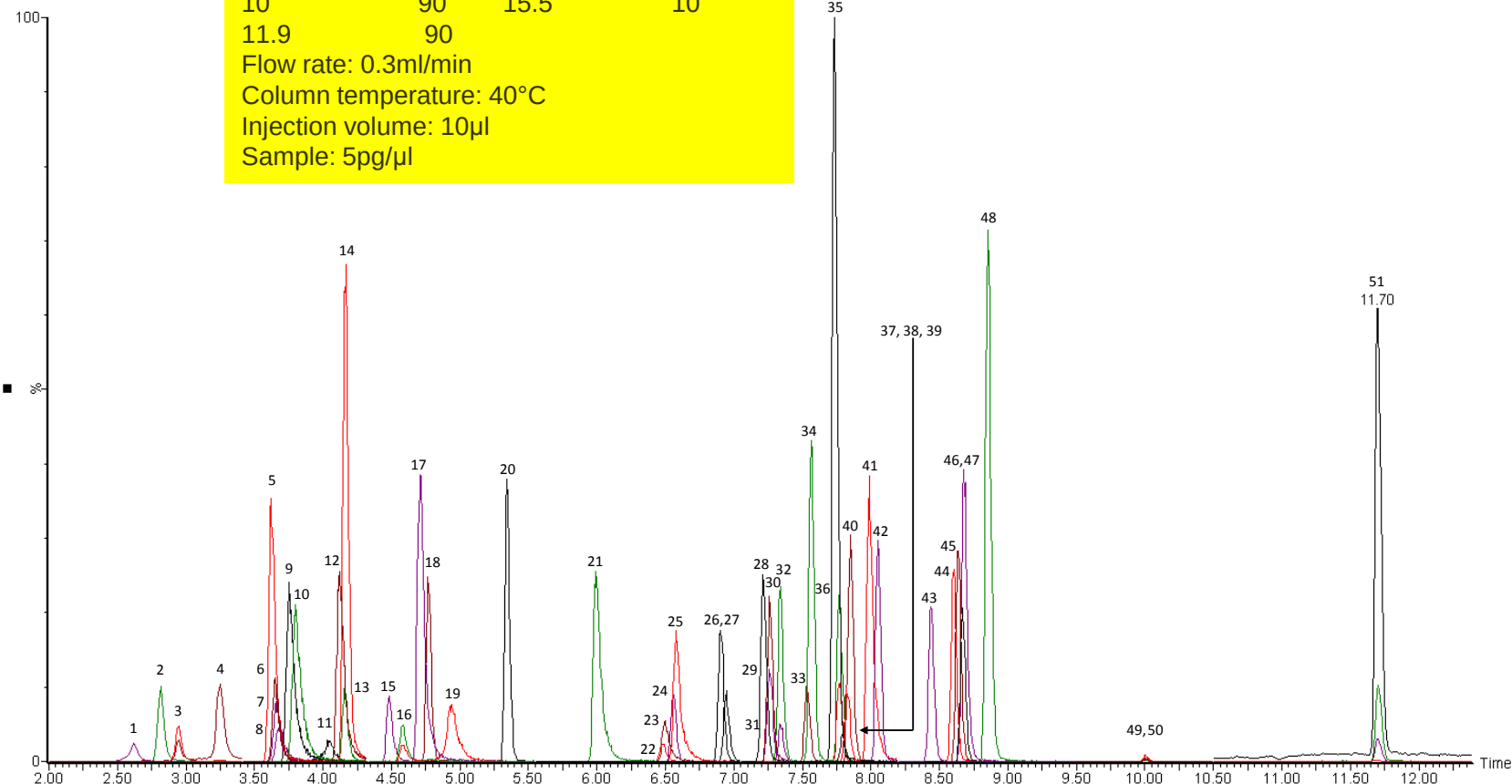
MS Quattro Premier XE triple quad

MRM, positive and negative mode ESI

Desolvation temperature: 450°C

Ion source temperature: 150°C

Collision gas pressure: 3.5 x 10⁻³ mbar



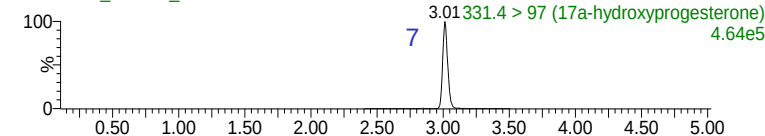


Drugs of Abuse Screen by UHPLC-MS/MS (II)

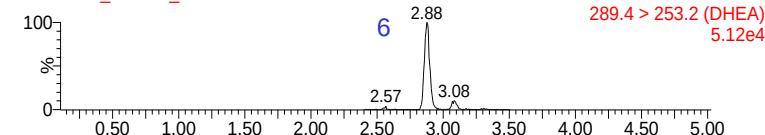
Peak	Analyte	Rt	Q1 (Da)	Q3 (Da)	Peak	Analyte	Rt	Q1 (Da)	Q3 (Da)
1	Oxymorphone	2.62	302.2	198.1	27	Bromazepam	6.95	316.1	182.1
2	Morphine-d3	2.82	289.2	201.0	28	Ketamine	7.21	238.1	124.9
3	Morphine	2.95	286.2	201.0	29	Clonazepam	7.26	316.1	270.1
4	Hydromorphone	3.25	286.2	185.1	30	Nitrazepam	7.26	282.2	236.1
5	Amphetamine-d5	3.62	141.0	123.9	31	Alpha-hydroxytriazolam	7.34	359.1	331.1
6	Amphetamine	3.65	136.0	118.9	32	Flunitrazepam	7.34	314.2	268.2
7	Dihydrocodeine	3.66	302.2	199.1	33	Alpha-hydroxyalprazolam	7.54	325.2	297.1
8	MDA	3.67	180.1	105.0	34	Estazolam	7.56	295.2	267.2
9	MDMA	3.75	194.1	163.0	35	Zolpidem	7.73	308.2	235.1
10	Methamphetamine	3.8	150.0	90.9	36	Triazolam	7.77	343.0	308.1
11	Oxycodone	4.03	316.2	241.2	37	2-Hydroxyethylflurazepam	7.77	333.2	109.0
12	MDEA	4.12	208.2	163.0	38	Lorazepam	7.80	321.1	275.1
13	BZE-d3	4.15	293.1	171.0	39	Oxazepam	7.82	287.2	241.0
14	BZE	4.17	290.1	168.0	40	Alprazolam	7.85	309.2	281.2
15	6-MAM	4.48	328.2	165.1	41	Methadone	7.99	310.2	265.2
16	Codeine	4.59	300.3	215.1	42	Temazepam	8.05	301.1	255.1
17	Norfentanyl	4.71	233.1	84.0	43	Nordiazepam	8.44	271.1	139.9
18	7-Amino-clonazepam	4.77	286.2	121.0	44	Midazolam	8.61	326.2	291.2
19	Hydrocodone	4.94	300.2	199.1	45	Diazepam-d5	8.63	290.2	154.0
20	7-Amino-flunitrazepam	5.34	284.2	135.0	46	Diazepam	8.67	285.2	154.0
21	Cocaine	5.99	304.2	182.0	47	Flurazepam	8.68	388.2	315.1
22	Norbuprenorphine	6.47	414.3	101.0	48	Fentanyl	8.85	337.3	105.0
23	PCP	6.49	244.2	159.9	49	THC-COOH-d3	9.98	348.2	302.2
24	Zaleplon	6.55	306.2	264.2	50	THC-COOH	10.01	345.2	299.2
25	EDDP	6.58	278.2	234.2	51	Buprenorphine	11.70	468.3	101.0
26	Norketamine	6.9	224.1	124.9					

Endogenous Steroid Hormones by LC-MS/MS

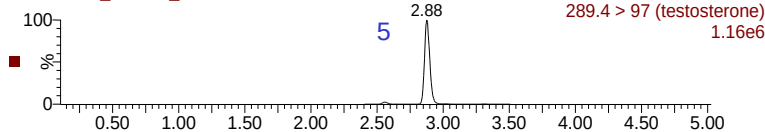
STERIODS_191114_015



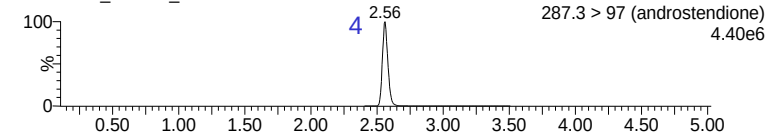
STERIODS_191114_015



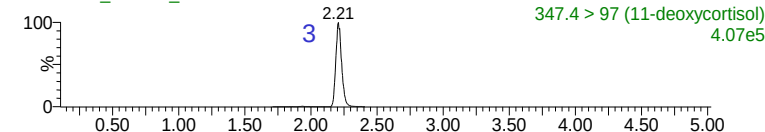
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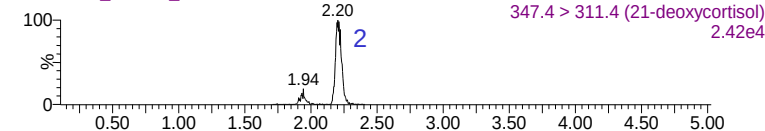
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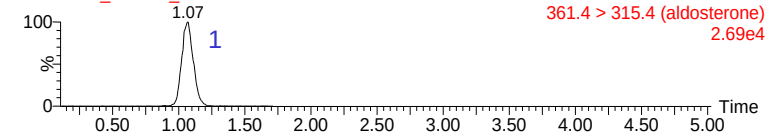
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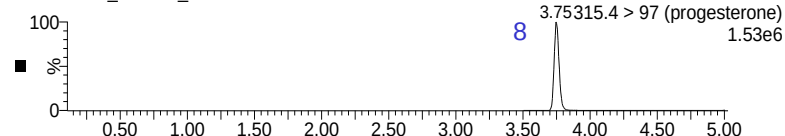
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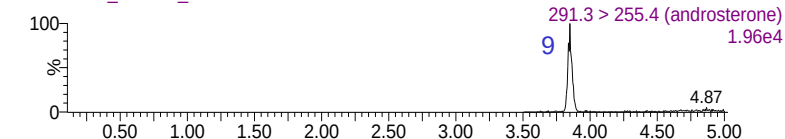
STERIODS_191114_015



STERIODS_191114_015



STERIODS_191114_015



Peak	Analyte	Q1 (Da)	Q3 (Da)
1	Aldosterone	361.4	315.4
2	21-Deoxycortisol	347.4	311.4
3	11-Deoxycortisol	347.4	97.0
4	Androstendione	287.3	97.0
5	Testosterone	289.4	97.0
6	DHEA	289.4	253.2
7	17α-Hydroxyprogesterone	331.4	97.0
8	Progesterone	315.4	97.0
9	Androsterone	291.3	255.4

ACE Excel 2 C18 2μm, 50 x 2.1mm

Gradient analysis

A: 0.1% formic acid in water

B: 0.1% formic acid in MeOH

Time (mins)	%B	Curve	Time (mins)	%B	Curve
0	50	0	5.3	100	6
0.8	50	6	5.8	50	6
4.2	81	6	6.8	50	6
4.3	100	6			

Flow rate: 0.4ml/min

Injection volume: 10μl

Column temperature: 40°C

Sample: 2ng/μl

Detection: MS/MS, positive mode ESI



Metabolite Profiling of Green Tea by LC-MS

3D plot from UHPLC with high resolution accurate mass MS

Sample:

Metabolites from green tea extracted into cold water by vortexing for 20 mins. Samples filtered prior to injection onto column and modular Accela LC system

ACE Excel C18-Amide 1.7 μ m, 100 x 2.1mm
Gradient analysis

A: 0.01% formic acid in water

B: 0.01% formic acid in MeCN

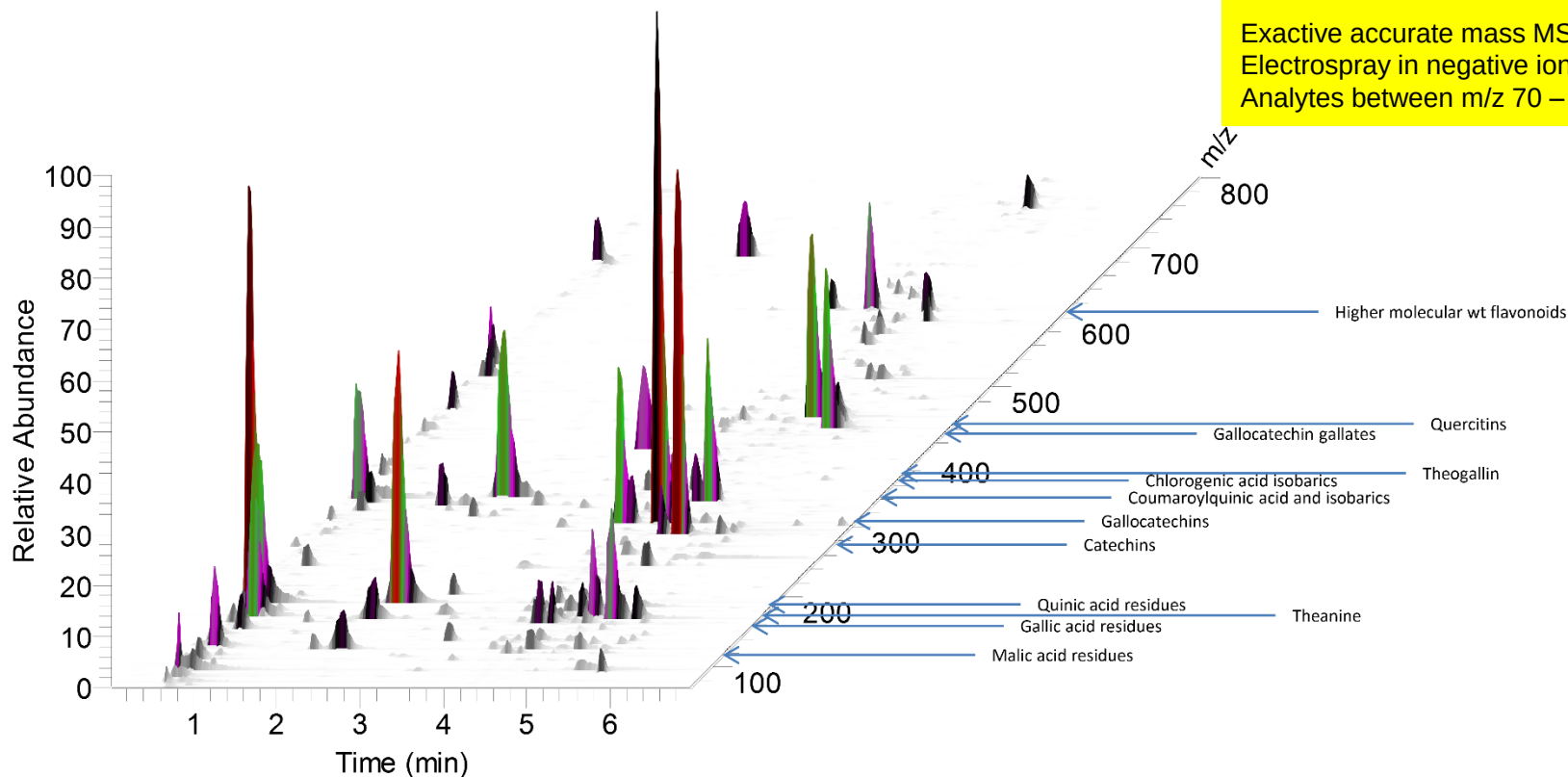
Time (mins)	%B	Time (mins)	%B
0	3	8.5	3
2.5	10	10	3
8	100		

Flow rate: 0.5ml/min

Exact accurate mass MS system

Electrospray in negative ion mode

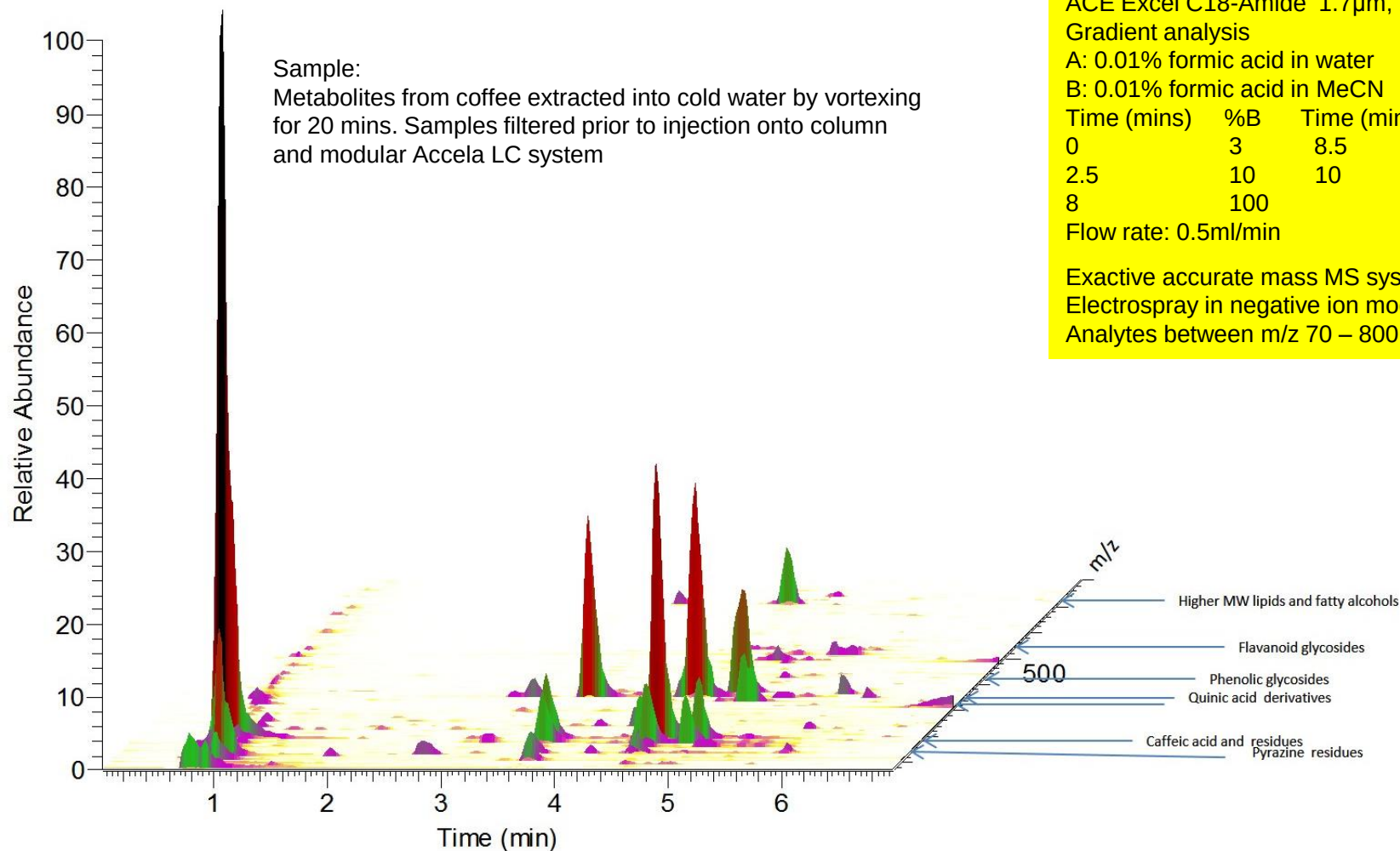
Analytes between m/z 70 – 800 monitored





Metabolite Profiling of Coffee by LC-MS

3D plot from UHPLC with high resolution accurate mass MS



ACE Excel C18-Amide 1.7 μ m, 100 x 2.1mm

Gradient analysis

A: 0.01% formic acid in water

B: 0.01% formic acid in MeCN

Time (mins)	%B	Time (mins)	%B
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0	3	8.5	3
---	---	-----	---

2.5	10	10	3
-----	----	----	---

8	100		
---	-----	--	--

Flow rate: 0.5ml/min

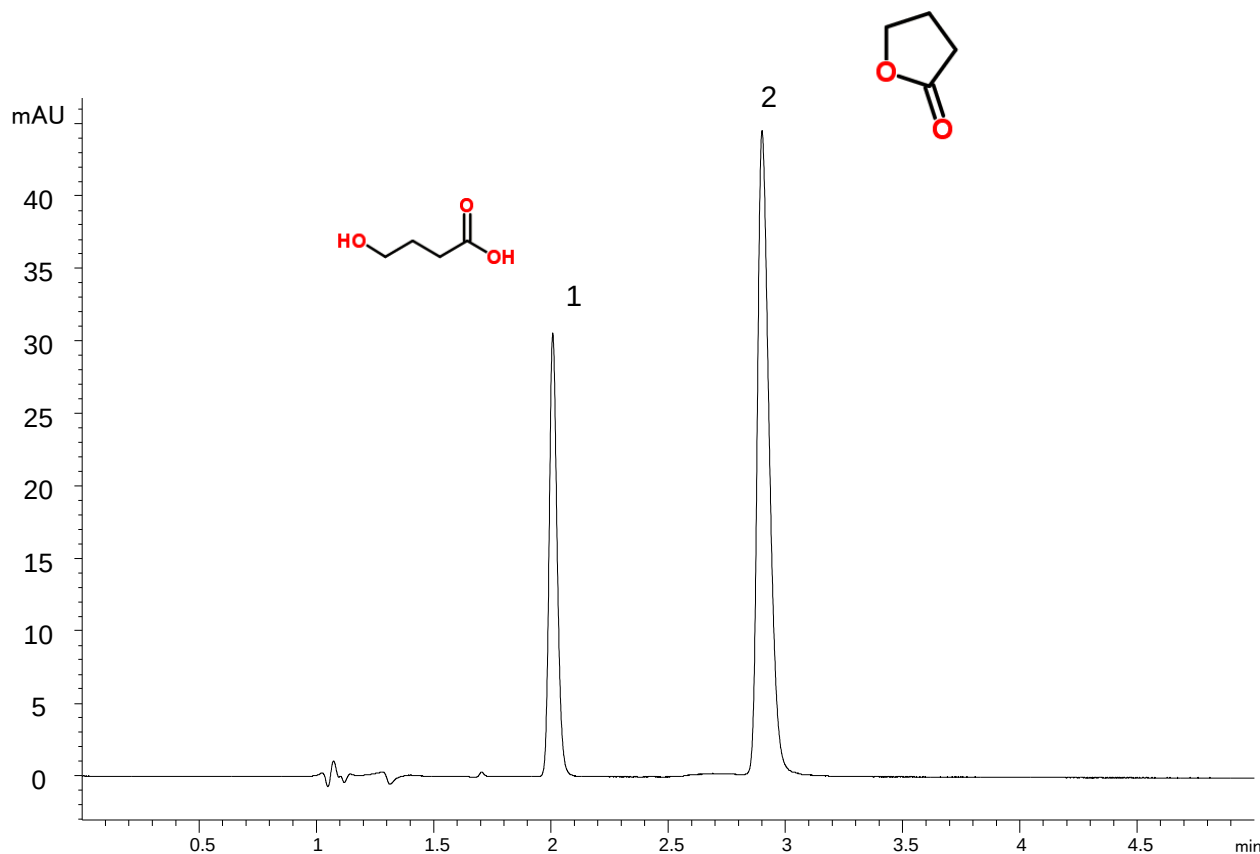
Exactive accurate mass MS system

Electrospray in negative ion mode

Analytes between m/z 70 – 800 monitored



ACE C18-AR: Separation GHB and GBL



ACE Excel 2 C18-AR
2 μ m, 100 x 3.0 mm
Isocratic analysis
20 mM KH₂PO₄ pH 2.5
Water/Acetonitrile (98:2 v/v)
Flow rate: 0.43 ml/min
Column temperature: 30°C
Detection: UV, 215 nm

1. GHB
2. GBL

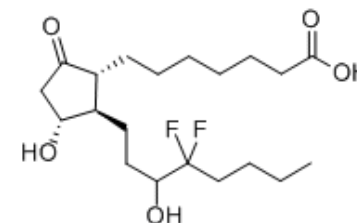


15-Hydroxy Lubiprostone in Human Plasma

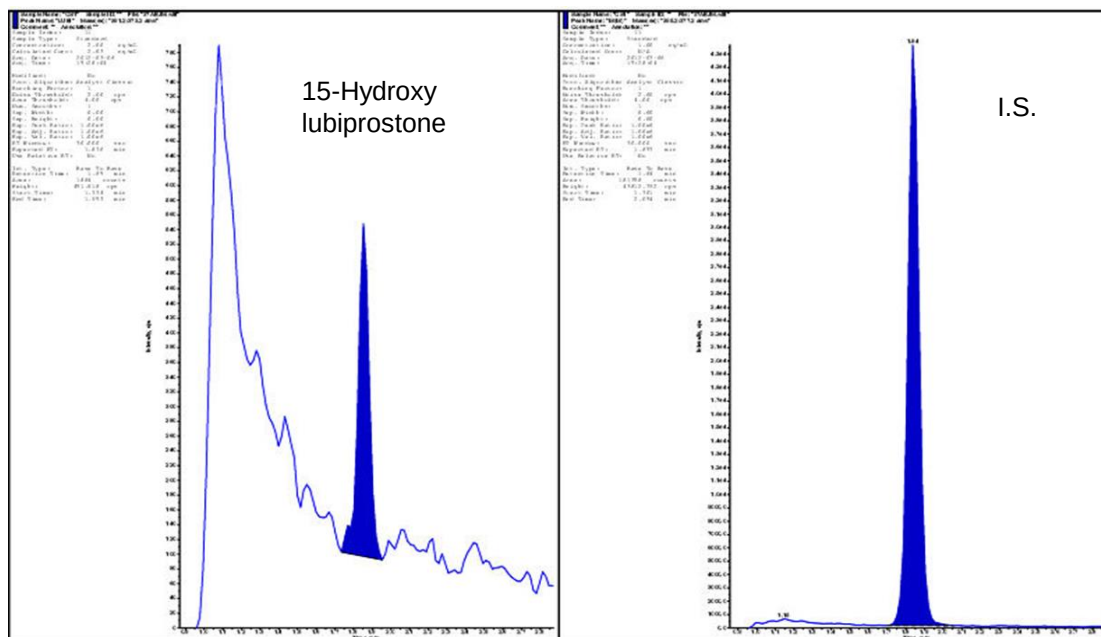
Lubiprostone, a fatty acid derived from prostaglandin E1, is rapidly metabolised to 15-hydroxy lubiprostone.

Quantitation is based on 15-hydroxy lubiprostone, with the d4 analogue as internal standard

Lowest calibration standard sample containing 2.0pg/ml in human EDTA K3 plasma



15-Hydroxy lubiprostone
MW 392.5



ACE Excel 2 C18 2 μ m, 50 x 3.0mm
Isocratic analysis
A = 0.1% formic acid in water
B = Acetonitrile
Flow rate: 0.65ml/min
Column temperature: 35°C
Injection volume: 15 μ l

MDS Sciex API 5000
TurbolonSpray negative mode
IonSpray voltage: -4500V
Source temperature: 450°C

Transitions monitored:
15-Hydroxy lubiprostone
391.2 $\rightarrow$ 373.2
I.S. (15-Hydroxy lubiprostone-d4)
395.2 $\rightarrow$ 377.2

**ACE**

HPLC / UHPLC Columns

Vitamin D2/D3

Key:

- 1 Ergocalciferol (D2)
- 2 Cholecalciferol (D3)

ACE Excel C18-Amide

2 μ m, 50 x 3.0 mm

Isocratic analysis

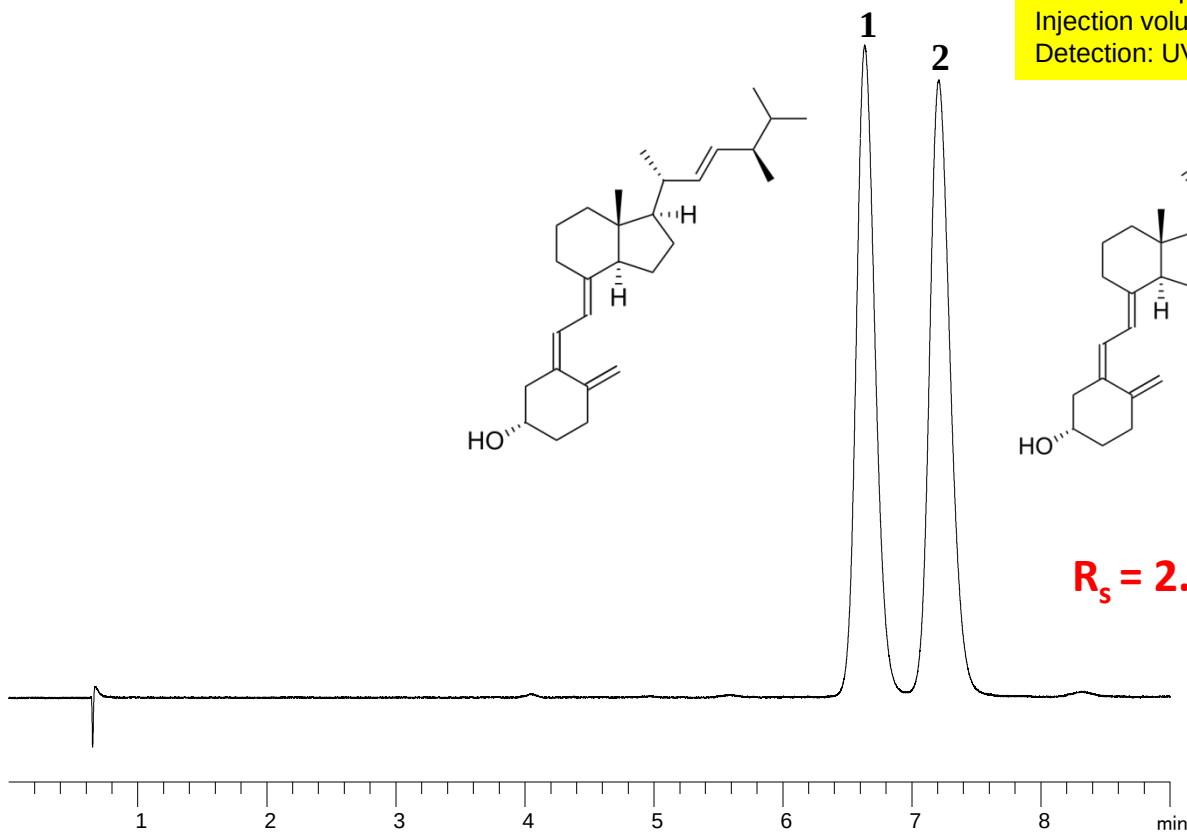
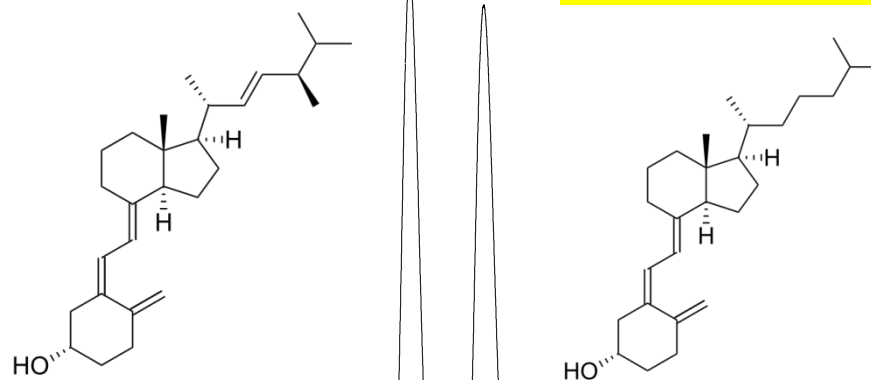
100% MeCN

Flow rate: 0.43 ml/min

Column temperature: 20°C

Injection volume: 3 μ l

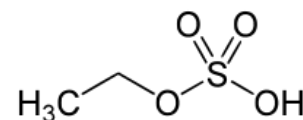
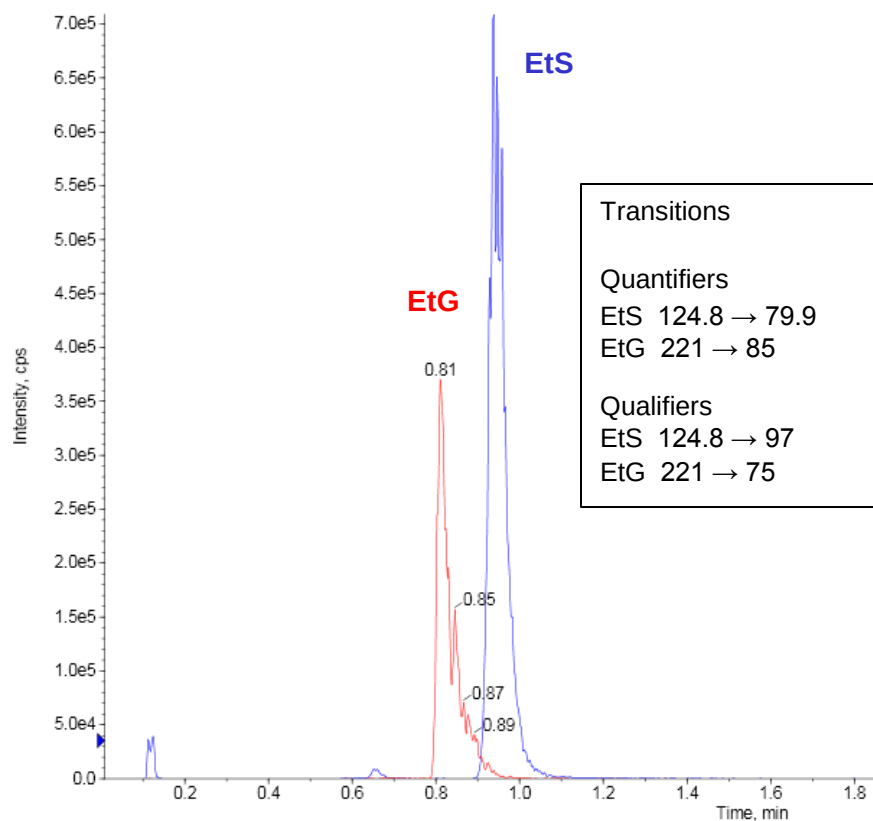
Detection: UV 265 nm

 **$R_s = 2.06$**

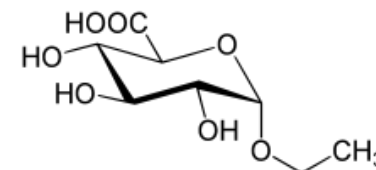


Alcohol Biomarkers by UHPLC-MS/MS

Extracted ion chromatogram



Ethyl sulphate (EtS)



Ethyl glucuronide (EtG)

ACE Excel 1.7 C18 1.7µm, 100 x 2.1mm
Gradient analysis

A = 1mM ammonium fluoride

B = Acetonitrile

Time (mins)	%B	Time (mins)	%B
0	0	2.0	100
0.5	20	4.0	100
1.5	20	4.5	0

Flow rate: 0.4ml/min

Column temperature: 40°C

Injection volume: 1µl

AB SCIEX triple quad 5500

Negative ESI MRM

Source temperature: 750°C

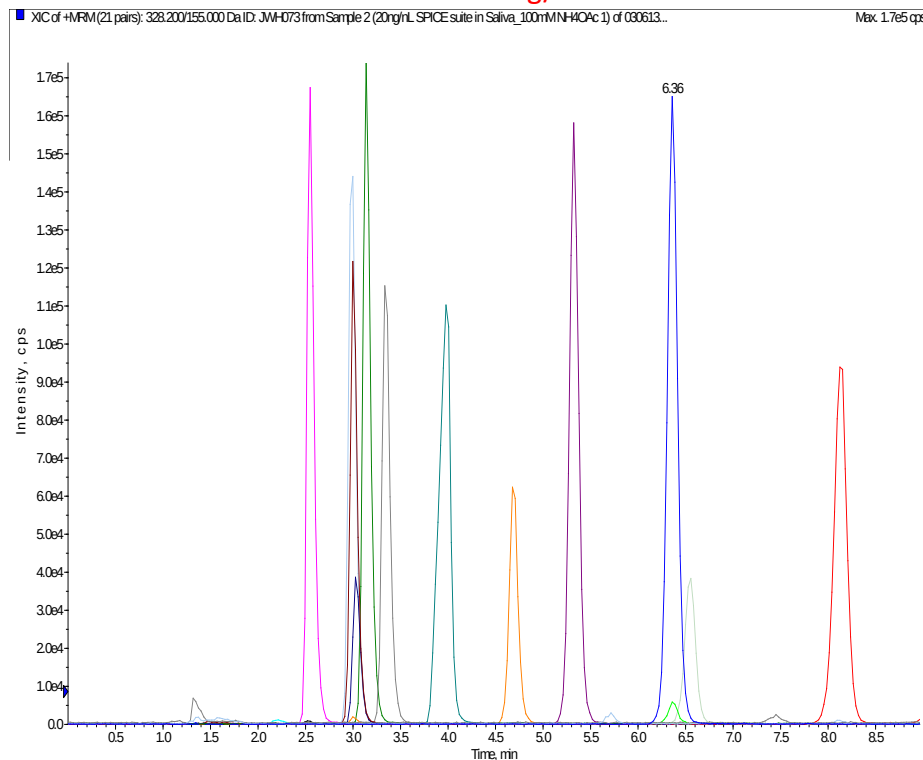
IonSpray voltage: -4500V

Fluoride counter-ion thought to enhance negative ESI response

Detection limit ~ 1ng/ml in oral fluid

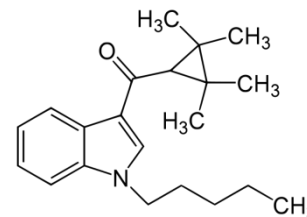
Synthetic Cannabinoids (SPICE) From Oral Fluid

Extracted ion chromatogram for SPICE analytes fortified in neat oral fluid at 20ng/mL

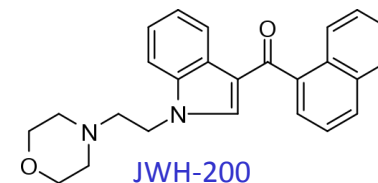


ACE Excel C18-AR 100x2.1mm, 2µm
Isocratic analysis
15:85 v/v A:B
A = 0.1% v/v formic acid (aq)
B = 0.1% v/v formic acid in MeOH
Ambient
0.3mL/min
Applied Biosystems / MDS Sciex 4000 Q-Trap
Positive mode Turbo Ionspray®

Retention Time (minutes)	Analyte	MRM Transition	Declustering Potential (DP)	Collision Energy (CE)	Cell Exit Potential (CXP)
2.55	JWH-250 N-(5-hydroxypentyl)	352>120.9	40	30	16
2.99	JWH-073 N-(3-hydroxybutyl)	344>155	40	30	16
3.00	UR-144 5-Hydroxy-pentyl	328.5>125	30	35	16
3.03	UR-144 Pentanoic Acid	342.5>125	30	35	16
3.14	d5-JWH-018 N-(4-hydroxypentyl)	363.5> 155	40	35	16
3.14	JWH-018 N-(4-hydroxypentyl)	358> 155	40	30	16
3.34	JWH-018 5-pentanoic acid	372>155	40	30	16
3.98	JWH-200	385>155	40	30	16
4.69	XLR-11	330>125	30	35	16
5.32	JWH-250	336>121	40	30	16
6.36	JWH-073	328>155	40	30	16
6.37	UR-144 5-Chloro-pentyl	346.9>125	30	35	16
6.55	UR-144	312.5>125	30	35	16
8.14	JWH-018	342>155	40	30	16



UR-144



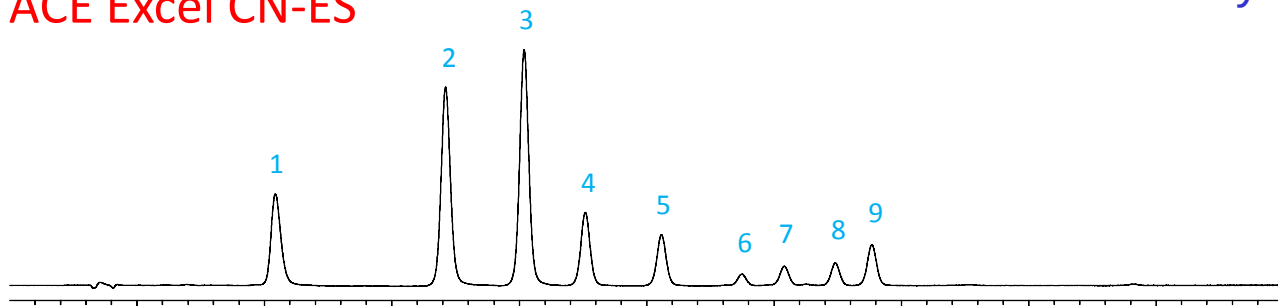
JWH-200



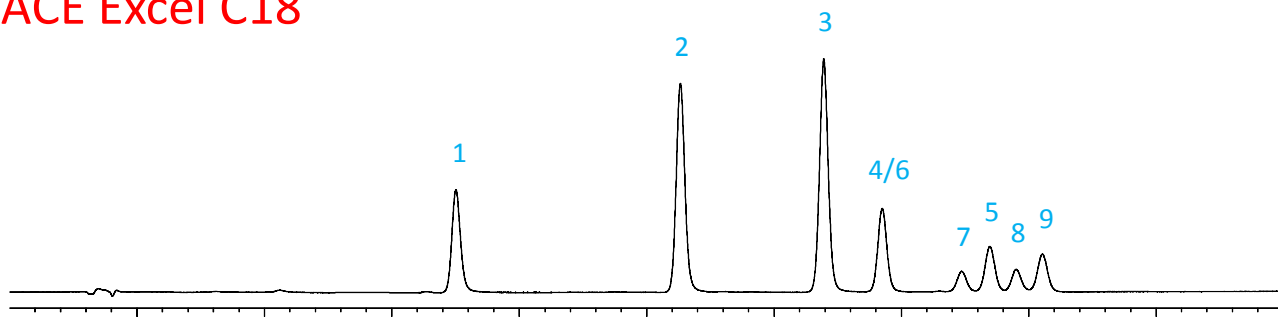
Steroids UHPLC-UV Analysis

ACE Excel CN-ES

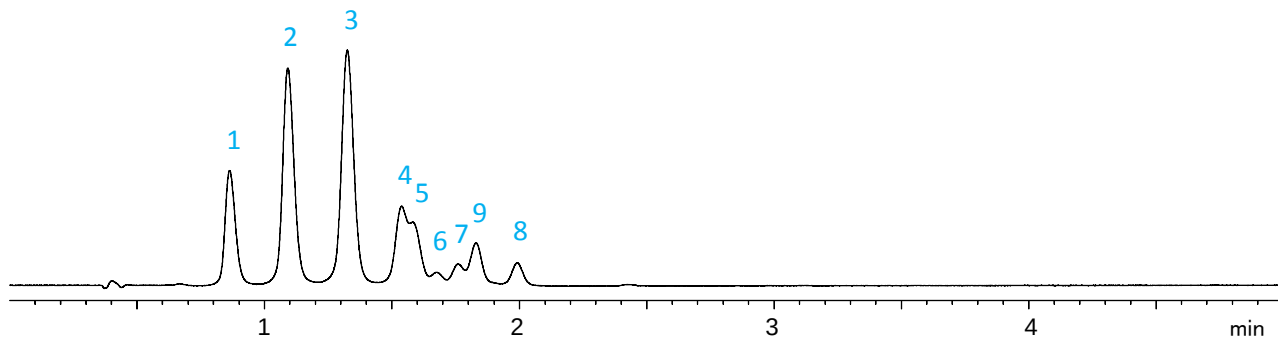
Alternative selectivity & full separation noted



ACE Excel C18



ACE Excel CN



50x2.1mm, 2 μ m
Gradient analysis

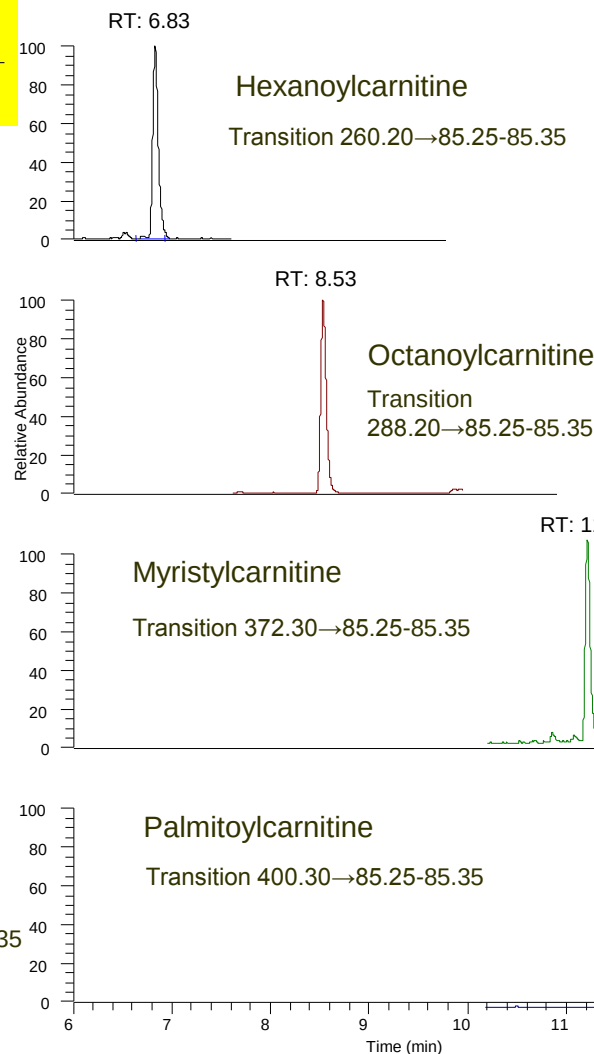
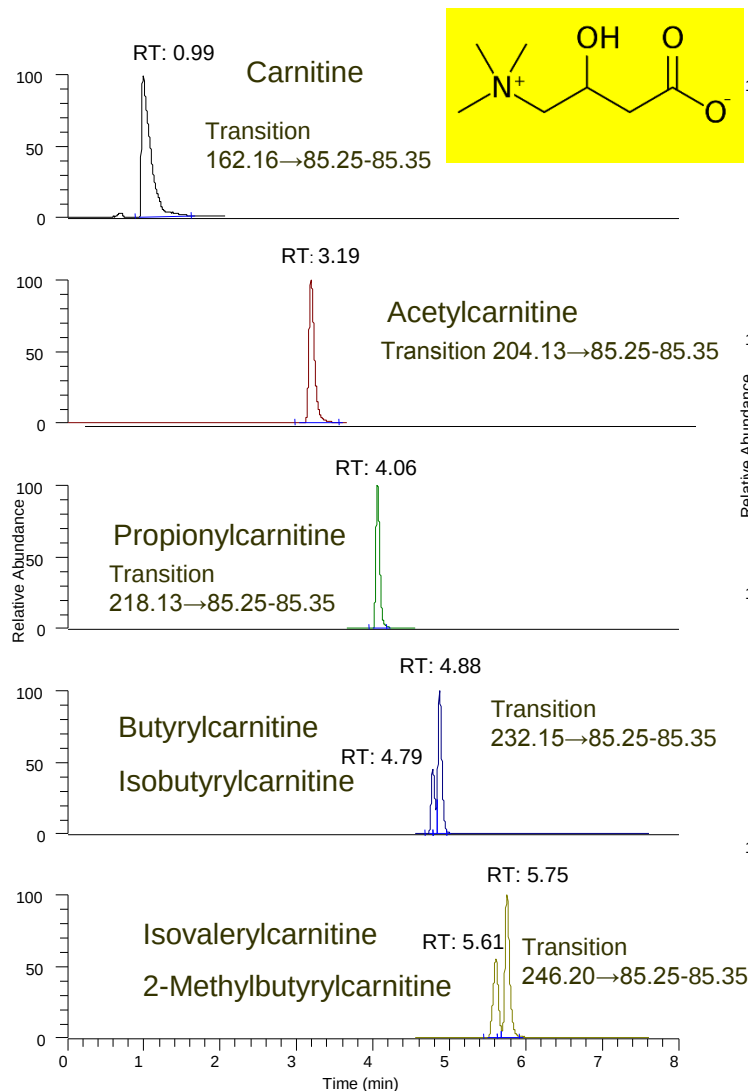
A = 0.1% formic acid
B = 0.1% FA in MeCN
40 °C
0.4 mL/min

T (min)	%B
0	25
10	80
10.5	80
11	25

1. Cortisone
2. Corticosterone
3. 11 α hydroxyprogesterone
4. cortisone-21-acetate
5. 11-ketoprogesterone
6. β -estradiol
7. 17 α estradiol
8. 17 α ethynylestradiol
9. estrone



UHPLC-MS/MS of Acylcarnitines



ACE Excel C18-PFP
2 μ m, 100 x 2.1mm
Gradient analysis
A = 0.1% formic acid in water
B = 0.1% formic acid in methanol

Time (Mins)	%B
0	0.5
0.5	0.5
9.0	90
13.0	90

Flow rate: 0.3ml/min

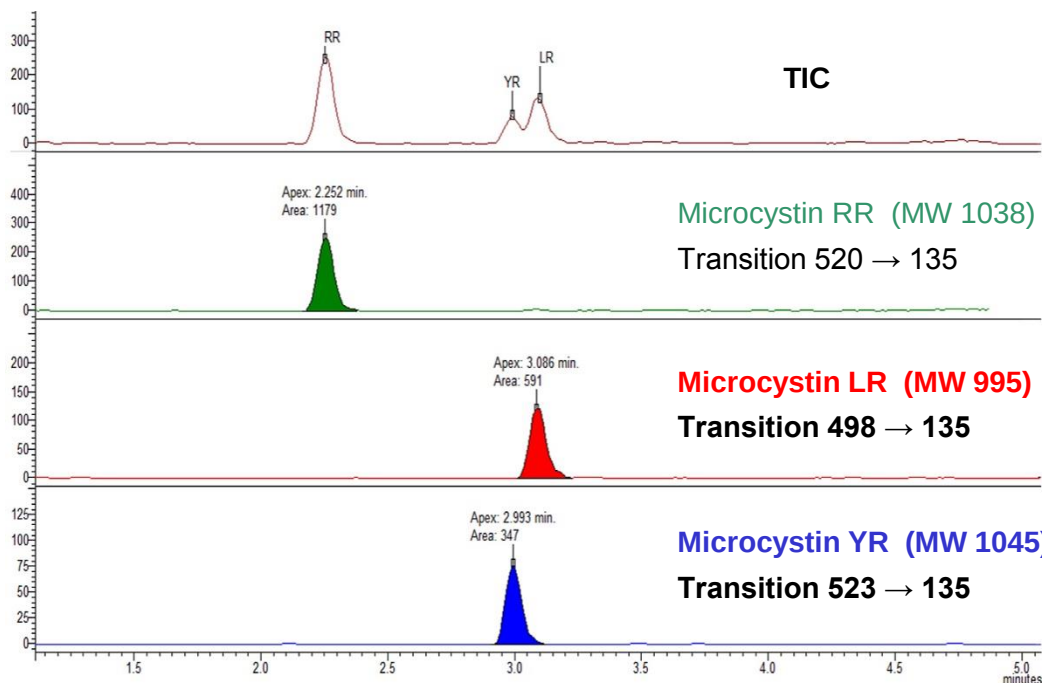
Positive mode ESI

Sample: Dried serum extract



Microcystins From Blue/Green Algae In Drinking Water

ACE Excel 2 μm C18, 100 x 2.1 mm



0.05 ppb each

Variants	R	L
MC-LR	Leucine	Arginine
MC-RR	Arginine	Arginine
MC-YR	Tyrosine	Arginine

Bruker Advance UHPLC system
ACE Excel 2 μm C18, 100 x 2.1mm
Gradient elution

A = 0.1% formic acid in water

B = Acetonitrile

T (mins) %B T (mins) %B

0 30 7.1 30

1 30 10 30

7 95

Flow rate: 0.4mL/min

Column temperature: 40°C

Injection volume: 50 μL

Concentration each microcystin: 0.05ppb

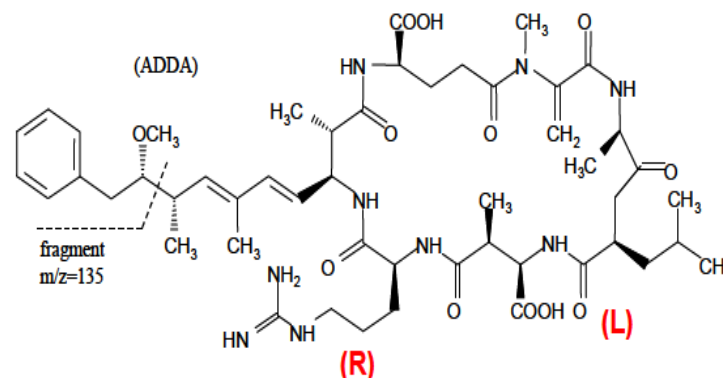
Bruker EVOQ Elite triple quad MS

VIP heated-ESI temperature: 350°C

Cone gas temperature: 200°C

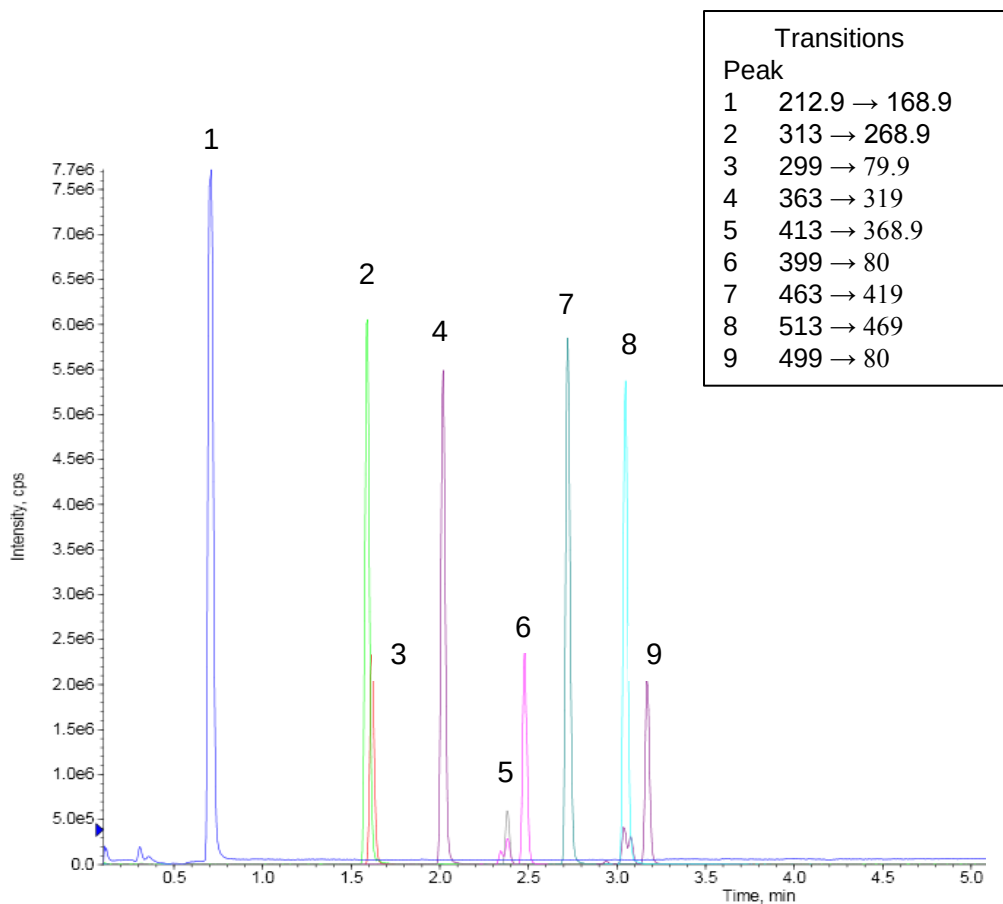
Spray voltage: 4500V (+)

Collision gas: argon 1.5mTorr





Perfluoro acids by LC-MS/MS



Peak ID	Analyte
1	Heptafluorobutyric acid
2	Perfluorohexanoic acid
3	Perfluorobutylsulphonic acid
4	Perfluoroheptanoic acid
5	Perfluorooctanoic acid
6	Perfluorohexylsulphonic acid
7	Perfluorononanoic acid
8	Perfluorodecanoic acid
9	Perfluorooctanesulphonic acid

ACE Excel 2 C18 2µm, 50 x 2.1mm

Gradient analysis

A = 2mM NH₄OAc, 0.1% acetic acid/CH₃CN (95:5)

B = 2mM NH₄OAc, 0.1% acetic acid/CH₃CN (5:95)

T (mins)	% B	T (mins)	%B
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0	25	7.5	95
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0.5	25	8.0	25
-----	----	-----	----

5.5	95	10.0	25
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Flow rate: 0.5ml/min

Column temperature: 40°C

Injection volume: 20µl

AB SCIEX triple quad 5500

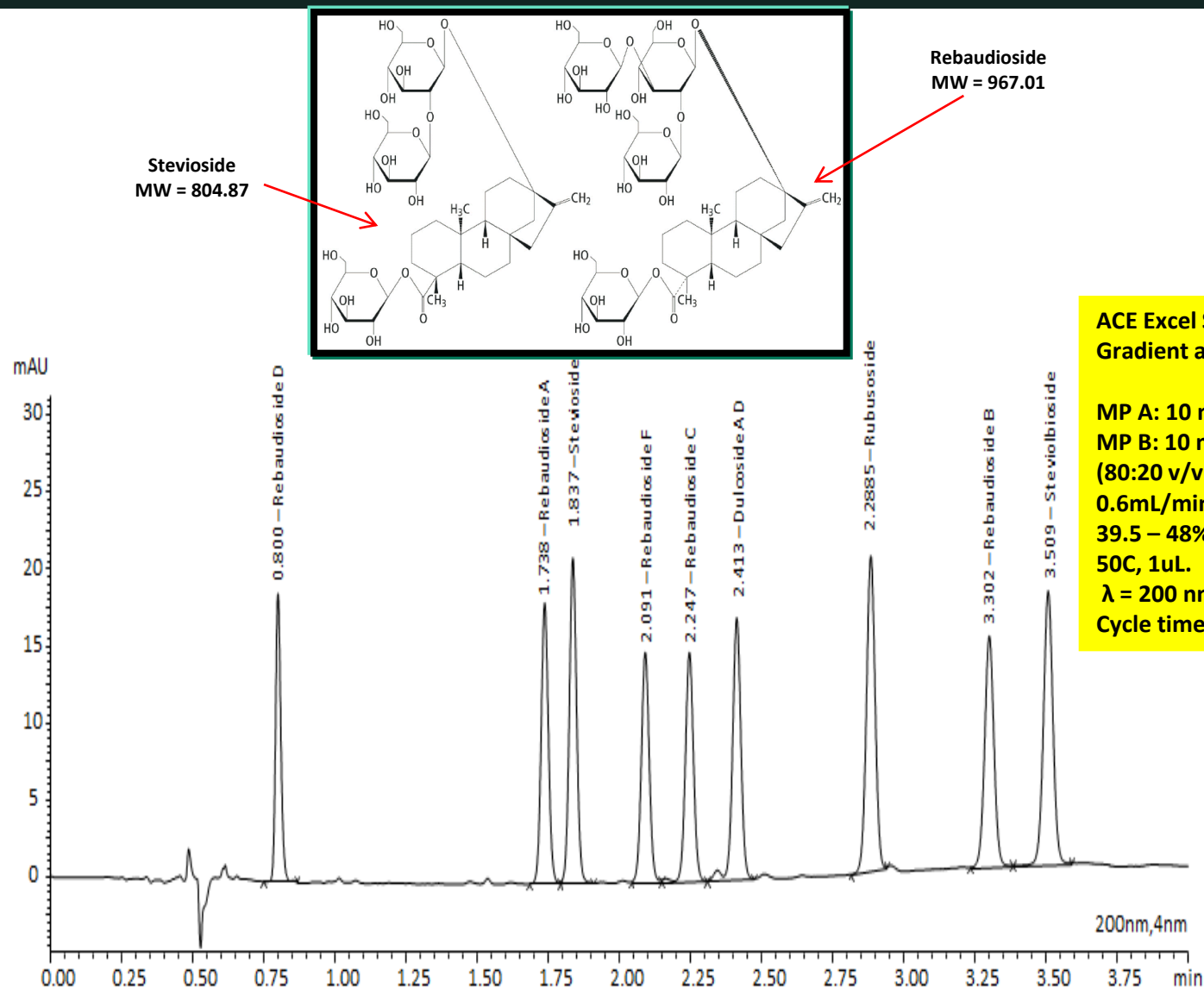
Negative ESI MRM

Source temperature: 450°C

IonSpray voltage: -2400V



Artificial Sweeteners (Stevia Glycosides)

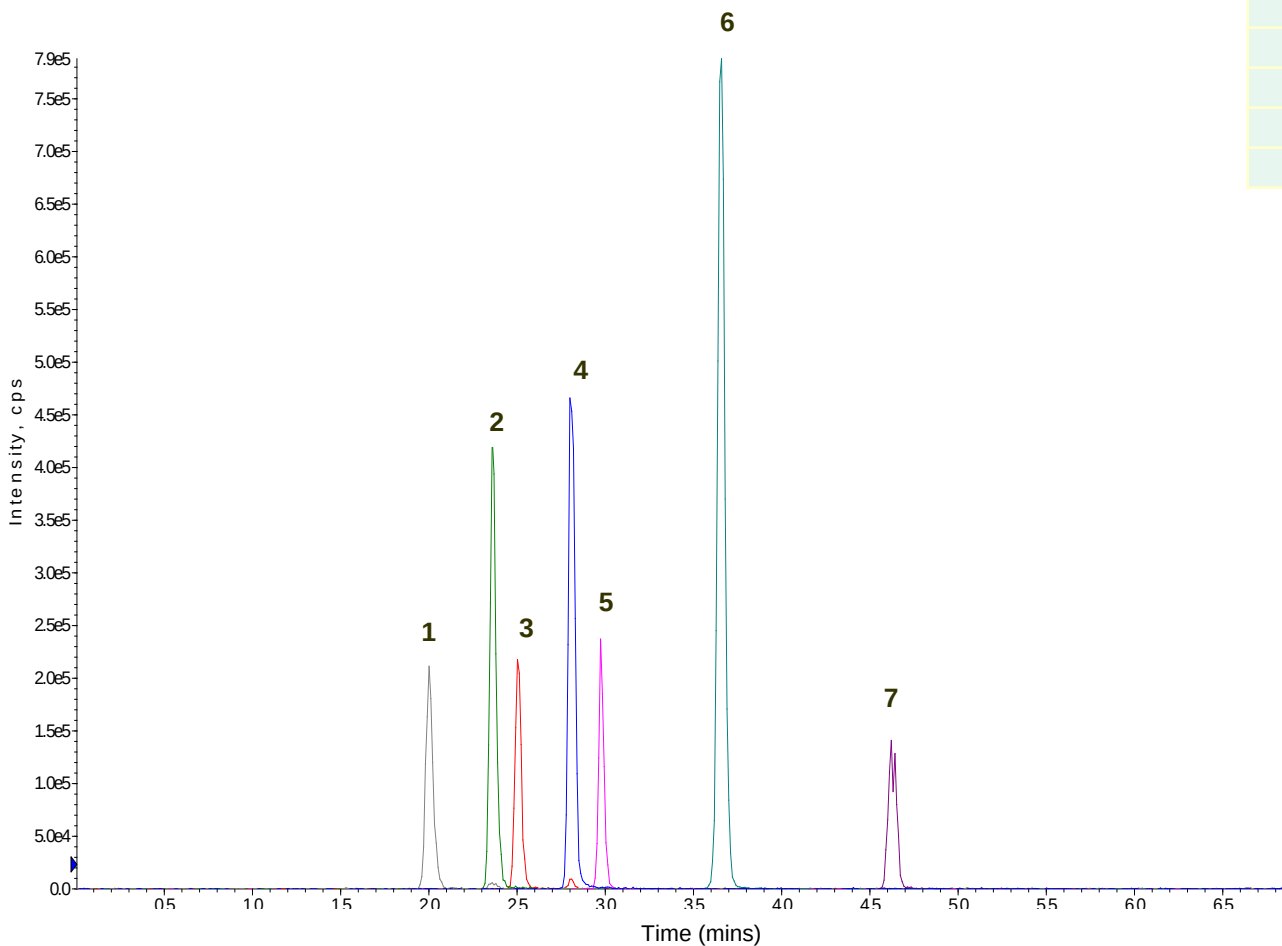


ACE Excel SuperC18, 2um, 150 x 2.1 mm
Gradient analysis

MP A: 10 mM NaH₂PO₄, pH 2.8 in H₂O.
MP B: 10 mM NaH₂PO₄, pH 2.8 in MeCN/H₂O (80:20 v/v).
0.6mL/min
39.5 – 48%B in 4 mins.
50C, 1uL.
λ = 200 nm.
Cycle time = 7 mins.



ACE C18-AR: Mycotoxins by LC-MS/MS



Peak	Analyte	Q1 (Da)	Q3 (Da)
1	Aflatoxin G2	331.1	313.1
2	Aflatoxin G1	329.0	243.1
3	Aflatoxin B2	315.1	287.0
4	Aflatoxin B1	313.1	285.0
5	HT-2 toxin	442.2	263.1
6	T-2 toxin	484.2	305.1
7	Ochratoxin A	404.1	239.0

ACE Excel 2 C18-AR 2 μ m, 50 x 2.1mm

Gradient analysis

A: 1mM NH₄OAc, 0.5% acetic acid

B: 1mM NH₄OAc, 0.5% acetic acid in 95% MeOH

Time (mins) %B

0 40

1 40

2.4 60

6.8 87

Flow rate: 0.6ml/min

Column temperature: 40°C

Injection volume: 20 μ l

AB SCIEX triple quad 5500

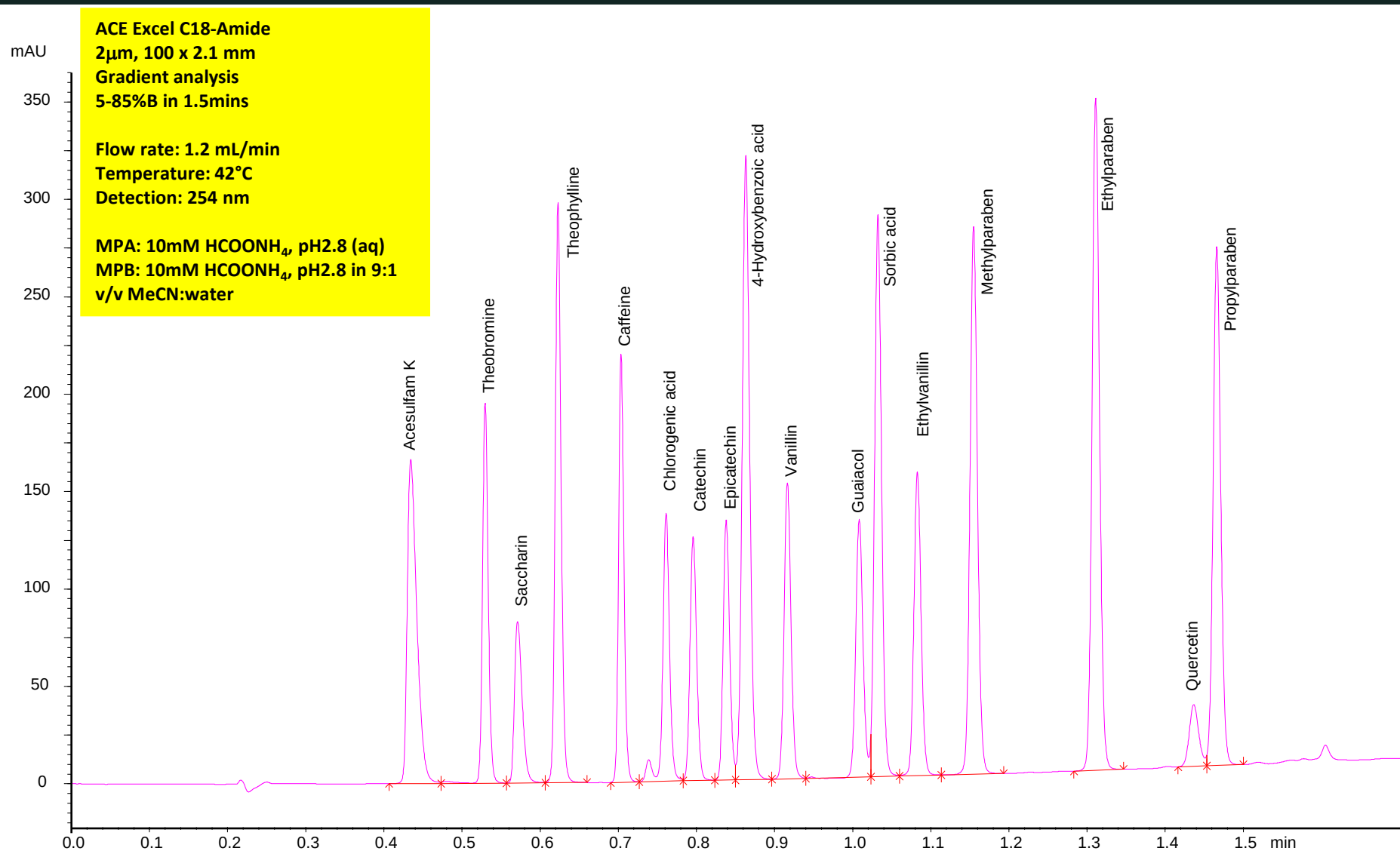
Positive mode ESI

Source temperature: 500°C

IonSpray voltage: 5000V



Chocolate Analysis



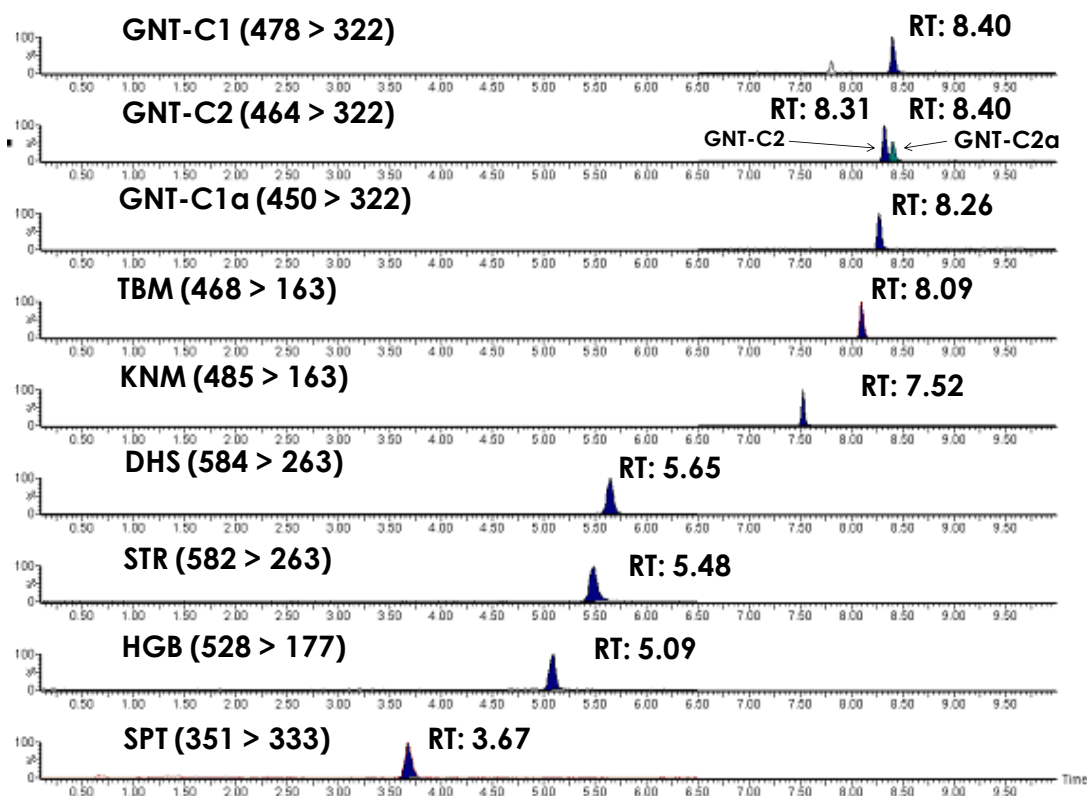


Aminoglycosides in Eggs

UHPLC-MS/MS method for aminoglycosides in eggs

Extraction at low pH, clean-up with WCX SPE cartridge

Egg sample spiked at 100µg/kg (CCα)



Key

GNT Gentamicin
 TBM Tobramycin
 KNM Kanamycin
 DHS Dihydrostreptomycin
 STR Streptomycin
 HGB Higromycin-B
 SPT Spectinomycin

ACE Excel C18-PFP, 2µm, 100 x 2.1mm

Gradient analysis

A = 20mM HFBA in H₂O/CH₃CN (98:2)

B = 20mM HFBA in CH₃CN/H₂O (98:2)

Time (mins) %B Curve

0	5	-
2	15	6
4.5	19	6
5.5	19.5	8
6	22	6
7	35	6
9	48	8
9.5	5	6

Flow rate: 0.4ml/min

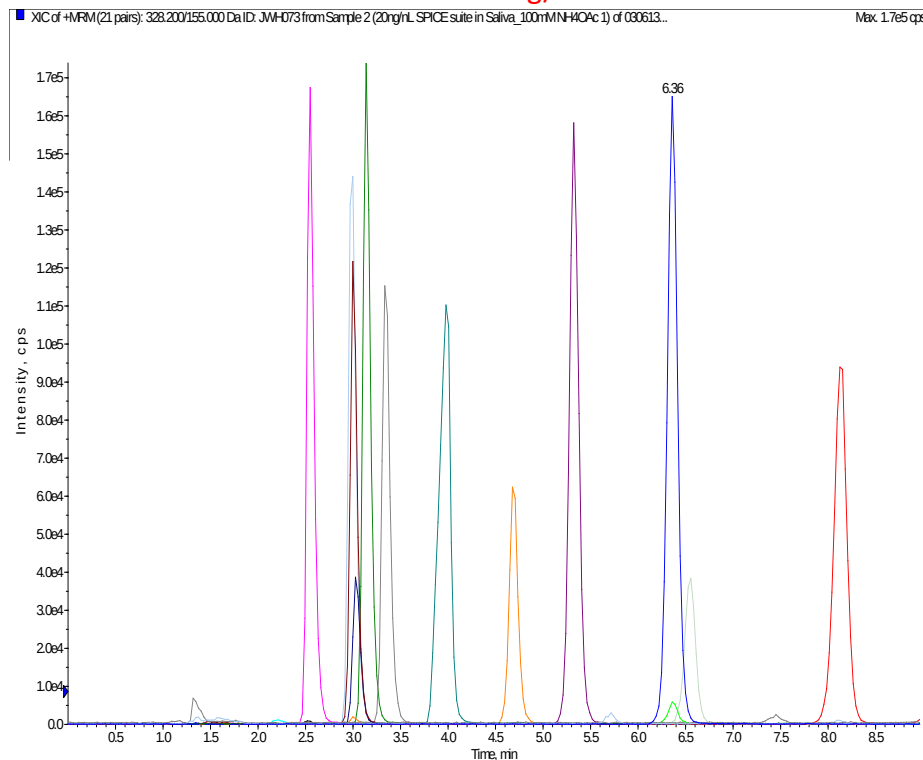
Column temperature: 40°C

Positive ESI MRM (transitions as shown)



Synthetic Cannabinoids (SPICE) From Oral Fluid

Extracted ion chromatogram for SPICE analytes fortified in neat oral fluid at 20ng/mL



ACE Excel C18-AR 100x2.1mm, 2µm
Isocratic analysis
15:85 v/v A:B

A = 0.1% v/v formic acid (aq)

B = 0.1% v/v formic acid in MeOH

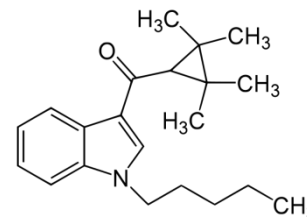
Ambient

0.3mL/min

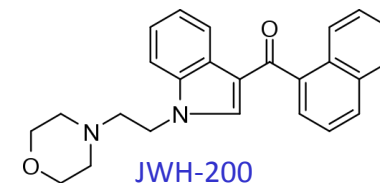
Applied Biosystems / MDS Sciex 4000 Q-Trap

Positive mode Turbo Ionspray®

Retention Time (minutes)	Analyte	MRM Transition	Declustering Potential (DP)	Collision Energy (CE)	Cell Exit Potential (CXP)
2.55	JWH-250 N-(5-hydroxypentyl)	352>120.9	40	30	16
2.99	JWH-073 N-(3-hydroxybutyl)	344>155	40	30	16
3.00	UR-144 5-Hydroxy-pentyl	328.5>125	30	35	16
3.03	UR-144 Pentanoic Acid	342.5>125	30	35	16
3.14	d5-JWH-018 N-(4-hydroxypentyl)	363.5>155	40	35	16
3.14	JWH-018 N-(4-hydroxypentyl)	358>155	40	30	16
3.34	JWH-018 5-pentanoic acid	372>155	40	30	16
3.98	JWH-200	385>155	40	30	16
4.69	XLR-11	330>125	30	35	16
5.32	JWH-250	336>121	40	30	16
6.36	JWH-073	328>155	40	30	16
6.37	UR-144 5-Chloro-pentyl	346.9>125	30	35	16
6.55	UR-144	312.5>125	30	35	16
8.14	JWH-018	342>155	40	30	16



UR-144

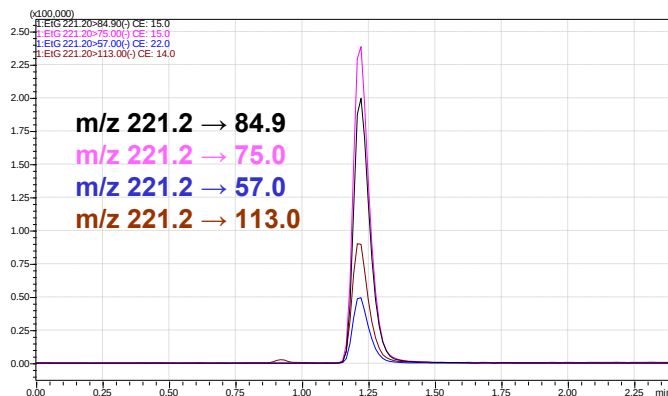


JWH-200

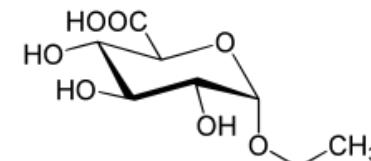
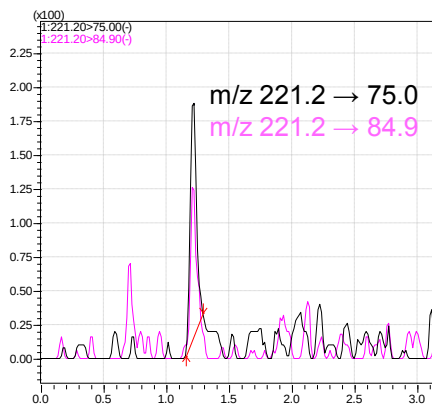


Ethyl Glucuronide in Water by LC-MS/MS

Ethyl glucuronide (EtG)



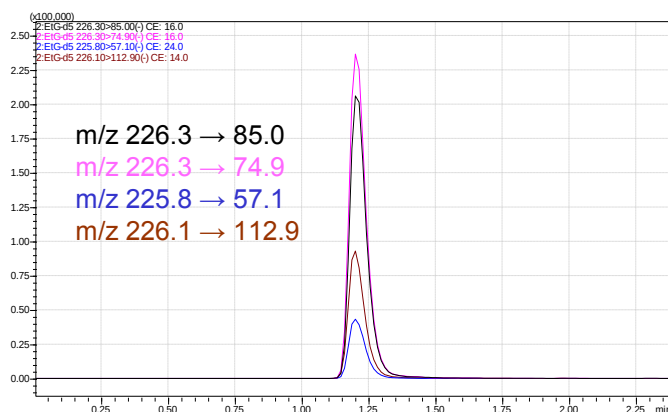
EtG 100pg/ml



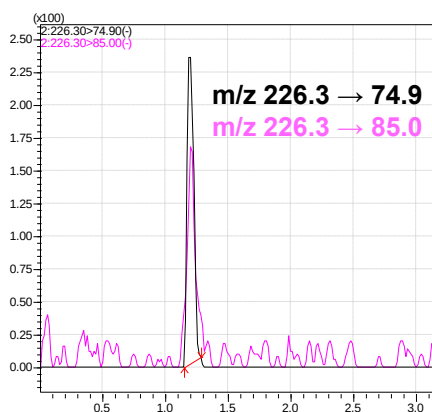
Ethyl glucuronide

**LOQ for EtG in water
= 300fg on-column**

Ethyl glucuronide-d5 (EtG-d5)



EtG-d5 100pg/ml



ACE Excel 2 C18-PFP

2µm, 100 x 2.1 mm

Gradient analysis

A = 0.05% formic acid in water

B = Methanol

T (mins)	%B	T (mins)	%B
4	70	7	95
6	95	7.01	5

Flow rate: 0.4 ml/min

Injection volume: 3 µl

Column temperature: 40°C

Shimadzu LCMS-8050

ESI voltage: -3kV

Desolvation line: 250°C

Interface heater: 380°C

Nebulizing gas: 3 l/min

Heat block: 400°C



USP Hydroquinone

Key:

1 Hydroquinone

ACE Excel C18, 2 μ m, 50 x 3.0 mm

Isocratic analysis

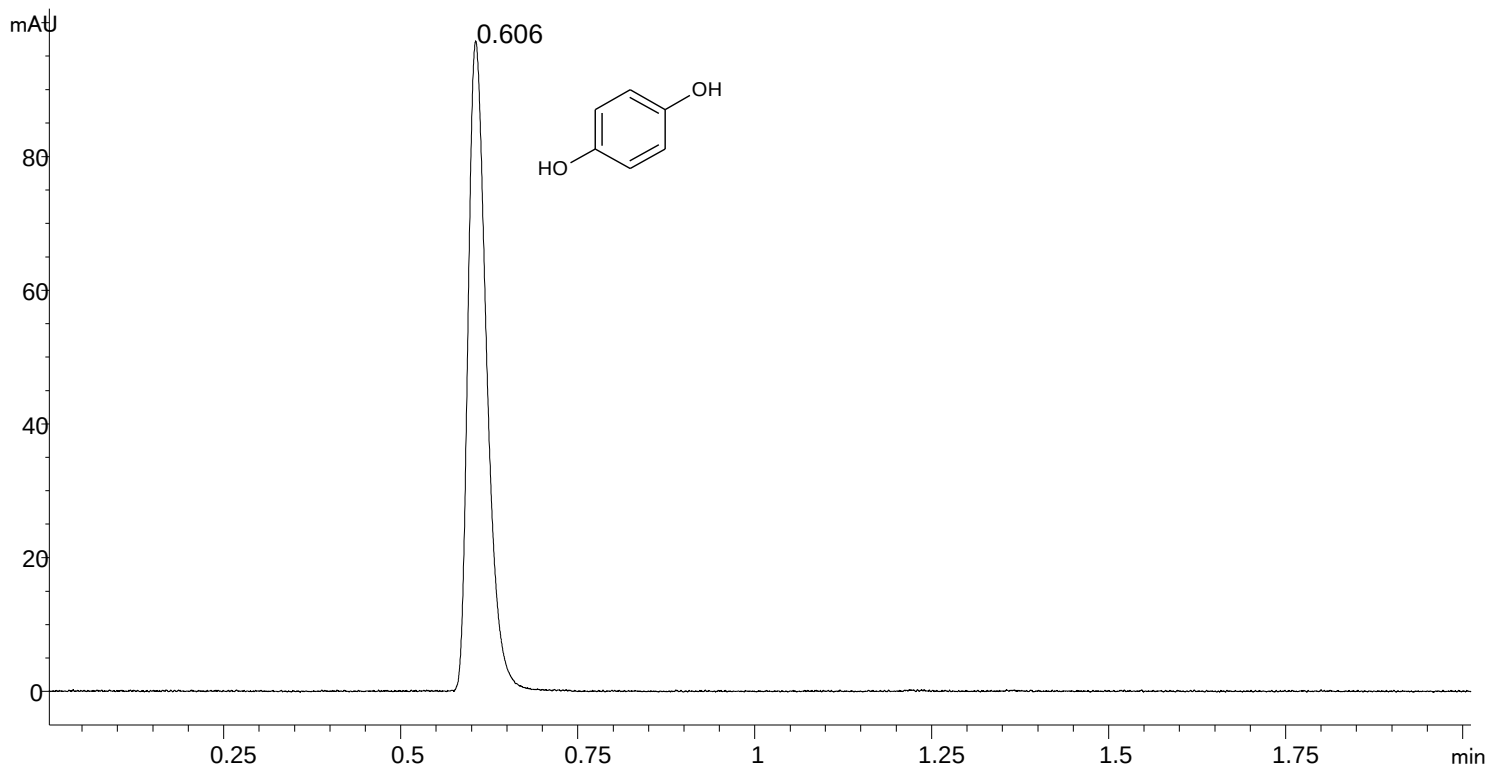
H₂O/MeOH (45:55 v/v)

Flow rate: 0.45 ml/min

Column temperature: Ambient (22°C)

Detection: UV at 280 nm

Injection volume: 0.9 μ l

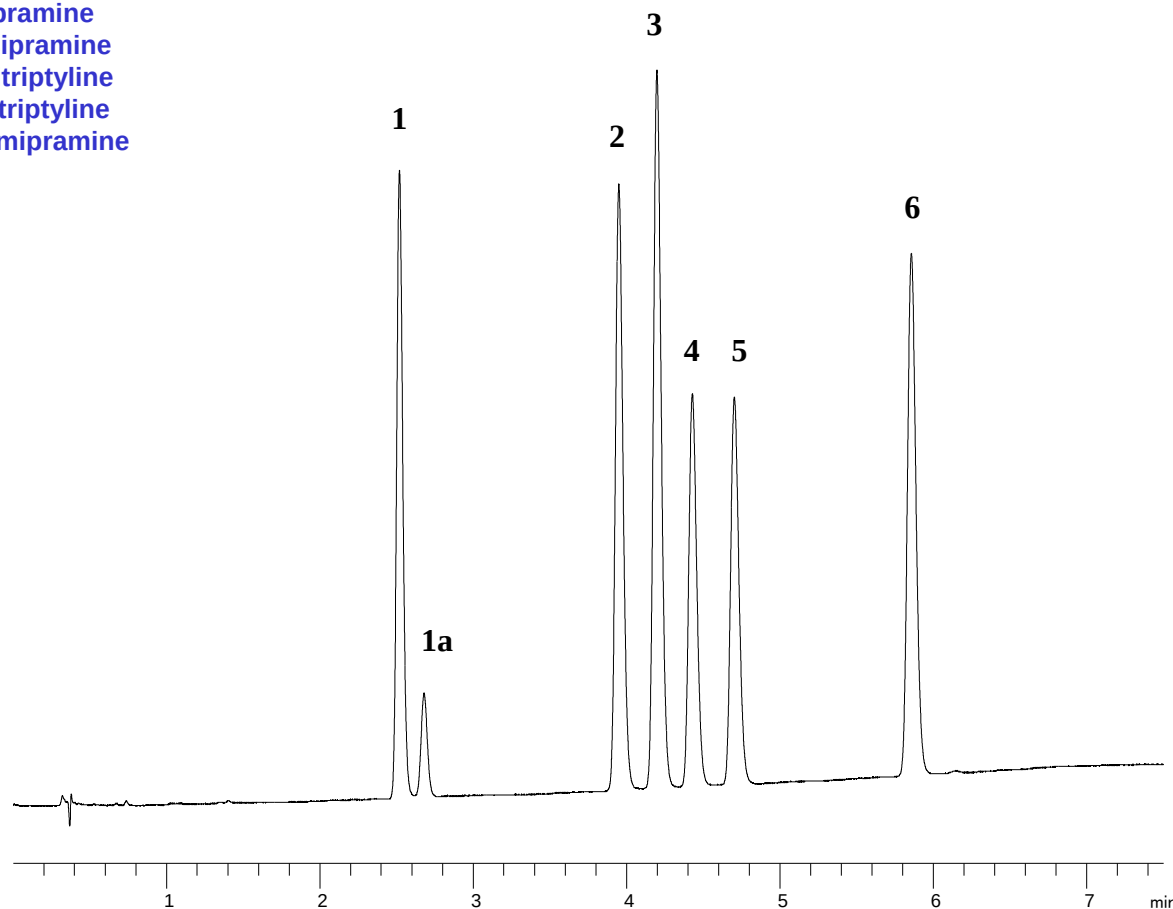




Tricyclic Antidepressants

Key:

- 1 Doxepin
- 1a Doxepin isomer
- 1 Imipramine
- 2 Desipramine
- 3 Amitriptyline
- 4 Nortriptyline
- 5 Clomipramine



ACE Excel SuperC18

2 μ m, 100 x 3.0mm

Gradient analysis

A = 20 mM ammonium formate pH 3.0

B = 20 mM ammonium formate pH 3.0
in MeOH:water 9:1 v/v

Time (mins)	%B
0	50
6	70
7	70
7.5	

Flow rate: 1.2ml/min

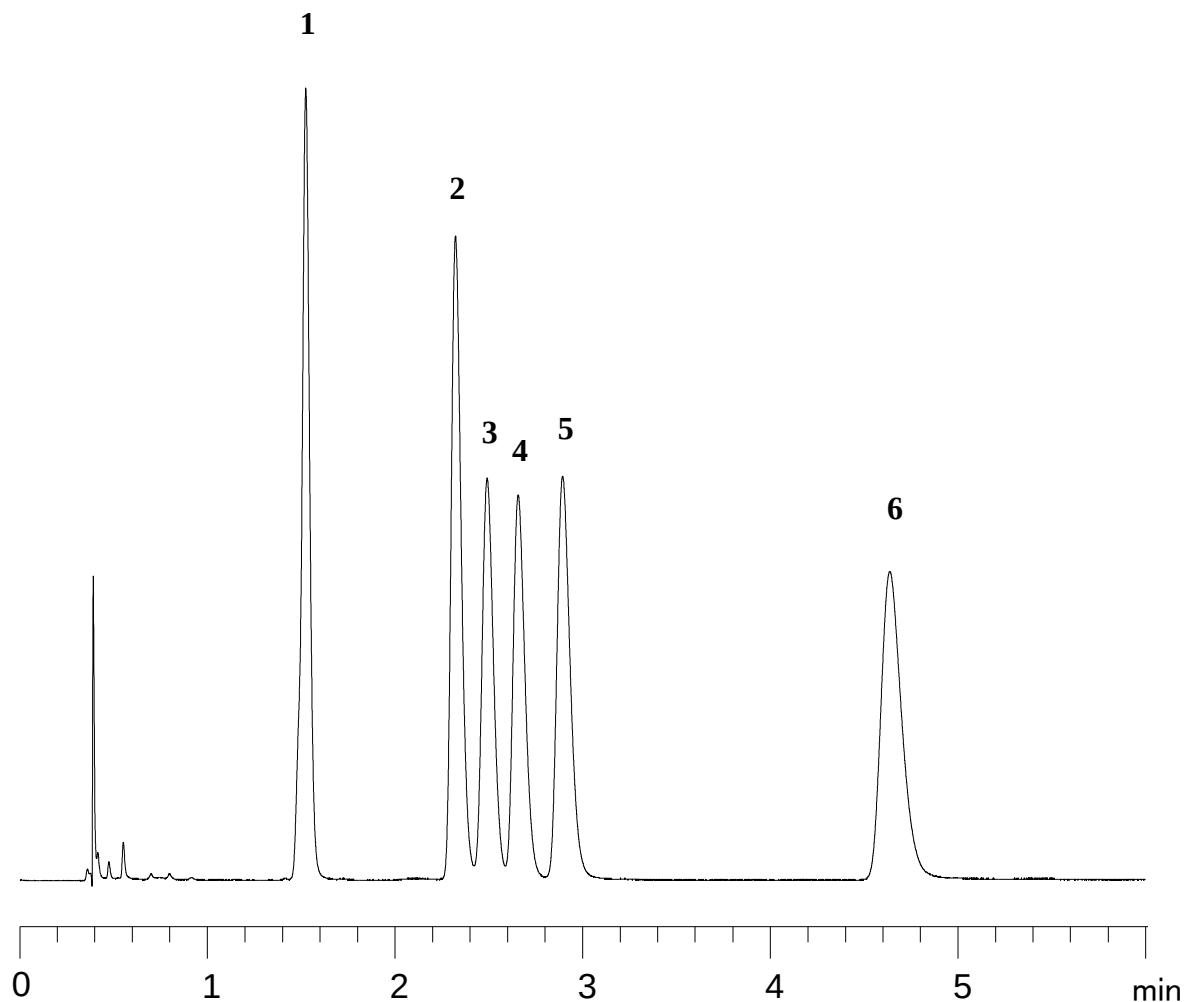
Column temperature: 40°C

Injection volume: 2 μ l

Detection: UV, 260nm



Tricyclic Antidepressants



ACE Excel C18-PFP

2 μ m, 100 x 3.0mm

Isocratic analysis

B = 20 mM ammonium formate pH 3.0
in MeOH:water 54:46 v/v

Flow rate: 1.2ml/min

Column temperature: 40°C

Injection volume: 2 μ l

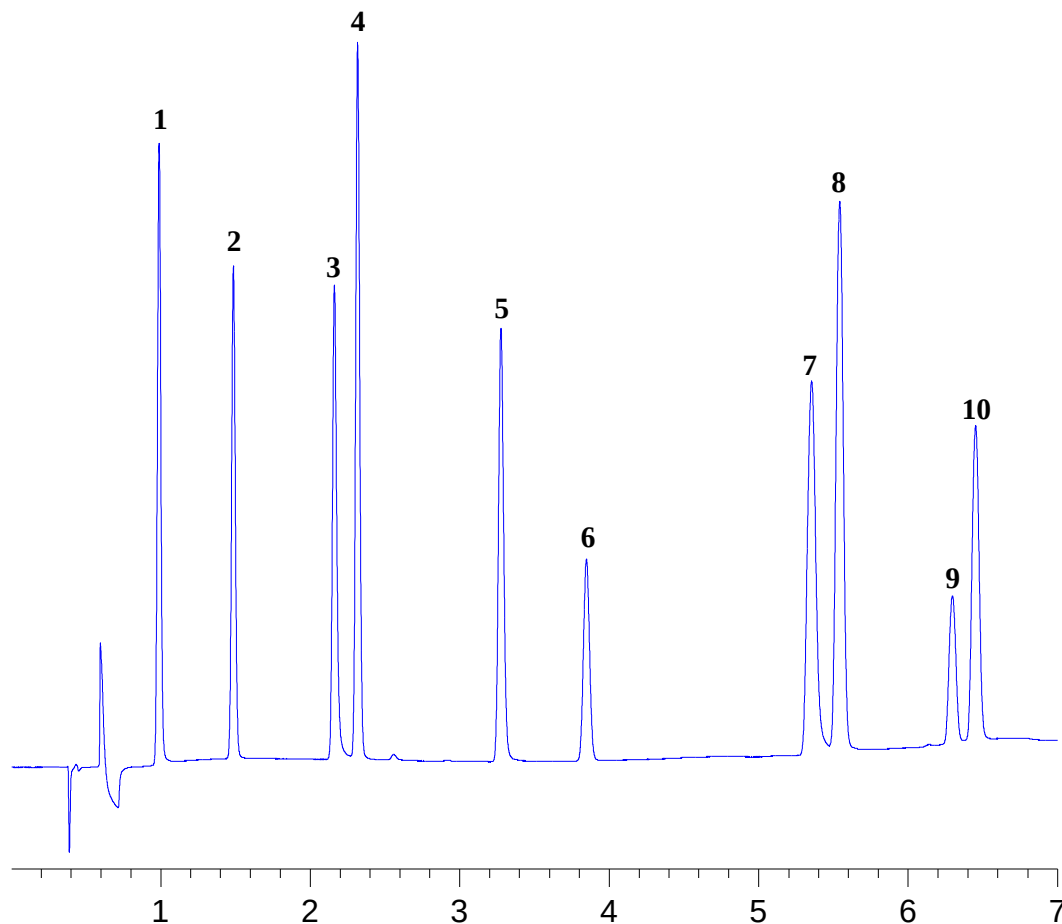
Detection: UV, 260nm

Key:

- 1 Doxepin
- 2 Imipramine
- 3 Protriptyline
- 4 Nortriptyline
- 5 Trimipramine
- 6 Clomipramine



Paracetamol and Related Compounds



ACE Excel C18-PFP

2 μ m, 100 x 3.0mm

Gradient analysis

A = 20 mM ammonium acetate pH 6.0

B = 20 mM ammonium acetate pH 6.0

in MeOH:water 9:1 v/v

Time (mins)	%B
0	9
5.5	63
6.5	63
7	9

Flow rate: 1.2ml/min

Column temperature: 27°C

Injection volume: 2 μ l

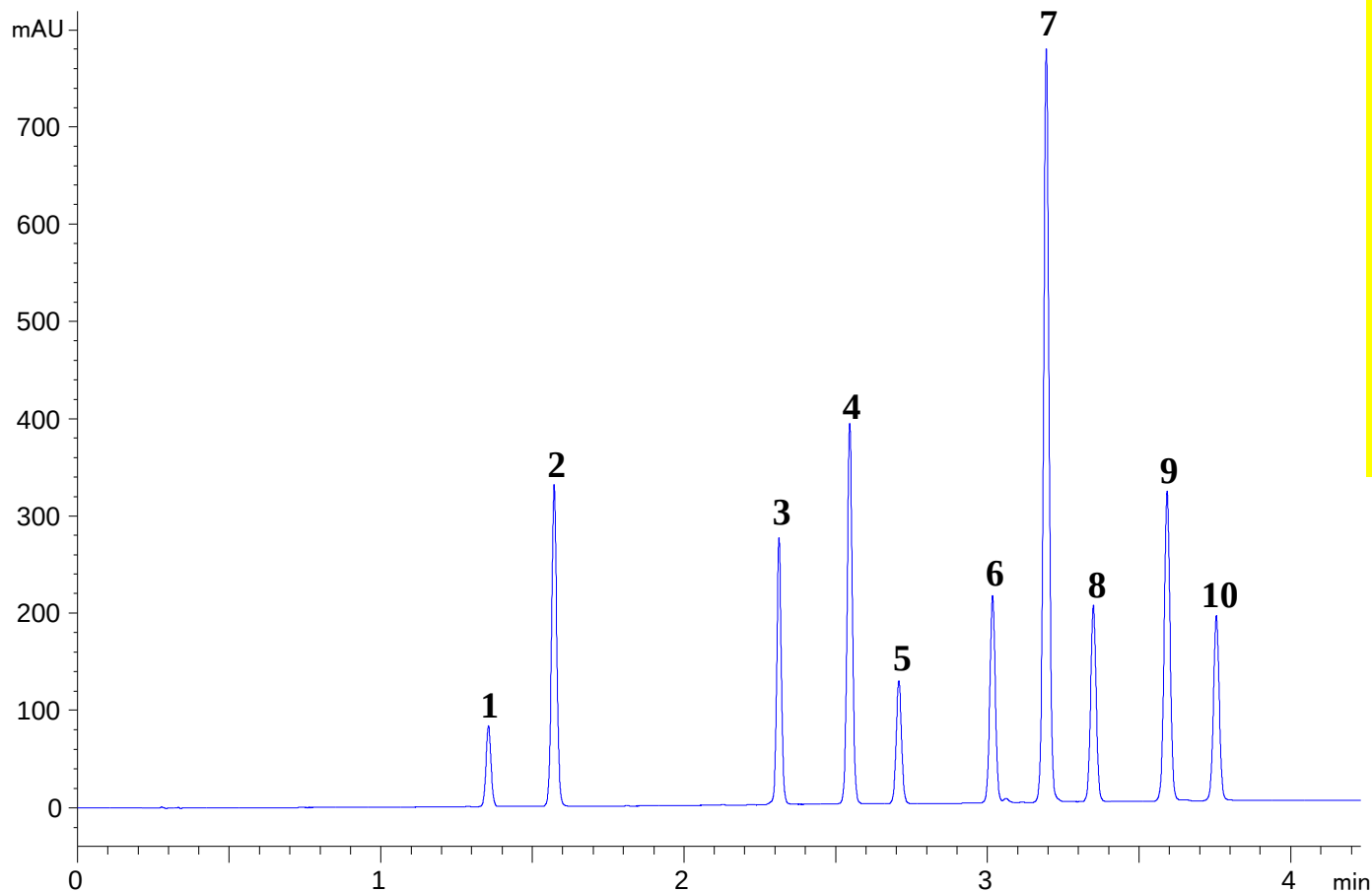
Detection: UV, 220nm

Key:

- 1 4-aminophenol
- 2 Hydroquinone
- 3 2-aminophenol
- 4 Paracetamol
- 5 2-acetamidophenol
- 6 Phenol
- 7 4-nitrophenol
- 8 2-nitrophenol
- 9 4-chloroacetanilide
- 10 4-chlorophenol



NSAIDS – Fast analysis



ACE Excel Super C18

2 μ m, 50 x 3.0 mm

Gradient analysis

A = 0.1% formic acid in water

B = 0.1% formic acid in acetonitrile

Time (mins)	%B
0	20
0.25	20
3.5	70
4	70
4.25	20

Flow rate: 0.86ml/min

Column temperature: 40°C

Injection volume: 1.4 μ l

Detection: UV, 254nm

Key:

1 Aspirin

2 Phenacetin

3 Sulindac

4 Tolmetin

5 Naproxen

6 Nimesulide

7 Flurbiprofen

8 Diclofenac

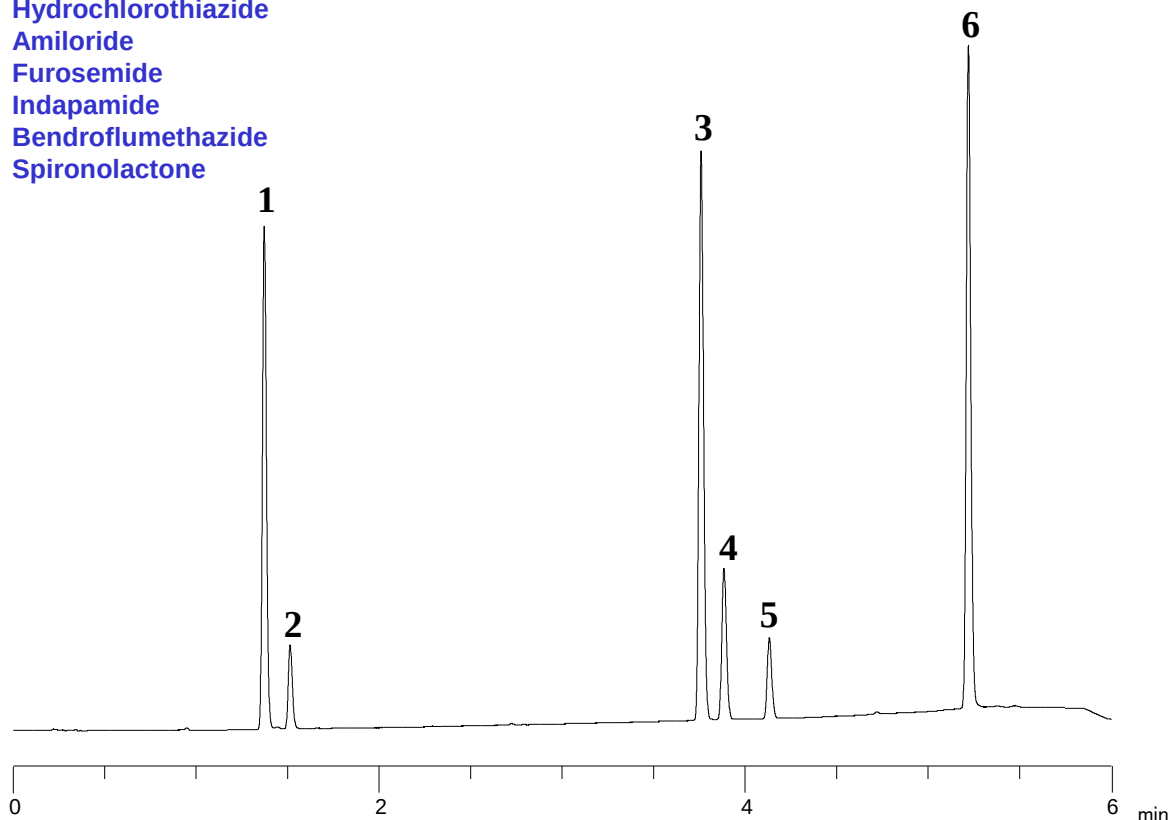
9 Phenylbutazone

10 meclofenamic acid

Diuretics

Key:

- 1 Hydrochlorothiazide
- 2 Amiloride
- 3 Furosemide
- 4 Indapamide
- 5 Bendroflumethazide
- 6 Spironolactone



ACE Excel C18-PFP

2µm, 50 x 3.0 mm

Gradient analysis

A = 10 mM ammonium formate pH 3.0

B = 10 mM ammonium formate pH 3.0

in MeOH:water 9:1 v/v

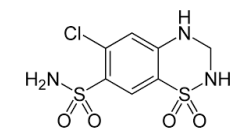
Time (mins)	%B
0	5
0.5	5
5	70
5.5	70
6	5

Flow rate: 1.0ml/min

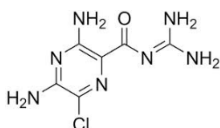
Column temperature: 60°C

Injection volume: 2µl

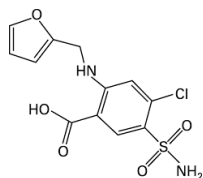
Detection: UV, 254nm



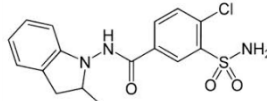
Hydrochlorothiazide



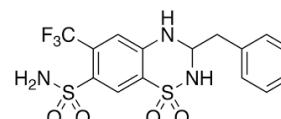
Amiloride



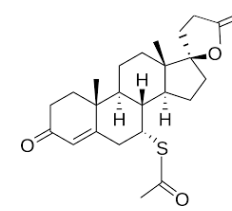
Furosemide



Indapamide



Bendroflumethazide



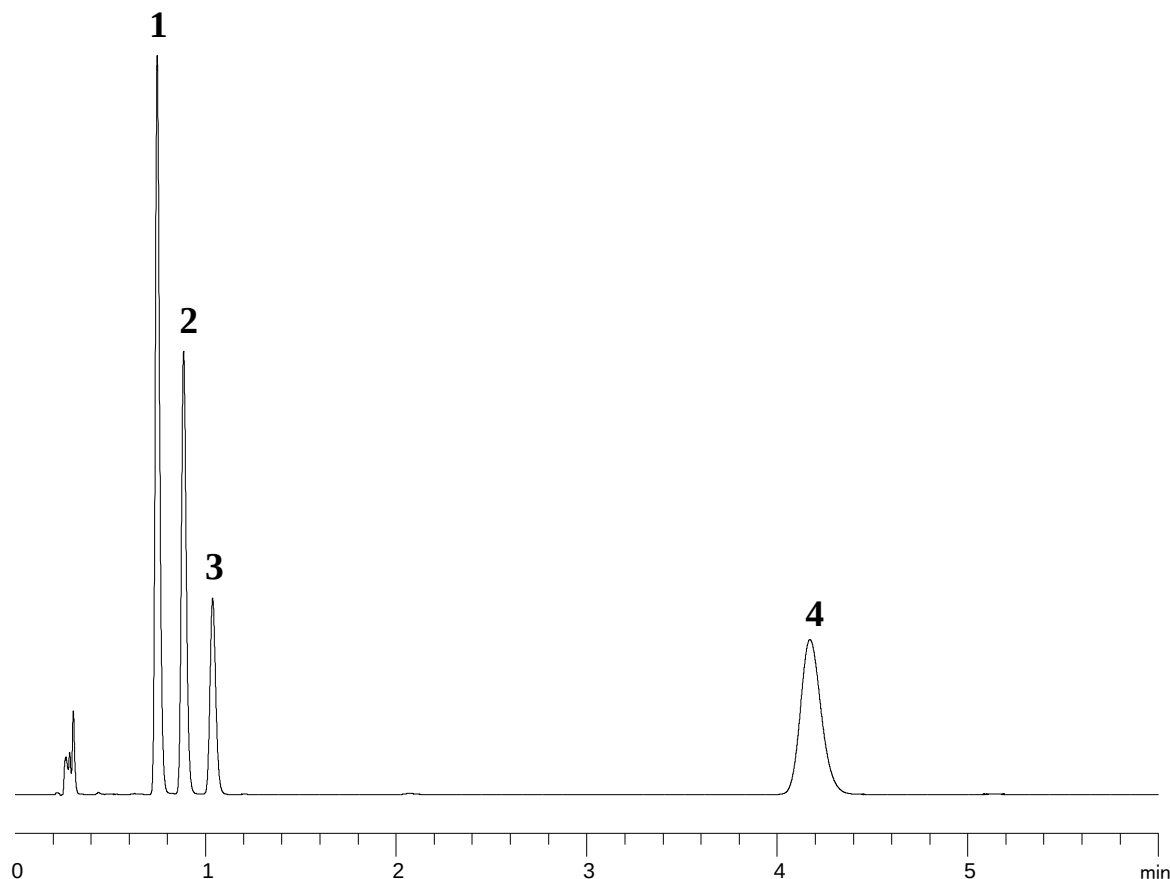
Spironolactone



Diuretics (Isocratic)

Key:

- 1 Furosemide
- 2 Indapamide
- 3 Bendroflumethazide
- 4 Spironolactone



ACE Excel C18-PFP

2 μ m, 50 x 3.0 mm

Isocratic analysis

10 mM ammonium formate pH 3.0

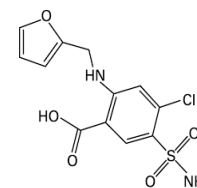
in methanol:water 45:55 v/v

Flow rate: 1.0ml/min

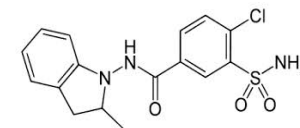
Column temperature: 60°C

Injection volume: 2 μ l

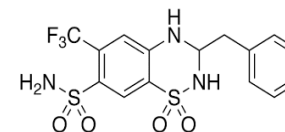
Detection: UV, 254nm



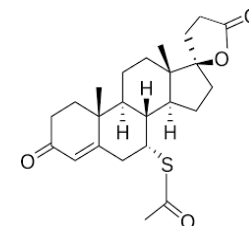
Furosemide



Indapamide



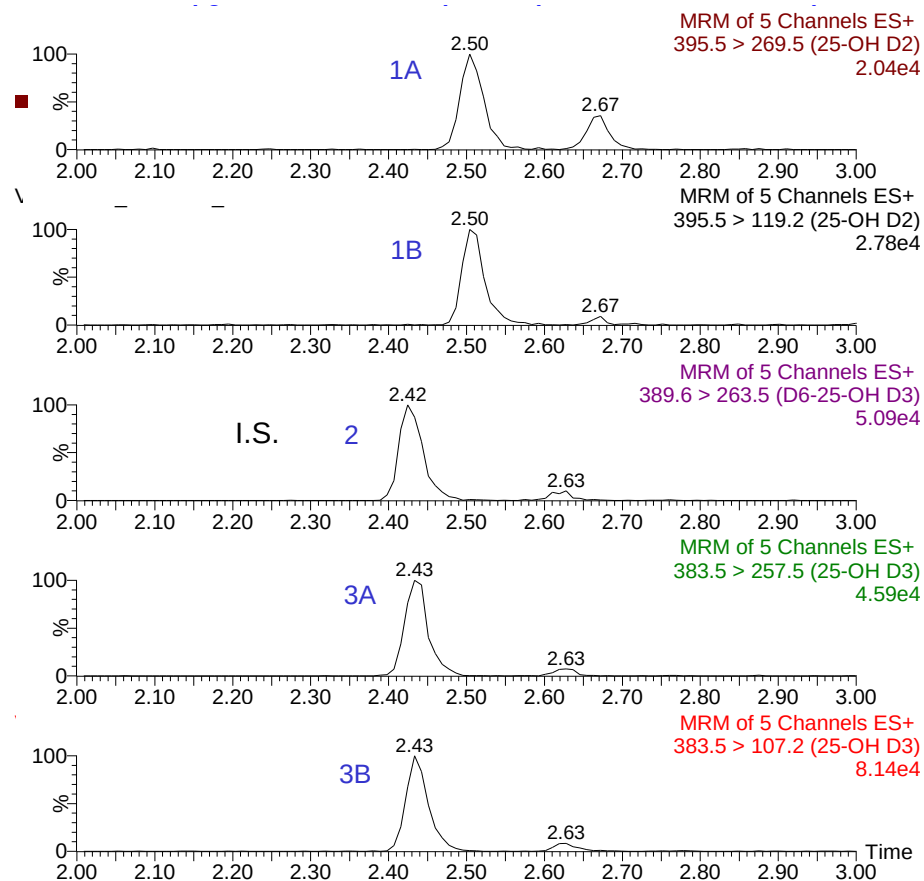
Bendroflumethazide



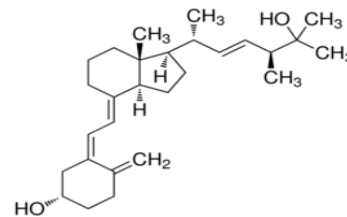
Spironolactone



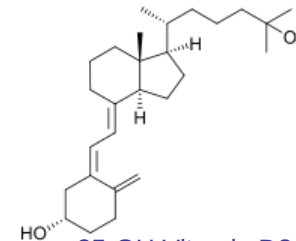
25-Hydroxy Vitamin D by LC-MS/MS



Chromatographic separation of 25-hydroxy vitamin D2 and D3 from spiked serum



25-OH Vitamin D2



25-OH Vitamin D3

Peak	Analyte	Q1 (Da)	Q3 (Da)
1A	25-OH D2	395.5	269.5
1B	25-OH D2	395.5	119.2
2	d6-25-OH D3	389.6	263.5
3A	25-OH D3	383.5	257.5
3B	25-OH D3	383.5	107.2

ACE Excel 2 C18-PFP 2μm, 100 x 2.1mm

Gradient analysis

A: 2mM NH₄OAc, 0.1% formic acid in water

B: 2mM NH₄OAc, 0.1% formic acid in MeOH

Time (mins) %B Curve

0 75 1

3.0 100 6

4.0 75 11

Flow rate: 0.4ml/min

Column temperature: 40°C

Injection volume: 15μl

Sample: calibrated serum containing 14.8 and 19.6 ng/ml 25-OH-D2 and 25-OH-D3

Quattro premier XE triple quad MS

MRM positive mode ESI

Desolvation temperature: 450°C

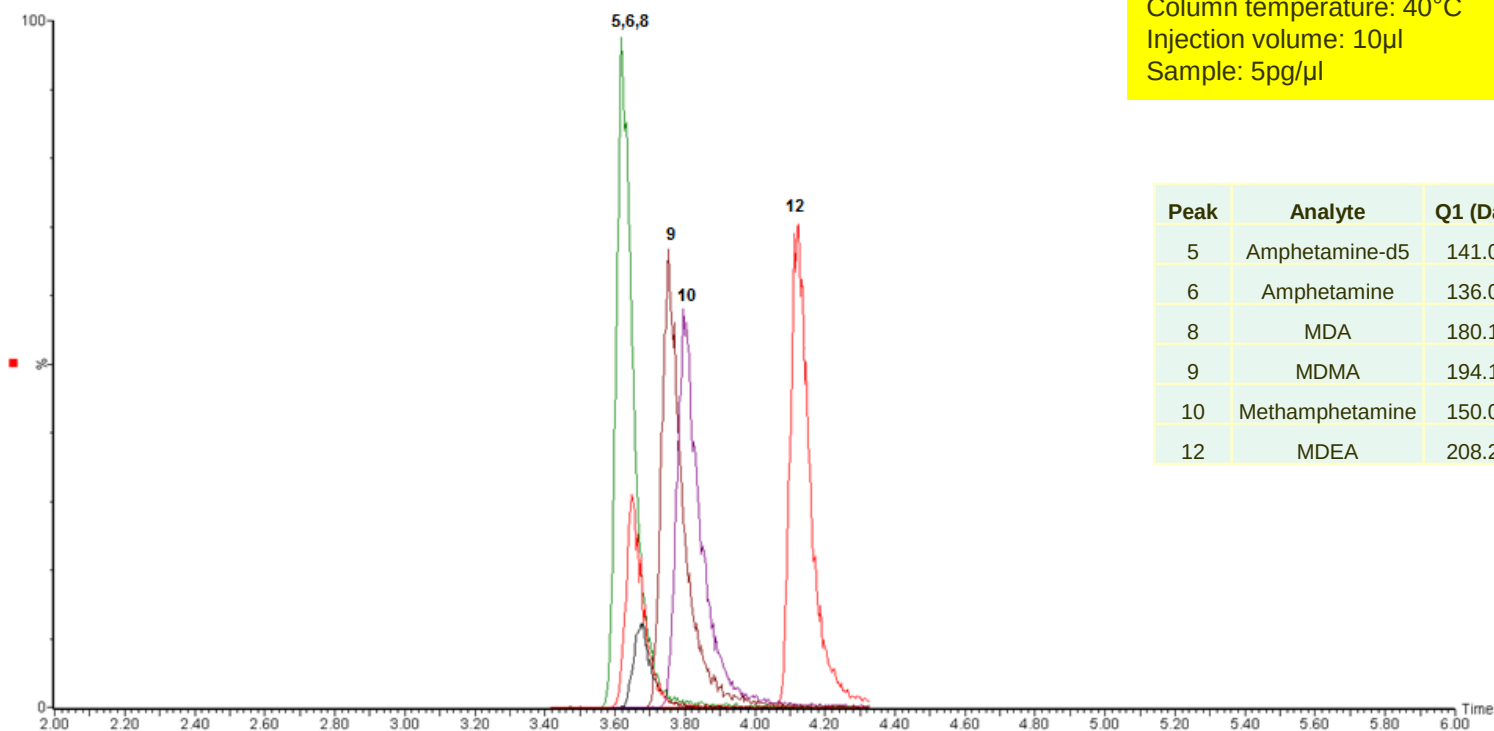
Ion source temperature: 150°C



Amphetamines from Drugs of Abuse Screen

MS Quattro Premier XE triple quad
MRM, positive and negative mode ESI
Desolvation temperature: 450°C
Ion source temperature: 150°C
Collision gas pressure: 3.5×10^{-3} mbar

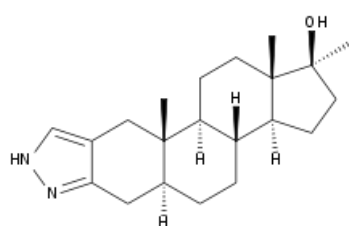
ACE Excel 1.7 C18 1.7 μ m, 100 x 2.1mm
Gradient analysis
A: 5mM NH₄OAc in water
B: 5mM NH₄OAc in MeOH
Time (mins) %B Time (mins) %B
0 10 13.4 10
10 90 15.5 10
11.9 90
Flow rate: 0.3ml/min
Column temperature: 40°C
Injection volume: 10 μ l
Sample: 5pg/ μ l



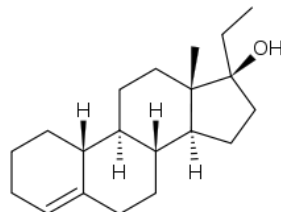
Peak	Analyte	Q1 (Da)	Q3 (Da)
5	Amphetamine-d5	141.0	123.9
6	Amphetamine	136.0	118.9
8	MDA	180.1	105.0
9	MDMA	194.1	163.0
10	Methamphetamine	150.0	90.9
12	MDEA	208.2	163.0



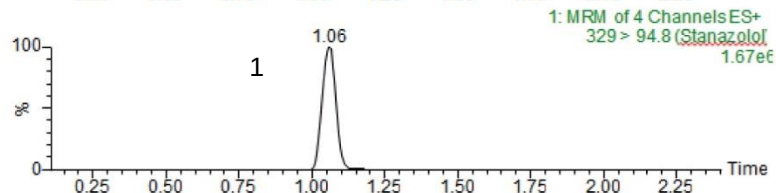
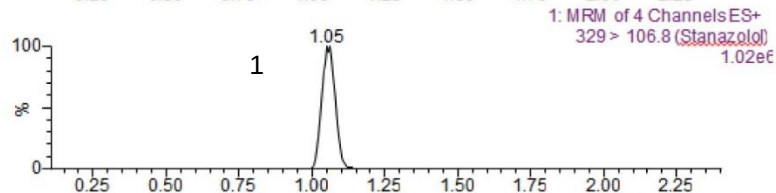
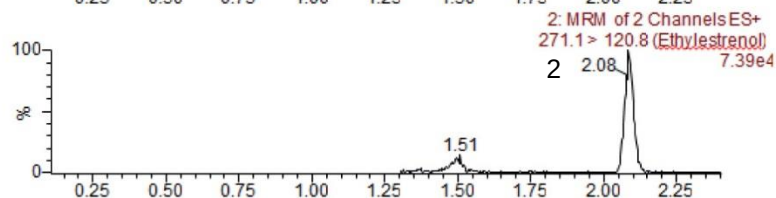
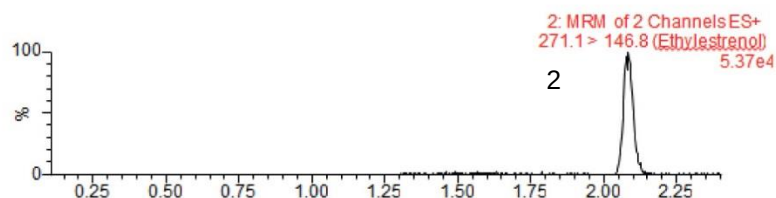
Anabolic Steroids from Horse Urine by LC-MS/MS



Stanozolol



Ethylestrenol



Sample preparation using Biotage ISOLUTE SLE+ protocol

ACE Excel 2 C18 2μm, 50 x 2.1mm

Gradient analysis

A: 2mM NH₄OAc, 0.1% formic acid (aq)

B: 2mM NH₄OAc, 0.1% formic acid in MeOH

T (mins) %B Curve T (mins) %B Curve

0 75 0 3.50 100 6

0.25 75 6 3.51 75 6

1.50 90 6 4.0 75 1

1.51 100 6

Flow rate: 0.4ml/min

Column temperature: 40°C

Injection volume: 10μl

Premier XE triple quad MS

MRM positive ion mode

Desolvation temperature: 450°C

Ion source temperature: 120°C

Peak	Analyte	MRM Transition	Cone Voltage (V)	Collision Energy (eV)
1	Stanozolol	329.0 > 94.8	50	40
	Stanozolol	329.0 > 106.8	50	40
2	Ethylestrenol	271.1 > 120.8	35	15
	Ethylestrenol	271.1 > 146.8	35	15



Benzodiazepines from Drugs of Abuse Screen

ACE Excel 1.7 C18 1.7 μ m, 100 x 2.1mm
Gradient analysis

A: 5mM NH₄OAc in water

B: 5mM NH₄OAc in MeOH

Time (mins)	%B	Time (mins)	%B
0	10	13.4	10
10	90	15.5	10
11.9	90		

Flow rate: 0.3ml/min

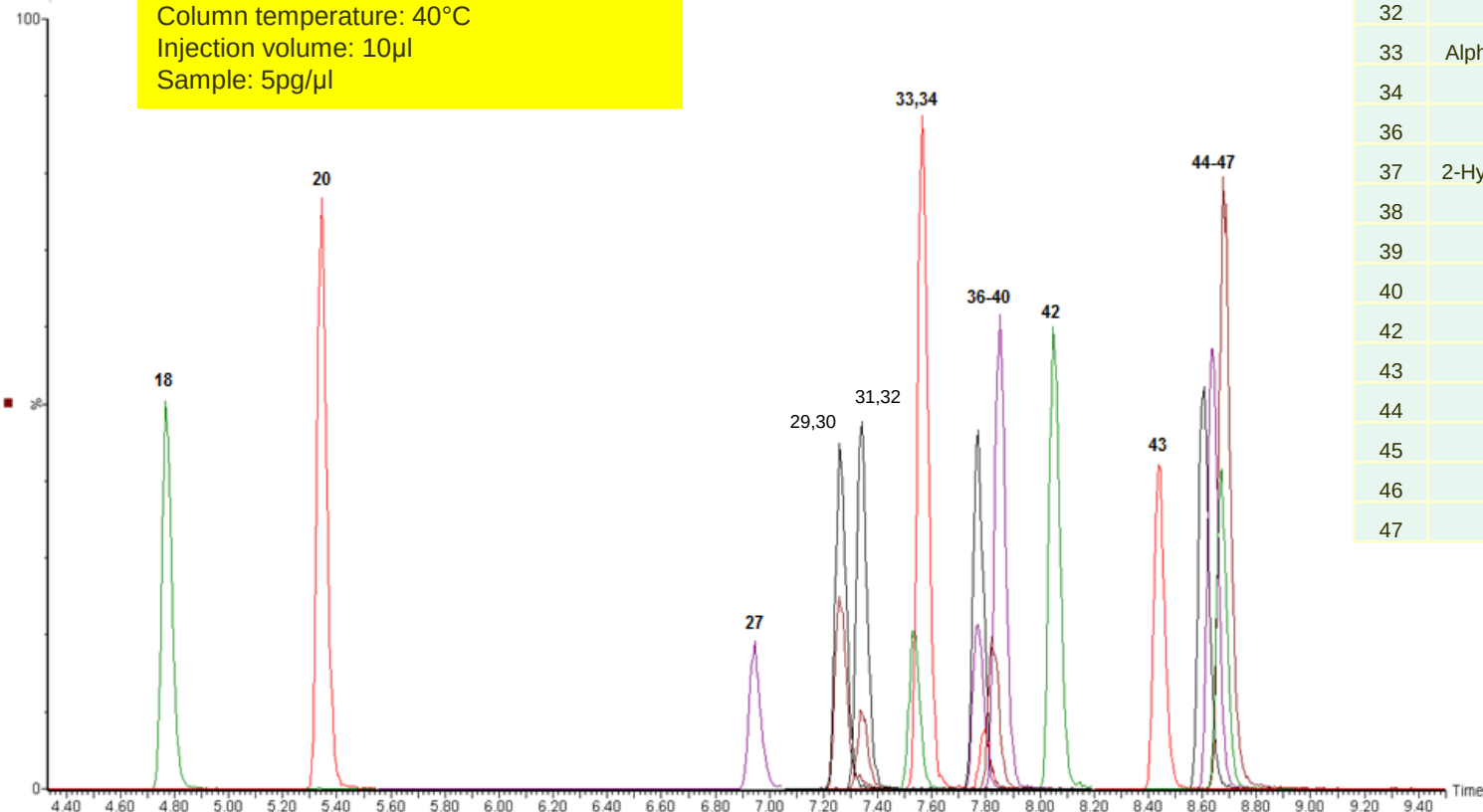
Column temperature: 40°C

Injection volume: 10 μ l

Sample: 5pg/ μ l

MS Quattro Premier XE triple quad
MRM, positive and negative mode ESI
Desolvation temperature: 450°C
Ion source temperature: 150°C
Collision gas pressure: 3.5 x 10⁻³ mbar

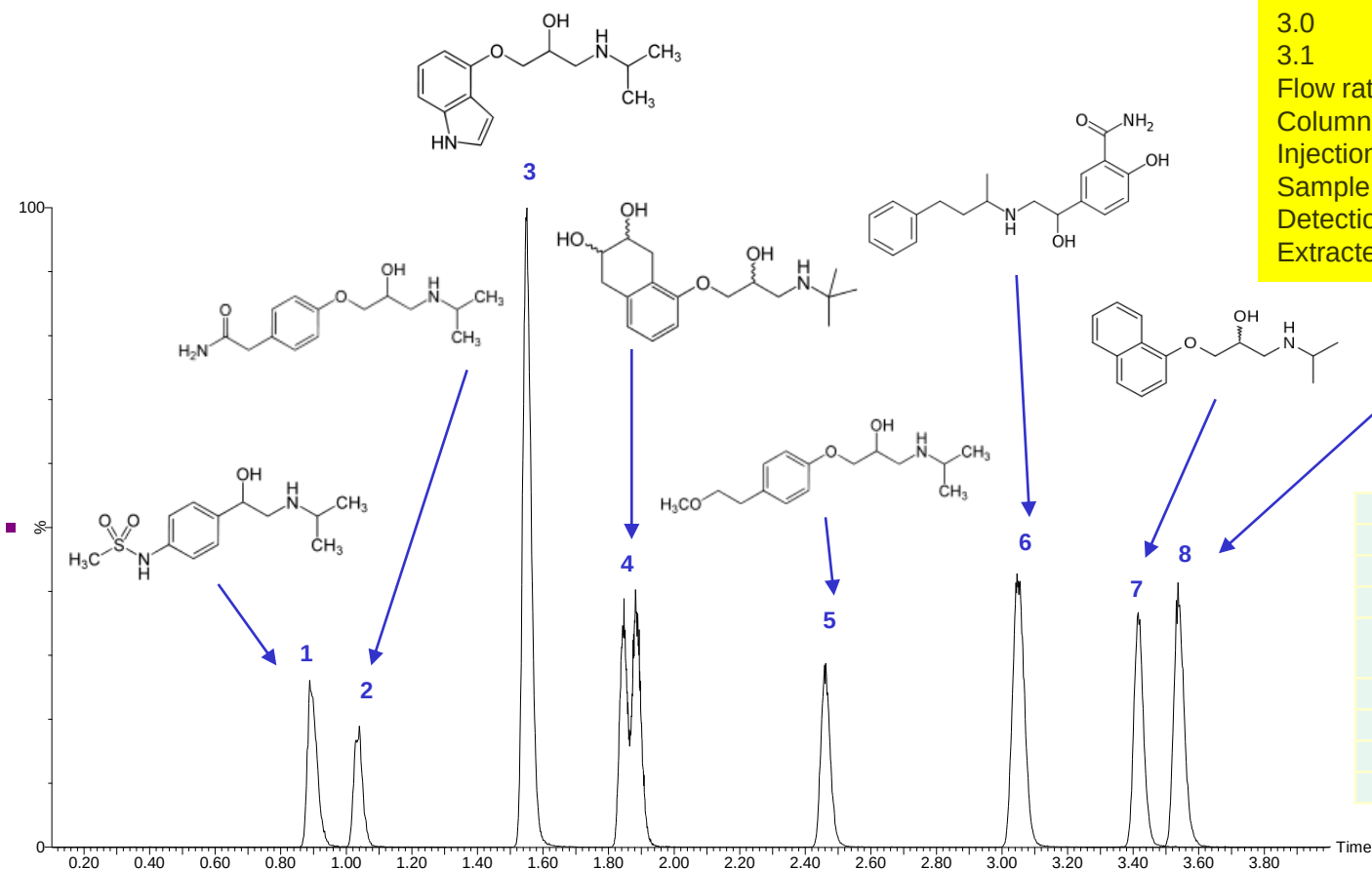
Peak	Analyte	Q1 (Da)	Q3 (Da)
18	7-Amino-clonazepam	286.2	121.0
20	7-Amino-flunitrazepam	284.2	135.0
27	Bromazepam	316.1	182.1
29	Clonazepam	316.1	270.1
30	Nitrazepam	282.2	236.1
31	Alpha-hydroxytriazolam	359.1	331.1
32	Flunitrazepam	314.2	268.2
33	Alpha-hydroxyalprazolam	325.2	297.1
34	Estazolam	295.2	267.2
36	Triazolam	343.0	308.1
37	2-Hydroxyethylflurazepam	333.2	109.0
38	Lorazepam	321.1	275.1
39	Oxazepam	287.2	241.0
40	Alprazolam	309.2	281.2
42	Temazepam	301.1	255.1
43	Nordiazepam	271.1	139.9
44	Midazolam	326.2	291.2
45	Diazepam-d5	290.2	154.0
46	Diazepam	285.2	154.0
47	Flurazepam	388.2	315.1





Beta-Blockers by LC-MS/MS

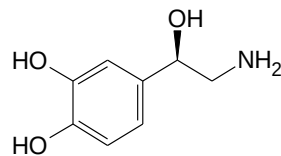
ACE Excel 2 C18 2 μ m, 50 x 2.1mm
 Gradient analysis
 A: 2mM NH₄OAc, 0.1% formic acid in water
 B: 2mM NH₄OAc, 0.1% formic acid in MeOH
 Time (mins) %B Curve
 0 10 0
 3.0 50 6
 3.1 10 6
 Flow rate: 0.4ml/min
 Column temperature: 40°C
 Injection volume: 10 μ l
 Sample: 2.5pg/ μ l
 Detection: MS/MS, positive mode ESI
 Extracted ion chromatogram



Peak	Analyte	Q1 (Da)	Q3 (Da)
1	Sotalolol	272.9	212.8
2	Atenolol	267.0	189.8
3	Pindolol	248.9	115.8
4	Nadolol diastereomers	310.0	253.9
5	Metoprolol	268.0	115.8
6	Labetalol	329.1	161.8
7	Propanolol	260.0	115.7
8	Alprenolol	250.0	115.8

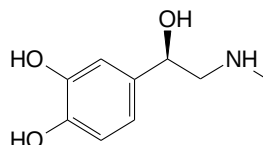


ACE Excel 2 C18-PFP: Catecholamines by LC-MS/MS



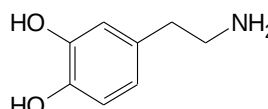
Norepinephrine

1



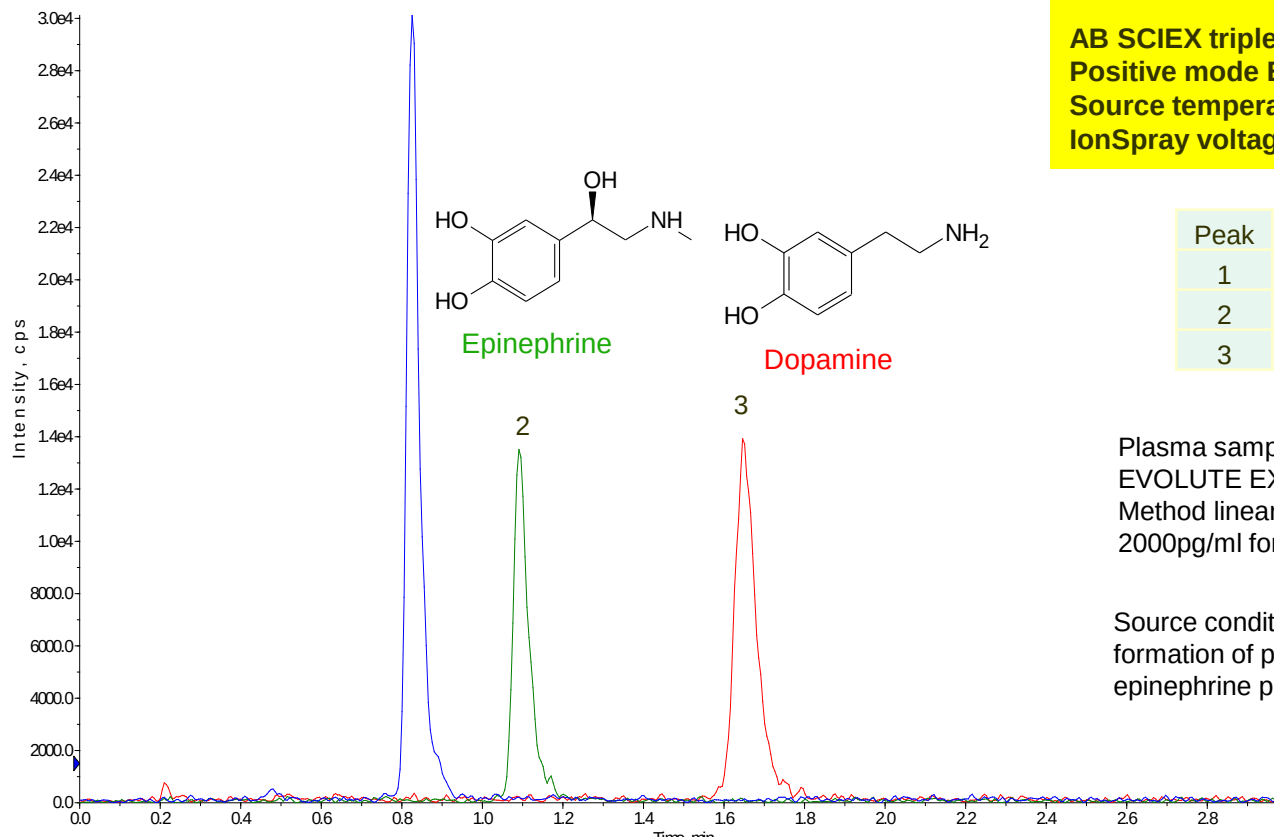
Epinephrine

2



Dopamine

3



ACE Excel 2 C18-PFP 2µm, 100 x 2.1mm

Isocratic analysis

Mobile phase: 2mM formate buffer, pH 3.2/MeOH (98:2)

Flow rate: 0.4ml/min

Column temperature: 40°C

Injection volume: 10µl

AB SCIEX triple quad 5500

Positive mode ESI

Source temperature: 700°C

IonSpray voltage: 5500V

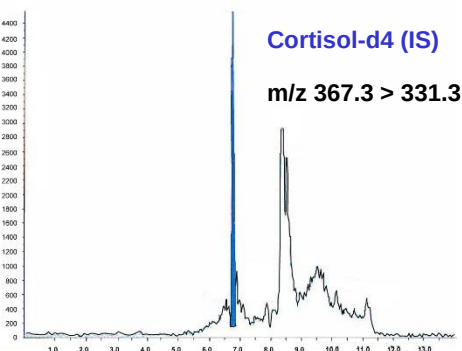
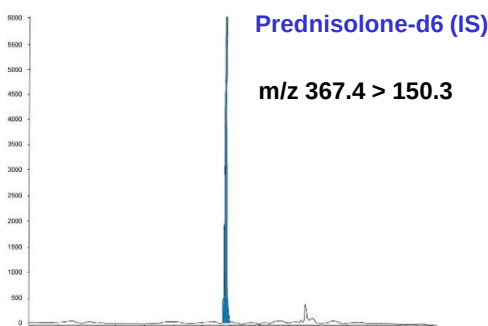
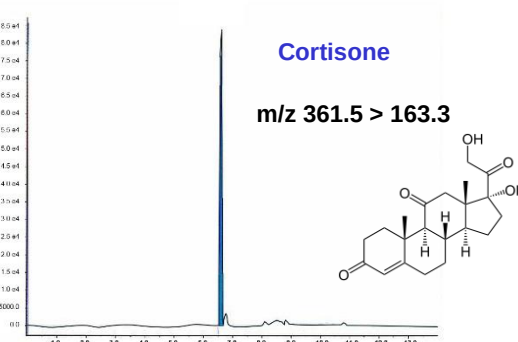
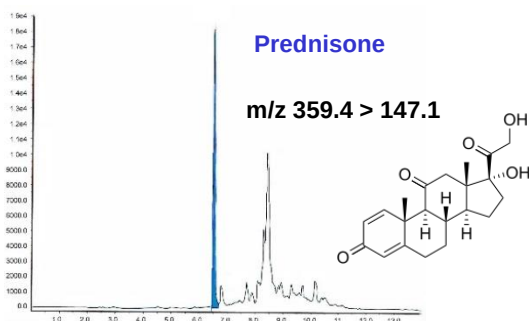
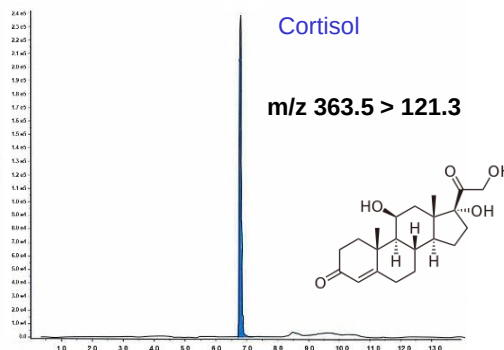
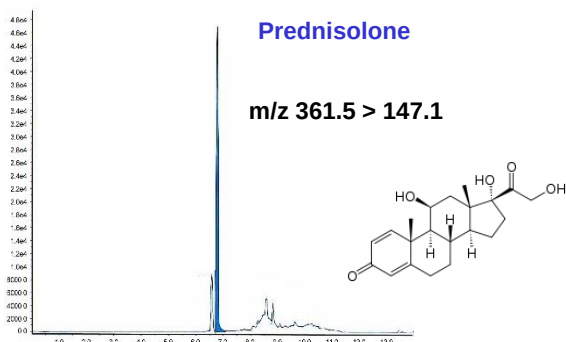
Peak	Analyte	Q1 (Da)	Q3 (Da)
1	Norepinephrine	152.1	107.1
2	Epinephrine	166.1	107.1
3	Dopamine	154.1	91.1

Plasma samples were extracted using Biotage EVOLUTE EXPRESS WCX methodology.

Method linear over concentration range 100 to 2000pg/ml for spiked human plasma

Source conditions were optimised to promote the formation of protonated, dehydrated norepinephrine and epinephrine precursors to enhance sensitivity

Serum Prednisolone, Prednisone, Cortisol and Cortisone by LC-MS/MS



Sample - Methanolic stock standard solutions spiked into steroid depleted serum to produce QC sample containing prednisolone (250nmol/l), cortisol (250nmol/l), prednisone (50nmol/l) and cortisone (50nmol/l)

Sample clean up – Diethyl ether extraction

ACE Excel C18 2 μ m, 100 x 2.1mm
Gradient analysis

A: 4mM NH₄OAc (aq)

B: 0.2% (v/v) HCO₂H in MeOH

Time (mins)	%B
0	30
0.25	30
3.9	70
6.0	70
6.01	95
7.0	95
7.01	100

Flow rate: 0.2ml/min

Column temperature: 50°C

Injection volume: 50 μ l

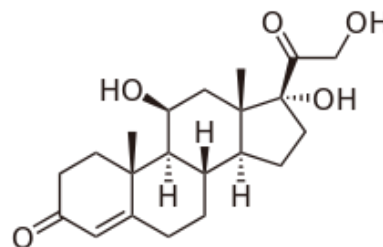
Applied Biosystems 5000 MS/MS
APCI in positive ion mode



Cortisol in Urine by LC-MS/MS

Sample clean up – zinc sulphate precipitation and online extraction

Sample – BioRad Liquichek Urine Quality Control standard (16nmol/l cortisol)

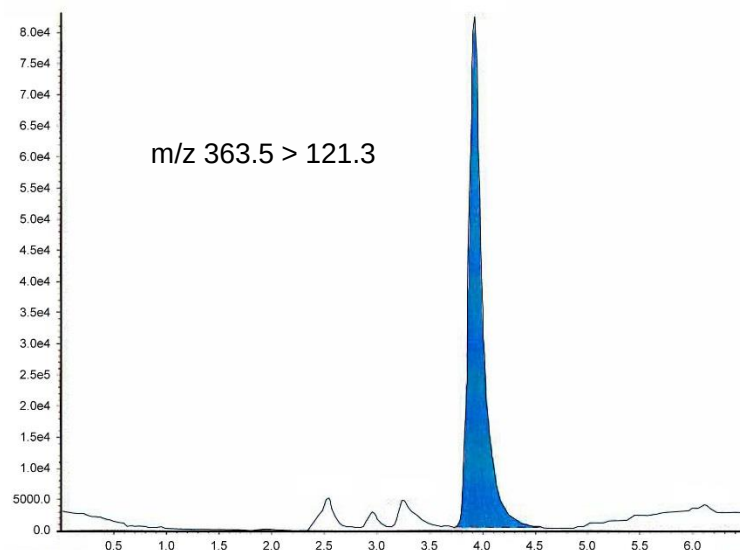


Cortisol

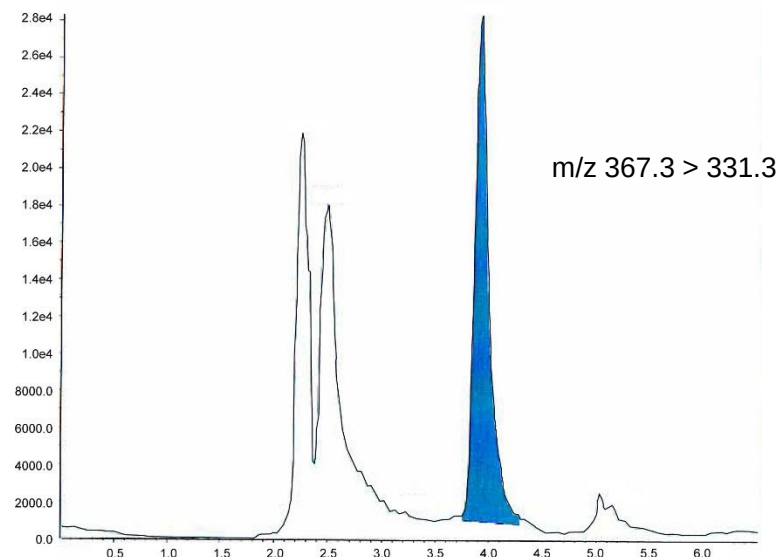
ACE Excel C18 2µm, 100 x 2.1mm
 A: 4mM ammonium acetate (aq)
 B: 0.2% (v/v) formic acid in methanol
 A/B = 71.5: 28.5
 Flow rate: 0.7ml/min
 Column temperature: 50°C
 Injection volume: 50µl

Applied Biosystems 5000 MS/MS
 APCI in positive ion mode

Cortisol



Cortisol-d4 (IS)





Metabolite Profiling of Human Urine by LC-MS

3D plot from UHPLC with high resolution accurate mass MS

Sample:

Urine of healthy adult volunteer, filtered prior to injection onto column and modular Accela LC system

ACE Excel C18-Amide 1.7 μ m, 100 x 2.1mm
Gradient analysis

A: 0.01% formic acid in water

B: 0.01% formic acid in MeCN

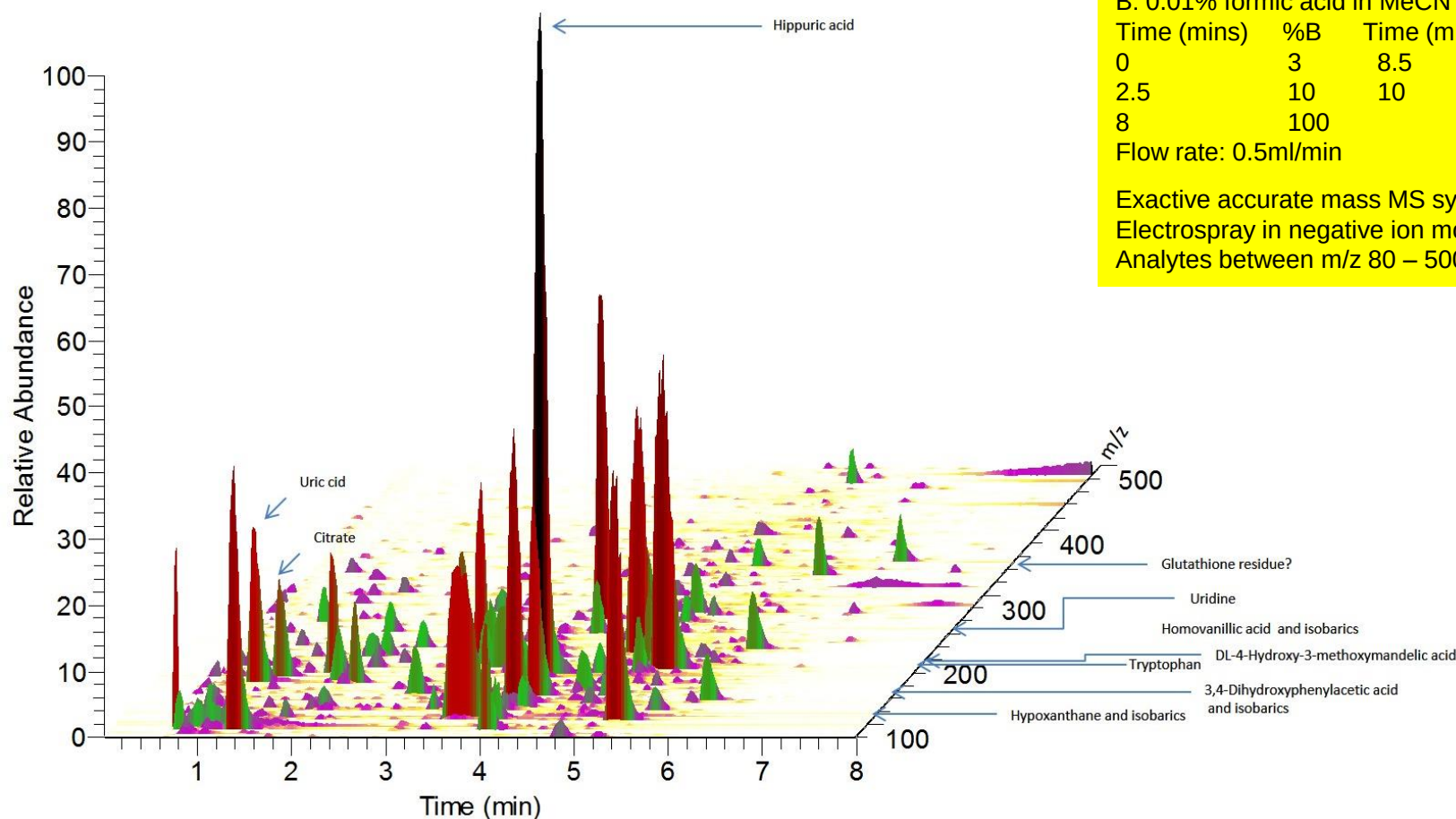
Time (mins)	%B	Time (mins)	%B
0	3	8.5	3
2.5	10	10	3
8	100		

Flow rate: 0.5ml/min

Exact accurate mass MS system

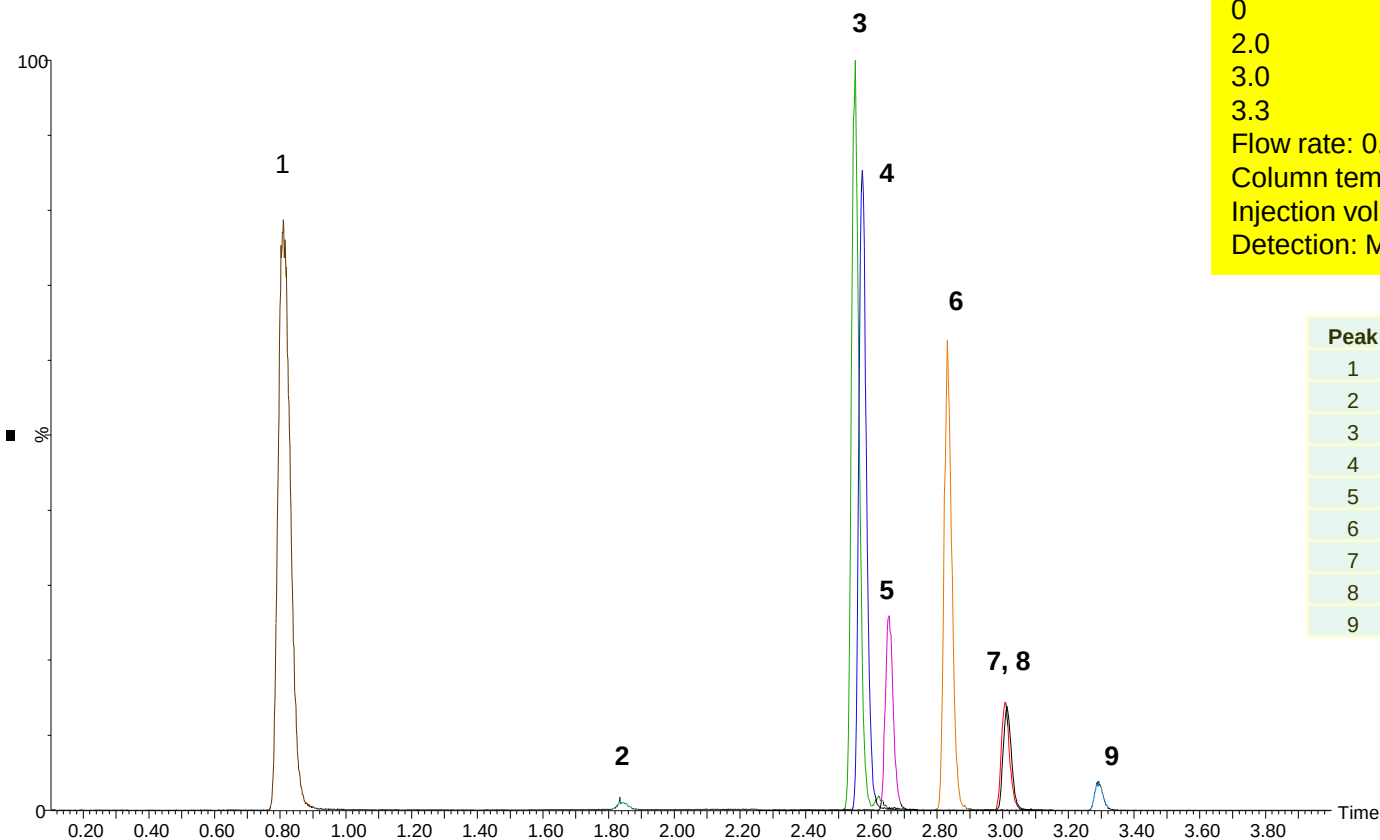
Electrospray in negative ion mode

Analytes between m/z 80 – 500 monitored





Non-Steroidal Anti-Inflammatory Drugs by LC-MS/MS



ACE Excel 2 C18 2 μ m, 50 x 2.1mm

Gradient analysis

A: 2mM NH₄OAc, 0.1% formic acid in water

B: 2mM NH₄OAc, 0.1% formic acid in MeOH

Time (mins) %B Curve

0 15 0

2.0 70 6

3.0 90 6

3.3 15 11

Flow rate: 0.4ml/min

Column temperature: 40°C

Injection volume: 10 μ l

Sample: 10pg/ μ l

Detection: MS/MS, positive mode ESI

Peak	Analyte	Q1 (Da)	Q3 (Da)
1	Acetaminophen	151.7	109.7
2	Salicylic acid	136.7	92.7
3	Sulindac	357	233.1
4	Ketoprofen	255	209
5	Naproxen	231	184.9
6	Phenylbutazone	309.1	119.8
7	Indomethacin	357.9	138.7
8	Diclofenac	295.8	213.9
9	Mefenamic acid	242	208.8



Opiates from Drugs of Abuse Screen

MS Quattro Premier XE triple quad
MRM, positive and negative mode ESI
Desolvation temperature: 450°C
Ion source temperature: 150°C
Collision gas pressure: 3.5×10^{-3} mbar

ACE Excel 1.7 C18 1.7µm, 100 x 2.1mm
Gradient analysis

A: 5mM NH₄OAc in water

B: 5mM NH₄OAc in MeOH

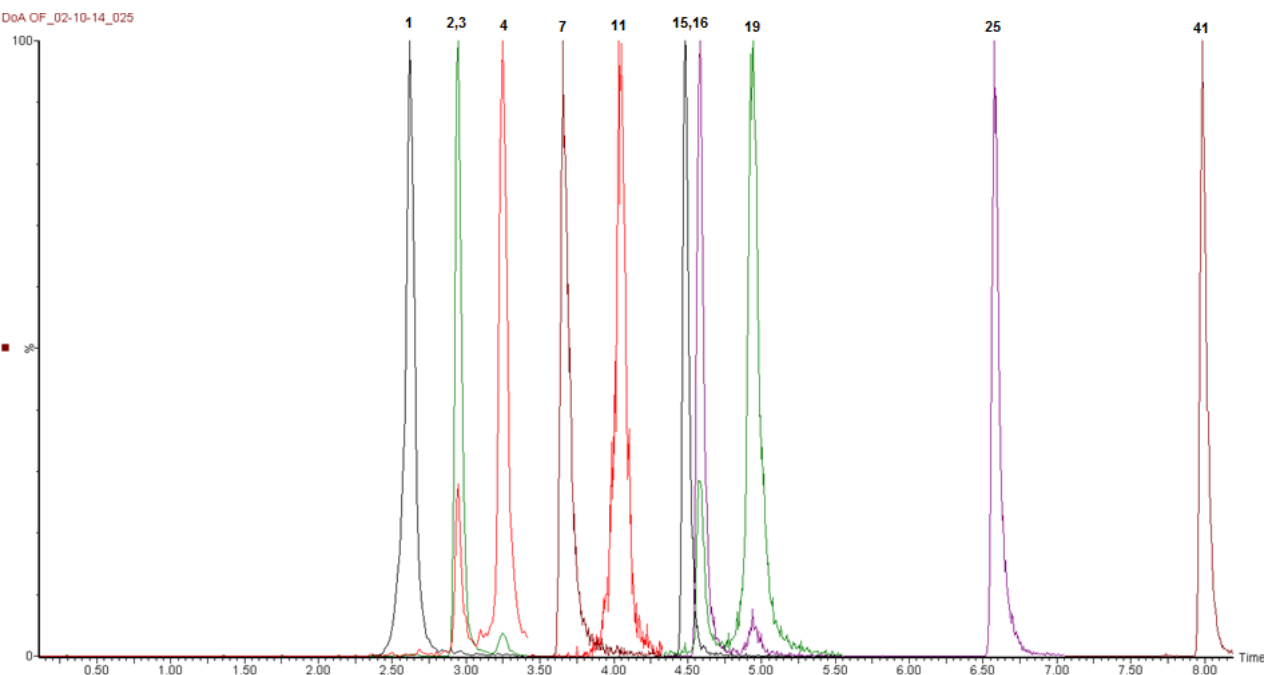
Time (mins)	%B	Time (mins)	%B
0	10	13.4	10
10	90	15.5	10
11.9	90		

Flow rate: 0.3ml/min

Column temperature: 40°C

Injection volume: 10µl

Sample: 5pg/µl



Peak	Analyte	Q1 (Da)	Q3 (Da)
1	Oxymorphone	302.2	198.1
2	Morphine-d3	289.2	201.0
3	Morphine	286.2	201.0
4	Hydromorphone	286.2	185.1
7	Dihydrocodeine	302.2	199.1
11	Oxycodone	316.2	241.2
15	6-MAM	328.2	165.1
16	Codeine	300.3	215.1
19	Hydrocodone	300.2	199.1
25	EDDP	278.2	234.2
41	Methadone	310.2	265.2



ACE Excel 2 C18-AR – Thyroid Hormones by LC-MS/MS

TIC

T3

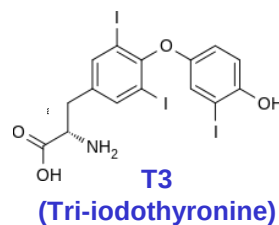
1.98

rT3

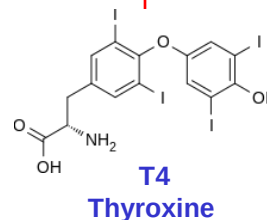
2.48

T4

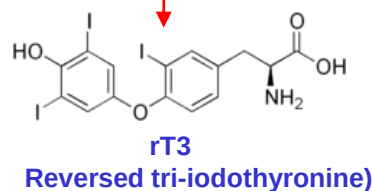
2.71



Enzymatic
De-iodination



Isobaric Minor
Metabolite



ACE Excel 2 C18-AR 2µm, 100 x 2.1mm

Gradient analysis

A: 2mM NH₄OAc, 0.1% formic acid in water

B: 2mM NH₄OAc, 0.1% formic acid in MeOH

Time (mins)	%B	Curve
0	60	1
3	77	6
3.1	60	1

Flow rate: 0.4ml/min

Column temperature: 40°C

Injection volume: 10µl

Sample: 2.5pg/µl

XEVO TQS triple quadrupole MS

Desolvation temperature: 500°C

Ion source temperature: 150°C

Positive mode ESI, MRM

Low level thyroid hormones quantified by LC-MS/MS using MRM, following extraction of serum samples using Biotage EVOLUTE EXPRESS AX methodology.

Method linear over concentration range of 25 – 1000pg/ml

Analyte	Q1 (Da)	Q3 (Da)	Analyte	Q1 (Da)	Q3 (Da)
T3	651.8	605.8	T3/rT3-d6 I.S.	657.8	611.8
	(651.8)	(507.8)	T4	777.7	731.7
	(651.8)	(478.9)		(777.7)	(351)
rT3	651.8	605.8		(777.7)	(633.8)
	(651.8)	(507.8)			
	(651.8)	(478.9)			