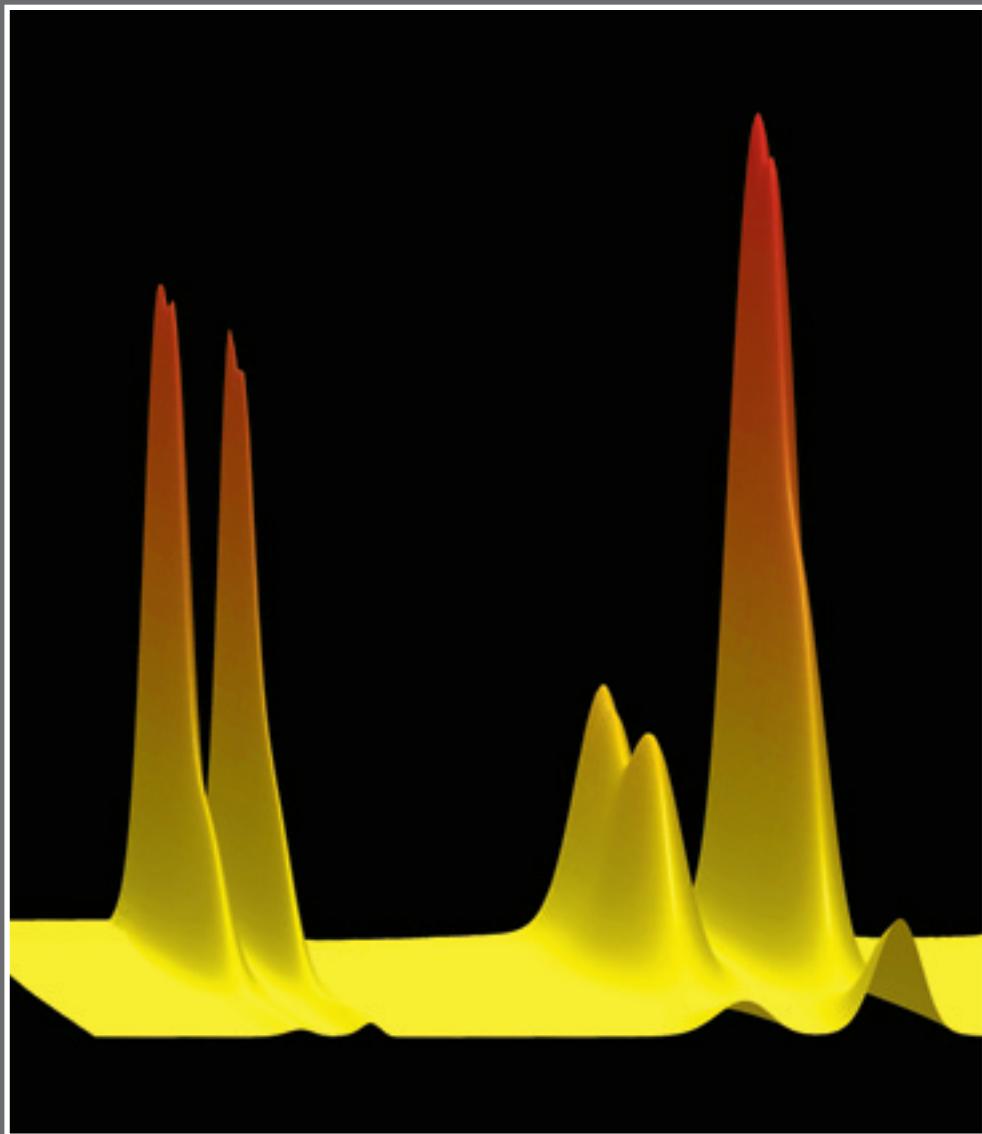


# *Kromasil application guide*



# Kromasil®

*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, NH<sub>2</sub>, 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

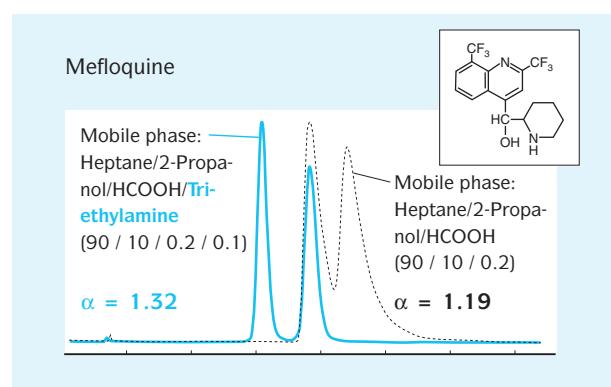


Figure 1 | Influence of the mobile phase additives on the separation of mefloquine.

Conditions: Column: 4.6 × 250 mm, Kromasil CHI-DMB, 5 µm  
Flow rate: 2 ml/min. Detection: UV 280 nm

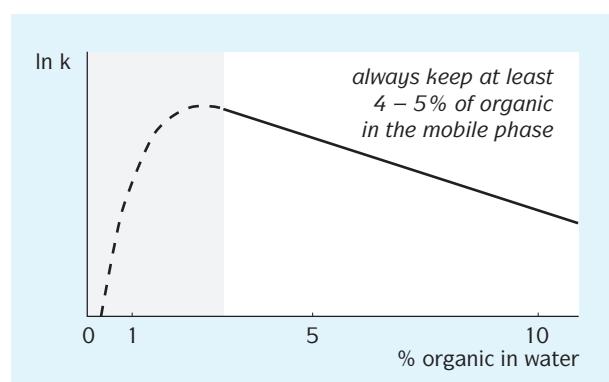


Figure 2 | The retention factor,  $k$ , vs. organic content. General retention behaviour at low organic content using high density RP phases.

Column length (mm)	Particle size (µm)	Flow rate (ml/min.)	Relative time	Relative $R_s$	Relative $\Delta P$
250	10	0.5	1	1	1
125	5	1.0	0.25	1	4
87.5	3.5	1.43	0.125	1	8

Table 1 | Relation between optimal flow rate, analysis time and back pressure,  $\Delta P$ , when going to smaller particles, and shorter columns. The comparisons are for a constant resolution,  $R_s$ .

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.

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:

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

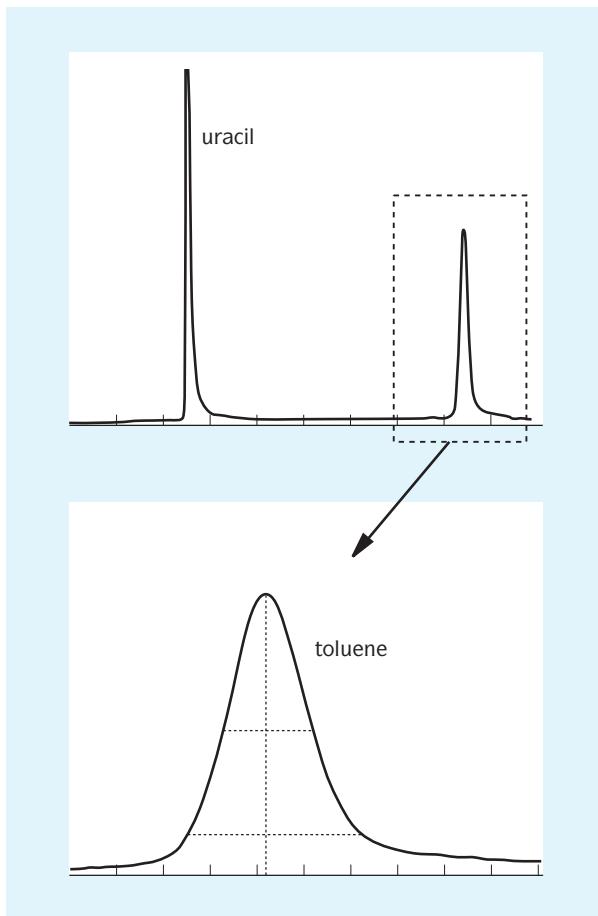
1. **The separation factor,  $\alpha$ , can be increased.** This can be done by optimizing stationary and mobile phase, i.e. choosing the best column and mobile phase composition for the specific application
2. **The number of theoretical plates can be increased.** This can be done by:
  - a. Increasing 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 asymmetry $_{0,1}$  was 1.19**

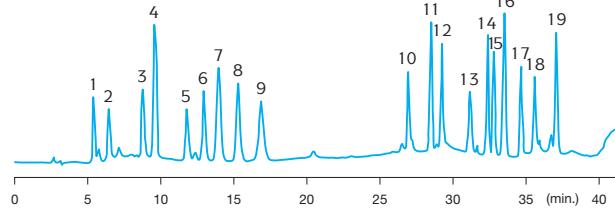
*Conditions: Packing material: 83 kg Kromasil 10 µm Bed length: 25 cm Eluent: acetonitrile/water (7/3) Flow rate: 20 lit./min. Sample: uracil and toluene*

# Applications

## Amino acids

### Amino acids, PTC derivatives

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



Phase: Kromasil 100 Å, 5 µm, C18

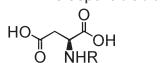
Column: 4.6 × 200 mm

Temperature: 38°C

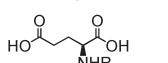
Eluent A: 3% ACN in 0.1M sodium acetate

Eluent B: ACN:water (80:20; v:v).

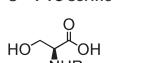
1 = PTC-aspartic acid



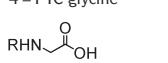
2 = PTC-glutamic acid



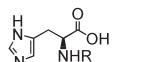
3 = PTC-serine



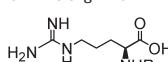
4 = PTC-glycine



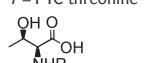
5 = PTC-histidine



6 = PTC-arginine



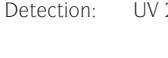
7 = PTC-threonine



8 = PTC-alanine



9 = PTC-proline



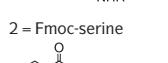
10 = PTC-tyrosine



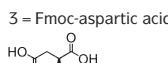
11 = PTC-valine



12 = PTC-methionine



13 = PTC-cysteine



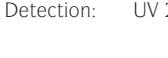
14 = PTC-isoleucine



15 = PTC-leucine



16 = PTC-norleucine (I. S.)



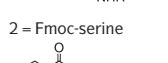
17 = PTC-phenylalanine



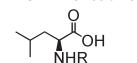
18 = PTC-tryptophan



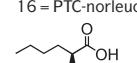
19 = PTC-lysine



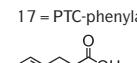
15 = PTC-leucine



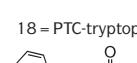
16 = PTC-norleucine (I. S.)



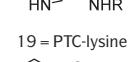
17 = PTC-phenylalanine



18 = PTC-tryptophan



19 = PTC-lysine



R = PTC derivative group



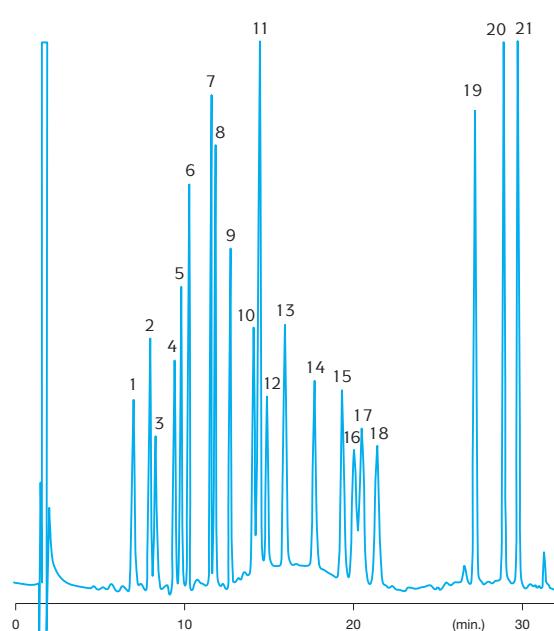
Gradient: Linear gradient elution. 0 min. 0% B, 13 min. 7% B, 23 min. 23% B, 29 min. 35% B, 35 min. 40% B, 40 min. 100% B, 45 min. 100% B, 47 min. 0% B

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 methylchloroformate) derivatization. (ref. 30)



Phase: Kromasil 100 Å, 5 µm, C8

Column: 4 × 250 mm

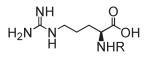
Temperature: 45°C

Eluent A: sodium acetate buffer (100 mM, pH 4.4):THF:ACN (75:15:10; v:v:v)

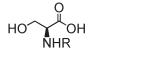
Eluent B: ACN:THF (85:15; v:v).

Gradient: 0 – 2.5 min. 0% B, 2.5 – 6.6 min. 7% B, 6.6 – 8.3

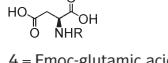
1 = Fmoc-arginine



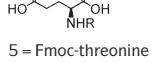
2 = Fmoc-serine



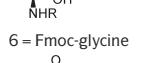
3 = Fmoc-aspartic acid



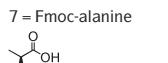
4 = Fmoc-glutamic acid



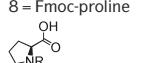
5 = Fmoc-threonine



6 = Fmoc-glycine



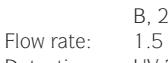
7 = Fmoc-alanine



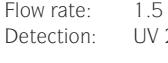
8 = Fmoc-proline



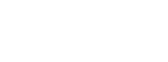
9 = Fmoc-tyrosine



10 = Fmoc-methionine



11 = Fmoc-OH



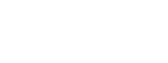
12 = Fmoc-NH<sub>2</sub>



13 = Fmoc-valine



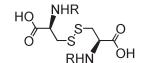
14 = Fmoc-phenylalanine



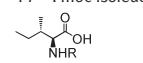
15 = Fmoc-tryptophane



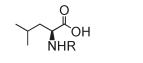
16 = Fmoc-cysteine



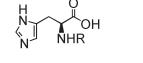
17 = Fmoc-isoleucine



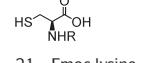
18 = Fmoc-leucine



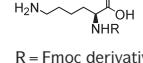
19 = Fmoc-histidine



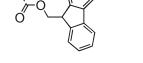
20 = Fmoc-cysteine



21 = Fmoc-lysine



R = Fmoc derivative group



min. 14% B, 8.3 – 8.4 min. 21% B, 8.4 – 10 min.

21% B, 10 – 10.1 min. 17% B, 10.1 – 20 min. 19%

B, 20 – 29 min. 55% B, 29 – 30 min. 100% B

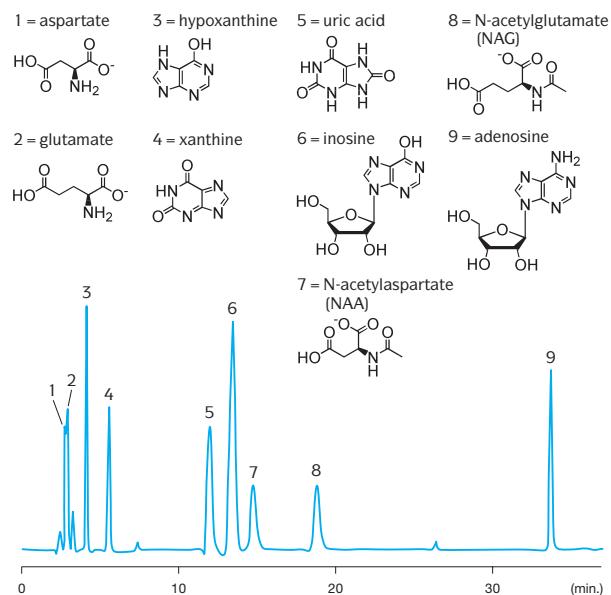
Flow rate: 1.5 ml/min.

Detection: UV 263 nm

# Amino acids

## Amino acids

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



Phase: Kromasil 100 Å, 5 µm, C18

Column: 4.6 × 250 mm

Temperature: 23°C

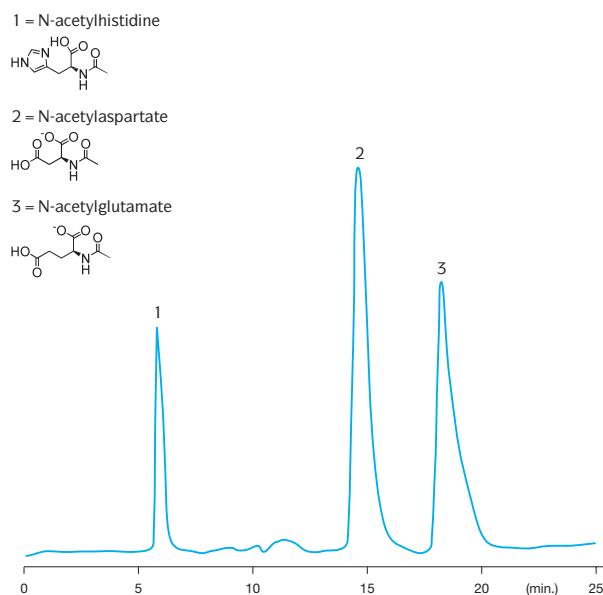
Eluent: 2.8 mM tetrabutylammonium hydroxide,  
25 mM KH<sub>2</sub>PO<sub>4</sub>, 1.25% MeOH (pH 7)

Flow rate: 1 ml/min.

Detection: UV 210 nm

## Amino acids, N-acetylated

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



Phase: Kromasil 100 Å, 5 µm, C18

Column: 4.6 × 250 mm

Temperature: 23°C

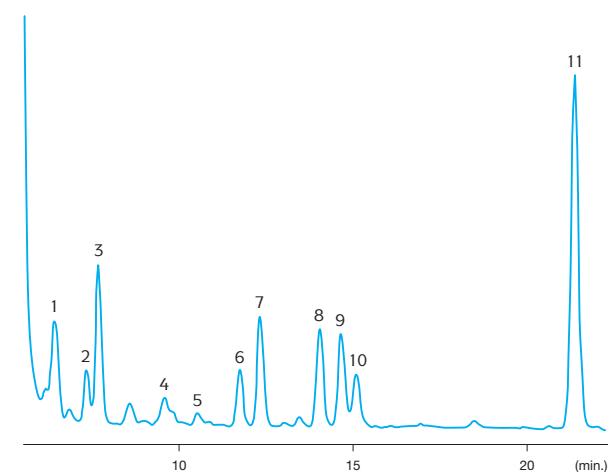
Eluent: tetrabutylammonium hydroxide 2.8 mM:  
KH<sub>2</sub>PO<sub>4</sub> 25 mM and 1.25% MeOH, pH 7

Flow rate: 1 ml/min.

Detection: UV 210 nm

## Amino acids, benzoylated

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



Phase: Kromasil 100 Å, 5 µm, C18

Column: 4 × 250 mm

Eluent: acetonitrile-water mixtures

Gradient: 70 – 95% ACN in 30 min.

Flow rate: 1 ml/min.

Detection: UV 274 nm

# Amino acids

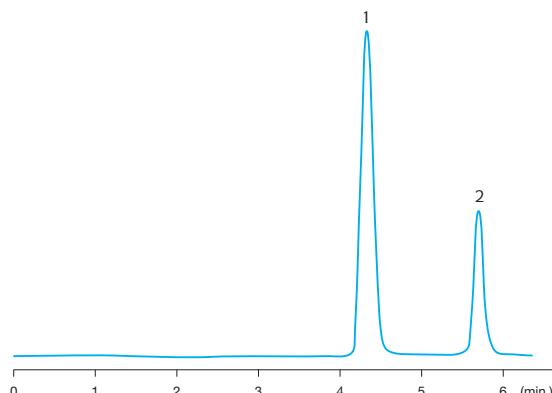
## Aminosalicylic acids

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 × 200 mm

Eluent: MeOH:phosphate buffer (35:65; v:v)

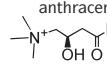
Flow rate: 1 ml/min.

Detection: UV 254 nm

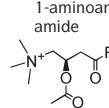
## Carnitines, aminoanthracene derivatives

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)

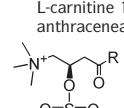
1 = L-carnitine 1-aminoanthraceneamide



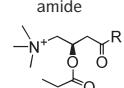
2 = acetyl-L-carnitine 1-aminoanthraceneamide



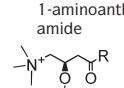
3 = methansulfonyl-L-carnitine 1-aminoanthraceneamide



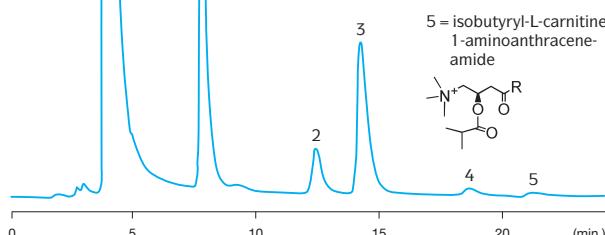
4 = propionyl-L-carnitine 1-aminoanthraceneamide



5 = isobutyryl-L-carnitine 1-aminoanthraceneamide



R = 1-aminoanthracene group



Phase: Kromasil 100 Å, 5 µm, C18

Column: 4.6 × 250 mm

Eluent: ACN:ammonium acetate (0.1 M, pH 3.5) (30:70; v:v)

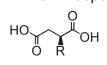
Flow rate: 1.3 ml/min.

Detection: spectrofluorimetric ( $\lambda_{ex}$  248 nm,  $\lambda_{em}$  418 nm)

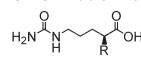
## Boronophenylalanine

Determination of boronophenylalanine in biological samples after precolumn derivatization with o-phthalaldehyde (OPA). (ref. 237)

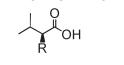
1 = OPA-aspartic acid



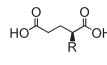
8 = OPA-citrulline



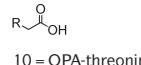
15 = OPA-valine



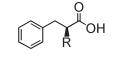
2 = OPA-glutamic acid



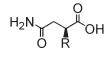
9 = OPA-glycine



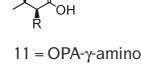
16 = OPA-phenylalanine



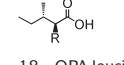
3 = OPA-asparagine



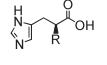
10 = OPA-threonine



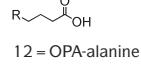
17 = OPA-isoleucine



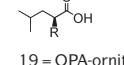
4 = OPA-histidine



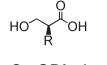
11 = OPA-γ-aminobutyric acid (GABA)



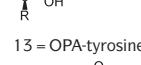
18 = OPA-leucine



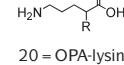
5 = OPA-serine



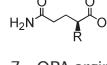
12 = OPA-alanine



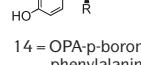
19 = OPA-ornithine



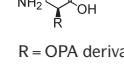
6 = OPA-glutamine



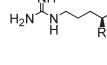
13 = OPA-tyrosine



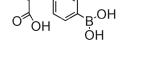
20 = OPA-lysine



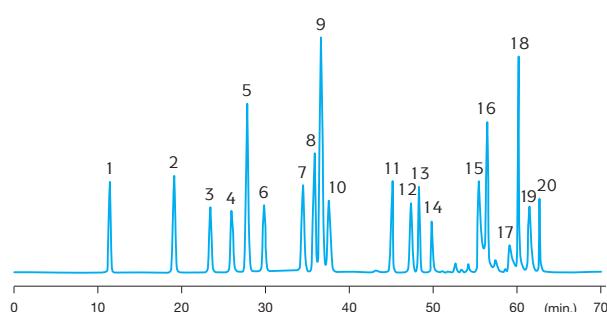
7 = OPA-arginine



14 = OPA-p-borophenylalanine



R = OPA derivative group



Phase: Kromasil 100 Å, 5 µm, C18

Column: 4.6 × 250 mm

Temperature: 23°C

Eluent A: 50 mM CH<sub>3</sub>COONa (pH 7.4) : 50 mM NaHPO<sub>4</sub> (pH 7.4) : MeOH : THF (48:48:2:2; v:v:v:v)

Eluent B: MeOH:water (65:35; v:v).

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.

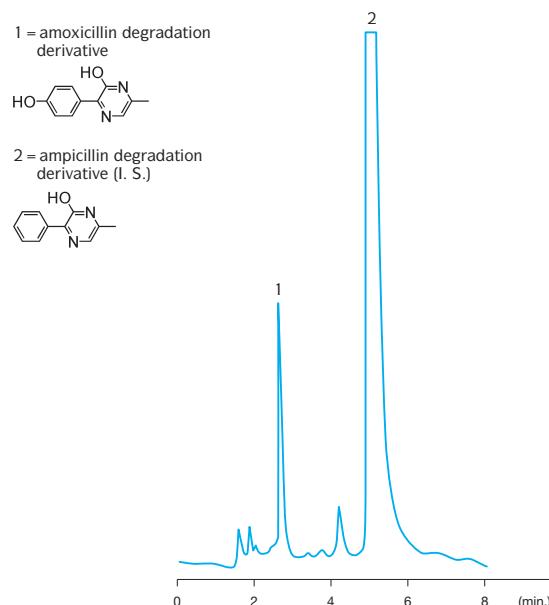
Flow rate: 1.2 ml/min.

Detection: spectrofluorimetric ( $\lambda_{ex}$  330 nm,  $\lambda_{em}$  430 nm)

# Drugs and metabolites

## Amoxicillin

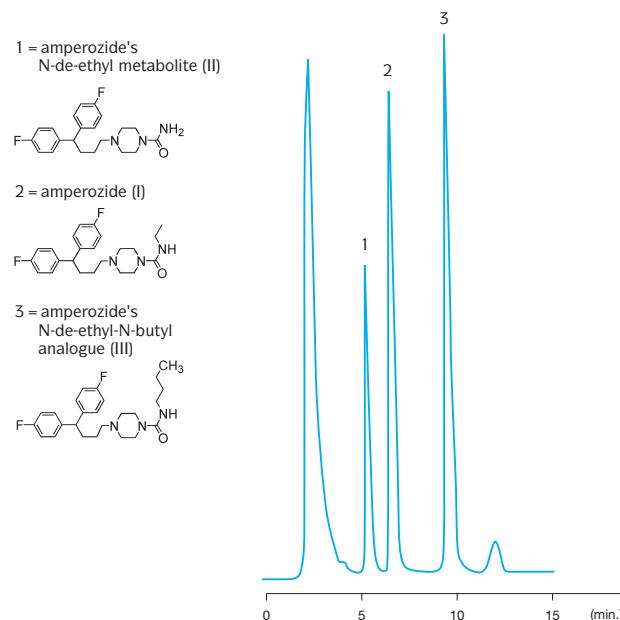
Measurement of amoxicillin in gastric tissue samples. (ref. 6)



Phase: Kromasil 100 Å, 5 µm, C18  
Column: 3.2 × 150 mm  
Temperature: 40°C  
Eluent: MeOH-water (55:45; v:v)  
Flow rate: 0.4 ml/min.  
Detection: fluorescence ( $\lambda_{\text{ex}}$  365 nm,  $\lambda_{\text{em}}$  445 nm)

## Amperozide

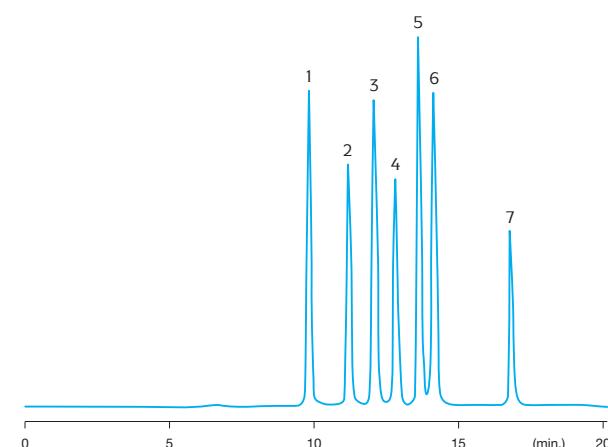
Separation of amperozide, derivate and metabolite. (ref. 45)



Phase: Kromasil 100 Å, 5 µm, C18  
Column: 2.1 × 200 mm  
Eluent: MeOH:ammonium phosphate buffer (pH 7.8) (78:22; v:v)  
Flow rate: 0.2 ml/min.  
Detection: UV 265 nm

## Anthocyanidins

Separation of cyanidin from 3-O-β-glycosylated anthocyanidins. (ref. 347)



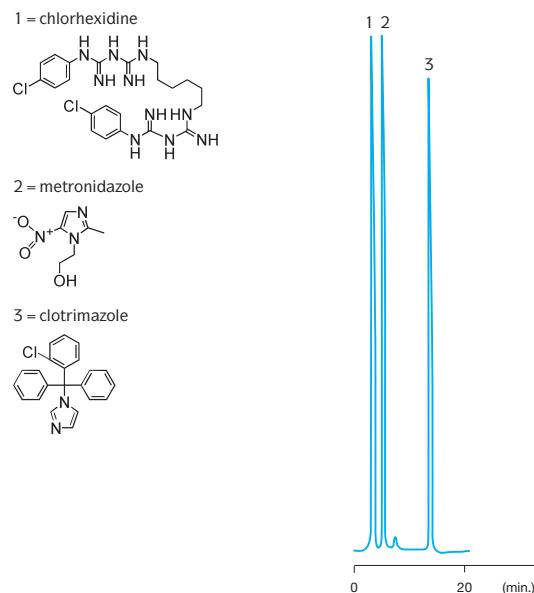
Phase: Kromasil 100 Å, 5 µm, C18  
Column: 4.6 × 250 mm  
Temperature: 23°C  
Eluent A: HCOOH:water (1:10; v:v)  
Eluent B: HCOOH:water:MeOH (1:9:10; v:v:v)  
Gradient: 0% – 60% A in 5 min., 60% – 45% A in 5 min., 45% – 0% A in 6 min., 0% A in 10 min.

Flow rate: 1.2 ml/min.  
Detection: 520 nm

# Drugs and metabolites

## Antibacterial drugs

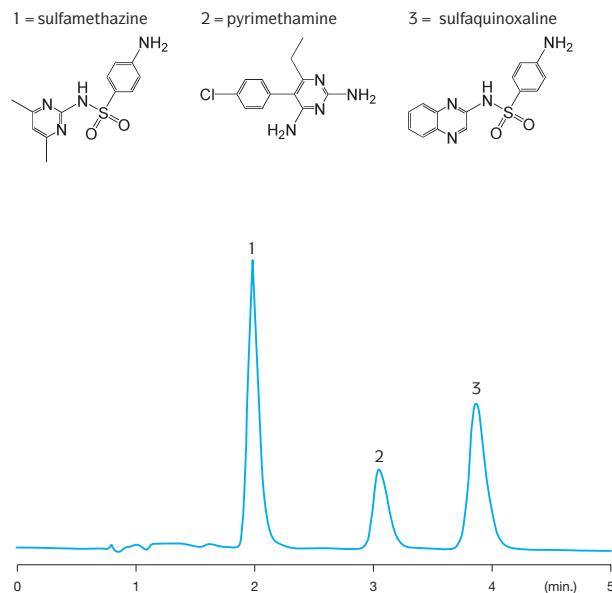
Determination of metronidazole, clotrimazole and chlorhexidine acetate in Shuangzuo effervescent tablets. (ref. 23)



Phase: Kromasil 100 Å, 5 µm, C18  
Column: 4.6 × 250 mm  
Eluent: MeOH:buffer (70:30; v:v) (NaAc 24.4 g, HAc 80 ml, (C<sub>4</sub>H<sub>9</sub>)NBr 4.83 g in 1000 ml water, pH 3.6)  
Flow rate: 1 ml/min.  
Detection: UV 260 nm

## Antibacterial drugs, veterinary

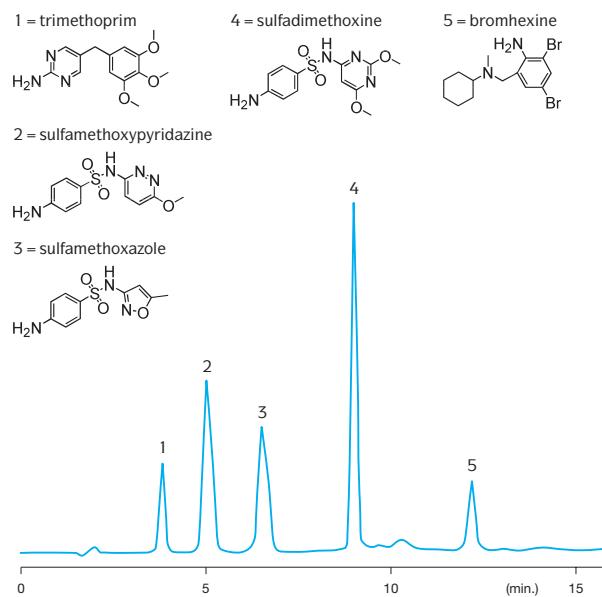
Simultaneous determination of sulfaquinoxaline, sulfamethazine and pyrimethamine. (ref. 246)



Phase: Kromasil 100 Å, 5 µm, C18  
Column: 4.6 × 150 mm  
Eluent: 40 mM phosphate buffer (pH 3 containing 10 mM ClO<sub>4</sub><sup>-</sup>) : ACN (65:35; v:v)  
Flow rate: 1.5 ml/min.  
Detection: UV 270 nm

## Antibacterials, sulfa drugs

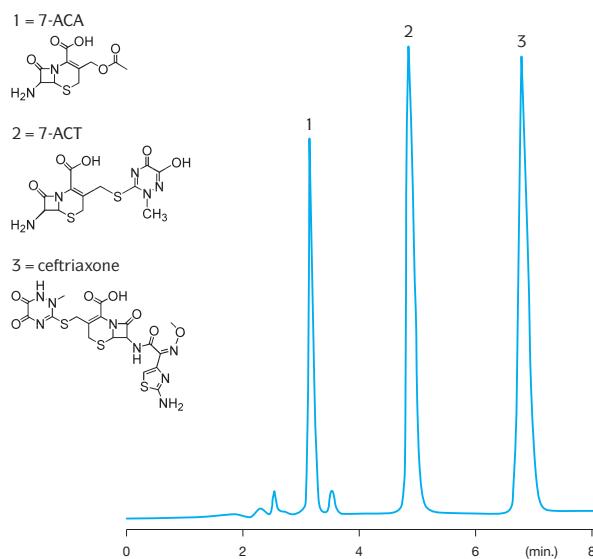
Determination of sulfamethoxypyridazine, sulfamethoxazole, sulfadimethoxine and associated compounds. (ref. 267)



Phase: Kromasil 100 Å, 5 µm, C18  
Column: 4.6 × 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-triazin-3-yl)-thio]methyl-cephalosporanic acid (7-ACT). (ref. 129)

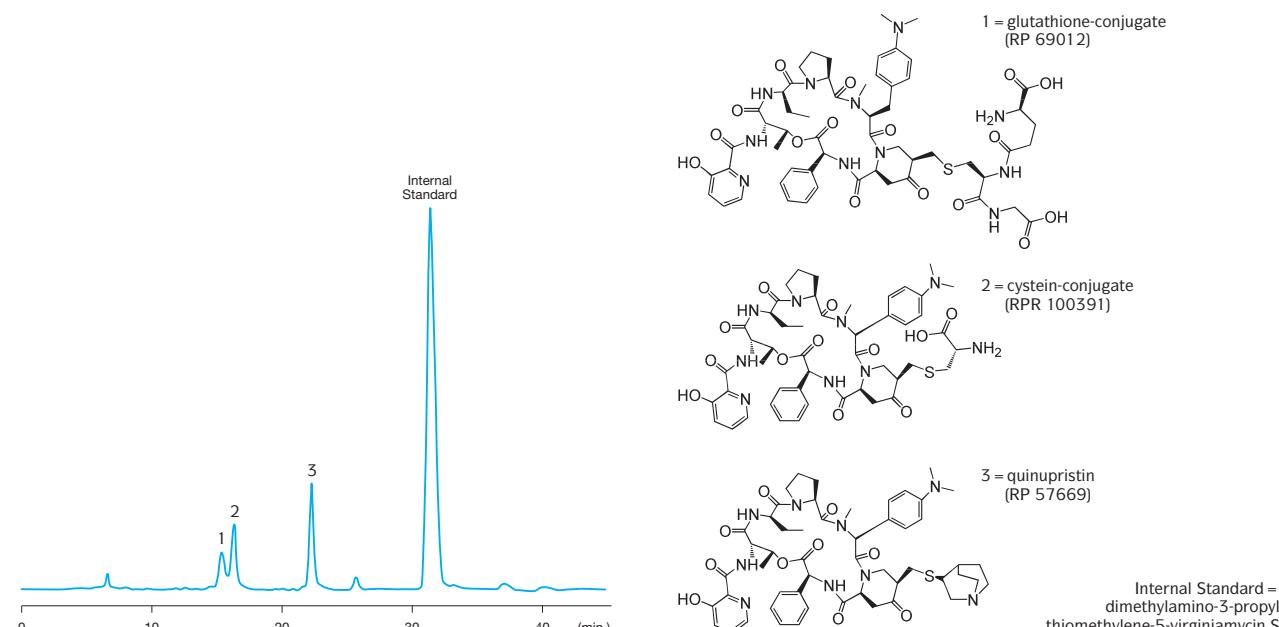


Phase: Kromasil 100 Å, 5 µm, C18  
Column: 4.6 × 200 mm  
Eluent: ACN:tetrabutyl ammonium bromide:phosphate buffer (pH 7):water (32:0.32:4.4:63.6; v:v:v:v)  
Flow rate: 1 ml/min.  
Detection: UV 270 nm

# Drugs and metabolites

## Antibiotics and metabolites

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

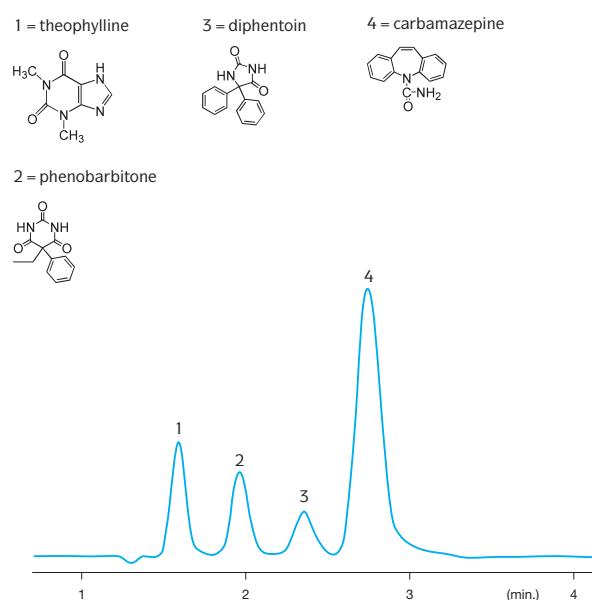


Phase: Kromasil 100 Å, 5 µm, C18  
Column: 4.6 × 125 mm  
Eluent A: 0.8 ml of 70% perchloric acid (PCA) / litre water  
Eluent B: ACN  
Gradient: 30% B for 11 min., 32% B from 11.1 to 15 min.,  
40% B from 15.6 to 16 min., 38% B from 16.1 to  
34 min., 80% B from 34.1 to 36 min.

Flow rate: 0 – 11 min: 0.5 ml/min., 11 – 36 min: 1 ml/min.  
Detection: fluorescence ( $\lambda_{\text{ex}}$  360 nm and  $\lambda_{\text{em}}$  410 nm)

## Anticonvulsants

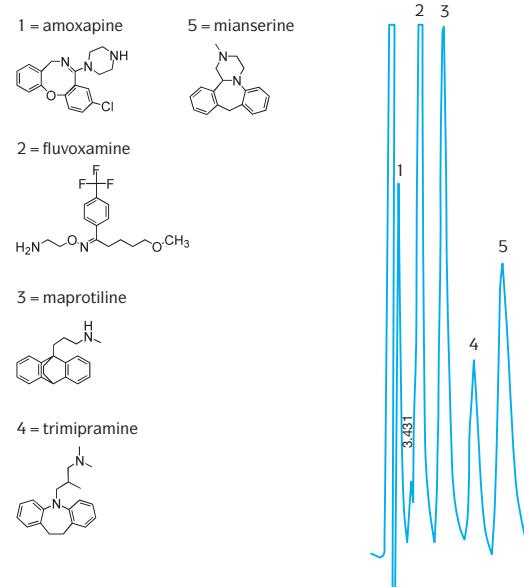
Determination of theophylline, phenobarbitone, diphenotoin and carbamazepine. (ref. 301b)



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

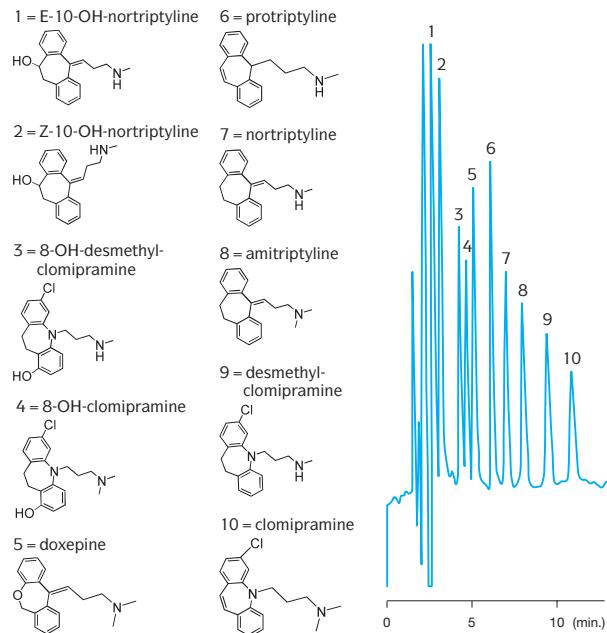
Determination of antidepressant drugs and metabolites. (ref. 49)



# Drugs and metabolites

## Antidepressants

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



Phase: Kromasil 100 Å, 5 µm, C8

Column: 4 × 250 mm

Temperature: ambient

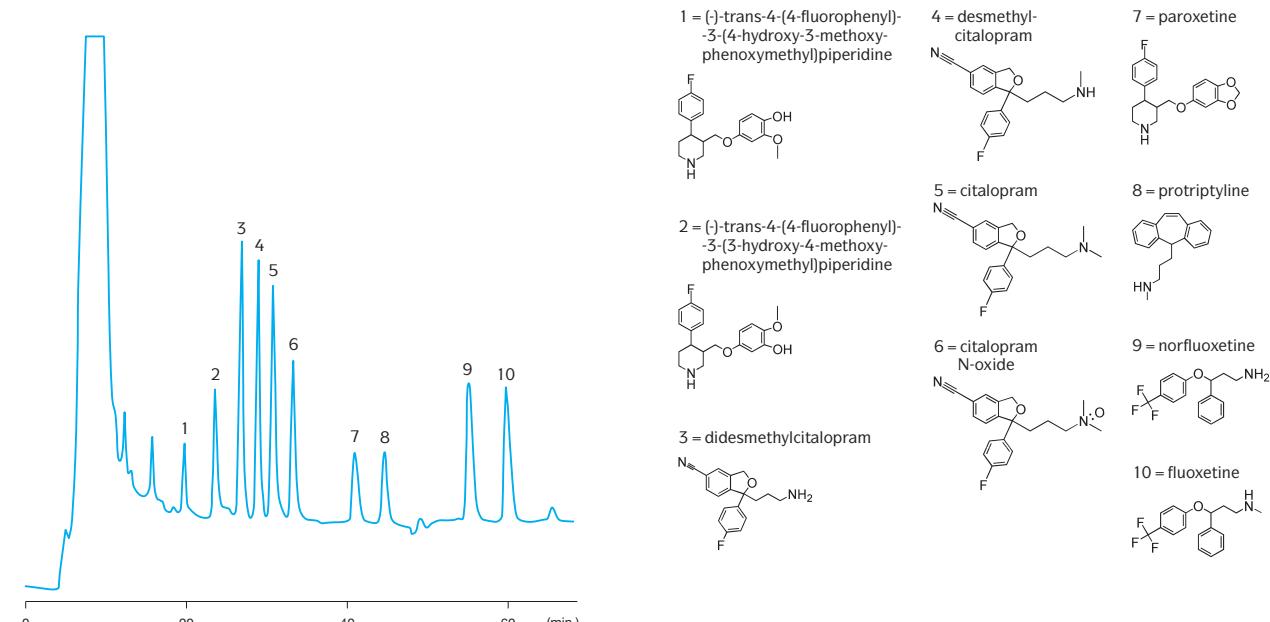
Eluent: ACN:KH<sub>2</sub>PO<sub>4</sub> (0.04 M) (40:60; v:v)

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)



Phase: Kromasil 100 Å, 3.5 µm, C18

Column: 0.32 × 300 mm

Temperature: gradient: 35 °C (3 min.) prior to ramp of 1.3 °C/min. to 100 °C (10 min.)

Eluent: ACN:NH<sub>4</sub>HCOO (45 mM, pH 4) (25:75; v:v)

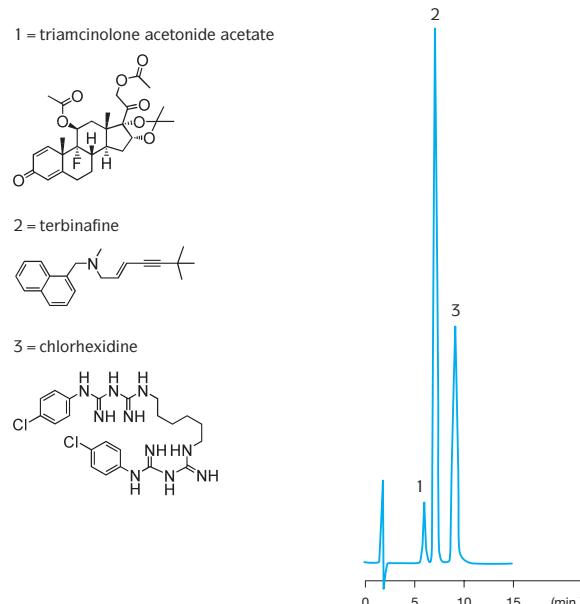
Flow rate: 5 µl/min

Detection: UV 230 nm

# Drugs and metabolites

## Antifungals

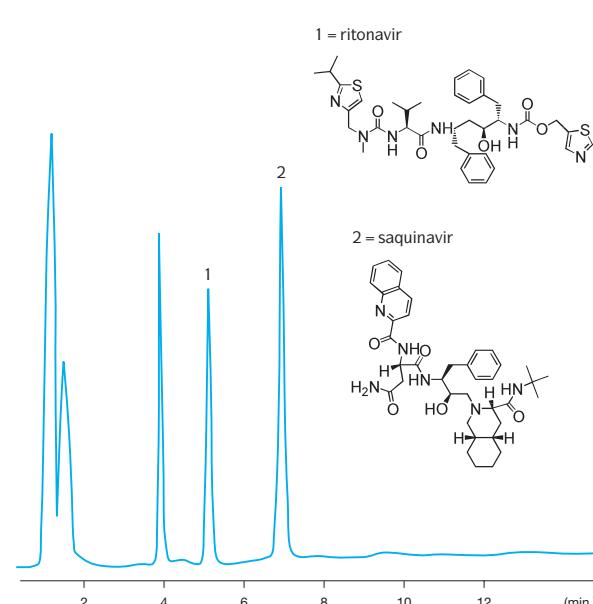
Determination of terbinafine hydrochloride, chlorhexidine and triamcinolone acetonide acetate. (ref. 110)



Phase: Kromasil 100 Å, 5 µm, C18  
Column: 4.6 × 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

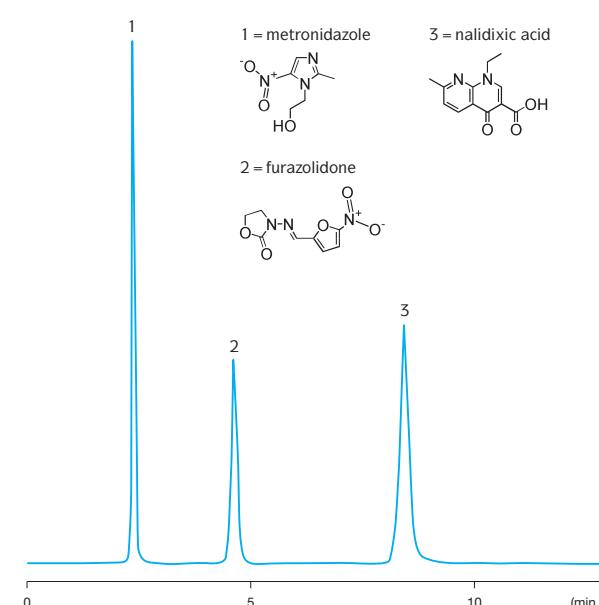
Simultaneous determination of ritonavir and saquinavir. (ref. 126)



Phase: Kromasil 100 Å, 5 µm, C8  
Column: 4.6 × 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

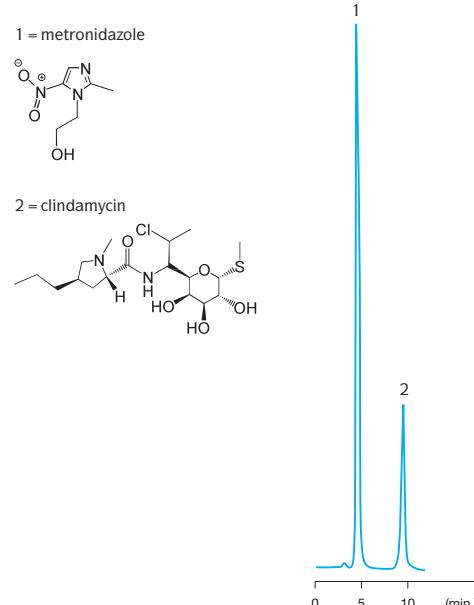
Determination of metronidazole and nalidixic acid. (ref. 156)



Phase: Kromasil 100 Å, 5 µm, C8  
Column: 4.6 × 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

Determination of metronidazole and clindamycin. (ref. 268)

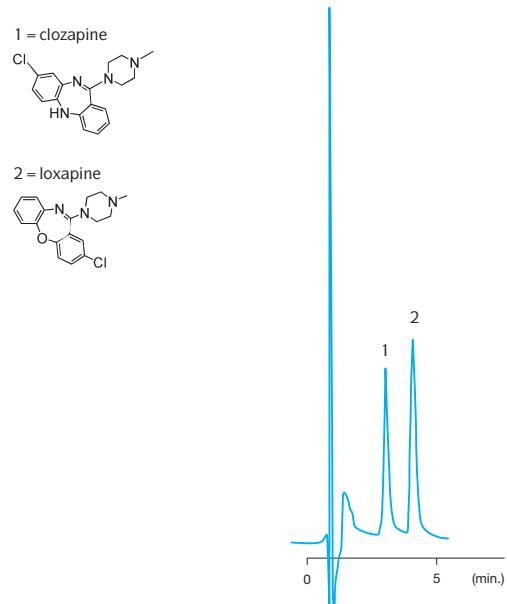


Phase: Kromasil 100 Å, 5 µm, C18  
Column: 4.6 × 250 mm  
Eluent: Potassium dihydrogen phosphate (pH 3.8), 0.05 M:ACN (79:21; v:v)  
Flow rate: 1 ml/min.  
Detection: UV 210 nm

# Drugs and metabolites

## Antipsychotics

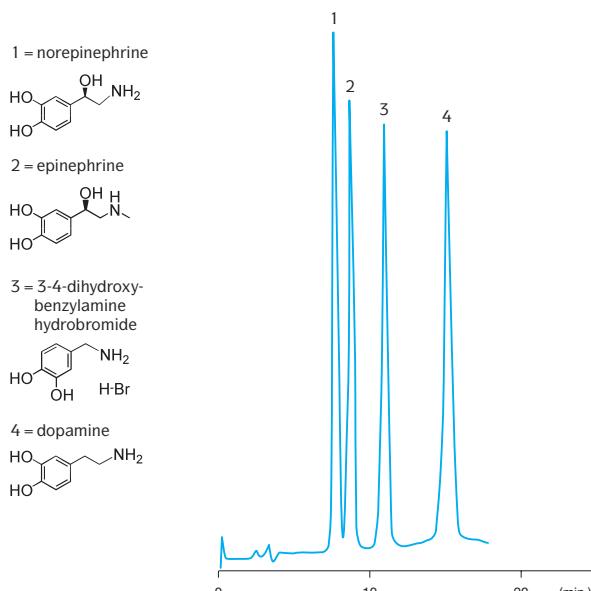
Determination of clozapine and loxapine. (ref. 64)



Phase: Kromasil 100 Å, 5 µm, C8  
Column: 4.6 × 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

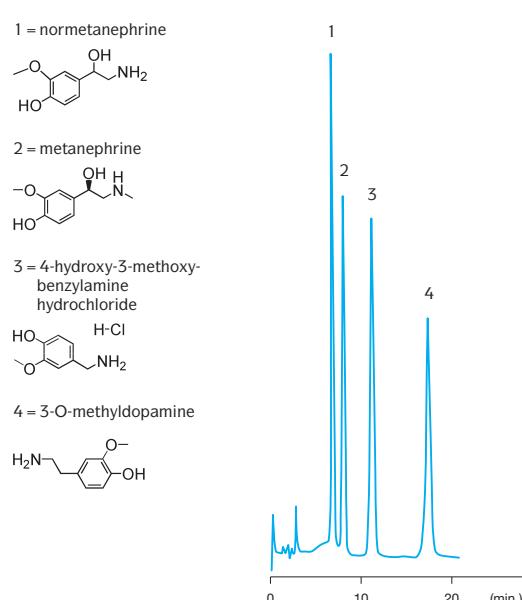
Determination of catecholamines in pig liver. (ref. 95a)



Phase: Kromasil 100 Å, 5 µm, C8  
Column: 4.6 × 150 mm  
Eluent: 300 ml 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: 0.6 ml/min.  
Detection: electrochemical potential + 0.65 V

## Catecholamines

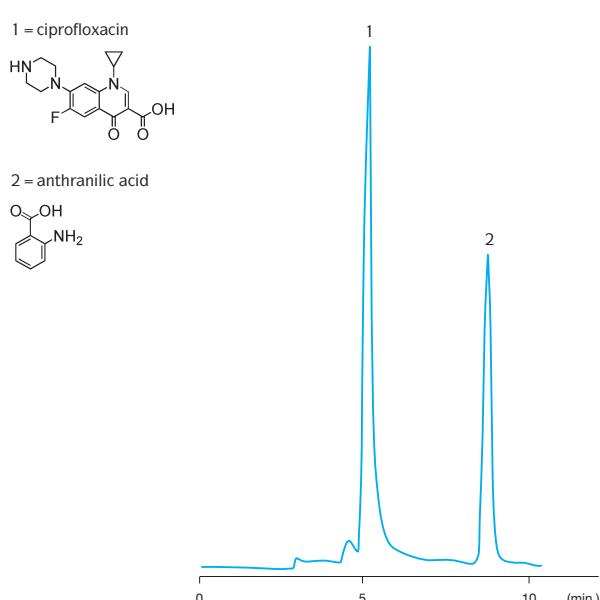
Determination of methoxycatecholamines in pig liver. (ref. 95b)



Phase: Kromasil 100 Å, 5 µm, C8  
Column: 4.6 × 150 mm  
Eluent: 300 ml 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 + 0.8 V

## Ciprofloxacin

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



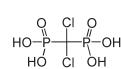
Phase: Kromasil 100 Å, 5 µm, C18  
Column: 4.6 × 250 mm  
Temperature: ambient  
Eluent: ACN:MeOH:acetate buffer (pH 3.6; 50 mM) (10:30:60; v:v:v) containing 1% v/v HAc  
Flow rate: 0.8 ml/min.  
Detection: fluorescence ( $\lambda_{\text{ex}}$  300 nm,  $\lambda_{\text{em}}$  458 nm)

# Drugs and metabolites

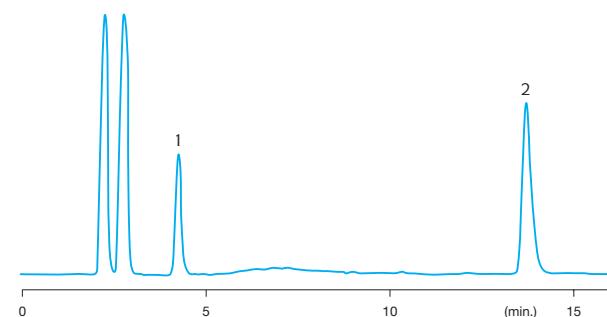
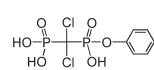
## Clodronate

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 × 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)

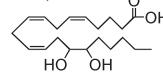
Flow rate: 1.2 ml/min.

Detection: ELS

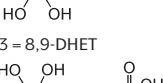
## Cytochrome P450 metabolites

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

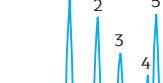
1 = 14,15-DHET



2 = 11,12-DHET



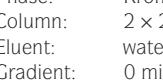
3 = 8,9-DHET



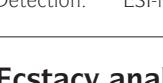
4 = 5,6-DHET



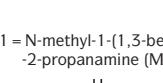
5 = 20-HETE



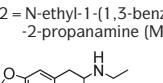
6 = 14,15-EET



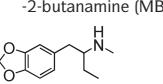
7 = 11,12-EET



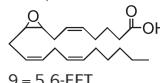
8 = 8,9-EET



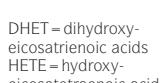
9 = 5,6-EET



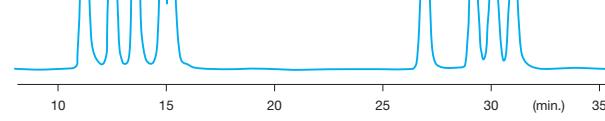
8 = 8,9-EET



9 = 5,6-EET



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



Phase: Kromasil 100 Å, 5 µm, C18

Column: 2 × 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

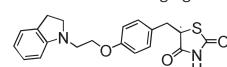
Flow rate: 0.2 ml/min.

Detection: ESI-MS

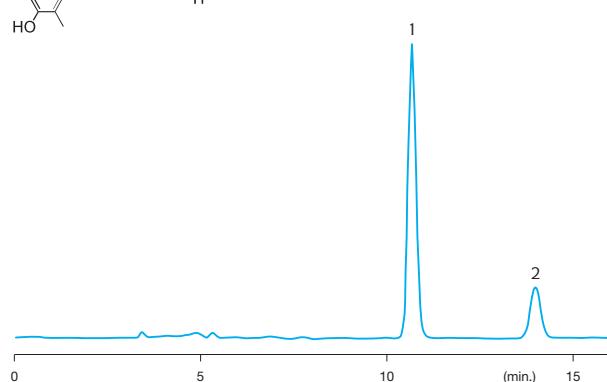
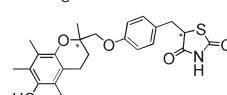
## DRF-2189

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, C18

Column: 4.6 × 250 mm

Eluent: 0.05 M NaH<sub>2</sub>PO<sub>4</sub>:ACN:MeOH (22.5:37.5:40; v:v:v)  
(pH 5.0)

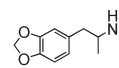
Flow rate: 1 ml/min.

Detection: fluorescence ( $\lambda_{\text{ex}}$  292 nm and  $\lambda_{\text{em}}$  325 nm)

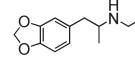
## Ecstasy analogues

Identification of a homologue derivative of "ecstasy". (ref. 170)

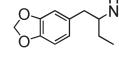
1 = N-methyl-1-(1,3-benzodioxol-5-yl)-2-propanamine (MDMA)



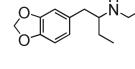
2 = N-ethyl-1-(1,3-benzodioxol-5-yl)-2-propanamine (MDEA)



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, C18

Column: 4.6 × 250 mm

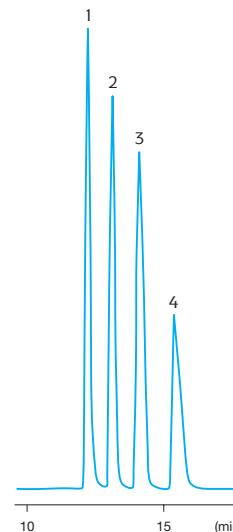
Temperature: ambient

Eluent: ACN:0.1 M triethylammonium acetate (aq) pH 7.3

Gradient: 5% to 80% ACN in 25 min.

Flow rate: 1 ml/min.

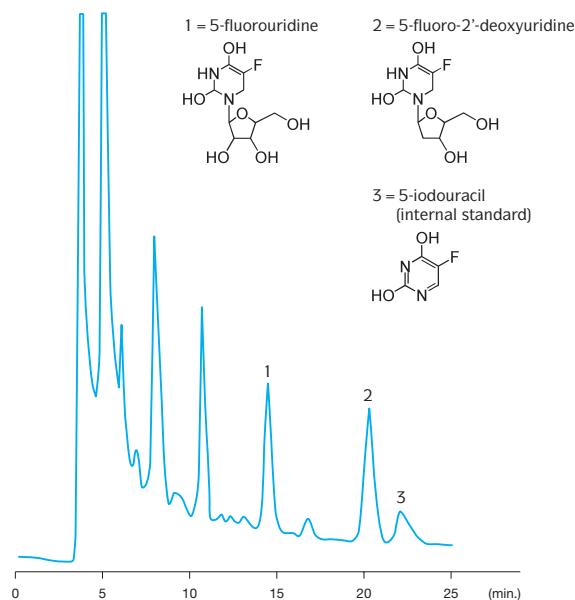
Detection: UV 280 nm



# Drugs and metabolites

## 5-fluorouracil metabolites

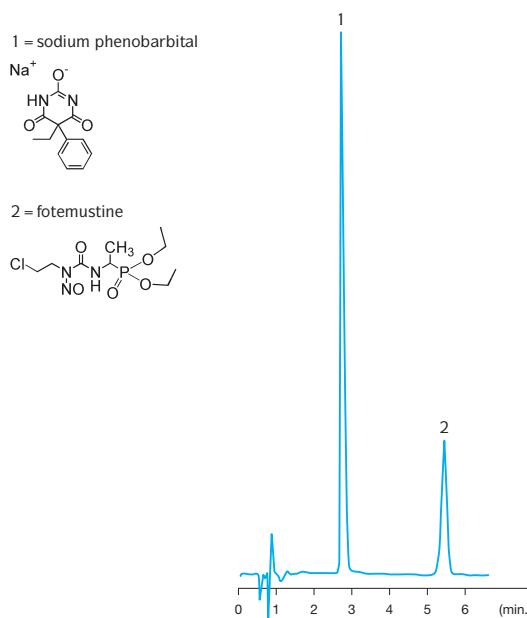
Determination of the main metabolites of 5-fluorouracil in plasma. (ref. 116)



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

## Fotemustine

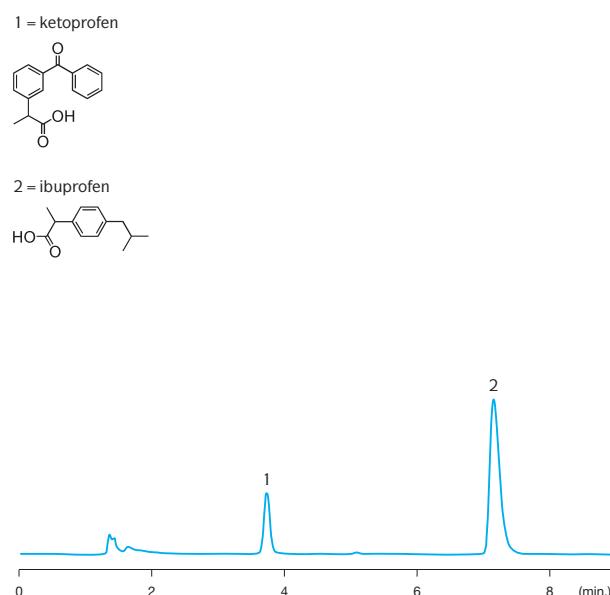
Stability study of fotemustine in PVC infusion bags. (ref. 124)



Phase: Kromasil 100 Å, 5 µm, C18  
Column: 4.6 × 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

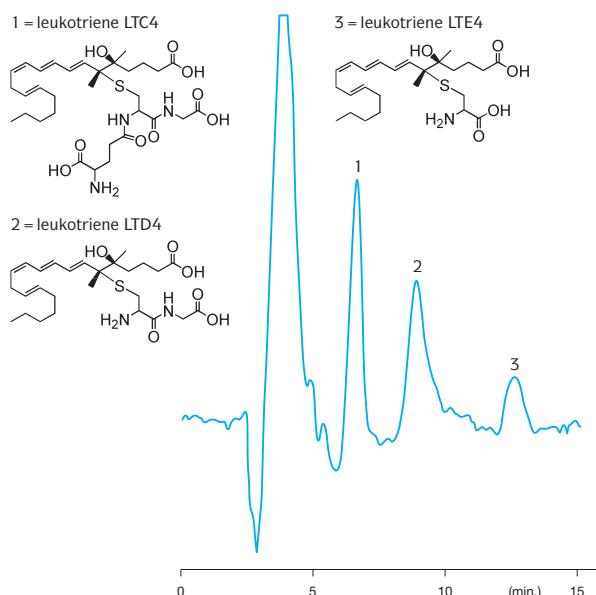
Determination of ketoprofen in vitro in rat skin. (ref. 247)



Phase: Kromasil 100 Å, 5 µm, C18  
Column: 4 × 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)

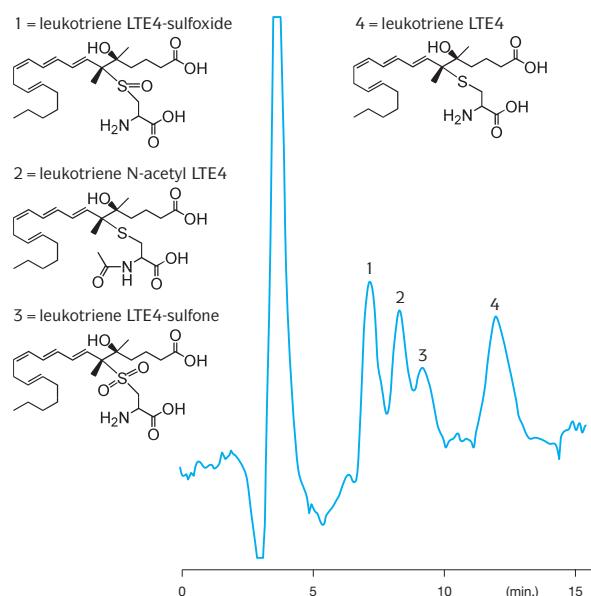


Phase: Kromasil 100 Å, 5 µm, C4  
Column: 2.1 × 100 mm  
Eluent: ACN:K<sub>2</sub>HPO<sub>4</sub> 10 mM (pH 7.4) (30:70; v:v)  
Flow rate: 0.2 ml/min.  
Detection: fluorescence ( $\lambda_{ex}$  544 nm,  $\lambda_{em}$  572 nm)

# Drugs and metabolites

## Leukotrienes, cross-reactive

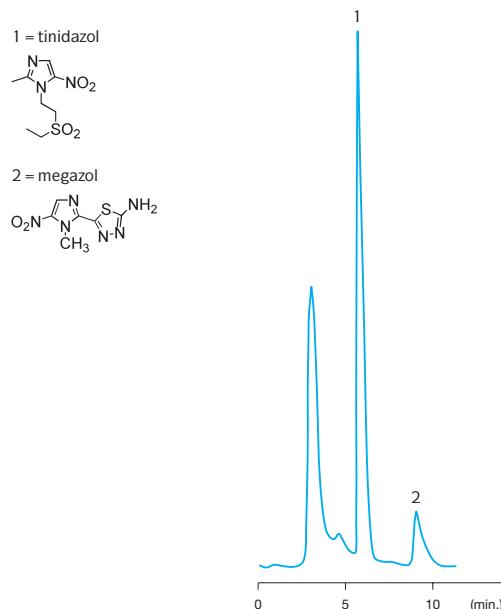
Determination of cross-reactive leukotrienes in biological matrices. (ref. 71b)



Phase: Kromasil 100 Å, 5 µm, C4  
Column: 2.1 × 100 mm  
Eluent: ACN:K<sub>2</sub>HPO<sub>4</sub> 10 mM (pH 7.4) (30:70; v:v)  
Flow rate: 0.2 ml/min.  
Detection: fluorescence ( $\lambda_{\text{ex}}$  544 nm,  $\lambda_{\text{em}}$  572 nm)

## Megazol

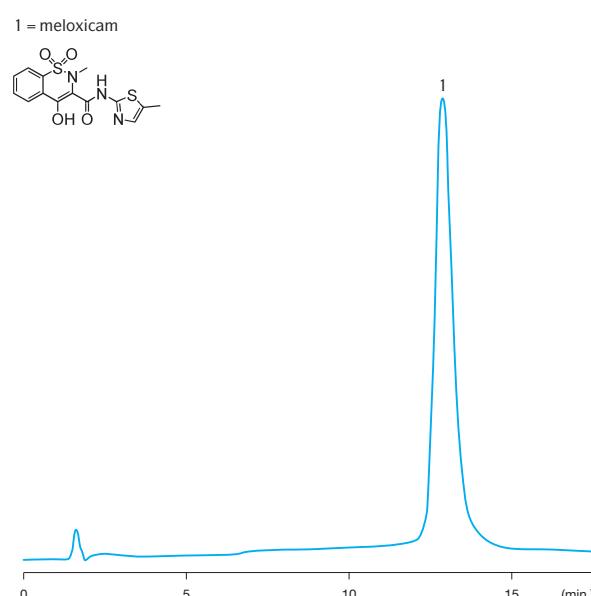
Analysis of megazol in human plasma. (ref. 113)



Phase: Kromasil 100 Å, 10 µm, C8  
Column: 4 × 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

## Meloxicam

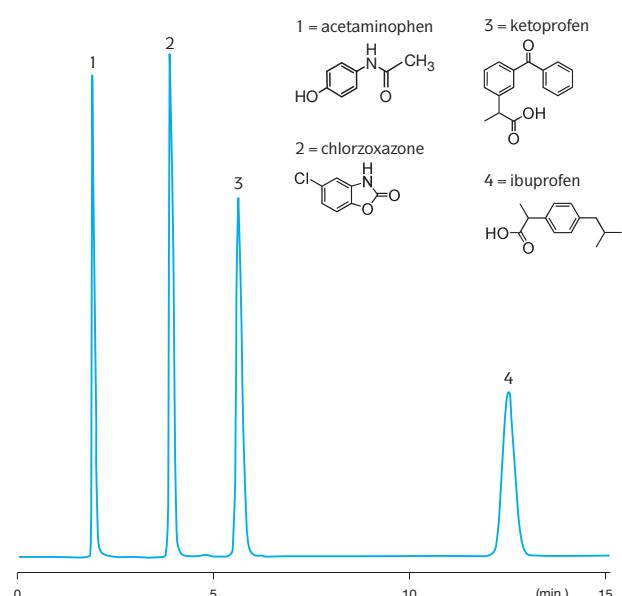
Determination of meloxicam in human plasma. (ref. 283)



Phase: Kromasil 100 Å, 5 µm, C18  
Column: 4.6 × 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

## Pain relievers

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

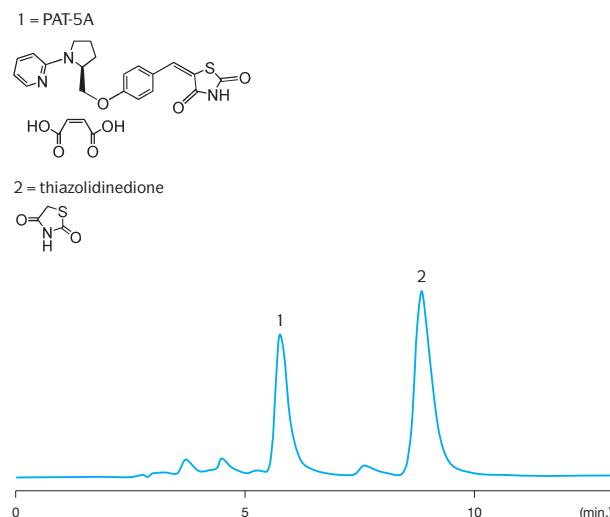


Phase: Kromasil 100 Å, 5 µm, C8  
Column: 4.6 × 250 mm  
Temperature: 20±1 °C  
Eluent: ACN:0.2% triethylamine (pH 3.2) (50:50; v:v)  
Flow rate: 1.5 ml/min.  
Detection: UV 215 nm

# Drugs and metabolites

## PAT-5A

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

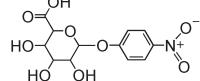


Phase: Kromasil 100 Å, 5 µm, C18  
Column: 4.6 × 250 mm  
Eluent: NaH<sub>2</sub>PO<sub>4</sub> (0.05 M, pH 4):MeOH (25:75; v:v)  
Flow rate: 1 ml/min.  
Detection: UV 345 nm

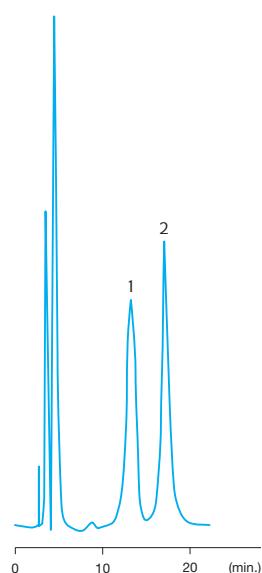
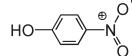
## Phenolics

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

1 = 4-nitrophenyl-β-D-glucuronide



2 = 4-nitrophenol

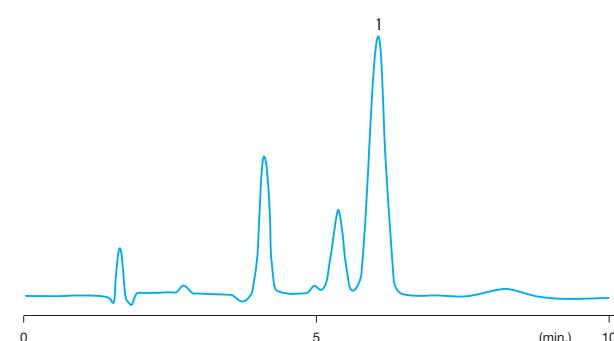
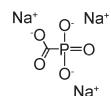


Phase: Kromasil 100 Å, 5 µm, C18  
Precolumn: Nucleosil 5µm, C4  
Column: 4.6 × 100 mm (precolumn: 4.6 × 50 mm)  
Temperature: ambient  
Eluent: 30 mM cetyltrimethylammonium bromide in 0.05 M 6-aminohexanoic acid (pH: 5) and 20% ACN (precolumn 7%) (v:v)  
Flow rate: 1 ml/min.  
Detection: UV 300 nm

## Phosphonoformate (foscarnet)

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

1 = phosphonoformate (foscarnet)

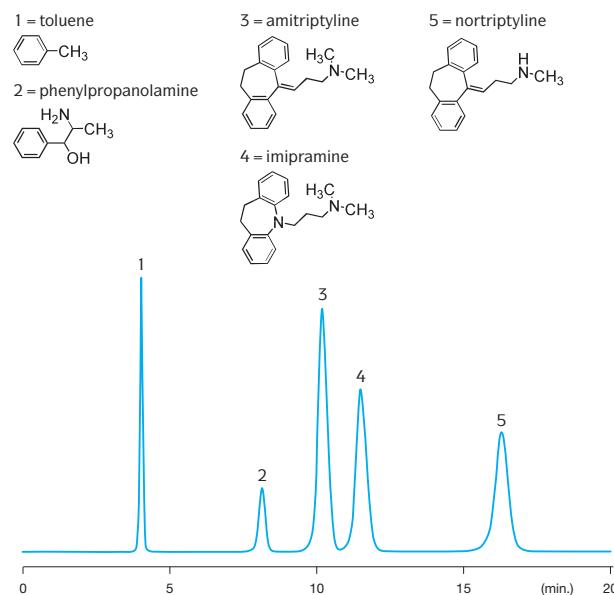


Phase: Kromasil 100 Å, 5 µm, C18  
Column: 4.6 × 150 mm  
Eluent: methanol : 40 mM Na<sub>2</sub>HPO<sub>4</sub>-buffer, pH 7.6 (adjusted with orthophosphoric acid), containing 0.25 mM THAHSO<sub>4</sub> (25:75; v:v)  
Flow rate: 1 ml/min.  
Detection: electrochemical (potential + 1.125 V)

# Drugs and metabolites

## QC test, tricyclic antidepressants

QC test of Kromasil CN. (ref. 342)



Phase: Kromasil 60 Å, 10 µm, CN

Column: 4.6 × 250 mm

Temperature: ambient

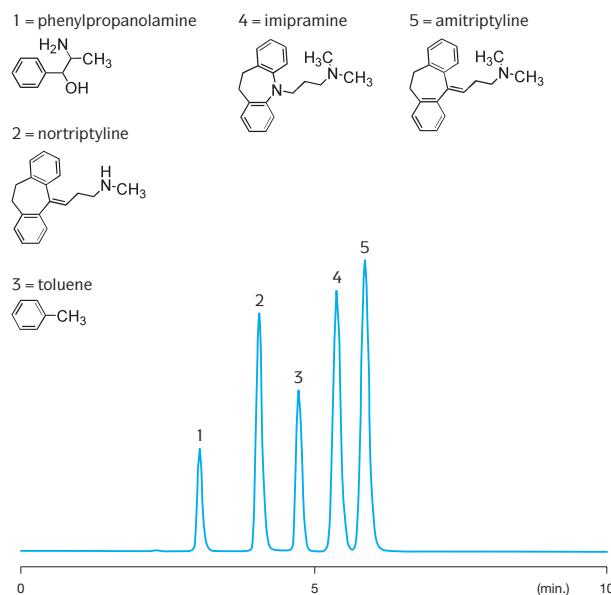
Eluent: MeOH:KH<sub>2</sub>PO<sub>4</sub> 25 mM pH 6.0 (80:20; v:v)

Flow rate: 1 ml/min.

Detection: UV 215 nm

## QC test, tricyclic antidepressants

QC test of Kromasil C4. (ref. 349)



Phase: Kromasil 100 Å, 5 µm, C4

Column: 4.6 × 250 mm

Temperature: ambient

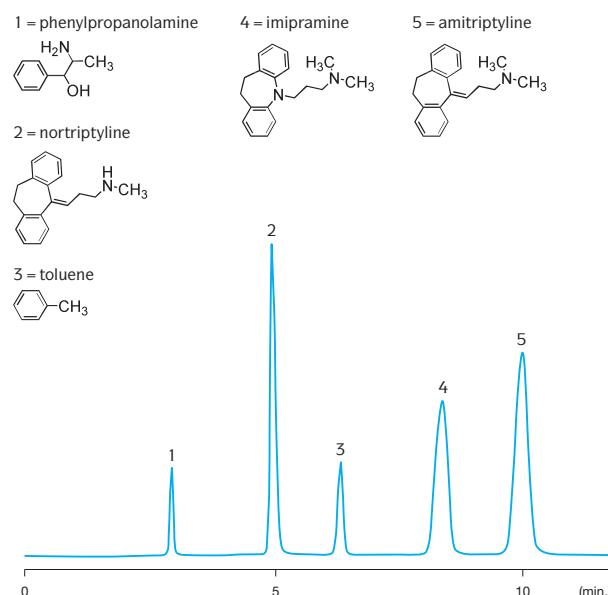
Eluent: MeOH:KH<sub>2</sub>PO<sub>4</sub> 25 mM pH 6.0 (80:20; v:v)

Flow rate: 1 ml/min.

Detection: UV 215 nm

## QC test, tricyclic antidepressants

QC test of Kromasil C8. (ref. 350)



Phase: Kromasil 100 Å, 5 µm, C8

Column: 4.6 × 250 mm

Temperature: ambient

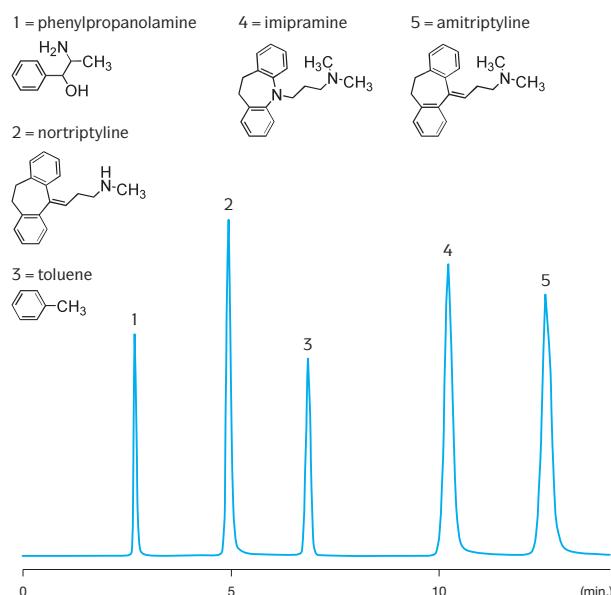
Eluent: MeOH:KH<sub>2</sub>PO<sub>4</sub> 25 mM pH 6.0 (80:20; v:v)

Flow rate: 1 ml/min.

Detection: UV 215 nm

## QC test, tricyclic antidepressants

QC test of Kromasil C18. (ref. 351)



Phase: Kromasil 100 Å, 5 µm, C18

Column: 4.6 × 250 mm

Temperature: ambient

Eluent: MeOH:KH<sub>2</sub>PO<sub>4</sub> 25 mM pH 6.0 (80:20; v:v)

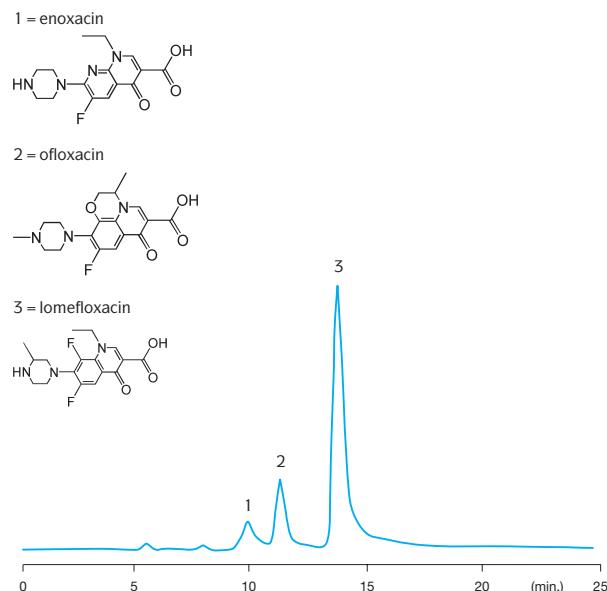
Flow rate: 1 ml/min.

Detection: UV 215 nm

# Drugs and metabolites

## Quinolinones

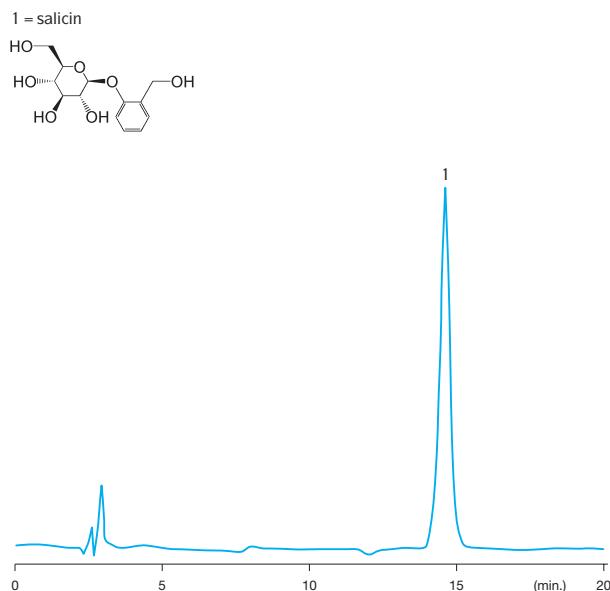
Determination of quinolinones in food. (ref. 119)



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

## Salicin

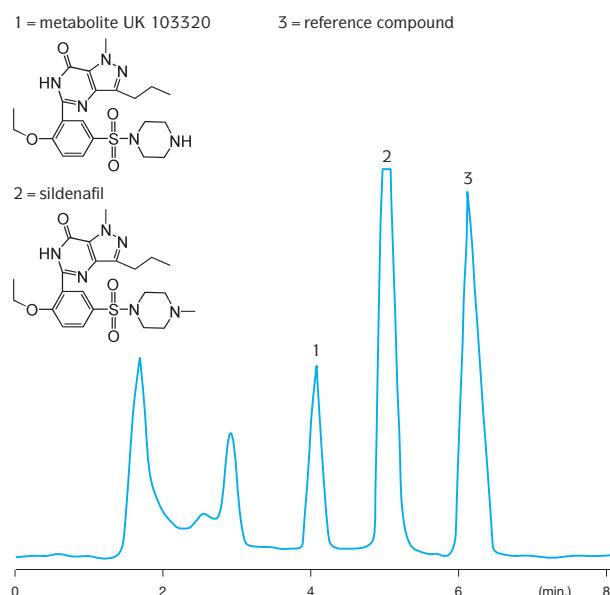
Determination of salicin in extract of willow bark. (ref. 262)



Phase: Kromasil 100 Å, 5 µm, C18  
 Column: 4.6 × 250 mm  
 Eluent: MeOH:KH<sub>2</sub>PO<sub>4</sub> buffer (pH 4.01, 0.01 M) (15:85; v:v)  
 Flow rate: 1 ml/min.  
 Detection: UV 265 nm

## Sildenafil

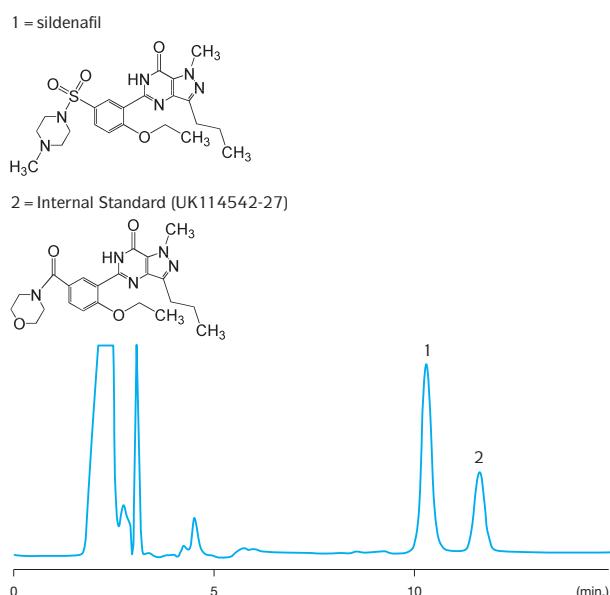
Determination of sildenafil (Viagra) and its metabolite (UK 103320) with ASTED equipment. (ref. 98)



Phase: Kromasil 100 Å, 5 µm, C4  
 Column: 4.6 × 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)

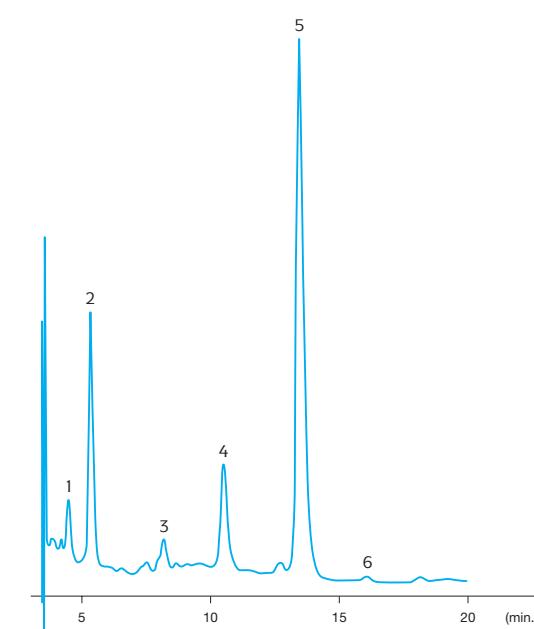


Phase: Kromasil 100 Å, 5 µm, C4  
 Column: 4.6 × 150 mm  
 Temperature: 40°C  
 Eluent: ACN : 0.5 M potassium phosphate buffer (pH 4.5; containing 10 mM diethylamine HCl) (32:68; v:v)  
 Flow rate: 0.7 ml/min.  
 Detection: UV 230 nm

# Drugs and metabolites

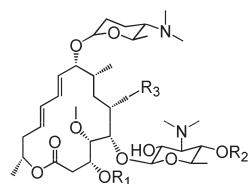
## Spiramycin

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

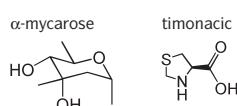
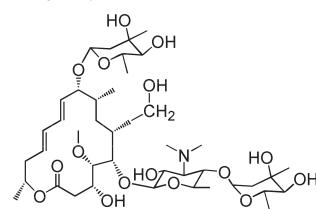


Phase: Kromasil 100 Å, 5 µm, C8  
 Column: 4.6 x 250 mm  
 Temperature: 60°C  
 Eluent: CH<sub>3</sub>CN:sodium phosphate buffer (0.05 M pH 2.3) (33:67; v:v) + 6 g/l NaClO<sub>4</sub>  
 Flow rate: 1.1 ml/min.  
 Detection: UV 232 nm

1, 2, 3, 4 and 6 = substituted base structure according to table



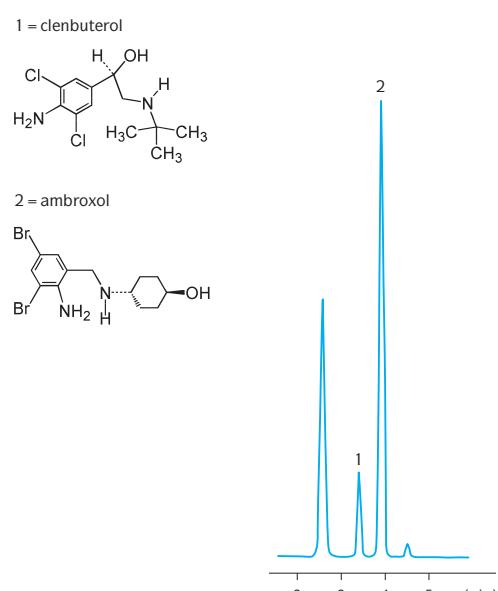
5 = spiramycin S



	R1	R2	R3
1. cysteyl neospiramycin I	H	H	timonacic
2. cysteyl spiramycin I	H	α-mycarose	timonacic
3. spiramycin I + cysteyl neospiramycin III	H	α-mycarose	COH
4. cysteyl spiramycin III	COCH <sub>2</sub> CH <sub>3</sub>	H	timonacic
5. spiramycin S	COCH <sub>2</sub> CH <sub>3</sub>	α-mycarose	timonacic
6. spiramycin III		α-mycarose	COH

## Steroids

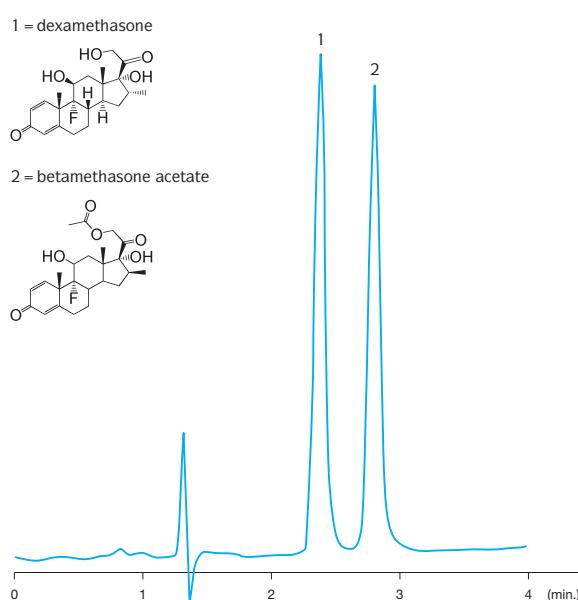
Analysis of clenbuterol hydrochloride and ambroxol hydrochloride. (ref. 331)



Phase: Kromasil 60 Å, 10 µm, CN  
 Column: 4.6 x 250 mm  
 Eluent: 1.8 g sodium decanesulphate + 3 g potassium phosphate monobasic + 600 ml water (pH 3.0) + 200 ml ACN + 200 ml MeOH  
 Flow rate: 1.5 ml/min.  
 Detection: UV 215 nm

## Steroids

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

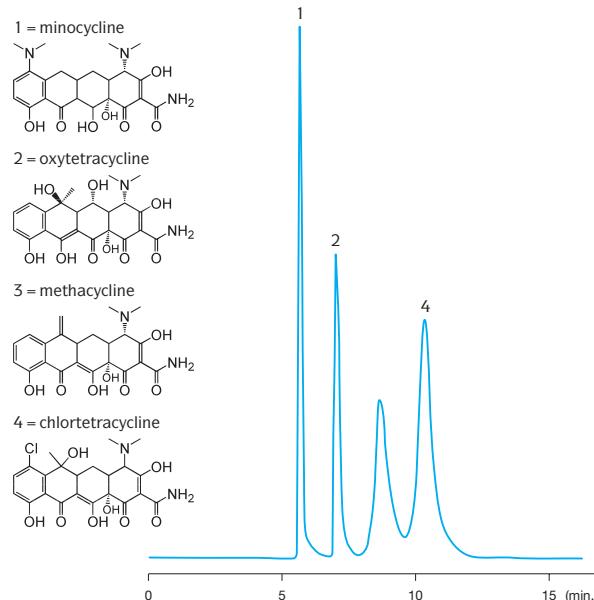


Phase: Kromasil 100 Å, 5 µm, C18  
 Column: 4 x 150 mm  
 Eluent: MeOH:water (80:20; v:v)  
 Flow rate: 1 ml/min.  
 Detection: UV 240 nm

# Drugs and metabolites

## Tetracyclines

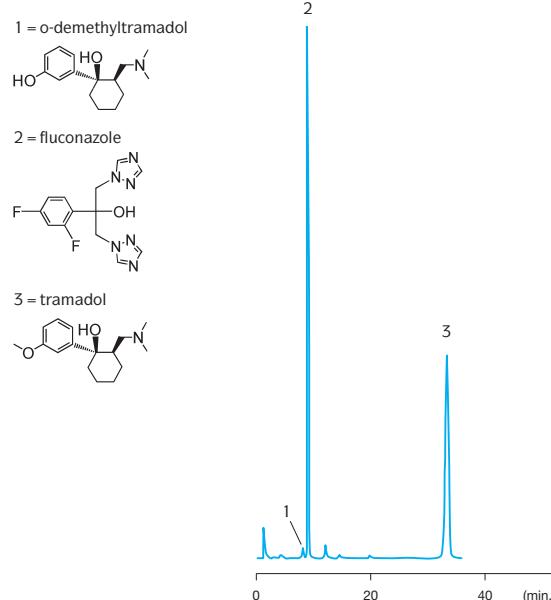
Determination of tetracyclines as chelates with aluminum(III).  
(ref. 273)



Phase: Kromasil 100 Å, 5 µm, C18  
Column: 4.6 × 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 ( $\lambda_{\text{ex}}$  380 nm and  $\lambda_{\text{em}}$  480 nm)

## Tramadol

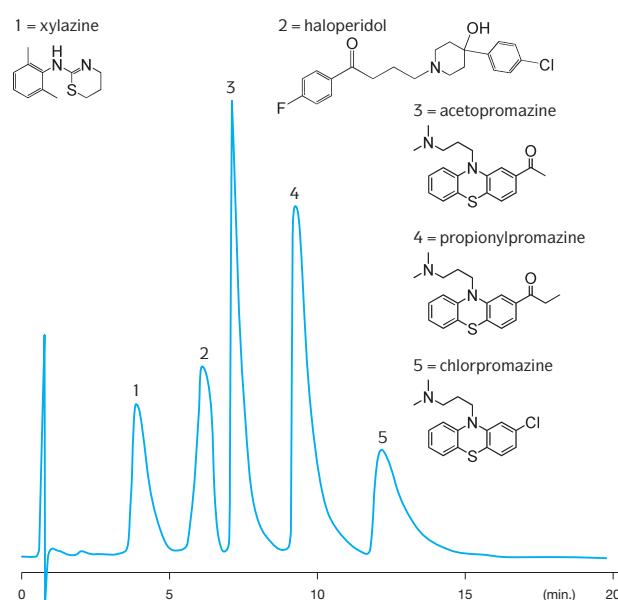
Determination of tramadol and its active metabolite in human plasma. (ref. 130)



Phase: Kromasil 100 Å, 5 µm, C18  
Column: 4 × 250 mm  
Temperature: 30°C ± 3°C  
Eluent: acetonitrile:water (19:81, v:v) cont. 0.06 M NaH<sub>2</sub>PO<sub>4</sub> and 0.05 M triethylamine, adjusted to pH 7.90  
Flow rate: 1 ml/min.  
Detection: fluorescence ( $\lambda_{\text{ex}}$  207 nm and  $\lambda_{\text{em}}$  300 nm)

## Tranquilizers, veterinary

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

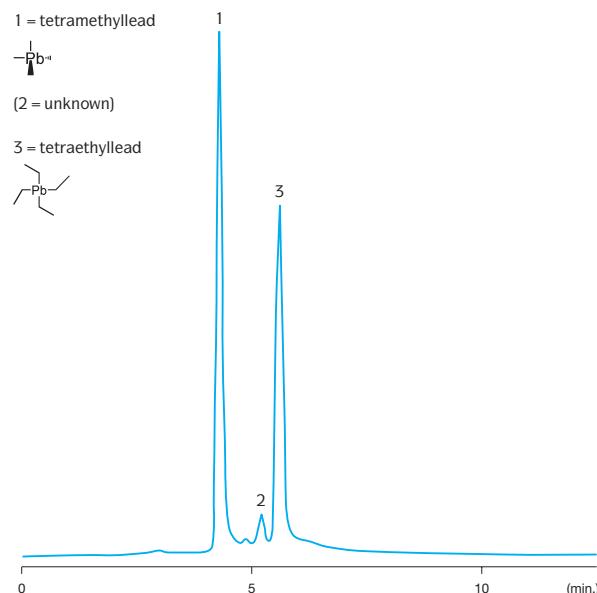


Phase: Kromasil 100 Å, 5 µm, C18  
Column: 4 × 150 mm  
Eluent: MeOH:water (80:20; v:v)  
Flow rate: 1 ml/min.  
Detection: UV 240 nm

## Environmental

**Alkyllead**

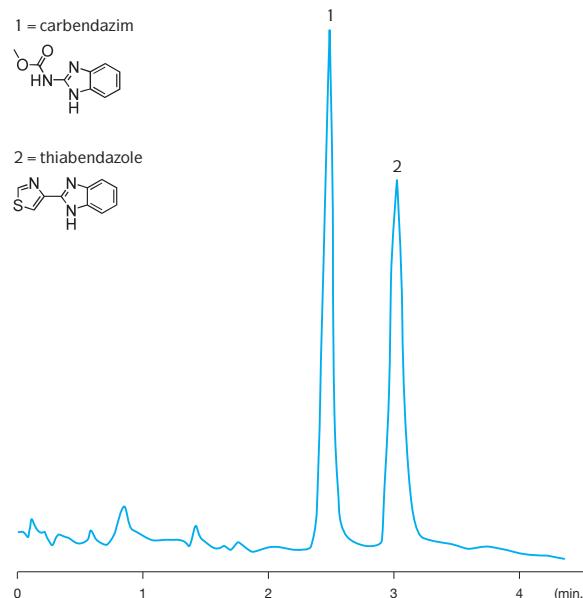
Determination of tetramethyllead and tetraethyllead. (ref. 198)



Phase: Kromasil 100 Å, 5 µm, C18  
Column: 0.32 × 230 mm  
Temperature: start: 50°C, ramp: 16°C/min., hold: 100°C  
Eluent: ACN  
Flow rate: 10 µl/min.  
Detection: ICP-MS

**Benzimidazole fungicides**

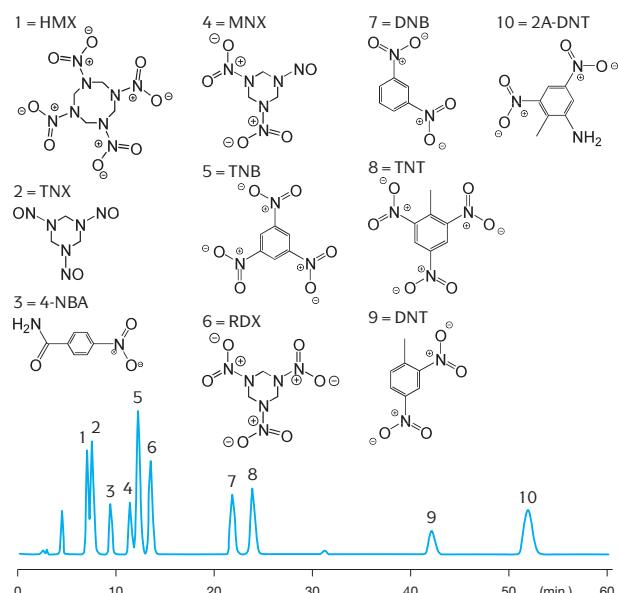
Determination of benzimidazole fungicides in fruits. (ref. 112)



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

**Explosives**

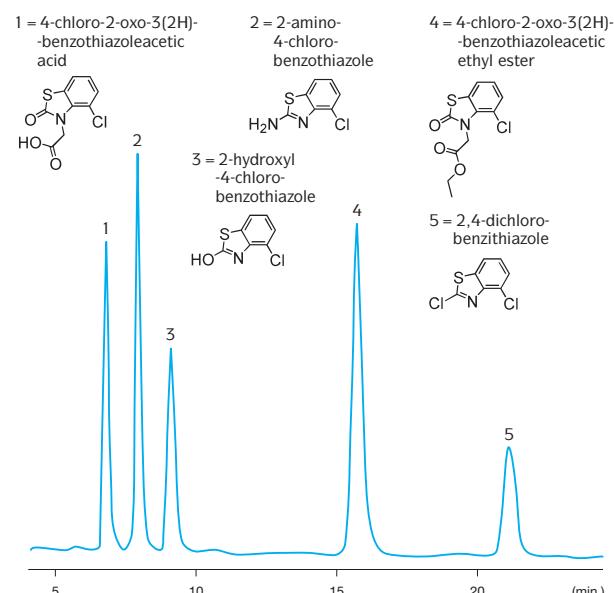
Sensitive determination of RDX, nitroso-RDX metabolites and other munitions in ground water. (ref. 175)



Phase: Kromasil 100 Å, C8  
Column: 2 × 250 mm  
Temperature: 32°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)

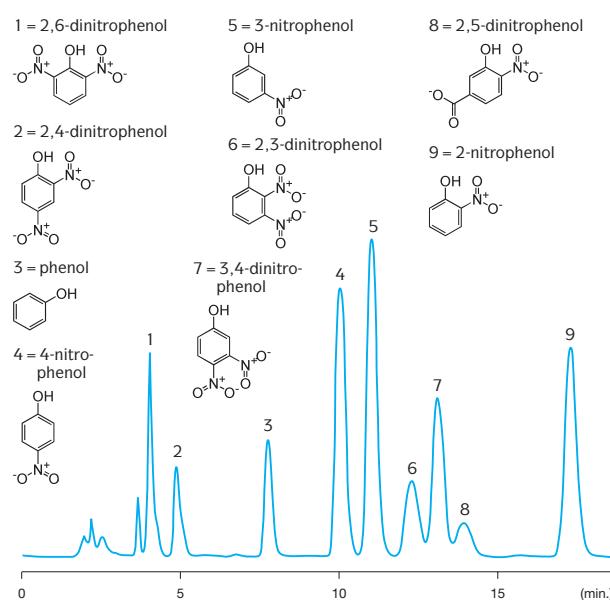


Phase: Kromasil 100 Å, 5 µm, C18  
Column: 4.6 × 200 mm  
Temperature: 30°C  
Eluent: MeOH:water:HAc (60:40:1; v:v:v)  
Flow rate: 0.7 ml/min.  
Detection: UV 254 nm

# Environmental

## Nitrophenols

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



Phase: Kromasil 100 Å, 5 µm, C18

Column: 4.4 × 250 mm

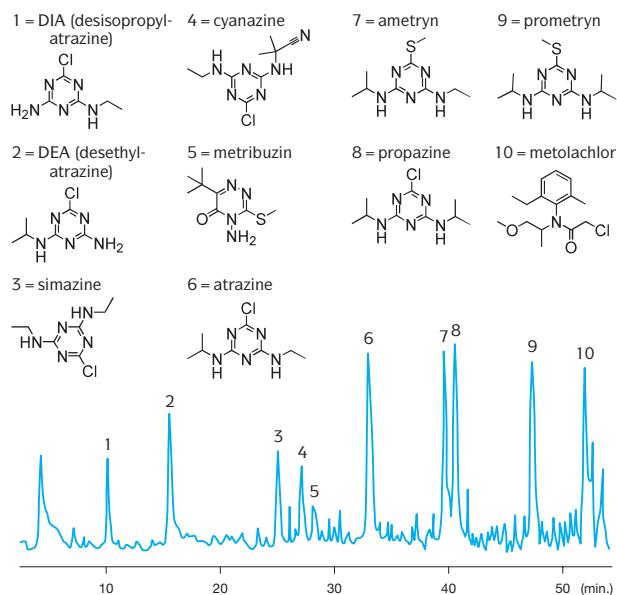
Eluent: A:B (55:45; v:v) A: 0.005 M KH<sub>2</sub>PO<sub>4</sub> (pH 4.5 with H<sub>3</sub>PO<sub>4</sub>):ACN (90:10; v:v) B: 0.005 M KH<sub>2</sub>PO<sub>4</sub> (pH 4.5 with H<sub>3</sub>PO<sub>4</sub>):MeOH (25:75; v:v)

Flow rate: 1 ml/min.

Detection: 230 nm

## Organonitrogen pesticides

Determination of organonitrogen pesticides in large volumes of surface water. (ref. 132)



Phase: Kromasil 100 Å, 5 µm, C18

Column: 4.6 × 250 mm

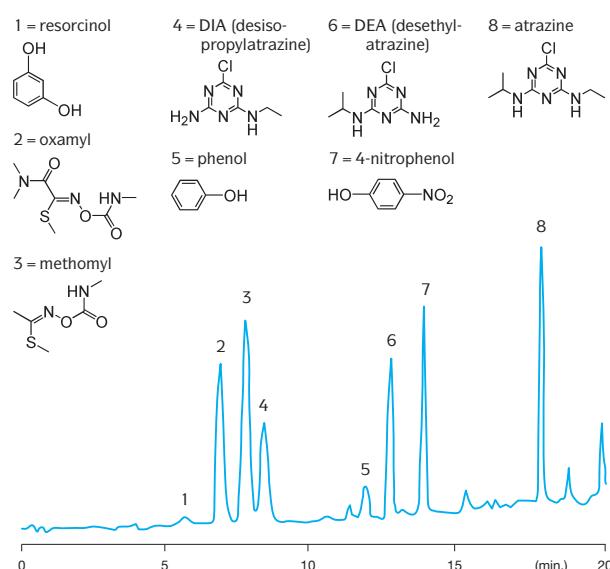
Eluent: Gradient, ACN:water, 15 – 60% ACN for 50 min, 60% for 15 min

Flow rate: 1 ml/min.

Detection: APCI-MS

## Pesticides and metabolites

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



Phase: Kromasil 100 Å, 5 µm, C18

Column: 4.6 × 250 mm

Temperature: 65°C

Eluent: ACN:water (pH 3 adjusted with sulfuric acid)

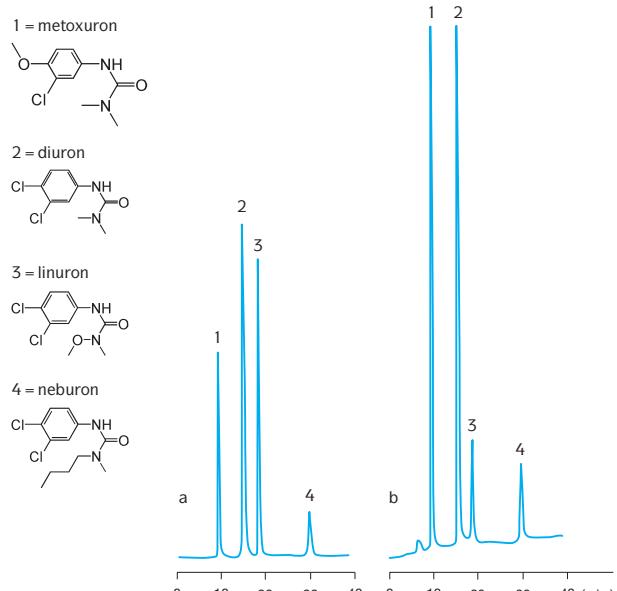
Gradient: From 15 to 25% ACN in 9.3 min., to 50% ACN in 4.3 min., to 100% ACN in 6 min. and then 2 min. isocratic elution at 100% ACN.

Flow rate: 1 ml/min.

Detection: UV 280 or 240 nm

## Phenylurea herbicides

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



Phase: Kromasil 100 Å, 5 µm, C18

Column: 1 × 300 mm

Eluent: MeOH:water (75:25; v:v) in 0.01 M lithium perchlorate at pH 5.5 (adjusted with 1% phosphoric acid)

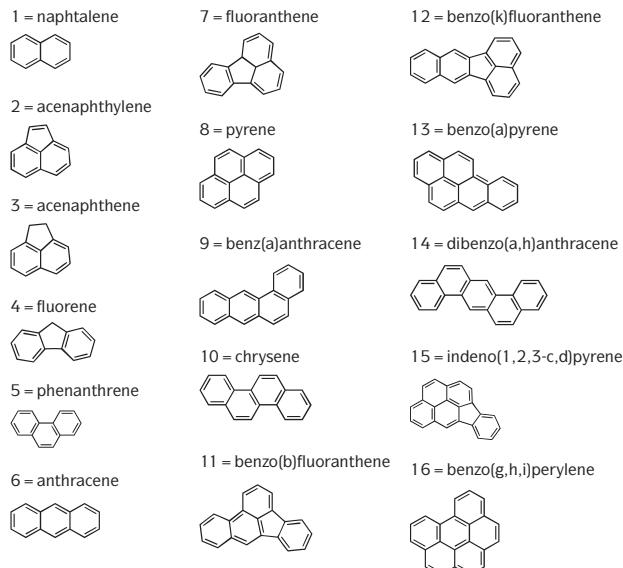
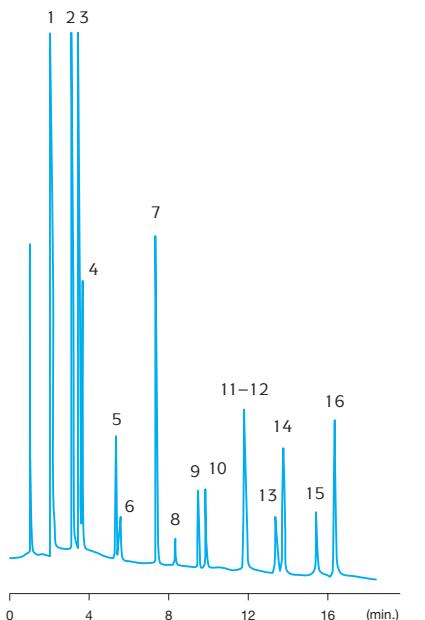
Flow rate: 20 – 40 µl/min

Detection: UV 254 nm and electrochemical (potential 1,35 V) respectively for the figures

## Environmental

**Polycyclic aromatic hydrocarbons**

Analysis of polycyclic aromatic hydrocarbons. (ref. 184)



Phase: Kromasil 100 Å, 5 µm, C18

Column: 4.6 × 250 mm

Temperature: 40°C

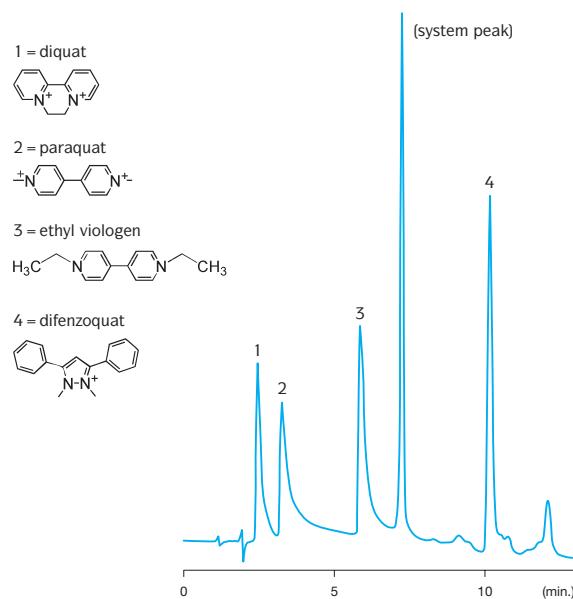
Eluent: CO<sub>2</sub>:ACNGradient: 0 min. 100% CO<sub>2</sub>, 20 min. 60% CO<sub>2</sub>,  
25 min. 60% CO<sub>2</sub>

Flow rate: 3 ml/min.

Detection: UV 210 nm

**Quaternary ammonium herbicides**

Determination of quaternary ammonium herbicides. (ref. 201)



Phase: Kromasil 100 Å, 5 µm, C8

Column: 2.1 × 200 mm

Temperature: 50°C

Eluent: Pentafluoropropionic acid in water (15 mM, pH 3.3) : ACN

Gradient: 0 min. 2% ACN, 5 min. 8.6% ACN, 5.01 min. 40%  
ACN, 13 min. 40% ACN

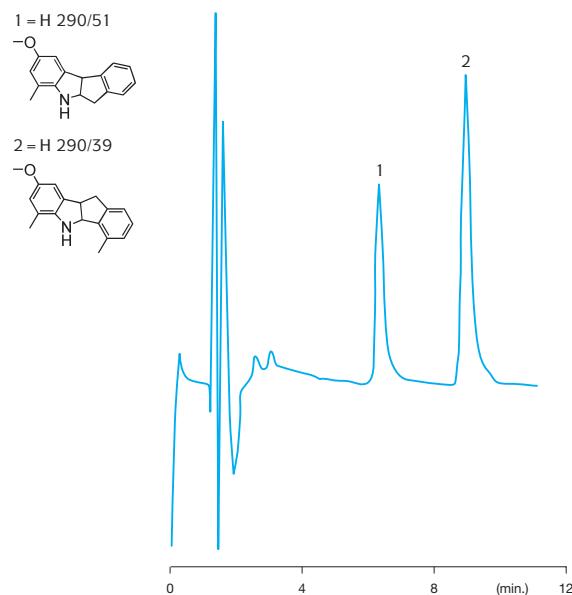
Flow rate: 200 µl/min.

Detection: UV

# Food and nutrition

## Antioxidants, lipophilic

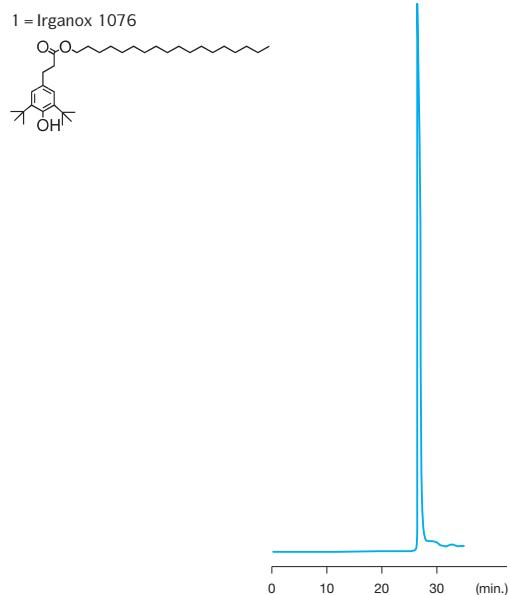
Determination of lipophilic antioxidants in plasma. (ref. 53)



Phase: Kromasil 100 Å, 5 µm, C8  
 Column: 4.6 × 150 mm  
 Eluent: Tris (50 mM), HCl (12 mM) and 65% ACN (pH 8.5)  
 Flow rate: 1 ml/min.  
 Detection: electrochemical, potential +0.70 V

## Irganox

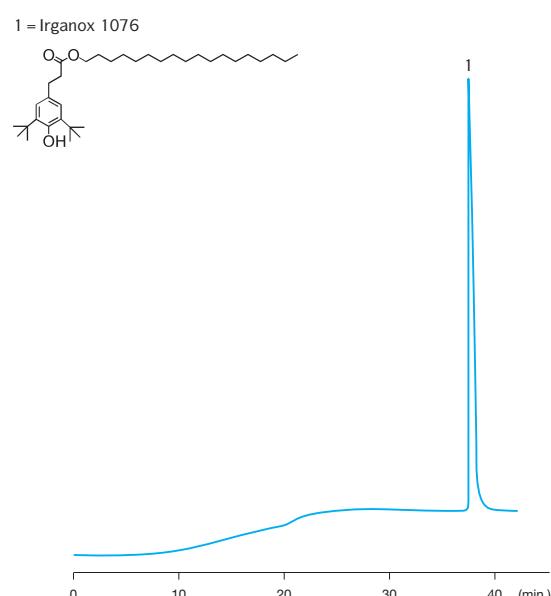
Determination of Irganox (an antioxidant). (ref. 20)



Phase: Kromasil 100 Å, 3.5 µm, C18  
 Column: 0.25 × 250 mm  
 Temperature: gradient: 5 °C/min. from 5 to 40 °C, 2 °C/min. from 40 to 80 °C, 5 °C/min. from 80 to 90 °C  
 Eluent: ACN + 10mM TEA + HCOOH  
 Flow rate: 5 µl/min.  
 Detection: ELS

## Irganox

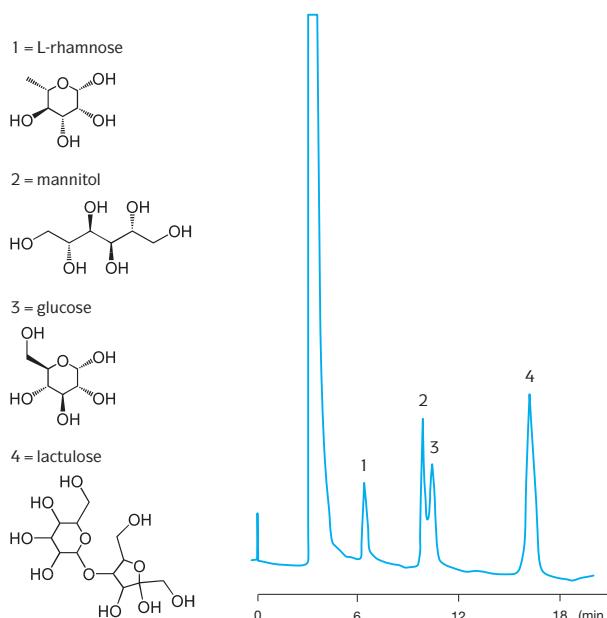
Determination of Irganox. (ref. 208)



Phase: Kromasil 100 Å, 5 µm, C18  
 Temperature: from 7 to 90 °C at 3 °C/min.  
 Column: 0.32 × 500 mm  
 Eluent: ACN  
 Flow rate: 5 µl/min.  
 Detection: UV 280 nm

## Sugars

Analysis of sugars in urine. (ref. 82)

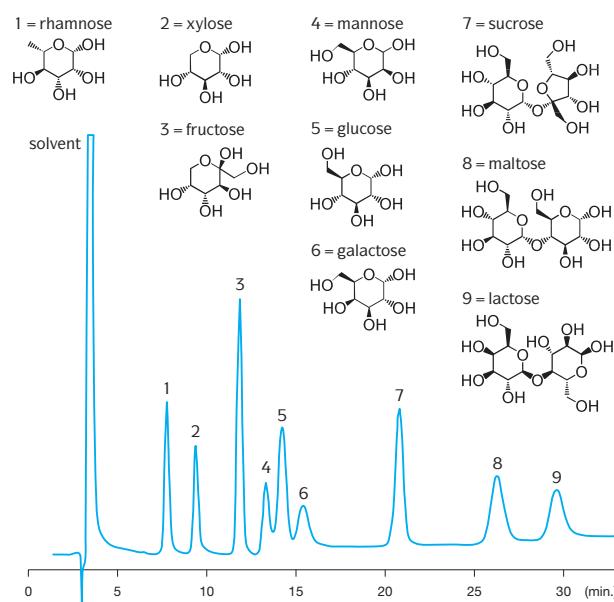


Phase: Kromasil 100 Å, 5 µm, NH<sub>2</sub>  
 Column: 4.6 × 250 mm  
 Temperature: ambient  
 Eluent: ACN:water (70:30; v:v)  
 Flow rate: 1 ml/min.  
 Detection: refractive index

## Food and nutrition

**Sugars**

Analysis of sugars. (ref. 315)



Phase: Kromasil 100 Å, 5 µm, NH2

Column: 4.6 × 250 mm

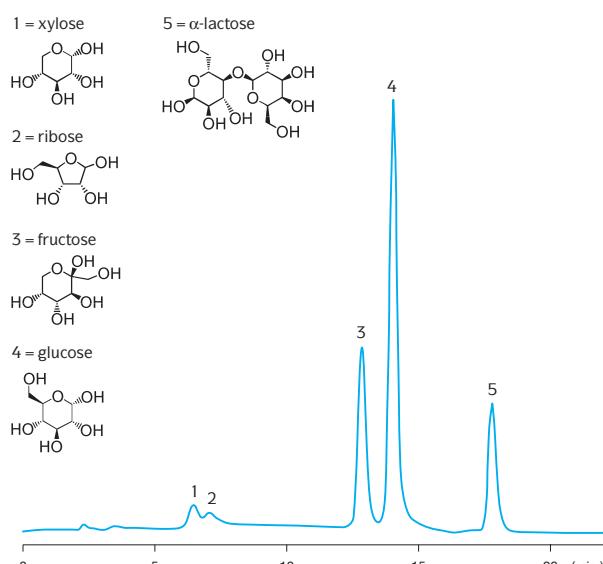
Eluent: ACN:water (75:25; v:v)

Flow rate: 1 ml/min.

Detection: RI

**Sugars, phosphorylated**

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



Phase: Kromasil 100 Å, 5 µm, NH2

Column: 4 × 250 mm

Eluent: ACN:water (85:15; v:v) at pH 4.8

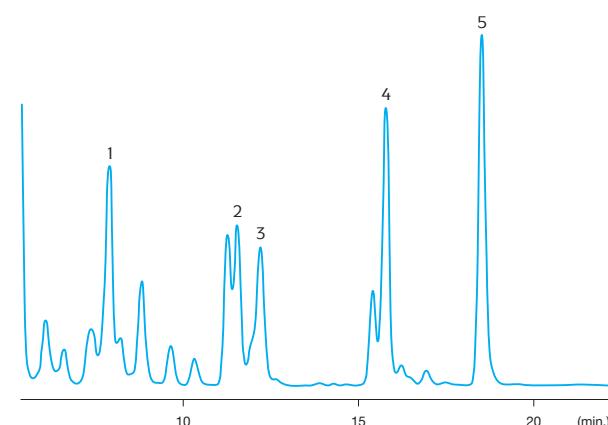
Flow rate: 1.4 ml/min.

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)



Phase: Kromasil 100 Å, 5 µm, C18

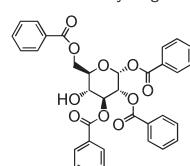
Column: 4 × 250 mm

Eluent: Gradient, ACN-water, 0 min. 70% ACN, 30 min. 95% ACN

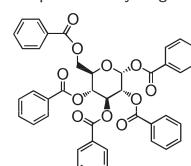
Flow rate: 1 ml/min.

Detection: UV 228 nm

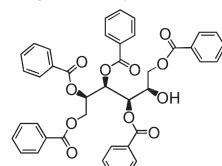
1 = tetrabenzoyl-D-glucose



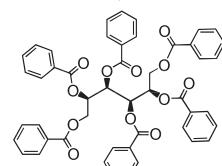
4 = pentabenzoyl-D-glucose



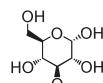
2 = pentabenzoyl mannitol



5 = hexabenzoyl mannitol



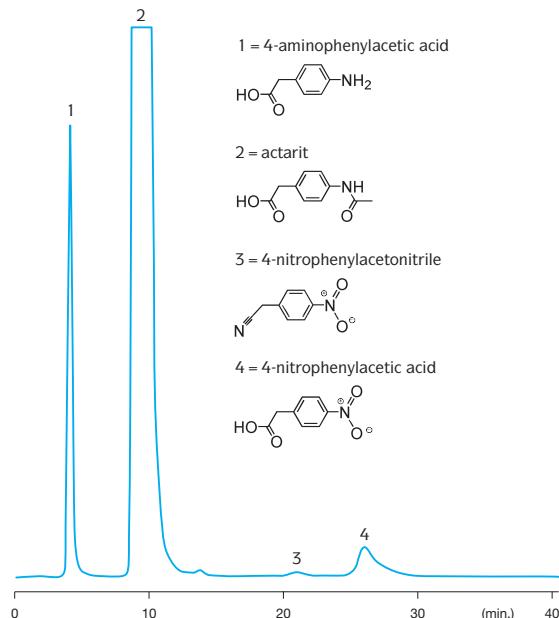
3 = 3-O-methyl-D-glucose



# Natural products

## Actarit

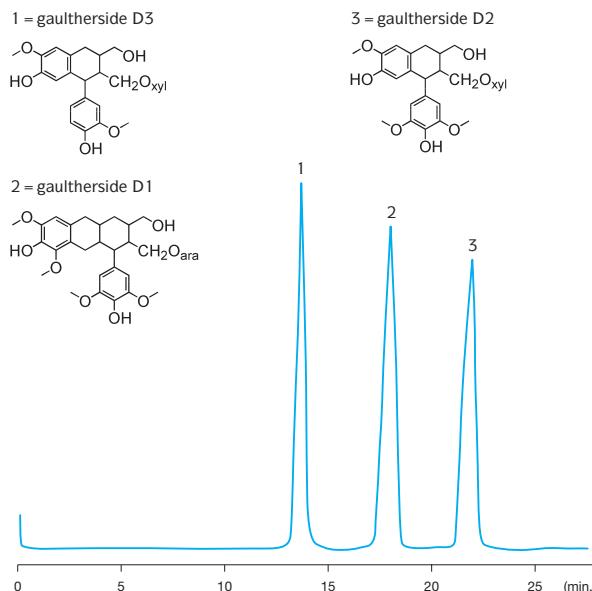
Determination of actarit and related compounds. (ref. 274)



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

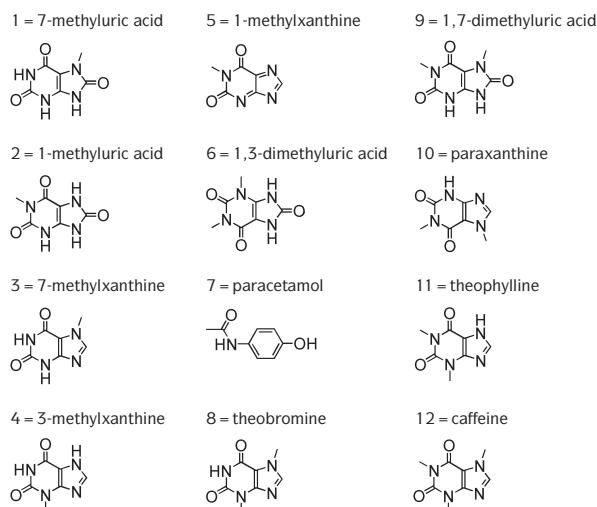
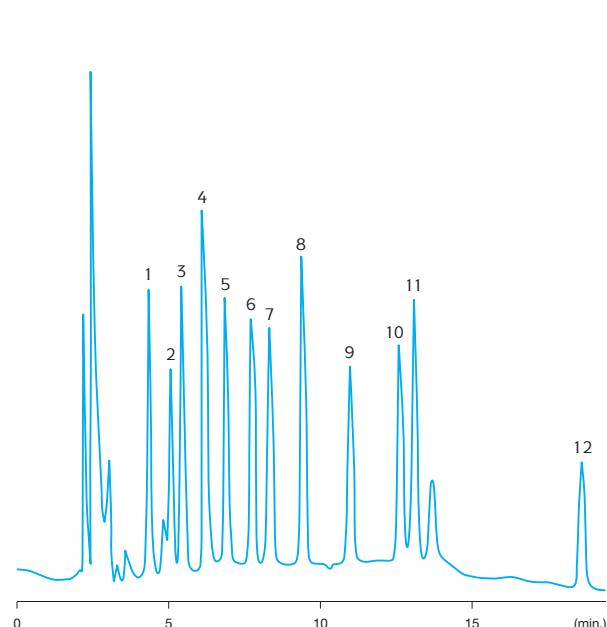
Determination of Gaulthersides in Yunnan wintergreen. (ref. 307)



Phase: Kromasil 100 Å, 5 µm, C18  
Column: 3.9 × 250 mm  
Temperature: ambient  
Eluent: MeOH:ACN:water (25:5:70; v:v:v) pH = 3.5 (adjusted with H<sub>3</sub>PO<sub>4</sub>)  
Flow rate: 0.7 ml/min.  
Detection: UV 220 nm

## Caffeine and metabolites

Quantitation of caffeine metabolism products. (ref. 271)

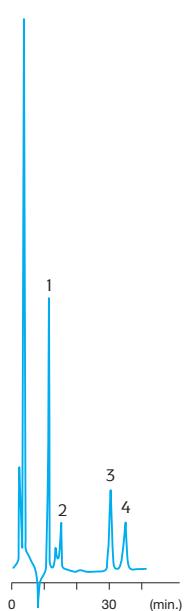
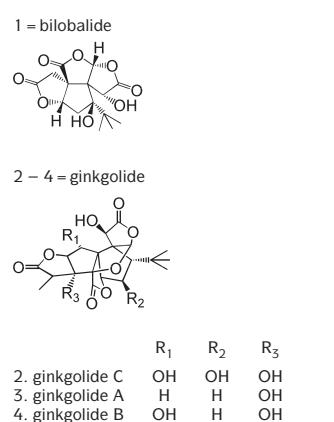


Phase: Kromasil 100 Å, 5 µm, C4  
Column: 4 × 250 mm  
Temperature: ambient  
Eluent: acetate buffer (pH 3.5) : MeOH (97:3; v:v)  
Gradient: 0 min. 3% MeOH, 20 min. 20% MeOH  
Flow rate: 1 ml/min.  
Detection: UV 275 nm

# Natural products

## Ginkgolides

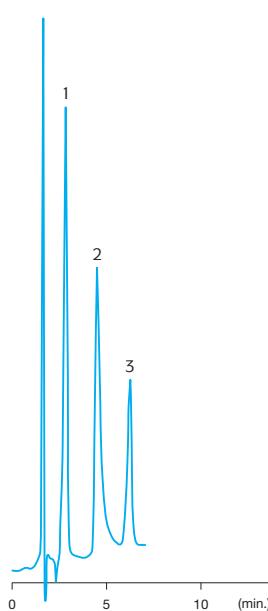
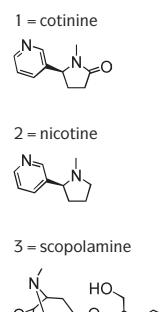
Determination of ginkgolides. (ref. 277)



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

## Nicotine

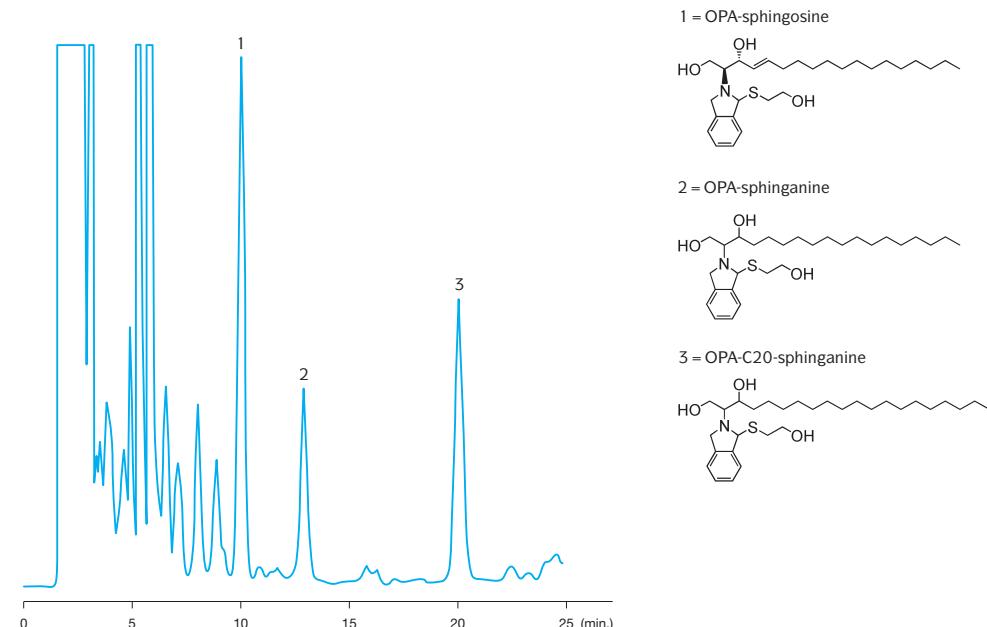
Clinical assay of nicotine and its metabolite, cotinine, in body fluids. (ref. 306)



Phase: Kromasil 100 Å, 5 µm, C8  
 Column: 4 × 250 mm  
 Temperature: ambient, 22 °C  
 Eluent: ammonium acetate (0.05 M):CH<sub>3</sub>OH (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)



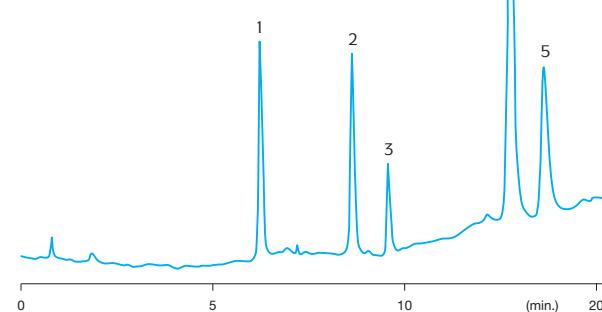
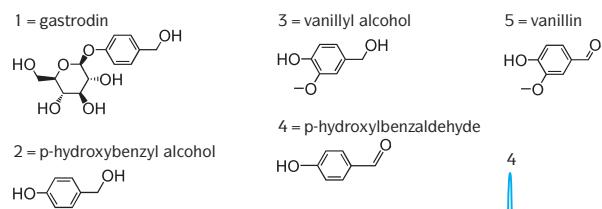
Phase: Kromasil 100 Å, 5 µm, C18  
 Column: 4.6 × 250 mm  
 Temperature: 45 °C  
 Eluent A: 0.07 M K<sub>2</sub>HPO<sub>4</sub> in MeOH (1:9; v:v)  
 Eluent B: MeOH  
 Gradient: 0 min. 0% B, 10 min. 0% B, 30 min. 40% B, 32 min. 100% B, 42 min. 100% B, 44 min. 0% B, 60 min. 0% B

Flow rate: 1.3 ml/min.  
 Detection: fluorescence ( $\lambda_{\text{ex}}$  340 nm,  $\lambda_{\text{em}}$  455 nm)

# Natural products

## TCM, Traditional Chinese Medicine

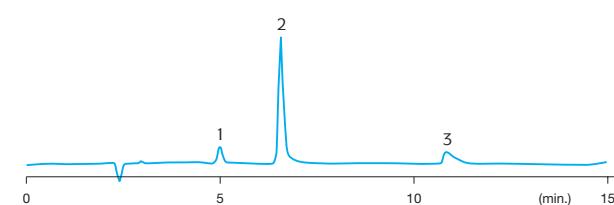
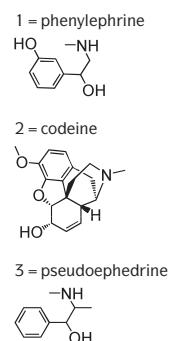
Determination of gastrodin, p-hydroxybenzyl alcohol, vanillyl alcohol, p-hydroxylbenzaldehyde and vanillin from TCM. (ref. 297)



Phase: Kromasil 100 Å, 5 µm, C18  
Column: 4.6 × 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)



Phase: Kromasil 100 Å, 5 µm, C18  
Column: 4.6 × 250 mm  
Temperature: 45°C  
Eluent: MeOH:water:acetic acid (40:60:2; v:v:v)  
+ 5 mM IPR-B<sub>8</sub>  
Flow rate: 1 ml/min.  
Detection: UV 245 nm

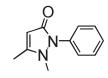
## TCM, Traditional Chinese Medicine

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

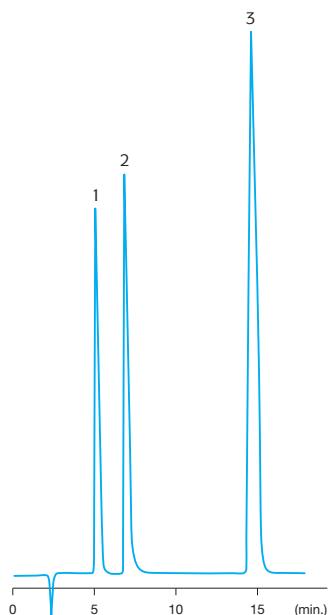
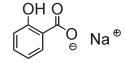
1 = caffeine



2 = antipyrine



3 = sodium salicylate

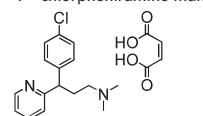


Phase: Kromasil 100 Å, 5 µm, C18  
Column: 4.6 × 250 mm  
Eluent: 20 mM potassium dihydrogen phosphate: MeOH:gla- cial acetic acid (55:25:0.4; v:v:v)  
Flow rate: 1 ml/min.  
Detection: UV 242 nm

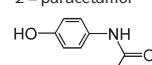
## TCM, Traditional Chinese Medicine

Determination of four components of Ganmaoling capsules. (ref. 258)

1 = chlorpheniramine maleate



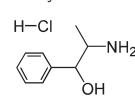
2 = paracetamol



3 = caffeine



4 = phenylpropanolamine hydrochloride



Phase: Kromasil 100 Å, 5 µm, C18

Column: 4.6 × 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

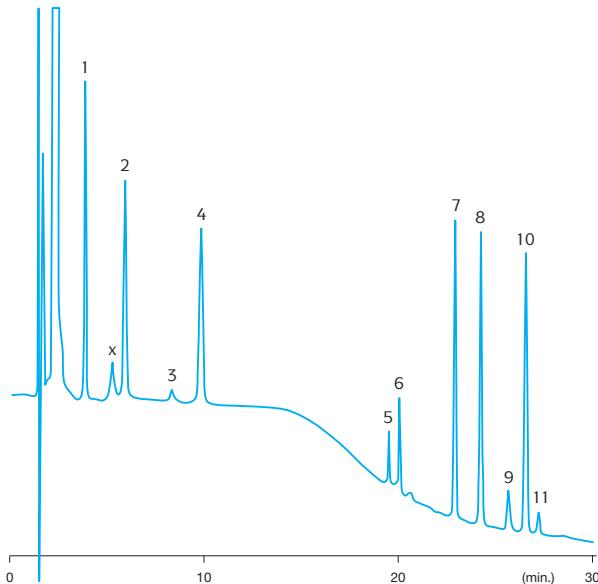
Flow rate: 1 ml/min.

Detection: UV 214 nm

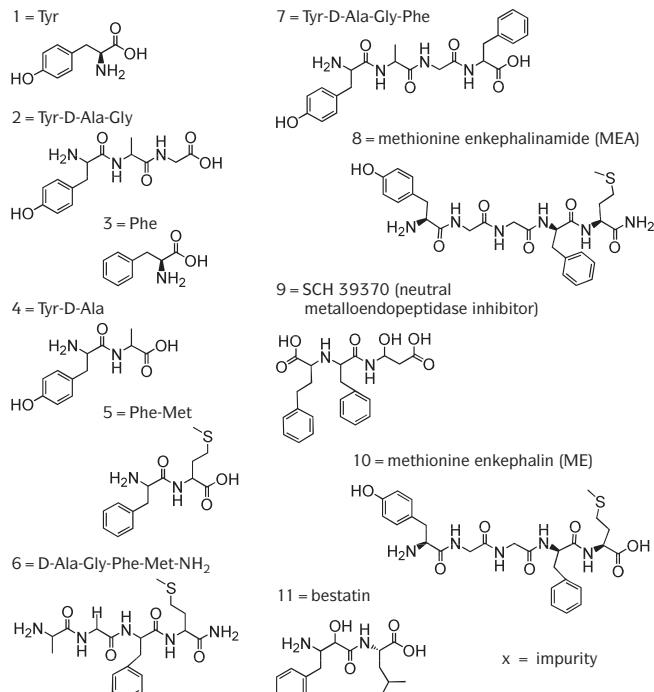
# Peptides

## Enkephalin peptides

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



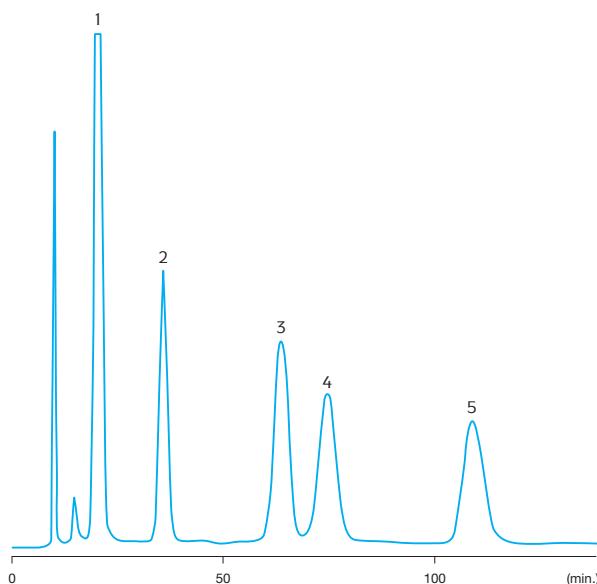
Phase: Kromasil 100 Å, 5 µm, C8  
 Column: 4.6 × 150 mm  
 Eluent A: 35 mM phosphate buffer (pH 2.1)  
 Eluent B: 59 mM phosphate buffer (pH 2.1) – ACN (60:40; v:v)  
 Gradient: ACN from 4 to 5% in 9 min., then to 15% in 5 min., finally to 30% in 21 min.



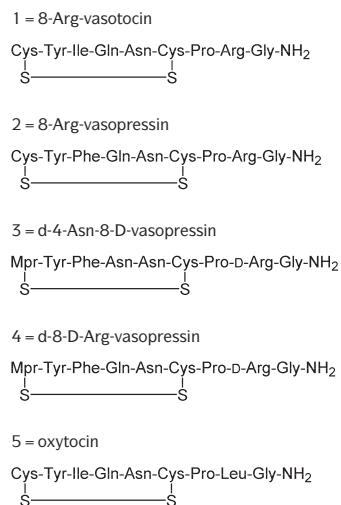
Flow rate: 1 ml/min.  
 Detection: UV 205 nm

## Nonapeptides

Analysis of five nonapeptides. (ref. 28)



Phase: Kromasil 100 Å, 12.5 µm, C18  
 Column: 10 × 250 mm  
 Eluent: ACN:phosphate buffer (pH 7) (18:82; v:v)  
 Flow rate: 4 ml/min.  
 Detection: UV 225 nm

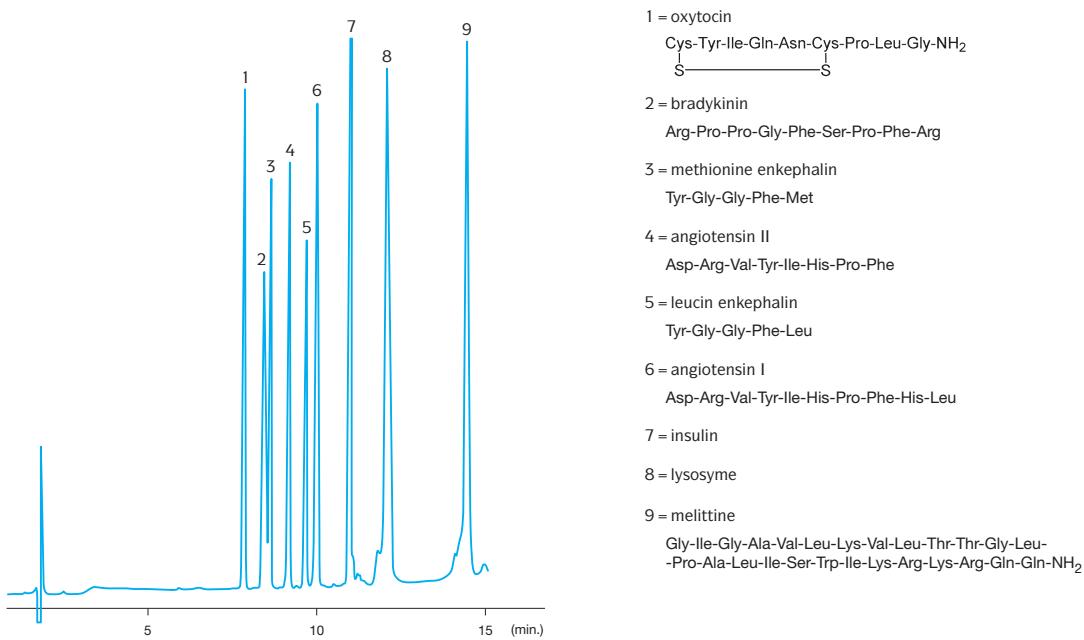


# Peptides

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## Peptides

Separation of 9 peptides. (ref. 316)



Phase: Kromasil 100 Å, 5 µm, C8

Column: 4.5 × 250 mm

Temperature: ambient

Eluent A: 0.1% TFA in 10% CH<sub>3</sub>CN and 90% water

Eluent B: 0.1% TFA in 90% CH<sub>3</sub>CN and 10% water

Gradient: 0 min. 0% B, 8 min. 25% B, 20 min. 75% B

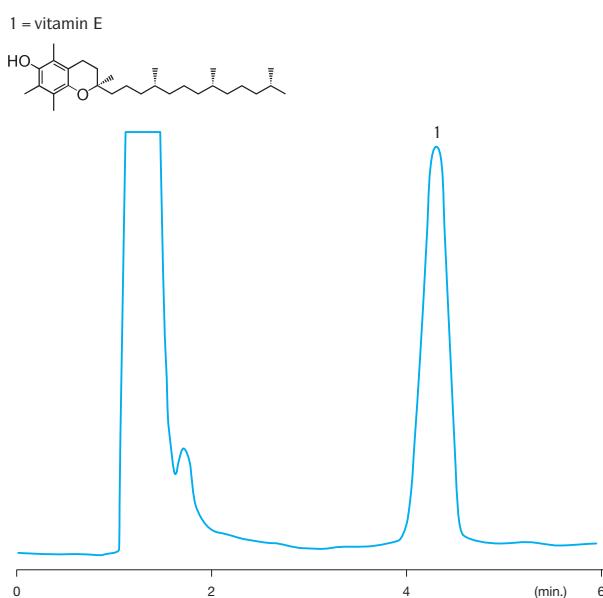
Flow rate: 2 ml/min.

Detection: UV 254 nm

# Vitamins

## Vitamin E

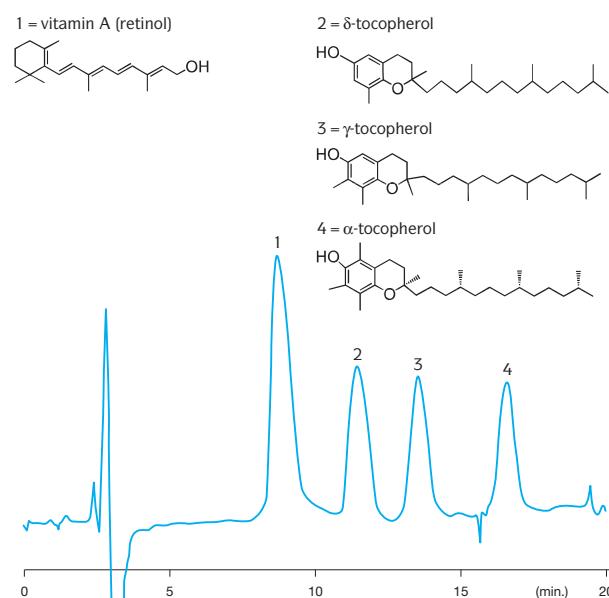
Determination of vitamin E in human plasma. (ref. 108)



Phase: Kromasil 100 Å, 5 µm, C1  
 Column: 4.6 × 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

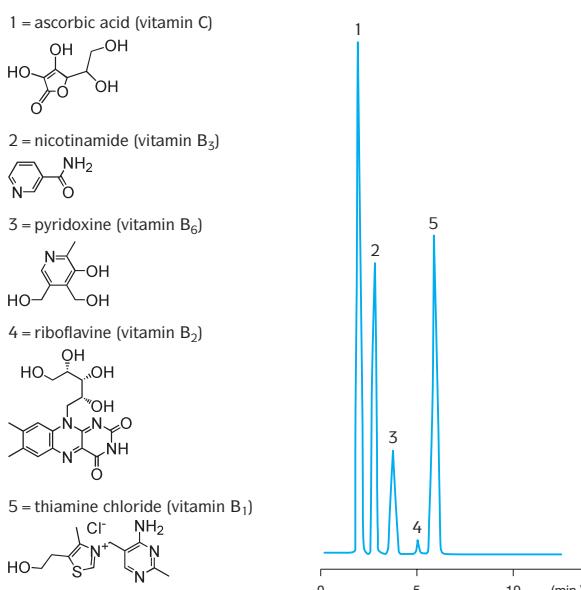
Determination of tocopherols and vitamin A in vegetable oils. (ref. 188)



Phase: Kromasil 100 Å, 5 µm, C18  
 Column: 0.2 × 800 mm  
 Temperature: 65°C  
 Eluent: CO<sub>2</sub> with 8% MeOH  
 Pressure: 180 atm  
 Detection: electrochemical (potential + 1.80 V versus Quasi-Reference Electrode)

## Vitamins

Analysis of soluble vitamins. (ref. 330)

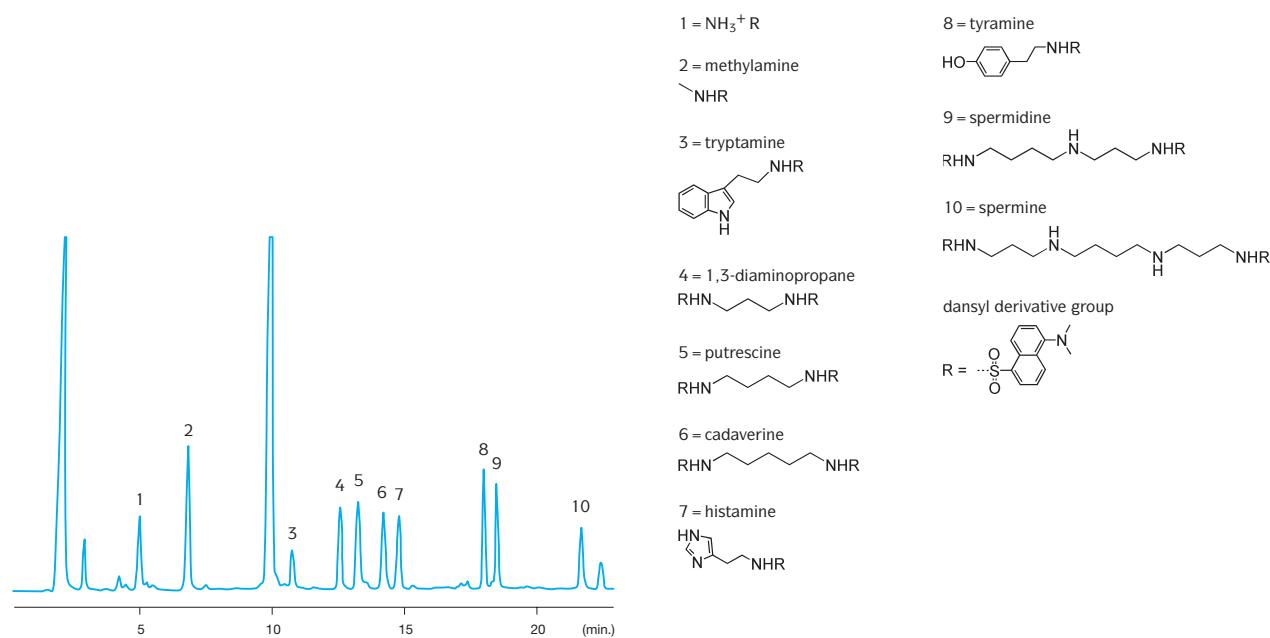


Phase: Kromasil 100 Å, 10 µm, NH2  
 Column: 4.6 × 250 mm  
 Eluent: 0.68 g sodium 1-hexanesulfonic acid + 0.8 g phosphoric acid + 720 ml water (pH 2.3) + 80 ml ACN + 200 ml MeOH  
 Flow rate: 1 ml/min.  
 Detection: UV 210 nm

# Other

## Amines

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

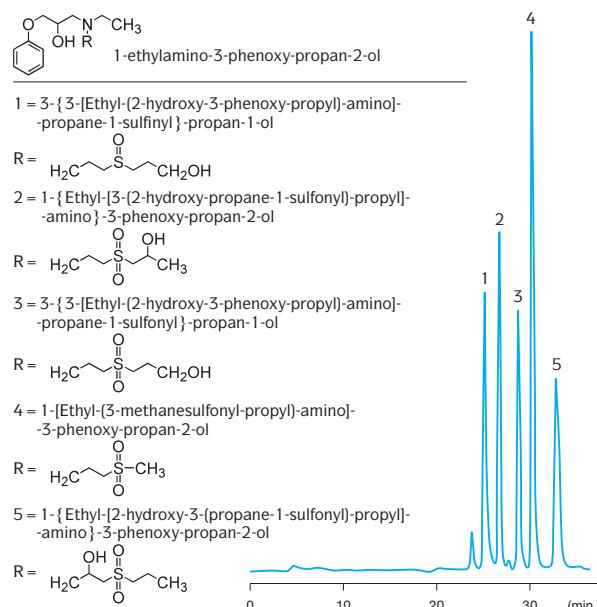


Phase: Kromasil 100 Å, 5 µm, C18  
 Column: 4.6 × 250 mm  
 Temperature: 25 °C  
 Eluent: ACN:water  
 Gradient: 0 min 60% ACN, 6 min 75% ACN, 8 min 75% ACN, 13 min 95% ACN, 20 min 95% ACN, 20.01 min 60% ACN

Flow rate: 1 ml/min.  
 Detection: UV 254 nm

## Amino alcohols

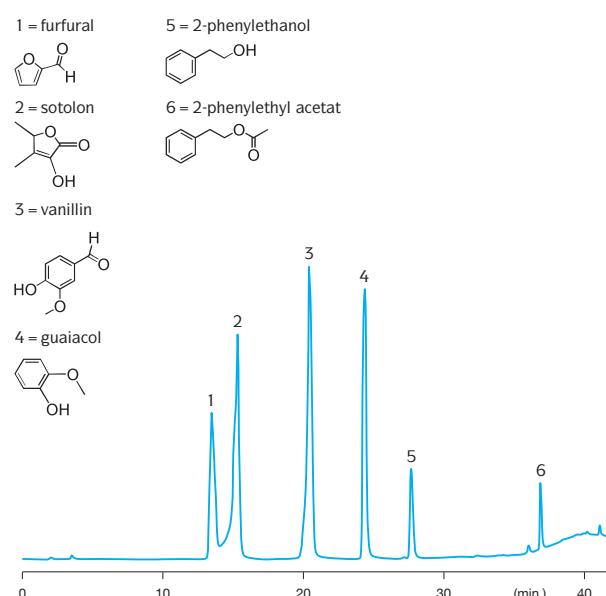
Separation of derivates of 1-ethylamino-3-phenoxy-propan-2-ol. (ref. 38)



Phase: Kromasil 100 Å, 5 µm, C8  
 Column: 0.2 × 900 mm  
 Eluent: ACN:ammonium acetate( 5 mM) (55:45; v:v)  
 Flow rate: 0.95 µl/min.  
 Detection: ESI-MS

## Aroma extracts in alcoholic beverages

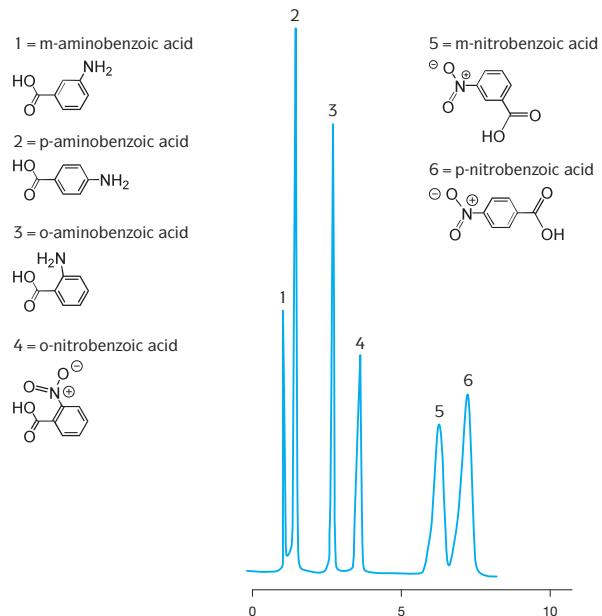
Separation of aroma extracts found in wine and other alcoholic beverages. (ref. 209)



Phase: Kromasil 100 Å, 5 µm, C18  
 Column: 10 × 250 mm  
 Eluent: water:ethanol  
 Gradient: 0 min. 100% water, 8 min. 80% water, 28 min. 50% water, 40 min. 0% water  
 Flow rate: 2 ml/min.  
 Detection: UV 220 nm

**Aromatics**

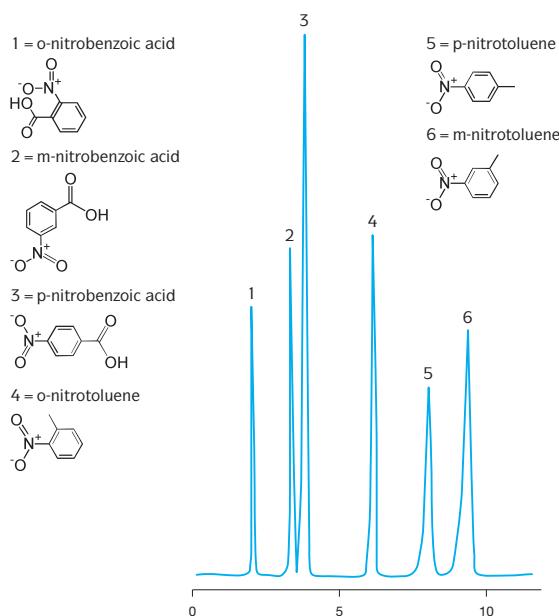
Separation of mixtures of nitrobenzoic acid and aminobenzoic acid isomers. (ref. 214)



Phase: Kromasil 100 Å, 5 µm, C18  
Column: 4.6 × 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**

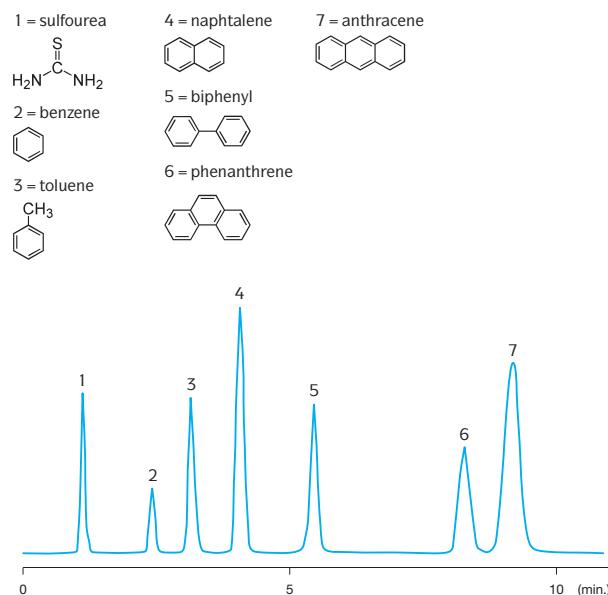
HPLC analysis of isomers of nitrotoluene and nitrobenzoic acid. (ref. 213)



Phase: Kromasil 100 Å, 5 µm, C18  
Column: 4.6 × 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**

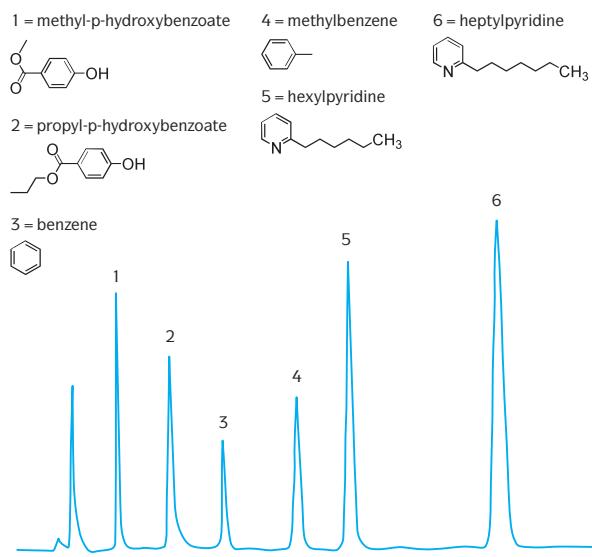
Determination of sulfonurea, benzene, toluene, naphtalene, biphenyl, phenanthrene, anthracene. (ref. 301a)



Phase: Kromasil 100 Å, 5 µm, C18  
Column: 0.8 × 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)

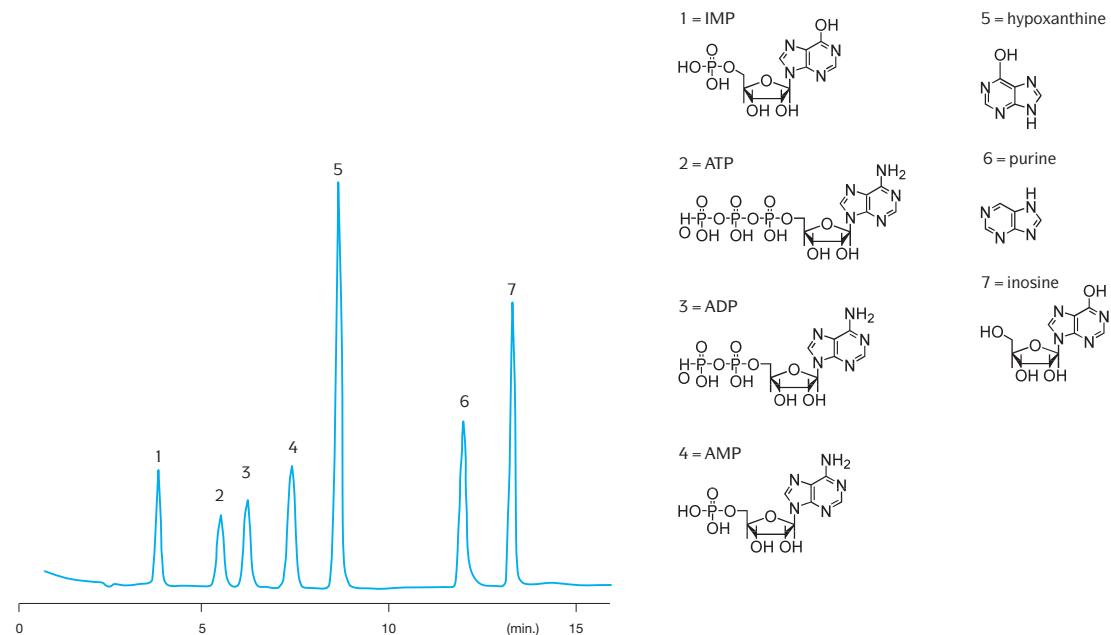


Phase: Kromasil 100 Å, 5 µm, C18  
Column: 4.6 × 150 mm  
Eluent: ACN:water (56.9:43.1; v:v)  
Flow rate: 1 ml/min.  
Detection: UV 254 nm

# Other

## ATP degradation products

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

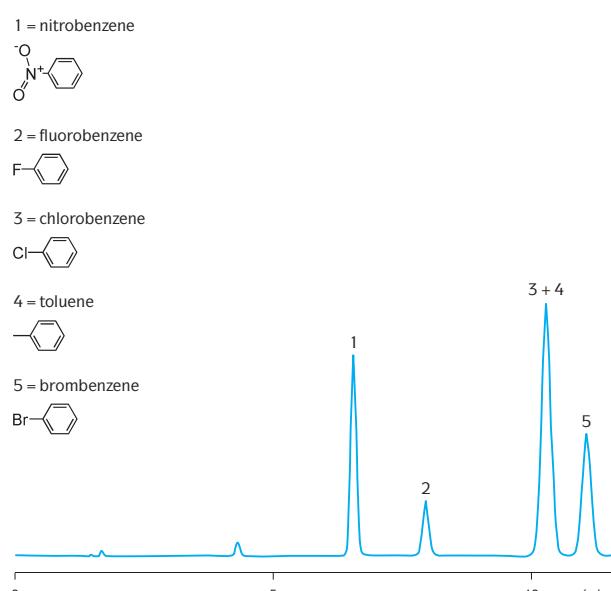


Phase: Kromasil 100 Å, 5 µm, C18  
Column: 4.6 × 250 mm  
Temperature: 25°C  
Eluents: Eluent A, ACN and eluent B, phosphate buffer (pH 7.00, 60 mM K<sub>2</sub>HPO<sub>4</sub> + 40 mM KH<sub>2</sub>PO<sub>4</sub>)

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

## Benzene, substituted

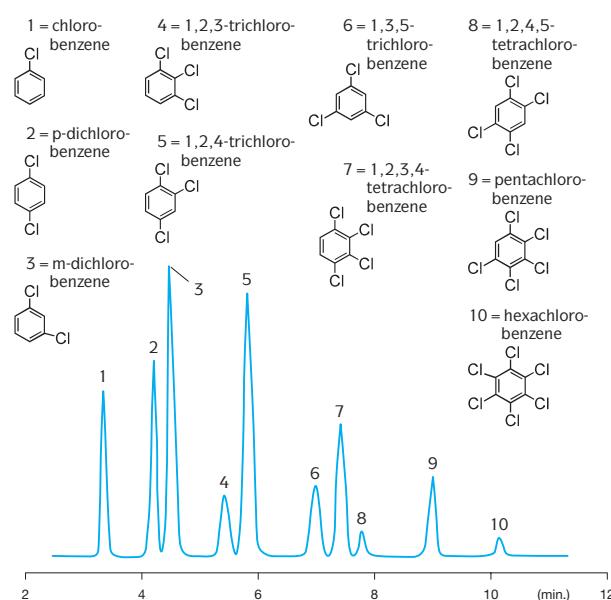
Separation of substituted benzene. (ref. 1)



Phase: Kromasil 100 Å, 5 µm, C8  
Column: 4.6 × 250 mm  
Temperature: 20°C  
Eluent: ACN:water (60:40; v:v)  
Flow rate: 1 ml/min.  
Detection: UV 210 nm

## Chlorinated benzenes

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

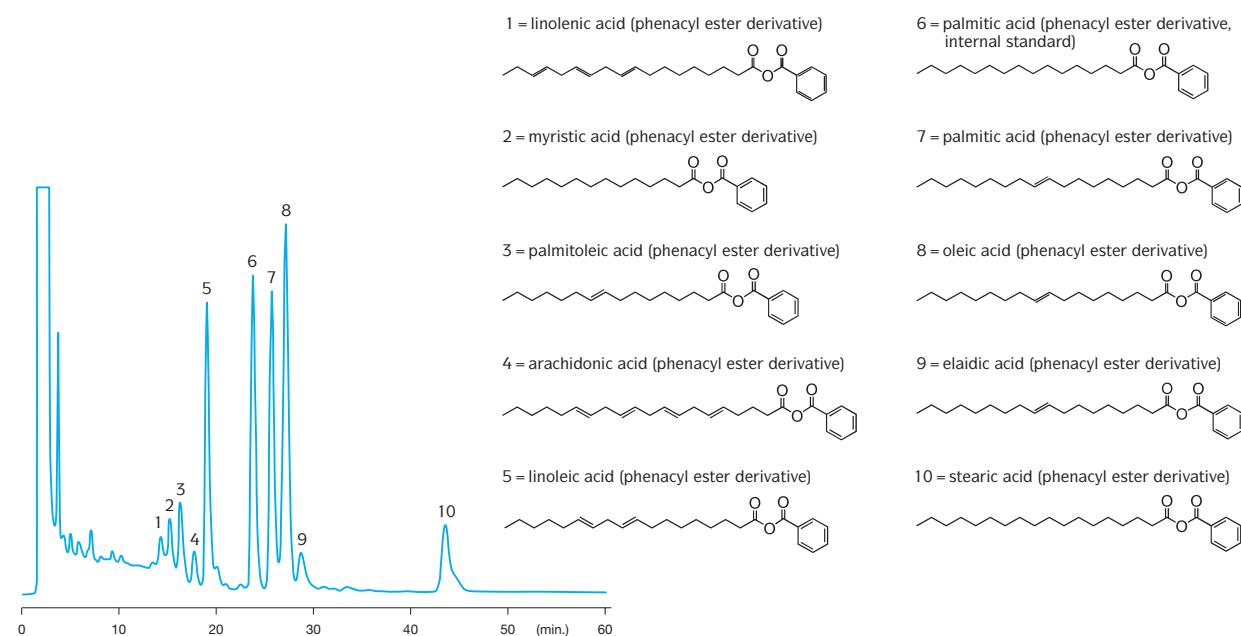


Phase: Kromasil 100 Å, 5 µm, C18  
Column: 0.8 × 150 mm  
Eluent: eluent A: ACN, eluent B: water  
Gradient: 0 min. 80% A, 5 min. 80% A, 10 min. 100% A  
Flow rate: 32 µl/min.  
Detection: UV 220 nm

# Other

## Fatty acids

Analysis of plasma fatty acids as their phenacyl esters. (ref. 193)



Phase: Kromasil 100 Å, 5 µm, C18

Column: 4.6 × 250 mm

Temperature: ambient

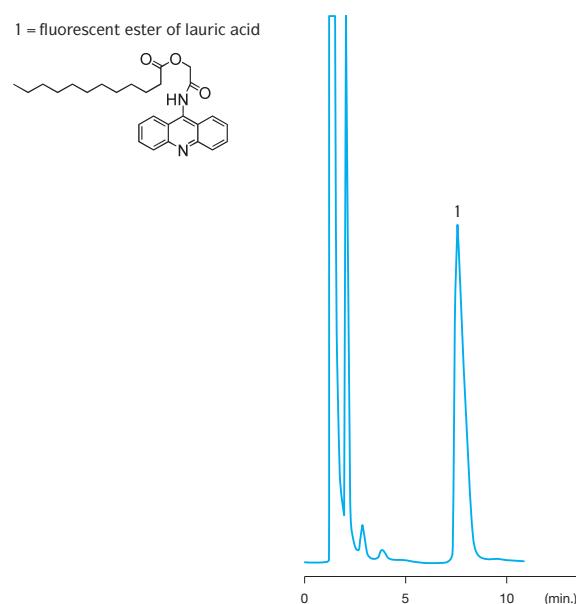
Eluent: MeOH:water (91:9; v:v)

Flow rate: 1.15 ml/min.

Detection: UV 254 nm

## Lauric acid

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



Phase: Kromasil 100 Å, 7 µm, C18

Column: 4.6 × 150 mm

Eluent: ACN:MeOH:water (55:10:35; v:v:v)

0.2% phosphoric acid added

Flow rate: 1 ml/min.

Detection: fluorescence ( $\lambda_{\text{ex}}$  357.5 nm and  $\lambda_{\text{em}}$  482 nm)

## Organic acids

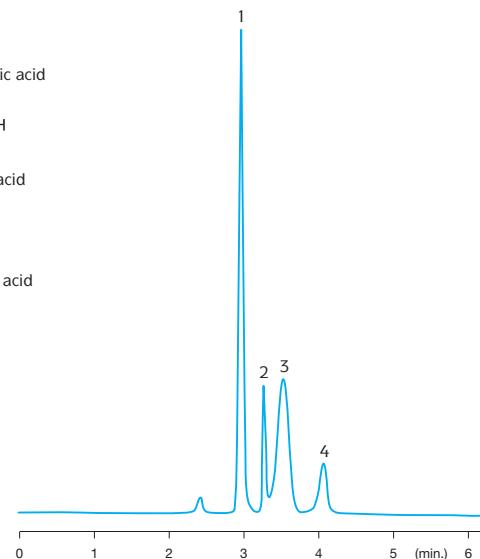
Separation of formic acid, benzoic acid, lactic acid, acetic acid. (ref. 344)

1 = formic acid  
O=[H]C(=O)O

2 = benzoic acid  
Oc1ccccc1

3 = lactic acid  
OC(O)C(O)C(=O)O

4 = acetic acid  
OC(=O)O



Phase: Kromasil 100 Å, 5 µm, C18

Column: 4.6 × 250 mm

Eluent: KH<sub>2</sub>PO<sub>4</sub>-buffer (10 mM, pH 2.5):ACN (95:5; v:v)

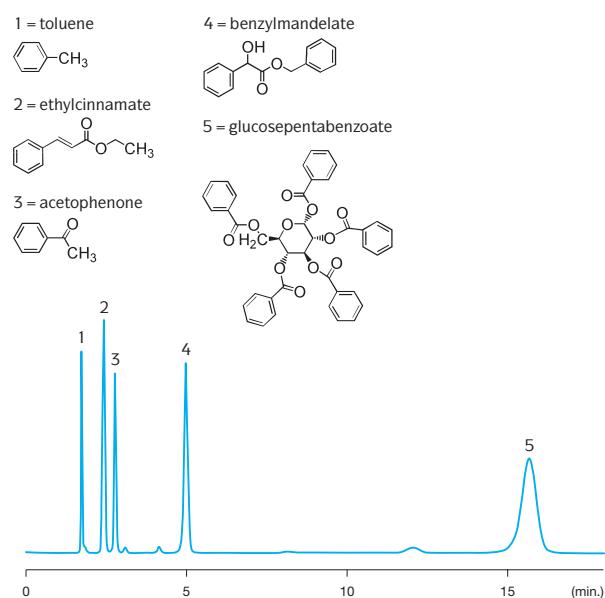
Flow rate: 38 µl/min.

Detection: UV 254 nm

# Other

## QC test, neutral compounds

QC test of Kromasil CN. (ref. 341)



Phase: Kromasil 60 Å, 10 µm, CN

Column: 4.6 × 250 mm

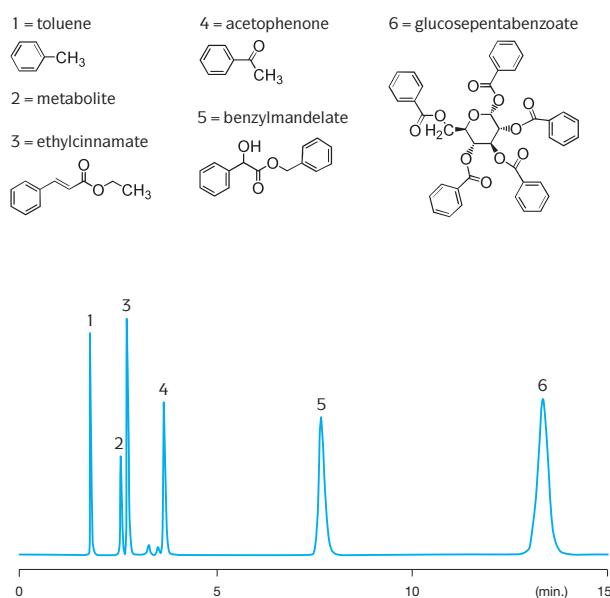
Eluent: hexane:ethylacetate (90:10; v:v)

Flow rate: 2 ml/min.

Detection: UV 254 nm

## QC test, neutral compounds

QC test of Kromasil SIL. (ref. 346)



Phase: Kromasil 60 Å, 5 µm, SIL

Column: 4.6 × 250 mm

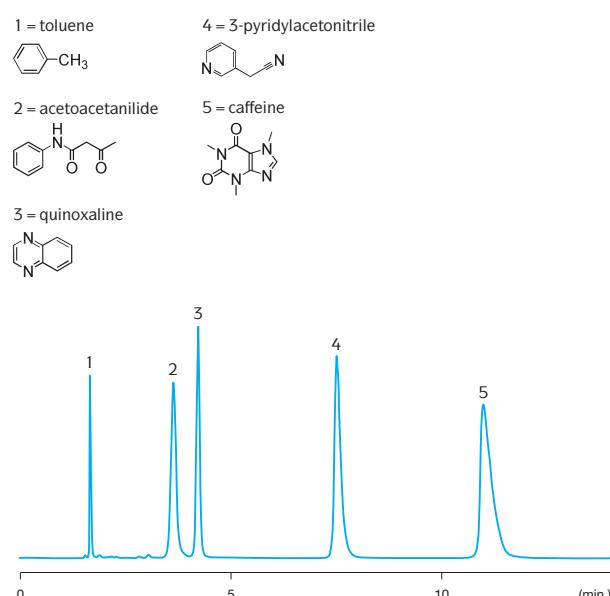
Eluent: hexane:ethylacetate (85:15; v:v)

Flow rate: 2 ml/min.

Detection: UV 254 nm

## QC test, silanophilic compounds

QC test of Kromasil SIL. (ref. 345)



Phase: Kromasil 60 Å, 5 µm, SIL

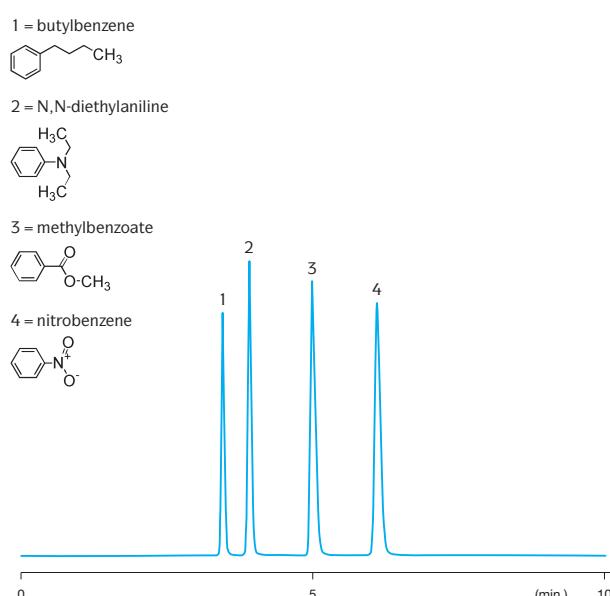
Column: 4.6 × 250 mm

Eluent: MeCl<sub>2</sub>:MeOH (98:2; v:v)

Flow rate: 2 ml/min.

Detection: UV 254 nm

## QC test, substituted aromatic compounds

QC test of Kromasil NH<sub>2</sub>. (ref. 343)Phase: Kromasil 100 Å, 5 µm, NH<sub>2</sub>

Column: 4.6 × 250 mm

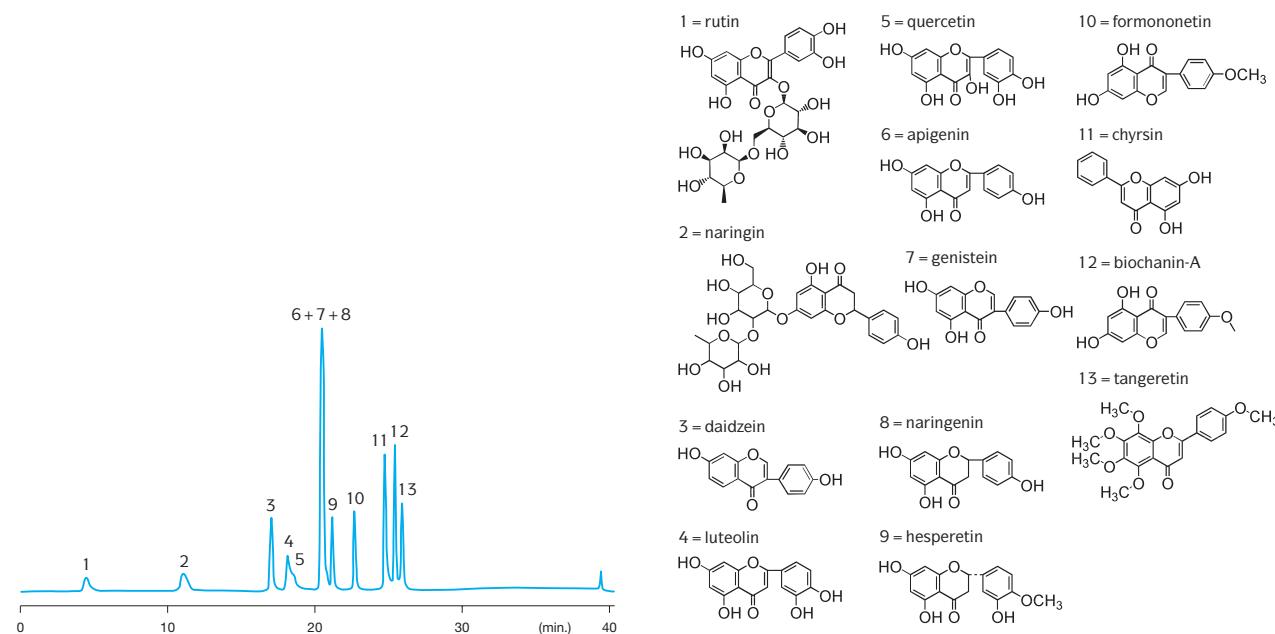
Eluent: hexane:MeCl<sub>2</sub> (97:3; v:v)

Flow rate: 1 ml/min.

Detection: UV 254 nm

## Flavonoid glycosides

Analysis of flavonoid glycosides. (ref. 100)

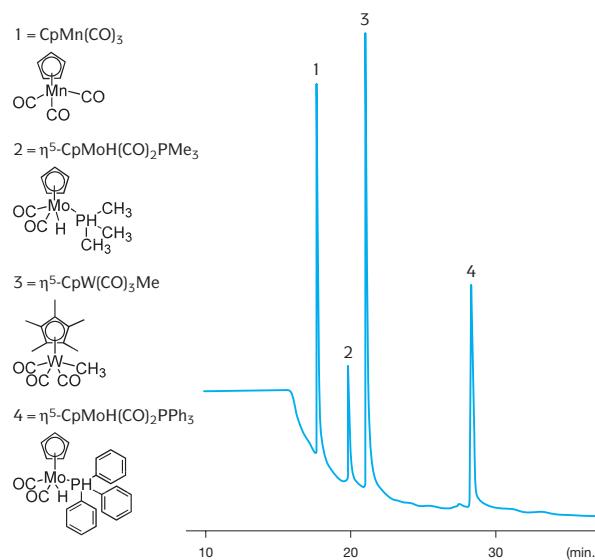


Phase: Kromasil 100 Å, 5 µm, C18  
 Column: 3.2 × 250 mm  
 Eluent: ACN:water  
 Gradient: 0 min. 20% ACN, 10 min. 20% ACN, 18 min. 40% ACN, 28 min. 75% ACN, 30 min. 100% ACN, 37 min. 100% ACN

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

## Organometallic catalysts

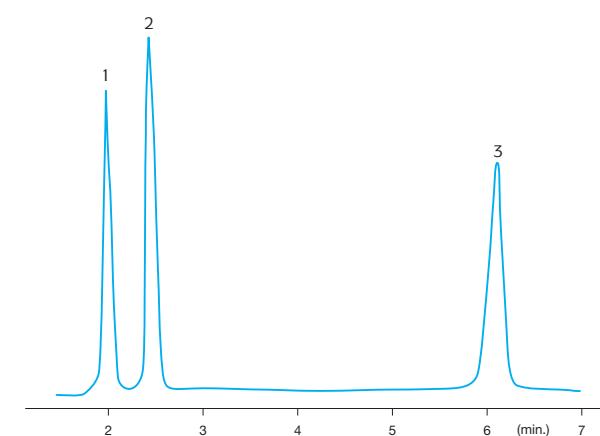
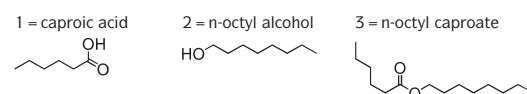
Purity testing of organometallic catalysts. (ref. 248)



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)

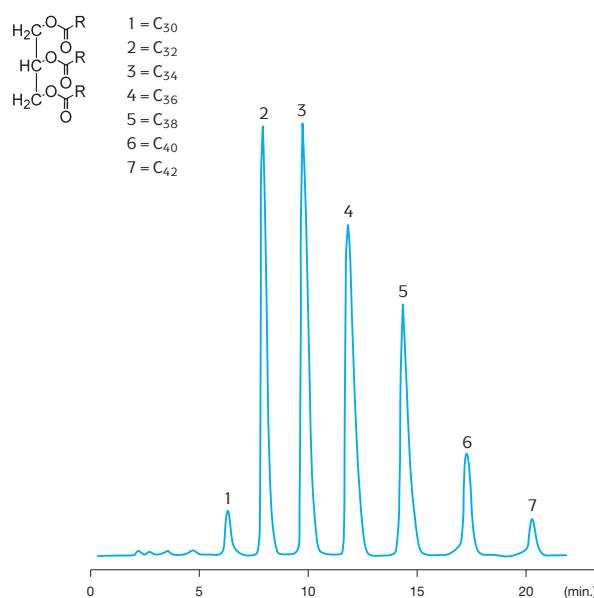


Phase: Kromasil 100 Å, 5 µm, C18  
 Temperature: 30°C  
 Column: 4.6 × 150 mm  
 Eluent: MeOH:water (95:5; v:v)  
 Flow rate: 1 ml/min.  
 Detection: refractive index

# Other

## Triacylglycerols

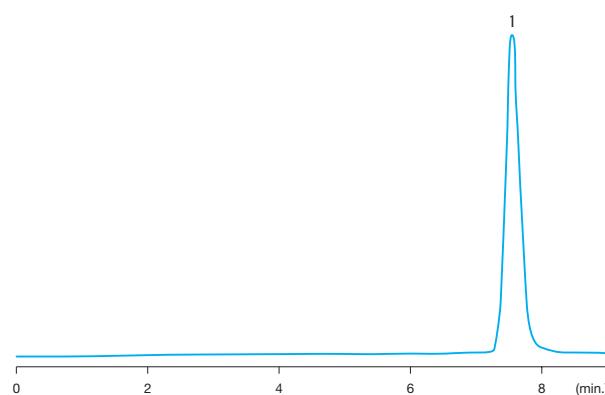
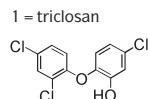
Analysis of seven triacylglycerols. (ref. 139)



Phase: Kromasil 100 Å, 5 µm, C18  
 Column: 0.7 × 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

## Triclosan

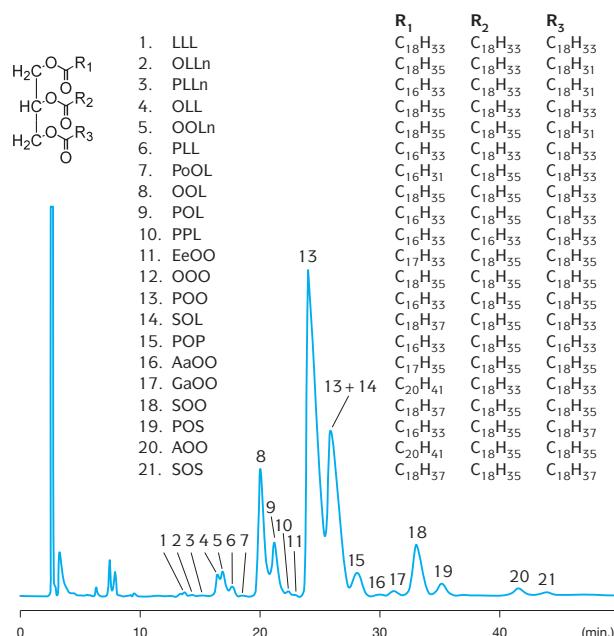
Determination and stability tests of triclosan in disinfectants.  
 (ref. 8)



Phase: Kromasil 100 Å, 7 µm, C18  
 Column: 4.6 × 200 mm  
 Eluent: MeOH:ACN:water (40:40:20; v:v:v)  
 containing 0.02 M KH<sub>2</sub>PO<sub>4</sub> (pH 2.7)  
 Flow rate: 1 ml/min.  
 Detection: UV 280 nm

## Triglycerides

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



Phase: Kromasil 100 Å, 5 µm, C18  
 Column: 4 × 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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paraquat	25	sildenafil (viagra)	20	tyramine	34
paraxanthine	28	simazine	24	Tyr-D-Ala	31
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PAT-5A	18	sodium salicylate	30	Tyr-D-Ala-Gly-Phe	31
pelargonidin-3-O- $\beta$ -glycoside	9	sotolon	34	tyrosine	7
pentabenzoyl mannitol	27	spermidine	34	tyrosine, Fmoc-	6
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Phe	31	spiramycin III	21	uric acid	7
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phenylalanine	7	sulfamethoxazole	10	vanillin	34
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piperidine, (-)-trans-4-(4-fluorophenyl)-		tetramethyllead	23	vitamin B <sub>3</sub>	33
3-(3-hydroxy-4-methoxyphenoxy)methyl)-	12	theobromine	28	vitamin B <sub>6</sub>	33
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propanamine (MDMA), N-methyl-		thiazolidinedione	18		
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propazine	24	threonine, PTC-	6		
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serine, OPA-	8	Tyr	31		
serine, PTC-	6				

# Kromasil

## Kromasil 60 Å bulk packings

Phases	Particle sizes, µm					
	3.5	5	7	10	13	16
SIL	□	■	■	■	■	■
CN	□	■	□	■	□	■

## Kromasil 100 Å bulk packings

Phases	Particle sizes, µm					
	3.5	5	7	10	13	16
SIL	■	■	■	■	■	■
C4	■	■	■	■	■	■
C8	■	■	■	■	■	■
C18	■	■	■	■	■	■
NH <sub>2</sub>	■	■	■	■	■	■
Chiral DMB	□	■	□	■	□	■
Chiral TBB	□	■	□	■	□	■

## Kromasil 300 Å bulk packings

Phases	Particle sizes, µm					
	3.5	5	7	10	13	16
SIL	□	■	□	■	□	■
C4	□	■	□	■	□	■
C8	□	■	□	■	□	■
C18	□	■	□	■	□	■

■ = available as standard product      □ = please inquire!

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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.

Kromasil is available in bulk, or in high-pressure slurry-packed columns. The development, production and marketing of Kromasil are ISO 9001 certified.

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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