Cytarabine and cytidine are isobaric.
Robust method with good separation achieved.
LLOQ = 1 ng/ml human plasma

**Gradient analysis**

<table>
<thead>
<tr>
<th>T (mins)</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>3.0</td>
<td>13</td>
</tr>
<tr>
<td>4.0</td>
<td>90</td>
</tr>
<tr>
<td>5.0</td>
<td>0</td>
</tr>
</tbody>
</table>

Flow rate: 0.7 ml/min

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

**ACE 3 C18 3μm, 50 x 2.1 mm**

Cytarabine (m/z: 244 > 112)

5-Fluorocytidine (IS) (m/z: 262 > 130)

Uracil arabinofuranoside (m/z: 245 > 113)

Reproduced with permission of Agilux Laboratories, USA
Tricyclic Antidepressants

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

**ACE Excel SuperC18**
2μm, 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

<table>
<thead>
<tr>
<th>Time (mins)</th>
<th>%B</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>50</td>
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<tr>
<td>6</td>
<td>70</td>
</tr>
<tr>
<td>7</td>
<td>70</td>
</tr>
<tr>
<td>7.5</td>
<td></td>
</tr>
</tbody>
</table>

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

Reproduced with permission of inVentiv Health Clinical, Quebec, Canada
Tricyclic Antidepressants

ACE Excel C18-PFP
2μm, 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: 2μl
Detection: UV, 260nm

Key:
1 Doxepin
2 Imipramine
3 Protriptyline
4 Nortriptyline
5 Trimipramine
6 Clomipramine
Paracetamol and Related Compounds

ACE Excel C18-PFP
2μm, 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

<table>
<thead>
<tr>
<th>Time (mins)</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>5.5</td>
<td>63</td>
</tr>
<tr>
<td>6.5</td>
<td>63</td>
</tr>
<tr>
<td>7</td>
<td>9</td>
</tr>
</tbody>
</table>

Flow rate: 1.2ml/min
Column temperature: 27°C
Injection volume: 2μl
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
TurboIonSpray – negative mode
Extracted ion chromatogram of MRM m/z 423.3 → 321.1

Reproduced with permission of Biotrial Bioanalytical Services, Laval, QC, Canada
### NSAIDs Analysis

**Column:** 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

**Conditions:**
- Time (mins) | %B
  - 0      | 20
  - 20     | 70
  - 25     | 70
  - 26     | 20

**Flow rate:** 1ml/min

**Column temperature:** 40°C

**Injection volume:** 10μl

**Detection:** UV, 254nm

### Key Peaks

1. Aspirin
2. Phenacetin
3. Sulindac
4. Tolmetin
5. Naproxen
6. Nimesulide
7. Flurbiprofen
8. Diclofenac
9. Phenylbutazone
10. Meclofenamic Acid
Pristinamycin antibiotic is a mixture of 2 components – pristinamycin IA and IIA
Virginiamycin used as internal standard

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

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
1.61 10 4 0

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

MDS Scix 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

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ACE Excel Super C18
2μm, 50 x 3.0 mm
Gradient analysis
A = 0.1% formic acid in water
B = 0.1% formic acid in acetonitrile

<table>
<thead>
<tr>
<th>Time (mins)</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
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</tr>
<tr>
<td>0.25</td>
<td>20</td>
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<tr>
<td>3.5</td>
<td>70</td>
</tr>
<tr>
<td>4</td>
<td>70</td>
</tr>
<tr>
<td>4.25</td>
<td>20</td>
</tr>
</tbody>
</table>

Flow rate: 0.86ml/min
Column temperature: 40°C
Injection volume: 1.4μl
Detection: UV, 254nm

Key:
1 Aspirin
2 Phenacetin
3 Sulindac
4 Tolmetin
5 Naproxen
6 Nimesulide
7 Flurbiprofen
8 Diclofenac
9 Phenybutazone
10 meclofenamic acid
Diuretics

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

ACE Excel C18-PFP
2 μm, 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

<table>
<thead>
<tr>
<th>Time (mins)</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>0.5</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>70</td>
</tr>
<tr>
<td>5.5</td>
<td>70</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
</tr>
</tbody>
</table>

Flow rate: 1.0 ml/min
Column temperature: 60°C
Injection volume: 2 μl
Detection: UV, 254nm

- Hydrochlorothiazide
- Amiloride
- Furosemide
- Indapamide
- Bendroflumethazide
- Spironolactone
Diuretics (Isocratic)

Key:
1. Furosemide
2. Indapamide
3. Bendroflumethazide
4. Spironolactone

ACE Excel C18-PFP
2μm, 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: 2μl
Detection: UV, 254nm
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

<table>
<thead>
<tr>
<th>Time (mins)</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7.5</td>
<td>100</td>
</tr>
<tr>
<td>8.5</td>
<td>100</td>
</tr>
<tr>
<td>9.0</td>
<td></td>
</tr>
</tbody>
</table>

Flow rate: 1.2ml/min
Column temperature: 25°C
Injection volume: 2μl
Detection: UV, 254nm
Analgesics / Cough & Cold Medicine Ingredients

1. paracetamol
2. 4-aminobenzoic acid
3. 4-hydroxybenzoic acid
4. caffeine
5. 2-acetamidophenol
6. 3-hydroxybenzoic acid
7. salicylamide
8. acetalanilide
9. phenol
10. aspirin
11. benzoic acid
12. sorbic acid
13. salicylic acid
14. phenacetin
15. salicylaldehyde

Gradient analysis
A = 0.1% v/v formic acid (aq)
B = 0.1% v/v formic acid in MeCN
40C
T (min) %B
0 5
9 35
14 35
15 5
Pharmaceutically Relevant Mixture

ACE UltraCore SuperC18: selectivity with pH:

- **pH 3**: Gradient analysis
  - A1 = 10mM HCOONH₄, pH 3 (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, pH 10.7 in MeCN/water 9:1 v/v
  - T: %B
    - 0: 3
    - 5: 100
    - 6: 100
  - 40°C
  - 0.60 mL/min
  - 254 nm

- **pH 10.7**: Gradient analysis
  - A1 = 10mM HCOONH₄, pH 3 (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, pH 10.7 in MeCN/water 9:1 v/v
  - T: %B
    - 0: 3
    - 5: 100
    - 6: 100
  - 40°C
  - 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
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:

- **pH 3**
  - 50x2.1mm, 2.5µm
  - Gradient analysis
  - A1 = 10mM HCOONH₄, pH 3 (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, pH 10.7 in MeOH/water 9:1 v/v
  - T %B
    - 0 3
    - 5 100
    - 7 100
  - 40 °C
  - 0.60 mL/min
  - 254 nm

- **pH 10.7**
  - Benzamide
  - Caffeine
  - Procainamide
  - N-acetylprocainamide
  - Propiophenone
  - Toluene
  - Remacemide
  - Ethylbenzene
  - Carvdilol
  - Nortriptyline
  - Clomipramine
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

Reproduced with permission of Mary Nguyen, Guys Hospital, London
Procaine and p-Aminobenzoic acid

**Procaine HCl linearity**

\[ y = 104546204.5777x \]
\[ R^2 = 0.9964 \]

**4-Aminobenzoic acid linearity**

\[ y = 2E+08x \]
\[ R^2 = 0.9975 \]

**Procaine – sterile manufactured product**

System suitability tests

ACE C18-PFP 3μm, 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
Gradient analysis

A = 20mM KH$_2$PO$_4$, pH 2.83 (aq)
B = 20mM KH$_2$PO$_4$, pH 2.83 in 1:1 v/v MeOH:water

40°C

205nm

1.5mL/min

T (min) %B
0  20
15  70

1. Pyridoxine (vitamin B6)
2. Pantothenic acid (vitamin B5)
3. p-Aminobenzoic acid
4. Folic acid (vitamin B9 or vitamin M)
5. D-Biotin (vitamin B7 or vitamin H)
6. Cyanocobalamin (vitamin B12)
7. Riboflavin (vitamin B2)
ACE 3 C18-AR

- **Isocratic analysis**
- **Flow rate**: 1.0 mL/min
- **Temperature**: 22°C
- **Detection**: UV 260 nm
- **Mobile Phase (MP)**: 96.5:3.5 v/v 0.1% H3PO4 (aq) : MeOH
Non-Steroidal Anti-Inflammatory Drugs

ACE 3 C18-AR

150x4.6mm, 3µm
Gradient analysis
A = 0.1% v/ formic acid (aq)
B = 0.1% v/v formic acid in MeOH
40°C
1.0mL/min
254nm

<table>
<thead>
<tr>
<th>T (min)</th>
<th>%B</th>
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<tbody>
<tr>
<td>0</td>
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</tr>
<tr>
<td>28</td>
<td>74</td>
</tr>
<tr>
<td>33</td>
<td>74</td>
</tr>
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</table>

**Chemicals**

1. Bendromethazide
2. Ketoprofen
3. Sulindac
4. Diclofenac
5. Ibuprofen
6. Naproxen
7. Indomethacin
8. Meclofenamic acid
9. Mefenamic acid

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Complex Steroid Mixture Separation

ACE 3 C18-AR

Separation of 12 Steroid Standards

1. Estriol
2. Prednisolone
3. Hydrocortisone
4. Cortisone
5. Corticosterone
6. 17β-Estradiol
7. Cortisone-21-acetate
8. 17α-Estradiol
9. 19-Norethindrone
10. 17α-Ethinylestradiol
11. 21-Hydroxyprogesterone
12. Estrone
ACE® C18-PFP™: Halogenated Positional Isomers

Chloroacetophenone halogenated isomers separation

ACE C18

ACE C18-AR

ACE C18-PFP

Regioisomer Selectivity
Polar Analytes – Nucleosides & Vitamins

100% Aqueous

Thiamine or vitamin B1

Nicotinamide

L-Ascorbic acid or vitamin C

Cytosine

Uracil

Pyridoxamine or Vitamin B6

Cytidine

2-Deoxycytidine

ACE C18-PFP, 3µm, 150 x 4.6 mm

Isocratic analysis

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

Mobile phase: 20 mM H₃PO₄ (aq)
Separation of Sulphonamides

ACE Excel C18-PFP
3μm, 150 x 4.6mm
Gradient analysis
A = Water
B = Acetonitrile
C = 10% formic acid

<table>
<thead>
<tr>
<th>Time (mins)</th>
<th>%A</th>
<th>%B</th>
<th>%C</th>
</tr>
</thead>
<tbody>
<tr>
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<td>15</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>74</td>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td>14</td>
<td>59</td>
<td>40</td>
<td>1</td>
</tr>
<tr>
<td>16</td>
<td>84</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>18</td>
<td>84</td>
<td>15</td>
<td>1</td>
</tr>
</tbody>
</table>

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

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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

Polar embedded + hydrophobic Interactions

ACE Excel C18-Amide

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

ACE Excel C18-PFP

ACE CN-ES
100x3mm, 3μm
Gradient analysis

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

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

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, 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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USP Guaifenesin

Key:
1 Guaifenesin
2 Benzoic acid

ACE UltraCore SuperC18, 2.5 μm, 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 μL

Translated method according to USP <621> guidance. Always check the latest and official method information from the relevant pharmacopoeia prior to analysis.
ACE Ultracore SuperC18, 2.5 µm, 100 x 4.6 mm
Isocratic analysis
MeCN/MeOH/H$_2$O (25:25:50 v/v)
Flow rate: 1 mL/min
Column temperature: Ambient (22°C)
Detection: UV at 253 nm
Injection volume: 5.8 µL

Translated method according to USP <621> guidance. Always check the latest and official method information from the relevant pharmacopoeia prior to analysis.
Key:
1 Ethylparaben
2 Estrone
3 Estradiol

ACE Ultracore SuperC18, 2.5 μm, 100 x 4.6 mm
Isocratic analysis
H₂O/MeCN (45:55 v/v)
Flow rate: 1.39 mL/min
Column temperature: Ambient (22°C)
Detection: UV at 205 nm
Injection volume: 10.1 μL

Translated method according to USP <621> guidance. Always check the latest and official method information from the relevant pharmacopoeia prior to analysis.
Key:
1 Ethylparaben
2 17α-Ethinylestradiol

ACE Ultracore SuperC18, 2.5 µm, 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 µL

Translated method according to USP <621> guidance. Always check the latest and official method information from the relevant pharmacopoeia prior to analysis.
**Key:**

1. Naproxen
2. Butyrophenone

**ACE Ultracore SuperC18, 2.5 μm, 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 μL

Translated method according to USP <621> guidance. Always check the latest and official method information from the relevant pharmacopoeia prior to analysis.
ACUltracore SuperC18, 2.5 μm, 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 μL

Translated method according to USP <621> guidance. Always check the latest and official method information from the relevant pharmacopoeia prior to analysis.
**Key:**

1. Hydroquinone

**ACE Excel C18, 2 μm, 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 μL

Translated method according to USP <621> guidance. Always check the latest and official method information from the relevant pharmacopoeia prior to analysis.
translated method according to usp <621> guidance. always check the latest and official method information from the relevant pharmacopoeia prior to analysis.
USP Metronidazole

Key:
1. Metronidazole

ACE C8, 3 μm, 75 x 4.6 mm
Isocratic analysis
H₂O/MeOH (4:1 v/v)
Flow rate 1.0 mL/min
Column temperature: 30°C
Detection: UV at 319 nm
Injection volume: 15 μL

Translated method according to USP <621> guidance. Always check the latest and official method information from the relevant pharmacopoeia prior to analysis.