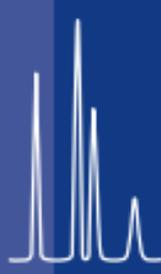


Quick  
Guide

# Applications



**KNAUER**

Established in Germany in 1962, KNAUER was one of the first companies to manufacture HPLC systems and chromatography columns.

With an emphasis on reliability and ease-of-use, we constantly strive to improve our product's performance: making your analyses faster, easier, and more economical.

With this Quick Guide to LC Applications, we aim to provide an overview of some of our most interesting fast LC and UHPLC separations, demonstrating the possibilities of modern LC columns and instrumentation.

**Can we help you with your LC application?**

**Our application specialists are at your service!**

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# Environmental



# Environmental

## Determination of 16 EPA PAH



### Separation column

BlueOrchid PAH, 50 x 2 mm

### Separation conditions

Eluent: A: MeOH/H<sub>2</sub>O 75:25  
B: ACN

Flow rate: 1.0 ml/min

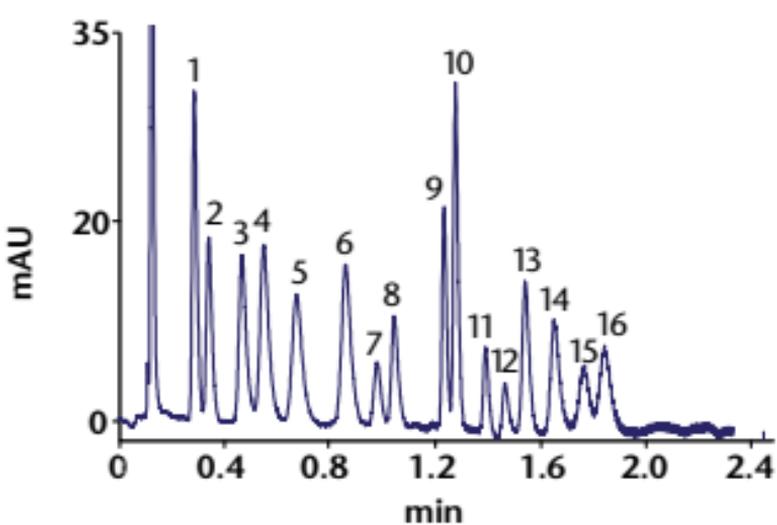
Mode: RP-Mode, gradient

Injection volume: 1 µl EPA standard

Pressure: < 500 bar (< 7250 psi)

Detection: 254 nm (100 Hz, 0.01 s)  
10 mm, 2 µl flow cell

Temperature: 25 °C



1	Naphthalene
2	Acenaphthalene
3	Acenaphthene
4	Fluorene
5	Phenanthrene
6	Anthracene
7	Fluoranthene
8	Pyrene
9	Benzo(a)anthracene
10	Chrysene

11	Benzo(b)fluoranthene
12	Benzo(k)fluoranthene
13	Benzo(a)pyrene
14	Dibenzo(a,h)anthracene
15	Benzo(g,h,i)perylene
16	Indeno(1,2,3-cd)pyrene



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# Environmental

## Separation of benzene derivatives



### Separation column

BlueOrchid C18 1.8 $\mu$ m, 100 x 2 mm

### Separation conditions

Eluent: ACN/H<sub>2</sub>O 85:15 (v/v)

Flow rate: 1.0 ml/min

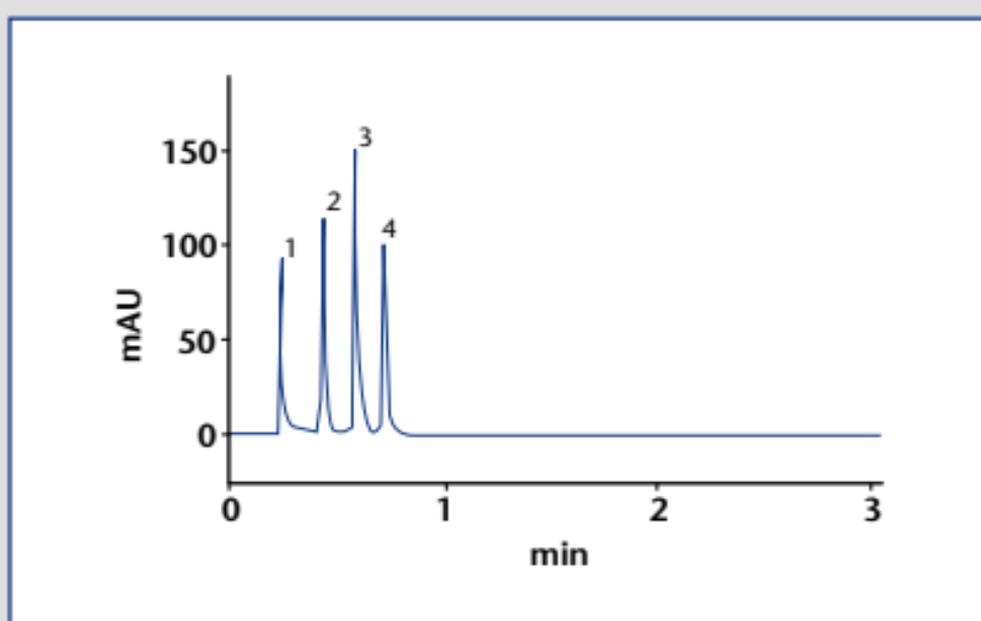
Mode: RP-Mode, isocratic

Injection volume: 1  $\mu$ l

Pressure: 750 bar (10150 psi)

Detection: MW-1, 254 nm (50Hz, 0.01 s)

Temperature: 35 °C



1 Thiourea

2 Toluene

3 Propylbenzene

4 Butylbenzene



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# Environmental

## Determination of DNPH carbonyls



### Separation column

BlueOrchid C18 A 1.8 $\mu$ m, 100 x 2mm

### Separation conditions

Eluent: A: H<sub>2</sub>O  
B: ACN

Gradient: 0–2 min 40% – 55% B  
2–4 min 55% – 100% B  
4–4.5 min 100% B

Flow rate: 0.8 ml/min

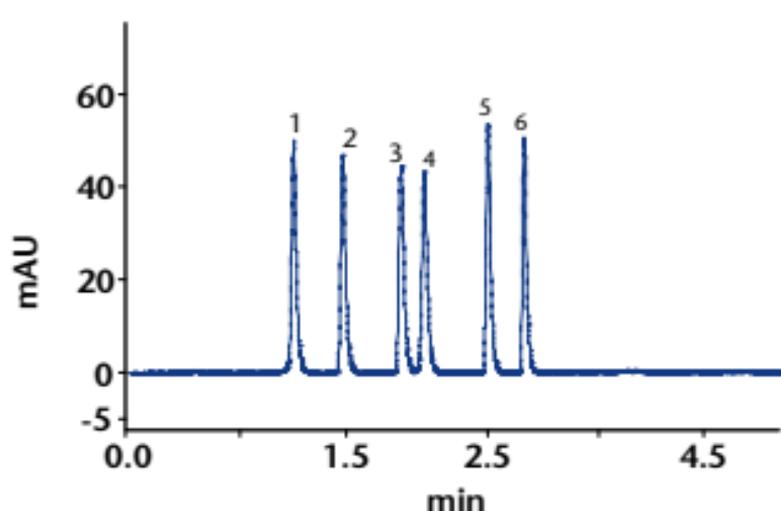
Mode: RP-Mode, gradient

Injection volume: 2 $\mu$ l standard (2 ng/ $\mu$ l)

Pressure: 680 bar (9860 psi)

Detection: 370 nm (50Hz, 0.1 s)  
50 mm flow cell

Temperature: 40 °C



- 1 Formaldehyde
- 2 Acetaldehyde
- 3 Acetone
- 4 Acrolein
- 5 Propionaldehyde
- 6 Crotonaldehyde



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# Environmental

## Determination of dispersion dyes



© Adam Borkowski

### Separation column

BlueOrchid PFP, 1.8 $\mu$ m, 100 x 2 mm

### Separation conditions

Eluent: H<sub>2</sub>O/MeOH 25:75 (v/v)

Gradient: isocratic

Flow rate: 0.55 ml/min

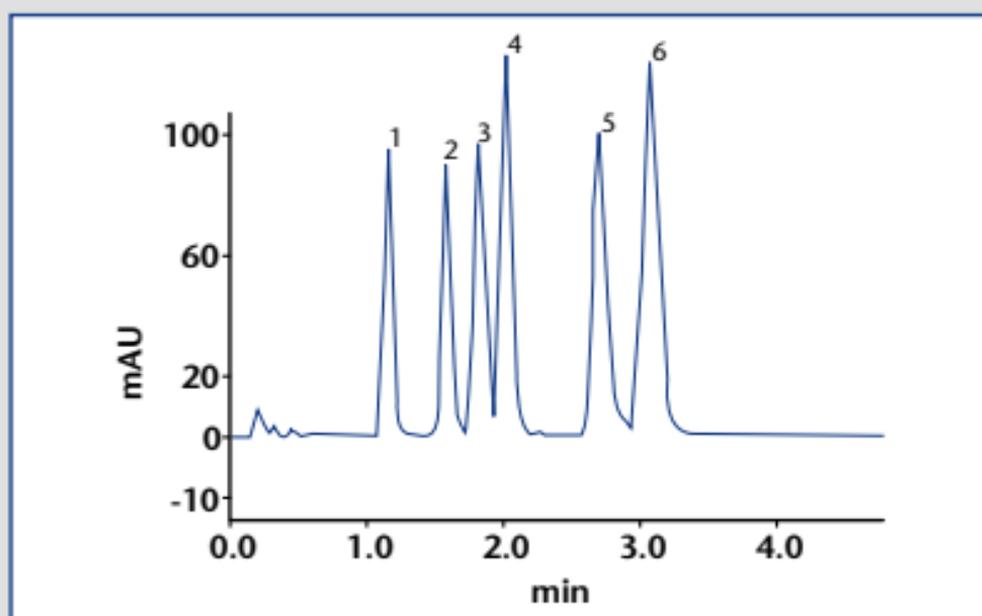
Mode: RP-Mode, isocratic

Injection volume: 1  $\mu$ l

Pressure: 620 bar (8990 psi)

Detection: PDA-1, (50Hz, 0.01 s)  
10mm, 2 $\mu$ l flow cell;  
wavelength program  
0.0 – 1.30 min 440 nm  
1.32 – 1.71 min 355 nm  
1.73 – 1.93 min 500 nm  
1.95 – 2.30 min 615 nm  
2.32 – 2.88 min 435 nm  
2.90 – 3.50 min 605 nm

Temperature: 30 °C



- |   |                    |
|---|--------------------|
| 1 | Disperse Orange    |
| 2 | Disperse Yellow    |
| 3 | Disperse Red 1     |
| 4 | Disperse Blue 106  |
| 5 | Disperse Orange 37 |
| 6 | Disperse Blue 124  |

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# Environmental

## Determination of pesticides I

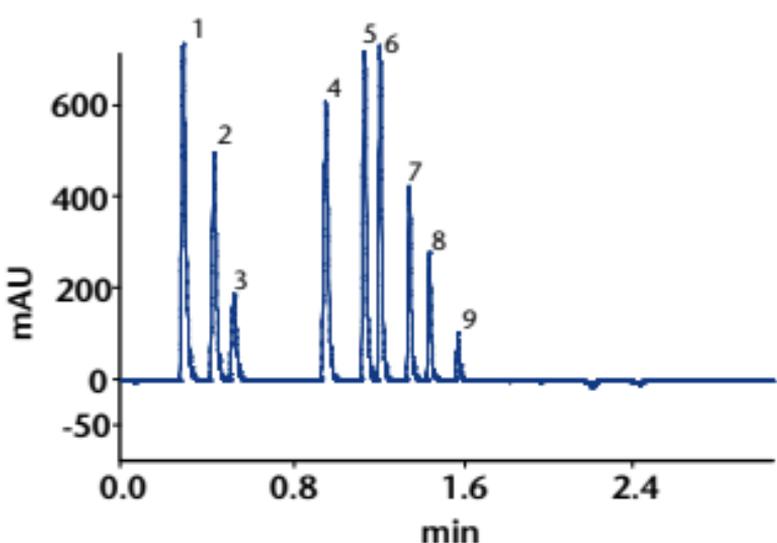


### Separation column

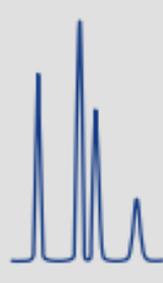
BlueOrchid C18 1.8 $\mu$ m, 50 x 2mm

### Separation conditions

Eluent:	A: H <sub>2</sub> O B: ACN	
Gradient:	0–0.5 min	35% B
	0.5–1.5 min	35%–100% B
	1.5–2.0 min	100% B
Flow rate:	0.6 ml/min	
Mode:	RP-Mode, gradient	
Injection volume:	1 $\mu$ l	
Pressure:	300 bar (4350 psi)	
Detection:	215 nm (50Hz, 0.05 s) 10 mm, 2 $\mu$ l flow cell	
Temperature:	40 °C	



- 1 Chloridazon
- 2 Metoxuron
- 3 Monuron
- 4 Diuron
- 5 Propazine
- 6 Linuron
- 7 Metolachlor
- 8 Parathion-ethyl
- 9  $\beta$ -Endosulfan



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# Environmental

## Determination of pesticides II



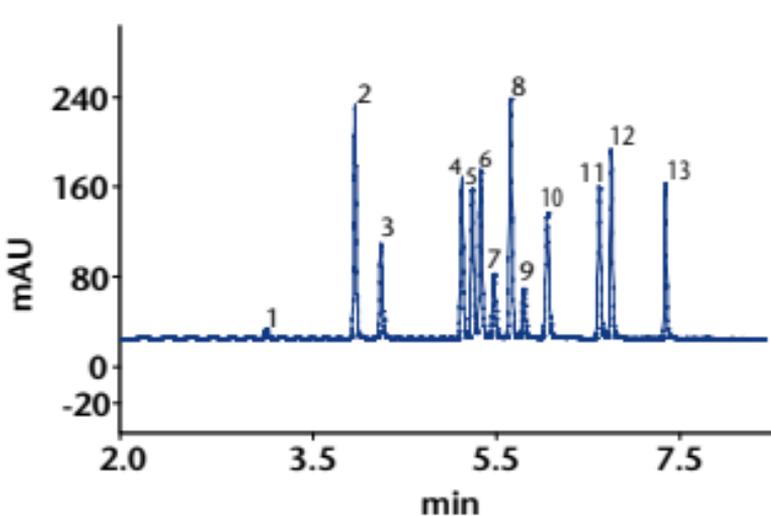
© felinda

### Separation column

BlueOrchid C18 1.8 $\mu$ m, 100 x 2 mm

### Separation conditions

Eluent:	A: H <sub>2</sub> O B: ACN
Gradient:	0.0–0.5 min 5% B 0.5–6.0 min 5%–45% B 6.8–8.0 min 45%–95% B
Flow rate:	0.6 ml/min
Mode:	RP-Mode, gradient
Injection volume:	2 $\mu$ l
Pressure:	520 bar (7540 psi)
Detection:	215 nm (50 Hz, 0.05 s) 10 mm, 2 $\mu$ l flow cell
Temperature:	40 °C



- |    |                    |
|----|--------------------|
| 1  | Desethylatrazine   |
| 2  | Metoxuron          |
| 3  | Simazine           |
| 4  | Methabenzthiazuron |
| 5  | Chlorotoluron      |
| 6  | Atrazine           |
| 7  | Monolinuron        |
| 8  | Isoproturon        |
| 9  | Metobromuron       |
| 10 | Metazachlor        |
| 11 | Terbutylazine      |
| 12 | Linuron            |
| 13 | Metolachlor        |



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# Environmental

## Determination of phenols

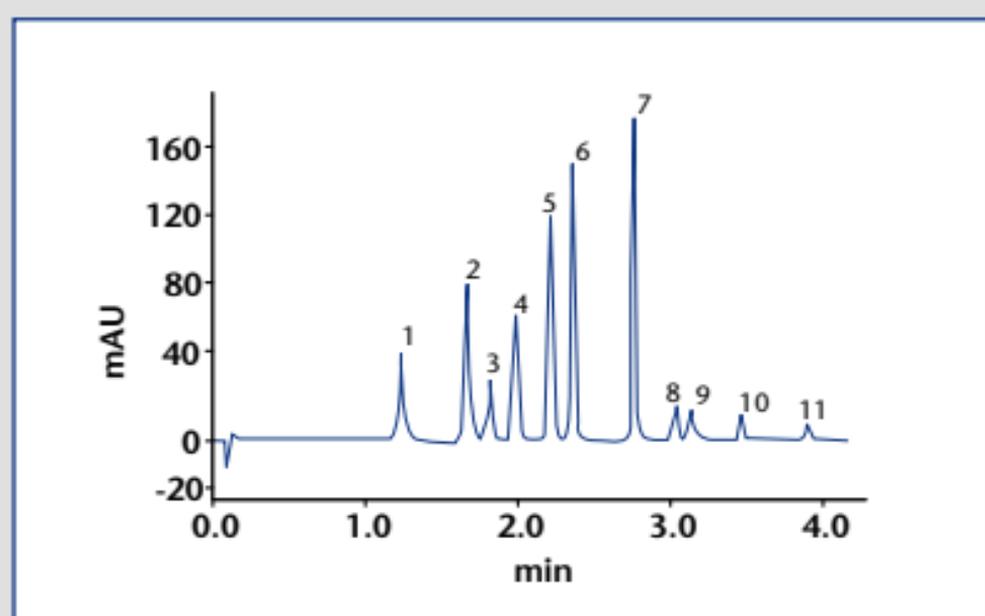


### Separation column

BlueOrchid C18 1.8 $\mu$ m, 50 x 2 mm

### Separation conditions

Eluent:	A: H <sub>2</sub> O (0.1 % HAc) B: MeOH (0.1 % HAc)
Gradient:	0.0–1.0 min 5% B 1.0–4.5 min 5%–95% B 4.5–5.0 min 95% B
Flow rate:	1.0 ml/min
Mode:	RP-Mode, gradient
Injection volume:	5 $\mu$ l
Pressure:	380 bar (5510 psi)
Detection:	275 nm/320 nm (50 Hz, 0.05 s)
Temperature:	50 °C



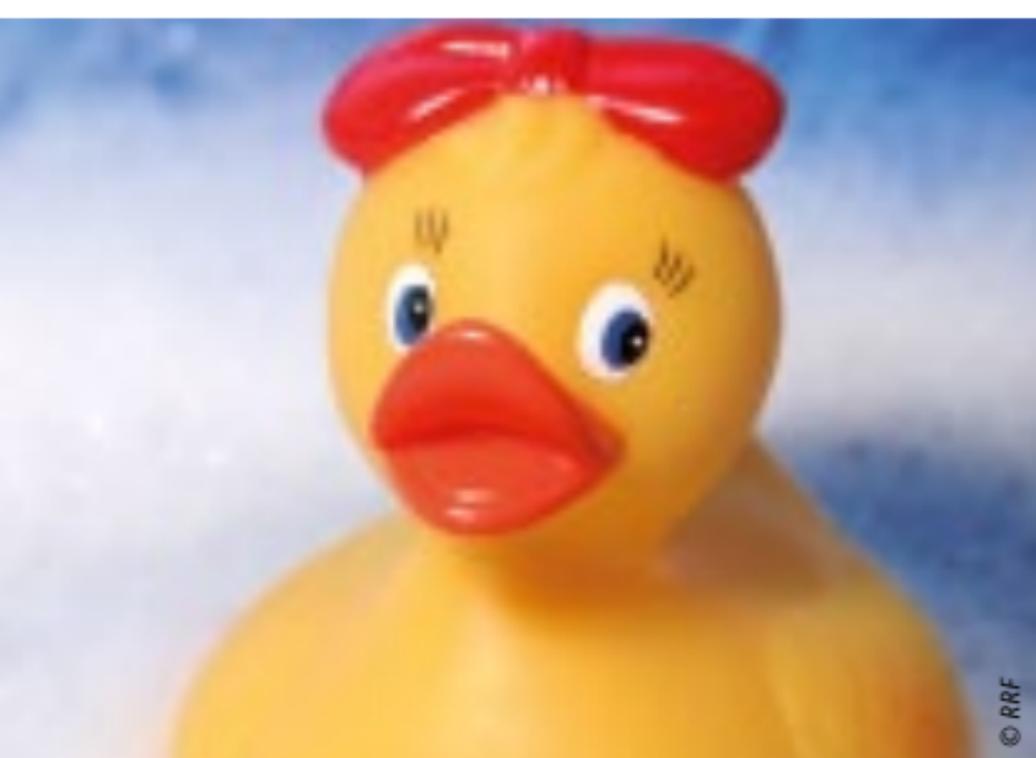
1	Phenol
2	4-Nitrophenol
3	2,4-Dinitrophenol
4	2-Nitrophenol
5	2-Chlorophenol
6	2,3-Dimethylphenol
7	2-Methyl-4,6-dinitrophenol
8	4-Chloro-3-Methylphenol
9	2,4-Dichlorophenol
10	2,4,6-Trichlorophenol
11	Pentachlorophenol

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# Environmental

## Determination of phthalates



### Separation column

BlueOrchid C18 1.8 $\mu$ m, 100 x 2 mm

### Separation conditions

Eluent: A: H<sub>2</sub>O / ACN 15:85  
B: ACN

Gradient: 0.0–1.2 min 0% B  
1.2–3.2 min 0%–100% B  
3.2–5.0 min 100% B

Flow rate: 0.5 ml/min

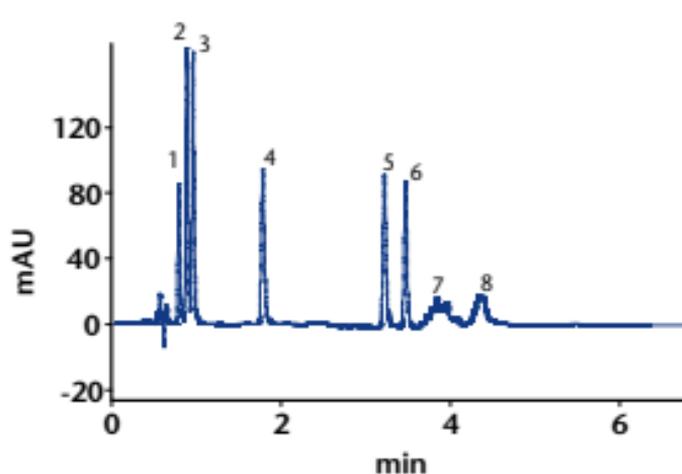
Mode: RP-Mode, gradient

Injection volume: 2 $\mu$ l

Pressure: 350 bar (5080 psi)

Detection: 225 nm (50 Hz, 0.05 s)  
10 mm, 2 $\mu$ l flow cell

Temperature: 30 °C



1 Benzylbenzoate (IS)

2 BBP

3 DBP

4 DHP

5 DEHP

6 DNOP

7 DINP

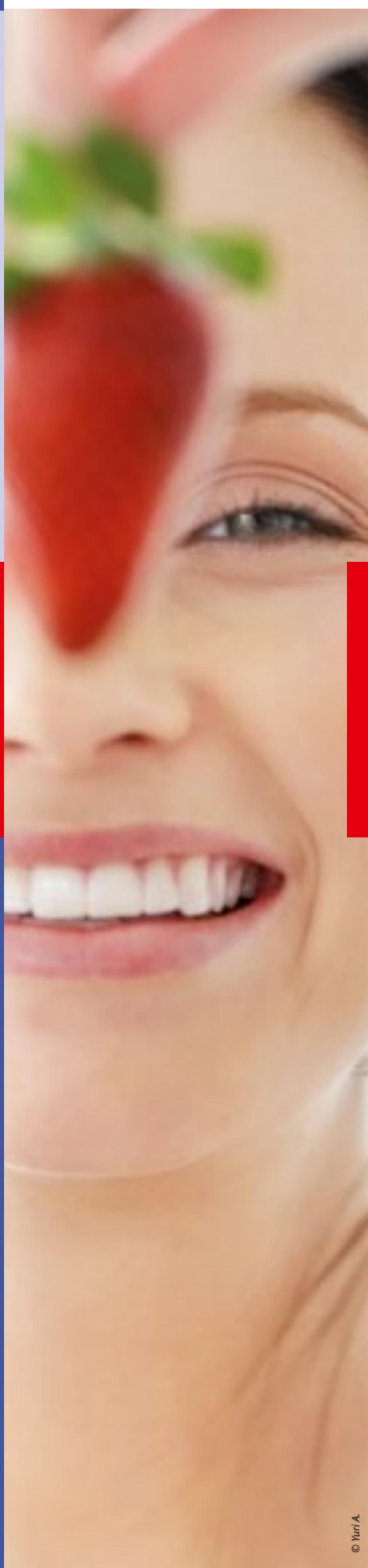
8 DIDP



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# Food



# Food

## Determination of benzoates

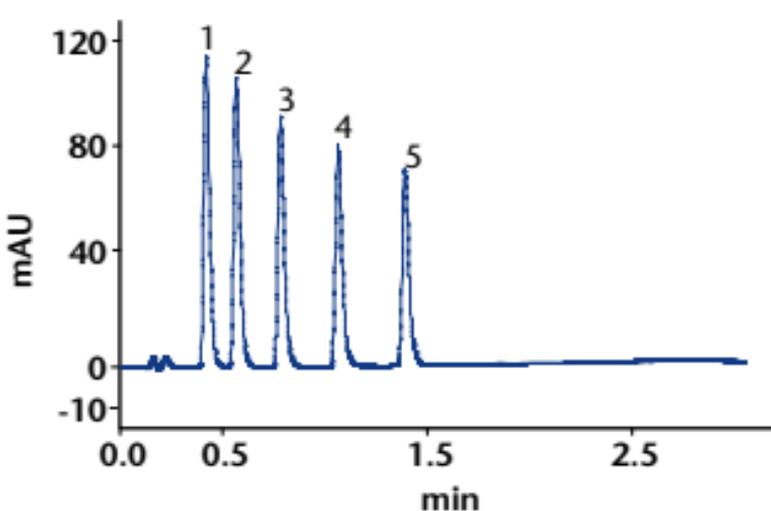


### Separation column

BlueOrchid C18 1.8 $\mu$ m, 50 x 2 mm

### Separation conditions

Eluent:	A: Water B: Methanol
Gradient:	0–2 min 60%–90% B 2–3 min 90% B 3–3.5 min 60% B
Flow rate:	0,6 ml/min
Mode:	RP-Mode, gradient
Injection volume:	1 $\mu$ l
Pressure:	620 bar (8990 psi)
Detection:	PDA-1, 254 nm (100 Hz; 0.005 s) 10 mm, 2 $\mu$ l flow cell
Temperature:	30 °C



- 1 Methyl benzoate
- 2 Ethyl benzoate
- 3 Propyl benzoate
- 4 Butyl benzoate
- 5 Pentyl benzoate



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# Food

## Determination of bisphenols I



© aidanccr

### Separation column

BlueOrchid C18 1.8 $\mu$ m, 50 x 2 mm

### Separation conditions

Eluent: H<sub>2</sub>O/ACN 55:45, (v/v)

Flow rate: 0.6 ml/min

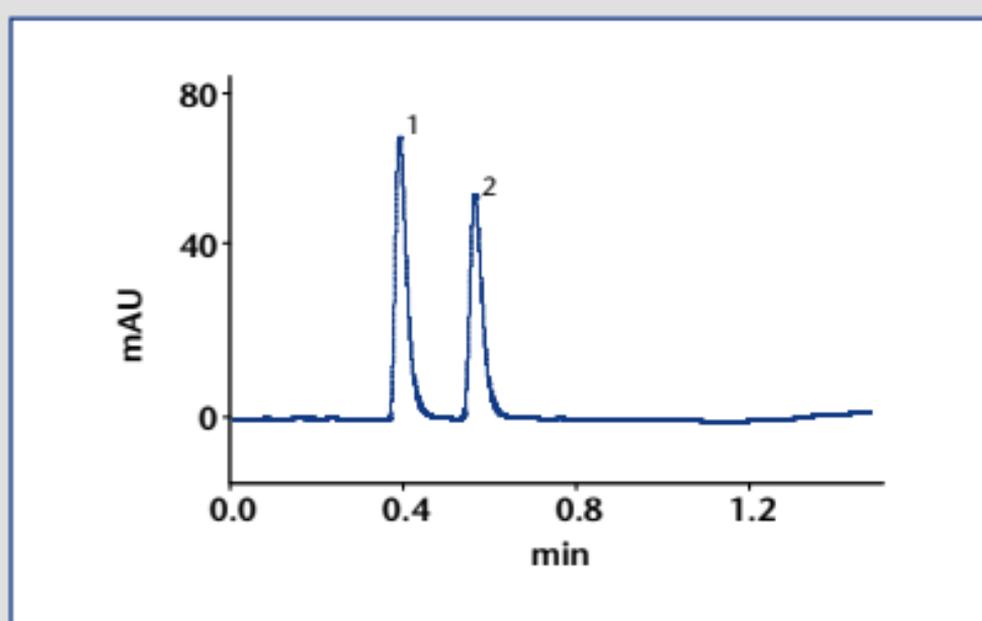
Mode: RP-Mode, isocratic

Injection volume: 2 $\mu$ l

Pressure: 550 bar (7980 psi)

Detection: PDA-1, 227 nm (50 Hz; 0.01 s)  
10 mm, 2 $\mu$ l flow cell

Temperature: 30 °C



1 Bisphenol F

2 Bisphenol A

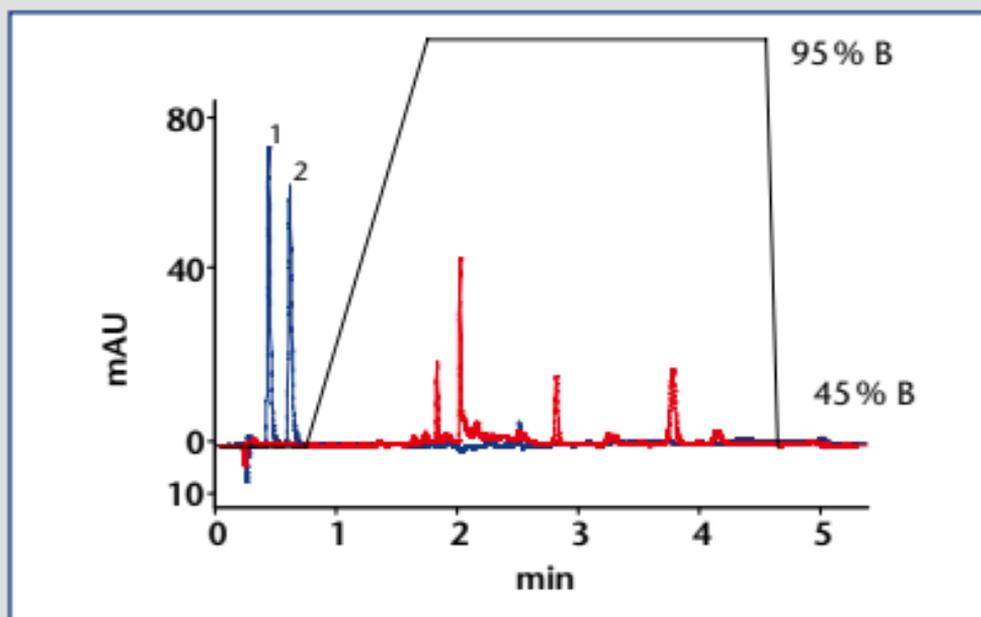
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**Food****Determination of bisphenols II**

© Foodlovers

**Separation column**BlueOrchid C18 1.8 $\mu$ m, 50 x 2mm**Separation conditions**

Eluent:	A: H <sub>2</sub> O B: ACN
Gradient:	0.0–0.7 min 45 % B 0.7–1.7 min 45–95 % B 1.7–4.5 min 95 % B 4.5–4.6 min 95–45 % B
Flow rate:	0.6 ml/min
Mode:	RP-Mode, gradient
Injection volume:	2 $\mu$ l
Pressure:	550 bar (7690 psi)
Detection:	PDA-1, 227 nm (50 Hz, 0.01 s) 10 mm, 2 $\mu$ l flow cell
Temperature:	30°C



- 1 Bisphenol F
- 2 Bisphenol A

standard = blue  
wrapping film extract = red

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# Food

## Determination of coumarin

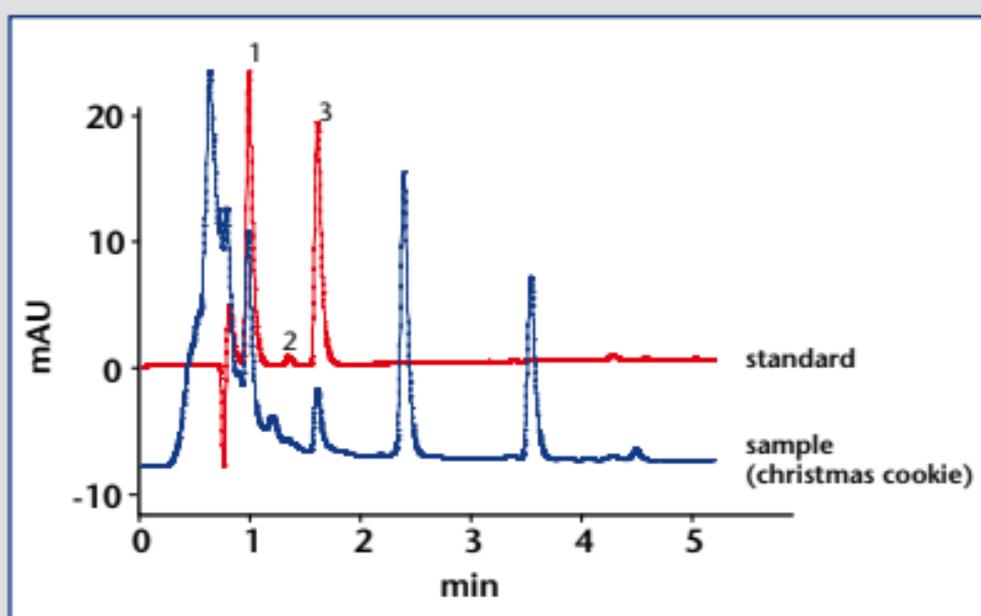


### Separation column

BlueOrchid C18 1.8 $\mu$ m, 100 x 2 mm

### Separation conditions

Eluent:	A: H <sub>2</sub> O B: MeOH
Gradient:	0.0 – 4.6 min 50–70% B 4.6 – 4.7 min 70–95% B 4.7 – 5.95 min 95% B 5.95–6.0 min 95–50% B
Flow rate:	0,3 ml/min
Mode:	RP-Mode, gradient
Injection volume:	2 $\mu$ l
Pressure:	520 bar (7540 psi)
Detection:	UV, 278 nm (50 Hz, 0,02 s) 10 mm, 2 $\mu$ l flow cell
Temperature:	30 °C



- 1 Impurity I
- 2 Impurity II
- 3 Coumarin



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# Food

## Determination of organic acids



© Carmen Steiner

### Separation column

BlueOrchid C18A 1.8 $\mu$ m, 100 x 2 mm

### Separation conditions

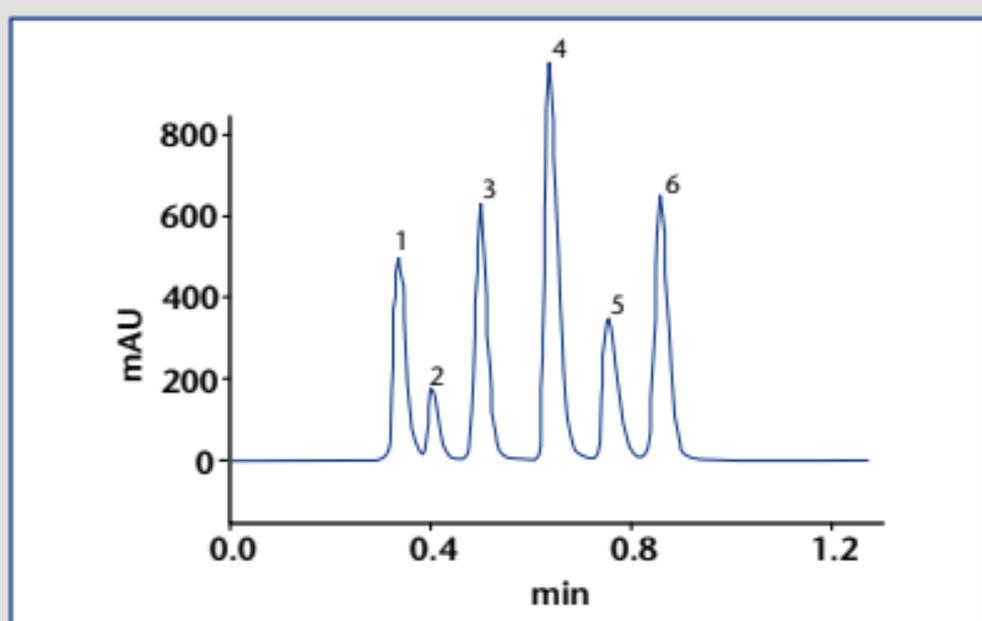
Eluent: 1.7 mMol NaH<sub>2</sub>PO<sub>4</sub> (pH 2.5)

Flow rate: 0.7 ml/min

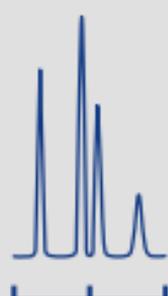
Mode: RP-Mode, isocratic

Injection volume: 1  $\mu$ lDetection: 210 nm (100 Hz; 0.001 s)  
10 mm, 2  $\mu$ l flow cell

Temperature: 30 °C



- 1 Oxalic acid
- 2 Malonic acid
- 3 Ascorbic acid
- 4 Malic acid
- 5 Citric acid
- 6 Fumaric acid

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# Food

## Determination of preservatives



© mars

### Separation column

BlueOrchid C18 1.8 $\mu$ m, 50 x 2mm

### Separation conditions

Eluent: A: Ammonium formiate buffer/  
MeOH 70:30

B: Ammonium formiate buffer/  
MeOH 40:60

Gradient: 0–0.1 min 100% A  
0.1–0.5 min 100%–60% A  
0.5–1.2 min 60% A  
1.2–2 min 0% A

Flow rate: 0.85 ml/min

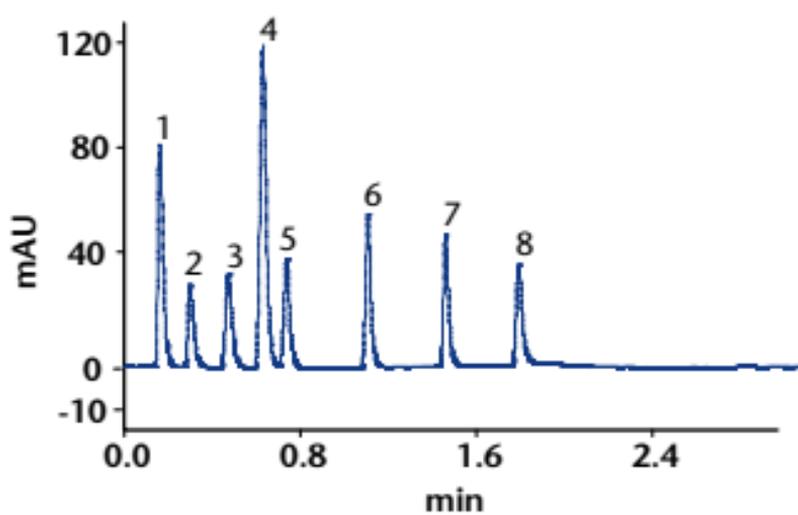
Mode: RP-Mode

Injection volume: 0.5  $\mu$ l

Pressure: –

Detection: PDA-1, 240nm (50Hz, 0.02s)  
10mm, 2 $\mu$ l flow cell

Temperature: 40 °C



- 1 4-Hydroxy benzoic acid
- 2 2-Methoxy benzoic acid
- 3 Benzoic acid
- 4 Sorbic acid
- 5 p-Hydroxy methyl benzoate
- 6 p-Hydroxy ethyl benzoate
- 7 p-Hydroxy propyl benzoate
- 8 p-Hydroxy butyl benzoate

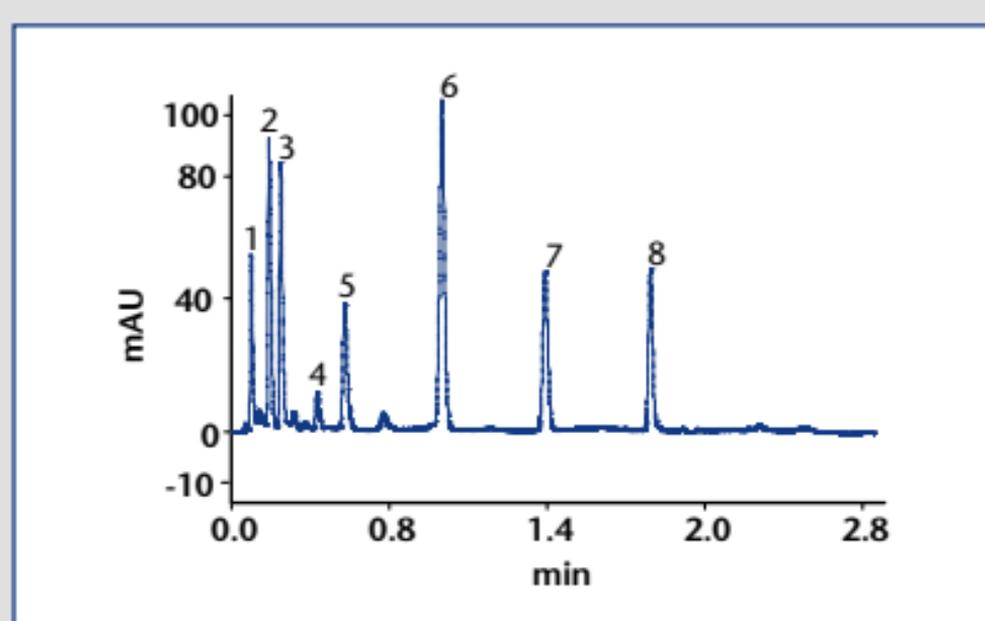


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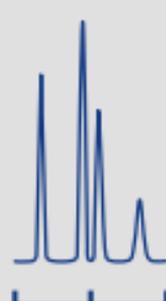
[applications@knauer.net](mailto:applications@knauer.net)

**Food****Separation of Sudan dyes****Separation column**BlueOrchid C18 1.8 $\mu$ m, 50 x 2mm**Separation conditions**

Eluent:	A: H <sub>2</sub> O (+ 0.1 % formic acid) B: ACN (+ 0.1 % formic acid)
Gradient:	0–0.5 min 70% B 0.5–1.5 min 100% B 1.5–2.0 min 100% B
Mode:	RP-Mode, gradient
Flow rate:	1.0 ml/min
Injection volume:	1 $\mu$ l
Pressure:	400 bar (5800 psi)
Detection:	PDA-1, 240 nm (50 Hz, 0.01 s) 10 mm, 2.4 $\mu$ l flow cell
Temperature:	25 °C



- |   |                        |
|---|------------------------|
| 1 | Impurity 1             |
| 2 | 4-Phenylazophenol      |
| 3 | $\alpha$ -Naphthyl red |
| 4 | Impurity 2             |
| 5 | Impurity 3             |
| 6 | Sudan II               |
| 7 | Sudan III              |
| 8 | Sudan IV               |

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# Food

## Quick determination of tocopherols



### Separation column

BlueOrchid Si 1.8  $\mu\text{m}$ , 50 x 2 mm

### Separation conditions

Eluent: Heptane/2-butanol 1000:4 (v/v)

Flow rate: 1.7 ml/min

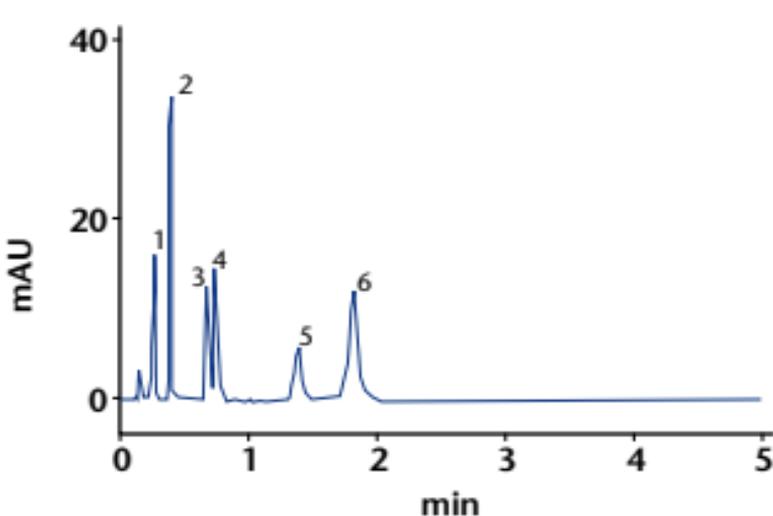
Mode: NP-Mode, isocratic

Injection volume: 1  $\mu\text{l}$

Pressure: 350 bar (5080 psi)

Detection: MW-1, 280 nm (50Hz, 0.01 s)  
10 mm, 2  $\mu\text{l}$  flow cell

Temperature: 25  $^{\circ}\text{C}$



- 1 Trans-retinol
- 2 Alpha-tocopherol
- 3 Beta-tocopherol
- 4 Gamma-tocopherol
- 5 Delta-tocopherol
- 6 Vitamin D<sub>2</sub>



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# Food

## Determination of water soluble vitamins

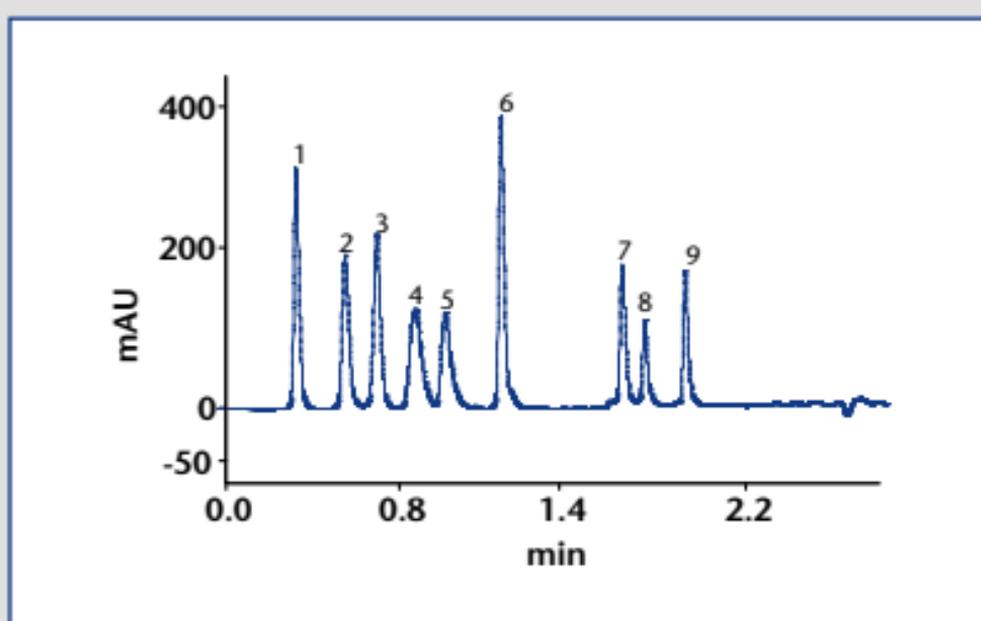


### Separation column

BlueOrchid C18 A 1.8 $\mu$ m, 100 x 2mm

### Separation conditions

Eluent:	A: 50 mM NaH <sub>2</sub> PO <sub>4</sub> pH 4 B: MeOH
Mode:	RP-Mode, gradient
Flow rate:	0.7 ml/min
Injection volume:	1 $\mu$ l
Pressure:	800 bar (11600 psi)
Detection:	PDA-1, 254 nm (50 Hz, 0.01 s) 10 mm, 2.4 $\mu$ l flow cell
Temperature:	30 °C



- |   |                 |
|---|-----------------|
| 1 | Ascorbic acid   |
| 2 | Thiamine        |
| 3 | Nicotinic acid  |
| 4 | Pyridoxal       |
| 5 | Pyridoxin       |
| 6 | Nicotinamid     |
| 7 | Folic acid      |
| 8 | Cyanocobalamine |
| 9 | Riboflavin      |



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# Food

## Determination of xanthines

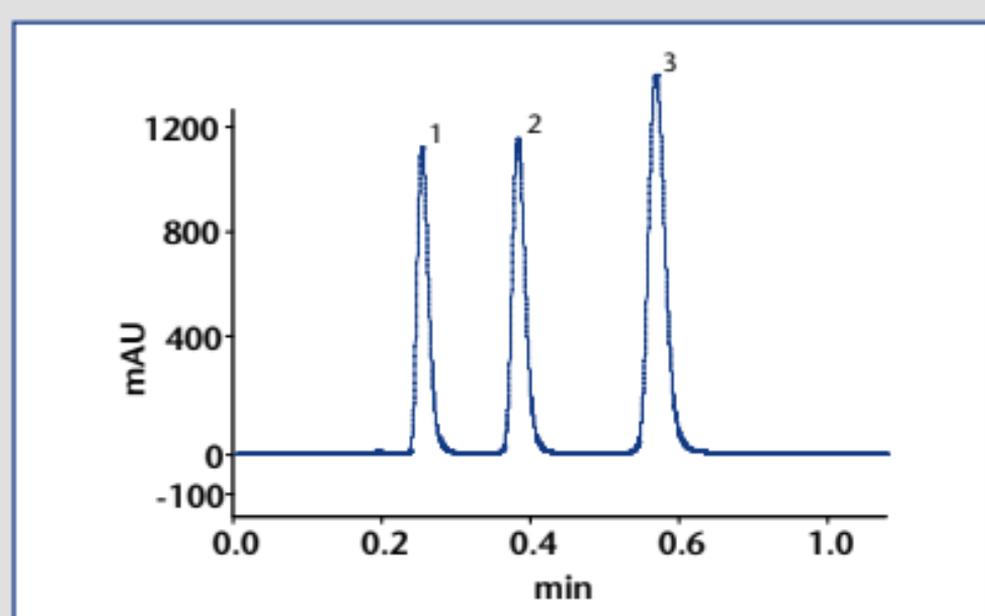


### Separation column

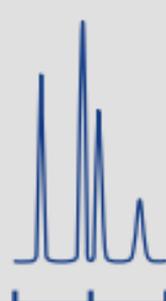
BlueOrchid C18 1.8 $\mu$ m, 50 x 2mm

### Separation conditions

Eluent:	A: H <sub>2</sub> O B: MeOH 80:20 (v/v)
Flow rate:	0.8 ml/min
Mode:	RP-Mode, isocratic
Injection volume:	1 $\mu$ l
Detection:	PDA-1, 275 nm (50 Hz; 0.01 s) 10 mm, 2 $\mu$ l flow cell
Temperature:	30 °C



- 1 Theobromine
- 2 Theophylline
- 3 Caffeine



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# Bio analytical



# Bioanalytical

## Determination of amino acids



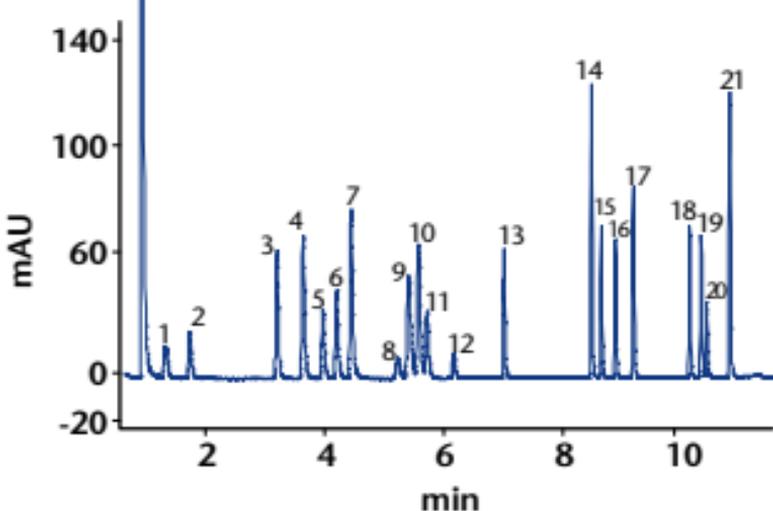
© Ireno2010

### Separation column

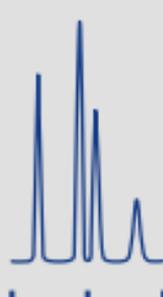
BlueOrchid C18 1.8 $\mu$ m, 100 x 2 mm

### Separation conditions

Eluent:	A: Sodium acetate B: ACN/Sodium acetate 60:40
Gradient:	0 min 92% A (0.6 ml/min) 0.0– 5.0 min 86% A (0.6 ml/min) 5.0– 6.0 min 86% A (0.6 ml/min) 6.0– 11.0 min 65% A (0.85 ml/min) 11.0– 12.0 min 65% A (0.85 ml/min)
Flow rate:	0.6 ml/min, 0.85 ml/min
Mode:	RP-Mode, gradient
Injection volume:	1 $\mu$ l
Pressure:	590 bar (8560 psi)
Detection:	PDA-1, 254 nm (100 Hz, 0.005 s) 10 mm, 2 $\mu$ l flow cell
Temperature:	40 $^{\circ}$ C



1	Aspartic acid	12	NH <sub>4</sub>
2	Glutamic acid	13	Proline
3	Serine	14	Tyrosine
4	Glycine	15	Cysteine
5	?	16	Vainel
6	?	17	Methionine
7	Histidine	18	Isoleucine
8	AMQ	19	Leucine
9	Threonine	20	Lysine
10	Alanine	21	Phenylalanine
11	Arginine		

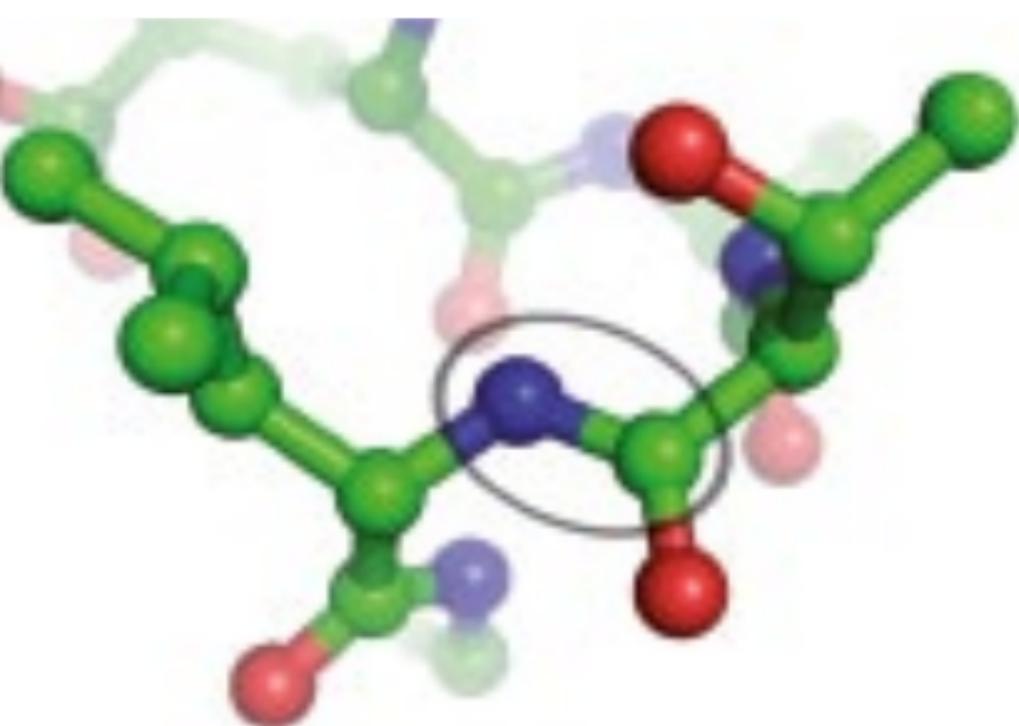


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# Bioanalytical

## Separation of mucopeptides

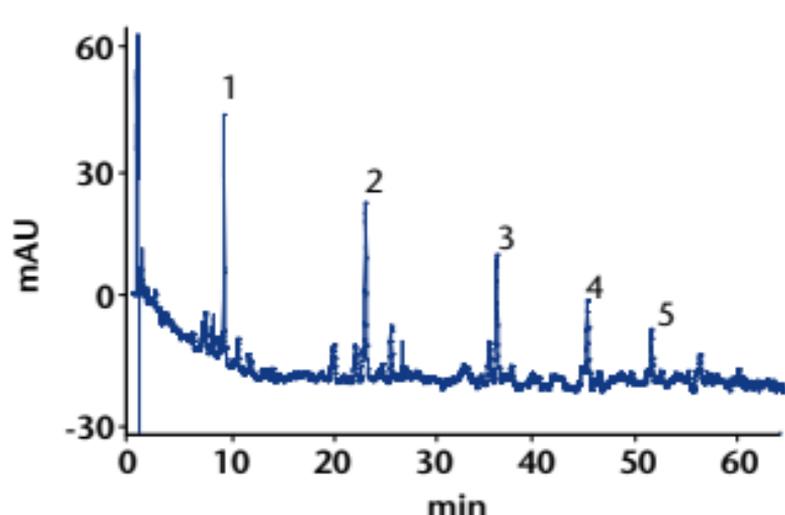


### Separation column

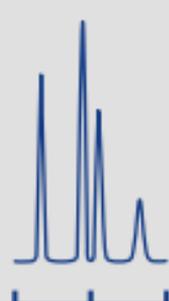
BlueOrchid C18 A 1.8 $\mu$ m, 100 x 2mm

### Separation conditions

Eluent:	A: 100 mM NaH <sub>2</sub> PO <sub>4</sub> pH 2.8 B: 100 mM NaH <sub>2</sub> PO <sub>4</sub> pH 2.8 + 30% MeOH (v/v)
Gradient:	0.0–12.5 min 0– 30% B 12.5–60.0 min 30–100% B
Flow rate:	0.5 ml/min
Mode:	RP-Mode, gradient
Injection volume:	5 $\mu$ l
Pressure:	580 bar (8410 psi)
Detection:	PDA-1, 206 nm (10Hz, 0,1 s) 10mm, 2 $\mu$ l flow cell
Temperature:	40 °C



- 1 Monomers
- 2 Dimers
- 3 Trimers
- 4 Tetramers
- 5 Oligomers

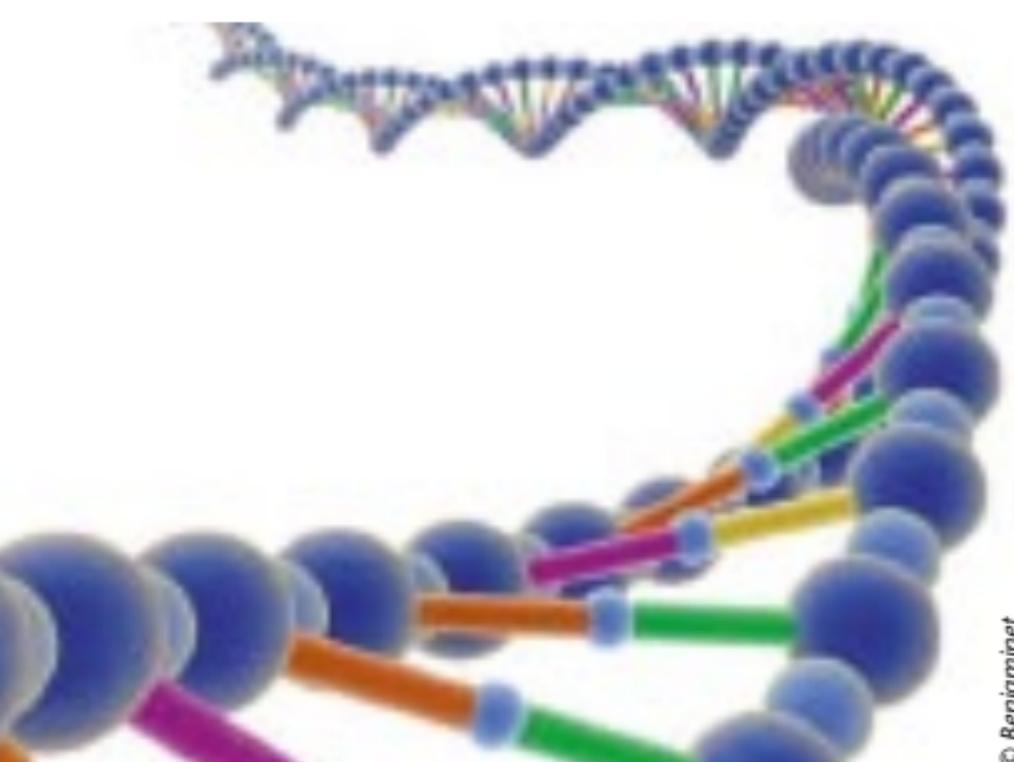


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# Bioanalytical

## Separation of nucleosides and bases



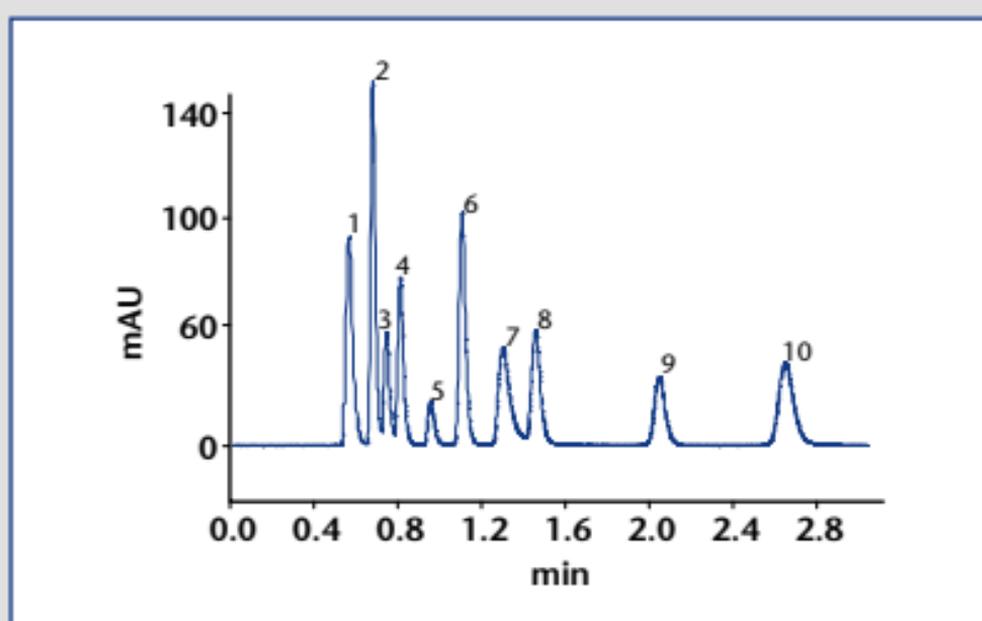
© Benjaminet

### Separation column

BlueOrchid PFP 1.8  $\mu$ m, 100 x 2 mm

### Separation conditions

Eluent:	20mM NH <sub>4</sub> Ac, pH 3.5 10% A/MeOH 90:10 (v/v)
Flow rate:	0.5 ml/min
Mode:	RP-Mode, isocratic
Injection volume:	1 $\mu$ l
Pressure:	570 bar (8270 psi)
Detection:	PDA-1, 210 nm (100 Hz, 0.001 s) 10 mm, 2 $\mu$ l flow cell
Temperature:	30 °C



- |    |           |
|----|-----------|
| 1  | Cytosine  |
| 2  | Uracil    |
| 3  | Cytidine  |
| 4  | Uridine   |
| 5  | Guanine   |
| 6  | Thymine   |
| 7  | Adenine   |
| 8  | Guanosine |
| 9  | Thymidine |
| 10 | Adenosine |

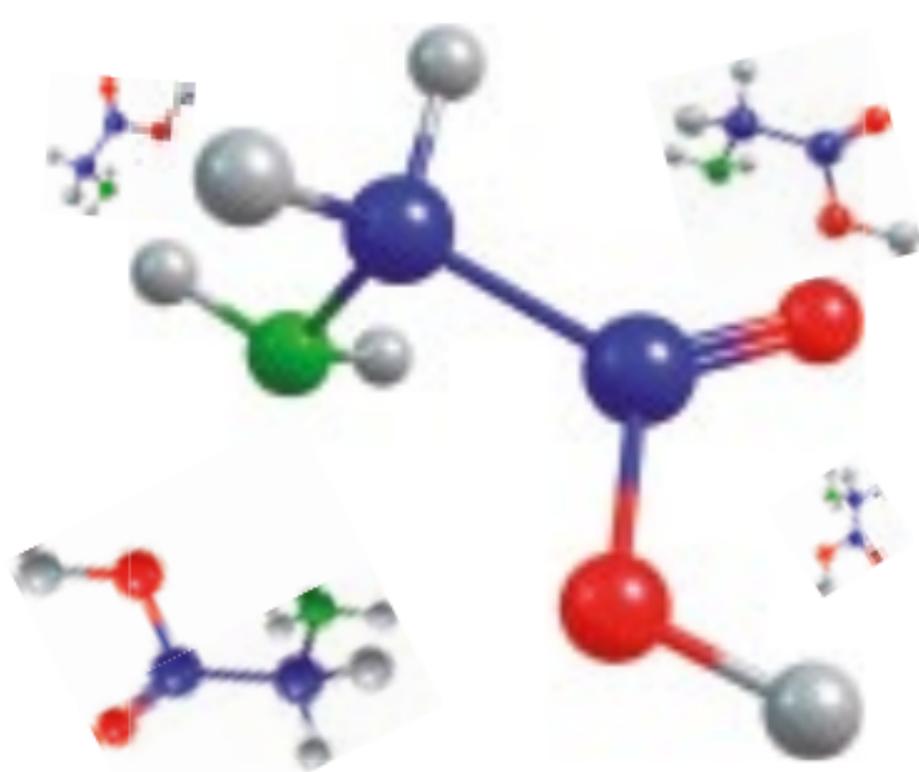


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# Bioanalytical

## Separation of peptides



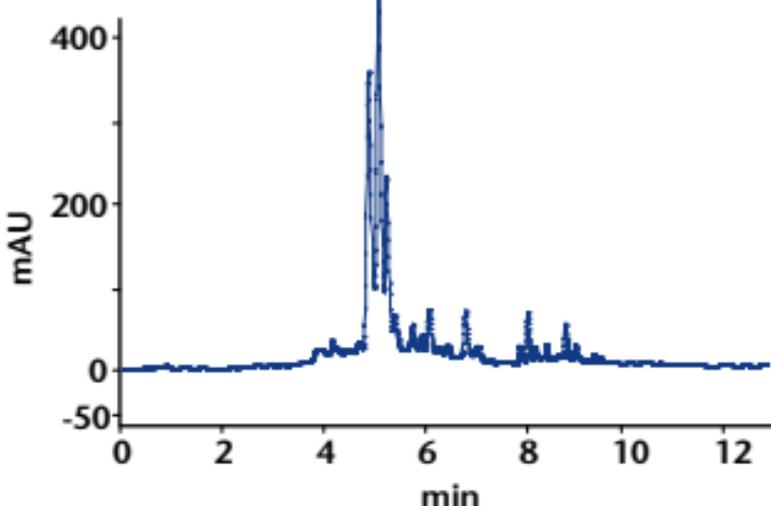
© Stanislav Pepeljaev

### Separation column

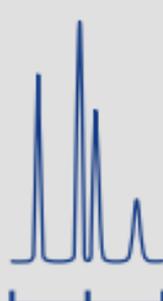
BlueOrchid C18 A 1.8 $\mu$ m, 100 x 2mm

### Separation conditions

Eluent:	A: H <sub>2</sub> O (0.1 % TFA) B: 60 % ACN (0.1 % TFA)
Flow rate:	0.5 ml/min
Gradient:	0.0–10.0 min 0–100 % B 10.0–13.0 min 100 % B
Mode:	RP-Mode, gradient
Injection volume:	5 $\mu$ l
Pressure:	540 bar (7830 psi)
Detection:	PDA-1, 210 nm (50 Hz, 0.01 s) 10 mm, 2 $\mu$ l flow cell
Temperature:	40 °C

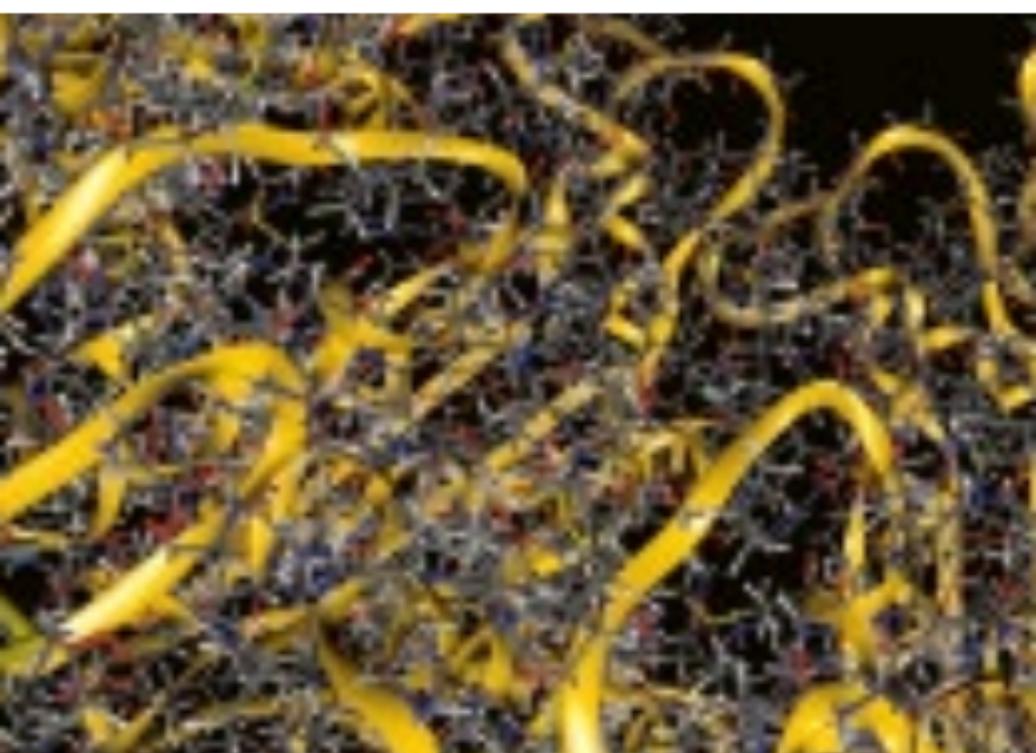


Peptide mixture  
(customer sample)

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# Bioanalytical

## Separation of proteins

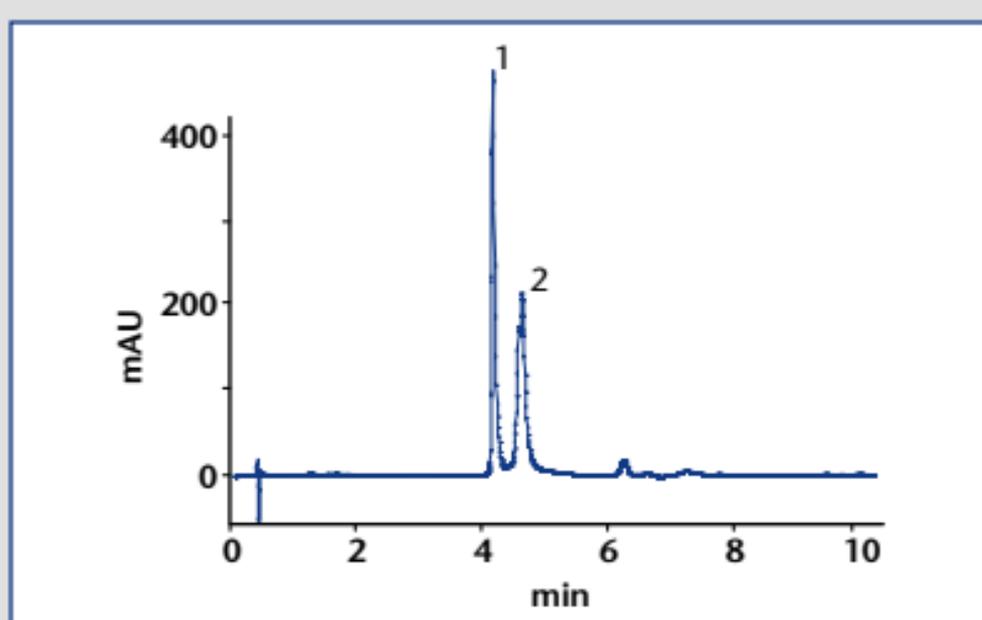


### Separation column

BlueOrchid C18 A 1.8 $\mu$ m, 100 x 2mm

### Separation conditions

Eluent:	A: H <sub>2</sub> O (0.1 % TFA) B: 60% ACN (0.1 % TFA)
Gradient:	0.0– 6min 30–100% B 6.0– 8.0min 100% B 8.0– 8.5min 100– 30% B 8.5–12.5min 30% B
Flow rate:	0.7 ml/min
Mode:	RP-Mode, gradient
Injection volume:	1 $\mu$ l/min
Pressure:	630 bar (9140 psi)
Detection:	PDA-1, 210 nm (50 Hz, 0.01 s) 10 mm, 2 $\mu$ l flow cell
Temperature:	40 °C



1  $\alpha$ -Lactalbumin

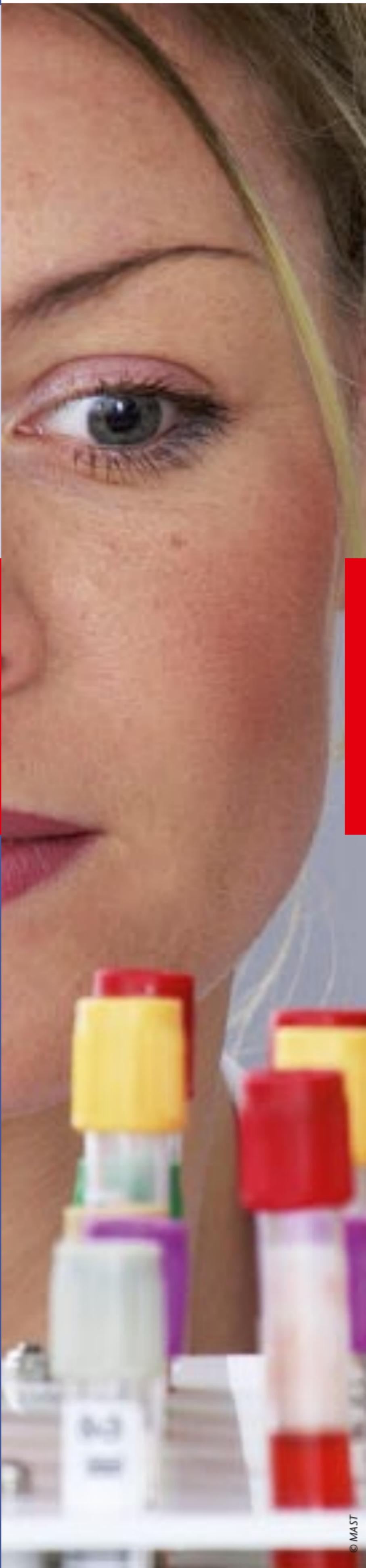
2  $\beta$ -Lactoglobulin



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# Clinical



# Clinical

Determination of sedatives,  
e.g. barbiturates



## Separation column

BlueOrchid C18 1.8 $\mu$ m, 50 x 2mm

## Separation conditions

Eluent: MeOH/H<sub>2</sub>O 60:40 (v/v)

Flow rate: 0.5 ml/min

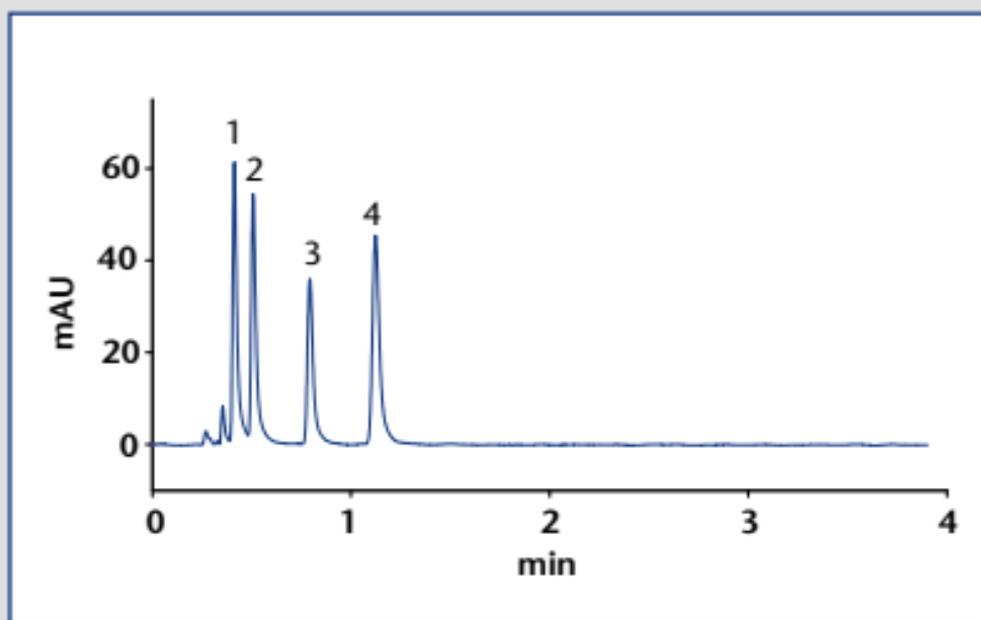
Mode: RP-Mode, isocratic

Injection volume: 1  $\mu$ l

Pressure: 826 bar (11980 psi)

Detection: MW-1, 254 nm (80Hz, 0.005 s)

Temperature: 40 °C

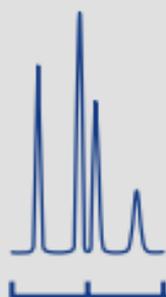


1 Barbital

2 Luminal

3 Prominal

4 Revonal



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# Clinical

## Separation of tt-muconic acid in urine (I)



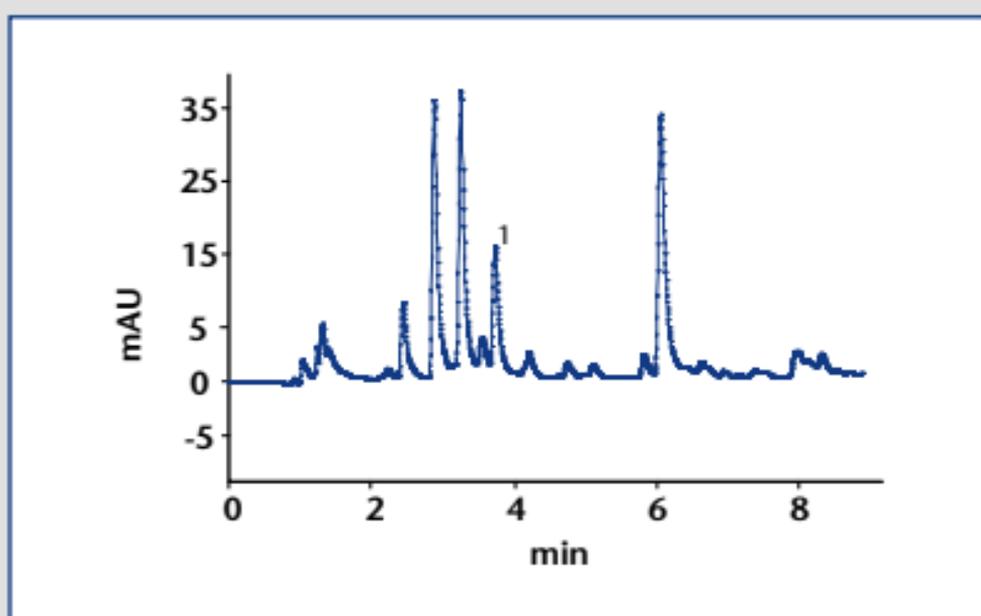
© Yahia LOUKKAL

### Separation column

BlueOrchid C18 A 1.8 $\mu$ m, 100 x 2mm  
Phenyl 1.8 $\mu$ m 100 x 2mm (column tandem)

### Separation conditions

Eluent:	A: H <sub>2</sub> O (1 % HAc) B: MeOH
Gradient:	0–7.5 min 5 % B–25 % B
	7.5–7.6 min 25 % B–90 % B
	7.6–9.5 min 90 % B
	9.5–9.6 min 90 % B– 5 % B
Flow rate:	0.4 ml/min
Mode:	RP-Mode, gradient
Injection volume:	10 $\mu$ l
Pressure:	590 bar (8560 psi)
Detection:	PDA-1, 259 nm (50Hz) 10mm, 2 $\mu$ l flow cell;
Temperature:	40 °C



urine sample

1 tt-Muconic acid



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# Clinical

## Separation of tt-muconic acid in urine (II)



### Separation column

Fused Core C18 1.7 $\mu$ m, 100 x 2mm

### Separation conditions

Eluent: A: H<sub>2</sub>O (1% HAc)  
B: MeOH

Gradient: 0–4.17 min 5% B-25% B  
4.17–4.19 min 25% B-90% B  
4.19–5.21 min 90% B  
5.21–5.23 min 90% B- 5% B

Flow rate: 0.4 ml/min

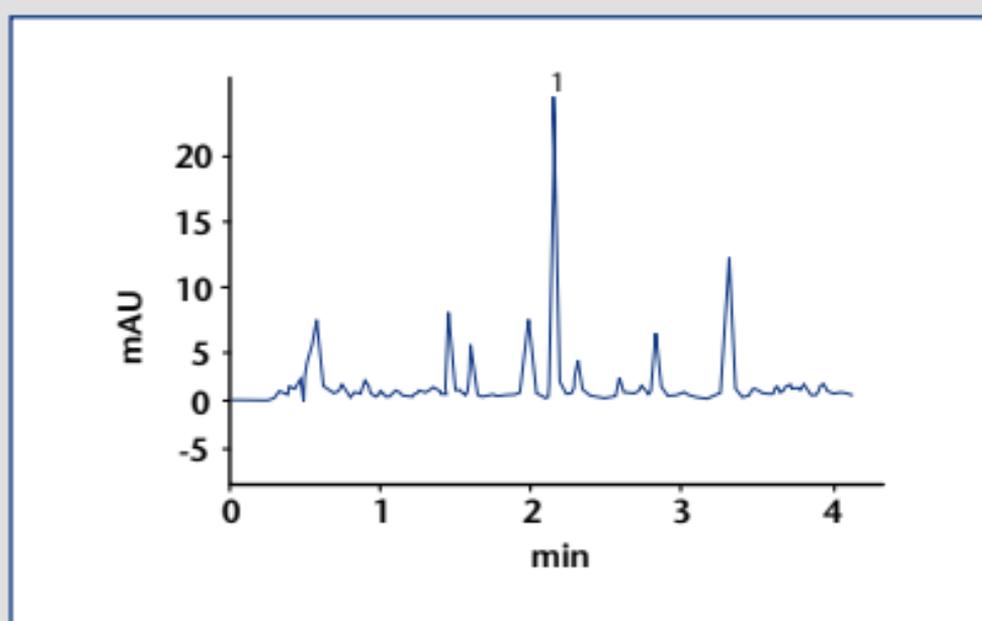
Mode: RP-Mode, gradient

Injection volume: 2 $\mu$ l

Pressure: 570 bar (8270 psi)

Detection: PDA-1, 259nm (50Hz)  
10mm, 2 $\mu$ l flow cell

Temperature: 30 °C



urine sample

1 tt-Muconic acid



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# Pharma- ceutical



# Pharmaceutical

## Separation of beta-blockers

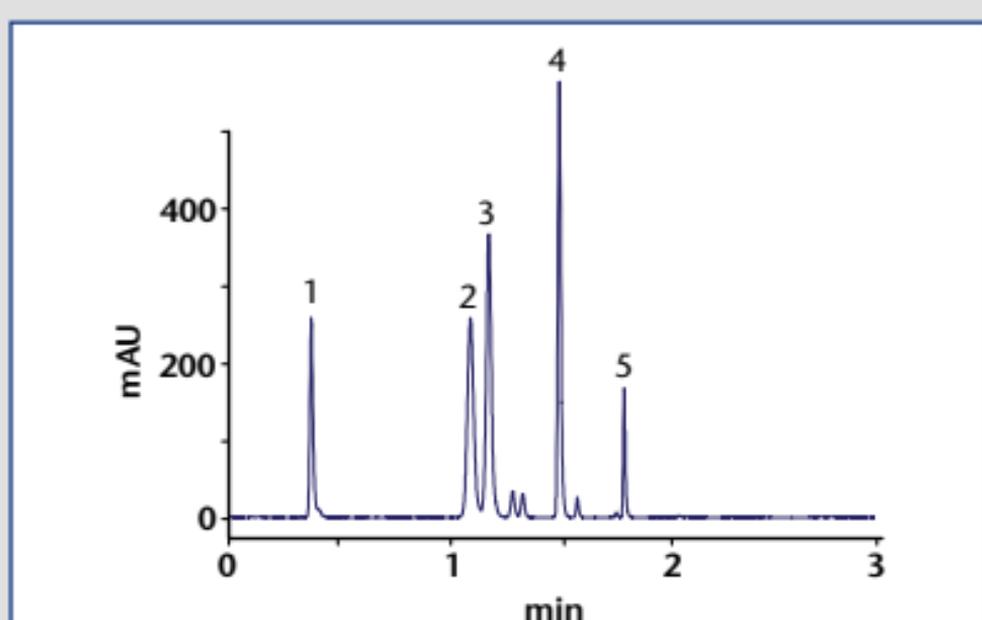


### Separation column

BlueOrchid C18 1.8 $\mu$ m, 50 x 2mm

### Separation conditions

Eluent:	A: 25 mM NaH <sub>2</sub> PO <sub>4</sub> pH 2.7 B: Eluent A / ACN 40:60 (v/v)
Gradient:	0.0–0.6 min 11 % B 0.6–2.0 min 11–75 % B
Flow rate:	1.0 ml/min
Mode:	RP-Mode, gradient
Injection volume:	0.5 $\mu$ l
Pressure:	650 bar (9430 psi)
Detection:	PDA-1, 254 nm (100 Hz, 0.005 s) 10 mm, 2 $\mu$ l flow cell
Temperature:	35 °C



- |   |            |
|---|------------|
| 1 | Atenolol   |
| 2 | Pindolol   |
| 3 | Nadolol    |
| 4 | Metoprolol |
| 5 | Alprenolol |



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# Pharmaceutical

## Separation of clindamycin (QC)



© Robert Kreschke

### Separation column

BlueOrchid C18 A 1.8 $\mu$ m, 100 x 2mm

### Separation conditions

Eluent: Buffer (13.6g/l  $\text{KH}_2\text{PO}_4$ ) /  
ACN 80:20 (v/v)

Flow rate: 0.7 ml/min

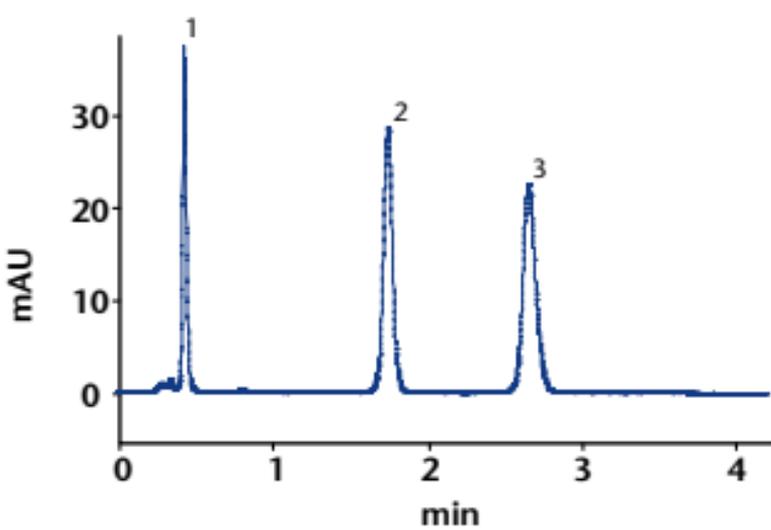
Mode: RP-Mode, isocratic

Injection volume: 5 $\mu$ l

Pressure: 855 bar (12400 psi)

Detection: PDA-1, 210nm (50Hz)  
10mm, 2 $\mu$ l flow cell

Temperature: 30 $^\circ$ C



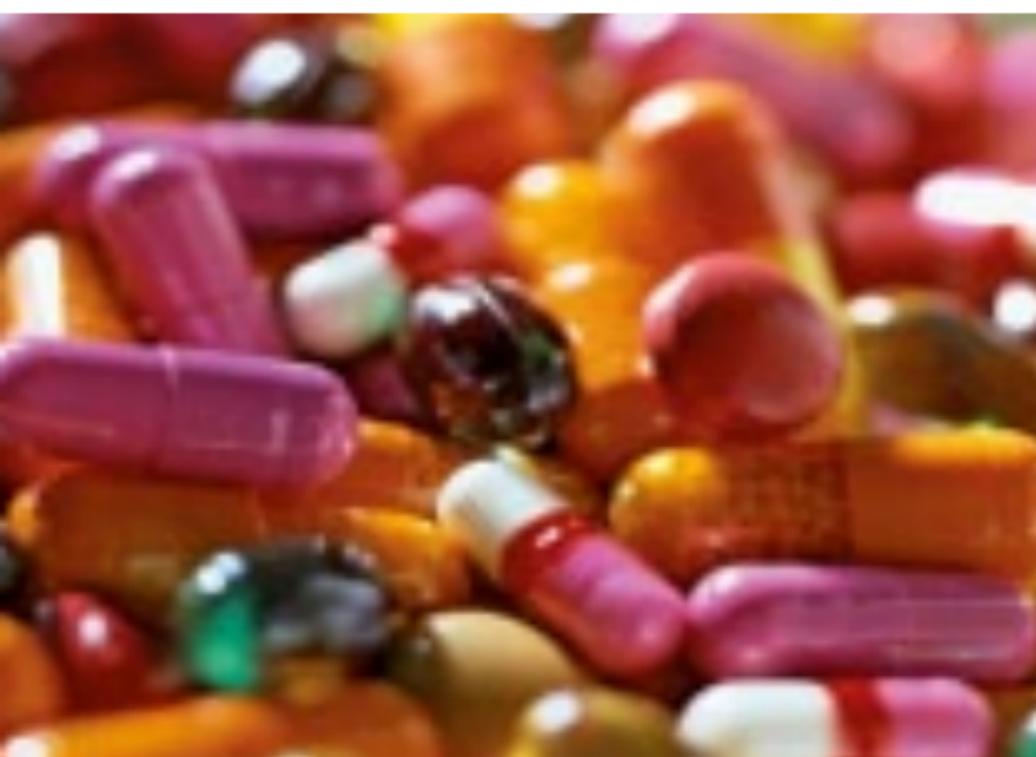
- 1 Impurity A
- 2 Clindamycin phosphate
- 3 Impurity E

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# Pharmaceutical

## Separation of paracetamol and common synthesis impurities

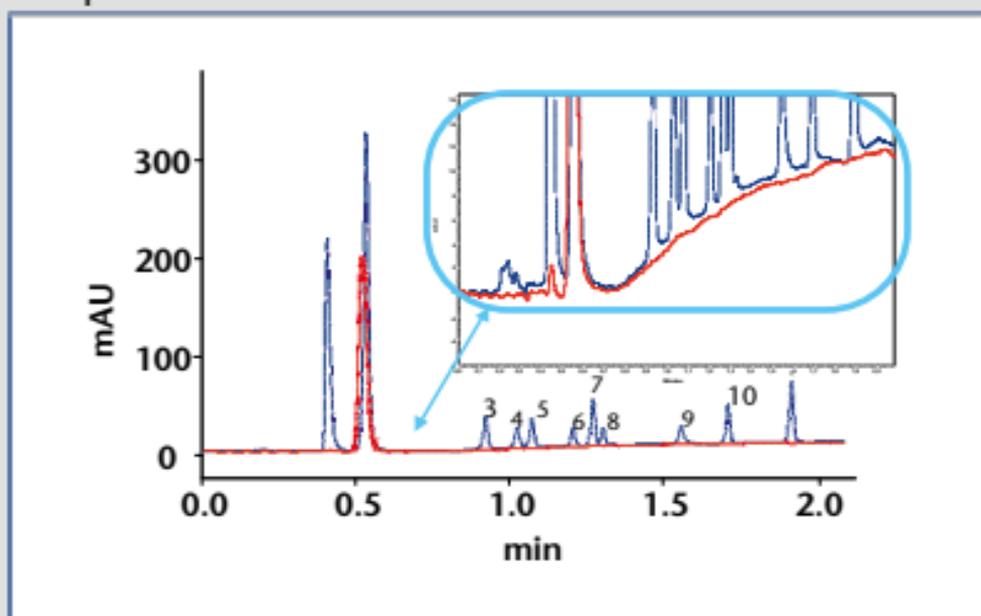


### Separation column

BlueOrchid C18 1.8 $\mu$ m, 100 x 2 mm

### Separation conditions

Eluent:	A: 1.7 mM NaH <sub>2</sub> PO <sub>4</sub> , pH 3.7 B: ACN
Gradient:	0.0–0.3 min 13% B 0.3–2 min 13–70% B 2–2.5 min 70% B
Flow rate:	0.85 ml/min
Mode:	RP-Mode, gradient
Injection volume:	1 $\mu$ l
Pressure:	950 bar (13800 psi)
Detection:	MW-1, 254 nm (100 Hz, 0.005 s) 10 mm, 2 $\mu$ l flow cell
Temperature:	50 °C



- 1 4-Aminophenol
- 2 Paracetamol
- 3 N-(4-hydroxyphenyl)propanamide
- 4 N-(2-hydroxyphenyl)acetamide
- 5 N-(3-chloro-4-hydroxyphenyl)acetamide
- 6 1-(4-Hydroxyphenyl)ethanoneoxime
- 7 N-phenylacetamide
- 8 4-(Acetylamino)phenyl acetate
- 9 4-Nitrophenol
- 10 Chloroacetanilide
- 11 1-(2-Hydroxyphenyl)ethanone



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# Pharmaceutical

## Separation of steroids



### Separation column

BlueOrchid C18 1.8 $\mu$ m, 50 x 2mm

### Separation conditions

Eluent: A: H<sub>2</sub>O

B: ACN

Gradient: 0–1,5 min 35%–75% B

1,5–2 min 75% B

Flow rate: 1.0 ml/min

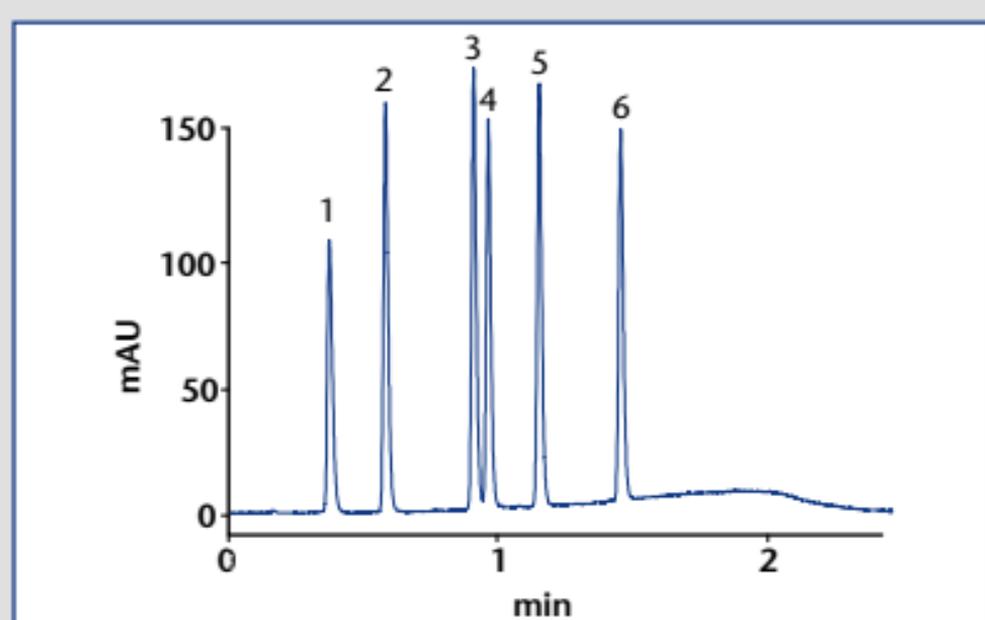
Mode: RP-Mode, gradient

Injection volume: 0.5  $\mu$ l

Pressure: 650 bar (9430 psi)

Detection: PDA-1, 254 nm (100 Hz, 0.005 s)  
10mm, 2 $\mu$ l flow cell

Temperature: 30 °C



1 Cortisone

2 Corticosterone

3 Testosterone

4 Deoxycorticosterone

5 Norgestrel

6 Progesterone

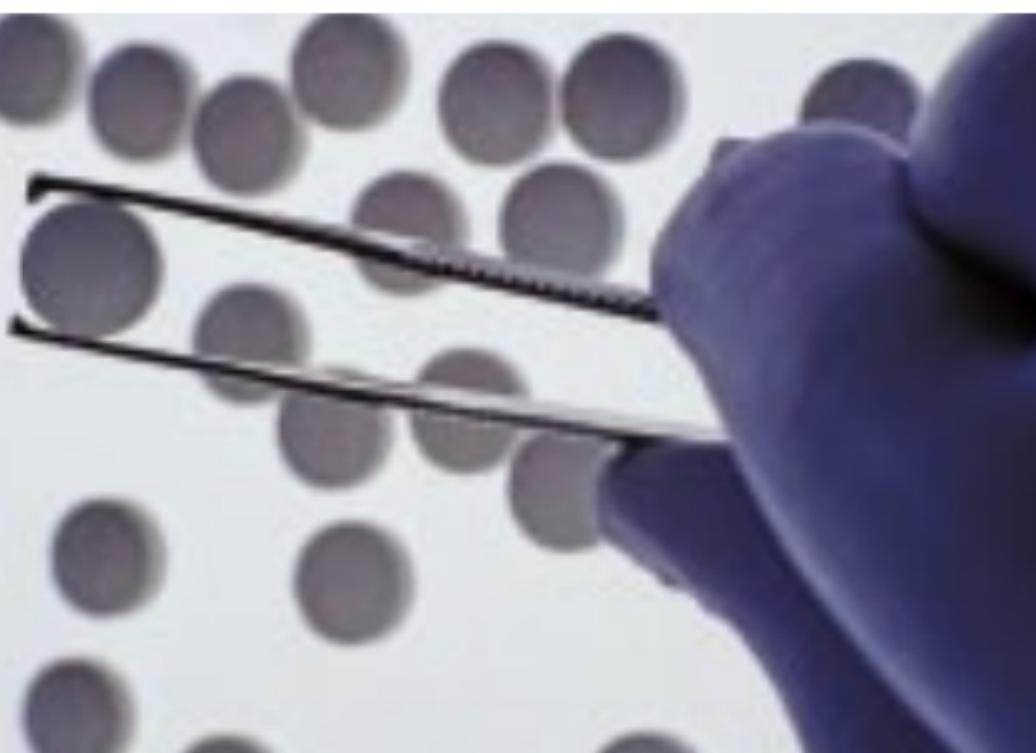


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# Pharmaceutical

## Separation of sulfa drugs

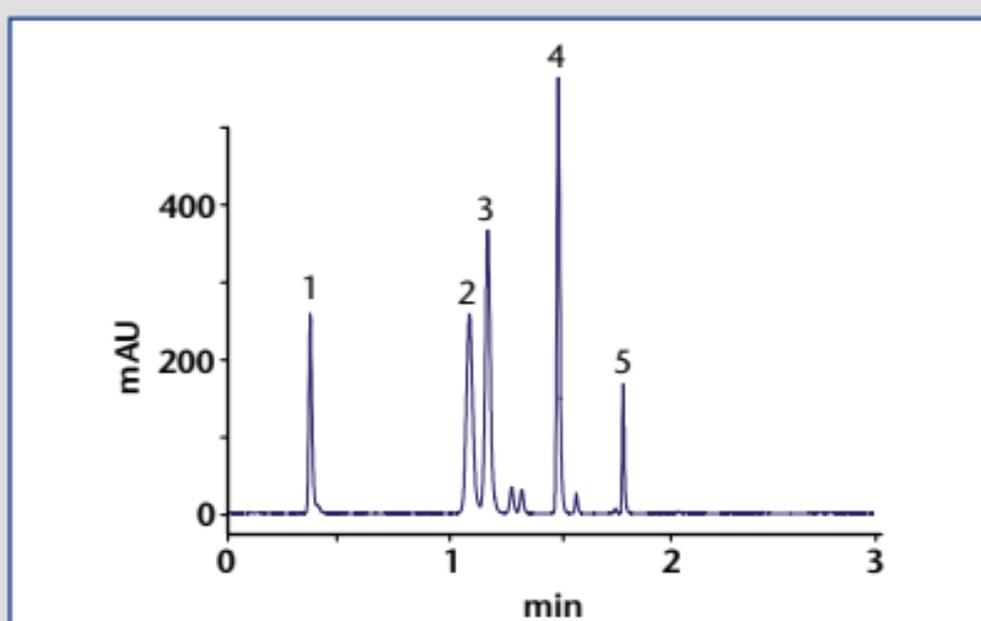


### Separation column

BlueOrchid C18 A 1.8 $\mu$ m, 50 x 2 mm

### Separation conditions

Eluent:	A: 1.7 mM NaH <sub>2</sub> PO <sub>4</sub> , pH 4
	B: ACN
Gradient:	0–0,8 min 8% B
	0.8–1.8 min 8%–40% B
Flow rate:	1.4 ml/min
Mode:	RP-Mode, gradient
Injection volume:	1 $\mu$ l
Pressure:	750 bar (10880 psi)
Detection:	PDA-1, 265 nm (100Hz, 0.005 s)
	10 mm, 2 $\mu$ l flow cell
Temperature:	40 °C



- 1 Sulfadiazine
- 2 Sulfamethiazole
- 3 Sulfamethoxypyridazine
- 4 Sulfamethoxazole
- 5 Sulfamethoxine



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# Pharmaceutical

## Separation of taxanes (I)



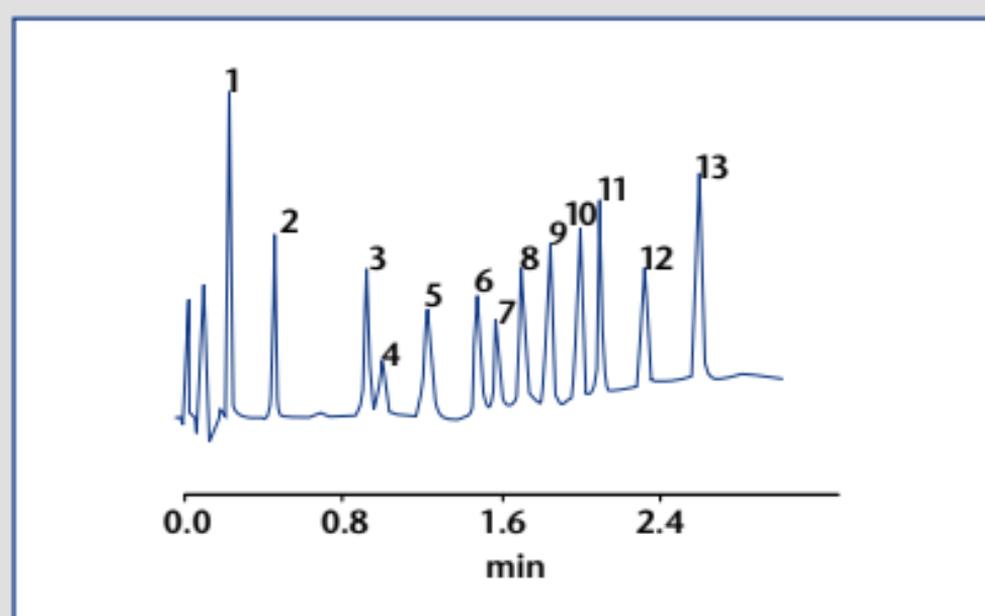
© Sebastian Kaulitzki

### Separation column

BlueOrchid PFP 1.8  $\mu\text{m}$ , 50 x 2 mm

### Separation conditions

Eluent:	A: H <sub>2</sub> O B: MeOH/ACN 7:93 (v/v)
Gradient:	0–1.2 min 35% B 1.2–4 min 58% B 4–5 min 58% B (2 min)
Flow rate:	0.6 ml/min
Mode:	RP-Mode, gradient
Injection volume:	2 $\mu\text{l}$
Detection:	PDA-1, 220 nm (100 Hz, 0.005 s) 10 mm 2 $\mu\text{l}$ flow cell
Temperature:	30 °C



1	10-Deacetyl baccatin
2	Baccatin III
3	10-Deacetyl-7-xylosyl taxol B
4	Taxinine M
5	10-Deacetyl-7-xylosyl taxol
6	10-Deacetyl taxol
7	10-Deacetyl-7-xylosyl taxol C
8	7-Xylosyl taxol
9	Cephalomanine
10	10-Deacetyl-7-epitaxol
11	Paclitaxel
12	Taxol C
13	7-Epitaxol



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# Chemical



# Selectivity test

Engelhardt test (modified)  
in less than 8 minutes



## Separation column

BlueOrchid C18 1.8 $\mu$ m, 100 x 2mm

## Separation conditions

Eluent: MeOH/H<sub>2</sub>O 55:45 (v/v)

Flow rate: 0.45 ml/min

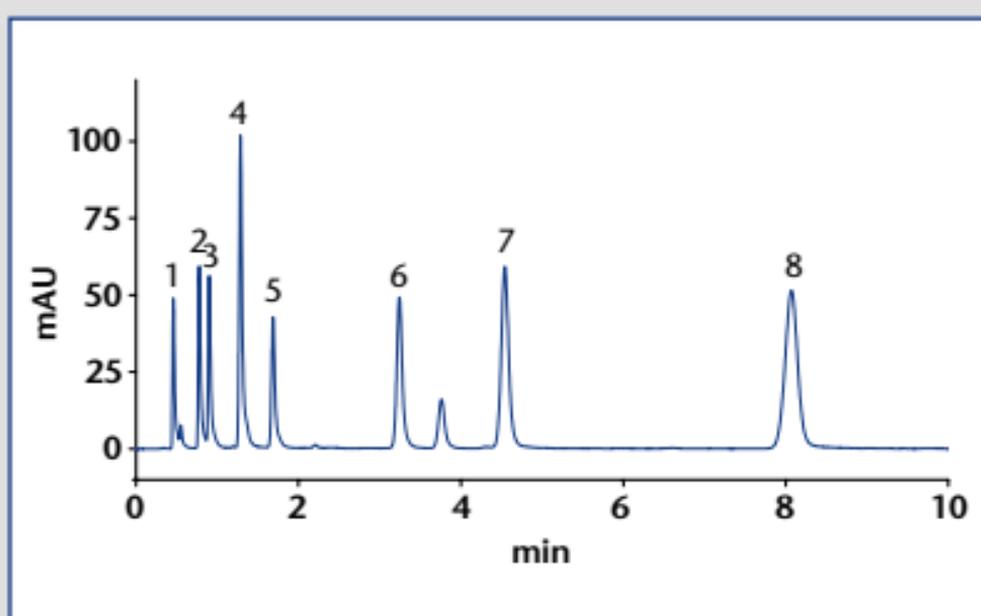
Mode: isocratic

Injection volume: 1  $\mu$ l

Pressure: 890 bar (12900 psi)

Detection: MW-1, 254 nm (80Hz, 0.005 s)

Temperature: 40 °C



- 1 Uracil
- 2 Aniline
- 3 Phenol
- 4 p-Ethylaniline
- 5 N,N-dimethylaniline
- 6 Benzoic acid ethyl ester
- 7 Toluene
- 8 Ethylbenzene



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# Blue Orchid

# Efficient

UHPLC columns



## BlueOrchid

Our sub- $2\mu\text{m}$  columns packed with  $1.8\mu\text{m}$  particles are specifically designed for UHPLC applications.

Their exceptional stability in both acidic and alkalic eluents make them universally applicable. BlueOrchid columns are available in 12 phase modifications and a variety of column dimensions.

# Economical

## BlueOrchid RP UHPLC column kits



### Advantages

- up to 10 times faster
- improved resolution
- enhanced sensitivity
- low back pressure
- excellent peak shape
- good value

These RP column kits, available in 50 and 100 mm, provide three different selectivities.

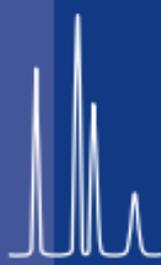
### Kit selectivities

- BlueOrchid C18 1.8  $\mu\text{m}$
- BlueOrchid C18 A 1.8  $\mu\text{m}$
- BlueOrchid C8 1.8  $\mu\text{m}$

Please refer to our BlueOrchid brochure for more details and ordering information.

**HPLC/UHPLC  
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