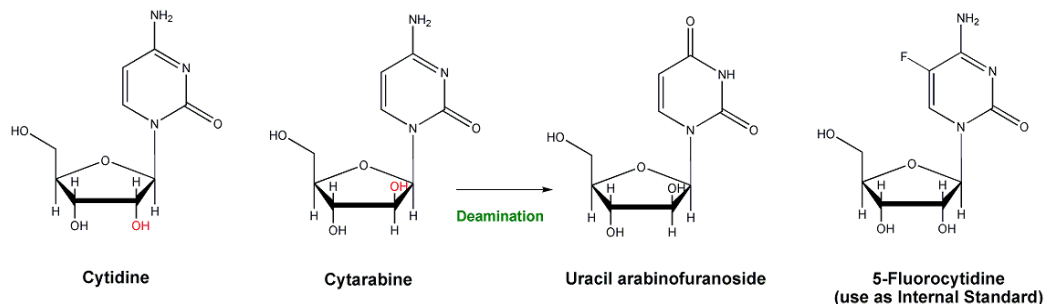


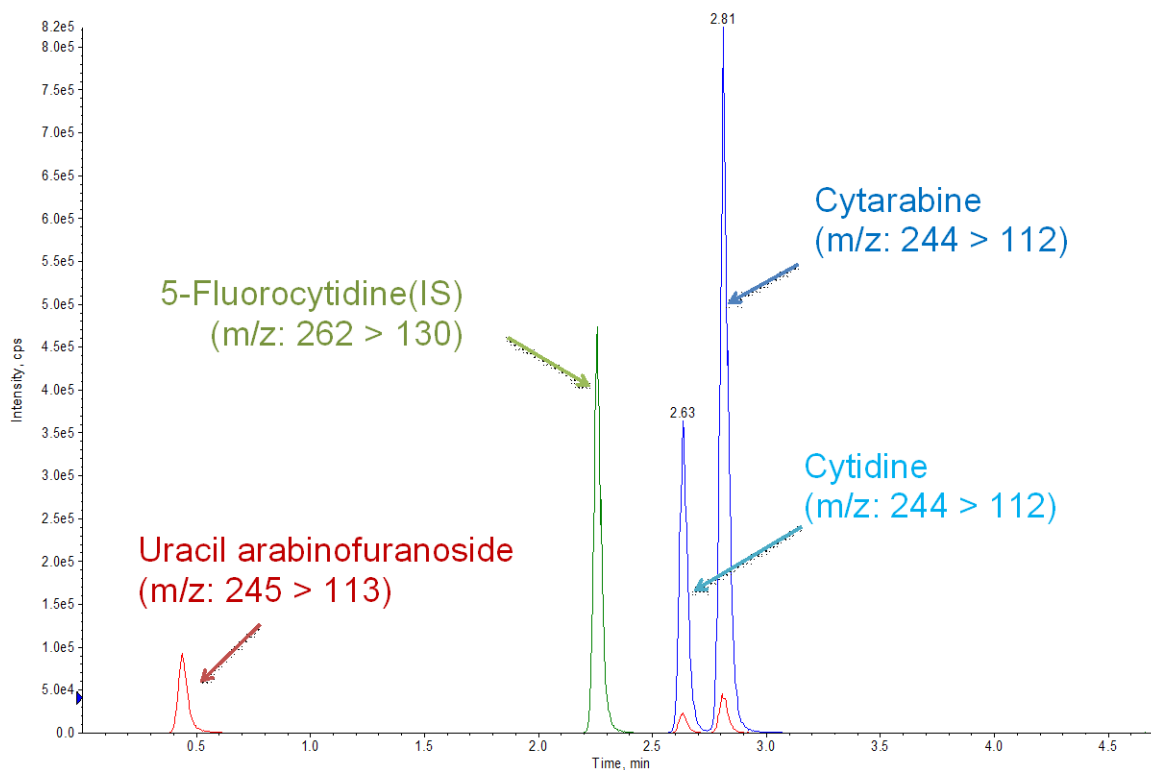
Cytarabine Analogues by Ion-Pairing LC-MS/MS



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

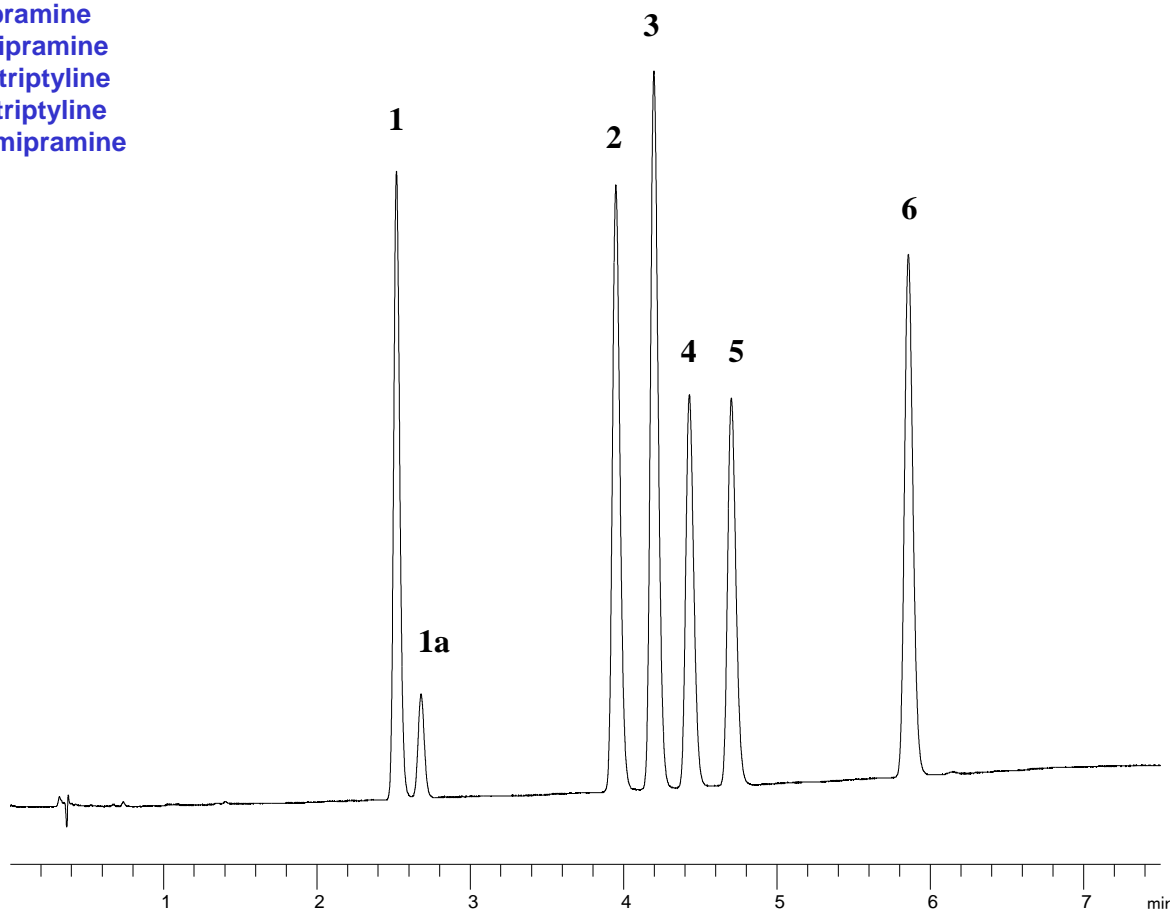
Flow rate: 0.7 ml/min
 API 4000 MS
 TurbolonSpray, positive mode
 Source temperature: 550°C



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	

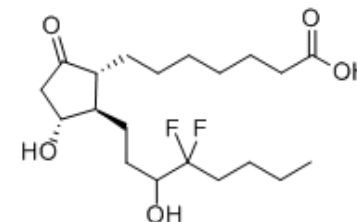
Flow rate: 1.2ml/min
Column temperature: 40°C
Injection volume: 2 μ l
Detection: UV, 260nm



15-Hydroxy Lubiprostone in Human Plasma

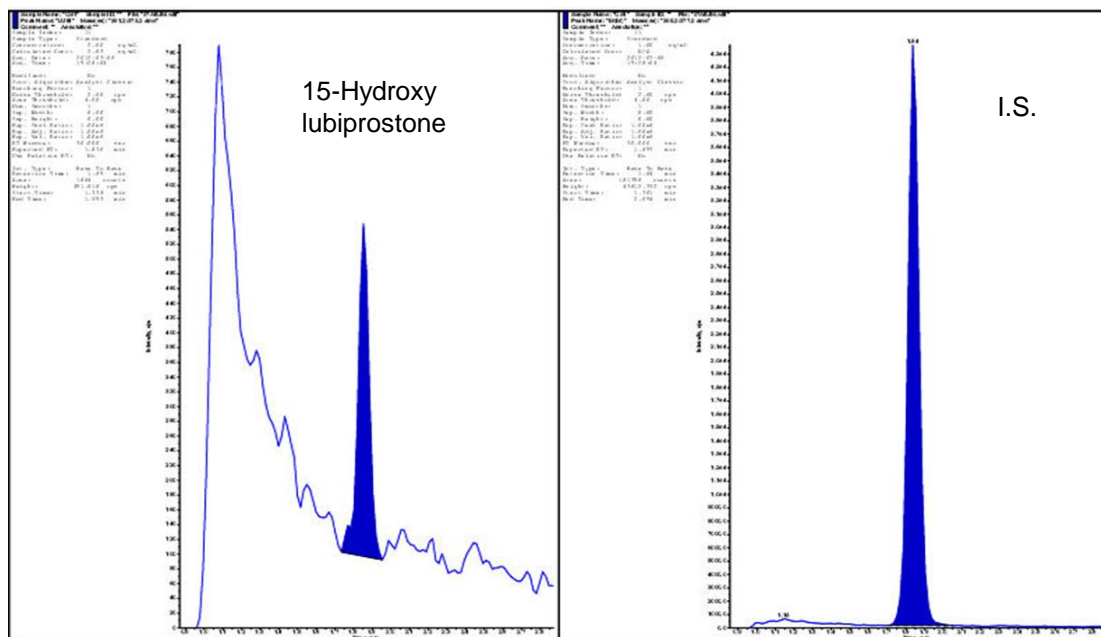
Lubiprostone, a fatty acid derived from prostaglandin E1, is rapidly metabolised to 15-hydroxy lubiprostone.

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

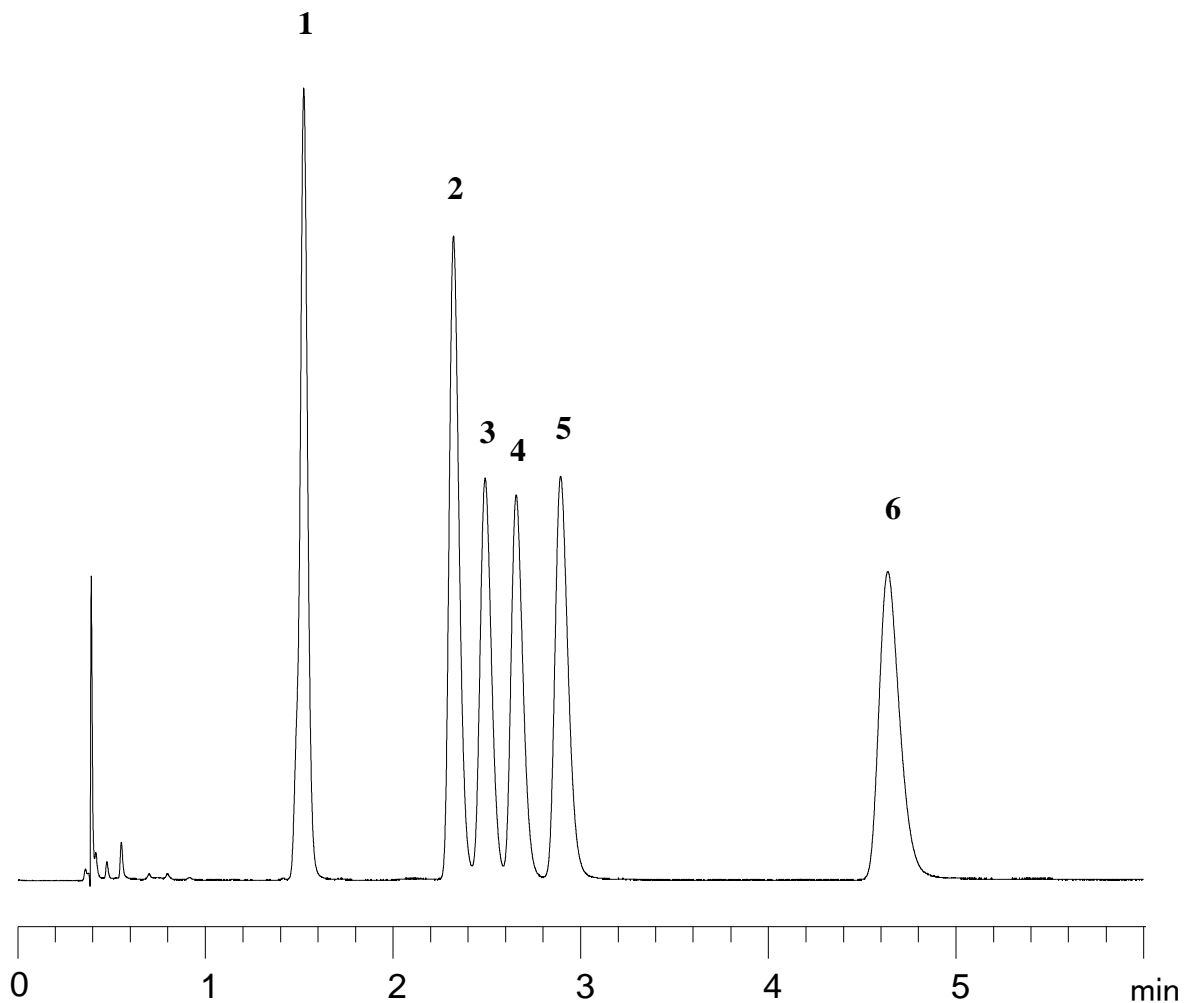


ACE Excel 2 C18 2 μ m, 50 x 3.0mm
Isocratic analysis
A = 0.1% formic acid in water
B = Acetonitrile
Flow rate: 0.65ml/min
Column temperature: 35°C
Injection volume: 15 μ l

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

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

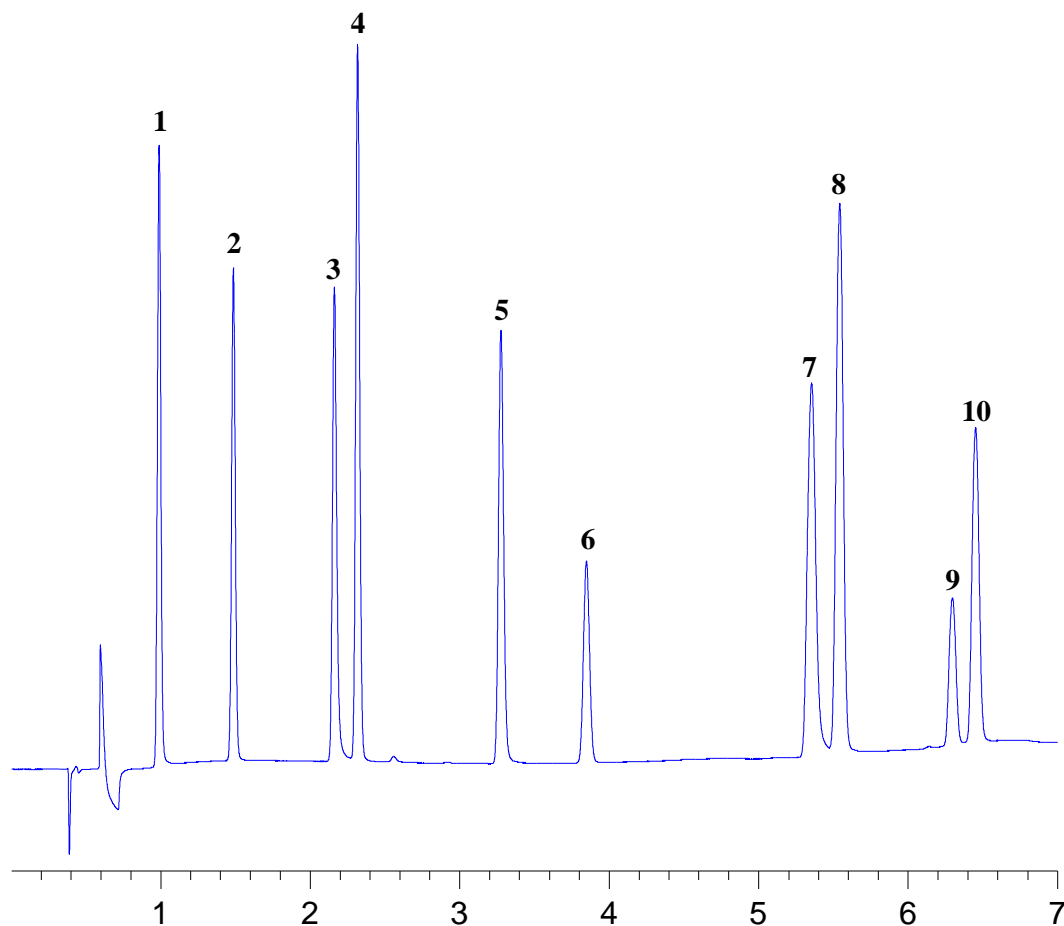
Flow rate: 1.2ml/min
Column temperature: 40°C
Injection volume: 2 μ l
Detection: UV, 260nm

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

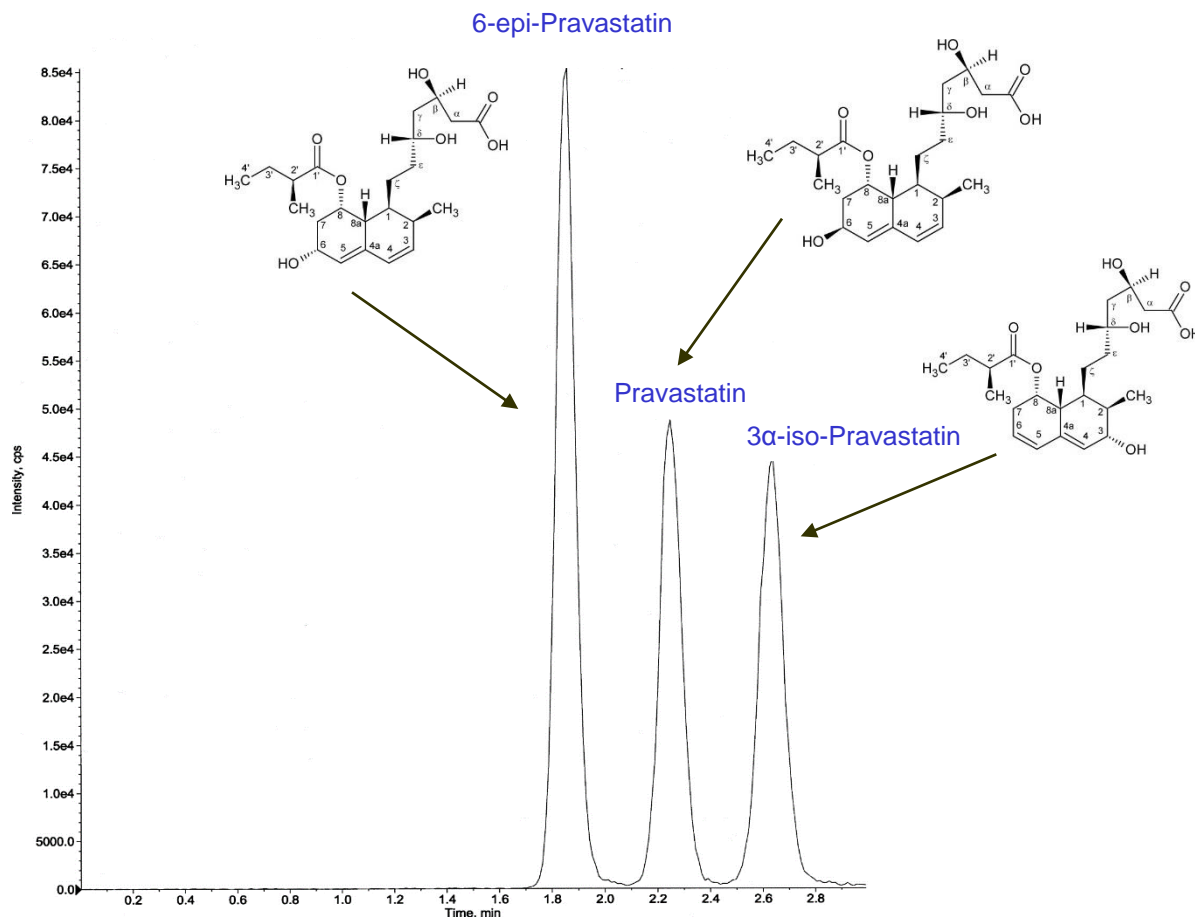
Detection: UV, 220nm

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



Pravastatin and Isomers by LC-MS/MS



All 3 compounds have MW 424

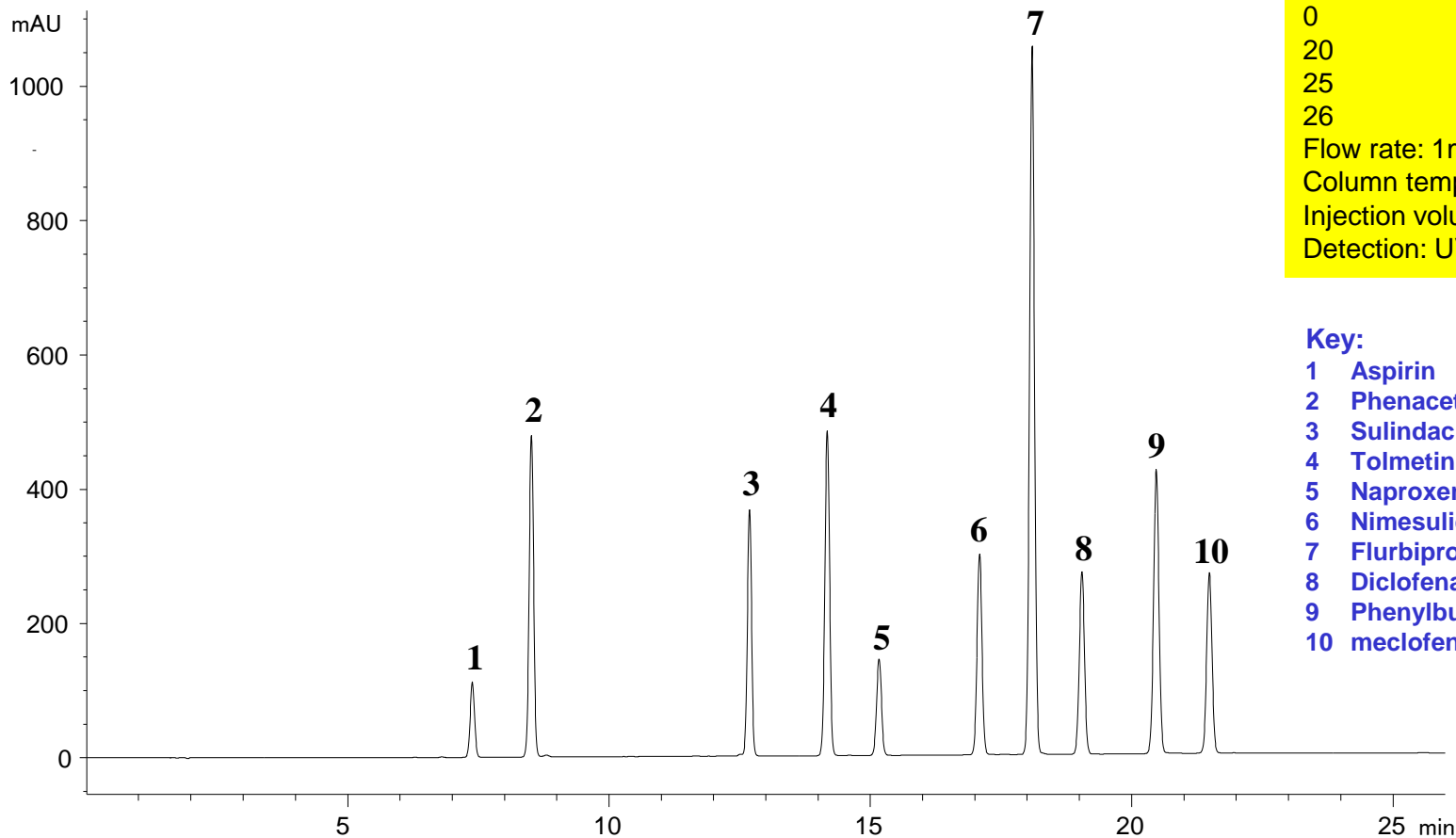
MS/MS conditions alone
insufficient for selective
quantitation

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
TurbolonSpray – negative mode
Extracted ion chromatogram of
MRM m/z 423.3 → 321.1

NSAIDs



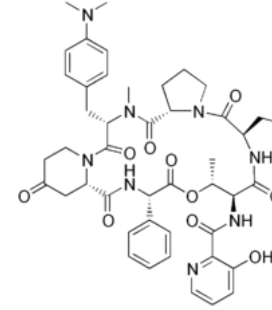
ACE 5 Super C18
 5µm, 150 x 4.6 mm
 Gradient analysis
 A = 0.1% formic acid in water
 B = 0.1% formic acid in acetonitrile
 Time (mins) %B
 0 20
 20 70
 25 70
 26 20
 Flow rate: 1 ml/min
 Column temperature: 40°C
 Injection volume: 10µl
 Detection: UV, 254nm

Key:

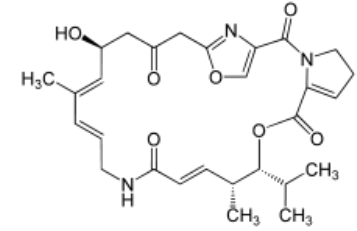
- 1 Aspirin
- 2 Phenacetin
- 3 Sulindac
- 4 Tolmetin
- 5 Naproxen
- 6 Nimesulide
- 7 Flurbiprofen
- 8 Diclofenac
- 9 Phenylbutazone
- 10 meclofenamic acid

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

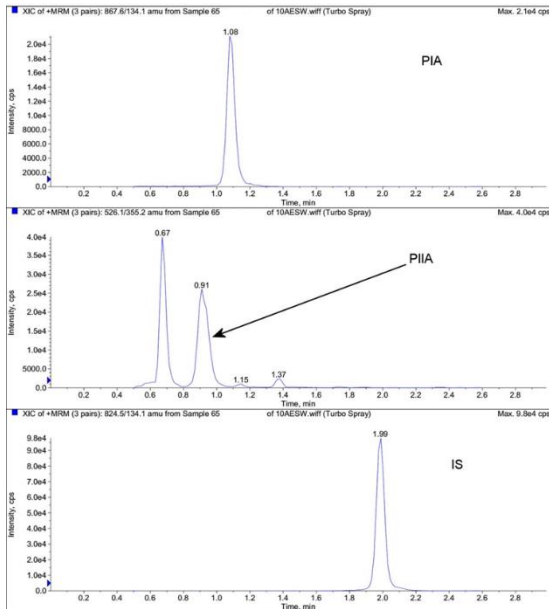


Pristinamycin IA

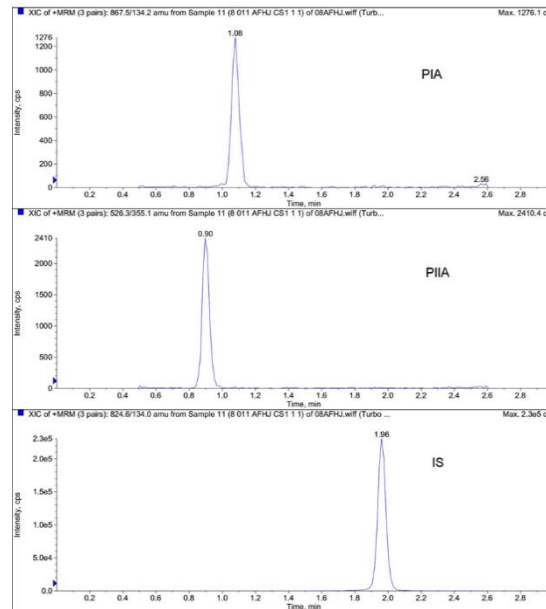


Pristinamycin IIA

Processed study sample containing
pristinamycin IA and IIA



Low calibration standard containing
2.5ng/ml each of pristinamycin IA and IIA
in human NaF/K₂C₂O₄ plasma



ACE 3 C18 3µm, 30 x 3.0mm
Gradient analysis
A = 1mM NH₄CO₂H + 0.1%
HCO₂H in 65:35 H₂O:CH₃CN
B = CH₃CN

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

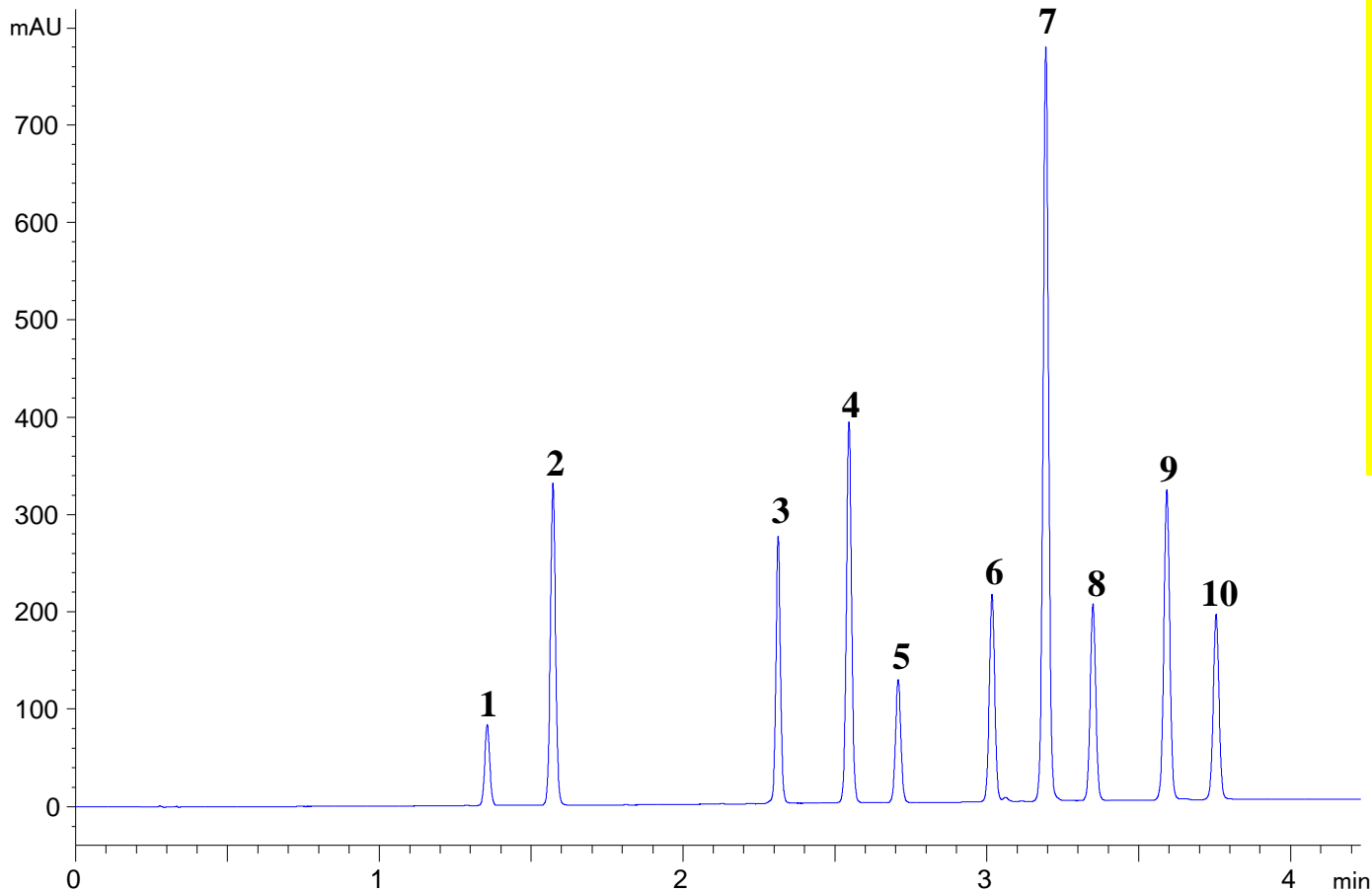
Flow rate: 1ml/min
Column temperature: 25°C
Injection volume: 10µl

MDS Sciex API 4000
TurbolonSpray positive mode

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



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:

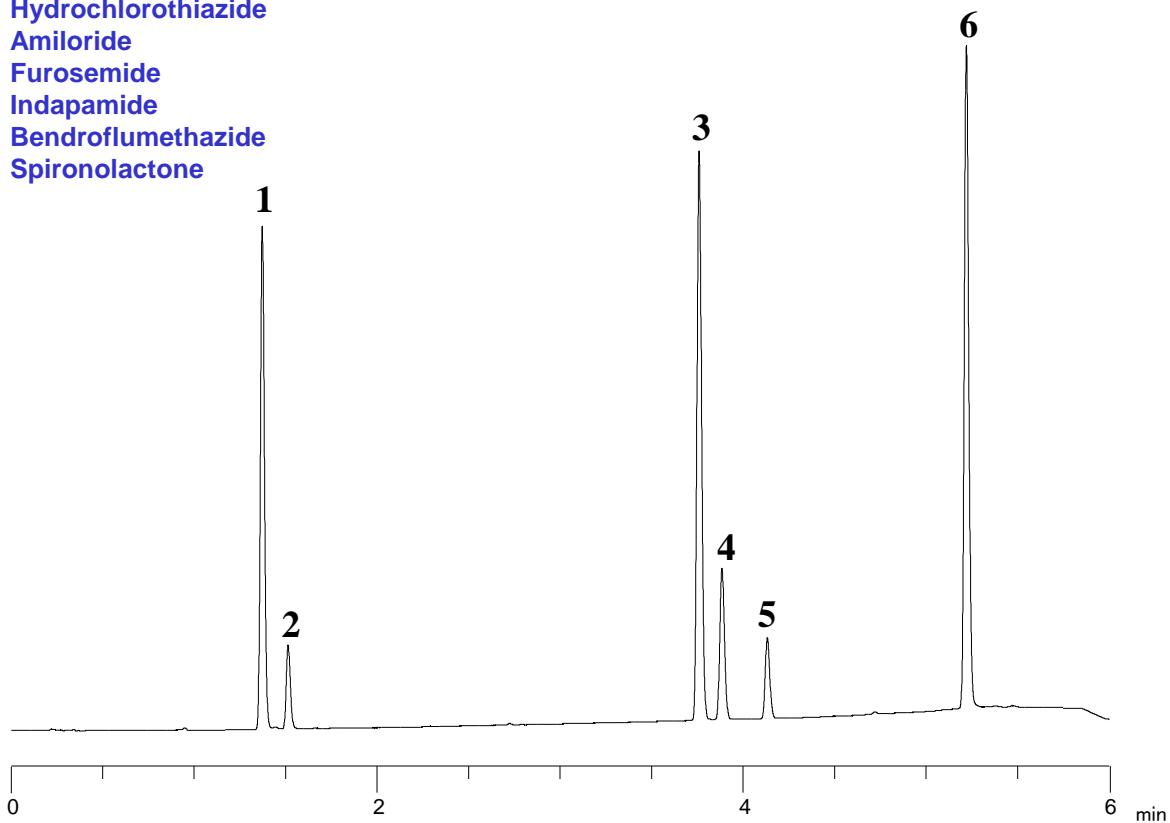
- 1 Aspirin
- 2 Phenacetin
- 3 Sulindac
- 4 Tolmetin
- 5 Naproxen
- 6 Nimesulide
- 7 Flurbiprofen
- 8 Diclofenac
- 9 Phenylbutazone
- 10 meclofenamic acid



Diuretics

Key:

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



ACE Excel C18-PFP

2 μ m, 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

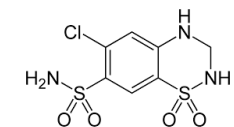
Time (mins)	%B
0	5
0.5	5
5	70
5.5	70
6	5

Flow rate: 1.0ml/min

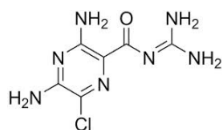
Column temperature: 60°C

Injection volume: 2 μ l

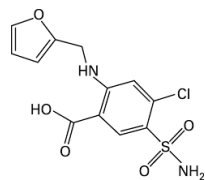
Detection: UV, 254nm



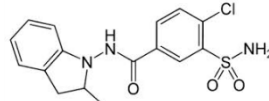
Hydrochlorothiazide



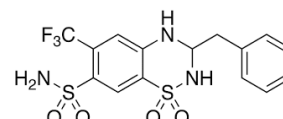
Amiloride



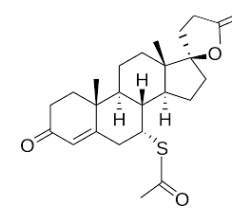
Furosemide



Indapamide



Bendroflumethazide



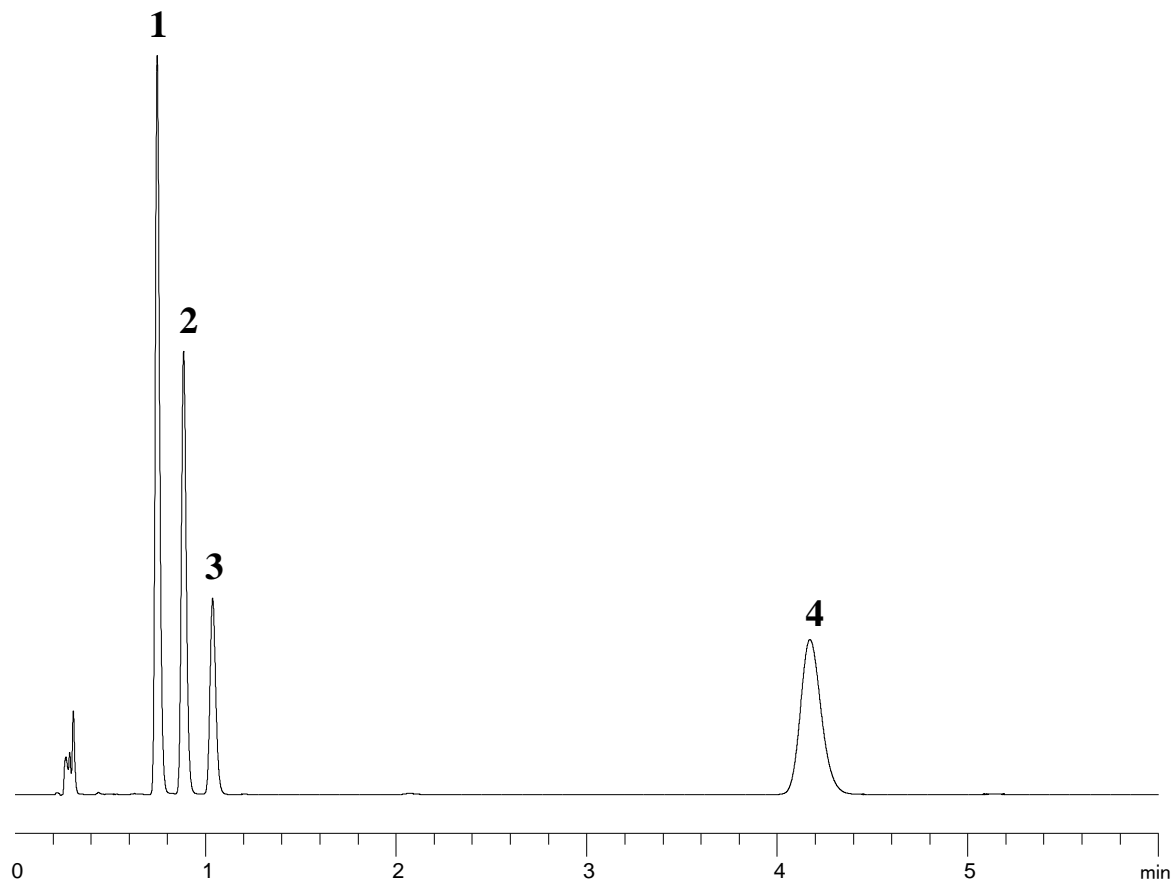
Spironolactone



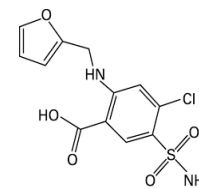
Diuretics (Isocratic)

Key:

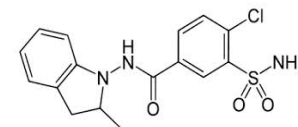
- 1 Furosemide
- 2 Indapamide
- 3 Bendroflumethazide
- 4 Spironolactone



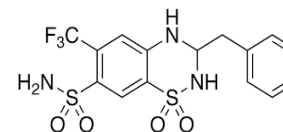
ACE Excel C18-PFP
2 μ m, 50 x 3.0 mm
Isocratic analysis
10 mM ammonium formate pH 3.0
in methanol:water 45:55 v/v
Flow rate: 1.0ml/min
Column temperature: 60°C
Injection volume: 2 μ l
Detection: UV, 254nm



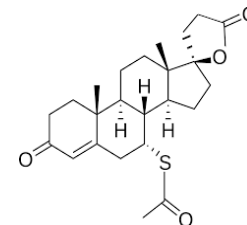
Furosemide



Indapamide



Bendroflumethazide



Spironolactone



Antihistamines

Key:

- 1 Pseudoephedrine
- 2 Scopolamine
- 3 Doxylamine
- 4 Chlorpheniramine
- 5 Triprolidine
- 6 Diphenhydramine
- 7 Acrivastine
- 8 Promethazine
- 9 Fexofenadine
- 10 Cetirizine
- 11 Loratadine

ACE UltraCore SuperC18

2.5µm, 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

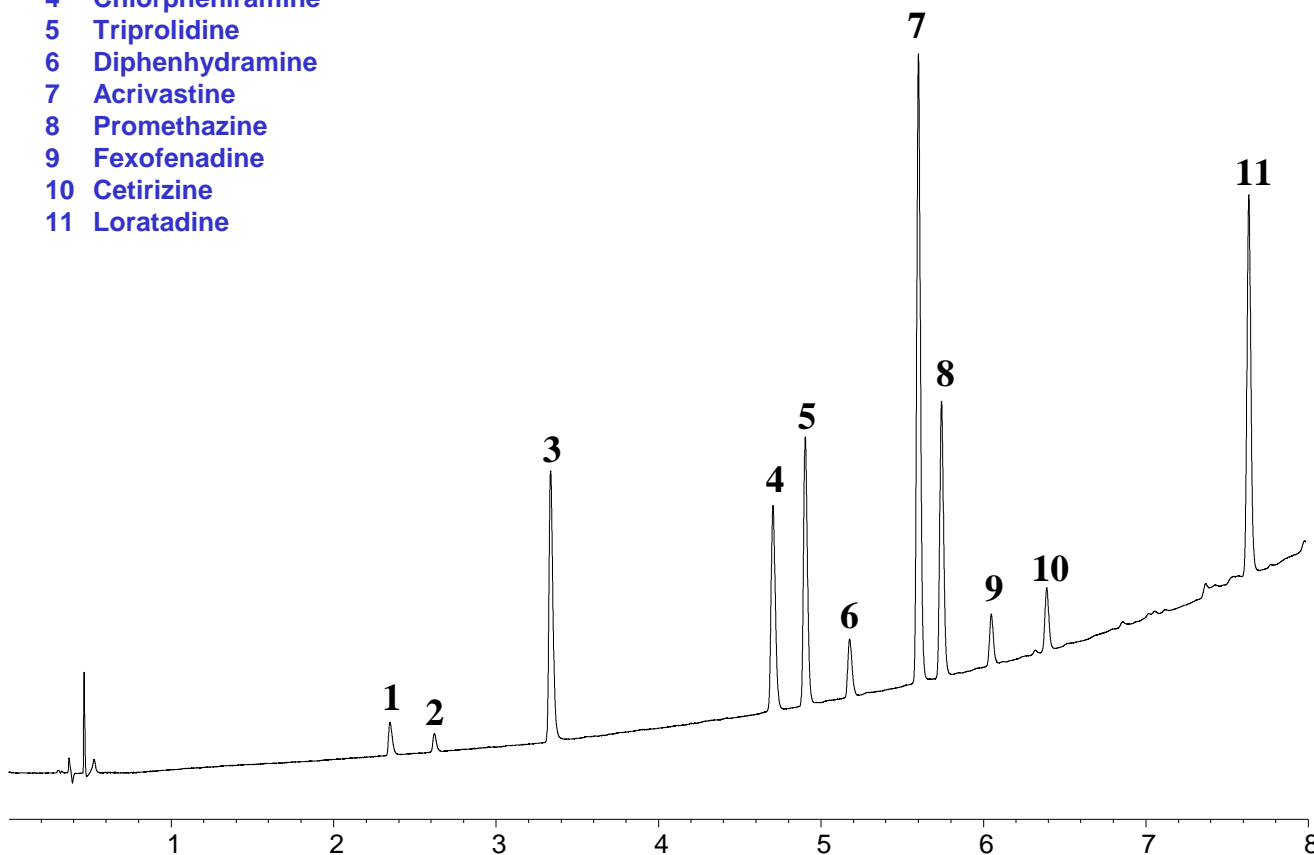
Time (mins)	%B
0	0
7.5	100
8.5	100
9.0	

Flow rate: 1.2ml/min

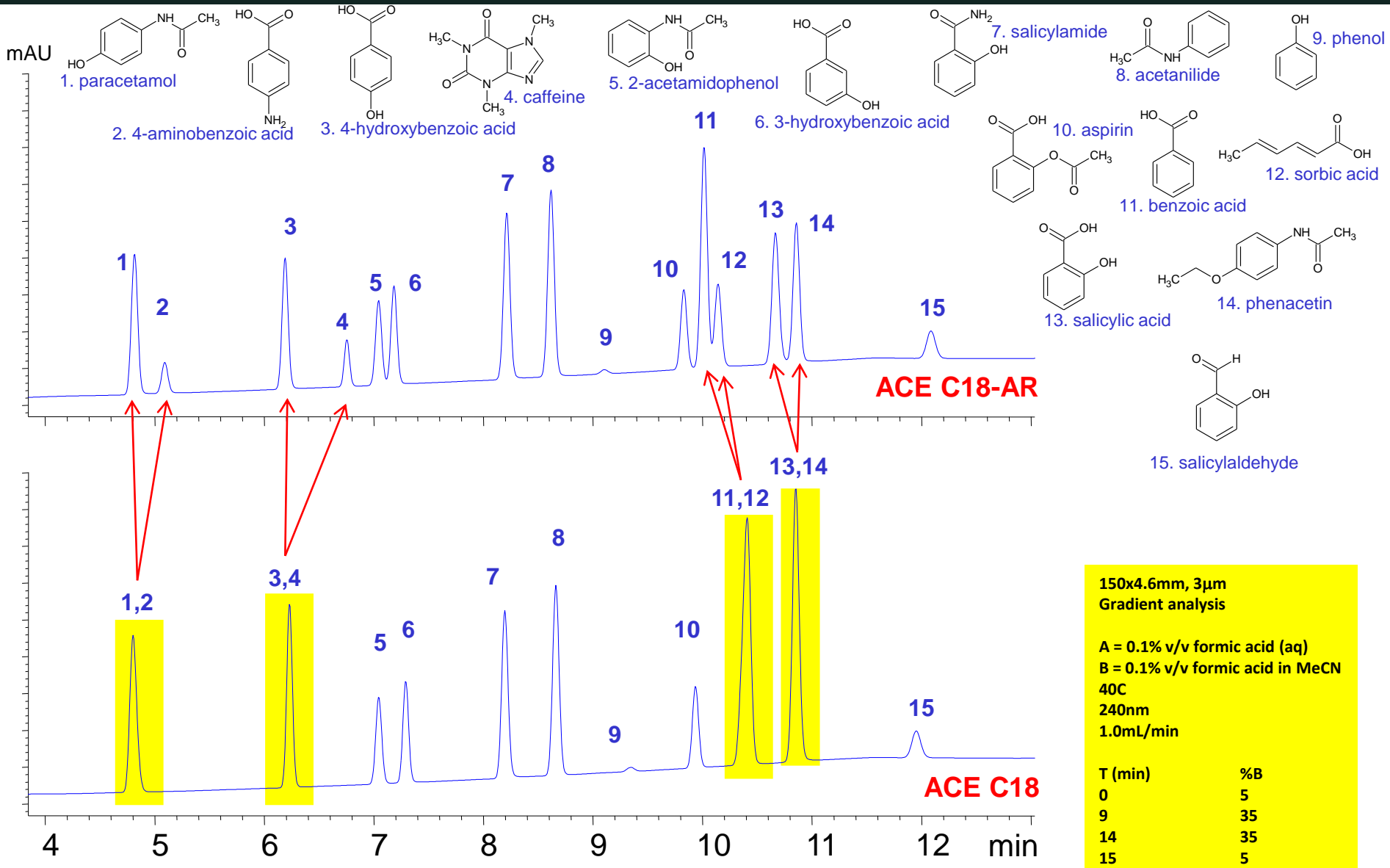
Column temperature: 25°C

Injection volume: 2µl

Detection: UV, 254nm



Analgesics / Cough & Cold Medicine Ingredients



150x4.6mm, 3µm
Gradient analysis

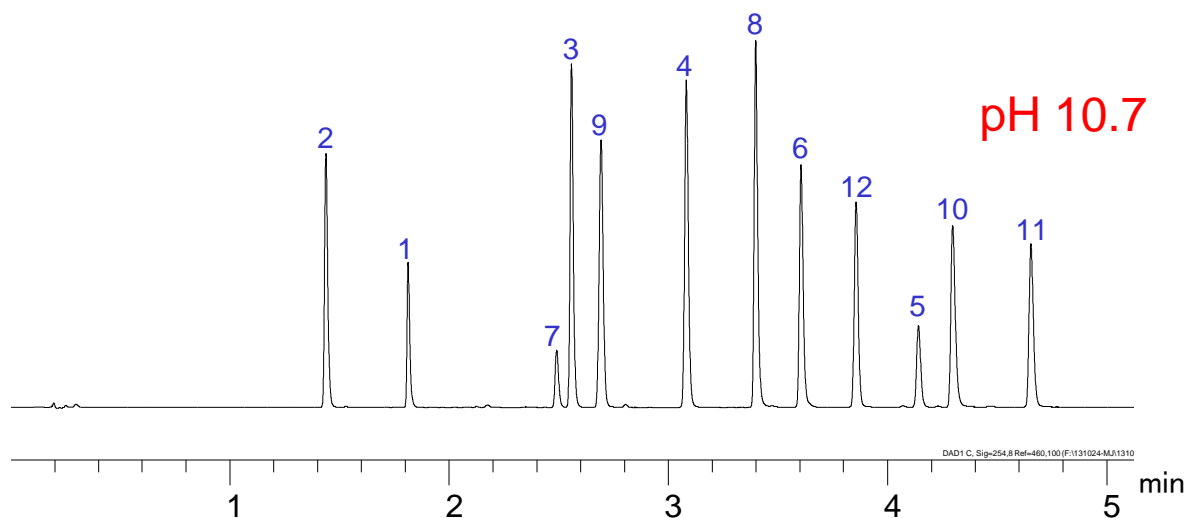
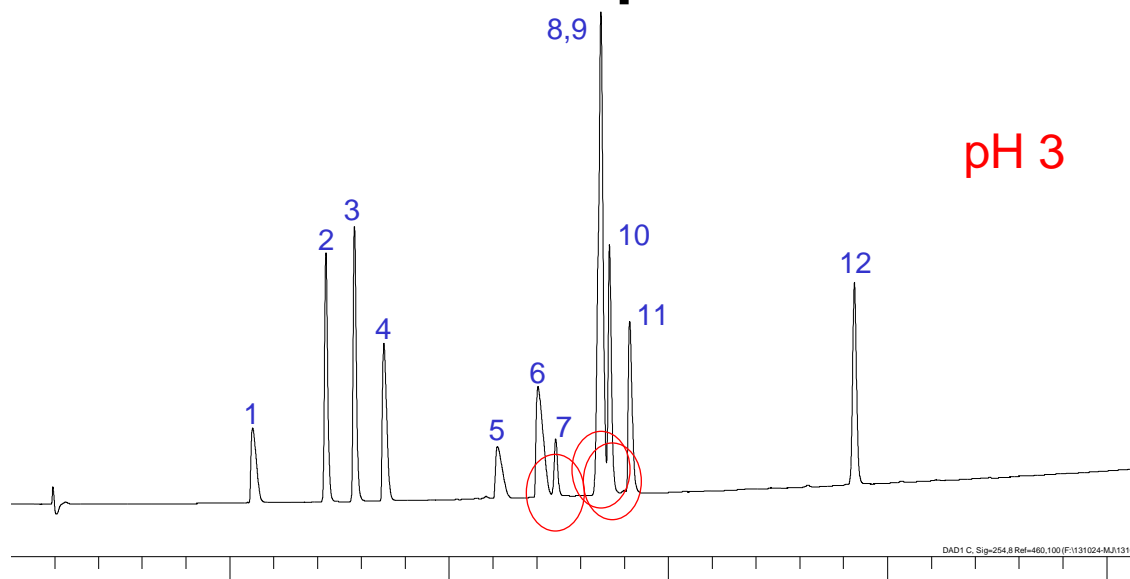
A = 0.1% v/v formic acid (aq)
B = 0.1% v/v formic acid in MeCN
40C
240nm
1.0mL/min

T (min)	%B
0	5
9	35
14	35
15	5



Pharmaceutically Relevant Mixture

◆ ACE UltraCore SuperC18: selectivity with pH:



50x2.1mm, 2.5µm
Gradient analysis

A1= 10mM HCOONH₄, pH3 (aq)
B1= 10mM HCOONH₄, pH 3 in
MeCN/water 9:1 v/v

A2= 0.1% NH₃, pH 10.7 (aq)
B2= 0.1% NH₃, pH10.7 in in
MeCN/water 9:1 v/v

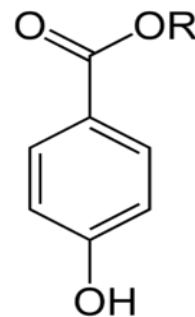
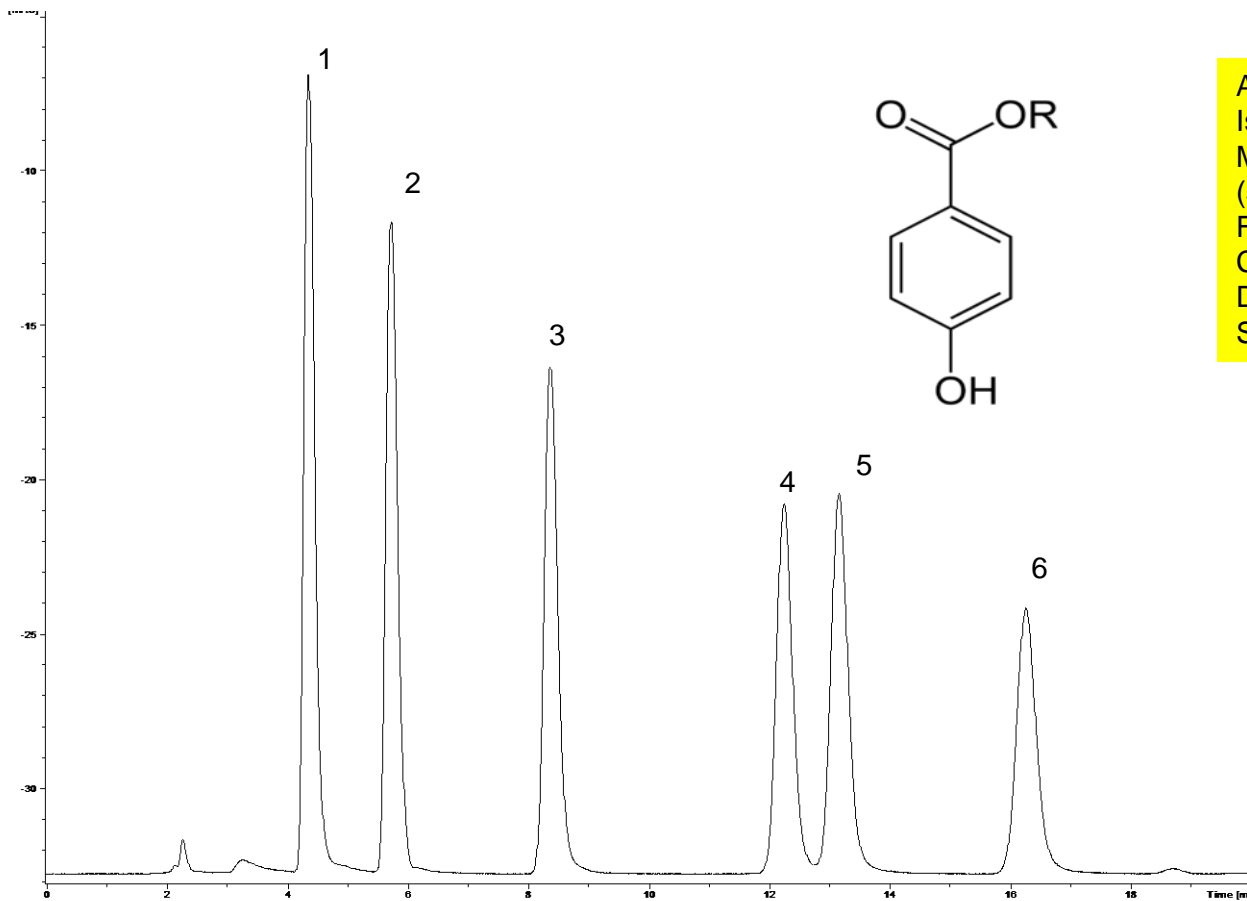
T	%B
0	3
5	100
6	100

40C
0.60 mL/min
254 nm

1. Atenolol
2. Methylphenylsulfoxide
3. Eserine
4. Prilocaine
5. Bupivacaine
6. Tetracaine
7. 1,2,3,4-Tetrahydro-1-naphthol
8. Carvedilol
9. Nitrobenzene
10. Methdilazine
11. Amitriptyline
12. Valerophenone



Paraben Preservatives

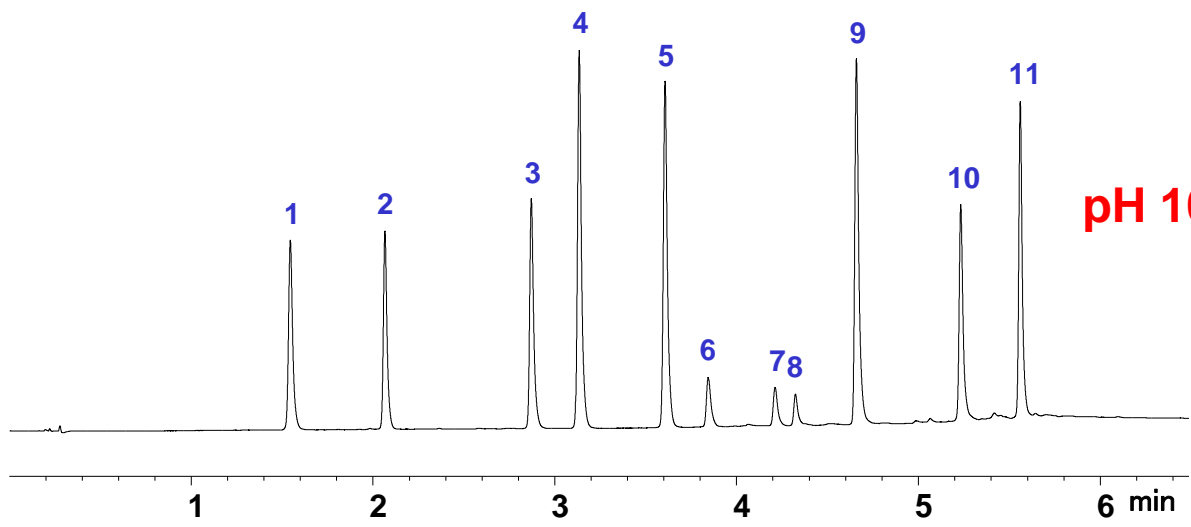
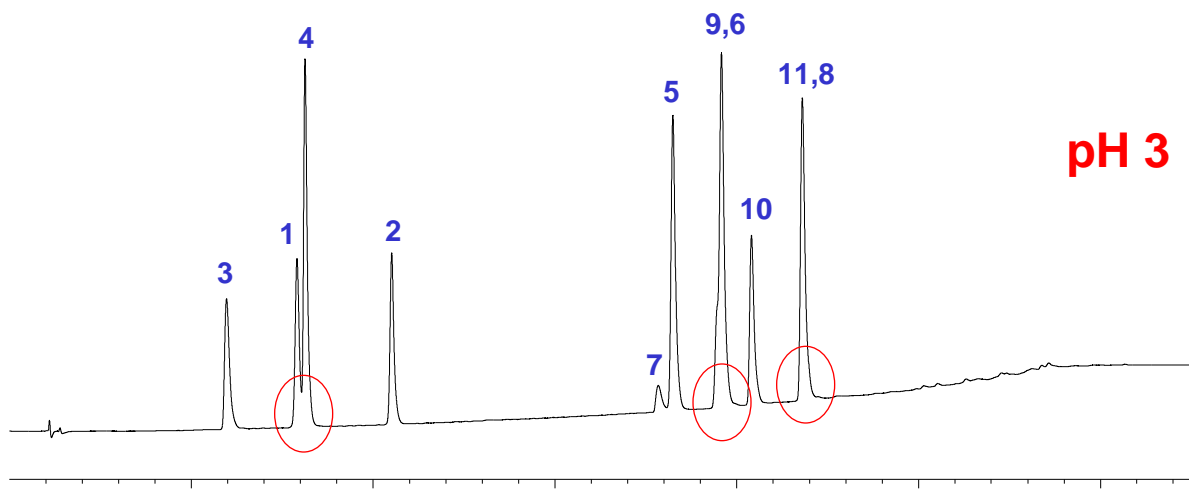


ACE 3 Phenyl, 150 x 2.1mm
Isocratic
Methanol – 25mM NH₄OAc
(50:50), pH 6.8
Flow rate: 0.2ml/min
Column temperature: 40°C
Detection: UV at 240nm
Sample volume: 2µl

1. Methylparaben
2. Ethylparaben
3. n-Propylparaben
4. i-Butylparaben
5. N-Butylparaben
6. Benzylparaben

Pharmaceutically Relevant mixture Separation

◆ ACE UltraCore SuperPhenylHexyl: selectivity with pH:



50x2.1mm, 2.5µm
Gradient analysis

A1= 10mM HCOONH₄, pH3 (aq)
B1= 10mM HCOONH₄, pH 3 in MeOH/water 9:1 v/v

A2= 0.1% NH₃, pH 10.7 (aq)
B2= 0.1% NH₃, pH10.7 in in MeOH/water 9:1 v/v

T	%B
0	3
5	100
7	100

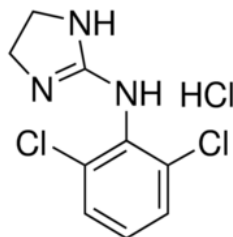
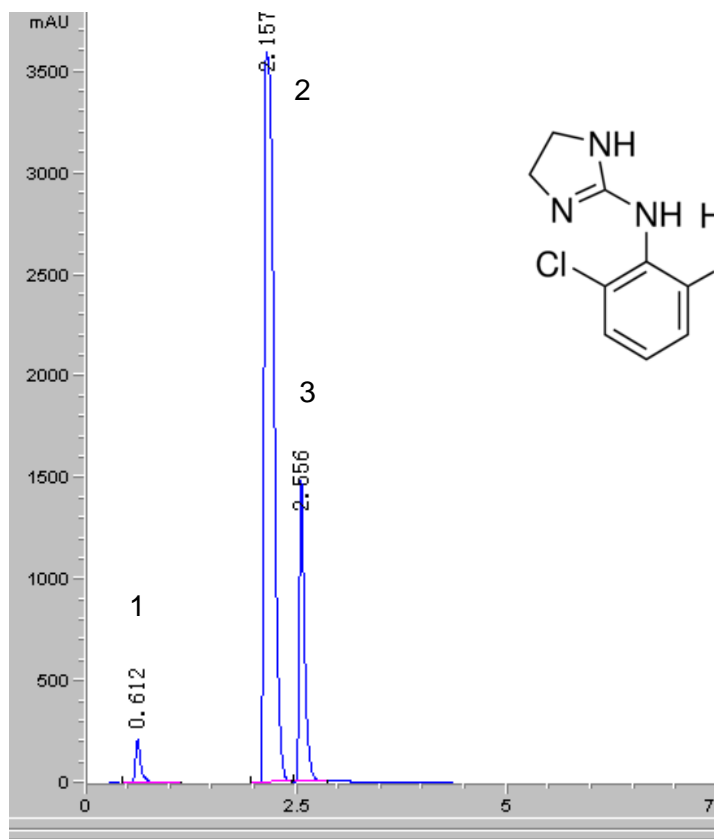
40 °C
0.60 mL/min
254 nm

1. Benzamide
2. Caffeine
3. Procainamide
4. N-acetylprocainamide
5. Propiophenone
6. Toluene
7. Remacemide
8. Ethylbenzene
9. Carvdilol
10. Nortriptyline
11. Clomipramine



Clonidine Hydrochloride

Clonidine hydrochloride oral solution containing preservatives



Concentration clonidine HCl = 10µg/ml
Concentration Preservatives ~ 1.5g/ml

1. Clonidine hydrochloride
2. Methyl hydroxybenzoate
3. Propyl hydroxybenzoate

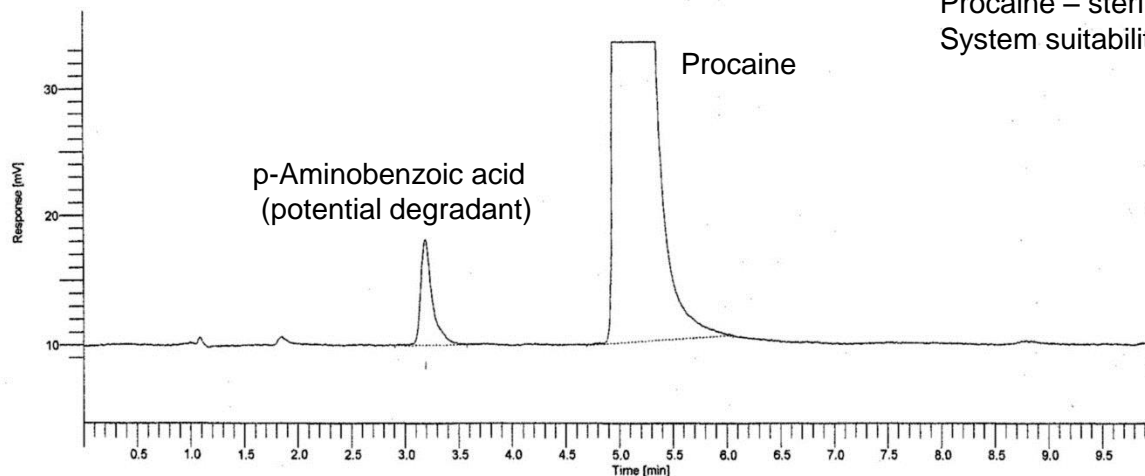
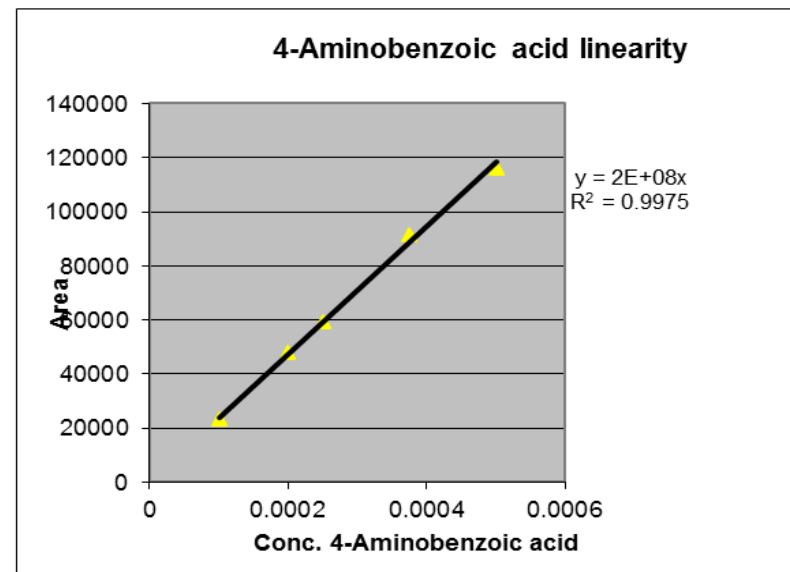
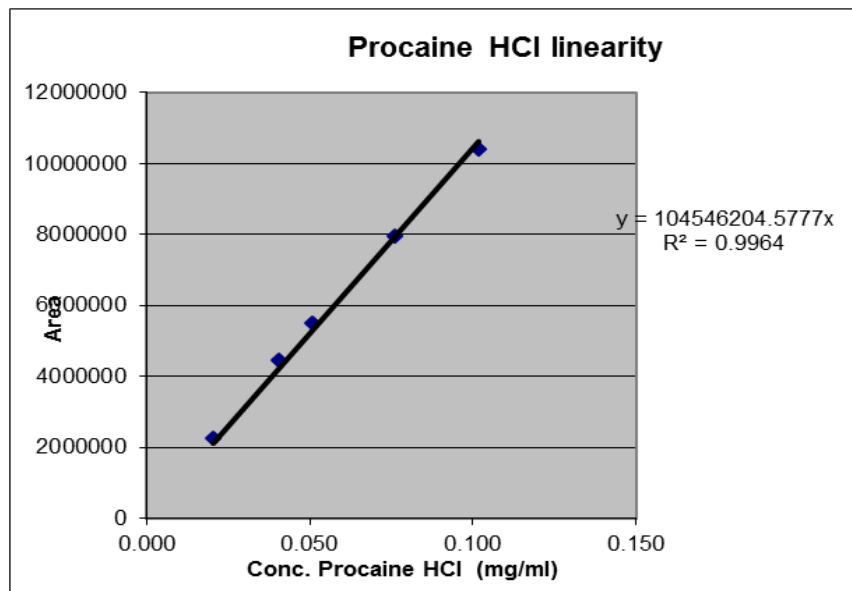
ACE UltraCore SuperC18
2.5µm, 50 x 4.6mm
Gradient analysis
A = 0.2% w/v phosphate buffer-CH₃OH-
CH₃CN (80:10:10)
B = CH₃CN

Time (mins)	%B
0	0
0.8	0
2.1	70
3.4	70
3.5	0

Flow rate: 2ml/min
Column temperature: 20°C
Injection volume: 100µl
Detection: UV, 220nm



Procaine and p-Aminobenzoic acid



Procaine – sterile manufactured product
System suitability tests

ACE C18-PFP 3 μ m, 100 x 4.6mm
Isocratic analysis
Methanol - 0.6% acetic acid (19:81)
(adjusted to pH 4.7 with 20% NaOH)
Flow rate: 1ml/min
Detection: UV, 179nm

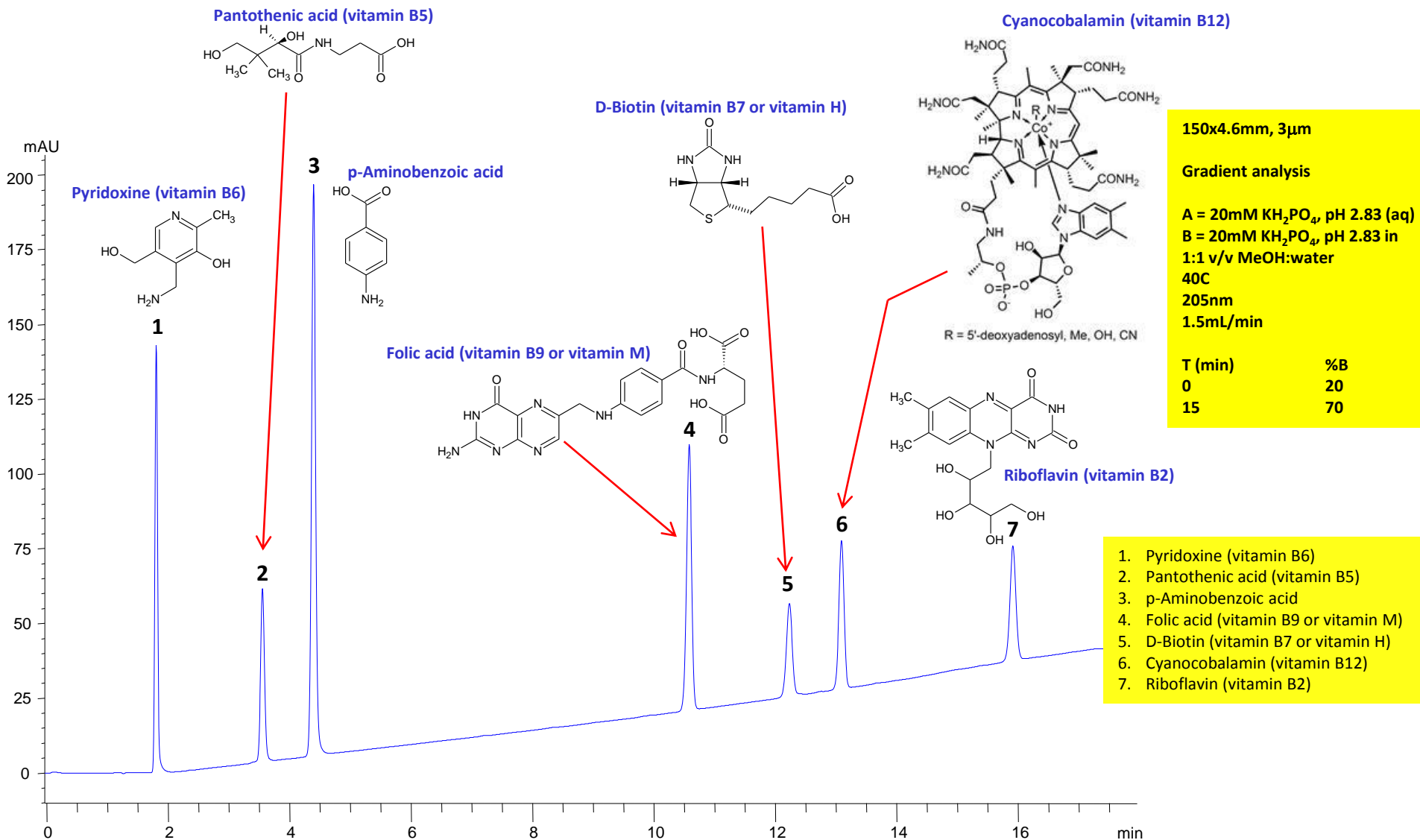
Detection limits for S/N = 10
Procaine = 0.0002mg/ml
p-Aminobenzoic acid = 0.00005mg/ml

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Water Soluble Vitamins

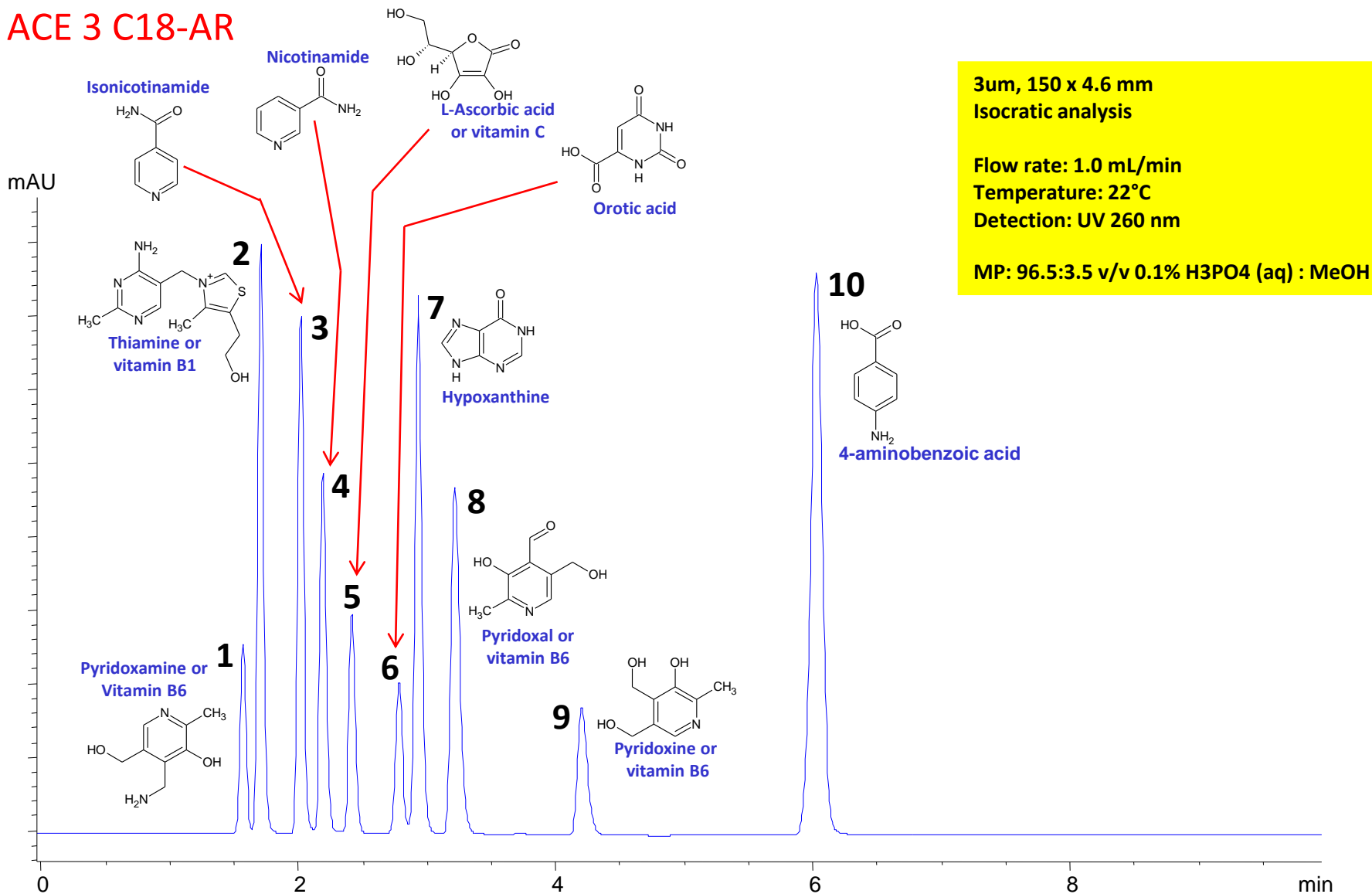
ACE 3 C18-AR





Water Soluble Vitamins / Polar Molecules

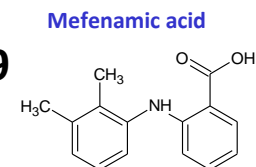
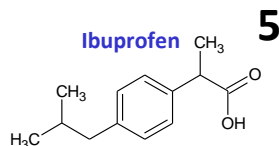
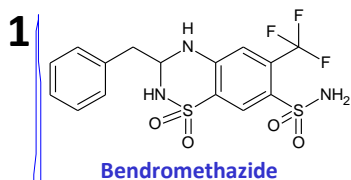
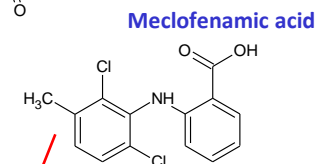
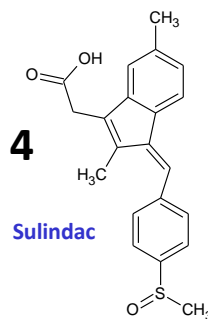
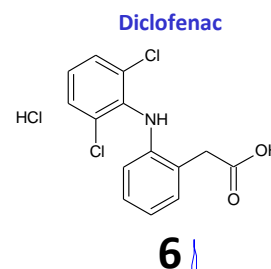
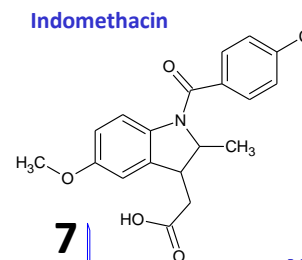
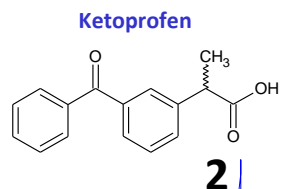
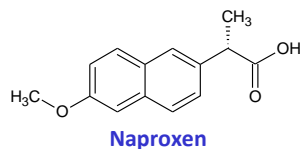
ACE 3 C18-AR





Non-Steroidal Anti-Inflammatory Drugs

ACE 3 C18-AR



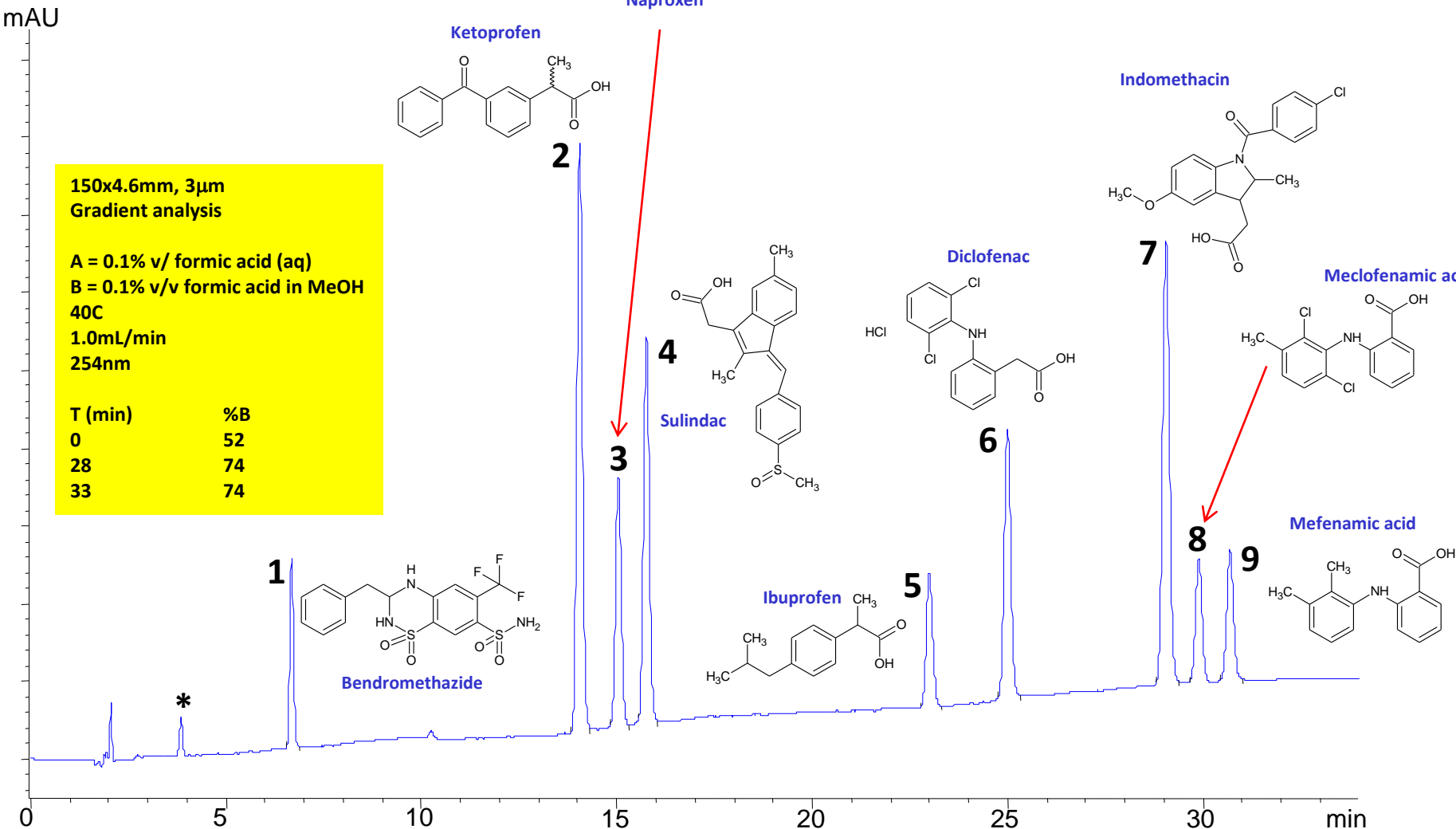
mAU

150x4.6mm, 3µm
Gradient analysis

A = 0.1% v/v formic acid (aq)
B = 0.1% v/v formic acid in MeOH
40C
1.0mL/min
254nm

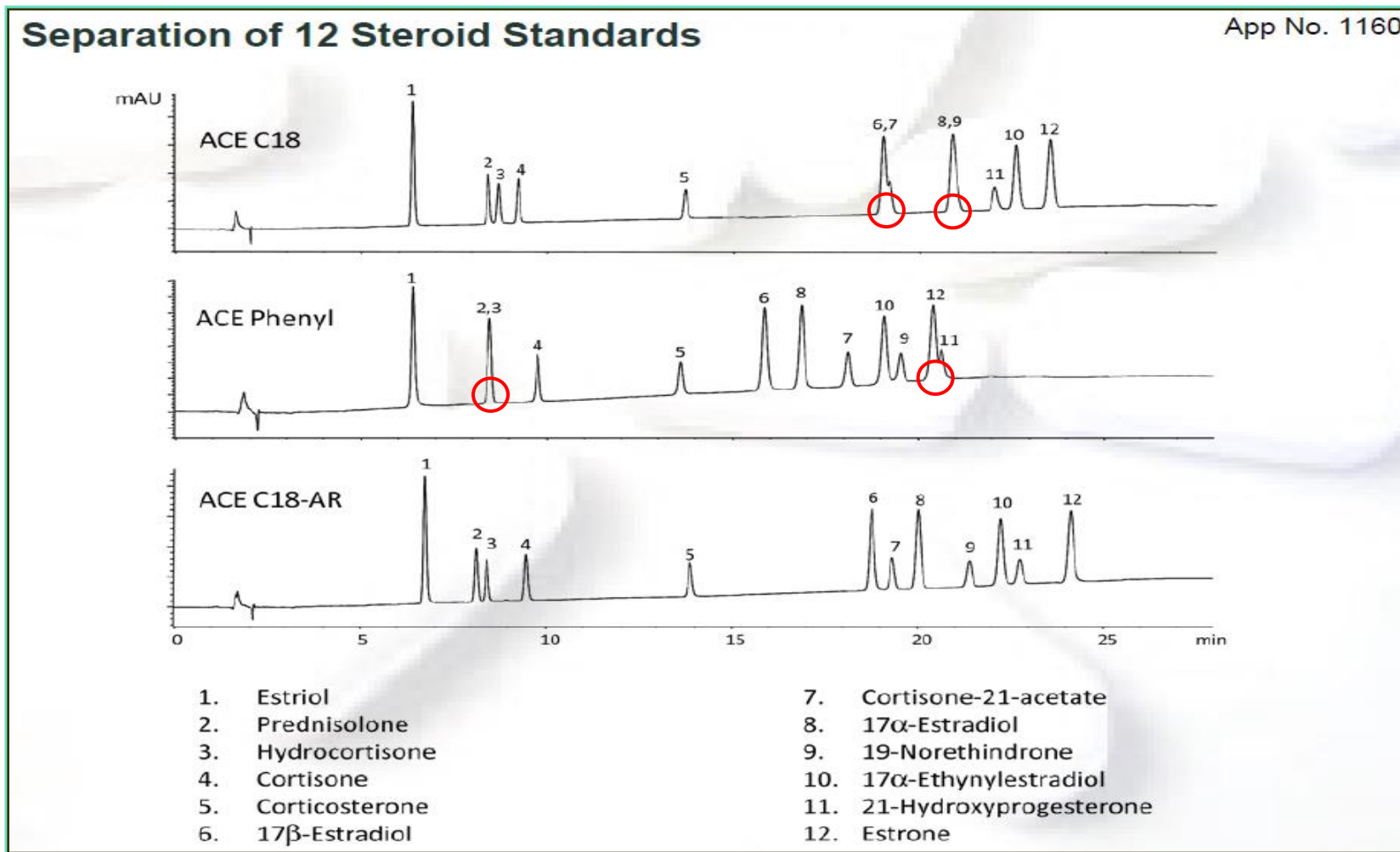
T (min)	%B
0	52
28	74
33	74

0 5 10 15 20 25 30 min



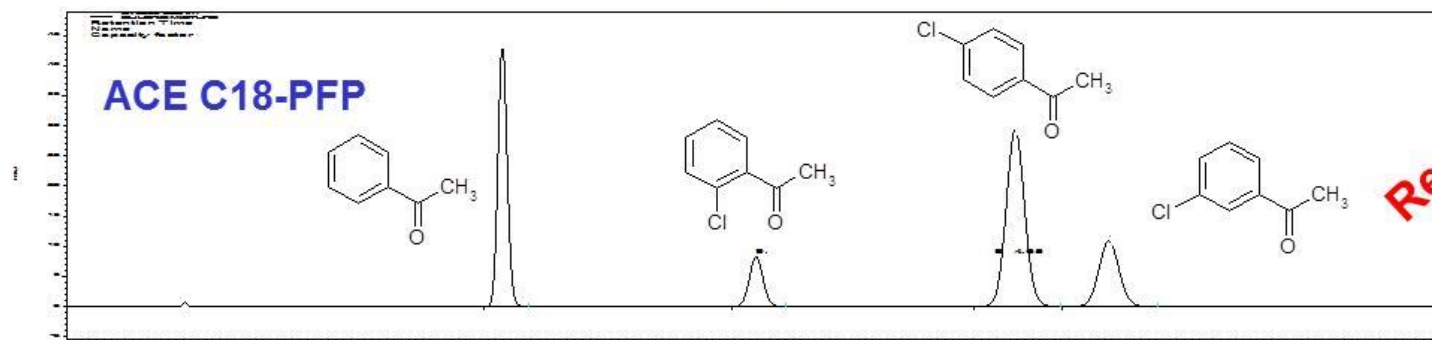
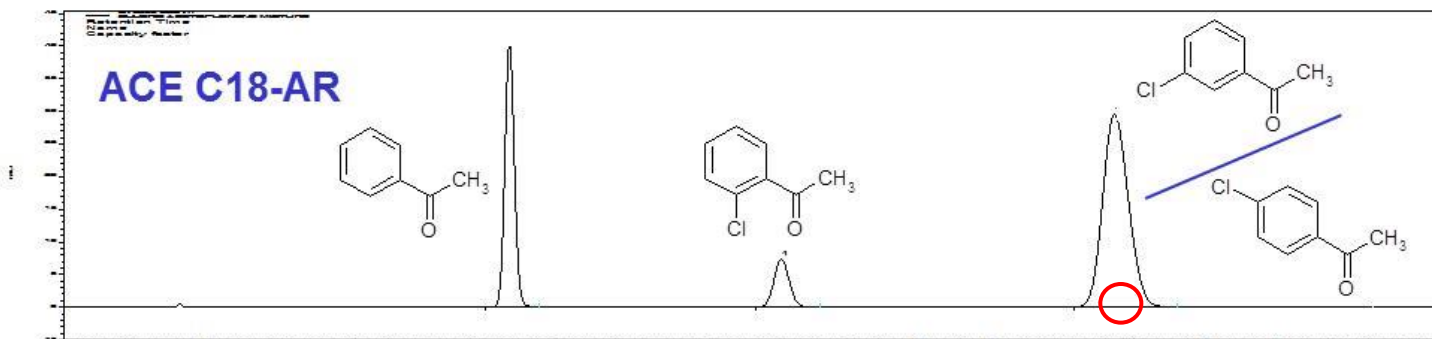
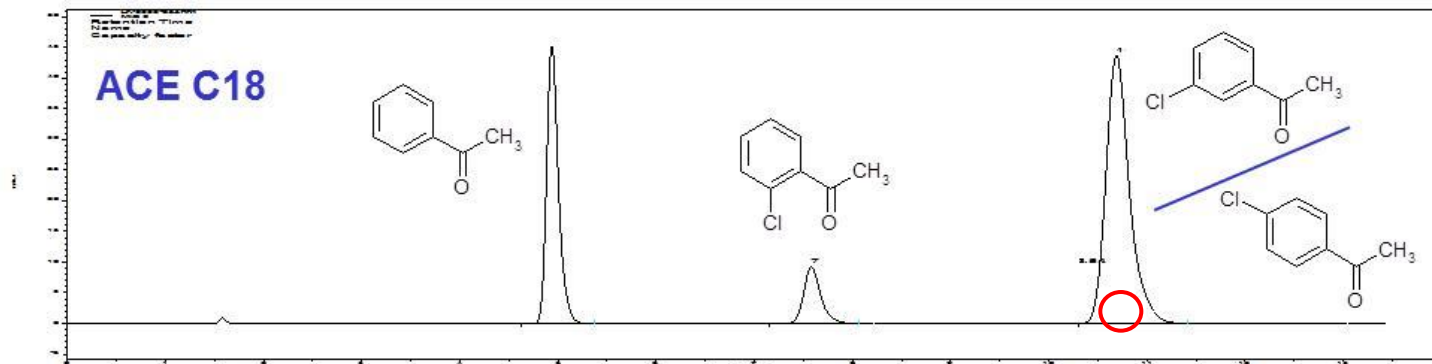
Complex Steroid Mixture Separation

ACE 3 C18-AR



ACE® C18-PFP™: Halogenated Positional Isomers

◆ Chloroacetophenone halogenated isomers separation

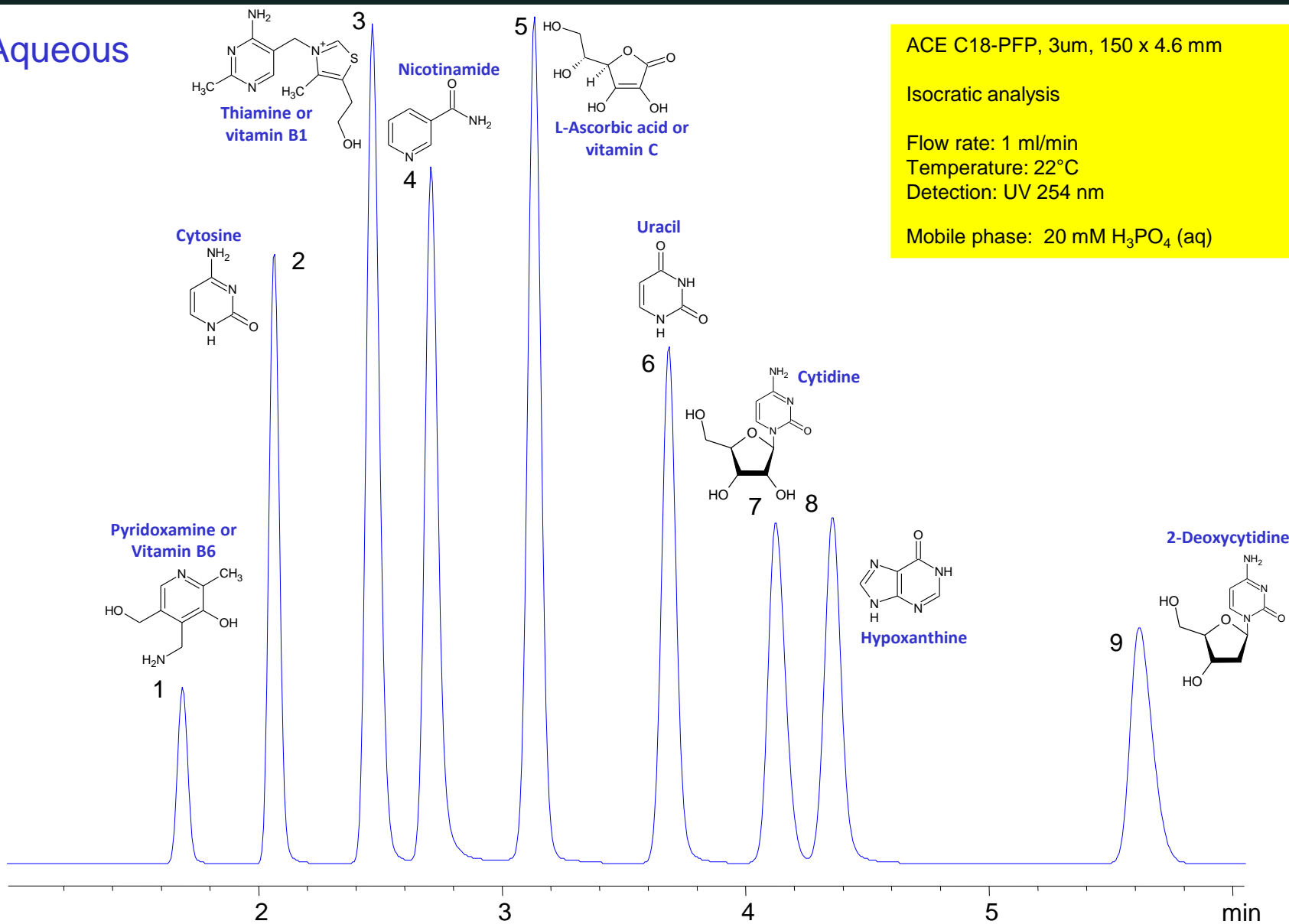


**Regioisomer
Selectivity**



Polar Analytes – Nucleosides & Vitamins

100% Aqueous





Separation of Sulphonamides

ACE Excel C18-PFP

3 μ m, 150 x 4.6mm

Gradient analysis

A = Water

B = Acetonitrile

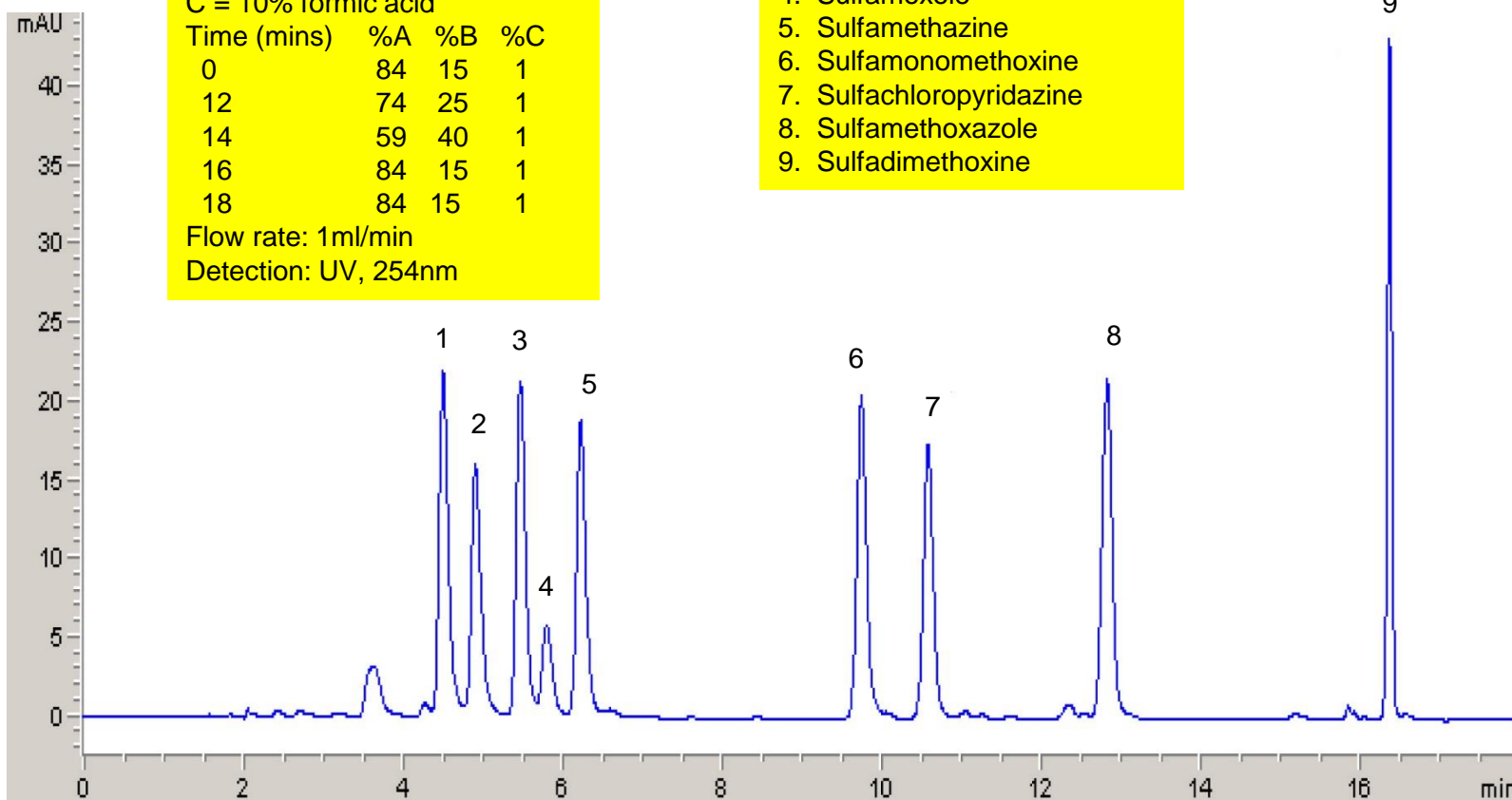
C = 10% formic acid

Time (mins)	%A	%B	%C
0	84	15	1
12	74	25	1
14	59	40	1
16	84	15	1
18	84	15	1

Flow rate: 1ml/min

Detection: UV, 254nm

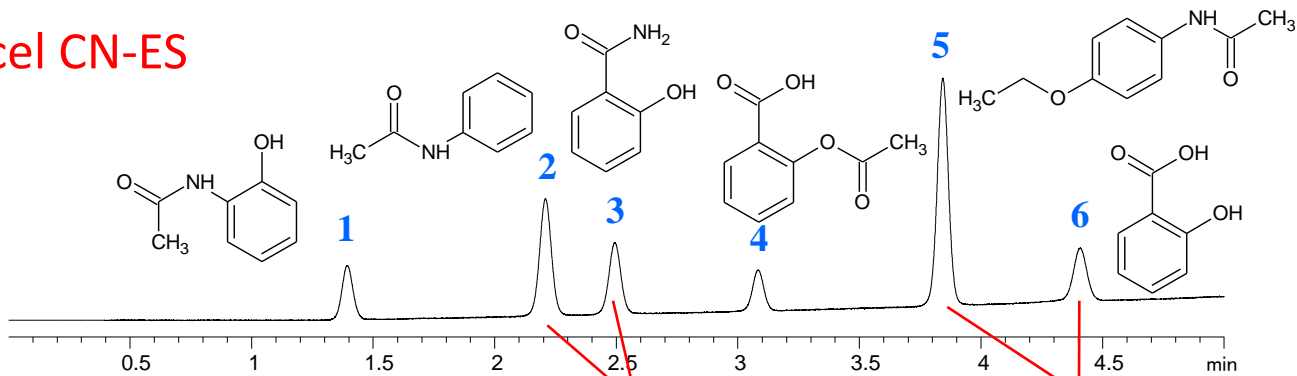
1. Sulfadiazine
2. Sulfapyridine
3. Sulfamerazine
4. Sulfamoxole
5. Sulfamethazine
6. Sulfamonomethoxine
7. Sulfachloropyridazine
8. Sulfamethoxazole
9. Sulfadimethoxine



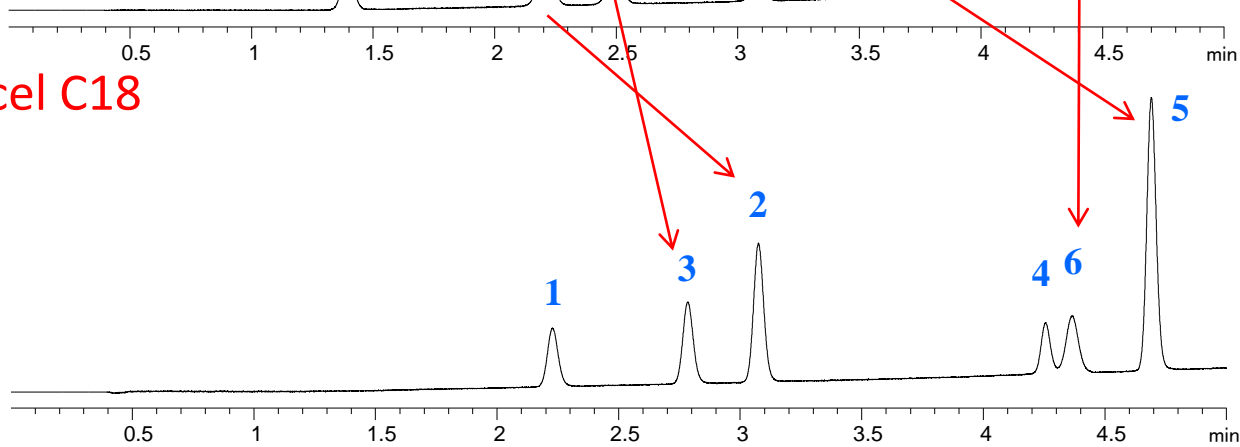
Aspirin And Related Substances

Alternative selectivity with MeOH containing eluents

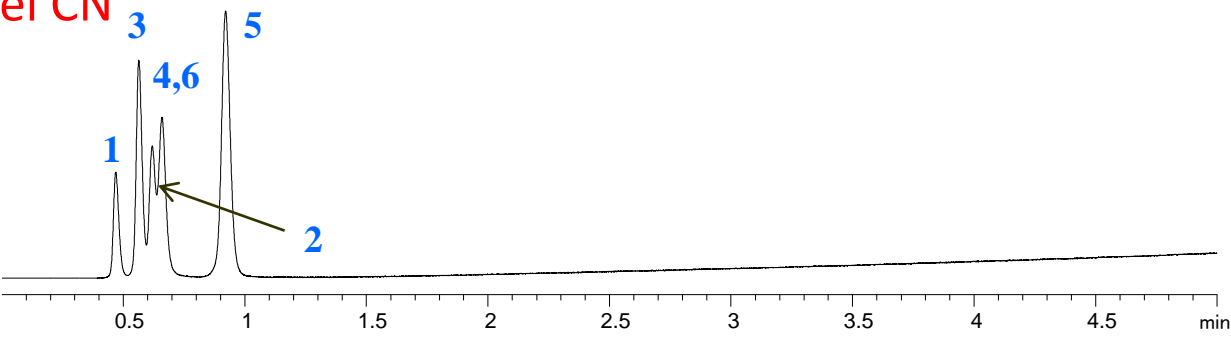
ACE Excel CN-ES



ACE Excel C18



ACE Excel CN



ACE CN-ES
 50x2.1mm, 3µm
 Gradient analysis

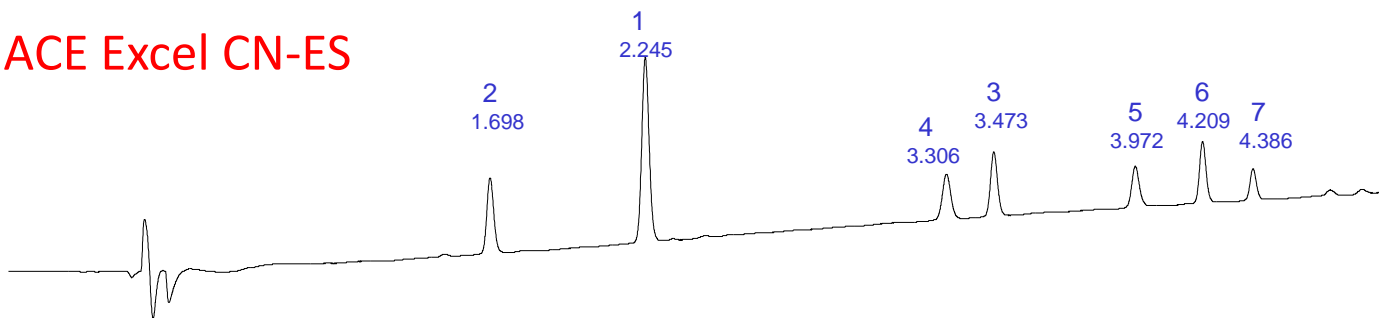
A = 0.1% formic acid
 B = 0.1% FA in MeOH
 40 °C
 0.6 mL/min
 240 nm
 5 – 38% B in 3.75 mins
 Hold at 38% B until 5 mins

1. 2-Acetamidophenol
2. Acetanilide
3. Salicylamide
4. Aspirin
5. Phenacetin
6. Salicylic acid

Small Pharmaceutically Relevant Analytes

Dipole + hydrophobic Interactions

ACE Excel CN-ES

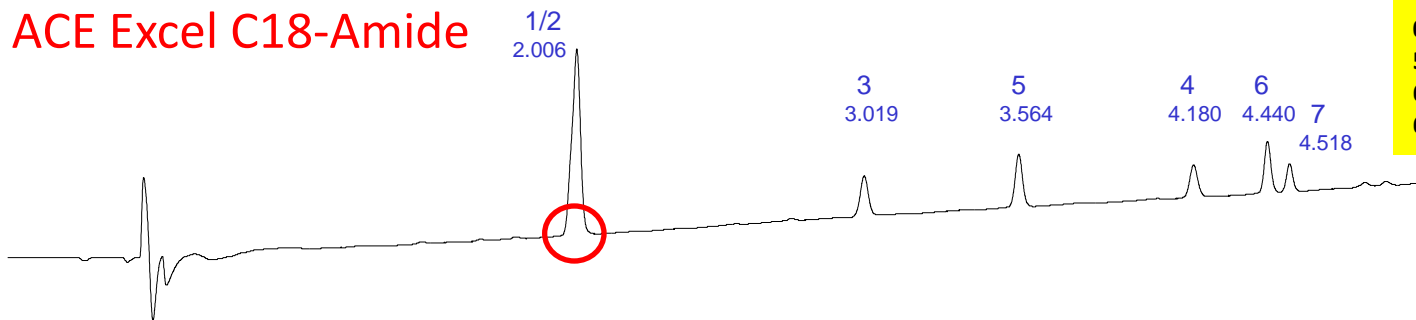


ACE CN-ES
100x3mm, 3µm
Gradient analysis

A = 20mM ammonium formate (aq)
B = 20mM ammonium formate in MeOH
40C
0.6 mL/min

Polar embedded + hydrophobic Interactions

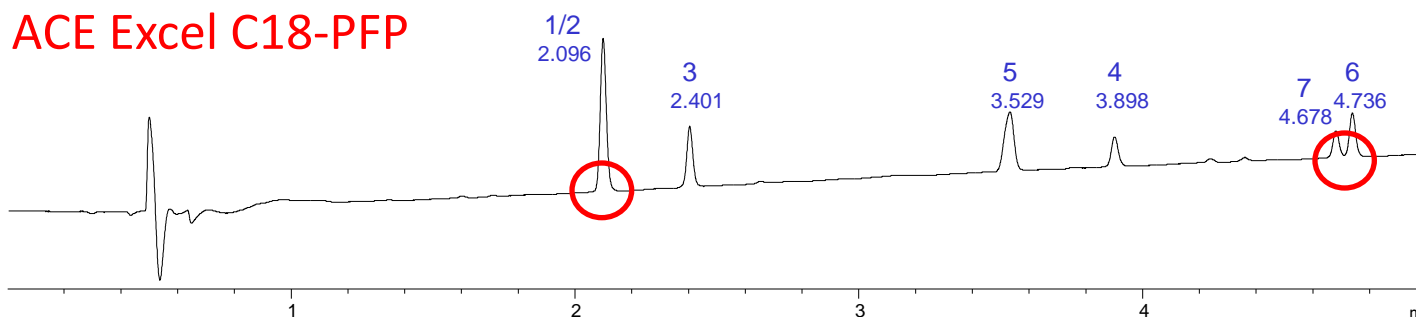
ACE Excel C18-Amide



T (min)	%B
0	3
5	100
6	100
6.5	3

Hydrophobic + π-π + dipole + shape / position Interactions

ACE Excel C18-PFP

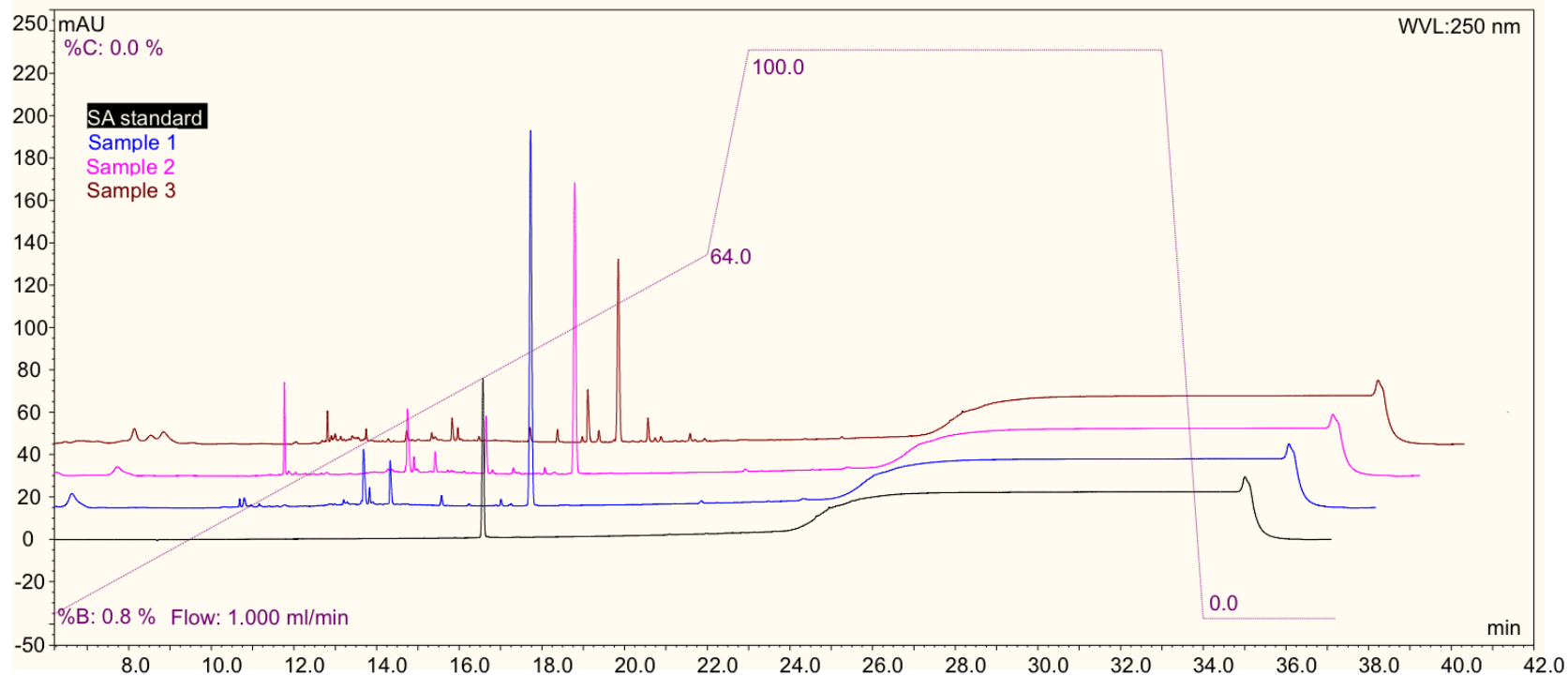


1. Hydrochlorothiazide
2. Methylphenylsulphoxide
3. 1,3,5 Trinitrobenzene
4. Myricetin
5. P-Cresol
6. Sulindac
7. Toluene



Salicylic acid in cell extracts

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



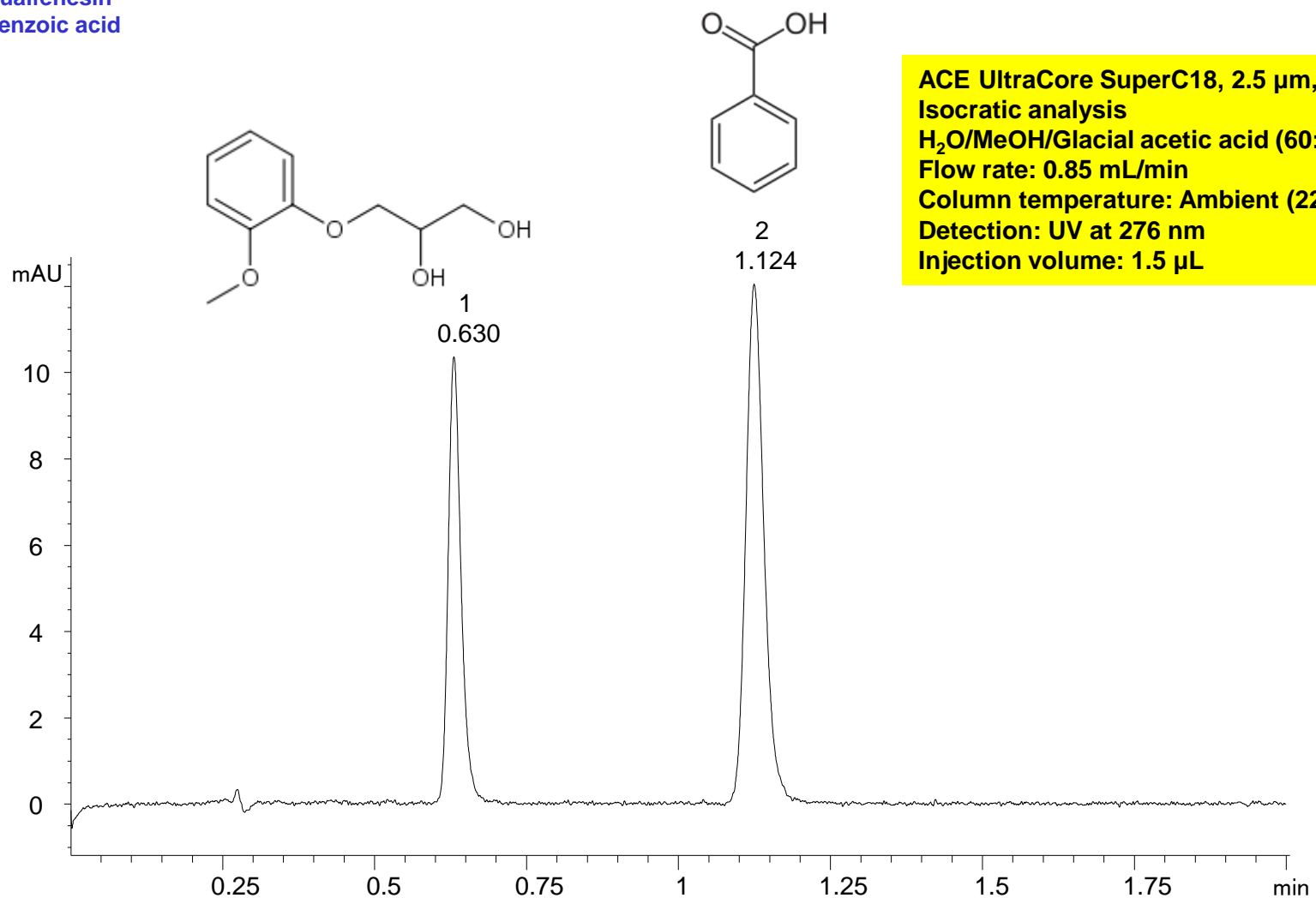
ACE UltraCore SuperC18, 5 μ m, 150 x 4.6mm
 Gradient analysis (shown above)
 A = 0.1% TFA in water
 B = 0.1% TFA in acetonitrile
 Flow rate: 1ml/min
 Detection: UV, 250nm



USP Guaifenesin

Key:

- 1 Guaifenesin
- 2 Benzoic acid



ACE UltraCore SuperC18, 2.5 μ m, 50 x 3.0 mm
Isocratic analysis
H₂O/MeOH/Glacial acetic acid (60:40:1.5 v/v)
Flow rate: 0.85 mL/min
Column temperature: Ambient (22°C)
Detection: UV at 276 nm
Injection volume: 1.5 μ L

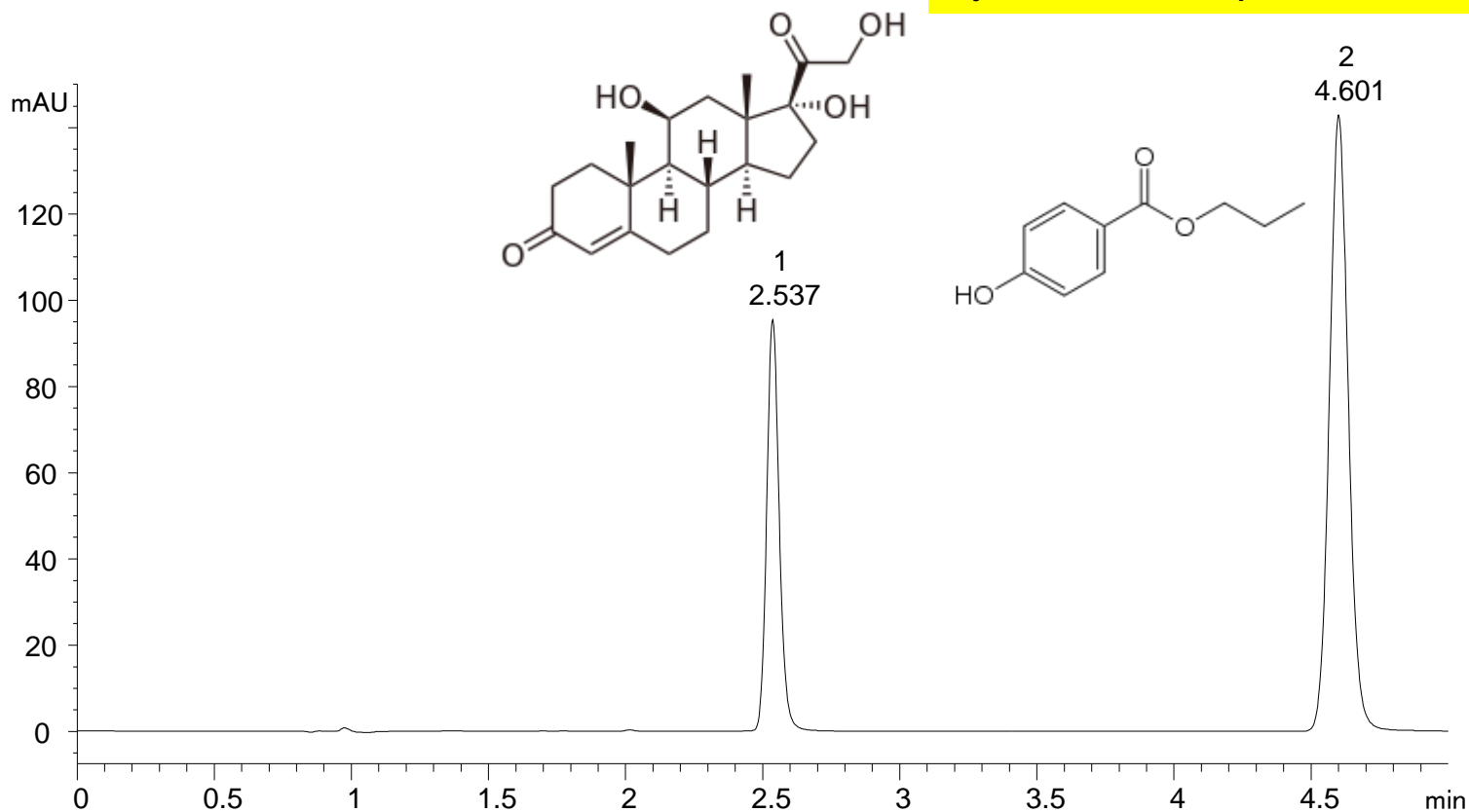


USP Hydrocortisone

Key:

- 1 Hydrocortisone
- 2 Propylparaben

ACE Ultracore SuperC18, 2.5 μ m, 100 x 4.6 mm
Isocratic analysis
MeCN/MeOH/H₂O (25:25:50 v/v)
Flow rate: 1 mL/min
Column temperature: Ambient (22°C)
Detection: UV at 253 nm
Injection volume: 5.8 μ L

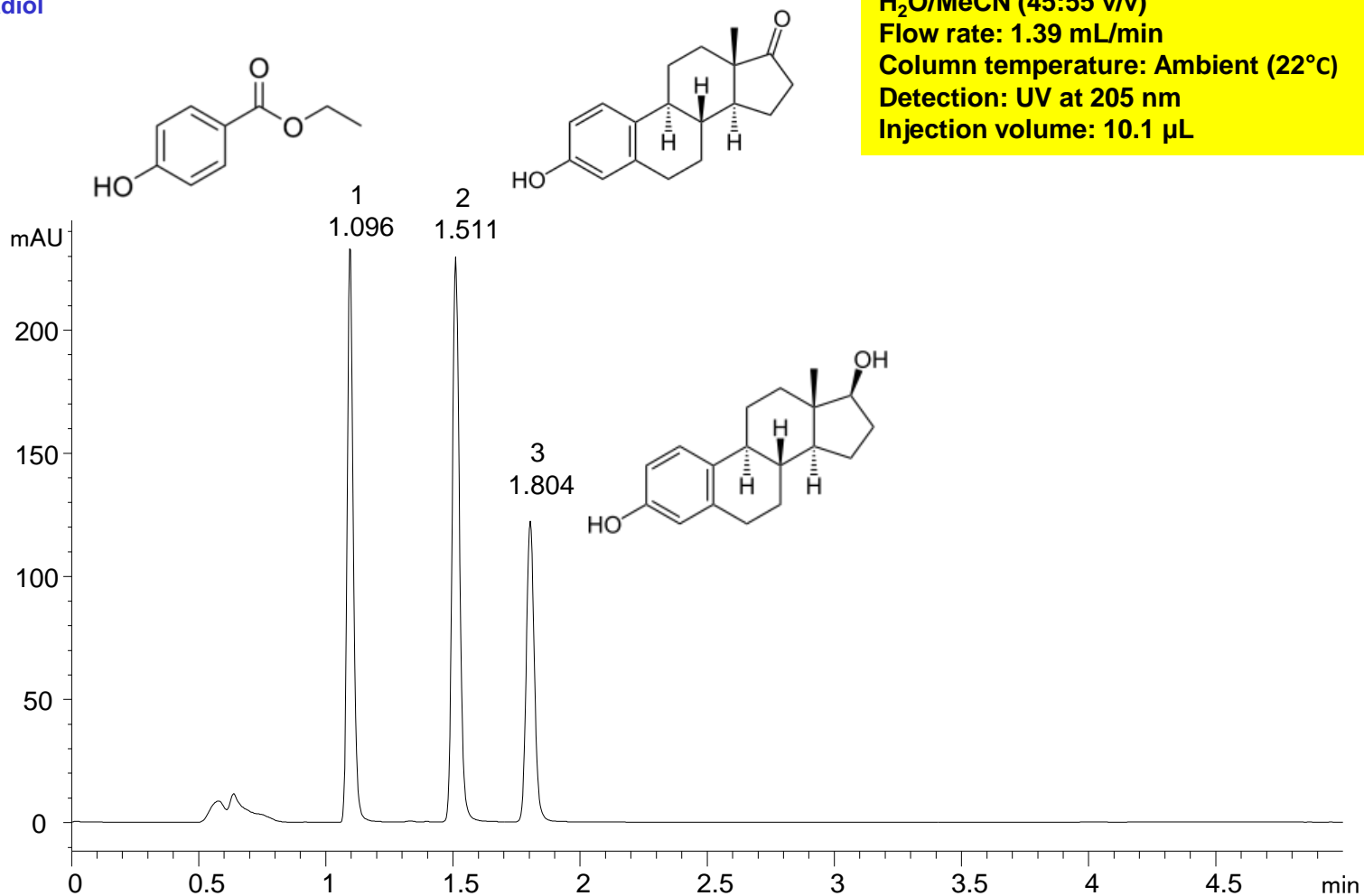




USP Estradiol

Key:

- 1 Ethylparaben
- 2 Estrone
- 3 Estradiol



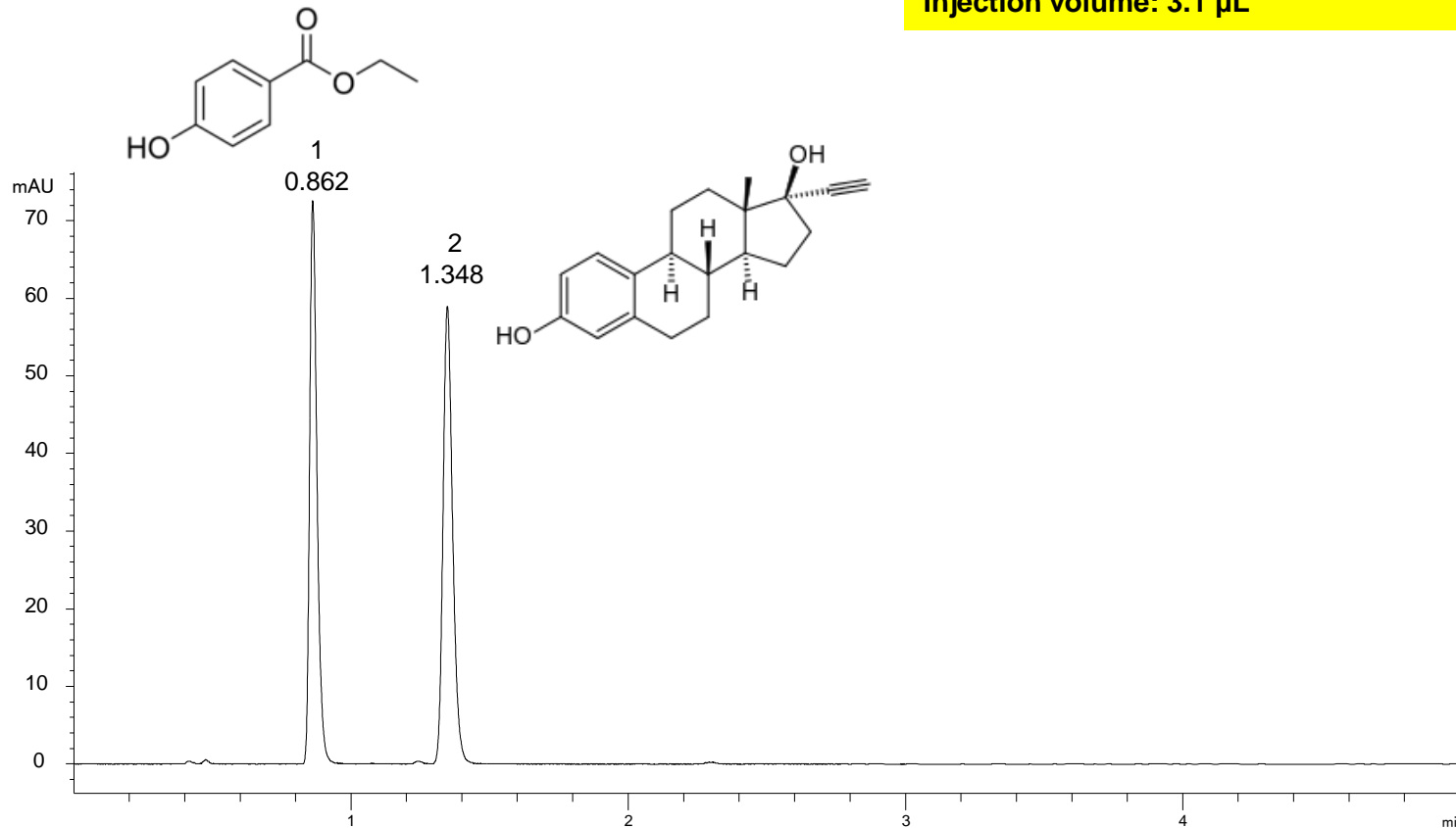


USP 17 α -Ethinylestradiol

Key:

- 1 Ethylparaben
- 2 17 α -Ethinylestradiol

ACE Ultracore SuperC18, 2.5 μ m, 50 x 3.0 mm
Isocratic analysis
MeCN/H₂O (50:50 v/v)
Flow rate: 0.43 mL/min
Column temperature: Ambient (22°C)
Detection: UV at 280 nm
Injection volume: 3.1 μ L



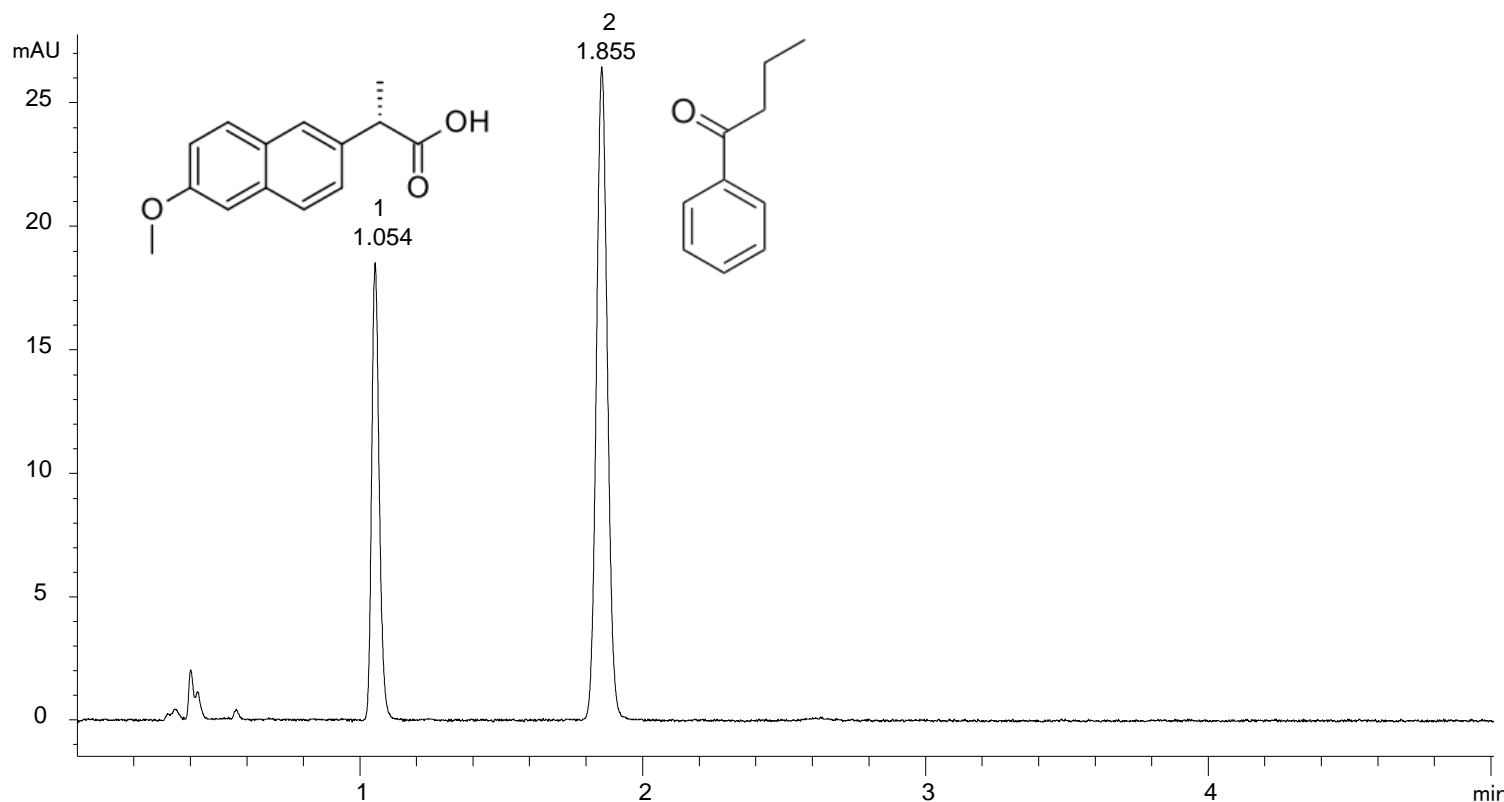


USP Naproxen

Key:

- 1 Naproxen
- 2 Butyrophenone

ACE Ultracore SuperC18, 2.5 μ m, 50 x 3.0 mm
Isocratic analysis
H₂O with glacial acetic acid (49:1)/MeCN (50:50 v/v)
Flow rate: 0.51 mL/min
Column temperature: Ambient (22°C)
Detection: UV at 254 nm
Injection volume: 2.8 μ L

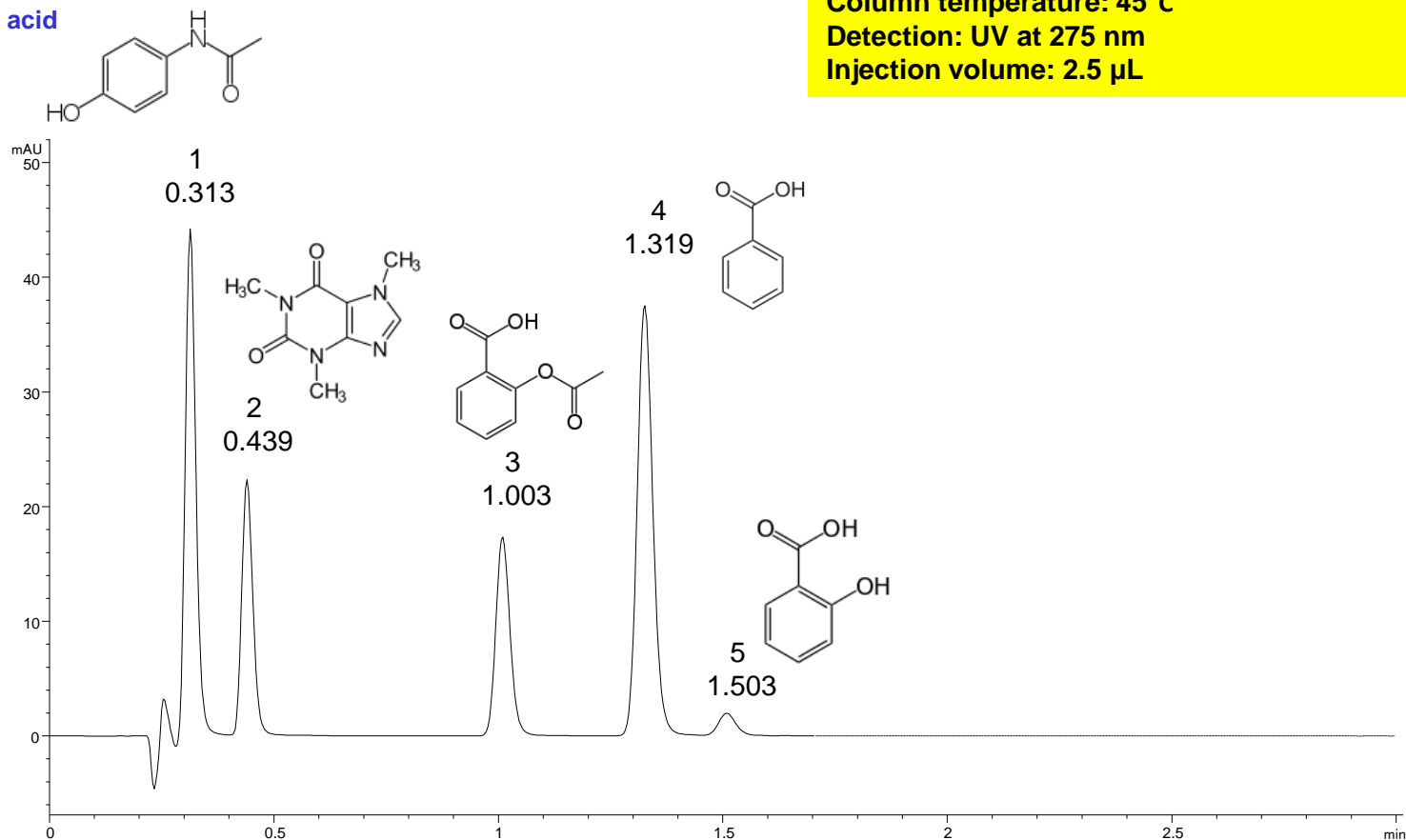




USP Paracetamol / Aspirin / Caffeine

Key:

- 1 Paracetamol
- 2 Caffeine
- 3 Aspirin
- 4 Benzoic acid
- 5 Salicylic acid



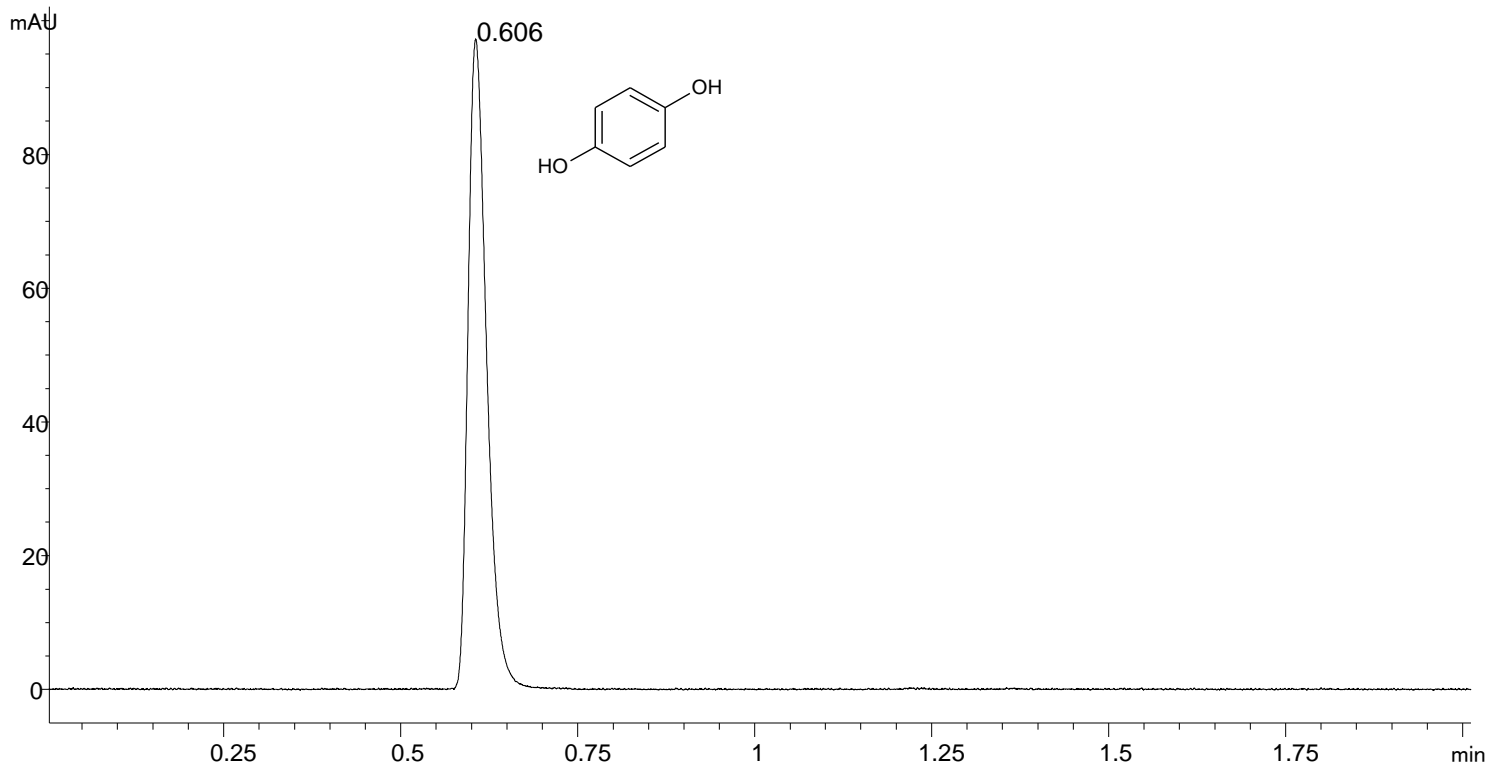
ACE Ultracore SuperC18, 2.5 μ m, 50 x 4.6 mm
 Isocratic analysis
 H₂O with glacial acetic acid (69:3)/MeOH (72:28 v/v)
 Flow rate: 2 mL/min
 Column temperature: 45°C
 Detection: UV at 275 nm
 Injection volume: 2.5 μ L



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**

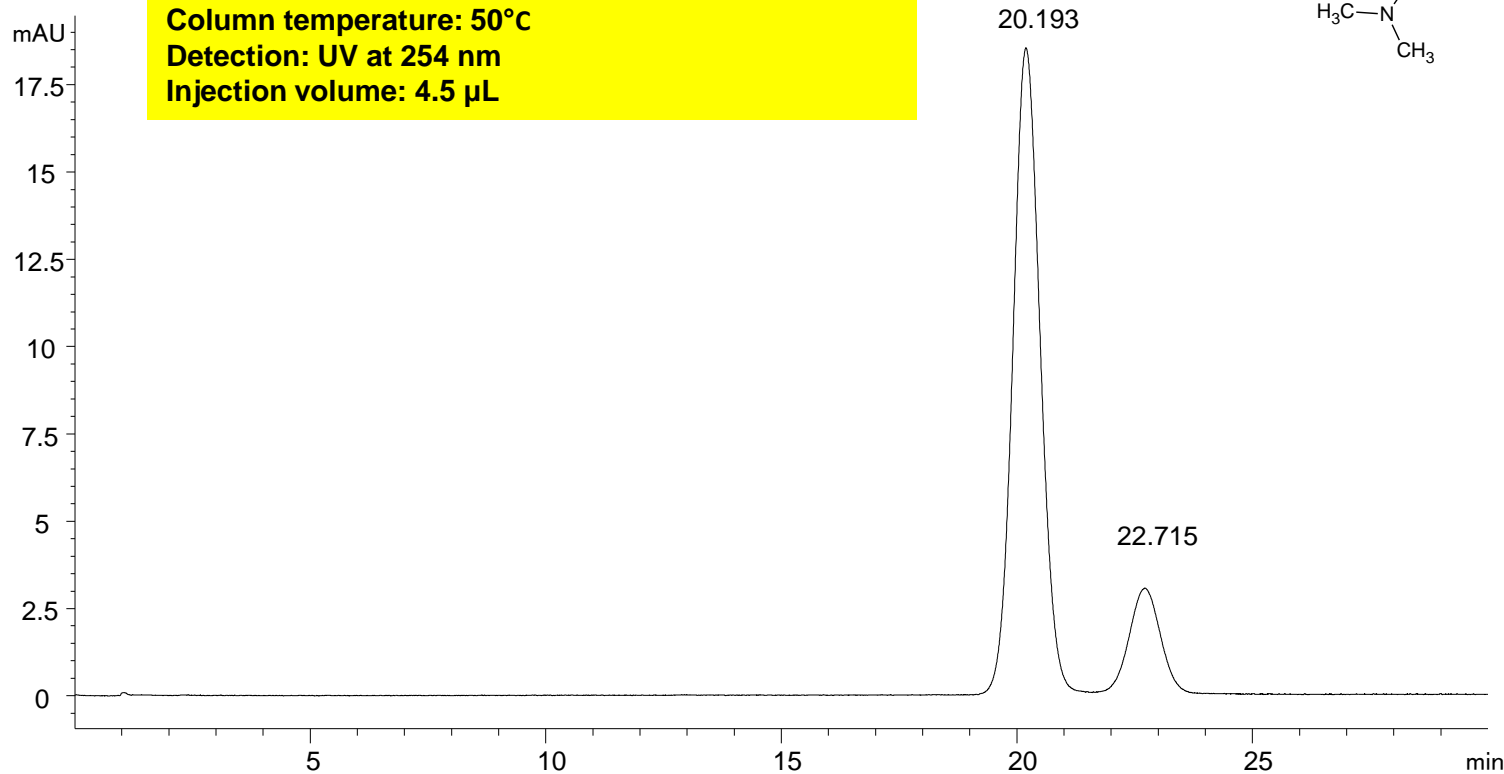
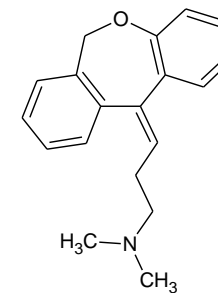


USP Doxepin

Key:

- 1 Doxepin isomer
- 2 Doxepin isomer

ACE C8, 3 μ m, 50 x 4.6 mm
Isocratic analysis
0.2 M Monobasic NaH_2PO_4 in water pH 2.5:MeOH
(30:70 v/v)
Flow rate: 0.56 mL/min
Column temperature: 50°C
Detection: UV at 254 nm
Injection volume: 4.5 μ L





USP Metronidazole

Key:

1 Metronidazole

