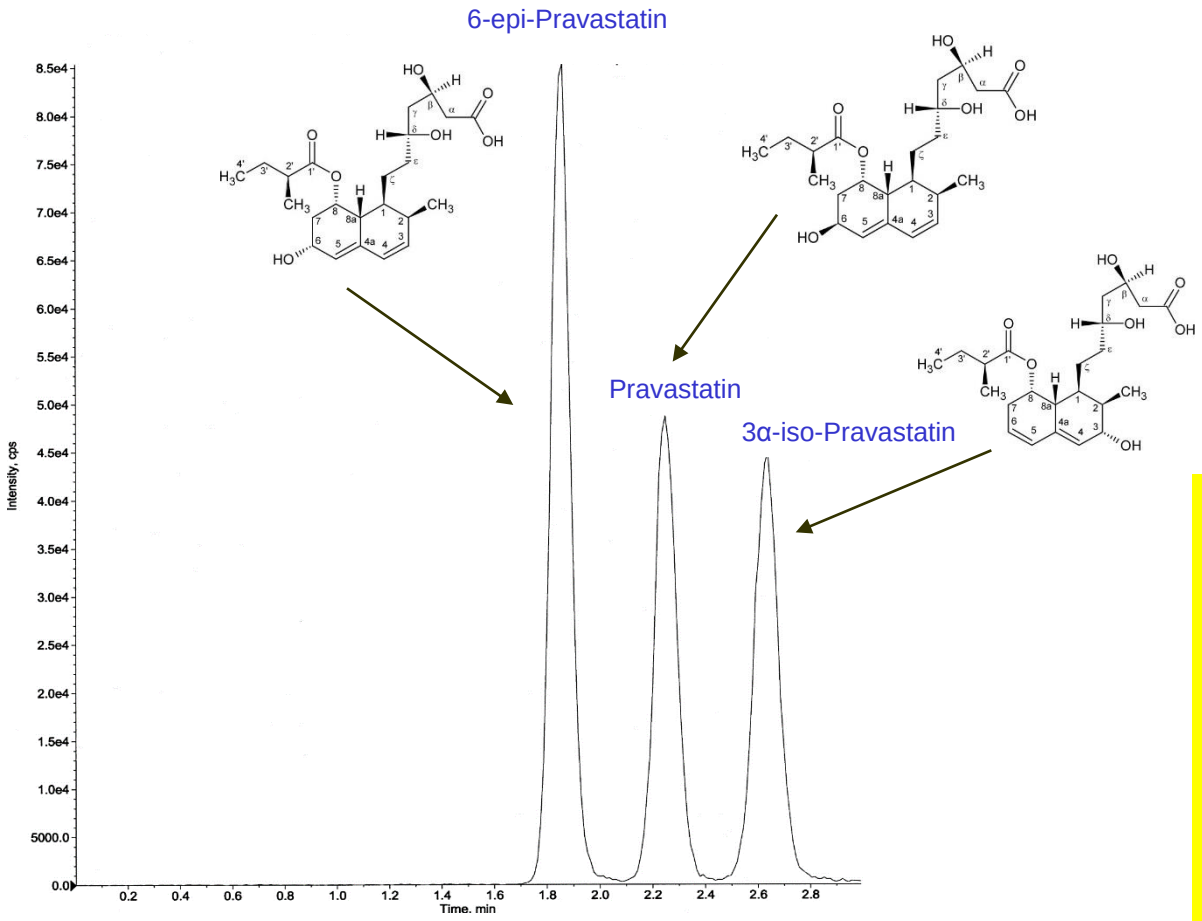
Pravastatin and Isomers by LC-MS/MS



All 3 compounds have MW 424

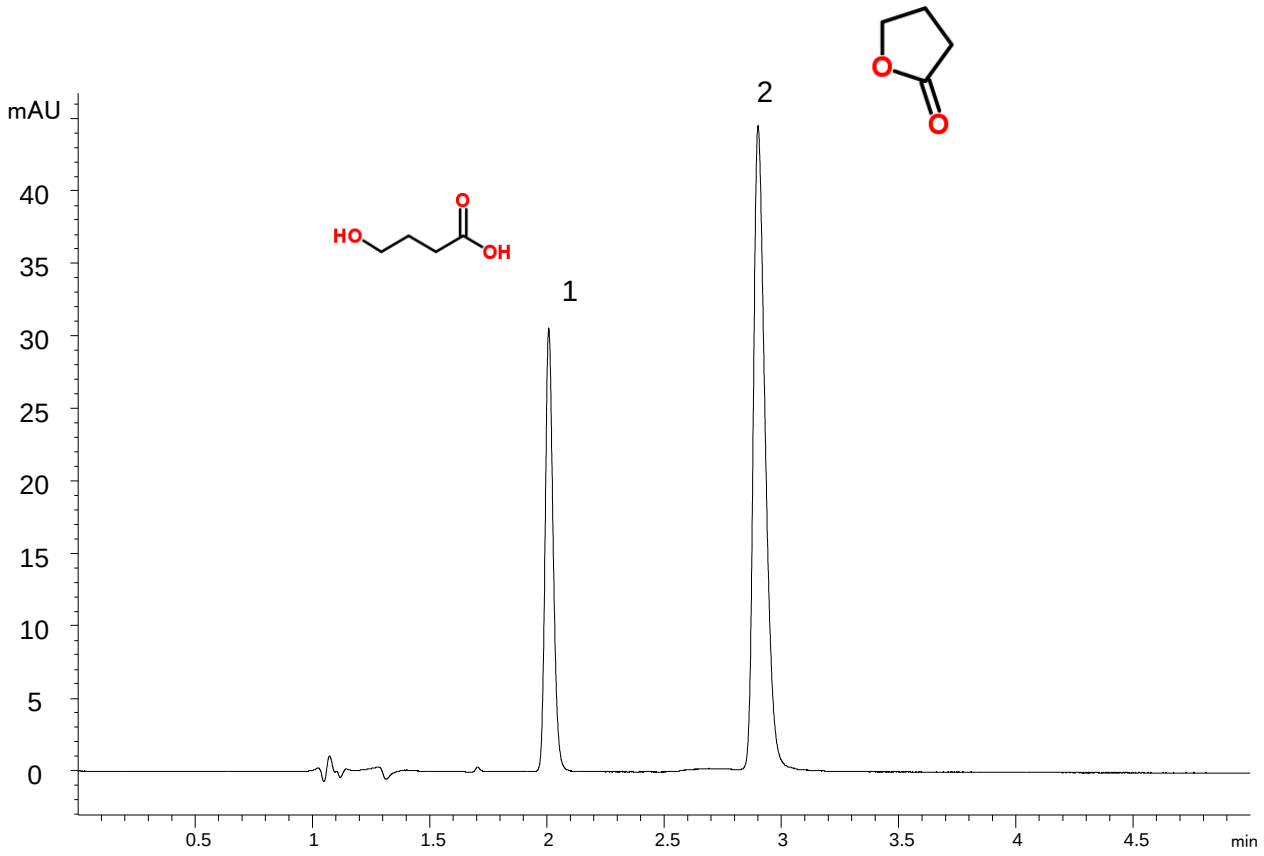
MS/MS conditions alone insufficient for selective quantitation

Baseline separation important

ACE C18 3 µm, 50 x 3.0 mm
Isocratic analysis
Acetonitrile-Methanol-THF-Water-Acetic acid (15:20:5:60:0.1)
Flow rate: 0.6 ml/min
Column temperature: Ambient
Injection volume: 2 µl
Sample: 1 µg/ml each isomer

API 3000 triple quad MS
TurboIonSpray – negative mode
Extracted ion chromatogram of MRM m/z 423.3 → 321.1

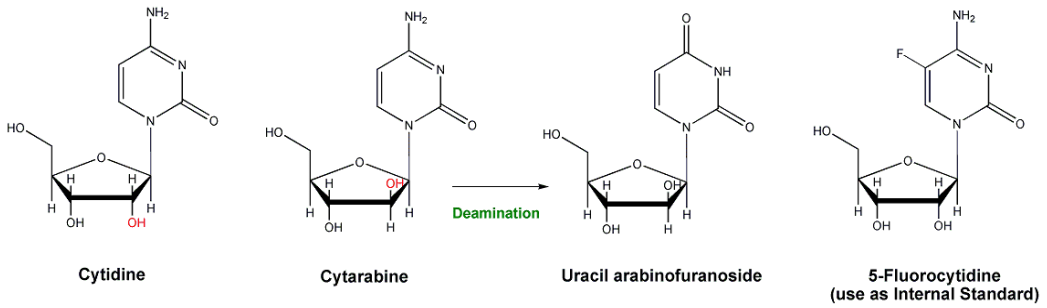
ACE C18-AR: Separation GHB and GBL



ACE Excel 2 C18-AR
5µm, 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

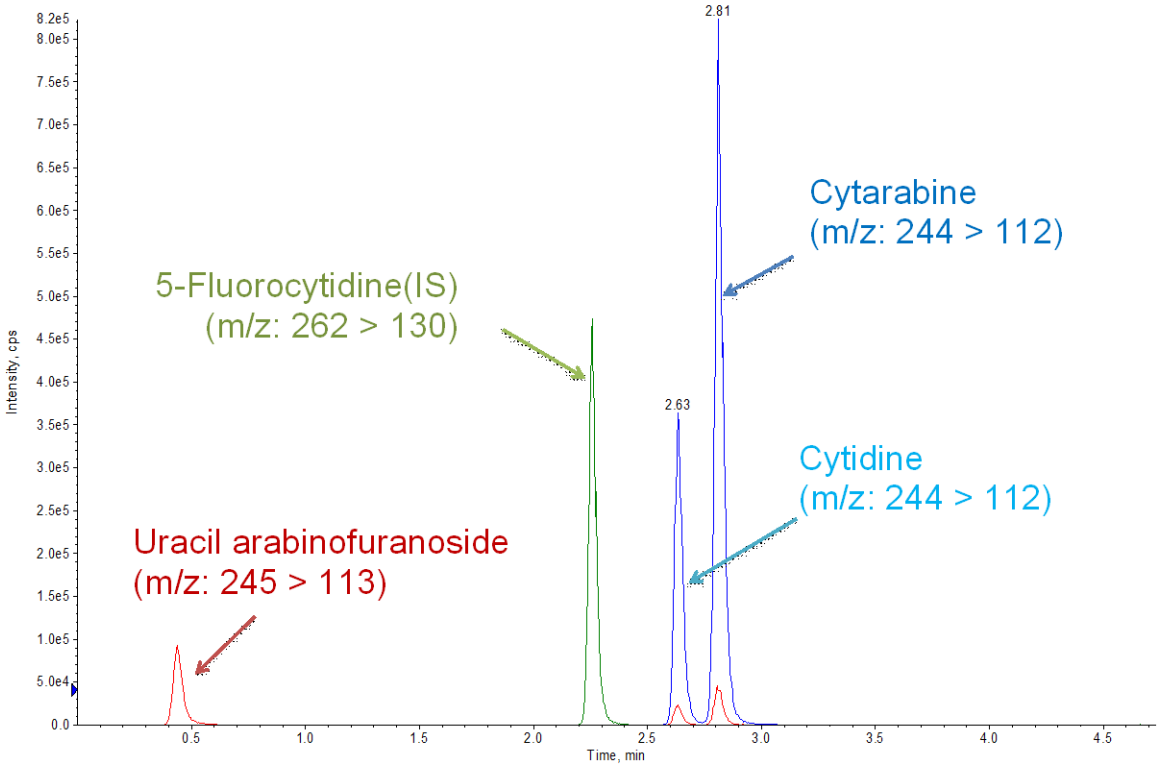
Cytarabine Analogues by Ion-Pairing LC-MS/MS



Cytarabine and cytidine are isobaric.

Robust method with good separation achieved.

LLOQ = 1 ng/ml human plasma



ACE 3 C18 3µm, 50 x 2.1 mm
Gradient analysis
A = 0.1% perfluoropentanoic acid + 0.1% formic acid in water
B = 0.1% perfluoropentanoic acid + 0.1% formic acid in acetonitrile

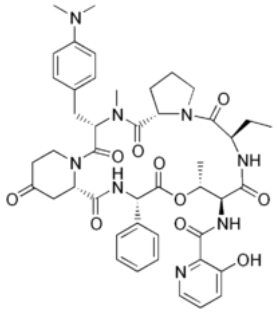
T (mins)	%B
0	0
0.5	0
3.0	13
4.0	90
5.0	0

Flow rate: 0.7 ml/min

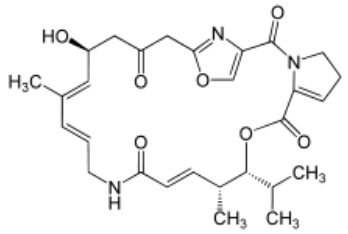
API 4000 MS
TurboIonSpray, positive mode
Source temperature: 550°C

Pristinamycin Components in Plasma by LC-MS/MS

Pristinamycin antibiotic is a mixture of 2 components – pristinamycin IA and IIA
Virginiamycin used as internal standard

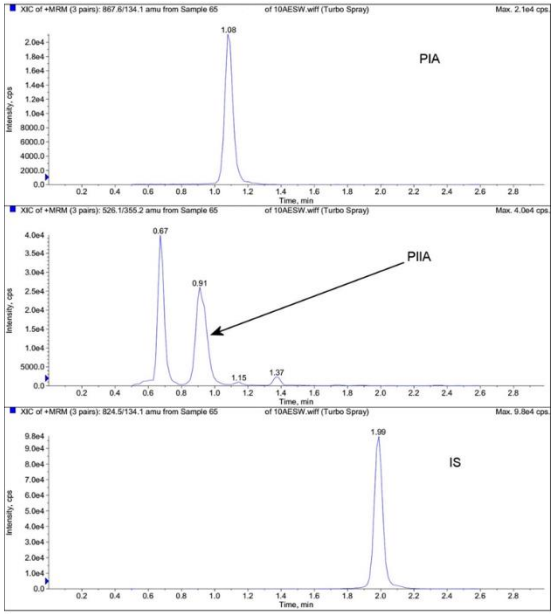


Pristinamycin IA

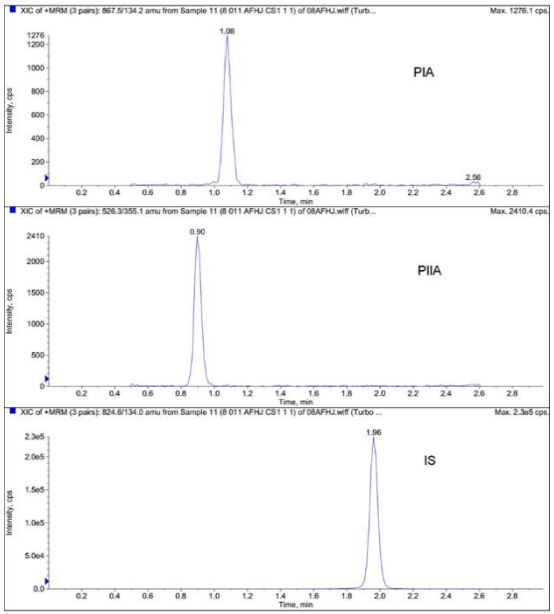


Pristinamycin IIA

Processed study sample containing pristinamycin IA and IIA



Low calibration standard containing 2.5ng/ml each of pristinamycin IA and IIA in human $NaF/K_2C_2O_4$ plasma



ACE 3 C18 3µm, 30 x 3.0mm
Gradient analysis
A = 1mM NH_4CO_2H + 0.1% HCO_2H in 65:35 H_2O:CH_3CN
B = CH_3CN

T (mins)	%B	T (mins)	%B
0	0	1.61	100
0.3	0	2.6	100
0.31	10	2.61	0
1.6	10	4	0

Flow rate: 1ml/min
Column temperature: 25°C
Injection volume: 10µl

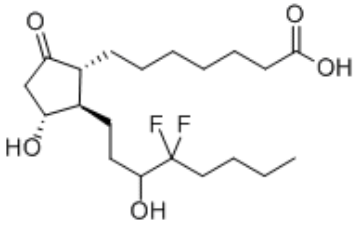
MDS Sciex API 4000
TurboIonSpray positive mode

Transitions monitored:
Pristinamycin IA 867.5 → 134.2
Pristinamycin IIA 526.3 → 355.1
I.S. (Virginiamycin) 824.6 → 134.0

15-Hydroxy Lubiprostone in Human Plasma

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

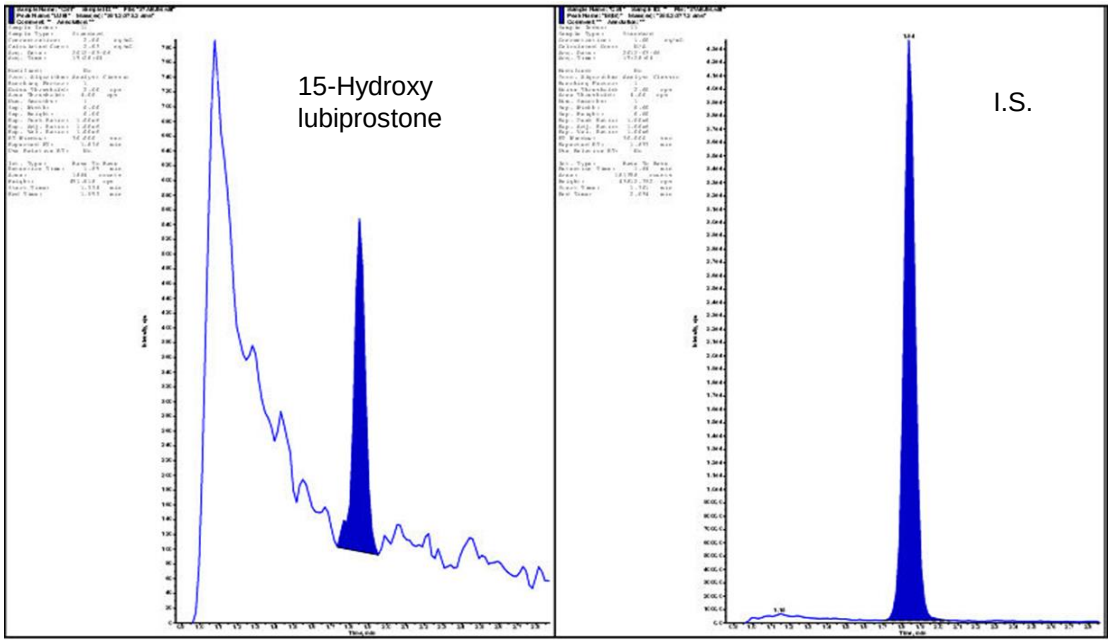
Quantitation is based on 15-hydroxy lubiprostone, with the d4 analogue as internal standard



15-Hydroxy lubiprostone

MW 392.5

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



ACE Excel 2 C18 2µm, 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: 15µl

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

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

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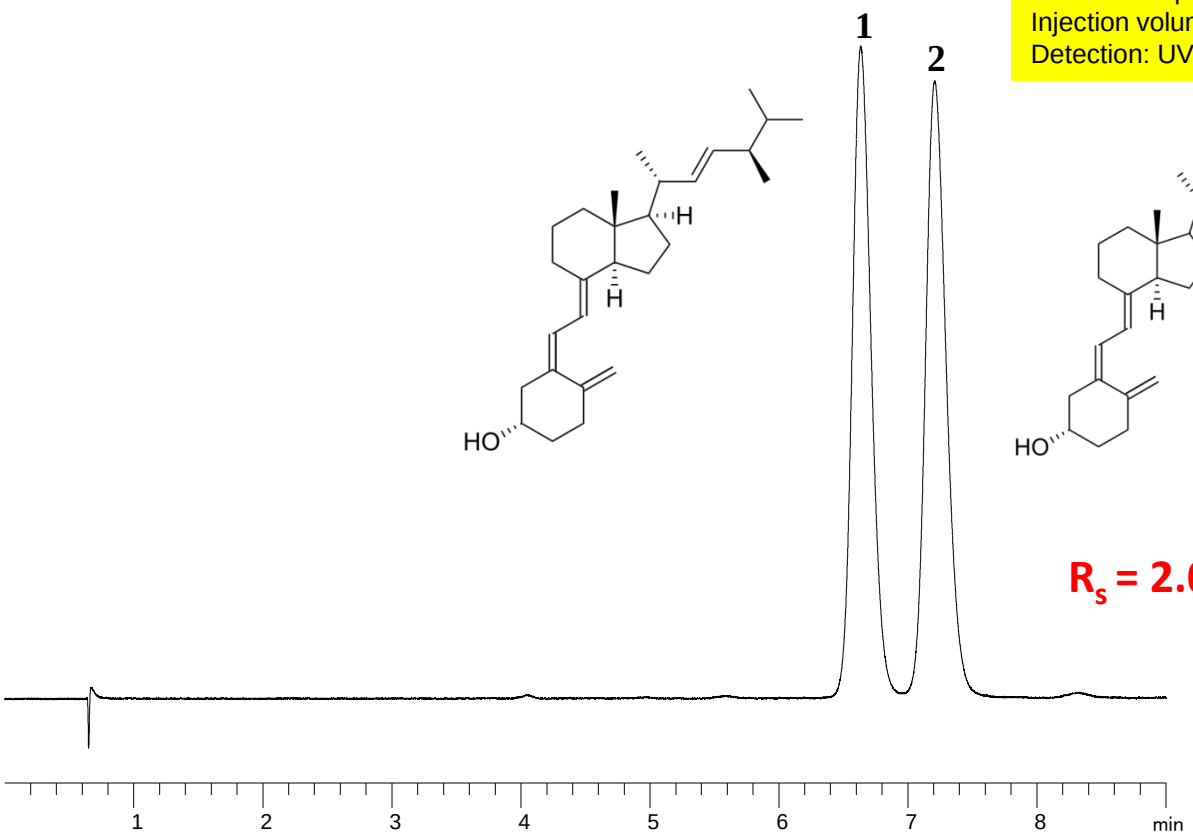
Vitamin D2/D3

Key:
1 Ergocalciferol (D2)
2 Cholecalciferol (D3)

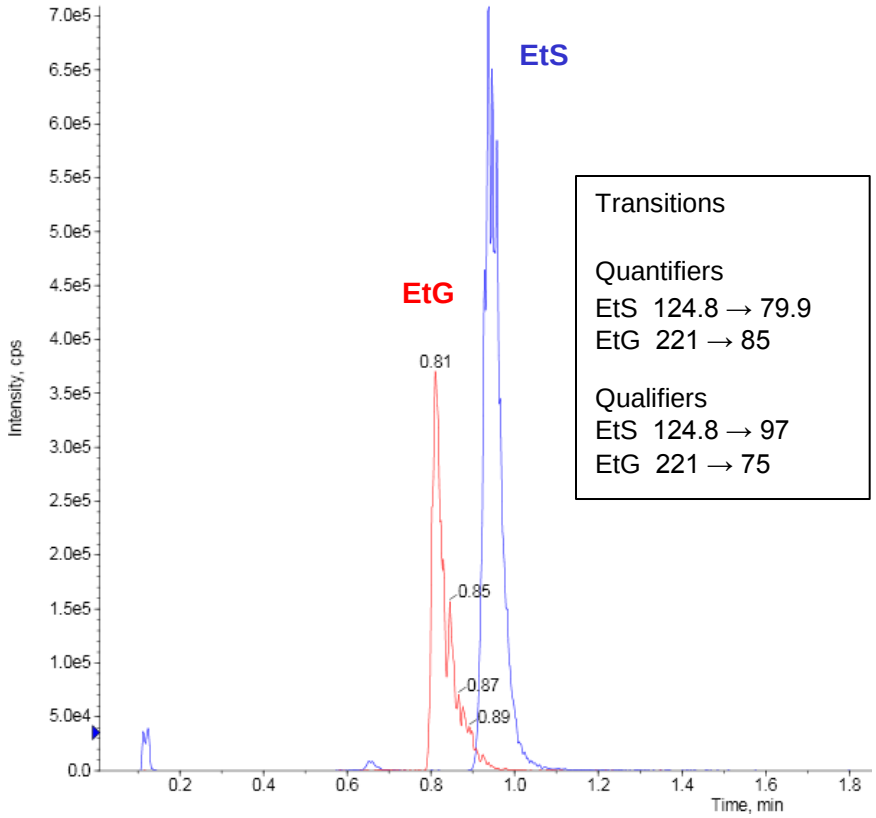
ACE Excel C18-Amide
2µm, 50 x 3.0 mm
Isocratic analysis
100% MeCN
Flow rate: 0.43 ml/min
Column temperature: 20°C
Injection volume: 3 µl
Detection: UV 265 nm



$R_s = 2.06$

Alcohol Biomarkers by UHPLC-MS/MS

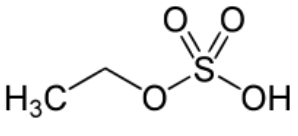
Extracted ion chromatogram



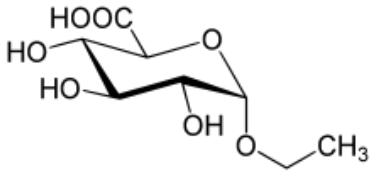
Transitions

Quantifiers
EtS 124.8 → 79.9
EtG 221 → 85

Qualifiers
EtS 124.8 → 97
EtG 221 → 75



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

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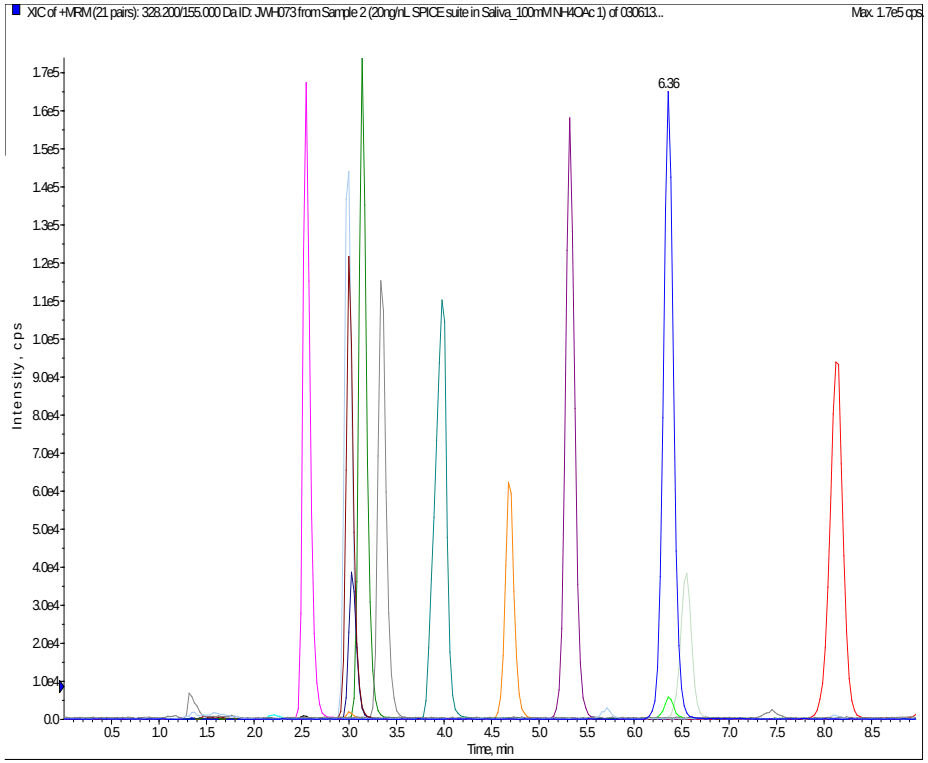
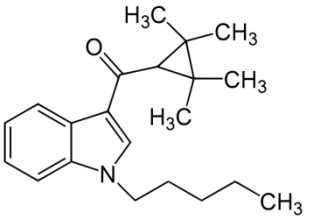
Synthetic Cannabinoids (SPICE) From Oral Fluid

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

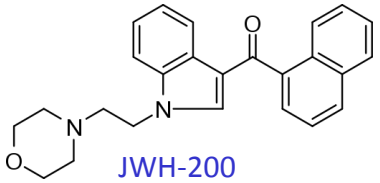


Retention Time (minutes)	Analyte	MRM Transition	Declustering Potential (DP)	Collision Energy (CE)	Cell Exit Potential (CXP)
2.55	JWH-250 N-(5-hydroxypentyl)	352>120.9	40	30	16
2.99	JWH-073 N-(3-hydroxybutyl)	344>155	40	30	16
3.00	UR-144 5-Hydroxy-pentyl	328.5>125	30	35	16
3.03	UR-144 Pentanoic Acid	342.5>125	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

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®



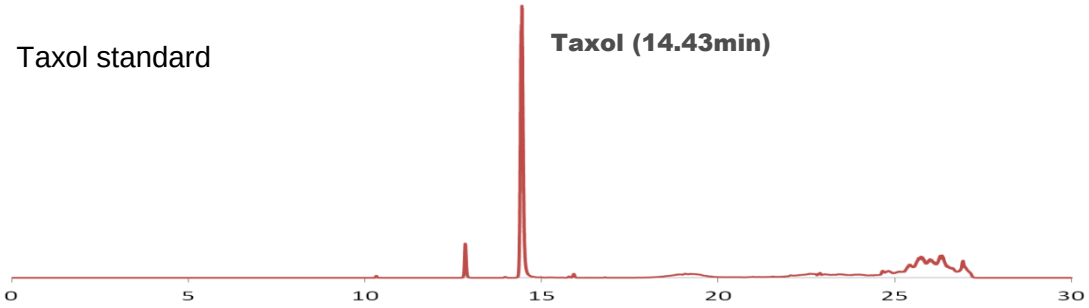
UR-144



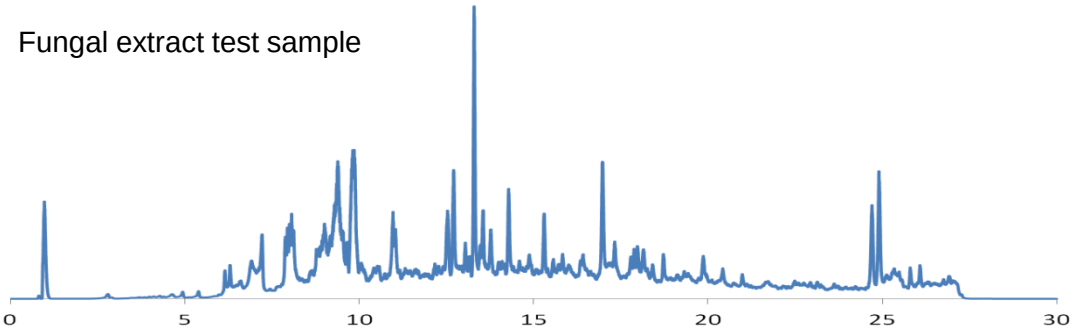
JWH-200

Taxol in Fungal Extract by LC-MS/MS

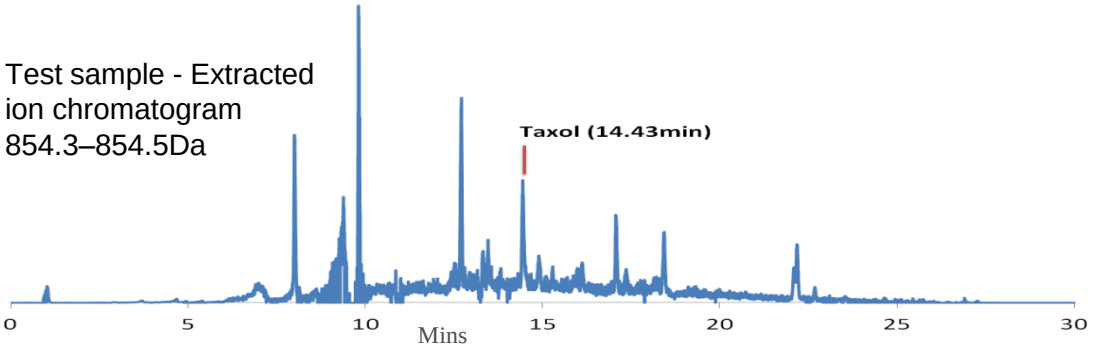
Taxol standard

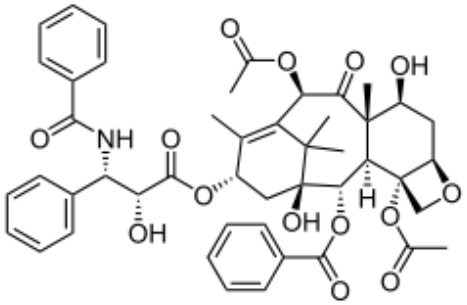


Fungal extract test sample



Test sample - Extracted ion chromatogram 854.3–854.5Da





Taxol (Paclitaxel)

ACE UltraCore SuperC18, 2.5µm, 150 x 2.1mm

Gradient analysis
A = 0.5% formic acid in water
B = 0.5% formic acid in acetonitrile

Time (mins)	%B
0	10
1	10
3	40
22	60
25	95

Flow rate: 0.35ml/min

Orbitrap Elite MS
FT positive ion mode
Collision induced dissociation –
Isolation width 5Da
Normalised collision energy 32eV
Activation Q 0.25
Activation time 10ms

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Microbial Extract by LC-MS

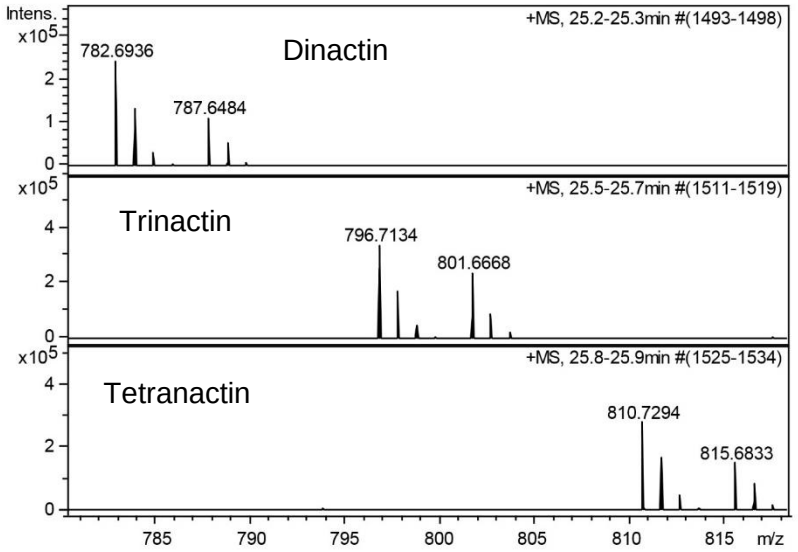
Dionex 3000RS UHPLC system coupled with Bruker MaXis Q-TOF MS

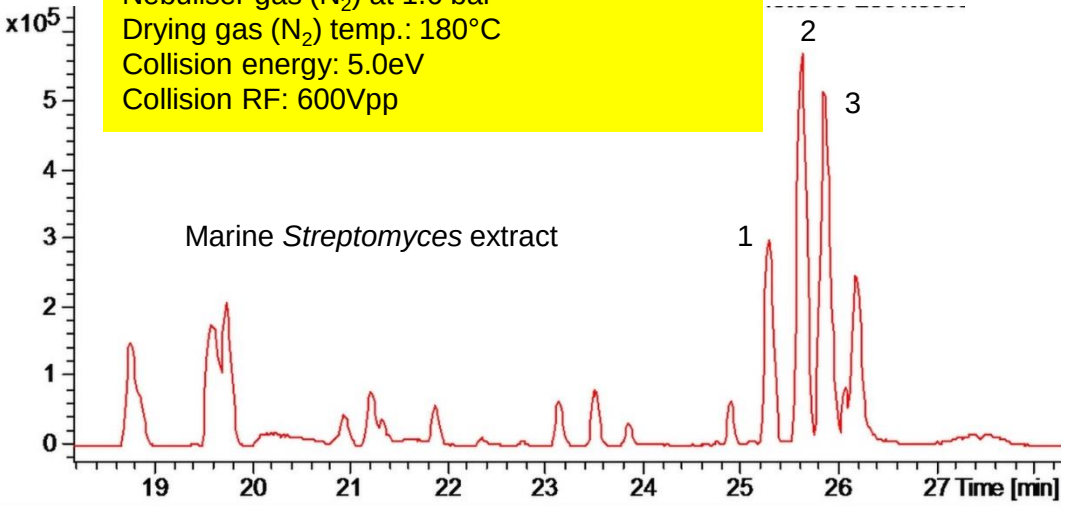
UltraCore SuperC18 2.5um, 150 x 2.1mm
Gradient analysis
A = 0.1% formic acid in water
B = 0.1% formic acid in acetonitrile

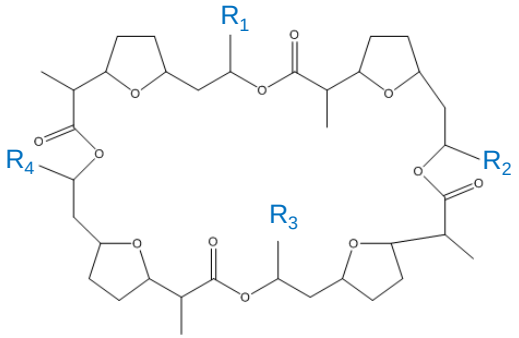
Time (mins)	%B	Time (mins)	%B
0	5	20	100
5	5	25	100

Flow rate: 0.2ml/min

Electrospray MS (positive mode)
Source: End plate offset: -500V,
Capillary: -4500V
Nebuliser gas (N_2) at 1.6 bar
Drying gas (N_2) temp.: 180°C
Collision energy: 5.0eV
Collision RF: 600Vpp







Macrotetralides

1. Dinactin $R_1 = R_3 = CH_2CH_3$, $R_2 = R_4 = CH_3$
2. Trinactin $R_1 = R_2 = R_3 = CH_2CH_3$, $R_4 = CH_3$
3. Tetranactin $R_1 = R_2 = R_3 = R_4 = CH_2CH_3$

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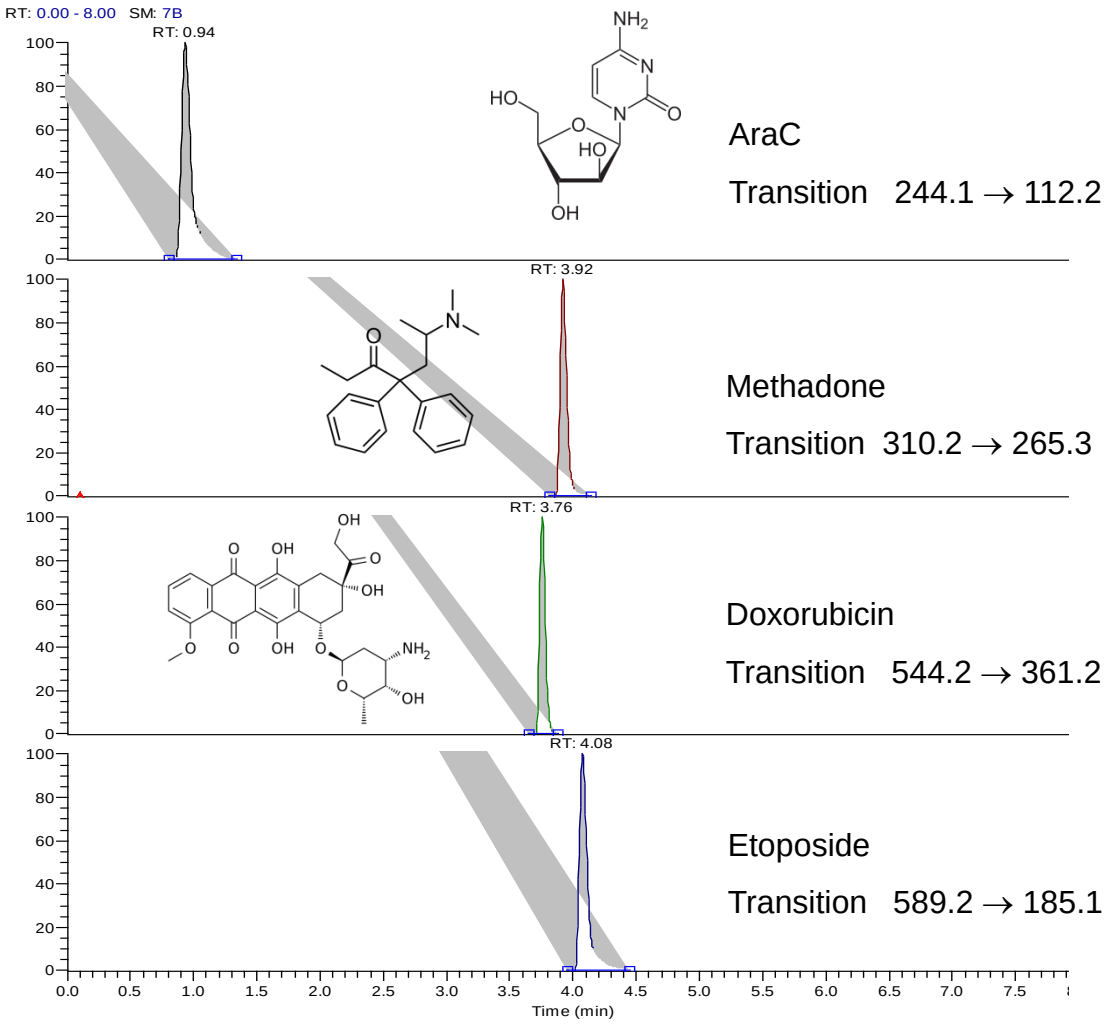
Cytotoxic Agents by UHPLC-MS/MS



AraC

Transition 244.1 → 112.2

Methadone

Transition 310.2 → 265.3

Doxorubicin

Transition 544.2 → 361.2

Etoposide

Transition 589.2 → 185.1

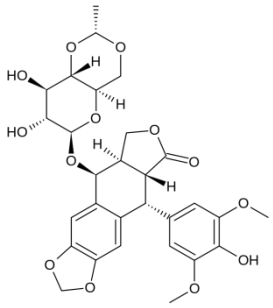
Thermo Scientific Accela UHPLC

ACE UltraCore SuperC18, 2.5µm, 100 x 2.1mm
Gradient analysis
A = 0.1% formic acid in water
B = 0.1% formic acid in acetonitrile

Time (mins)	%B
0	2
1	2
3	80
5	80
5.1	2
8	2

Flow rate: 0.25ml/min

Thermo Vantage triple quadrupole MS
MRM +ve ESI mode
Spray voltage: 3500V
Nitrogen sheath and auxiliary gas
CID with argon at 1.5 mTorr



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Human Defensins in Saliva

Sample preparation: SPE on C18

ACE UltraCore SuperC18
2.5µm, 50 x 3mm
Gradient analysis
A: 0.1% formic acid in water
B: 0.1% formic acid in CH_3CN

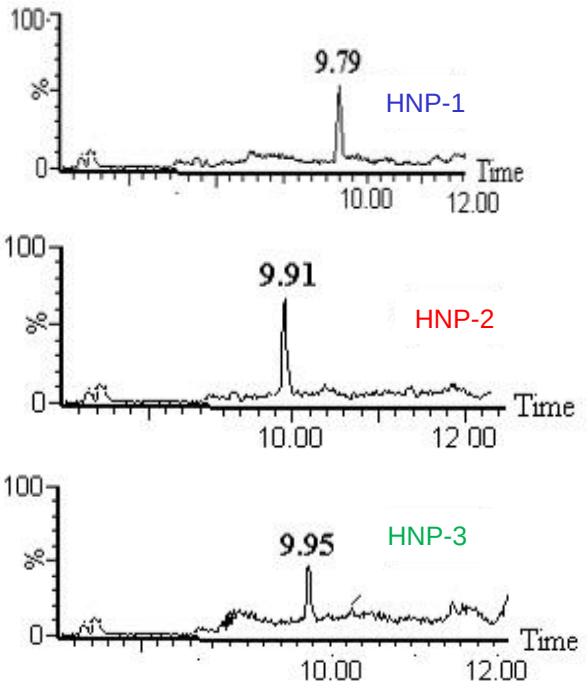
Time (mins)	%B
0	2
2	2
17	50
19	95
20	95

Flow rate: 0.6ml/min

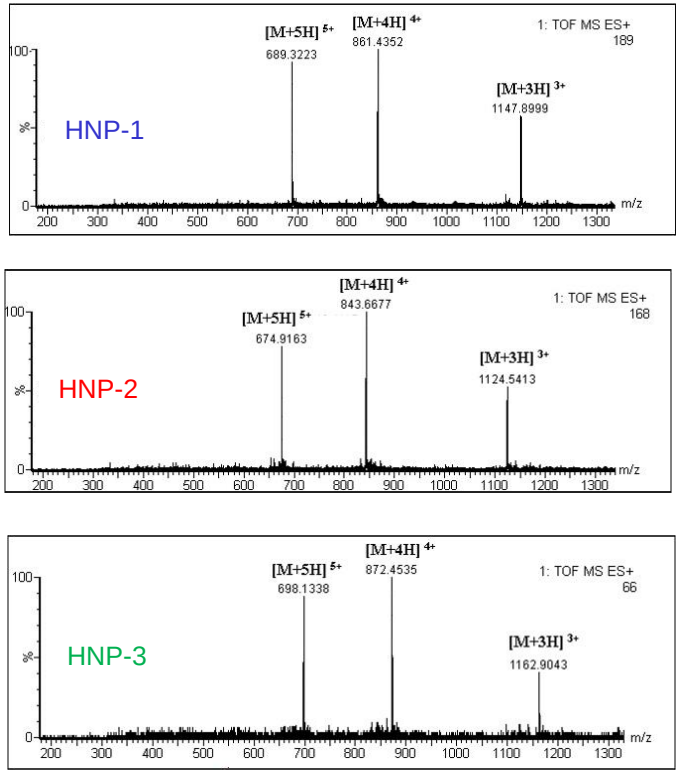
Synapt G1 QToF +ESI MS
Sampling cone voltage: 40V
Source temperature: 150°C
Capillary voltages: 4.8kV
Extraction cone voltages: 41kV
Desolvation temperature: 500°C
Acquisition: 100-2000 m/z

Extracted ion current chromatograms

(sum of multiply protonated ions $[M+3H]^{3+}$, $[M+4H]^{4+}$ and $[M+5H]^{5+}$)

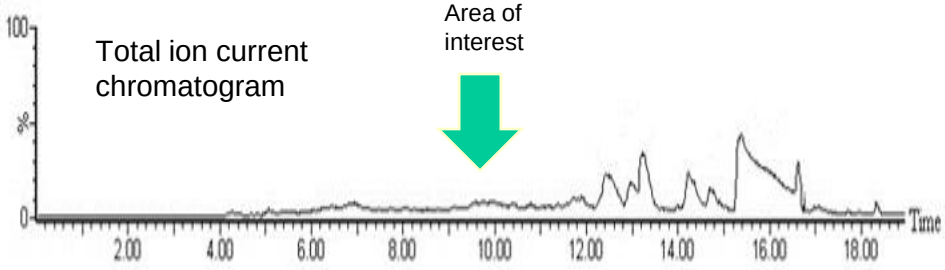


Mass spectra



Total ion current chromatogram

Area of interest



Defensin Human Neutrophil Peptides

HNP-1 30 amino acid residues
HNP-2 29 amino acid residues
HNP-3 30 amino acid residues

Detection limit ~ 2.1ng/µl

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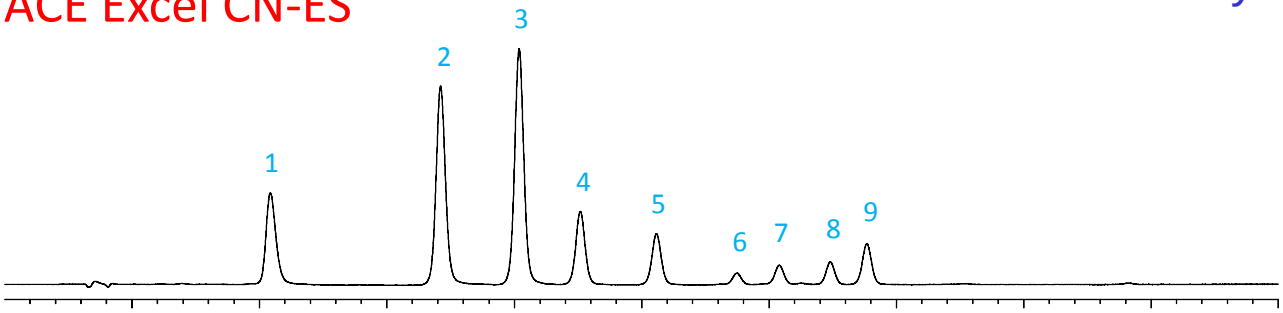
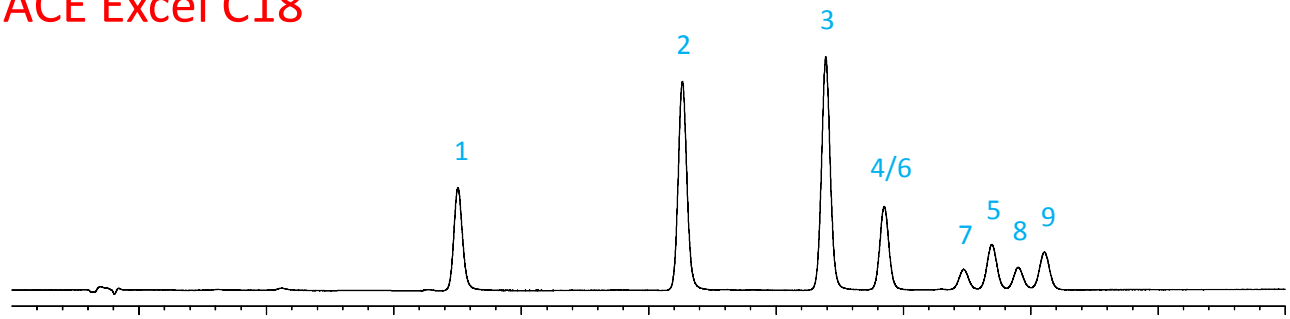
Steroids UHPLC-UV Analysis

Alternative selectivity & full separation noted

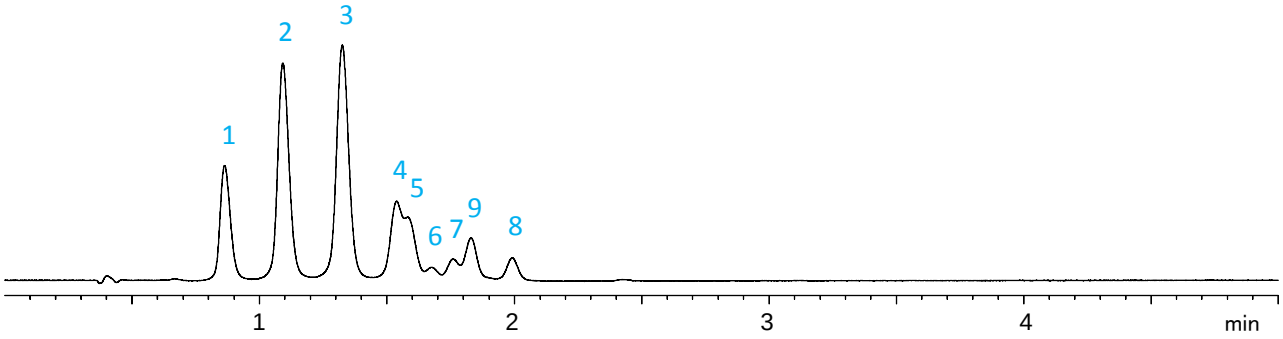
ACE Excel CN-ES



ACE Excel C18



ACE Excel CN



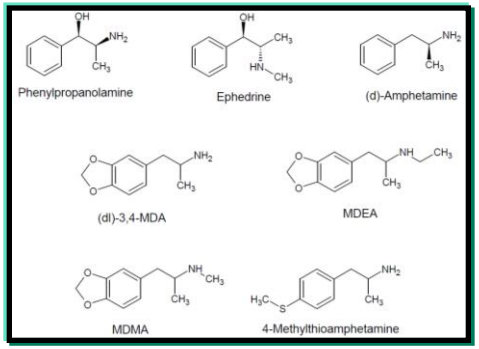
50x2.1mm, 2µm
Gradient analysis

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

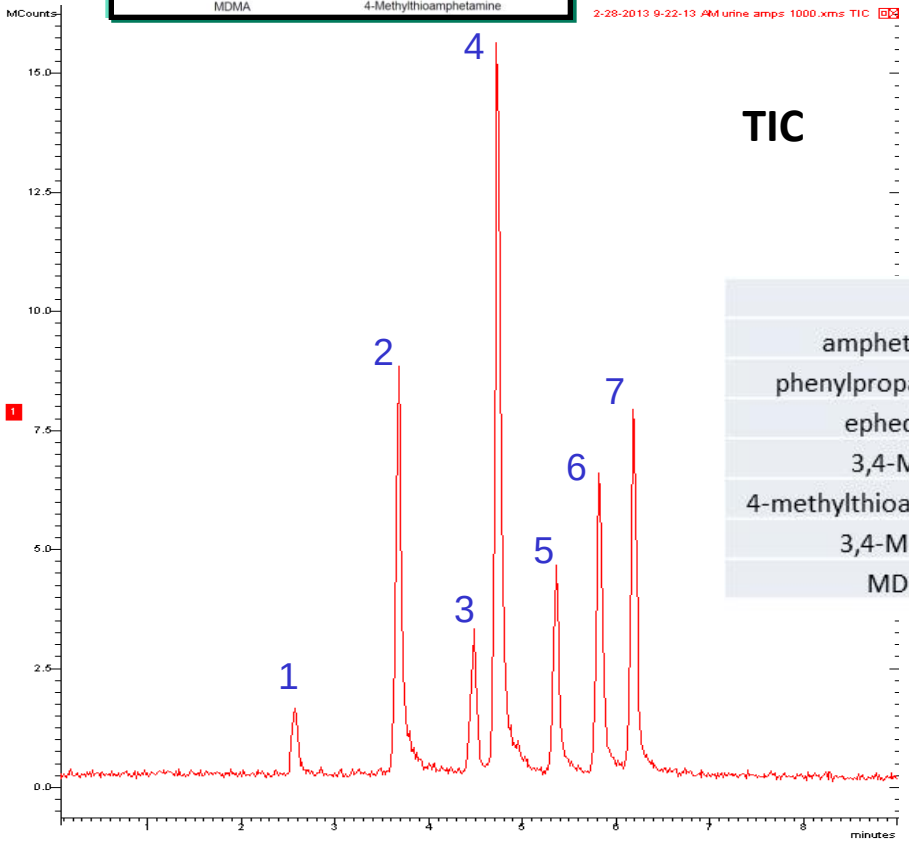
T (min)	%B
0	25
10	80
10.5	80
11	25

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

Amphetamines In Urine by LC-MS/MS



1. Phenylpropanolamine
2. (l)-Ephedrine
3. (dl)-3,4-MDA
4. (d)-Amphetamine
5. (dl)-3,4-MDMA
6. 4-methylthioamphetamine
7, (±)-MDEA



TIC

	LOD (est)
amphetamine	4 ppb
phenylpropanolamine	4 ppb
ephedrine	2 ppb
3,4-MDA	30 ppb
4-methylthioamphetamine	10 ppb
3,4-MDMA	2 ppb
MDEA	1 ppb

ACE Excel SuperC18, 3um, 75 x 2.1 mm
Gradient analysis

MP A: 5mM Ammonium Hydroxide, pH 10.8.
MP B: 5mM Ammomnium Hydroxide, pH 10.8 in 1:9 v/v H2O:MeOH.
0.6mL/min

T	%B
0	30
8	95

60C, 2uL.

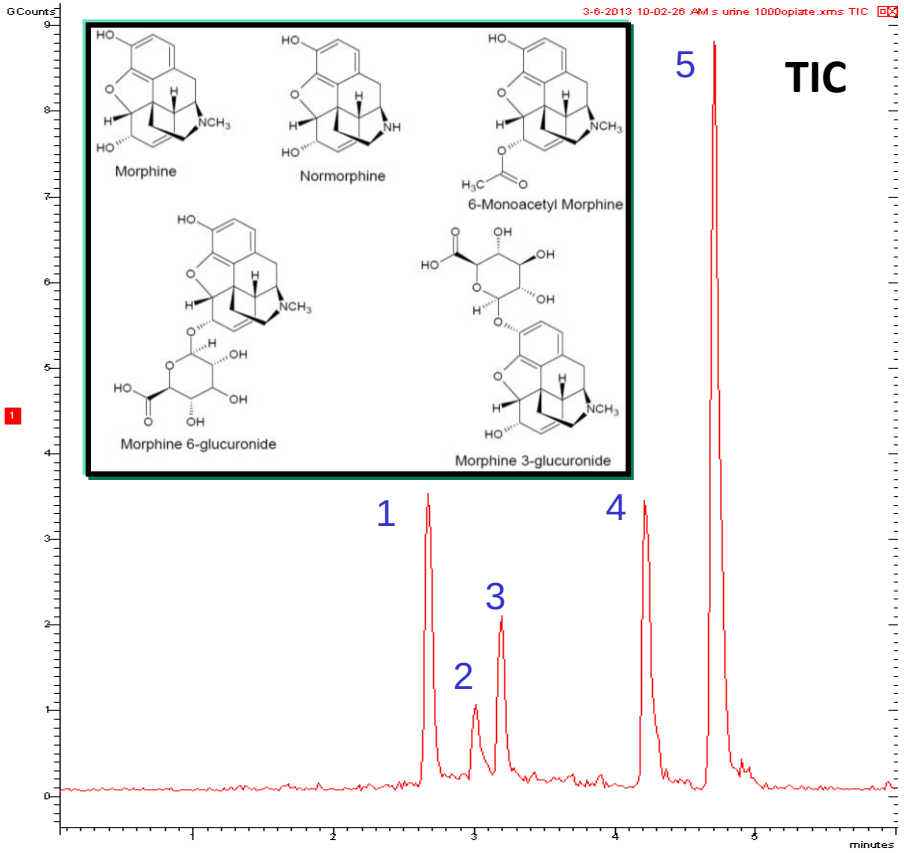
Varian 320 Triple Quadrupole MS
Electrospray voltage: +5 kV
Inlet capillary voltage: 30 V
CID with argon at 1.5 mTorr; Collision cell potential ranges from 5 to 17 V
Drying gas (nitrogen) temperature: 325 C
Nebulizing gas (nitrogen) pressure: 35 psi
Extended Dynamic Range

Compound	Q1 Mass	Q3 Mass
(dl)-3,4-MDMA	193.7	163.0
Phenylpropanolamine	151.6	134.0
(d)-Amphetamine	135.8	90.9
(l)-Ephedrine	166.2	148.0
(dl)-3,4-MDA	179.7	163.0
(±)-MDEA	207.7	163.0
4-methylthioamphetamine	182.2	165.0

Opiates In Urine by LC-MS/MS

1. Morphine 3-β-D-glucuronide
2. Normorphine
3. Morphine 6-β-D-glucuronide
4. Morphine
5. 6-Acetylmorphine

	LOD (est)
Normorphine	100 ppb
Morphine	20 ppb
6-acetylmorphone	10 ppb
Morphine 3-β-DG	100ppb
Morphine 6-β-DG	100ppb



ACE Excel SuperC18, 3um, 75 x 2.1 mm + guard
Gradient analysis

MP A: 5mM Ammonium Hydroxide, pH 10.8.
MP B: 5mM Ammomnium Hydroxide, pH 10.8 in 1:9 v/v H2O:MeOH.
0.6mL/min

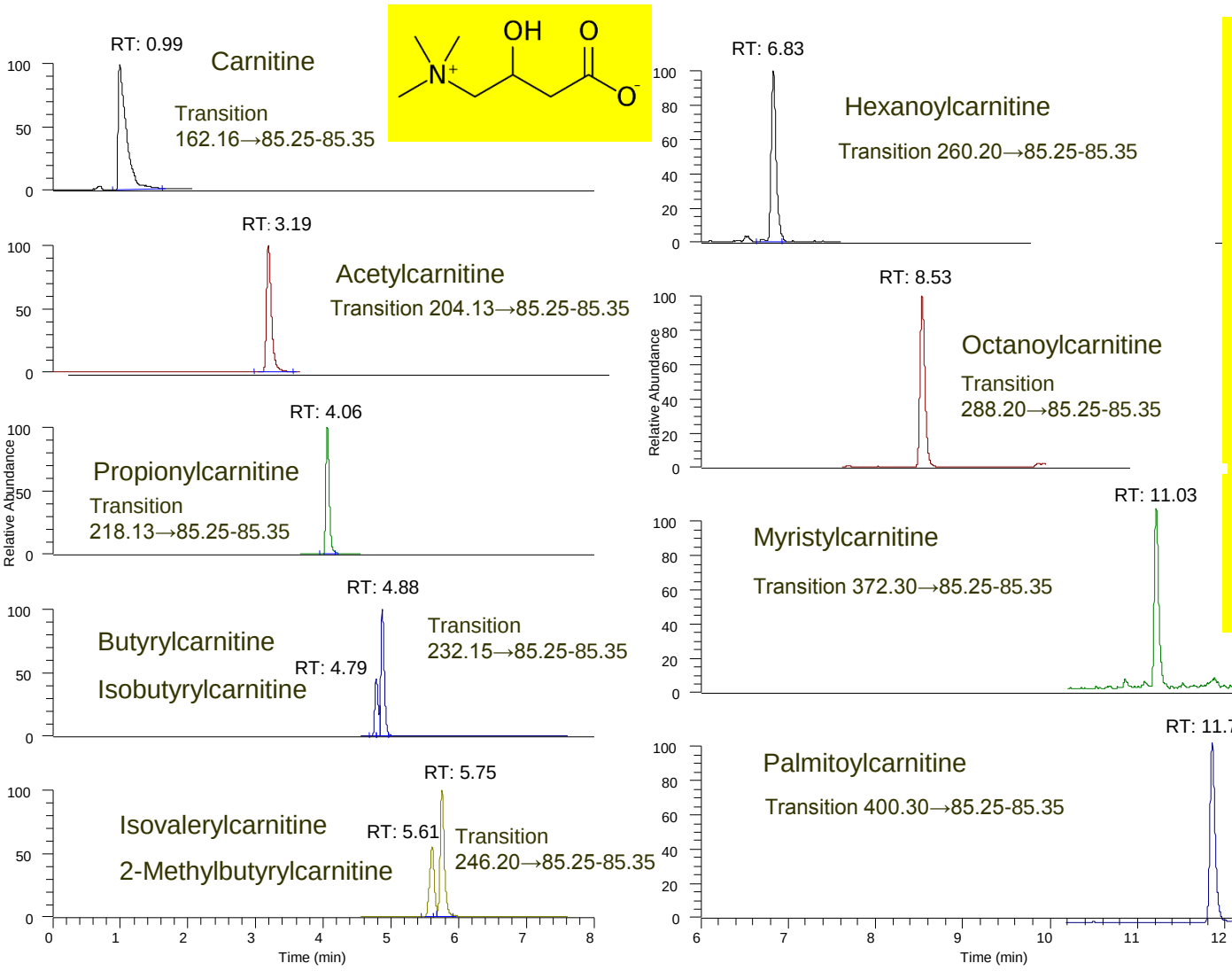
T	%B
0	5
5	95

60C, 2uL.

Varian 320 Triple Quadrupole MS
Electrospray voltage: +5 kV
Inlet capillary voltage: 30 V
CID with argon at 1.5 mTorr; Collision cell potential ranges from 5 to 17 V
Drying gas (nitrogen) temperature: 325 C
Nebulizing gas (nitrogen) pressure: 35 psi
Extended Dynamic Range

Compound	Q1 Mass	Q3 Mass
morphine 3-β-D glucuronide	462.0	285.9
Normorphine	272.0	165.0
morphine 6-β-D glucuronide	462.0	285.9
6-acetylmorphine	328.0	164.9
morphine	286.0	200.9

UHPLC-MS/MS of Acylcarnitines



ACE Excel C18-PFP
2µm, 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

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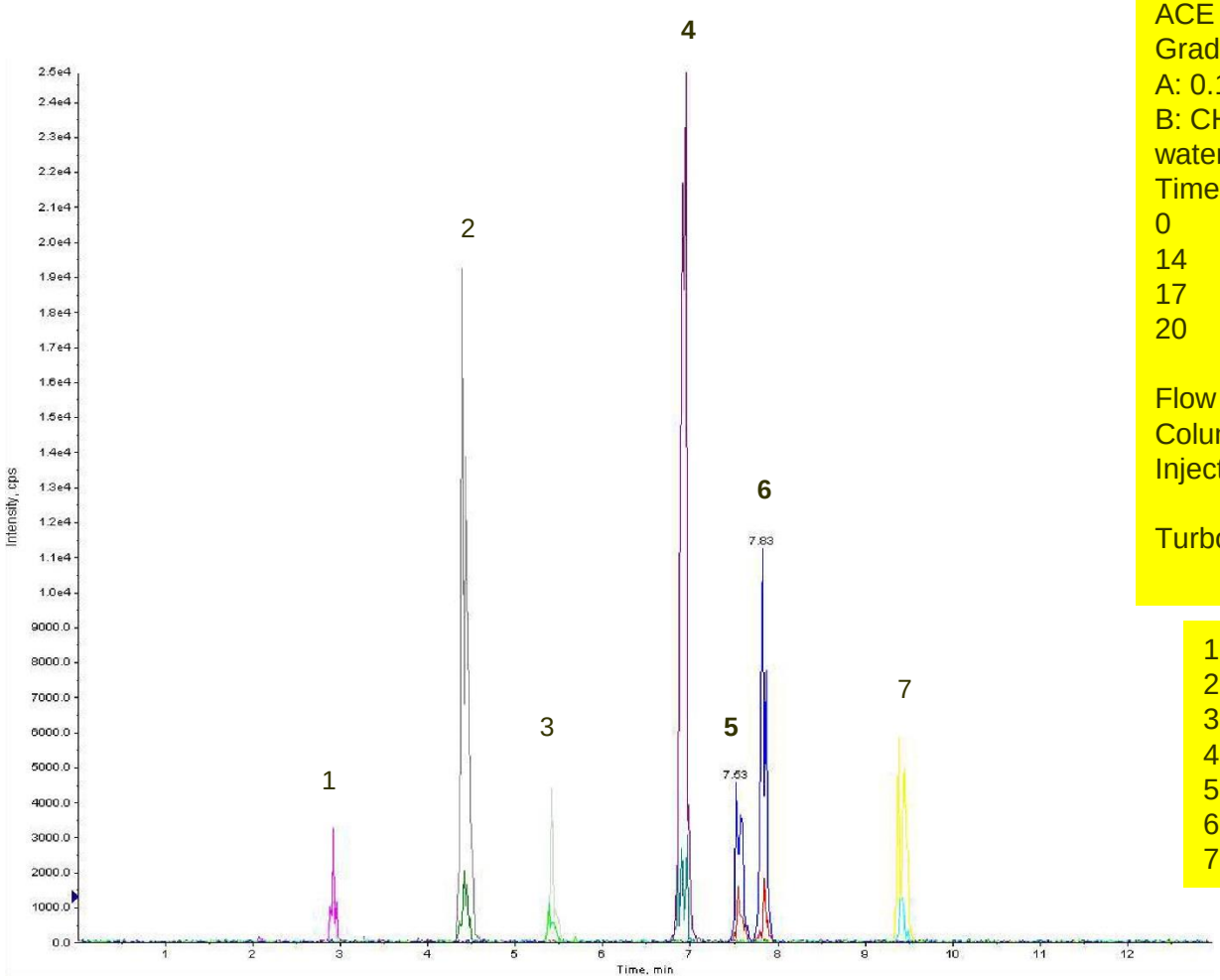
Corticosteroids by LC-MS/MS



ACE C18-PFP, 3µm, 150 x 2.1mm
Gradient analysis
A: 0.1% formic acid in water
B: CH_3CN – 0.1% formic acid in water

Time (mins)	%B
0	30
14	50
17	95
20	30

Flow rate: 0.3ml/min
Column temperature: 15°C
Injection volume: 25µl

Turbospray, MRM

1. Triamcinolone
2. Prednisolone
3. Fluoroprednisolone
4. Methylprednisolone
5. Betamethasone
6. Dexamethasone
7. Flumethasone

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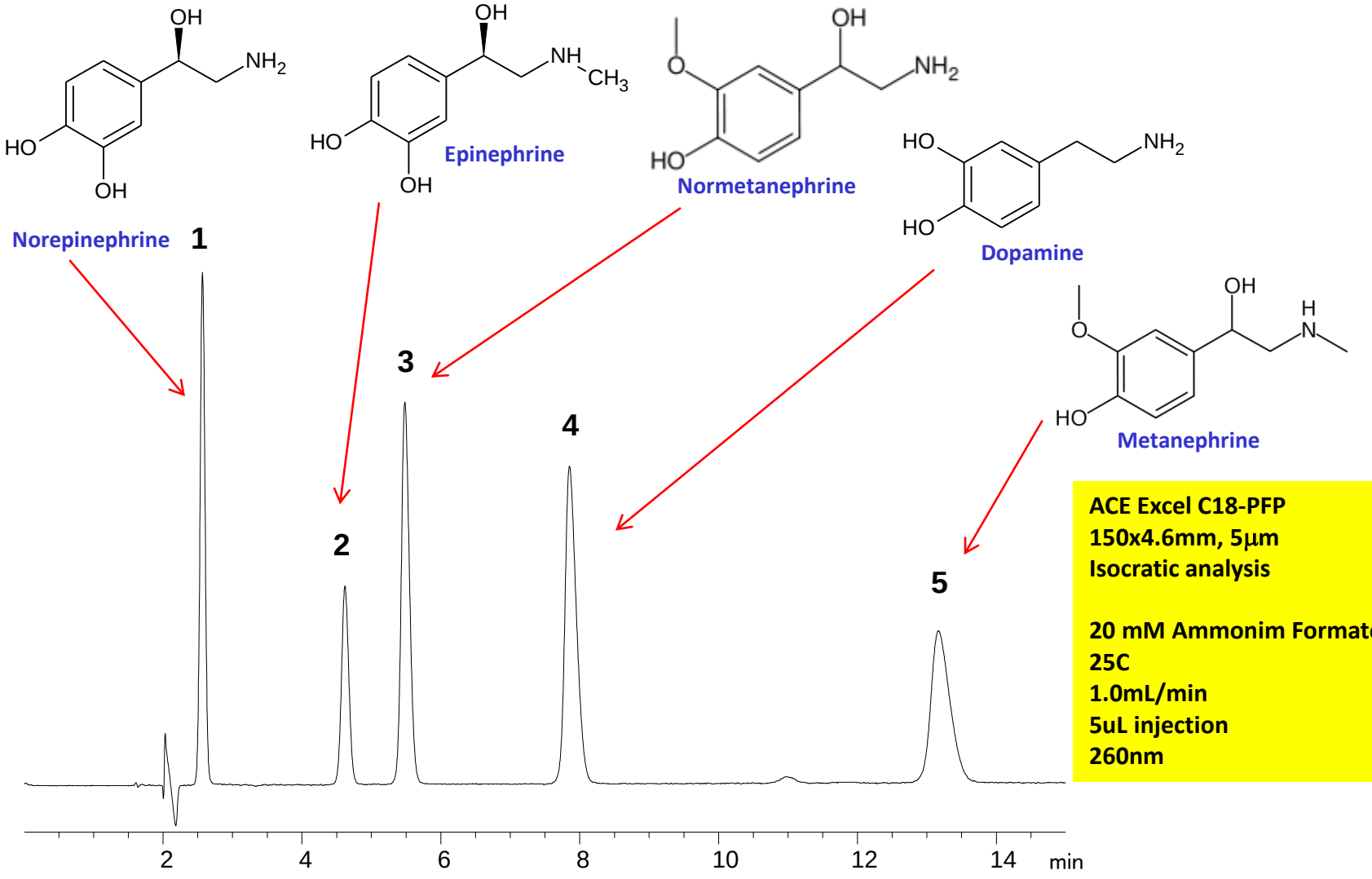
Catecholamines: Isocratic Analysis

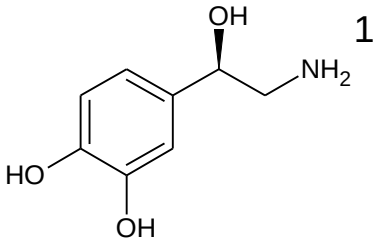


ACE Excel C18-PFP
150x4.6mm, 5µm
Isocratic analysis

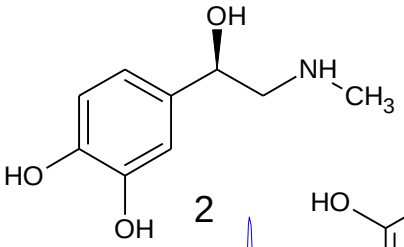
20 mM Ammonim Formate, pH 3 (aq)
25C
1.0mL/min
5uL injection
260nm

Catecholamine Analysis

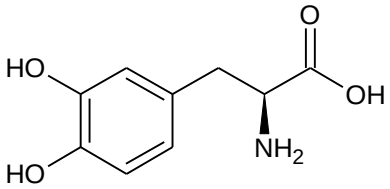
100% Aqueous



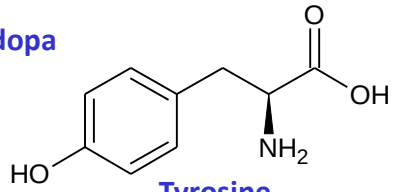
1 Norepinephrine



2 Epinephrine



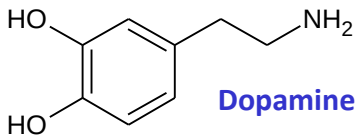
3 Levodopa



Tyrosine

4

5



Dopamine

ACE C18-PFP, 5µm, 150 x 4.6 mm

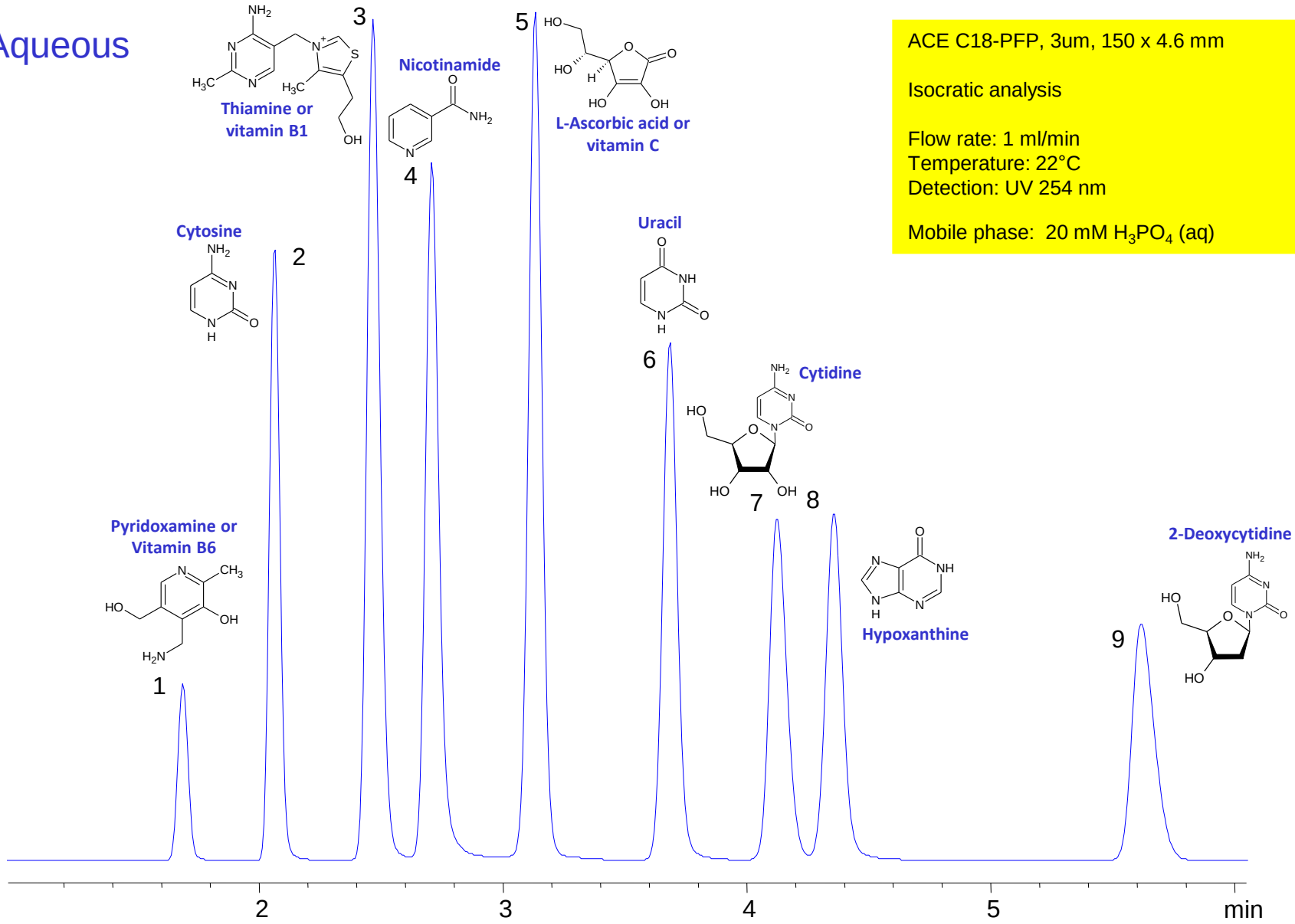
Isocratic analysis

Flow rate: 1.0 mL/min
Temperature: 22°C
Detection: UV 266 nm

MP: 12.5mM Ammonium formate, pH 3.0 (aq)

Polar Analytes – Nucleosides & Vitamins

100% Aqueous



ACE C18-PFP, 3um, 150 x 4.6 mm

Isocratic analysis

Flow rate: 1 ml/min
Temperature: 22°C
Detection: UV 254 nm

Mobile phase: 20 mM H_3PO_4 (aq)

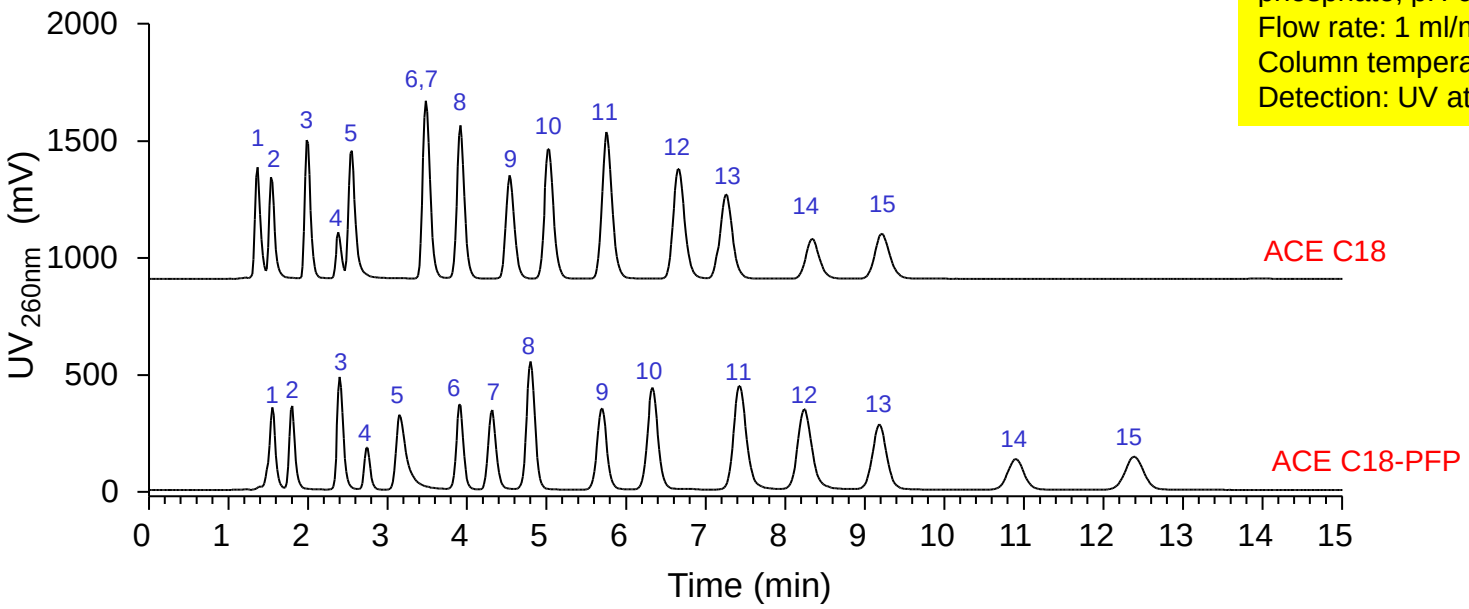
Disease Biomarker Profiling

Nucleotide, Nucleoside & Nucleobase Analysis

Analytes

1. dATP
2. dADP
3. dAMP
4. 5-Fluorodeoxyuridine
5. Adenine
6. Thymidine
7. 2-Fluorodeoxyuridine
8. Adenine arabinoside
9. 2'-C-methyladenosine
10. Adenosine
11. Deoxyadenosine
12. Cordycepin
13. 2-Fluoroadenine arabinoside
14. 2-Fluorodeoxyadenosine
15. 2-Fluoroadenosine

ACE C18 and ACE C18-PFP
3 µm, 100 x 4.6 mm
Isocratic analysis
Solvent = 12% methanol, 33 mM potassium phosphate, pH 6.2 with KOH
Flow rate: 1 ml/min
Column temperature: Ambient
Detection: UV at 260 nm



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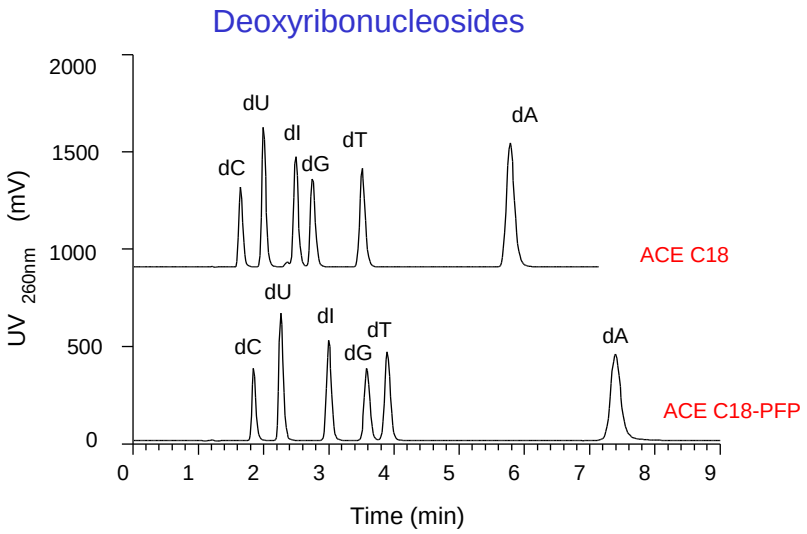
Disease Biomarker Profiling (II)

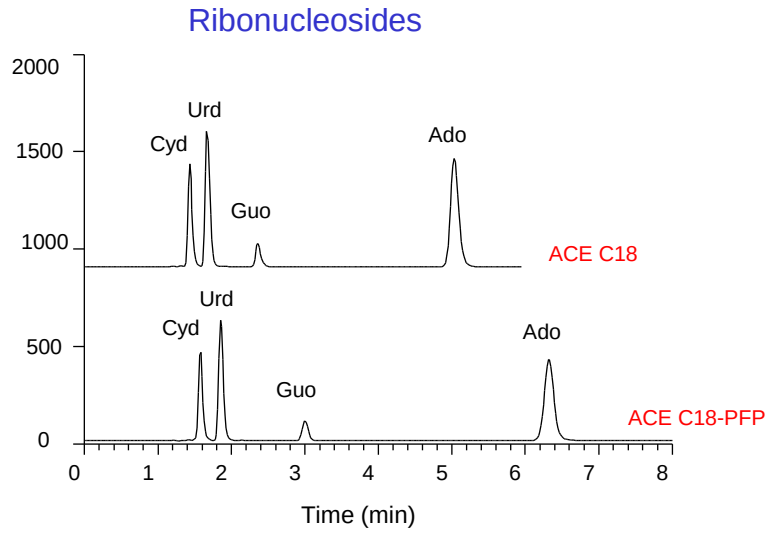
ACE C18 and ACE C18-PFP
3 µm, 100 x 4.6 mm
Isocratic analysis
Mobile phase: 12% methanol, 33 mM potassium phosphate, pH 6.2 with KOH
Flow rate: 1 ml/min
Column temperature: Ambient
Detection: UV at 260nm

Deoxyribonucleosides and Ribonucleosides

Key
dC deoxycytidine
dU deoxyuridine
dI deoxyinosine
dG deoxyguanosine
dT thymidine
dA deoxyadenosine

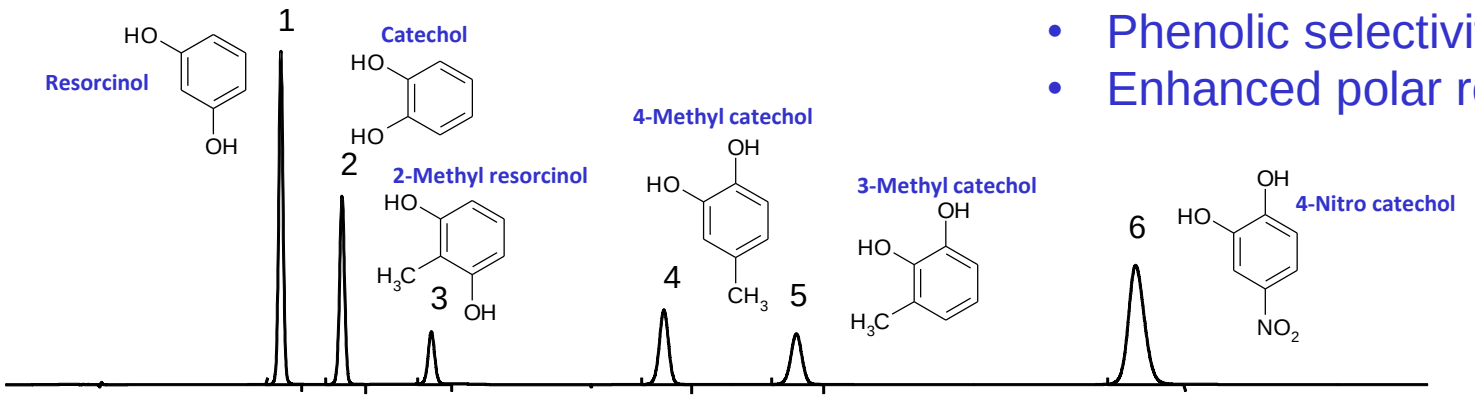
Cyd cytidine
Urd uridine
Guo guanosine
Ado adenosine





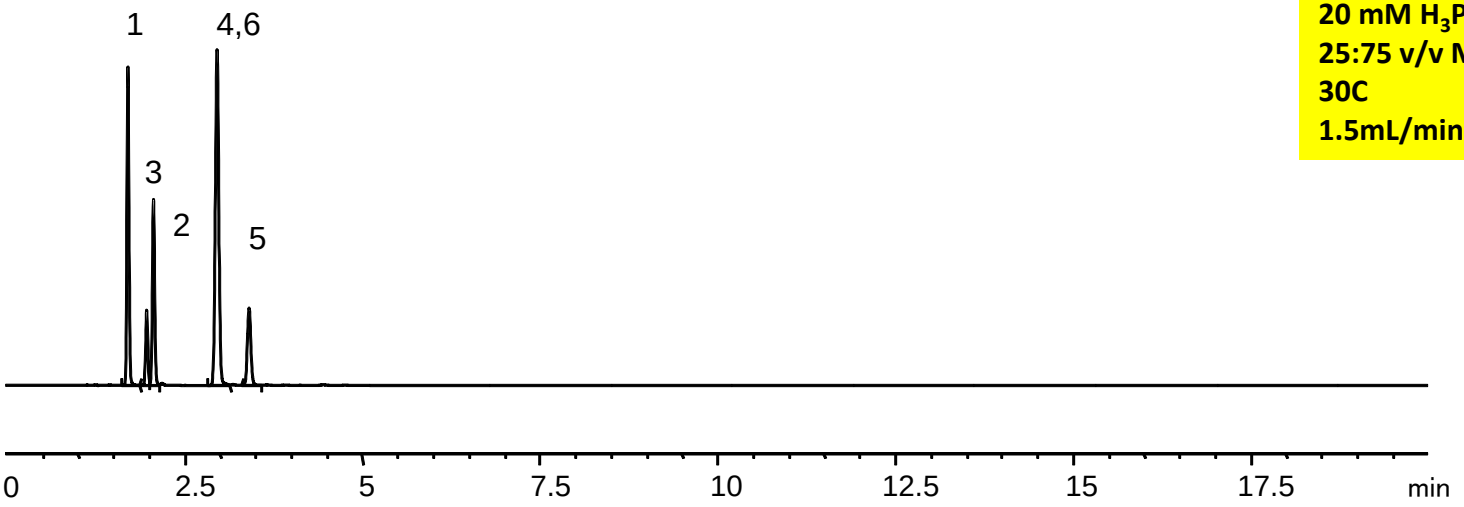
Catechols and Resorcinols

ACE Excel C18-Amide



- Phenolic selectivity
- Enhanced polar retention

ACE C18



150Ax4.6mm, 3µm
Isocratic analysis

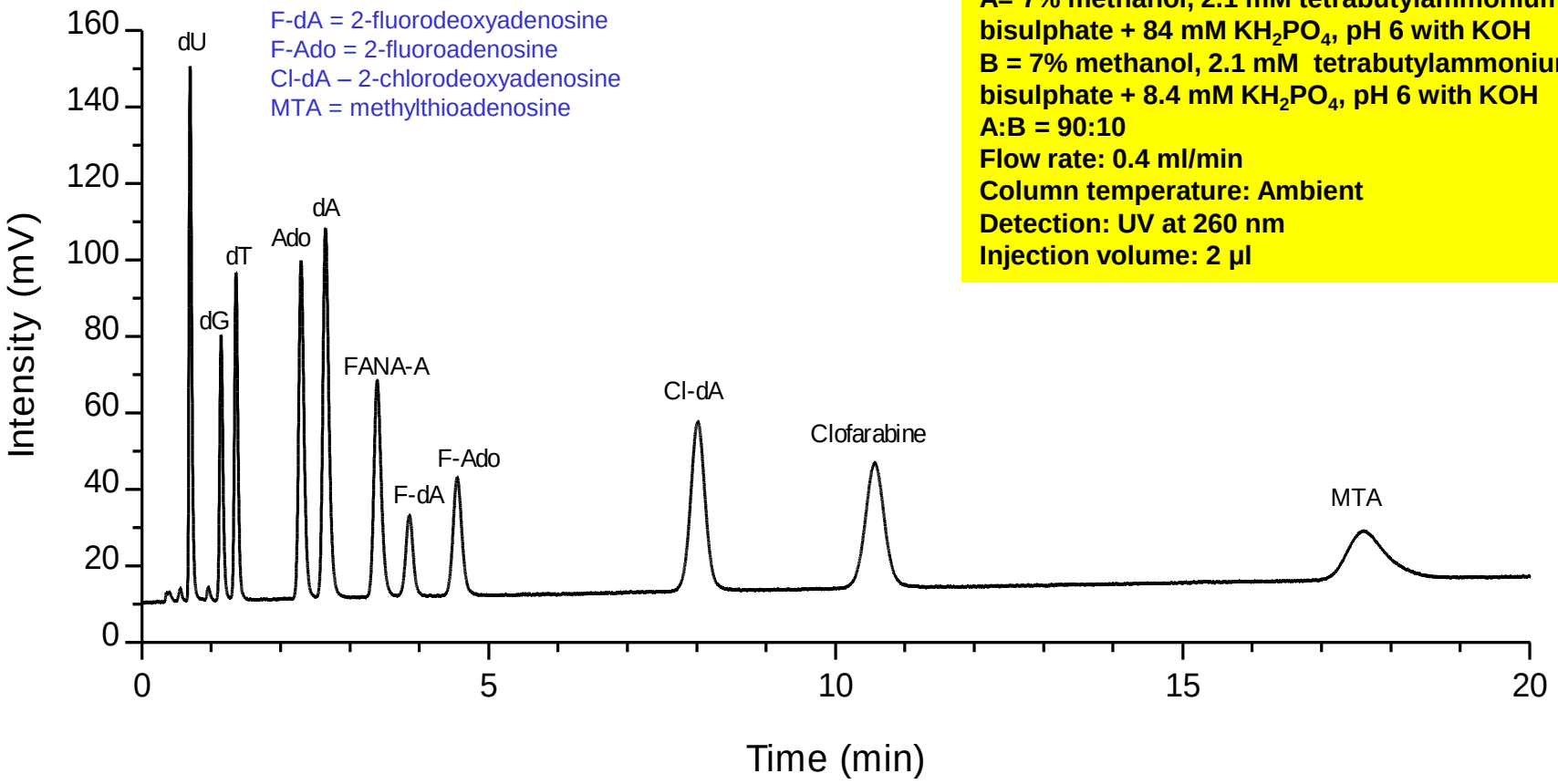
20 mM H_3PO_4 in
25:75 v/v MeCN/H_2O
30C
1.5mL/min

Biomarker Profiling

Deoxyribonucleosides

Key:
Ado = adenosine
FANA-A = 2-fluoro-2'-fluoroadenine arabinoside
F-dA = 2-fluorodeoxyadenosine
F-Ado = 2-fluoroadenosine
Cl-dA – 2-chlorodeoxyadenosine
MTA = methylthioadenosine

ACE UltraCore SuperC18, 2.5 µm, 50 x 2.1 mm
Isocratic analysis
A= 7% methanol, 2.1 mM tetrabutylammonium bisulphate + 84 mM KH_2PO_4, pH 6 with KOH
B = 7% methanol, 2.1 mM tetrabutylammonium bisulphate + 8.4 mM KH_2PO_4, pH 6 with KOH
A:B = 90:10
Flow rate: 0.4 ml/min
Column temperature: Ambient
Detection: UV at 260 nm
Injection volume: 2 µl



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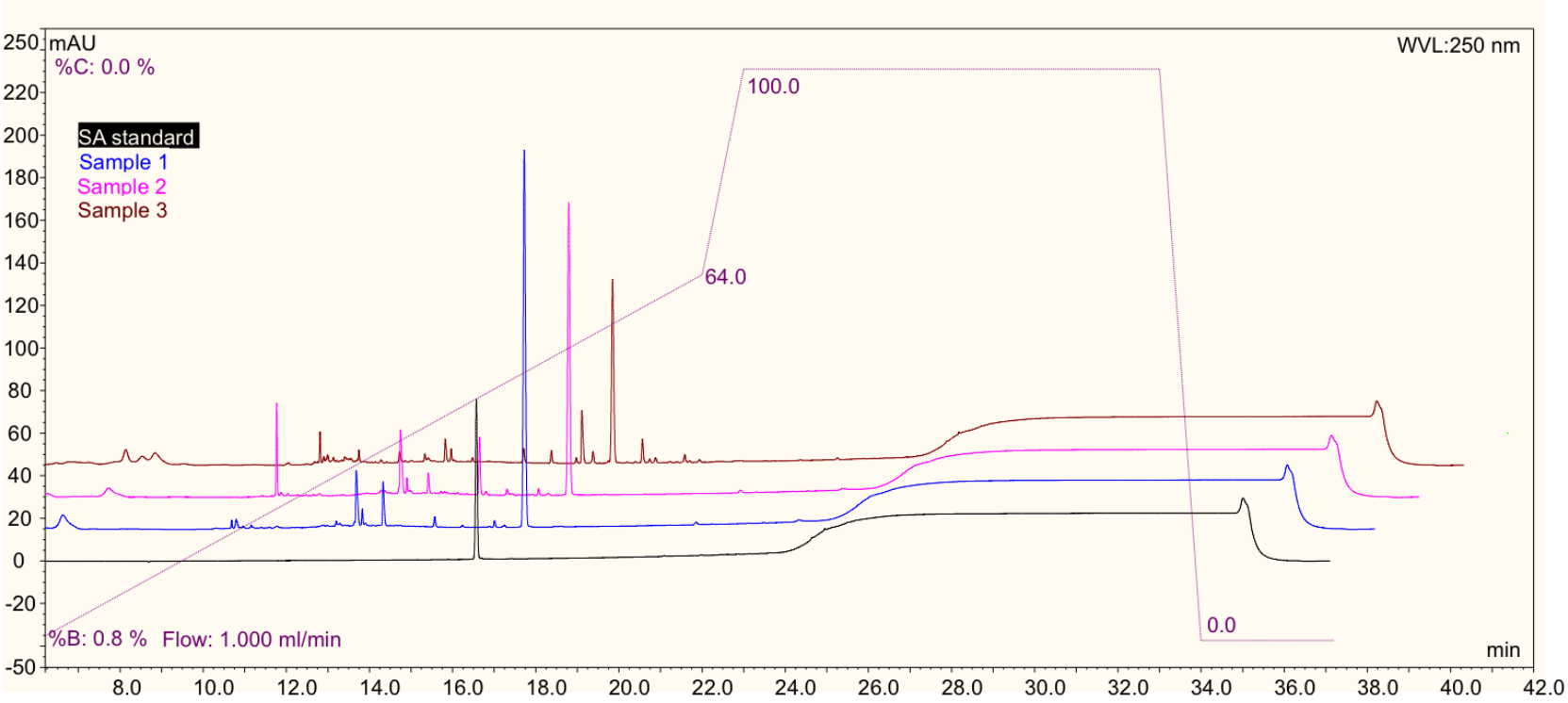
Salicylic acid in cell extracts

Extract from genetically engineered bacteria cells – targeted analysis for salicylic acid



ACE UltraCore SuperC18, 5µm, 150 x 4.6mm
Gradient analysis (shown above)
A = 0.1% TFA in water
B = 0.1% TFA in acetonitrile
Flow rate: 1ml/min
Detection: UV, 250nm

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Non-Steroidal Anti-Inflammatory Drugs by LC-UV

ACE 3 C18-AR