Kromasil®

Kromasil application guide

The way to peak performance in liquid chromatography
Kromasil applications – from our lab and the literature

The Kromasil packings and columns have been used over the years for demanding separations all over the world. In this guide we have collected examples of a variety of chromatographic separations, from small synthetic pharmaceuticals, up to peptides and larger molecules.

We hope this guide will be a useful tool when developing new HPLC separation methods in your lab.

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Cover figure shows a 3D chromatographic separation, with UV absorption at different wavelengths vs. time.

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The Kromasil packings – some hints for the best performance

The Kromasil family of packing materials is developed to be the perfect choice from analytical to process scale. Kromasil is presently available as bare silica, C4, C8, C18, NH2, CN or as Kromasil Chiral for separation of optical isomers. Pore sizes are 60 Å, 100 Å, and 300 Å, and particle sizes 3.5, 5, 7, 10, 13 and 16 µm. Slurry-packed columns are available from analytical up to 2” inner diameter, all with analytical efficiencies. For larger preparative and industrial scale columns bulk packing is provided.

To learn more about the properties of Kromasil silica please consult our other technical information.

Choice of mobile phase

Normal Phase conditions
Choose mixtures of hexane or heptane, and polar modifiers like alcohols, ethyl acetate, methylene chloride, etc. to adjust retention. The optimum retention factor range is normally $2 \leq k \leq 5$ for a two-component sample, but can be wider for a multi-component sample.

Acidic and basic additives can improve the chromatographic performance. In most cases small amounts of acetic acid or formic acid (0.05 – 0.10%) improve peak shape for acidic or basic solutes. In some cases the combination of acid and an organic amine (e.g. triethylamine) is necessary, for difficult solutes. The acid should always be in excess relative to the amine, in order to operate at a pH where the silanol groups on the silica are protonated.

Reversed Phase conditions
Choose mixtures of water or buffer, and water miscible solvents like alcohols, acetonitrile, THF, etc. to adjust retention factor $k$ to an optimal range. The pH can be controlled by using a buffer, and in order to minimize the ionization of the silica and the solutes. In order to control peak shape for very basic solutes an additive like TEA (triethylamine) can be added, if necessary. Kromasil is a fully hydroxylated ultra-pure silica, making the surface less acidic, resulting in good peak shape also for basic compounds.

For the C4, C8 and C18 phases, due to the very hydrophobic nature of the surface, it is important to always keep at least 4 – 5% of organic in the mobile phase, both when flushing or running the chromatographic separation. The reason is that in the case of a 100% aqueous mobile phase there is a risk that the surface within the porous system in the Kromasil particles is “dewetted”, resulting in a total or partial loss of retention.

This phenomenon of dewetting is more pronounced for high quality, high coverage materials, where the bonding procedure has been very efficient. This will result not only in higher retention times because

\[ \text{In } k \]

always keep at least 4 – 5% of organic in the mobile phase

% organic in water

\[ \alpha = 1.32 \]

Figure 1 | Influence of the mobile phase additives on the separation of mefloquine.
Conditions: Column: 4.6 x 250 mm, Kromasil CHI-DMB, 5 µm
Flow rate: 2 ml/min. Detection: UV 280 nm

\[ \alpha = 1.19 \]

Figure 2 | The retention factor, $k$, vs. organic content. General retention behaviour at low organic content using high density RP phases.
of a higher coverage and hence hydrophobicity, but also in a higher hydrolytic stability, and a longer lifetime of the column.

If a 100% aqueous mobile phase has been used accidentally, and the stationary phase has been dewetted, the column can easily be regenerated. Just flush the column with a mobile phase consisting of 40 – 50% or more of organic for 2 – 5 column volumes. After this the column can be equilibrated again with the mobile phase, and the original retention times should be seen.

We also recommend to always filter buffer solutions in order to remove small particulates. It is also preferable to premix aqueous/organic solutions, in order to avoid problems with gas formation in the mobile phase, or a temperature increase or decrease as an effect of endo- or exothermic mixing heats.

The recommended pH range for our RP phases is between pH 1.5 up to 9.5. However, in some applications mobile phases with pH above 11 have been used for continuous chromatography, for several thousands of column volumes.

### How to improve speed of separation

There is today a strong driving force towards faster separations, and hence smaller particles and shorter columns. A smaller particle will give a higher efficiency at a higher flow rate; for example will a 5 µm particle give twice the efficiency compared to 10 µm, at twice the flow rate. And if the resolution is to be kept constant one can also reduce column length by 50%. Table 1 gives the relation between the critical parameters when going to smaller particles, and shorter columns.

<table>
<thead>
<tr>
<th>Column length (mm)</th>
<th>Particle size (µm)</th>
<th>Flow rate (ml/min.)</th>
<th>Relative time</th>
<th>Relative Rs</th>
<th>Relative ΔP</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>10</td>
<td>0.5</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>125</td>
<td>5</td>
<td>1.0</td>
<td>0.25</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>87.5</td>
<td>3.5</td>
<td>1.43</td>
<td>0.125</td>
<td>1</td>
<td>8</td>
</tr>
</tbody>
</table>

It can be seen that the combination of smaller particles, shorter columns and higher flow rate will result in much faster analyses. The only drawback is the back pressure, which will increase significantly as particle size goes down.

All in all, one will save a factor 2 in analysis time by going from 5 to 3.5 µm for example, but also experience twice the back pressure.

### How to improve resolution

A good resolution in a short period of time is usually a requirement in analytical HPLC. One has essentially three ways to improve the resolution, as can be seen in the equation below:

$$R_s = \frac{1}{4} \left( \alpha - 1 \right) \cdot \sqrt{N} \cdot \left( \frac{k_1}{1 + k} \right)$$

1. **The separation factor, α, can be increased.** This can be done by optimizing stationary and mobile phase, i.e. choosing the best column and mobile phase composition for the specific application
2. **The number of theoretical plates can be increased.** This can be done by:
   a. Increasing the column length
   b. Decreasing the particle size
   c. Optimizing the flow rate. The optimum for small particles is at a higher flow rate than for larger particles (inverse linear relationship)
3. **The retention factor, k, can be increased, if it is too low.** This can be done by adjusting the elution strength of the mobile phase
Scale-up

The analytical separation is very often the start for a scale-up to semi-prep, prep, or large industrial scale chromatography. In the case of Kromasil there is the possibility of seamless scale-up from analytical to semi-prep and prep, and even large diameter Dynamic Axial Compression (DAC) columns. All Kromasil columns independent of diameter are delivered with the same, high efficiency guarantee, and even the large DAC columns can be packed giving the same performance.

We recommend that the method development for the preparative separation is performed using analytical columns, or possibly 10 mm ID if larger volumes of the sample fractions are needed. A small column will make the development work easier, and since the performance is identical in small and large diameter columns, the result in large scale can easily be predicted from the work in small scale. 10 µm particles are recommended, since the efficiency, back pressure, etc. then will be close to the large scale separation. For large scale 10 – 16 µm particles are usually a good choice. However, the performance using a different particle size can easily be predicted.

The optimal running conditions in large industrial scale can also be found by applying a software program, KromaGuide, developed by the Kromasil group. The KromaGuide optimization is part of our technical support to current Kromasil customers, or potential users.

Figure 3 | Scale-up to an 80 cm ID dynamic axial compression (DAC) column, showing analytical performance. Efficiency was 42,000 plates/meter (h = 2.38) and asymmetry₀₁ was 1.19

Sample: uracil and toluene
Applications

Amino acids

Amino acids, PTC derivatives
18 amino acids as phenylthiocarbamyl (PTC) derivatives. (ref. 7)

Phase: Kromasil 100 Å, 5 µm, C18
Column: 4.6 x 200 mm
Temperature: 38°C
Eluent A: 3% ACN in 0.1M sodium acetate
Eluent B: ACN/water (80:20; v:v)
Flow rate: 1 ml/min.
Detection: UV 254 nm

Amino acids, Fmoc-derivatives
Amino-acid analysis for protein and peptide hydrolysates with precolumn Fmoc (9-fluorenyl metylchloroformate) derivatization. (ref. 30)

Phase: Kromasil 100 Å, 5 µm, C8
Column: 4 x 250 mm
Temperature: 45°C
Eluent A: sodium acetate buffer (100 mM, pH 4.4):THF:ACN (75:15:10; v:v:v)
Eluent B: ACN:THF (85:15; v:v)
Flow rate: 1.5 ml/min.
Detection: UV 263 nm
**Amino acids**

Detection of N-acetylaspartate and N-acetylglutamate in cerebral tissue extracts. (ref. 228)

1 = aspartate  
2 = glutamate  
3 = hypoxanthine  
4 = xanthine  
5 = uric acid  
6 = inosine  
7 = N-acetylaspartate (NAA)  
8 = N-acetylglutamate (NAG)  
9 = adenosine

**Amino acids, N-acetylated**

Separation of N-acetylated amino acids. (ref. 348)

1 = N-acetylhistidine  
2 = N-acetylaspartate  
3 = N-acetylglutamate

**Amino acids, benzoylated**

Analysis of benzoylated amino acids. (ref. 51a)

1 = lysine (oxazol derivative)  
2 = glycine (oxazol derivative)  
3 = alanine (oxazol derivative)  
4 = methionine (benzoylated)  
5 = glutamic acid (benzoylated)  
6 = phenylalanine (oxazol derivative)  
7 = valine (benzoylated)  
8 = leucine (oxazol derivative)  
9 = isoleucine (benzoylated)  
10 = tyrosine (benzoylated)  
11 = cyclohexylalanine (benzoylated)
Aminosalicylic acids
Determination of 5-aminosalicylic acid and 3-aminosalicylic acid. (ref. 279)

1 = 5-aminosalicylic acid
2 = 3-aminosalicylic acid

Phase: Kromasil 100 Å, 5 µm, C18
Column: 4.6 x 200 mm
Eluent: MeOH:phosphate buffer (35:65, v:v)
Flow rate: 1 ml/min.
Detection: UV 254 nm

Carnitines, aminoanthracene derivatives
Determination of L-carnitine, acetyl-L-carnitine and propionyl-L-carnitine in human plasma by HPLC with post-column derivatization with 1-aminoanthracene. (ref. 66)

1 = L-carnitine 1-aminoanthraceneamide
2 = acetyl-L-carnitine 1-aminoanthraceneamide
3 = methansulfonyl-L-carnitine 1-aminoanthraceneamide
4 = propionyl-L-carnitine 1-aminoanthraceneamide
5 = isobutyryl-L-carnitine 1-aminoanthraceneamide

Phase: Kromasil 100 Å, 5 µm, C18
Column: 4.6 x 250 mm
Eluent: ACN:ammonium acetate (0.1 M, pH 3.5) (30:70, v:v)
Flow rate: 1.3 ml/min.
Detection: spectrofluorimetric (λex 248 nm, λem 418 nm)

Boronophenylalanine
Determination of boronophenylalanine in biological samples after precolumn derivatization with o-phthalaldehyde (OPA). (ref. 257)

1 = OPA-aspartic acid
2 = OPA-glutamic acid
3 = OPA-asparagine
4 = OPA-histidine
5 = OPA-serine
6 = OPA-glutamine
7 = OPA-arginine
8 = OPA-citrulline
9 = OPA-glycine
10 = OPA-threonine
11 = OPA-γ-aminobututaric acid (GABA)
12 = OPA-alanine
13 = OPA-tyrosine
14 = OPA-p-boronophenylalanine
15 = OPA-valine
16 = OPA-phenylalanine
17 = OPA-isoleucine
18 = OPA-leucine
19 = OPA-ornitine
20 = OPA-lysine
R = OPA derivative group

Phase: Kromasil 100 Å, 5 µm, C18
Column: 4.6 x 250 mm
Eluent: ACN:ammonium acetate (0.1 M, pH 3.5) (30:70, v:v)
Flow rate: 1.3 ml/min.
Detection: spectrofluorimetric (λex 330 nm, λem 430 nm)

Gradient: 80% A in 3 min, 80% – 70% A in 12 min,
70% – 50% A in 15 min, 50% – 45% A in 10 min,
45% – 20% A in 10 min, 20% – 15% A in 5 min,
15% – 10% A in 3 min, 10% – 0% A in 2 min,
0% A in 15 min.
Flow rate: 1.2 ml/min.
Detection: spectrofluorimetric (λex 248 nm, λem 418 nm)
**Amoxicillin**
Measurment of amoxicillin in gastric tissue samples. ([ref. 6](#))

1 = amoxicillin degradation derivative
2 = amoxicillin degradation derivative (I.S.)

**Amperozide**
Separation of amperozide, derivate and metabolite. ([ref. 45](#))

1 = amperozide’s N-de-ethyl metabolite (II)
2 = amperozide (I)
3 = amperozide’s N-de-ethyl N-butyl analogue (III)

**Anthocyanidins**
Separation of cyanidin from 3-O-β-glycosylated anthocyanidins. ([ref. 347](#))

1 = petunidin-3-O-β-glycoside
2 = delphinidin-3-O-β-glycoside
3 = pelargonidin-3-O-β-glycoside
4 = malvidin-3-O-β-glycoside
5 = cyanidin-3-O-β-glycoside
6 = peonidin-3-O-β-glycoside
7 = cyanidin

**Drugs and metabolites**
Drugs and metabolites

Antibacterial drugs
Determination of metronidazole, clotrimazole and chlorhexidine acetate in Shuangzo effervescent tablets. (ref. 23)

1 = chlorhexidine
2 = metronidazole
3 = clotrimazole

Phase: Kromasil 100 Å, 5 µm, C18
Column: 4.6 x 250 mm
Eluent: MeOH:buffer (70:30; v:v) (NaAc 24.4 g, HAc 80 ml, (C4H9)NBr 4.83 g in 1000 ml water, pH 3.6)
Flow rate: 1 ml/min.
Detection: UV 260 nm

Antibacterial drugs, veterinary
Simultaneous determination of sulfaquinoxaline, sulfamethazine and pyrimethamine. (ref. 246)

1 = sulfamethazine
2 = pyrimethamine
3 = sulfaquinoxaline

Phase: Kromasil 100 Å, 5 µm, C18
Column: 4.6 x 150 mm
Eluent: 40 mM phosphate buffer (pH 3 containing 10 mM ClO4–) : ACN (65:35; v:v)
Flow rate: 1.5 ml/min.
Detection: UV 270 nm

Antibacterials, sulfa drugs
Determination of sulfamethoxypyridazine, sulfamethoxazole, sulfadimethoxine and associated compounds. (ref. 267)

1 = trimethoprim
2 = sulfamethoxypyridazine
3 = sulfamethoxazole
4 = sulfadimethoxine
5 = bromhexine

Phase: Kromasil 100 Å, 5 µm, C18
Column: 4.6 x 150 mm
Eluent: 10 mM citrate buffer (pH 3):MeOH
Gradient: 0 min. 31% MeOH, 4 min. 69% MeOH, 14 min. 69% MeOH, 16 min. 31% MeOH
Flow rate: 1 ml/min.
Detection: UV 255 nm

Antibiotics and intermediates
Determination of ceftriaxone, 7-aminocephalosporanic acid (7-ACA) and 7-amino-3-[[2,5-dihydro-6-hydroxy-2-methyl-5-oxo-1,2,4-trizin-5-yl]-thio)methyl]-cephalosporanic acid (7-ACT). (ref. 129)

1 = 7-ACA
2 = 7-ACT
3 = ceftriaxone

Phase: Kromasil 100 Å, 5 µm, C18
Column: 4.6 x 200 mm
Eluent: ACN:tetrabutyl ammonium bromide:phosphate buffer (pH 7) water (52.0:32.4:4.63.6; v:v:v:v)
Flow rate: 1 ml/min.
Detection: UV 270 nm
Antibiotics and metabolites

Determination of quinupristin and its main metabolites in human plasma. (ref. 143)

Phase: Kromasil 100 Å, 5 µm, C18
Column: 4.6 × 125 mm
Eluent A: 0.8 ml of 70% perchloric acid (PCA) / litre water
Eluent B: ACN
Gradient: 30% B for 11 min., 32% B from 11.1 to 15 min., 40% B from 15.6 to 16 min., 58% B from 16.1 to 34 min., 80% B from 34.1 to 36 min.

Flow rate: 0 – 11 min: 0.5 ml/min., 11 – 36 min: 1 ml/min.
Detection: fluorescence (λ<sub>ex</sub> 360 nm and λ<sub>em</sub> 410 nm)

Anticonvulsants

Determination of theophylline, phenobarbitone, diphentoin and carbamazepine. (ref. 301b)

1 = theophylline
2 = phenobarbitone
3 = diphentoin
4 = carbamazepine

Phase: Kromasil 100 Å, 5 µm, C18
Column: 0.8 × 150 mm
Eluent: MeOH:water (70:30; v:v)
Flow rate: 35 µl/min
Detection: UV 210 nm

Antidepressants

Determination of antidepressant drugs and metabolites. (ref. 49)

1 = amoxapine
2 = fluvoxamine
3 = maprotiline
4 = trimipramine
5 = mianserine

Phase: Kromasil 100 Å, 5 µm, C18
Column: 2.1 × 150 mm
Eluent: ACN phosphate buffer (40:60; v:v) (pH 6.5)
Flow rate: 0.35 ml/min
Detection: UV 220 nm
**Drugs and metabolites**

### Antidepressants

**Analysis of amitriptyline and nortriptyline in plasma.** (ref. 58)

1 = E-10-OH-nortriptyline
2 = Z-10-OH-nortriptyline
3 = 8-OH-desmethyl-clomipramine
4 = 8-OH-clomipramine
5 = doxepine
6 = protriptyline
7 = nortriptyline
8 = amitriptyline
9 = desmethyl-clomipramine
10 = clomipramine

**Phase:** Kromasil 100 Å, 5 µm, C8  
**Column:** 4 x 250 mm  
**Temperature:** ambient  
**Eluent:** ACN:KH$_2$PO$_4$ (0.04 M) (40:60; v:v)  
**Flow rate:** 1 ml/min.  
**Detection:** UV 240 nm

### Antidepressants and metabolites

**Simultaneous determination of citalopram, fluoxetine, paroxetine and their metabolites in plasma.** (ref. 309)

1 = (-)-trans-4-(4-fluorophenyl)-3-(4-hydroxy-3-methoxy-phenoxymethyl)piperidine  
2 = (-)-trans-4-(4-fluorophenyl)-3-(3-hydroxy-4-methoxy-phenoxymethyl)piperidine  
3 = didesmethylcitalopram  
4 = desmethyl-citalopram  
5 = citalopram  
6 = citalopram N-oxide  
7 = paroxetine  
8 = protriptyline  
9 = norfluoxetine  
10 = fluoxetine

**Phase:** Kromasil 100 Å, 3.5 µm, C18  
**Column:** 0.32 x 300 mm  
**Temperature:** gradient: 35°C (3 min.) prior to ramp of 1.3°C/min. to 100°C (10 min.)  
**Eluent:** ACN-NH$_4$HCOO (45 mM, pH 4) (25:75; v:v)  
**Flow rate:** 5 µl/min  
**Detection:** UV 230 nm
Antifungals
Determination of terbinafine hydrochloride, chlorhexidine and triamcinolone acetonide acetate. (ref. 110)

1 = triamcinolone acetonide acetate
2 = terbinafine
3 = chlorhexidine

Phase: Kromasil 100 Å, 5 µm, C18
Column: 4.6 x 200 mm
Eluent: 0.3% sodium heptanesulphonate in MeOH:water (73:27; v:v), pH 3.2
Flow rate: 1 ml/min.
Detection: UV 248 nm

Anti-HIV
Simultaneous determination of ritonavir and saquinavir. (ref. 126)

1 = ritonavir
2 = saquinavir

Phase: Kromasil 100 Å, 5 µm, C8
Column: 4.6 x 150 mm
Eluent: ACN : 5 mM potassium phosphate monobasic buffer, pH 8 (55:45; v:v)
Flow rate: 1 ml/min.
Detection: UV 240 nm

Antimicrobials
Determination of metronidazole and nalidixic acid. (ref. 156)

1 = metronidazole
2 = furazolidone
3 = nalidixic acid

Phase: Kromasil 100 Å, 5 µm, C18
Column: 4.6 x 250 mm
Temperature: 20°C ± 1°C
Eluent: ACN 0.2% triethylamine (pH 3.5) (35:65; v:v)
Flow rate: 1.5 ml/min.
Detection: UV 320 nm

Antimicrobials
Detection of metronidazole and clindamycin. (ref. 268)

1 = metronidazole
2 = clindamycin

Phase: Kromasil 100 Å, 5 µm, C18
Column: 4.6 x 250 mm
Eluent: Potassium dihydrogen phosphate (pH 3.8, 0.05 M):ACN (79:21; v:v)
Flow rate: 1 ml/min.
Detection: UV 210 nm
Drugs and metabolites

**Antipsychotics**
Determination of clozapine and loxapine. (ref. 64)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Retention Time (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 = clozapine</td>
<td><img src="image" alt="Clozapine structure" /></td>
<td>1</td>
</tr>
<tr>
<td>2 = loxapine</td>
<td><img src="image" alt="Loxapine structure" /></td>
<td>2</td>
</tr>
</tbody>
</table>

**Phase:** Kromasil 100 Å, 5 µm, C8  
**Column:** 4.6 x 150 mm  
**Temperature:** 31 °C  
**Eluent:** ACN:water (70:30; v:v) 25 mg ammonium acetate /100 ml mobile phase  
**Flow rate:** 1.4 ml/min.  
**Detection:** UV 210 nm

**Catecholamines**
Determination of catecholamines in pig liver. (ref. 95a)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Retention Time (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 = norepinephrine</td>
<td><img src="image" alt="Norepinephrine structure" /></td>
<td>1</td>
</tr>
<tr>
<td>2 = epinephrine</td>
<td><img src="image" alt="Epinephrine structure" /></td>
<td>2</td>
</tr>
<tr>
<td>3 = 3,4-dihydroxy-benzylamine hydrobromide</td>
<td><img src="image" alt="3,4-Dihydroxy-benzylamine hydrobromide structure" /></td>
<td>3</td>
</tr>
<tr>
<td>4 = dopamine</td>
<td><img src="image" alt="Dopamine structure" /></td>
<td>4</td>
</tr>
</tbody>
</table>

**Phase:** Kromasil 100 Å, 5 µm, C8  
**Column:** 4.6 x 150 mm  
**Temperature:** ambient  
**Eluent:** ACN:MeOH:acetate buffer (pH 3.6; 50 mM) (10:30:60; v:v:v) containing 1% v/v HAc  
**Flow rate:** 0.8 ml/min.  
**Detection:** fluorescence (λex 300 nm, λem 458 nm)

**Catecholamines**
Determination of methoxycatecholamines in pig liver. (ref. 95b)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Retention Time (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 = normetanephrine</td>
<td><img src="image" alt="Normetanephrine structure" /></td>
<td>1</td>
</tr>
<tr>
<td>2 = metanephrine</td>
<td><img src="image" alt="Metanephrine structure" /></td>
<td>2</td>
</tr>
<tr>
<td>3 = 4-hydroxy-3-methoxy-benzylamine hydrochloride</td>
<td><img src="image" alt="4-Hydroxy-3-Methoxy-Benzylamine Hydrochloride structure" /></td>
<td>3</td>
</tr>
<tr>
<td>4 = 3-O-methyldopamine</td>
<td><img src="image" alt="3-O-Methyldopamine structure" /></td>
<td>4</td>
</tr>
</tbody>
</table>

**Phase:** Kromasil 100 Å, 5 µm, C8  
**Column:** 4.6 x 150 mm  
**Eluent:** MeOH + 1.5 ml 1-octanesulfonic acid (200 mg/ml) + 100 ml 1 M NaAc + about 1 litre water (pH 3.8). Volume adjusted to 2 litres with water.  
**Flow rate:** 1.1 ml/min.  
**Detection:** electrochemical potential +0.65 V

**Ciprofloxacin**
Determination of ciprofloxacin in pharmaceutical preparations and biological fluids. (ref. 26)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Retention Time (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 = ciprofloxacin</td>
<td><img src="image" alt="Ciprofloxacin structure" /></td>
<td>1</td>
</tr>
<tr>
<td>2 = anthranilic acid</td>
<td><img src="image" alt="Anthranilic acid structure" /></td>
<td>2</td>
</tr>
</tbody>
</table>

**Phase:** Kromasil 100 Å, 5 µm, C18  
**Column:** 4.6 x 250 mm  
**Temperature:** ambient  
**Eluent:** ACN:MeOH:acetate buffer (pH 3.6; 50 mM) (10:30:60; v:v:v) containing 1% v/v HAc  
**Flow rate:** 0.8 ml/min.  
**Detection:** fluorescence (λex 300 nm, λem 458 nm)
**Drugs and metabolites**

### Clodronate

Simultaneous determination of clodronate and its partial ester derivative. (ref. 97)

1 = clodronate

2 = clodronate monophenylester

Phase: Kromasil 100 Å, 5 µm, C8
Column: 4.6 x 250 mm
Eluent: MeOH:ammonium acetate buffer (0.1 M + 0.23 M butylamine, pH 4.6)
Gradient: linear gradient elution: methanol from 3 to 40 – 60% for between 1.0 and 6.0 min. (not specified)
Flow rate: 1.2 ml/min.
Detection: ELS

### Cytochrome P450 metabolites

Analysis of cytochrome P450 metabolites of arachidonic acid. (ref. 10)

1 = 14,15-DHET

2 = 11,12-DHET

3 = 8,9-DHET

4 = 5,6-DHET

5 = 20-HETE

6 = 14,15-EET

7 = 11,12-EET

8 = 8,9-EET

9 = 5,6-EET

DHET = dihydroxy-eicosatrienoic acids
HETE = hydroxy-eicosatetraenoic acids
EET = epoxy-eicosatrienoic acids

Phase: Kromasil 100 Å, 5 µm, C18
Column: 4.6 x 250 mm
Eluent: water/ACN with 0.005% HAc
Gradient: 0 min. 60% ACN, 30 min. 80% ACN, 35 min. 100% ACN 40 min. 100% ACN
Flow rate: 0.2 ml/min.
Detection: ESI-MS

### DRF-2189

Determination of the insulin sensitizing agent DRF-2189 in rat plasma. (ref. 161)

1 = insulin sensitizing agent DRF-2189

2 = troglitazone

Phase: Kromasil 100 Å, 5 µm, C8
Column: 4.6 x 250 mm
Eluent: 0.05 M NaH₂PO₄:ACN:MeOH (22.5:37.5:40; v:v:v) (pH 5.0)
Gradient: linear gradient elution: methanol from 3 to 40 – 60% for between 1.0 and 6.0 min. (not specified)
Flow rate: 1 ml/min.
Detection: fluorescence (λₑ 292 nm and λₑ 325 nm)

### Ecstasy analogues

Identification of a homologue derivative of “ecstasy”. (ref. 170)

1 = N-methyl-1-(1,3-benzodioxol-5-yl)-2-propanamine (MDMA)

2 = N-ethyl-1-(1,3-benzodioxol-5-yl)-2-propanamine (MDEA)

3 = N-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (MBDB)

4 = N-ethyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (EBDB)

Phase: Kromasil 100 Å, 5 µm, C8
Column: 4.6 x 250 mm
Temperature: ambient
Eluent: ACN 0.1 M triethylammonium acetate (aq) pH 7.3
Gradient: 5% to 80% ACN in 25 min.
Flow rate: 1 ml/min.
Detection: UV 280 nm
Drugs and metabolites

5-fluorouracil metabolites
Determination of the main metabolites of 5-fluorouracil in plasma. (ref. 116)

- 1 = 5-fluorouridine
- 2 = 5-fluoro-2'-deoxyuridine
- 3 = 5-iodouracil (internal standard)

**Phase:** Kromasil 100 Å, 5 µm, C18  
**Column:** 4.6 x 150 mm  
**Temperature:** 20°C (ambient)  
**Eluent:** MeOH:water (3:97; v:v)  
**Flow rate:** 0.6 ml/min.  
**Detection:** UV 275 nm

Fotemustine
Stability study of fotemustine in PVC infusion bags. (ref. 124)

- 1 = sodium phenobarbital
- 2 = fotemustine

**Phase:** Kromasil 100 Å, 5 µm, C18  
**Column:** 4.6 x 150 mm  
**Temperature:** ambient  
**Eluent:** ACN:ammonium acetate buffer (0.05 M, pH 4.5) (30:70; v:v)  
**Flow rate:** 1 ml/min.  
**Detection:** UV 230 nm

Ketoprofen
Determination of ketoprofen in vitro in rat skin. (ref. 247)

- 1 = ketoprofen
- 2 = ibuprofen

**Phase:** Kromasil 100 Å, 5 µm, C18  
**Column:** 4 x 250 mm  
**Temperature:** 40°C  
**Eluent:** ACN:0.01 M potassium phosphate (pH 1.5) (60:40; v:v)  
**Flow rate:** 1 ml/min.  
**Detection:** UV 260 nm

Leukotrienes, cross-reactive
Determination of cross-reactive leukotrienes in biological matrices. (ref. 71a)

- 1 = leukotriene LTC4
- 2 = leukotriene LTD4
- 3 = leukotriene LTE4

**Phase:** Kromasil 100 Å, 5 µm, C4  
**Column:** 2.1 x 100 mm  
**Eluent:** ACN-K2HPO4 10 mM (pH 7.4) (30:70; v:v)  
**Flow rate:** 0.2 ml/min.  
**Detection:** fluorescence ($\lambda_{ex}$ 544 nm, $\lambda_{em}$ 572 nm)
**Leukotrienes, cross-reactive**

Determination of cross-reactive leukotrienes in biological matrices. (ref. 71b)

1 = leukotriene LTE4-sulfoxide
2 = leukotriene N-acetyl LTE4
3 = leukotriene LTE4-sulfone
4 = leukotriene LTE4

**Megazol**

Analysis of megazol in human plasma. (ref. 113)

1 = tinidazol
2 = megazol

**Meloxicam**

Determination of meloxicam in human plasma. (ref. 283)

1 = meloxicam

**Pain relievers**

Determination of acetaminophen, ibuprofen and chlorzoxazone. (ref. 154)

1 = acetaminophen
2 = chlorzoxazone
3 = ketoprofen
4 = ibuprofen

**Phase:** Kromasil 100 Å, 5 µm, C4
**Column:** 2.1 x 100 mm
**Eluent:** ACN-KHPO₄ 10 mM (pH 7.4) (30:70; v:v)
**Flow rate:** 0.2 ml/min.
**Detection:** fluorescence (λₑx 544 nm, λₑm 572 nm)

**Phase:** Kromasil 100 Å, 10 µm, C8
**Column:** 4 x 250 mm
**Temperature:** ambient
**Eluent:** phosphate buffer (0.068 M, pH 3):MeOH:ACN (65:20:15; v:v:v)
**Flow rate:** 0.7 ml/min.
**Detection:** UV 360 nm

**Phase:** Kromasil 100 Å, 5 µm, C18
**Column:** 4.6 x 150 mm
**Eluent:** MeOH-water:ACN:HAc (600:500:50:20; v:v:v:v) + 1.01 g sodium heptanesulfonate
**Flow rate:** 1 ml/min.
**Detection:** UV 355 nm

**Phase:** Kromasil 100 Å, 5 µm, C8
**Column:** 4.6 x 250 mm
**Temperature:** 20 ± 1°C
**Eluent:** ACN 0.2% triethylamine (pH 3.2) (50:50; v:v)
**Flow rate:** 1.5 ml/min.
**Detection:** UV 215 nm
Drugs and metabolites

**PAT-5A**
Determination of PAT-5A ([4-[N-(2-pyridyl)-2s]-pyrrolidine-2-methoxyphenylmethylene]thiazolidine-2,4-dione, maleic acid salt), an insulin sensitizing agent, in rat plasma. (ref. 244)

![Chemical structure of PAT-5A and thiazolidinedione]

- **Phase:** Kromasil 100 Å, 5 µm, C18
- **Column:** 4.6 x 250 mm
- **Eluent:** NaH₂PO₄ (0.05 M, pH 4):MeOH (25:75; v:v)
- **Flow rate:** 1 ml/min.
- **Detection:** UV 345 nm

**Phenolics**
Separation of phenolic compounds and corresponding glucuronides. (ref. 103)

- **1 = 4-nitrophenyl-D-glucuronide**
- **2 = 4-nitrophenol**

![Chemical structure of 4-nitrophenyl-D-glucuronide and 4-nitrophenol]

**Phosphonoformate (foscarnet)**
Determination of phosphonoformate (foscarnet) in human serum. (ref. 217)

- **1 = phosphonoformate (foscarnet)**

![Chemical structure of phosphonoformate (foscarnet)]

**Phosphonoformate (foscarnet)**
Determination of phosphonoformate (foscarnet) in human serum. (ref. 217)

- **Phase:** Kromasil 100 Å, 5 µm, C18
- **Precolumn:** Nucleosil 5µm, C4
- **Column:** 4.6 x 100 mm (precolumn: 4.6 x 50 mm)
- **Temperature:** ambient
- **Eluent:** 30 mM cetyltrimethylammonium bromide in 0.05 M 6-aminohexanoic acid (pH: 5) and 20% ACN (precolumn 7%) (v:v)
- **Flow rate:** 1 ml/min.
- **Detection:** UV 300 nm
QC test, tricyclic antidepressants
QC test of Kromasil CN. (ref. 342)

1 = toluene
2 = phenylpropanolamine
3 = amitriptyline
4 = imipramine
5 = nortriptyline

Phase: Kromasil 60 Å, 10 µm, CN
Column: 4.6 x 250 mm
Temperature: ambient
Eluent: MeOH:KH2PO4 25 mM pH 6.0 (80:20; v:v)
Flow rate: 1 ml/min.
Detection: UV 215 nm

QC test, tricyclic antidepressants
QC test of Kromasil C4. (ref. 349)

1 = phenylpropanolamine
2 = nortriptyline
4 = imipramine
5 = amitriptyline
3 = toluene

Phase: Kromasil 100 Å, 5 µm, C4
Column: 4.6 x 250 mm
Temperature: ambient
Eluent: MeOH:KH2PO4 25 mM pH 6.0 (80:20; v:v)
Flow rate: 1 ml/min.
Detection: UV 215 nm

QC test, tricyclic antidepressants
QC test of Kromasil C8. (ref. 350)

1 = phenylpropanolamine
2 = nortriptyline
3 = toluene
4 = imipramine
5 = amitriptyline

Phase: Kromasil 100 Å, 5 µm, C8
Column: 4.6 x 250 mm
Temperature: ambient
Eluent: MeOH:KH2PO4 25 mM pH 6.0 (80:20; v:v)
Flow rate: 1 ml/min.
Detection: UV 215 nm

QC test, tricyclic antidepressants
QC test of Kromasil C18. (ref. 351)

1 = phenylpropanolamine
2 = nortriptyline
3 = toluene
4 = imipramine
5 = amitriptyline

Phase: Kromasil 100 Å, 5 µm, C18
Column: 4.6 x 250 mm
Temperature: ambient
Eluent: MeOH:KH2PO4 25 mM pH 6.0 (80:20; v:v)
Flow rate: 1 ml/min.
Detection: UV 215 nm
Drugs and metabolites

**Quinolinones**
Determination of quinolinones in food. [ref. 119]

1 = enoxacin
2 = ofloxacin
3 = lomefloxacin

Phase: Kromasil 100 Å, 5 µm, C8
Column: 3.2 x 250 mm
Eluent: oxalic acid (0.01M):ACN:MeOH (6:3:1; v:v:v)
Flow rate: 0.5 ml/min.
Detection: fluorescence ($\lambda_{ex}$ 445 nm, $\lambda_{em}$ 278 nm)

**Salicin**
Determination of salicin in extract of willow bark. [ref. 262]

1 = salicin

Phase: Kromasil 100 Å, 5 µm, C18
Column: 4.6 x 250 mm
Eluent: MeOH:KH$_2$PO$_4$ buffer (pH 4.01, 0.01 M) (15:85; v:v)
Flow rate: 1 ml/min.
Detection: UV 265 nm

**Sildenafil**
Determination of sildenafil (Viagra) and its metabolite (UK 103520) with ASTED equipment. [ref. 98]

1 = metabolite UK 103520
2 = sildenafil
3 = reference compound

Phase: Kromasil 100 Å, 5 µm, C4
Column: 4.6 x 100 mm
Temperature: 40°C
Eluent: ACN:potassium phosphate buffer (0.5 M, pH 4.5, containing 10 mM diethylamine HCl):water (28:4:68; v:v:v)
Flow rate: 1.5 ml/min.
Detection: UV 230 nm

**Sildenafil**
Determination of sildenafil citrate (Viagra). [ref. 254]

1 = sildenafil
2 = Internal Standard (UK114542-27)

Phase: Kromasil 100 Å, 5 µm, C4
Column: 4.6 x 150 mm
Temperature: 40°C
Eluent: ACN: 0.5 M potassium phosphate buffer (pH 4.5; containing 10 mM diethylamine HCl):water (32:68; v:v)
Flow rate: 0.7 ml/min.
Detection: UV 230 nm
Spiramycin

Determination of spiramycin in pig liver. (ref. 94)

1. cysteyl neospiramycin I
2. cysteyl spiramycin I
3. spiramycin I
4. cysteyl spiramycin III
5. spiramycin S
6. spiramycin III

R1 R2 R3
1. cysteyl neospiramycin I_H_H_timonacic
2. cysteyl spiramycin I_H_α-mycarose_timonacic
3. spiramycin I
4. cysteyl spiramycin III_COCH3_H_timonacic
5. spiramycin S
6. spiramycin III_COCH3_α-mycarose_COH

Spiramycin

Determination of spiramycin in pig liver. (ref. 94)

Steroids

Analysis of clenbuterol hydrochloride and ambroxol hydrochloride. (ref. 351)

1 = clenbuterol
2 = ambroxol

Steroids

Analysis of dexamethasone and betamethasone acetate in bovine liver. (ref. 272a)

1 = dexamethasone
2 = betamethasone acetate
Drugs and metabolites

**Tetracyclines**

Determination of tetracyclines as chelates with aluminum(III). (ref. 273)

- 1 = minocycline
- 2 = oxytetracycline
- 3 = methacycline
- 4 = chlortetracycline

**Phase:** Kromasil 100 Å, 5 µm, C18
**Column:** 4.6 x 250 mm
**Eluent:** ACN:DMF:0.05 M citric acid-sodium citrate buffer (pH 2.5) (5:20:75; v:v:v)
**Flow rate:** 0.7 ml/min.
**Detection:** fluorescence ($\lambda_{ex}$ 380 nm and $\lambda_{em}$ 480 nm)

**Tramadol**

Determination of tramadol and its active metabolite in human plasma. (ref. 130)

- 1 = o-demethyltramadol
- 2 = fluconazole
- 3 = tramadol

**Phase:** Kromasil 100 Å, 5 µm, C18
**Column:** 4 x 250 mm
**Temperature:** 30°C ± 3°C
**Eluent:** acetonitrile:water (19:81, v:v) cont. 0.06 M NaH$_2$PO$_4$ and 0.05 M triethylamine, adjusted to pH 7.90
**Flow rate:** 1 ml/min.
**Detection:** fluorescence ($\lambda_{ex}$ 207 nm and $\lambda_{em}$ 300 nm)

**Tranquilizers, veterinary**

Analysis of xylazine, haloperidol, acetopromazine, propionylpromazine and chlorpromazine in bovine liver. (ref. 272b)

- 1 = xylazine
- 2 = haloperidol
- 3 = acetopromazine
- 4 = propionylpromazine
- 5 = chlorpromazine

**Phase:** Kromasil 100 Å, 5 µm, C18
**Column:** 4 x 150 mm
**Eluent:** MeOH:water (80:20; v:v)
**Flow rate:** 1 ml/min.
**Detection:** UV 240 nm
Alkyllead
Determination of tetramethyllead and tetraethyllead. (ref. 198)

1 = tetramethyllead
(2 = unknown)
3 = tetraethyllead

Phase: Kromasil 100 Å, 5 µm, C18
Column: 0.32 x 250 mm
Temperature: start: 50°C, ramp: 16°C/min., hold: 100°C
Eluent: ACN
Flow rate: 10 µl/min
Detection: ICP-MS

Benzimidazole fungicides
Determination of benzimidazole fungicides in fruits. (ref. 112)

1 = carbenzadim
2 = thiabendazole

Phase: Kromasil 100 Å, 5 µm, C18
Column: 4 x 150 mm
Temperature: 55°C
Eluent: MeOH-water (50:50; v:v)
Flow rate: 1 ml/min.
Detection: UV 285 nm

Explosives
Sensitive determination of RDX, nitroso-RDX metabolites and other munitions in ground water. (ref. 175)

1 = HMX
2 = TNX
3 = 4-NBA
4 = MNX
5 = TNB
6 = RDX
7 = DNB
10 = 2A-DNT

Phase: Kromasil 100 Å, C8
Column: 2 x 250 mm
Temperature: 52°C
Eluent: isopropanol:water:0.5 M ammonium formate (pH 8 adjusted by ammonium hydroxide) (20:78:2; v:v:v)
Flow rate: 0.2 ml/min.
Detection: UV

Herbicides
Analysis of 4-chloro-2-oxo-3(2H)-benzothiazoleacetic ethyl ester and related compounds. (ref. 286)

1 = 4-chloro-2-oxo-3(2H)-benzothiazoleacetic acid
2 = 2-amino-4-chloro-benzothiazole
3 = 2-hydroxy-4-chloro-benzothiazole
4 = 4-chloro-2-oxo-3(2H)-benzothiazoleacetic ethyl ester
5 = 2,4-dichloro-benzothiazole

Phase: Kromasil 100 Å, 5 µm, C18
Column: 4.6 x 200 mm
Temperature: 30°C
Eluent: MeOH:water:HAc (60:40:1; v:v:v)
Flow rate: 0.7 ml/min.
Detection: UV 254 nm
Environmental

Nitrophenols
Determination of toxic nitrophenols in the atmosphere. (ref. 183)

<table>
<thead>
<tr>
<th>1</th>
<th>2,6-dinitrophenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2,4-dinitrophenol</td>
</tr>
<tr>
<td>3</td>
<td>phenol</td>
</tr>
<tr>
<td>4</td>
<td>4-nitrophenol</td>
</tr>
</tbody>
</table>

Phase: Kromasil 100 Å, 5 µm, C18
Column: 4.6 x 250 mm
Eluent: A:B (55:45; v:v) A: 0.005 M KH₂PO₄ (pH 4.5 with H₃PO₄) : ACN (90:10; v:v) B: 0.005 M KH₂PO₄ (pH 4.5 with H₃PO₄):MeOH (25:75; v:v)
Flow rate: 1 ml/min.
Detection: 250 nm

Organonitrogen pesticides
Determination of organonitrogen pesticides in large volumes of surface water. (ref. 152)

<table>
<thead>
<tr>
<th>1</th>
<th>dia (desisopropylatrazine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>DEA (desethylatrazine)</td>
</tr>
<tr>
<td>3</td>
<td>simazine</td>
</tr>
<tr>
<td>4</td>
<td>cyanazine</td>
</tr>
</tbody>
</table>

Phase: Kromasil 100 Å, 5 µm, C18
Column: 4.6 x 250 mm
Eluent: Gradient, ACN:water, 15 – 60% ACN for 50 min, 60% for 15 min
Flow rate: 1 ml/min.
Detection: APCI-MS

Pesticides and metabolites
Analysis of polar phenolic compounds, pesticides and metabolites in water. (ref. 167)

<table>
<thead>
<tr>
<th>1</th>
<th>resorcinol</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>oxamyl</td>
</tr>
<tr>
<td>3</td>
<td>methomyl</td>
</tr>
</tbody>
</table>

Phase: Kromasil 100 Å, 5 µm, C18
Column: 4.6 x 250 mm
Temperature: 65°C
Eluent: ACN water (pH 3 adjusted with sulfuric acid)
Gradient: From 15 to 25% ACN in 9.3 min., to 50% ACN in 4.3 min., to 100% ACN in 6 min. and then 2 min. isocratic elution at 100% ACN.
Flow rate: 1 ml/min.
Detection: UV 280 or 240 nm

Phenylurea herbicides
Determination of phenylurea herbicides in water. (ref. 32)

<table>
<thead>
<tr>
<th>1</th>
<th>metoxuron</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>diuron</td>
</tr>
<tr>
<td>3</td>
<td>linuron</td>
</tr>
<tr>
<td>4</td>
<td>neburon</td>
</tr>
</tbody>
</table>

Phase: Kromasil 100 Å, 5 µm, C18
Column: 1 x 300 mm
Eluent: MeOH water (75:25; v:v) in 0.01 M lithium perchlorate at pH 5.5 (adjusted with 1% phosphoric acid)
Flow rate: 20 – 40 µl/min
Detection: UV 254 nm and electrochemical (potential 1.35 V) respectively for the figures
**Polycyclic aromatic hydrocarbons**

Analysis of polycyclic aromatic hydrocarbons. (ref. 184)

| 1 | naphtalene |
| 2 | acenaphthylene |
| 3 | acenaphthene |
| 4 | fluorene |
| 5 | phenanthrene |
| 6 | anthracene |
| 7 | fluoranthene |
| 8 | pyrene |
| 9 | benzo(a)anthracene |
| 10 | chrysene |
| 11 | benzo[b]fluoranthene |
| 12 | benzo[k]fluoranthene |
| 13 | benzo[a]pyrene |
| 14 | dibenzo[a,h]anthracene |
| 15 | indeno(1,2,3-c,d)pyrene |
| 16 | benzo[g,h,i]perylene |

Phase: Kromasil 100 Å, 5 µm, C18
Column: 4.6 x 250 mm
Temperature: 40°C
Eluent: CO2:ACN
Gradient: 0 min. 100% CO2, 20 min. 60% CO2, 25 min. 60% CO2
Flow rate: 3 ml/min
Detection: UV 210 nm

---

**Quaternary ammonium herbicides**

Determination of quaternary ammonium herbicides. (ref. 201)

| 1 | diquat |
| 2 | paraquat |
| 3 | ethyl viologen |
| 4 | difenzoquat |

Phase: Kromasil 100 Å, 5 µm, C8
Column: 2.1 x 200 mm
Temperature: 50°C
Eluent: Pentafluoropropionic acid in water (15 mM, pH 3.5) : ACN
Gradient: 0 min. 2% ACN, 5 min. 8.6% ACN, 5.01 min. 40% ACN, 13 min. 40% ACN
Flow rate: 200 µl/min.
Detection: UV
Food and nutrition

**Antioxidants, lipophilic**

Determination of lipophilic antioxidants in plasma. (ref. 55)

**Irganox**

Determination of Irganox (an antioxidant). (ref. 20)

**Irganox**

Determination of Irganox. (ref. 208)

**Sugars**

Analysis of sugars in urine. (ref. 82)
**Sugars**

Analysis of sugars. (ref. 315)

1 = rhamnose
2 = xylose
3 = fructose
4 = mannose
5 = glucose
6 = galactose
7 = sucrose
8 = maltose
9 = lactose

Phase: Kromasil 100 Å, 5 µm, NH2
Column: 4.6 x 250 mm
Eluent: ACN:water (75:25; v:v)
Flow rate: 1 ml/min.
Detection: RI

**Sugars, phosphorylated**

Determination of reducing sugars in beef sirloin, with post-column reduction. (ref. 27)

1 = xylose
2 = ribose
3 = fructose
4 = glucose
5 = o-lactose

Phase: Kromasil 100 Å, 5 µm, NH2
Column: 4 x 250 mm
Eluent: ACN water (85:15; v:v) at pH 4.8
Flow rate: 1.4 ml/min.
Post column: Post-column reduction at 95°C with tetrazolium blue (0.7 mM in distilled water and 0.16 M NaOH, 15% EtOH, 0.047M Na-K-tartrate, pH 12.7) before detection.
Detection: 550 nm

**Sugars and polyols, benzoylated**

Analysis of benzoylated sugars and polyols. (ref. 51b)

1 = tetrabenzoyl-D-glucose
2 = pentabenzoyl mannitol
3 = 3-O-methyl-D-glucose
4 = pentabenzoyl-D-glucose
5 = hexabenzoyl mannitol

Phase: Kromasil 100 Å, 5 µm, C18
Column: 4 x 250 mm
Eluent: Gradient, ACN-water, 0 min. 70% ACN, 30 min. 95% ACN
Flow rate: 1 ml/min.
Detection: UV 228 nm
Natural products

**Actarit**
Determination of actarit and related compounds. (ref. 274)

- **Phase:** Kromasil 100 Å, 5 µm, C18
- **Column:** 4.6 × 250 mm
- **Eluent:** MeOH:water (70:30; v:v) + 1% tetrabutylammonium bromide
- **Flow rate:** 1 ml/min.
- **Detection:** UV 245 nm

**Gaulthersides**
Determination of Gaulthersides in Yunnan wintergreen. (ref. 307)

- **Phase:** Kromasil 100 Å, 5 µm, C18
- **Column:** 3.9 × 250 mm
- **Eluent:** MeOH:ACN:water (25:5:70; v:v:v) pH=3.5 (adjusted with H₃PO₄)
- **Flow rate:** 0.7 ml/min.
- **Detection:** UV 220 nm

**Caffeine and metabolites**
Quantitation of caffeine metabolism products. (ref. 271)

- **Phase:** Kromasil 100 Å, 5 µm, C4
- **Column:** 4 × 250 mm
- **Temperature:** ambient
- **Eluent:** acetate buffer (pH 3.5): MeOH (97:3; v:v)
- **Gradient:** 0 min. 3% MeOH, 20 min. 20% MeOH
- **Flow rate:** 1 ml/min.
- **Detection:** UV 275 nm
**Natural products**

**Ginkgolides**

Determination of ginkgolides. (ref. 277)

1 = bilobalide
2 – 4 = ginkgolide

<table>
<thead>
<tr>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>OH</td>
<td>OH</td>
<td>OH</td>
</tr>
<tr>
<td>OH</td>
<td>H</td>
<td>OH</td>
</tr>
<tr>
<td>OH</td>
<td>H</td>
<td>OH</td>
</tr>
</tbody>
</table>

Phase: Kromasil 100 Å, 5 µm, C18
Column: 4.6 x 250 mm
Eluent: water:MeOH (77:33; v:v)
Flow rate: 1 ml/min.
Detection: refractive index

**Nicotine**

Clinical assay of nicotine and its metabolite, cotinine, in body fluids. (ref. 306)

1 = cotinine
2 = nicotine
3 = scopolamine

Phase: Kromasil 100 Å, 5 µm, C8
Column: 4 x 250 mm
Temperature: ambient, 22°C
Eluent: ammonium acetate (0.05 M):CH₃OH (60:40; v:v)
Flow rate: 1.4 ml/min.
Detection: UV 262 nm

**Sphingoids**

Analysis of sphinganine and sphingosine from urine with precolumn o-phthaldialdehyde (OPA) derivatization. (ref. 87)

1 = OPA-sphingosine
2 = OPA-sphinganine
3 = OPA-C₂₀-sphinganine

Phase: Kromasil 100 Å, 5 µm, C18
Column: 4.6 x 250 mm
Temperature: 45°C
Eluent A: 0.07 M K₂HPO₄ in MeOH (1:9; v:v)
Eluent B: MeOH
Gradient: 0 min. 0% B, 10 min. 0% B, 30 min. 40% B, 32 min. 100% B, 42 min. 100% B, 44 min. 0% B, 60 min. 0% B
Flow rate: 1.3 ml/min.
Detection: fluorescence (λₑₓ 340 nm, λₑᵣ 455 nm)
Natural products

TCM, Traditional Chinese Medicine
Determination of gastrodin, p-hydroxybenzyl alcohol, vanillyl alcohol, p-hydroxybenzaldehyde and vanillin from TCM. (ref. 297)

1 = gastrodin
2 = p-hydroxybenzyl alcohol
3 = vanillyl alcohol
4 = p-hydroxybenzaldehyde
5 = vanillin

Phase: Kromasil 100 Å, 5 µm, C18
Column: 4.6 x 150 mm
Temperature: ambient
Eluents: Eluent A: water, eluent B: MeOH
Gradient: 0 min 5% B, 9 min 44% B, 12 min 65% B, 15 min 65% B
Flow rate: 1 ml/min.
Detection: UV 270 nm

TCM, Traditional Chinese Medicine
Determination of three components in a Chinese doctor-cough syrup. (ref. 210)

1 = phenylephrine
2 = codeine
3 = pseudoephedrine

Phase: Kromasil 100 Å, 5 µm, C18
Column: 4.6 x 250 mm
Temperature: 45°C
Eluent: MeOH:water:acetic acid (40:60:2; v:v:v)
+ 5 mM IPR-B₈
Flow rate: 1 ml/min.
Detection: UV 245 nm

TCM, Traditional Chinese Medicine
Analysis of caffeine, antipyrine and sodium salicylate in Satongfeng injection. (ref. 215)

1 = caffeine
2 = antipyrine
3 = sodium salicylate

Phase: Kromasil 100 Å, 5 µm, C18
Column: 4.6 x 250 mm
Eluent: 20 mM potassium dihydrogen phosphate: MeOH:glacial acetic acid (55:25:0.4; v:v:v)
Flow rate: 1 ml/min.
Detection: UV 242 nm

TCM, Traditional Chinese Medicine
Determination of four components of Ganmaoling capsules. (ref. 258)

1 = chlorpheniramine maleate
2 = paracetamol
3 = caffeine
4 = phenylpropanolamine hydrochloride

Phase: Kromasil 100 Å, 5 µm, C18
Column: 4.6 x 250 mm
Temperature: 30°C
Eluent: ACN:diammonium hydrogen phosphate
(pH 3.1, 0.03 M) (12:88; v:v) containing 0.75 – 5 mM sodium sulfonic heptane
Flow rate: 1 ml/min.
Detection: UV 214 nm
**Enkephalin peptides**

Analysis of enkephalin peptides, their metabolites and enzyme inhibitors. (ref. 104)

**Nonapeptides**

Analysis of five nonapeptides. (ref. 28)
Peptides

Separation of 9 peptides. (ref. 316)

1 = oxytocin
   Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH₂

2 = bradykinin
   Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg

3 = methionine enkephalin
   Tyr-Gly-Phe-Met

4 = angiotensin II
   Asp-Arg-Val-Tyr-Ile-His-Pro-Phe

5 = leucin enkephalin
   Tyr-Gly-Phe-Leu

6 = angiotensin I
   Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu

7 = insulin

8 = lysozyme

9 = melittine

Flow rate: 2 ml/min.
Detection: UV 254 nm
Vitamin E
Determination of vitamin E in human plasma. (ref. 108)

1 = vitamin E

Phase: Kromasil 100 Å, 5 µm, C1
Column: 4.6 x 100 mm
Temperature: ambient
Eluent: MeOH:ACN:water (50:35:15; v:v:v)
Flow rate: 1.5 ml/min.
Detection: UV 292 nm

Vitamins
Determination of tocopherols and vitamin A in vegetable oils. (ref. 188)

1 = vitamin A (retinol)
2 = d-tocopherol
3 = γ-tocopherol
4 = α-tocopherol

Phase: Kromasil 100 Å, 5 µm, C18
Column: 0.2 x 800 mm
Temperature: 65°C
Eluent: CO2 with 8% MeOH
Pressure: 180 atm
Detection: electrochemical (potential +1.80 V versus Quasi-Reference Electrode)

Vitamins
Analysis of soluble vitamins. (ref. 330)

1 = ascorbic acid (vitamin C)
2 = niacinamide (vitamin B3)
3 = pyridoxine (vitamin B6)
4 = riboflavin (vitamin B2)
5 = thiamine chloride (vitamin B1)

Phase: Kromasil 100 Å, 10 µm, NH2
Column: 4.6 x 250 mm
Eluent: 0.68 g sodium 1-hexanesulfonic acid + 0.8 g phosphoric acid + 720 ml water (pH 2.3) + 80 ml ACN + 200 ml MeOH
Flow rate: 1 ml/min.
Detection: UV 210 nm
### Amines

Determination of amines from fish decomposition by dansyl chloride derivatisation. (ref. 73)

### Amino alcohols

Separation of derivates of 1-ethylamino-3-phenoxyl-propan-2-ol. (ref. 38)

### Aroma extracts in alcoholic beverages

Separation of aroma extracts found in wine and other alcoholic beverages. (ref. 209)
Aromatics
Separation of mixtures of nitrobenzoic acid and aminobenzoic acid isomers. (ref. 214)

1 = m-aminobenzoic acid
2 = p-aminobenzoic acid
3 = o-aminobenzoic acid
4 = o-nitrobenzoic acid

Phase: Kromasil 100 Å, 5 µm, C18
Column: 4.6 x 200 mm
Temperature: 35 °C
Eluent: MeOH:water:THF (55:44:1; v:v:v) with β-cyclodextrin at pH 3.0
Flow rate: 0 – 4 min. 2 ml/min., 4 – 10 min. 2.6 ml/min.
Detection: UV 254 nm

Aromatics
HPLC analysis of isomers of nitrotoluene and nitrobenzoic acid. (ref. 213)

1 = o-nitrobenzoic acid
2 = m-nitrobenzoic acid
3 = p-nitrobenzoic acid
4 = o-nitrotoluene
5 = p-nitrotoluene
6 = m-nitrotoluene

Phase: Kromasil 100 Å, 5 µm, C18
Column: 4.6 x 200 mm
Temperature: 35 °C
Eluent: MeOH:water:THF (55:44:1; v:v:v) with β-cyclodextrin at pH 3.0
Flow rate: 0 – 4 min. 2 ml/min., 4 – 10 min. 2.6 ml/min.
Detection: UV 254 nm

Aromatics
Determination of sulfonurea, benzene, toluene, naphtalene, biphenyl, phenanthrene, anthracene. (ref. 301a)

1 = sulfourea
2 = benzene
3 = toluene
4 = naphtalene
5 = biphenyl
6 = phenanthrene
7 = anthracene

Phase: Kromasil 100 Å, 5 µm, C18
Column: 0.8 x 150 mm
Eluent: MeOH:water (80:20; v:v)
Flow rate: 38 µl/min.
Detection: UV 254 nm

Aromatics
Separation of benzene and pyridine derivates. (ref. 40)

1 = methyl-p-hydroxybenzoate
2 = propyl-p-hydroxybenzoate
4 = methylbenzene
5 = hexylpyridine
6 = heptylpyridine

Phase: Kromasil 100 Å, 5 µm, C18
Column: 4.6 x 150 mm
Eluent: ACN:water (56.9:43.1; v:v)
Flow rate: 1 ml/min.
Detection: UV 254 nm
**ATP degradation products**

Determination of ATP degradation products from fish decomposition. (ref. 159)

Gradient: 0 min. 100% B, 4 min. 98% B, 5 min. 97% B, 8 min. 96% B, 15 min. 96% B, 15.01 min. 100% B

Flow rate: 1 ml/min.

Detection: UV 254 nm

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**Benzene, substituted**

Separation of substituted benzene. (ref. 1)

1 = nitrobenzene
2 = fluorobenzene
3 = chlorobenzene
4 = toluene
5 = bromobenzene

Gradient: 0 min. 100% A, 5 min. 100% A, 10 min. 100% A

Flow rate: 32 µl/min.

Detection: UV 220 nm

---

**Chlorinated benzenes**

Determination of chlorobenzene and derivates. (ref. 301c)

1 = chlorobenzene
2 = p-dichlorobenzene
3 = m-dichlorobenzene
4 = 1,3,5-trichlorobenzene
5 = 1,2,4-trichlorobenzene
6 = 1,3,5-trichlorobenzene
7 = 1,2,3,4-tetrachlorobenzene
8 = 1,2,4,5-tetrachlorobenzene
9 = pentachlorobenzene
10 = hexachlorobenzene

Gradient: 0 min. 80% A, 5 min. 80% A, 10 min. 100% A

Flow rate: 52 µl/min.

Detection: UV 220 nm
**Fatty acids**
Analysis of plasma fatty acids as their phenacyl esters. (ref. 193)

1 = linoleic acid (phenacyl ester derivative)
2 = myristic acid (phenacyl ester derivative)
3 = palmitoleic acid (phenacyl ester derivative)
4 = arachidonic acid (phenacyl ester derivative)
5 = linoleic acid (phenacyl ester derivative)
6 = palmitic acid (phenacyl ester derivative, internal standard)
7 = palmitic acid (phenacyl ester derivative)
8 = oleic acid (phenacyl ester derivative)
9 = elaidic acid (phenacyl ester derivative)
10 = stearic acid (phenacyl ester derivative)

**Organic acids**
Separation of formic acid, benzoic acid, lactic acid, acetic acid. (ref. 344)

1 = formic acid
2 = benzoic acid
3 = lactic acid
4 = acetic acid

**Lauric acid**
Detection of ester of lauric acid. (ref. 35)

1 = fluorescent ester of lauric acid

**Other**
---

Phase: Kromasil 100 Å, 5 µm, C18
Column: 4.6 x 250 mm
Temperature: ambient
Eluent: MeOH:water (91:9; v:v)
Flow rate: 1.15 ml/min.
Detection: UV 254 nm

---

Phase: Kromasil 100 Å, 7 µm, C18
Column: 4.6 x 150 mm
0.2% phosphoric acid added
Flow rate: 1 ml/min.
Detection: fluorescence ($\lambda_{ex}$ 357.5 nm and $\lambda_{em}$ 482 nm)

---

Phase: Kromasil 100 Å, 5 µm, C18
Column: 4.6 x 250 mm
Eluent: KH$_2$PO$_4$-buffer (10 mM, pH 2.5):ACN (95:5; v:v)
Flow rate: 38 µl/min.
Detection: UV 254 nm
QC test, neutral compounds

QC test of Kromasil CN. (ref. 341)

1 = toluene
2 = ethylcinnamate
3 = acetophenone
4 = benzylmandelate
5 = glucosepentabenzoate

Phase: Kromasil 60 Å, 10 µm, CN
Column: 4.6 x 250 mm
Eluent: hexane:ethylacetate (90:10; v:v)
Flow rate: 2 ml/min.
Detection: UV 254 nm

QC test, neutral compounds

QC test of Kromasil SIL. (ref. 346)

1 = toluene
2 = metabolite
3 = ethylcinnamate
4 = acetophenone
5 = benzylmandelate
6 = glucosepentabenzoate

Phase: Kromasil 60 Å, 5 µm, SIL
Column: 4.6 x 250 mm
Eluent: hexane:ethylacetate (85:15; v:v)
Flow rate: 2 ml/min.
Detection: UV 254 nm

QC test, silanophilic compounds

QC test of Kromasil SIL. (ref. 345)

1 = toluene
2 = acetoacetanilide
3 = quinoxaline
4 = 3-pyridylacetonitrile
5 = caffeine

Phase: Kromasil 60 Å, 5 µm, SIL
Column: 4.6 x 250 mm
Eluent: MeCl₂:MeOH (98:2; v:v)
Flow rate: 2 ml/min.
Detection: UV 254 nm

QC test, substituted aromatic compounds

QC test of Kromasil NH₂. (ref. 343)

1 = butylbenzene
2 = N,N-diethylaniline
3 = methylbenzoate
4 = nitrobenzene

Phase: Kromasil 100 Å, 5 µm, NH₂
Column: 4.6 x 250 mm
Eluent: hexane:MeCl₂ (97:3; v:v)
Flow rate: 1 ml/min.
Detection: UV 254 nm
Flavonoid glycosides
Analysis of flavonoid glycosides. (ref. 100)

Phase: Kromasil 100 Å, 5 µm, C18
Column: 3.2 × 250 mm
Eluent: ACN:water
Gradient: 0 min. 20% ACN, 10 min. 20% ACN, 18 min. 40% ACN, 28 min. 75% ACN, 30 min. 100% ACN, 37 min. 100% ACN

Flow rate: 0.75 ml/min.
Detection: UV 280 nm

Organometallic catalysts
Purity testing of organometallic catalysts. (ref. 248)

Phase: Kromasil 100 Å, 5 µm, C18
Column: 0.32 × 450 mm
Temperature: 60°C
Eluent: carbon dioxide
Flow rate: 7.2 µl/min.
Pressure: 100 bar (hold 10 min.) then 10 bar/min. until 180 bar (hold 1 min.), then 10 bar/min. until 300 bar (hold 1 min.), then 10 bar/min. until 400 bar (hold 10 min.)
Detection: FID

Surfactants
Determination of caproic acid, n-octyl alcohol and n-octyl caproate. (ref. 285)

Phase: Kromasil 100 Å, 5 µm, C18
Temperature: 30°C
Column: 4.6 × 150 mm
Eluent: MeOH : water (95:5; v:v)
Flow rate: 1 ml/min.
Detection: refractive index
**Triacylglycerols**

Analysis of seven triacylglycerols. (ref. 139)

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Phase: Kromasil 100 Å, 5 µm, C18
Column: 0.7 x 120 mm
Eluent: (A):ACN, (B):acetone
Gradient: stepwise: 0 – 5 min. 90% A, 5 – 25 min. 70% A, after 25 min. 40%A.
Flow rate: 5 – 100 µl/min (not specified)
Detection: ELS

---

**Triglycerides**

Analysis of triglyceride profiles in Cretan olive oils. (ref. 96)

|------|--------|--------|-------|--------|-------|-------|-------|-------|-------|--------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|

Phase: Kromasil 100 Å, 5 µm, C18
Column: 4 x 250 mm
Temperature: 40°C
Eluent: acetone:ACN (60:40; v:v)
Flow rate: 0.7 ml/min.
Detection: refractive index

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**Triclosan**

Determination and stability tests of triclosan in disinfectants. (ref. 8)

Phase: Kromasil 100 Å, 7 µm, C18
Column: 4.6 x 200 mm
Eluent: MeOH:ACN:water (40:40:20; v:v:v) containing 0.02 M KH2PO4 (pH 2.7)
Flow rate: 1 ml/min.
Detection: UV 280 nm
REFERENCES


90 B. Lu, M. Koirnur, D. Westerlund, Chromatographia 46(1-2) (1997) 72-78


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fluoranthene
fluorene
fluoranyl methylchloroformate, 9-
fluoro-2'-deoxyuridine, 5-
fluorobenzene
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fluvoxamine
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formic acid
formononetin
foscarnet
fotemustine
fructose
furazolidone
furfural
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galactose
gastrodin
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gaultereside D2
gaultereside D3
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ginkgolide A
ginkgolide B
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 glucose pentabenzoate
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glutamic acid
 glutamate, Fmoc-
glutamic acid, OPA-
glutamic acid, PTC-
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 glycine
 glycine, Fmoc-
glycine, OPA-
glycine, PTC-
guaiacol
H₂90-51
H₂90-39
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heptylpyridine
hesperetin
HETE, 20-
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hexachlorobenzene
hexylpyridine
histamine
histidine, Fmoc-
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histidine, OPA-
histidine, PTC-
HMX
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hydroxybenzaldehyde, p-
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hypoxanthine
ibuprofen
imipramine
indeno(1,2,3-c,d)pyrene
inosine
insulin
insulin sensitizing agent DRF-2189
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leucine, PTC-
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mannitol
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melittin
meloxicam
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methacycline
methansulfonyl-L-carnitine
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methyl tricarbaryl cyclopentadienyl tungsten
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methyl dopamine, 5-O-
methyl-p-hydroxybenzoate
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Availability of Kromasil

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■ = available as standard product    □ = please inquire!

Kromasil HPLC columns

Kromasil high pressure slurry-packed columns are available in dimensions from 2.1 mm up to 50.8 mm (2") inner diameter, all columns packed with analytical performance. For detailed information on availability please consult our column brochure, or contact us directly.
The moment you adopt our Kromasil High Performance Concept, you join thousands of chromatographers who share a common goal: to achieve better separations when analyzing or isolating pharmaceuticals or other substances.

Not only will you benefit from our patented silica technology, but you gain a strong partner with a reliable track record in the field of silica products. For the past 60 years, Eka Chemicals has pioneered new types of silica. Our long experience in the field of silica chemistry is the secret behind the development of Kromasil, and the success of our Separation Products Group.

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Eka Chemicals is a global company with 3,000 people in 30 countries. It is a business unit within Akzo Nobel, one of the world’s largest chemical groups, with more than 67,000 employees in 80 countries.

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