

# Application Guide



Ultra  
Fast  
HPLC

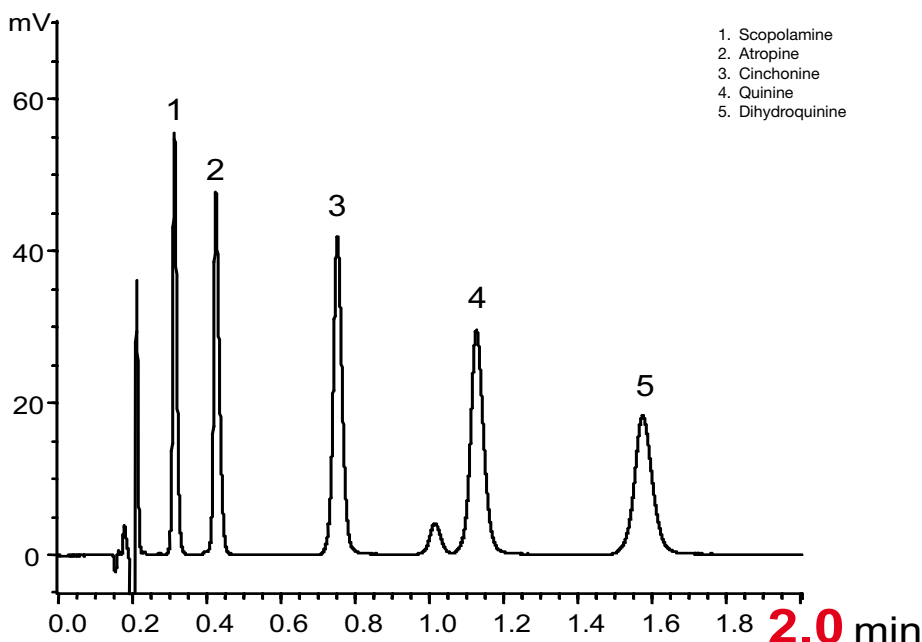
## HPLC Columns for Ultra Fast LC

Nowadays the need for Ultra Fast LC and Rapid Resolution is still growing, especially in the pharmaceutical industry, due to the continuous demand for high throughput analysis in research & development and quality control.

As a column and bulk media supplier with many years of practical chromatographic experience, YMC found it unacceptable that the use of novel separation media is often restricted to dedicated equipment and not applicable to the large installed base of “conventional” HPLC systems with a standard operating pressure rating of less than 400 bar. For this reason, specifications for YMC-UltraHT columns were designed to provide powerful chromatographic improvements, in terms of velocity and resolution, with conventional operating conditions as well as ultra-high pressure systems. Since YMC-UltraHT columns provides a substantially lower pressure drop than most competitive 2  $\mu\text{m}$  or sub-2  $\mu\text{m}$  media, high flow rates can be achieved without generating excessive back pressure and without the need for specialised equipment.

For effective high throughput separations, YMC offer a wide range of high performance HPLC columns, which allow Ultra Fast analytical HPLC separations using conventional equipment. Due to the down-scalability of the majority of YMC’s stationary phases, the time needed for a single analysis can be reduced to less than 60 seconds, depending on the sample conditions.

### YMC-UltraHT Pro C18 (2 $\mu\text{m}$ , 12nm) 50 x 2.0mm ID



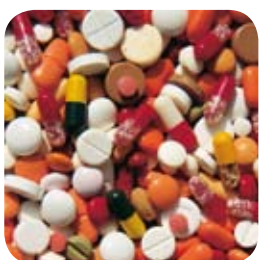
Part No.: AS12S020502WT  
Eluent: 20mM CH<sub>3</sub>COOH-CH<sub>3</sub>COONH<sub>4</sub> (pH 4.9) / acetonitrile (80/20)  
Flow rate: 0.6 ml/min (3.18 mm/sec)  
Temperature: 40 °C  
Detection: UV at 220 nm  
Pressure: 292 bar



Downscaling of methods.....page 4-5



Food & Beverages.....page 6-13



Pharmaceuticals.....page 14-23



Environmental.....page 24-25



Bioseparations .....page 26-27

## How to downscale for ultra-fast methods

Ever since the beginning of HPLC, more-demanding analytical problems have required progressive improvement in separation efficiency.

The challenges include ever more complex analytes and the reduction in analysis times to keep up with increasing numbers of samples. In addition to reducing the column dimensions and increasing flow rates, the implementation of small particles is a powerful tool to increase efficiency. In order to adapt the benefits of these technical advances it is necessary to adjust existing methods carried out under HPLC conditions to ultra-fast LC conditions.

### Downscaling of existing methods

In most cases the conventional HPLC method is carried out on 250 x 4.6 mm ID or 150 x 4.6 mm ID columns. However, for ultra-fast LC columns with 2.0, 2.1 or 3.0 mm ID combined with 50 mm or 100 mm length packed with 2 µm or sub-2 µm particles are chosen. Consequently, the following parameters need to be adjusted:

- Flow rate
- Injection volume
- Gradient conditions (unless isocratic conditions)
- Column volume

#### Flow rate:

$$F_{\text{Fast}} = F_{\text{HPLC}} \times (d_{\text{Fast}})^2 / (d_{\text{HPLC}})^2$$

Where  $d_{\text{HPLC}}$  and  $d_{\text{Fast}}$  are the column diameters and  $F_{\text{HPLC}}$  and  $F_{\text{Fast}}$  the flow rates.

#### Injection volume:

The injection volume is reduced by the ratio of the corresponding volumes of the two columns:

$$V_{\text{Fast}} = V_{\text{HPLC}} \times [(r_{\text{Fast}}^2 \times L_{\text{Fast}}) / (r_{\text{HPLC}}^2 \times L_{\text{HPLC}})]$$

Where  $r_{\text{HPLC}}^2$  and  $r_{\text{Fast}}^2$  are the radii of the columns  $L_{\text{HPLC}}$  and  $L_{\text{Fast}}$  are the lengths of the columns, and  $V_{\text{HPLC}}$  and  $V_{\text{Fast}}$  are the injection volumes.

#### Gradient:

The gradient is adjusted from HPLC to Ultra Fast LC using:

$$t_{\text{gFast}} = t_{\text{gHPLC}} \times L_{\text{Fast}} / L_{\text{HPLC}}$$

Where  $L_{\text{HPLC}}$  and  $L_{\text{Fast}}$  are the lengths of the HPLC and Ultra Fast LC columns, and  $t_{\text{gHPLC}}$  and  $t_{\text{gFast}}$  are the times of each gradient step respectively.

#### Column volume:

In order to keep the column volumes proportional, the gradient has to be re-adjusted for the new flow rate and column dimension according to:

$$t_{\text{g3}} = (F_{\text{Fast}} \times t_{\text{gFast}}) / F_3$$

Where  $F_{\text{Fast}}$  and  $t_{\text{gFast}}$  are the flow rate and gradient time of the geometrically scaled values (typically 650 µl/min for small molecules on a 2.1 mm id column) and  $F_3$  and  $t_{\text{g3}}$  are the optimised values.

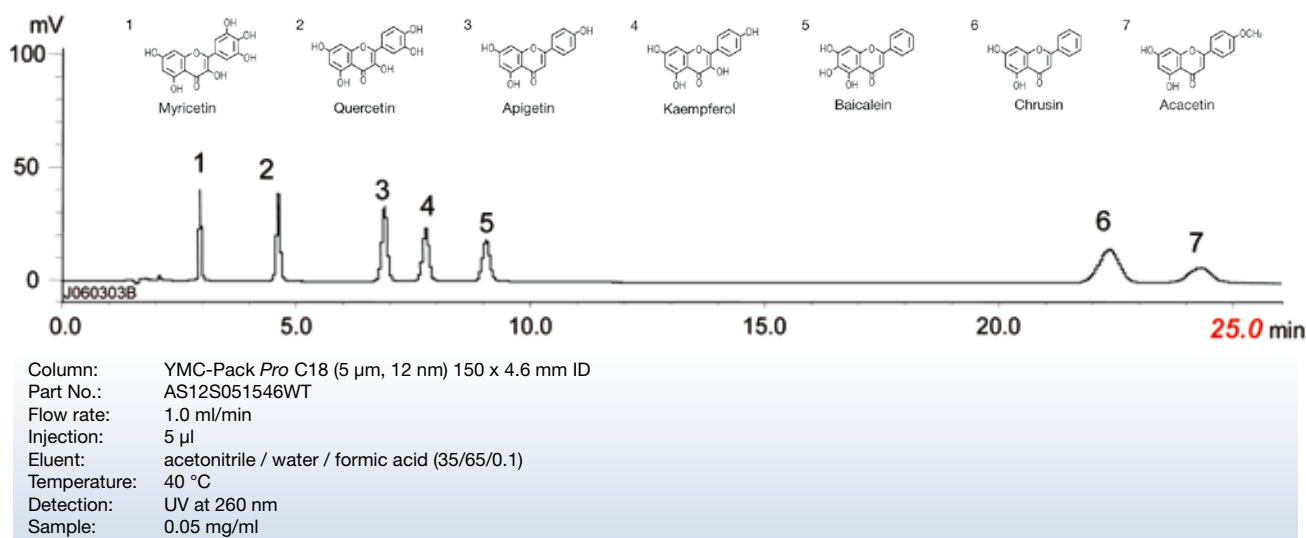


### How does it work in practice?

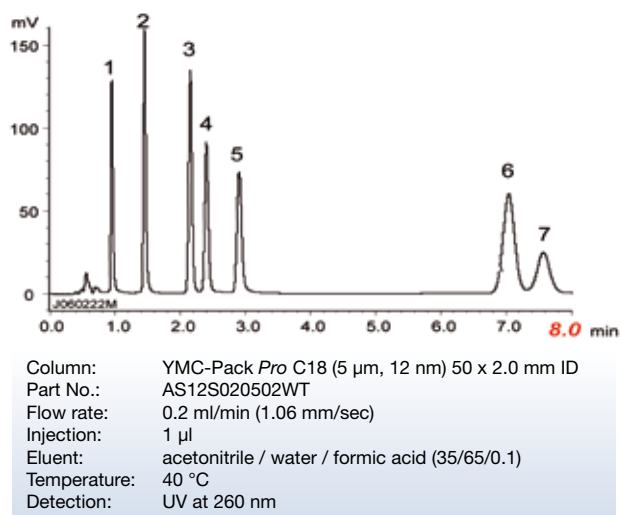
The example below shows how easy it is to down-scale an existing method. The compounds of interest are flavonoids.

The conventional HPLC is carried out on 150 x 4.6 mm ID packed with 5  $\mu\text{m}$ . The last eluting peak appears after 25 minutes.

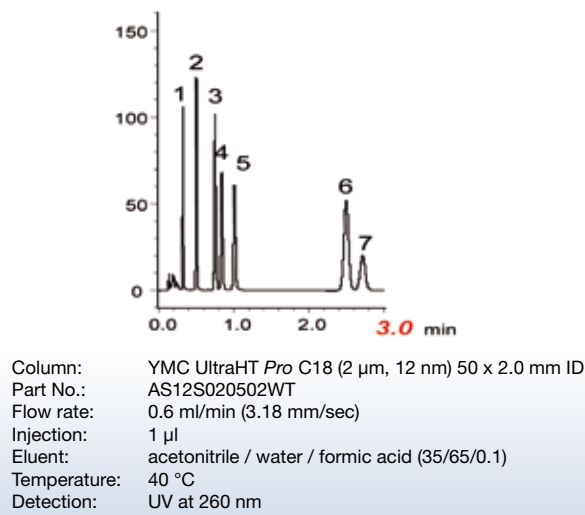
### Conventional LC method



### Fast LC method



### Ultra-fast LC method with increased flow



### Conclusion

The method transfer from a classical HPLC method to an ultra-fast HPLC method for the analysis of 7 flavonoids has been demonstrated. The reduction of column length and increased efficiency of a 2  $\mu\text{m}$  YMC-Pack *Pro* C18 column makes it possible to reduce the analysis time by a factor of almost 10, with even greater solvent savings, compared to the original method.

# Fast analysis of isoflavonoids in food

As soy is the most important source of vegetable oil worldwide, it contributes essentially to a balanced diet. Secondary components such as isoflavonoids have a significant positive effect on the hormonal balance. However, adverse effects can occur. The following method for a fast and robust separation of isoflavonoids will facilitate the analysis of these food ingredients.

## Structures of 12 isoflavones in soybeans

glycosides				aglycones			
Compound	(abbr.)	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	Compound (abbr.)	R <sub>1</sub>	R <sub>2</sub>
Daidzin	(D)	H	H	H	Daidzein (De)	H	H
Glycitin	(Gl)	H	OCH <sub>3</sub>	H	Glycitein (Gle)	H	OCH <sub>3</sub>
Genistin	(G)	OH	H	H	Genistein (Ge)	OH	H
6''-O-Acetyldaidzin	(AD)	H	H	COCH <sub>3</sub>			
6''-O-Acetylglycitin	(AGl)	H	OCH <sub>3</sub>	COCH <sub>3</sub>			
6''-O-Acetylgenistin	(AG)	OH	H	COCH <sub>3</sub>			
6''-O-Malonyldaidzin	(MD)	H	H	COCH <sub>2</sub> COOH			
6''-O-Malonylglycitin	(MGl)	H	OCH <sub>3</sub>	COCH <sub>2</sub> COOH			
6''-O-Malonylgenistin	(MG)	OH	H	COCH <sub>2</sub> COOH			

Figure 1

## Influence of acetic acid concentration on soy isoflavone separation

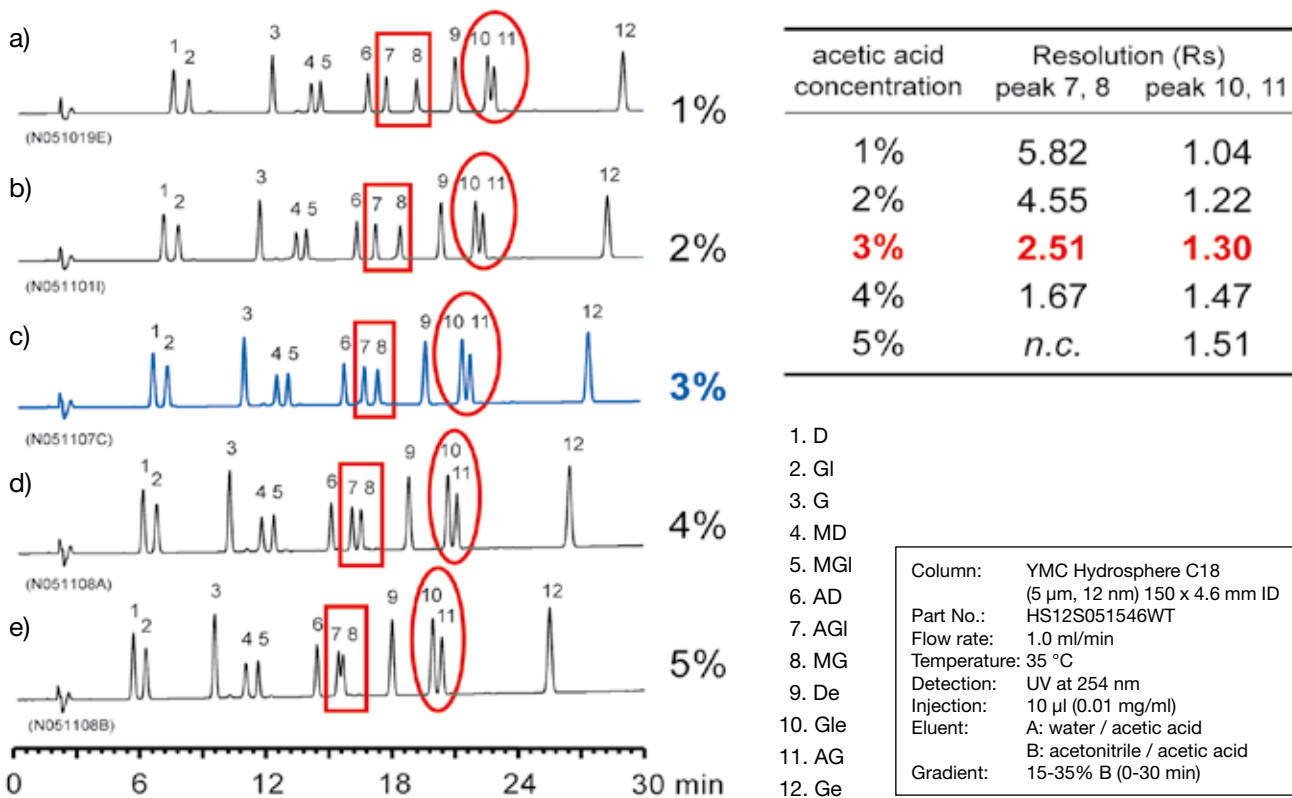


Figure 2

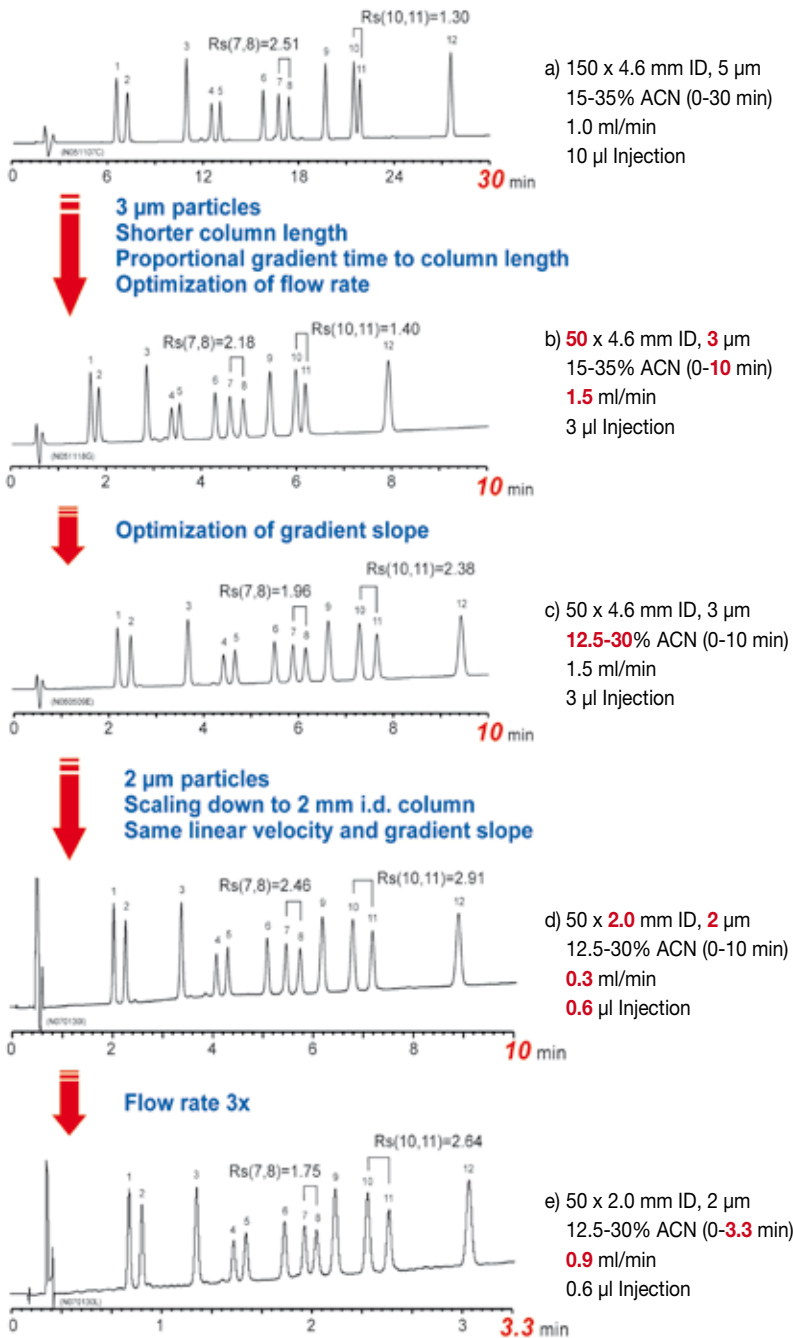
The isoflavonoids were extracted from the crude matrix by stirring with a 50:50 water/ethanol mixture at room temperature for one hour. After filtration (filter paper No. 5A) the samples were prepared for HPLC analysis by use of a syringe filter (0.2 µm). Initial experiments showed very quickly that the method would be successful using gradient elution with water/acetonitrile with acetic acid to control pH (see figure 2, chromatogram a). Further

optimisation was achieved by varying the acetic acid content. Peaks 10 and 11 (Glycitein and 6''-O-acetylgenistein) were baseline separated with a high percentage of acetic acid. However, under these conditions the resolution of peaks 7 and 8 (6''-O-Acetylglycitine and 6''-OMalonylgenistein) was poor. Reduction of the acetic acid to 3% resulted in near baseline separation of all 7 compounds (see figure 2, chromatogram c).





**Method transfer from conventional LC to ultra-fast LC**



The analysis time of 30 min could be reduced substantially by conventional means of reducing particle size and column dimension (3  $\mu$ m, 50 x 4.6 mm ID). To get the same results in terms of the chromatographic behaviour it is of importance to keep a constant gradient volume. Figures 3a and b show the method transfer to a 50 x 4.6 mm ID column. Increasing the flow rate to 1.5 ml/min was necessary to maintain the resolution and elution profile. Adjusting the gradient profile (figures 3b and c) led to a baseline resolution of the critical peak pair 10 and 11. This conventional method was then transferred to ultra-fast analysis on a JASCO high pressure system using 2  $\mu$ m particles. After modifying the chromatographic parameters the flow rate was again increased which reduced the analysis time in total by a factor of 10 (see figures 3d and e).

- 1. D
- 2. GI
- 3. G
- 4. MD
- 5. MGI
- 6. AD
- 7. AGI
- 8. MG
- 9. De
- 10. Gle
- 11. AG
- 12. Ge

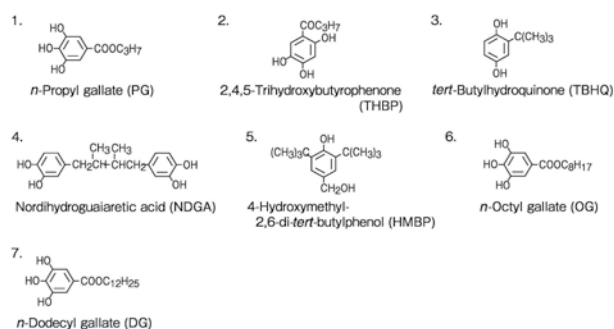
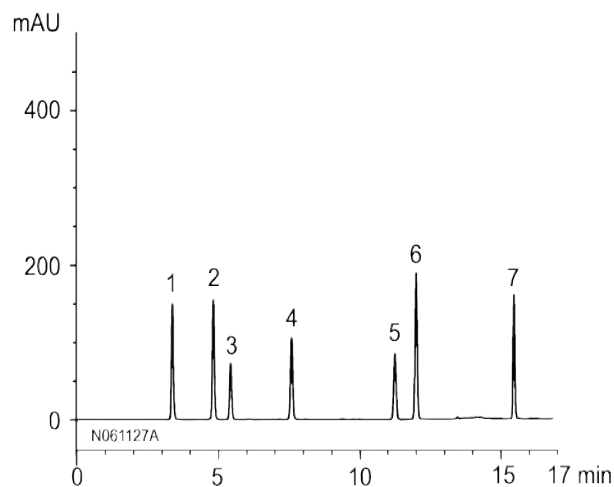
Column:	Hydrosphere C18 (12 nm)
Temp.:	35 °C
Detection:	UV at 254 nm
Eluent:	A: water / acetic acid (100/3) B: acetonitrile / acetic acid (100/3)

Figure 3

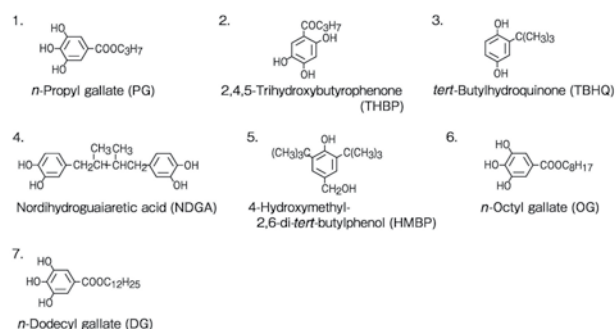
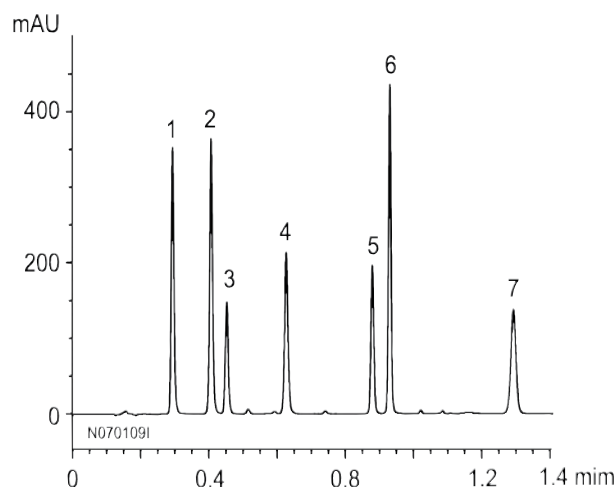
**Conclusion**

The aim of this study was the development of an ultra-fast method for the determination of isoflavonoids in soy-containing foods. The method transfer from conventional to ultra-fast HPLC systems was successful when using YMC UltraHT Hydrosphere C18 with 2  $\mu$ m particle size.

## Conventional and Ultra-fast LC method for antioxidants



Column: YMC-Pack *Pro* C18 (5  $\mu$ m, 12 nm) 150 x 4.6 mm ID  
 Part No.: AS12S051546WT  
 Flow rate: 1.0 ml/min  
 Eluent: A: water / TFA (100/0.1)  
 B: acetonitrile / methanol / TFA (75/25/0.1)  
 Gradient: 45-70% B (0-9 min)  
 70-95% B (9-12 min)  
 95% B (12-17 min)  
 Injection: 5  $\mu$ l  
 Temperature: 30  $^{\circ}$ C  
 Detection: UV at 280 nm  
 Sample: 0.05 ~ 0.3 mg/ml



Column: YMC-UltraHT *Pro* C18 (2  $\mu$ m, 12 nm) 50 x 2.0 mm ID  
 Part No.: AS12S020502WT  
 Flow rate: 0.8 ml/min  
 Eluent: A: water / TFA (100/0.1)  
 B: acetonitrile / methanol/TFA (75/25/0.1)  
 Gradient: 45-70% B (0-0.75 min)  
 70-95% B (0.75-1 min)  
 95% B (1-1.4 min)  
 Injection: 1  $\mu$ l  
 Temperature: 30  $^{\circ}$ C  
 Detection: UV at 280 nm  
 Sample: 0.05 ~ 0.3 mg/ml

The original HPLC method has been transferred to ultra-fast LC by employing a 2  $\mu$ m material and a column size of 50 x 2.0 mm ID. The ultra-fast LC method allows the same chromatographic performance within 1.4 minutes, with the resolution remaining constant, due to the full scalability of YMC-Pack *Pro* C18 .

## Conclusion

Transfer of this conventional method to an ultra-fast method resulted in solvent and time savings of about 90% with total retention of the resolution of the different antioxidants.

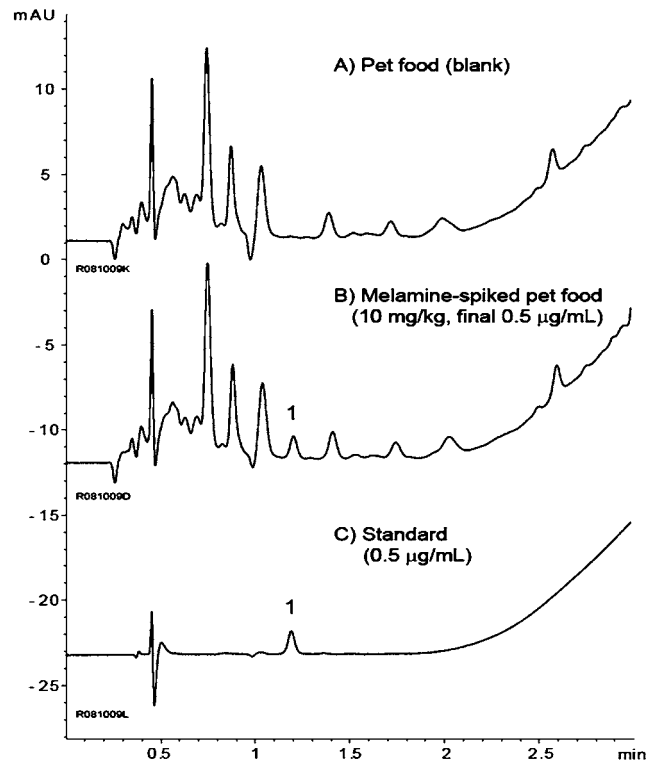
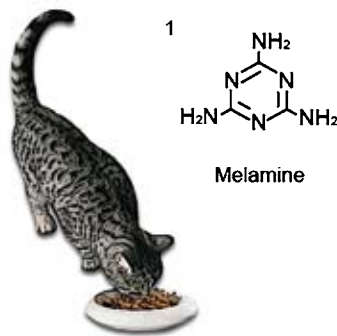




### Melamine in pet-food

#### Sample preparation method

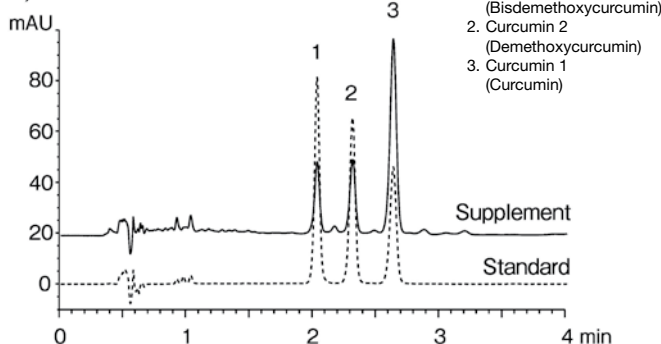
- Pet food (1 g)
- ← 50% aqueous acetonitrile (5 mL)
- Sonication for 30min
- Centrifugation at 10000 rpm for 10 min
- Filtration
- Dilution 4 times with eluent A
- Filtration
- Injection



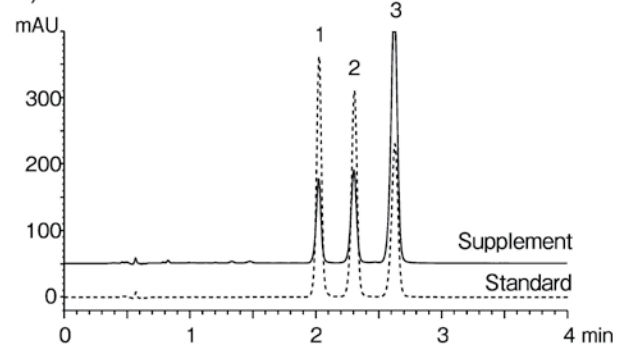
Column: YMC-UltraHT Hydrosphere C18 (2 µm, 12 nm) 50 x 2.0 mm ID  
 Part No.: HS12S020502WT  
 Eluent: A: water / heptafluorobutyric acid (100/0.1); B: methanol / heptafluorobutyric acid (100/0.1)  
 Gradient: 5% B (0-0.17 min), 5-90% B (0.17-3 min)  
 Flow rate: 0.4 ml/min  
 Temperature: 40 °C  
 Detection: UV at 240 nm  
 Injection: 1 µl

### Curcuminoids in a commercial turmeric supplement

A) UV at 250 nm



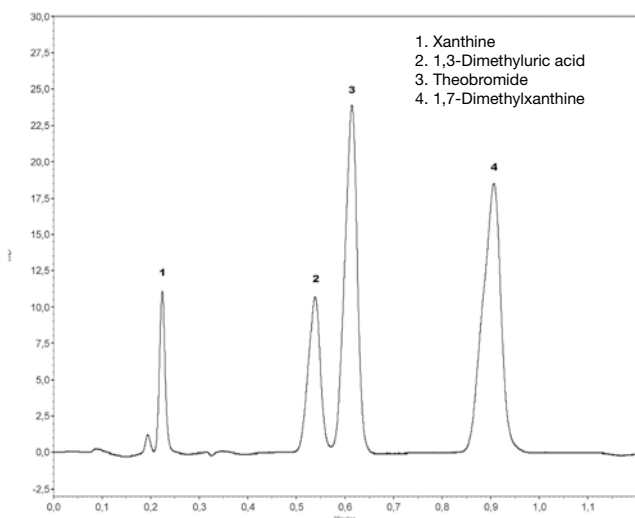
B) UV at 420 nm



Column: YMC-UltraHT Pro C18 (2 µm, 12 nm) 100 x 2.0 mm ID  
 Part No.: AS12S021002WT  
 Eluent: acetonitrile / water / formic acid (50/50/0.1)  
 Flow rate: 0.4 ml/min  
 Temperature: 40 °C  
 Detection: A) UV at 250 nm; B) UV at 420 nm  
 Injection: Standard 1 µl (0.1 mg/ml)  
               Supplement 1 µl (6.9 mg/ml)  
 Sample: methanol extract of a commercially available turmeric supplement

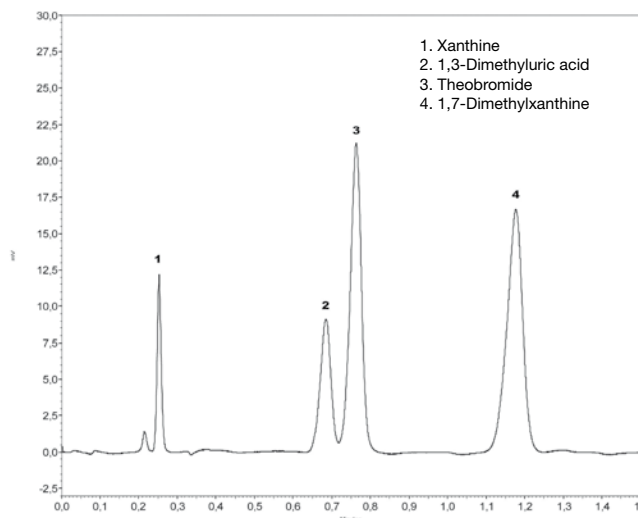
# Selected separations of food and beverages

## Separation of caffeine metabolites using acetonitrile eluent



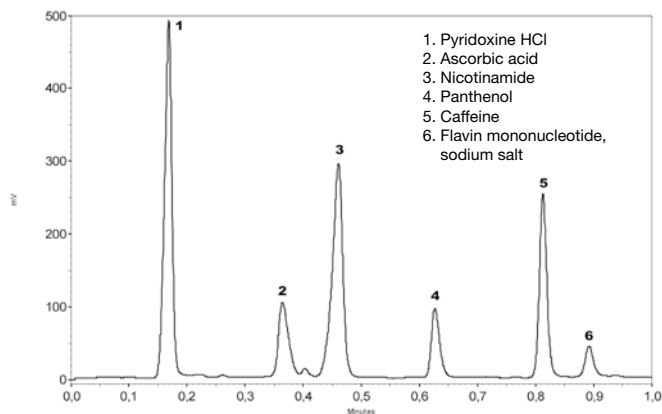
Column: YMC-UltraHT Hydrosphere C18 (2  $\mu$ m, 12 nm)  
50 x 2.0 mm ID  
Part No.: HS12S020502WT  
Mobile Phase: acetonitrile / acetic acid (pH 2.5) (06/94)  
Flow rate: 0.82 ml/min  
Detection: 254 nm  
Pressure: 420 bar  
Injection: 1  $\mu$ L  
Temperature: 40  $^{\circ}$ C

## Separation of caffeine metabolites using methanol eluent



Column: YMC-UltraHT Hydrosphere C18 (2  $\mu$ m, 12 nm)  
50 x 2.0 mm ID  
Part No.: HS12S020502WT  
Mobile Phase: methanol / acetic acid (pH 2.5) (08/92)  
Flow rate: 0.82 ml/min  
Detection: 254 nm  
Pressure: 465 bar  
Injection: 1  $\mu$ L  
Temperature: 40  $^{\circ}$ C

## Separation of water-soluble vitamins

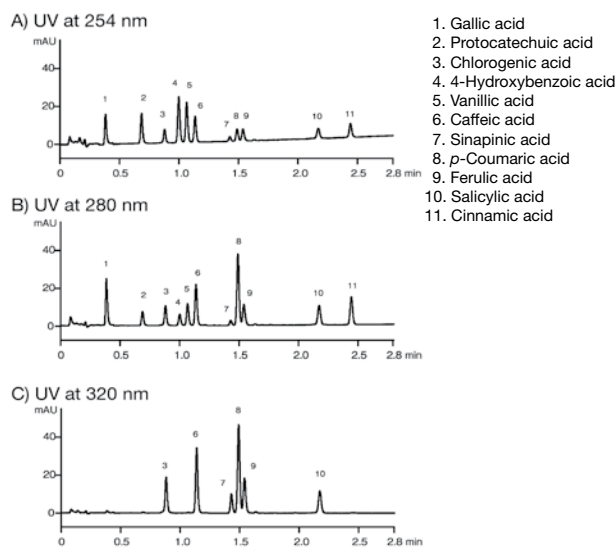


Column: YMC-UltraHT Hydrosphere C18 (12 nm, 2  $\mu$ m)  
50 x 2.0 mm ID  
Part No.: HS12S020502WT  
Mobile Phase: A: acetic acid (pH 3.5)  
B: acetonitrile / acetic acid (pH 3.5) (20/80)  
Gradient:  

Time	A (in %)	B (in %)
0.0	10	90
0.2	0	100
1.0	0	100
1.2	10	90
2.0	10	90

  
 Flow rate: 0.76 ml/min  
 Detection: 210 nm  
 Pressure: 450 bar  
 Injection: 1  $\mu$ L  
 Temperature: 40  $^{\circ}$ C

## Aromatic carboxylic acids in foods

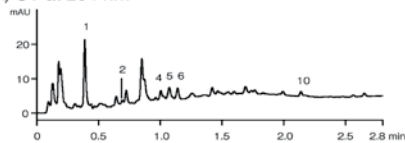


Column: YMC-UltraHT Pro C18 (2  $\mu$ m, 12 nm) 50 x 2.0 mm I.D.  
Part No.: AS12S020502WT  
Eluent: A: water / HCOOH (200/0.4)  
B: acetonitrile / methanol / THF / water / HCOOH (43.5/43.5/13/100/0.4)  
20-100%B (0-2.8 min)  
Flow rate: 0.5 ml/min  
Temperature: 40  $^{\circ}$ C  
Injection: 2  $\mu$ L (2.530  $\mu$ g/ml)



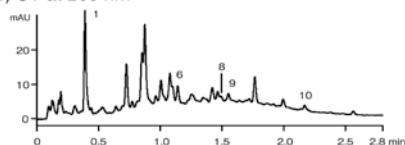
### Aromatic carboxylic acids in red wine

A) UV at 254 nm



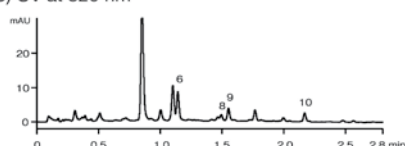
1. Gallic acid
2. Protocatechuic acid
3. Chlorogenic acid
4. 4-Hydroxybenzoic acid
5. Vanillic acid
6. Caffeic acid
7. Sinapinic acid
8. *p*-Coumaric acid
9. Ferulic acid
10. Salicylic acid

B) UV at 280 nm



11. Cinnamic acid

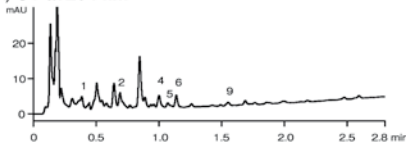
C) UV at 320 nm



Column: YMC-UltraHT *Pro* C18 (2  $\mu$ m, 12 nm) 50 x 2.0 mm ID  
 Part No.: AS12S020502WT  
 Eluent: A: water / HCOOH (200/0.4)  
 B: acetonitrile / methanol / THF / water / HCOOH (43.5/43.5/13/100/0.4)  
 Gradient: 20-100%B (0-2.8 min)  
 Flow rate: 0.5 ml/min  
 Temperature: 40 °C  
 Injection: 2  $\mu$ l  
 Sample: 5 times dilution of a red wine with water

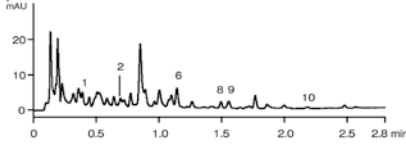
### Aromatic carboxylic acids in white wine

A) UV at 254 nm



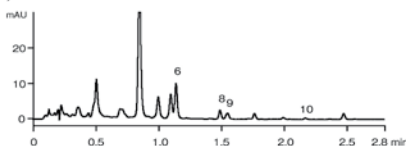
1. Gallic acid
2. Protocatechuic acid
3. Chlorogenic acid
4. 4-Hydroxybenzoic acid
5. Vanillic acid
6. Caffeic acid
7. Sinapinic acid
8. *p*-Coumaric acid
9. Ferulic acid
10. Salicylic acid
11. Cinnamic acid

B) UV at 280 nm



11. Cinnamic acid

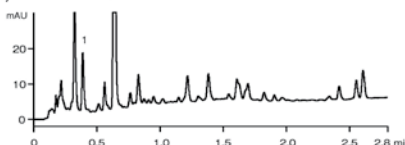
C) UV at 320 nm



Column: YMC-UltraHT *Pro* C18 (2  $\mu$ m, 12 nm) 50 x 2.0 mm ID  
 Part No.: AS12S020502WT  
 Eluent: A: water / HCOOH (200/0.4)  
 B: acetonitrile / methanol / THF / water / HCOOH (43.5/43.5/13/100/0.4)  
 Gradient: 20-100%B (0-2.8 min)  
 Flow rate: 0.5 ml/min  
 Temperature: 40 °C  
 Injection: 2  $\mu$ l  
 Sample: 2 times dilution of a white wine with water

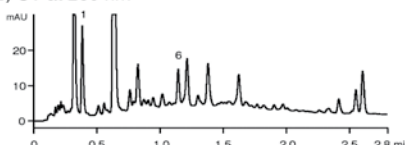
### Aromatic carboxylic acids in a tea extract

A) UV at 254 nm



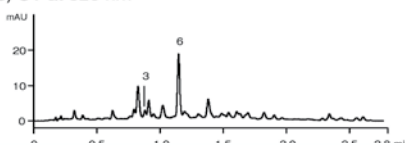
1. Gallic acid
2. Protocatechuic acid
3. Chlorogenic acid
4. 4-Hydroxybenzoic acid
5. Vanillic acid
6. Caffeic acid
7. Sinapinic acid
8. *p*-Coumaric acid
9. Ferulic acid
10. Salicylic acid
11. Cinnamic acid

B) UV at 280 nm



11. Cinnamic acid

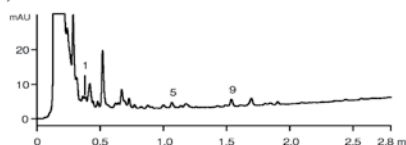
C) UV at 320 nm



Column: YMC-UltraHT *Pro* C18 (2  $\mu$ m, 12 nm) 50 x 2.0 mm ID  
 Part No.: AS12S020502WT  
 Eluent: A: water / HCOOH (200/0.4)  
 B: acetonitrile / methanol / THF / water / HCOOH (43.5/43.5/13/100/0.4)  
 Gradient: 20-100%B (0-2.8 min)  
 Flow rate: 0.5 ml/min  
 Temperature: 40 °C  
 Injection: 2  $\mu$ l  
 Sample: hot water extract of tea leaves (5 mg/ml)

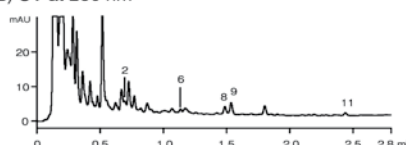
### Aromatic carboxylic acids in a beer

A) UV at 254 nm



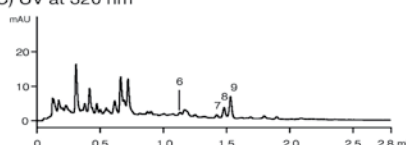
1. Gallic acid
2. Protocatechuic acid
3. Chlorogenic acid
4. 4-Hydroxybenzoic acid
5. Vanillic acid
6. Caffeic acid
7. Sinapinic acid
8. *p*-Coumaric acid
9. Ferulic acid
10. Salicylic acid
11. Cinnamic acid

B) UV at 280 nm



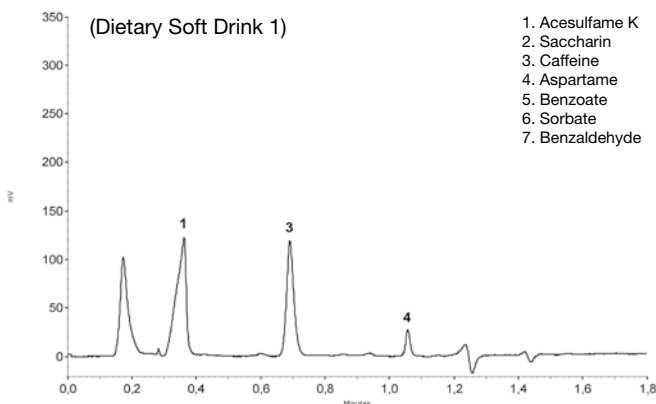
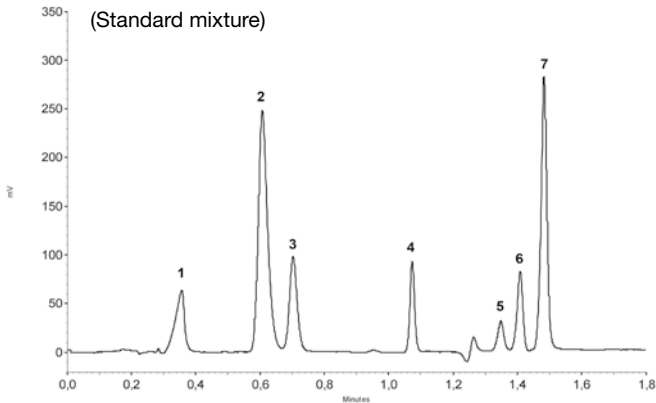
11. Cinnamic acid

C) UV at 320 nm

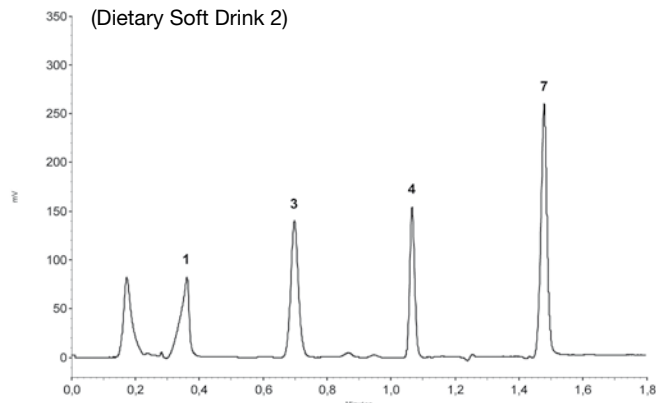


Column: YMC-UltraHT *Pro* C18 (2  $\mu$ m, 12 nm) 50 x 2.0 mm ID  
 Part No.: AS12S020502WT  
 Eluent: A: water / HCOOH (200/0.4)  
 B: acetonitrile / methanol / THF / water / HCOOH (43.5/43.5/13/100/0.4)  
 Gradient: 20-100%B (0-2.8 min)  
 Flow rate: 0.5 ml/min  
 Temperature: 40 °C  
 Injection: 2  $\mu$ l  
 Sample: 2 times dilution of a beer with water

**Separation of 6 key compounds in soft drinks**



- 1. Acesulfame K
- 2. Saccharin
- 3. Caffeine
- 4. Aspartame
- 5. Benzoate
- 6. Sorbate
- 7. Benzaldehyde

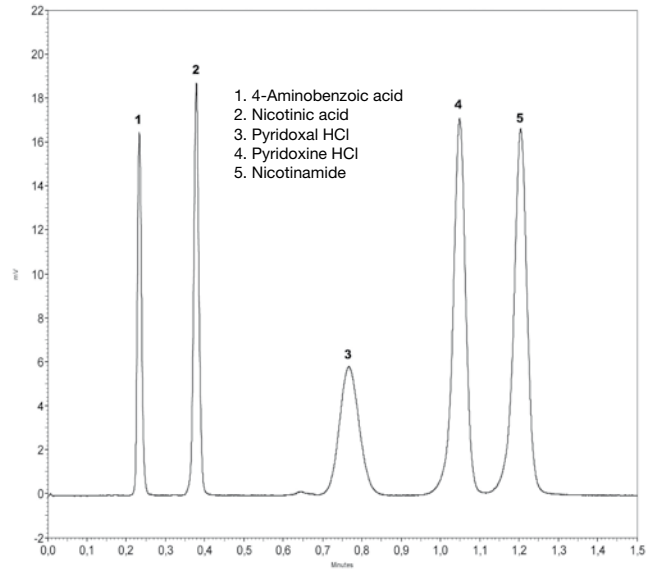


Column: YMC-UltraHT Hydrosphere C18 (2 µm, 12 nm)  
 50 x 2.0 mm ID  
 Part No.: HS12S020502WT  
 Mobile Phase: A: acetonitrile / MeOH / THF (20/60/20)  
 B: TFA (pH 2.15)  
 Gradient: 

Time	A (in %)	B (in %)
0	10	90
0.05	10	90
0.55	25	75
0.60	54	46
2.0	54	46
2.5	10	90

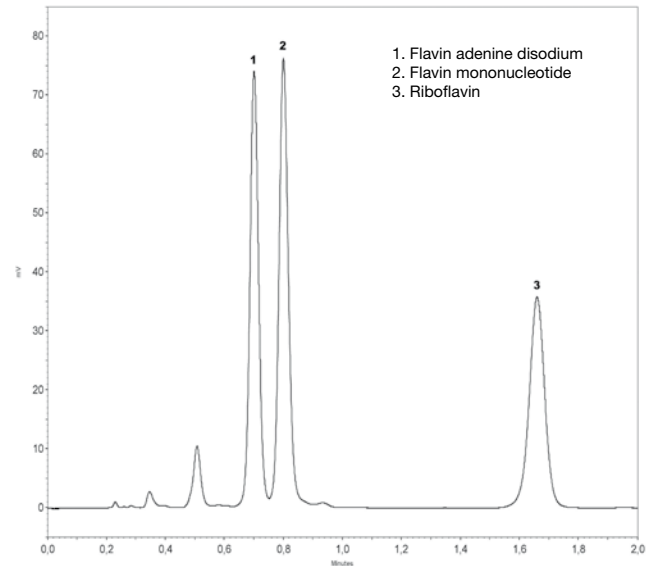
  
 Flow rate: 0.68 ml/min  
 Detection: 254 nm  
 Pressure: 460 bar  
 Injection: 1 µl  
 Temperature: 40 °C

**Separation of water soluble vitamins**



Column: YMC-UltraHT Hydrosphere C18 (2 µm, 12 nm)  
 50 x 2.0 mm ID  
 Part No.: HS12S020502WT  
 Mobile Phase: 10mM NH<sub>4</sub>CH<sub>3</sub>COO  
 Flow rate: 0.8 ml/min  
 Detection: UV at 254 nm  
 Pressure: 390 bar  
 Injection: 1 µl  
 Temperature: 40 °C

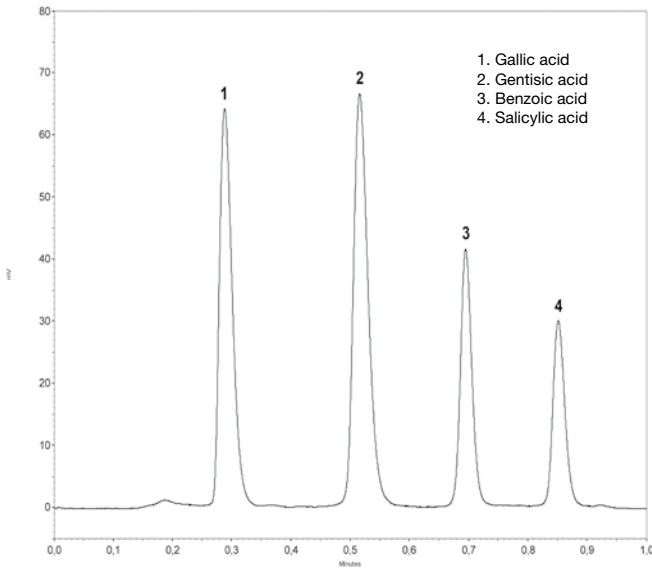
**Riboflavin and related compounds**



Column: YMC-UltraHT Hydrosphere C18 (2 µm, 12 nm)  
 50 x 2.0 mm ID  
 Part No.: HS12S020502WT  
 Mobile Phase: MeOH / 50mM NH<sub>4</sub>CH<sub>3</sub>COO (25/75)  
 Flow rate: 0.55 ml/min  
 Detection: UV at 260nm  
 Pressure: 400 bar  
 Injection: 1 µl  
 Temperature: 40 °C



### Separation of phenolic acids



- 1. Gallic acid
- 2. Gentisic acid
- 3. Benzoic acid
- 4. Salicylic acid

Column: YMC-UltraHT Hydrosphere C18 (12 nm, 2 µm)  
50 x 2.0 mm ID

Part No.: HS12S020502WT

Mobile Phase: A: methanol; B: formic acid (0.15%/pH 2.5)

Gradient:	Time	A (in %)	B (in %)
	0	50	50
	0.3	90	10
	0.9	90	10
	1.2	50	50

Flow rate: 0.5 ml/min

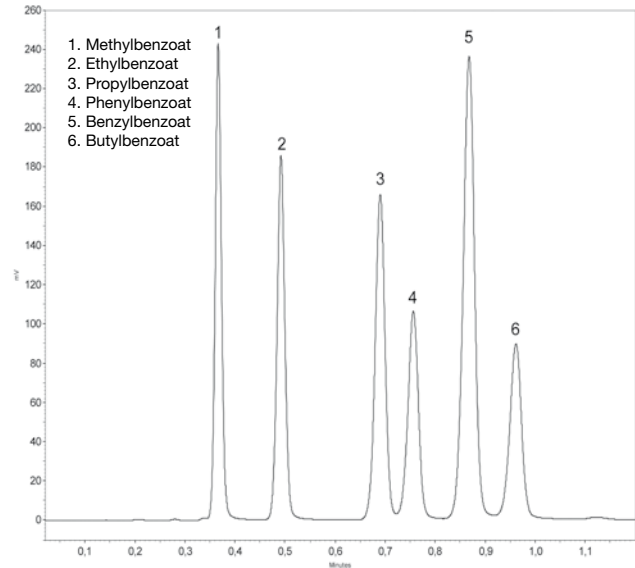
Detection: 254 nm

Pressure: 410 bar

Injection: 1 µl

Temperature: 40 °C

### Separation of benzoates



- 1. Methylbenzoat
- 2. Ethylbenzoat
- 3. Propylbenzoat
- 4. Phenylbenzoat
- 5. Benzylbenzoat
- 6. Butylbenzoat

Column: YMC-UltraHT Pro C18 (12 nm, 2 µm)  
50 x 2.0 mm ID

Part No.: AS12S020502WT

Mobile Phase: A: acetonitrile; B: water

Gradient:	Time	A (in %)	B (in %)
	0	60	40
	0.2	60	40
	1.0	72	28
	1.2	72	28

Flow rate: 0.8 ml/min

Detection: 254 nm

Pressure: 380 bar

Injection: 1 µl

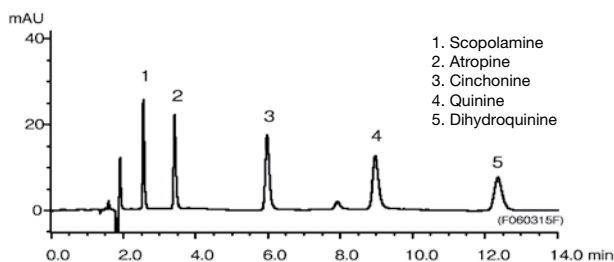
Temperature: 40 °C

# Alkaloids

The term "alkaloid" is used to describe any nitrogen-containing base, but this classification is too general for the wide variety of compound which occurs. True alkaloids are naturally occurring chemical compounds containing at least one basic nitrogen atom in a ring system derived from ami-

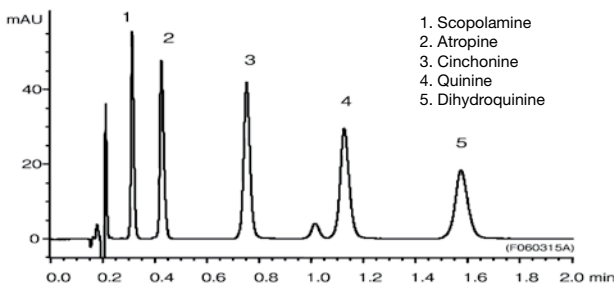
no acids (except phenylalanine). Alkaloids derived from other bases are classified as protoalkaloids or pseudoalkaloids. They are widely distributed in the natural world and produced by a large variety of organisms.

## Conventional LC method



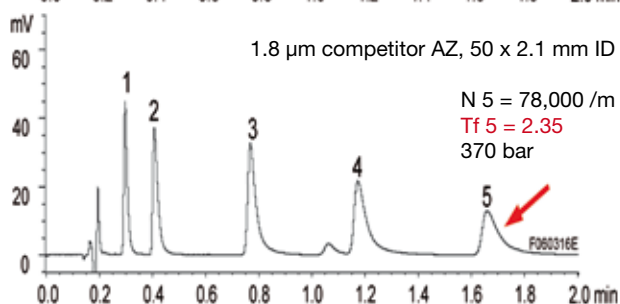
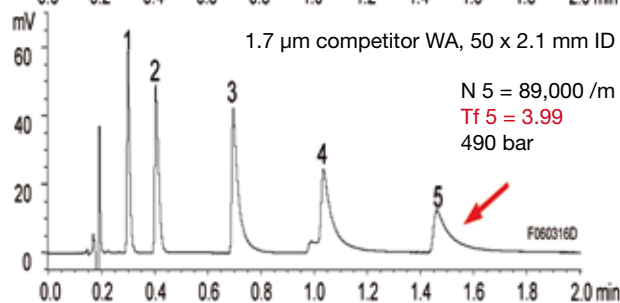
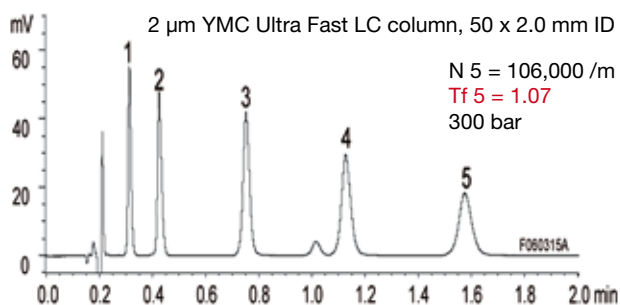
Column: YMC-Pack Pro C18 (5  $\mu$ m, 12 nm) 150 x 4.6 mm ID  
 Part No.: AS12S051546WT  
 Flow rate: 1.0 ml/min  
 Eluent: 20mM CH<sub>3</sub>COOH-CH<sub>3</sub>COONH<sub>4</sub> (pH 4.9) / acetonitrile (80/20)  
 Temperature: 40 °C  
 Detection: UV at 220 nm  
 Injection: 5  $\mu$ l  
 Sample: 0.02 ~ 0.1 mg/ml

## Ultra-fast LC method



Column: YMC UltraHT Pro C18 (2  $\mu$ m, 12 nm) 50 x 2.0 mm ID  
 Part No.: AS12S020502WT  
 Flow rate: 0.6 ml/min  
 Eluent: 20mM CH<sub>3</sub>COOH-CH<sub>3</sub>COONH<sub>4</sub> (pH 4.9) / acetonitrile (80/20)  
 Temperature: 40 °C  
 Detection: UV at 220 nm  
 Injection: 1  $\mu$ l  
 Sample: 0.02 ~ 0.1 mg/ml

## Comparison of YMC-UltraHT with alternative sub-2- $\mu$ m products



Eluent: 20mM CH<sub>3</sub>COOH-CH<sub>3</sub>COONH<sub>4</sub> (pH 4.9) / acetonitrile (80/20)  
 Flow rate: 0.6 ml/min  
 Temperature: 40 °C  
 Detection: UV at 220 nm

## Conclusion

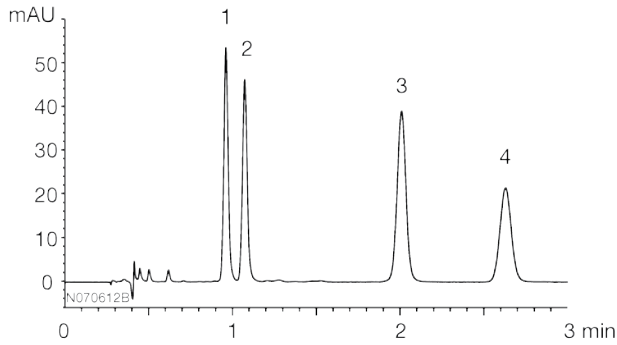
The method transfer from a conventional to an ultra-fast analysis for alkaloids has shown that the use of a YMC-UltraHT Pro C18 2  $\mu$ m column and adjustment of flow parameters for the smaller column dimensions can reduce the analysis time by about 85% accompanied by a significant solvent saving whilst increasing the sample throughput in the laboratory.



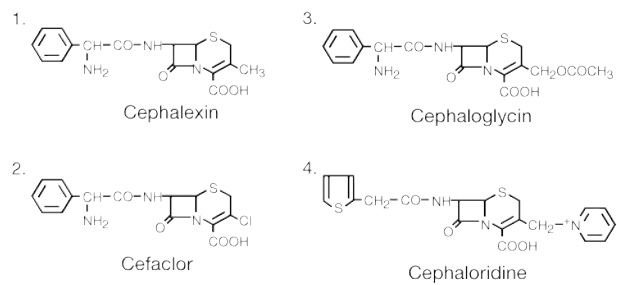
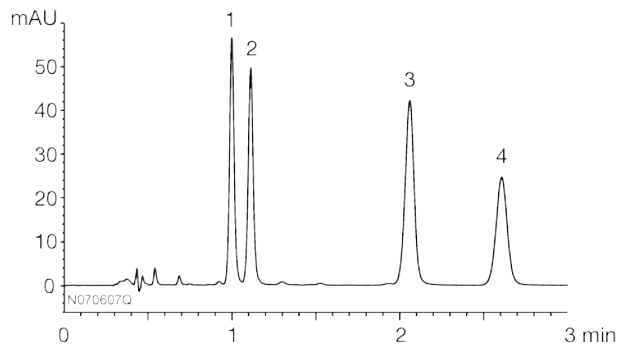


### Cephalosporin antibiotics

Column: 50 x 3.0 mm ID  
 Part No.: AS12S020503WT  
 Flow rate: 0.8 ml/min  
 Injection: 2 µl

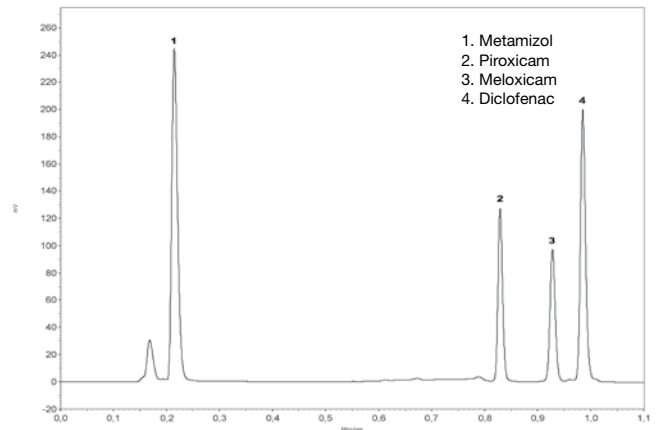


Column: 50 x 2.0 mm ID  
 Part No.: AS12S020502WT  
 Flow rate: 0.36 ml/min  
 Injection: 1 µl



Column: YMC-UltraHT Pro C18 (2 µm, 12 nm)  
 Eluent: acetonitrile / 20mM KH<sub>2</sub>PO<sub>4</sub> (10/90)  
 Temperature: 37 °C  
 Detection: UV at 260 nm  
 Sample: 0.1 ~ 0.2 mg/ml

### Separation of non steroidal anti inflammatory drugs (NSAIDs)



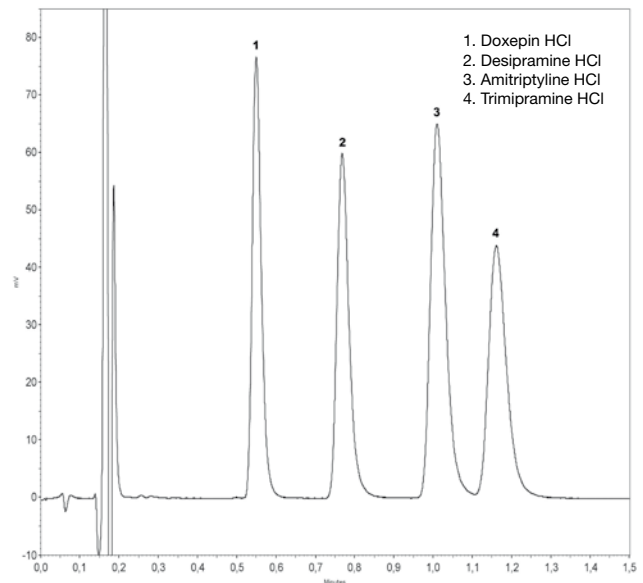
#### Method (Gradient method, with 4 NSAIDs)

Column: YMC-UltraHT Pro C18 (2 µm, 12 nm) 50 x 2.0 mm ID  
 Part No.: AS12S020502WT  
 Mobile Phase: A: acetonitrile; B: formic acid (0,15%)  
 Gradient:

Time	A (in %)	B (in %)
0.0	20	80
0.1	20	80
0.4	90	10
1.2	90	10

Flow rate: 0.8 ml/min  
 Detection: UV at 260 nm  
 Pressure: 420 bar  
 Injection: 1 µl  
 Temperature: 40 °C

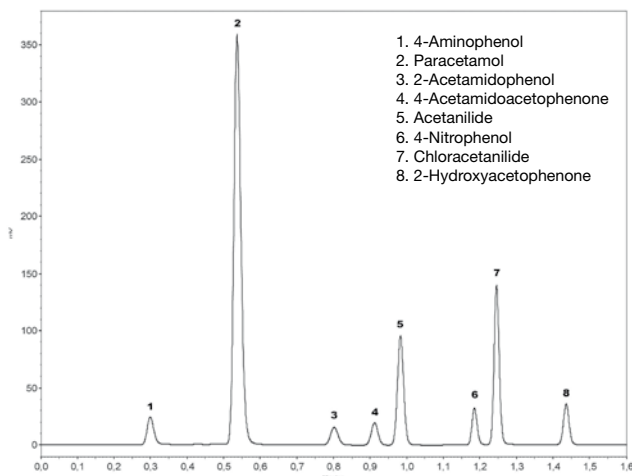
### Separation of tricyclic antidepressants



Column: YMC-UltraHT Pro C18 (2 µm, 12 nm) 50 x 2.0 mm ID  
 Part No.: AS12S020502WT  
 Mobile Phase: ACN / 20mM NH<sub>4</sub>CH<sub>2</sub>COO + CH<sub>3</sub>COOH (pH 4.6) (35/65)  
 Flow rate: 0.7 ml/min  
 Detection: UV at 215 nm  
 Pressure: 440 bar  
 Injection: 1 µl  
 Temperature: 40 °C

# Selected separations of pharmaceuticals

## Paracetamol and impurities

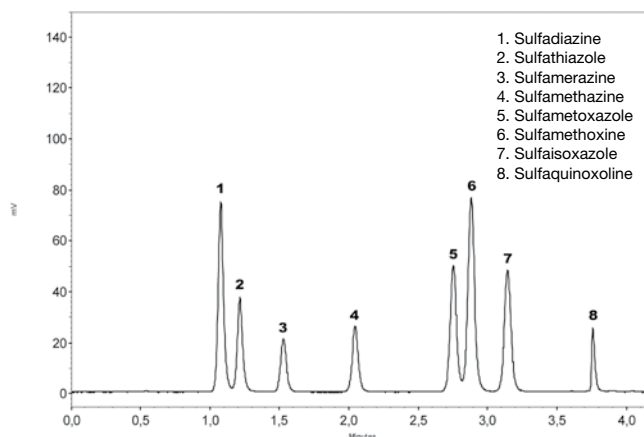


Column: YMC-UltraHT Pro C18 (2  $\mu$ m, 12 nm) 75 x 2.0 mm ID  
 Part No.: AS12S02L502WT  
 Mobile Phase: A: acetonitrile; B: formic acid (pH 2.8)  
 Gradient: 

Time	A (in %)	B (in %)
0.0	42	58
0.1	42	58
0.5	80	20
1.5	80	20

  
 Flow rate: 0.5 ml/min  
 Detection: UV at 254 nm  
 Pressure: 420 bar  
 Injection: 1  $\mu$ l  
 Temperature: 40 °C

## Sulfa drugs

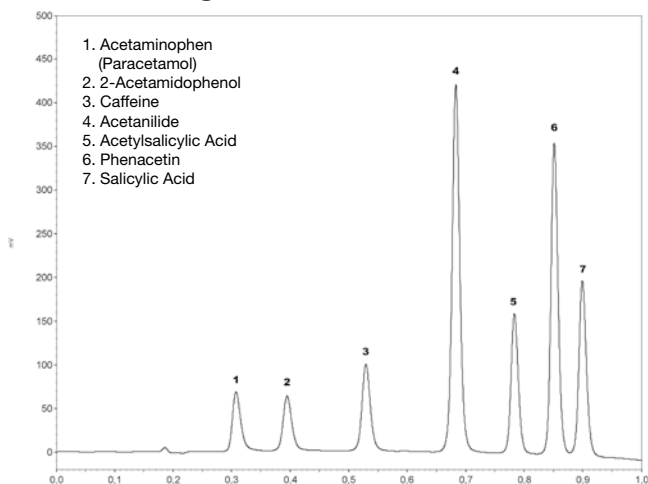


Column: YMC-UltraHT Hydrosphere C18 (2  $\mu$ m, 12 nm) 75 x 2.0 mm ID  
 Part No.: HS12S02L502WT  
 Mobile Phase: A: methanol; B: formic acid (pH 2.5)  
 Gradient: 

Time	A (in %)	B (in %)
0.0	18	82
0.1	18	82
2.8	42	56
2.9	100	0
4.0	100	0

  
 Flow rate: 0.45 ml/min  
 Detection: UV at 280 nm  
 Pressure: 460 bar  
 Injection: 1  $\mu$ l  
 Temperature: 40 °C

## Seven analgics

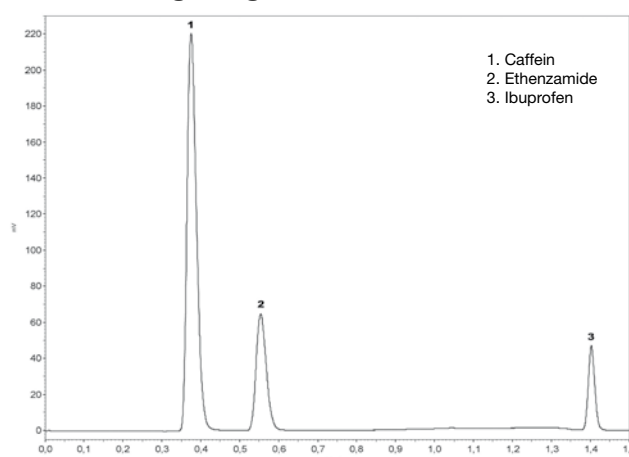


Column: YMC-UltraHT Hydrosphere C18 (2  $\mu$ m, 12 nm) 50 x 2.0 mm ID  
 Part No.: HS12S020502WT  
 Mobile Phase: A: acetonitrile; B: formic acid (pH 2.5)  
 Gradient: 

Time	A (in %)	B (in %)
0.0	25	75
1.0	90	10
1.2	90	10

  
 Flow rate: 0.8 ml/min  
 Detection: UV at 240 nm  
 Pressure: 350 bar  
 Injection: 1  $\mu$ l  
 Temperature: 40 °C

## Pain killing drugs



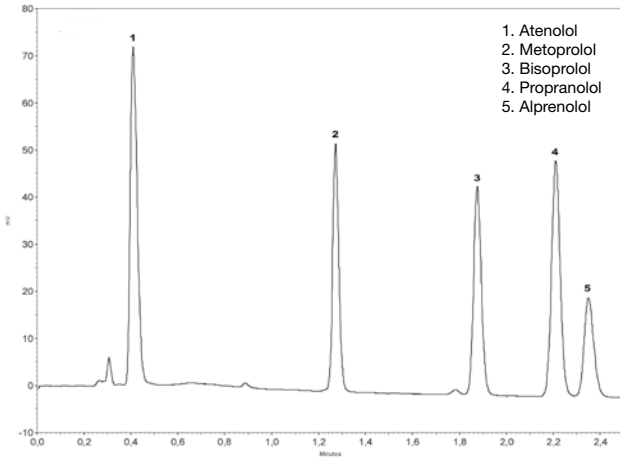
Column: YMC-UltraHT Pro C18 (2  $\mu$ m, 12 nm) 50 x 2.0 mm ID  
 Part No.: AS12S020502WT  
 Mobile Phase: A: methanol; B: formic acid pH (0.15%)  
 Gradient: 

Time	A (in %)	B (in %)
0.0	62	38
0.2	62	38
0.5	100	0
1.5	100	0
2.0	62	38

  
 Flow rate: 0.5 ml/min  
 Detection: UV at 254 nm  
 Pressure: 390 bar  
 Injection: 1  $\mu$ l  
 Temperature: 40 °C



**Beta-blockers**

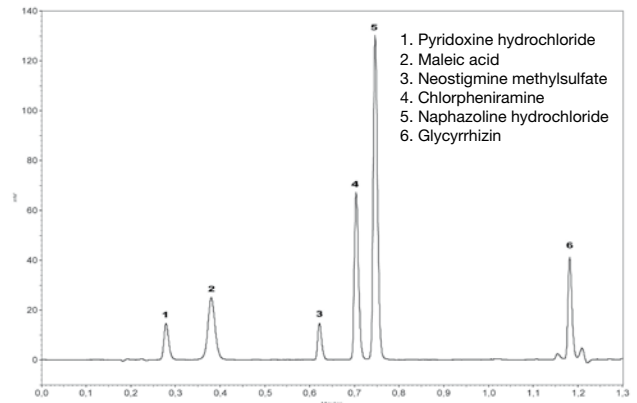


Column: YMC-UltraHT Pro C18 (2 µm, 12 nm) 50 x 2.0 mm ID  
 Part No.: AS12S020502WT  
 Mobile Phase: A: methanol; B: trifluoroacetic acid (0.05%)  
 Gradient: 

Time	A (in %)	B (in %)
0.0	30	70
0.1	30	70
0.2	55	45
2.5	55	45
3.0	30	70

  
 Flow rate: 0.5 ml/min  
 Detection: UV at 254 nm  
 Pressure: 390 bar  
 Injection: 1 µl  
 Temperature: 40 °C

**Ingredients in an eye drop formulation**

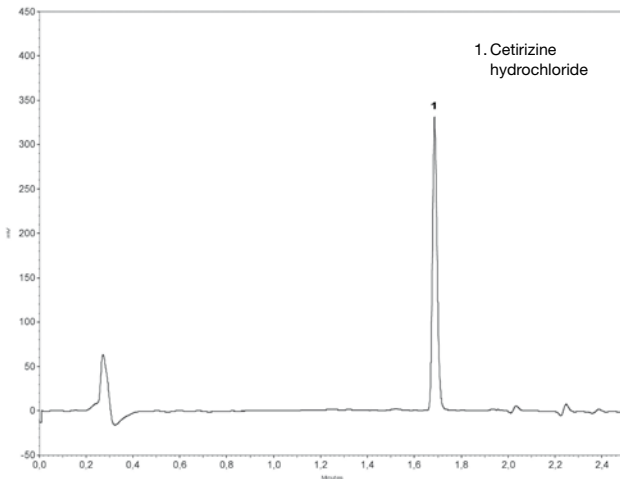


Column: YMC-UltraHT Hydrosphere C18 (2 µm, 12 nm) 50 x 2.0 mm ID  
 Part No.: HS12S020502WT  
 Mobile Phase: A: acetonitrile; B: 0.05% trifluoroacetic acid  
 Gradient: 

Time	A (in %)	B (in %)
0.0	10	90
0.1	10	90
0.3	50	50
0.8	50	50
0.9	90	10
1.3	90	10

  
 Flow rate: 0.85 ml/min  
 Detection: UV at 265 nm  
 Pressure: 390 bar  
 Injection: 1 µl  
 Temperature: 40 °C

**Analysis of Zyrtec® – Gradient Method**

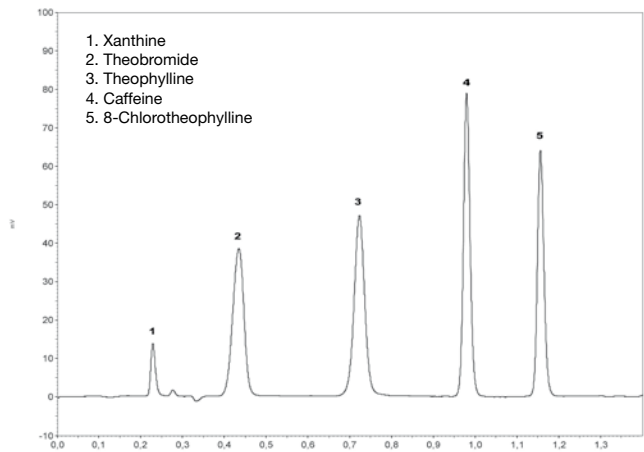


Column: YMC-UltraHT Pro C18 (2 µm, 12 nm) 50 x 2.0 mm ID  
 Part No.: AS12S020502WT  
 Mobile Phase: A: methanol; B: formic acid (pH 2.8)  
 Gradient: 

Time	A (in %)	B (in %)
0.0	30	70
0.2	30	70
0.7	90	10
2.0	90	10

  
 Flow rate: 0.5 ml/min  
 Detection: UV at 230 nm  
 Pressure: 380 bar  
 Injection: 1 µl  
 Temperature: 40 °C

**Xanthines**

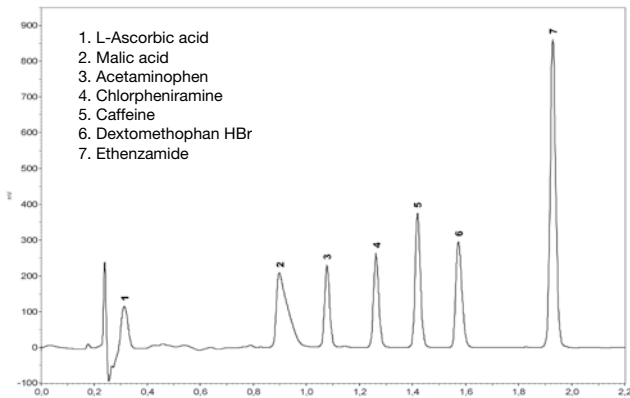


Column: YMC-UltraHT Hydrosphere C18 (2 µm, 12 nm) 50 x 2.0 mm ID  
 Part No.: HS12S020502WT  
 Mobile Phase: A: methanol; B: formic acid (pH 2.5)  
 Gradient: 

Time	A (in %)	B (in %)
0.0	20	80
0.4	20	80
0.7	70	30
1.3	70	30

  
 Flow rate: 0.72 ml/min  
 Detection: UV at 254 nm  
 Pressure: 470 bar  
 Injection: 1 µl  
 Temperature: 40 °C

**Ingredients of a cough cold medication**



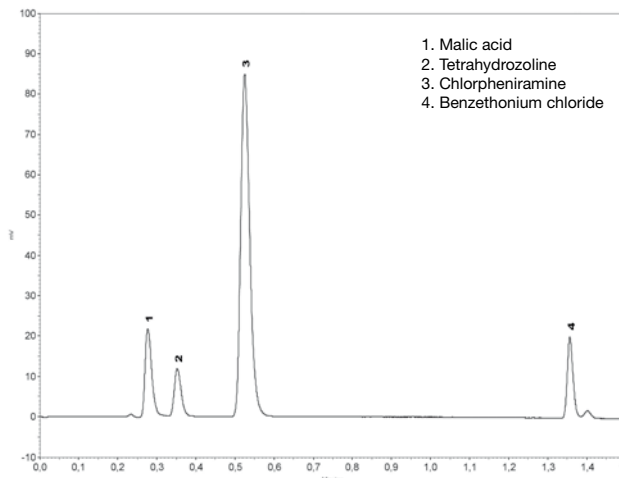
1. L-Ascorbic acid
2. Malic acid
3. Acetaminophen
4. Chlorpheniramine
5. Caffeine
6. Dextromethophan HBr
7. Ethenzamide

Column: YMC-UltraHT Hydrosphere C18 (2 µm, 12 nm)  
 50 x 2.0 mm ID  
 Part No.: HS12S020502WT  
 Mobile Phase: A: methanol + 0.15% formic acid  
 B: formic acid (0,15%)  
 Gradient: 

Time	A (in %)	B (in %)
0.0	10	90
0.6	55	45
1.2	55	45
2.0	90	10
2.2	90	10

  
 Flow rate: 0.65 ml/min  
 Detection: UV at 230 nm  
 Pressure: 400 bar  
 Injection: 1 µl  
 Temperature: 40 °C

**Ingredients of a nasal spray**



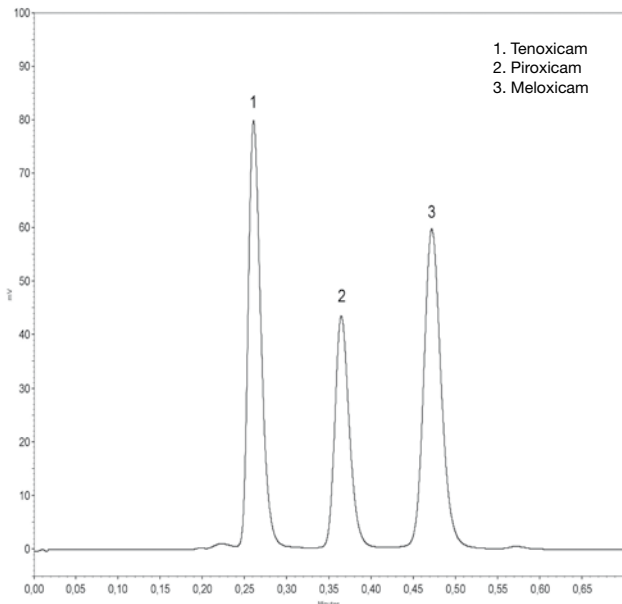
1. Malic acid
2. Tetrahydrozoline
3. Chlorpheniramine
4. Benzethonium chloride

Column: YMC-UltraHT Pro C18 (2 µm, 12 nm) 50 x 2.0 mm ID  
 Part No.: AS12S020502WT  
 Mobile Phase: A: methanol; B: trifluoroacetic acid (0,05%)  
 Gradient: 

Time	A (in %)	B (in %)
0.0	50	50
0.2	50	50
0.5	90	10
1.8	90	10

  
 Flow rate: 0.6 ml/min  
 Detection: UV at 260 nm  
 Pressure: 450 bar  
 Injection: 1 µl  
 Temperature: 40 °C

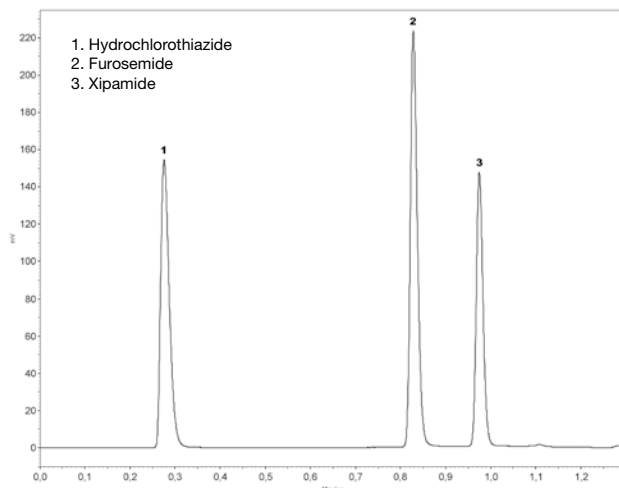
**Separation of 3 NSAIDs of the oxicam type**



1. Tenoxicam
2. Piroxicam
3. Meloxicam

Column: YMC-UltraHT Pro C18 (2 µm, 12 nm) 50 x 2.0 mm ID  
 Part No.: AS12S020502WT  
 Mobile Phase: methanol / formic acid (0.15%) (75/25)  
 Flow rate: 0.7 ml / min  
 Detection: UV at 260 nm  
 Pressure: 460 bar  
 Injection: 1 µL  
 Temperatur: 40 °C

**Diuretics**



1. Hydrochlorothiazide
2. Furosemide
3. Xipamide

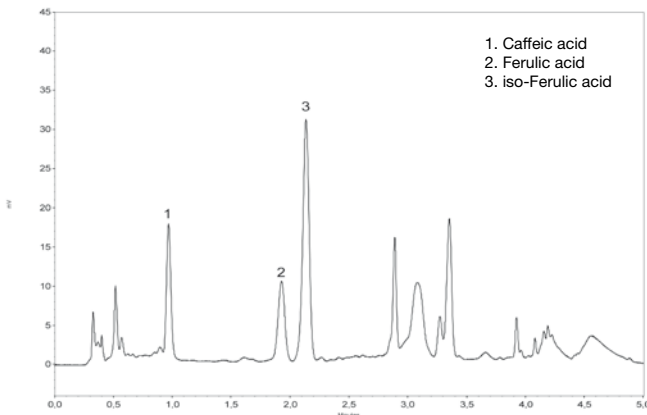
Column: YMC-UltraHT Pro C18 (12 nm, 2 µm) 50 x 2.0 mm ID  
 Part No.: AS12S020502WT  
 Mobile Phase: A: methanol; B: acetic acid 1% (pH 2.7)  
 Gradient: 

Time	A (in %)	B (in %)
0.0	50	50
0.1	50	50
0.3	95	05
1.1	95	05

  
 Flow rate: 0.6 ml/min  
 Detection: UV at 280 nm  
 Pressure: 440 bar  
 Injection: 1 µl  
 Temperature: 40 °C



**Phenolic acids in a herbal drug**

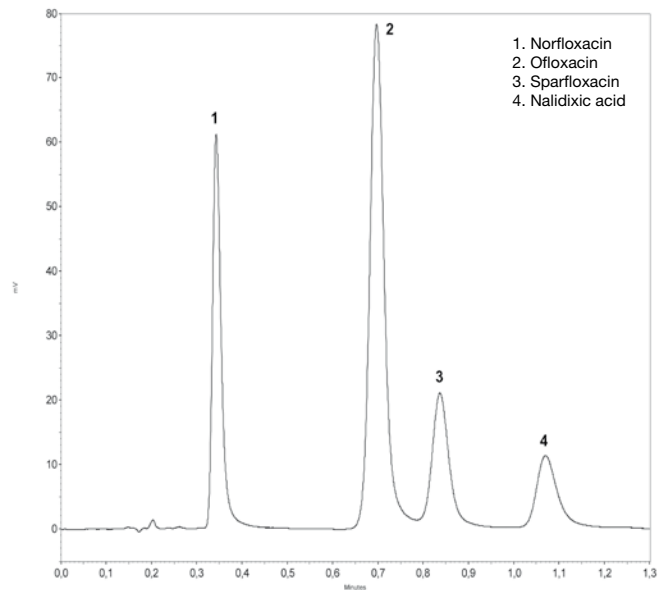


Column: YMC-UltraHT Pro C18 (12 nm, 2 μm)  
75 x 2.0 mm ID  
Part No.: AS12S02L502WT  
Mobile Phase: A: acetonitrile; B: acetic acid (0.1%/pH 3.25)  
Gradient:

Time	A (in %)	B (in %)
0.0	20	80
0.5	20	80
1.0	45	55
3.0	45	55
3.5	90	10
5.0	90	10

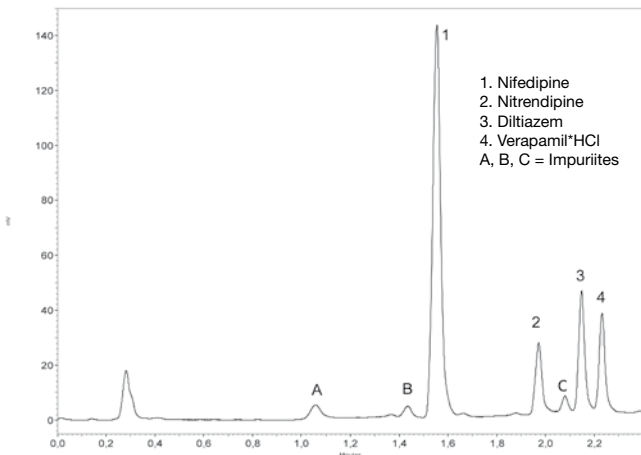
Flow rate: 0.4 ml/min  
Detection: UV at 320 nm  
Pressure: 450 bar  
Injection: 1 μl  
Temperature: 30 °C

**Antibiotics of the quinolone type**



Column: YMC-UltraHT Pro C18 (12 nm, 2 μm) 50 x 2.0 mm ID  
Part No.: AS12S020502WT  
Mobile Phase: acetonitrile / 20mM K<sub>2</sub>HPO<sub>4</sub> \* KH<sub>2</sub>PO<sub>4</sub> (pH 7.5) (20/80)  
Flow rate: 0.75 ml/min  
Detection: UV at 260 nm  
Pressure: 430 bar  
Injection: 1 μl  
Temperature: 40 °C

**Calcium antagonist**

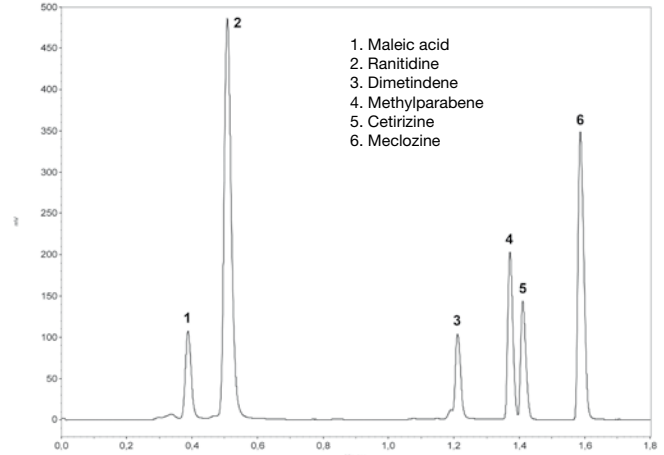


Column: YMC-UltraHT Hydrosphere C18 (12 nm, 2 μm)  
50 x 2.0 mm ID  
Part No.: HS12S020502WT  
Mobile Phase: A: methanol; B: 0.02M Na<sub>2</sub>HPO<sub>4</sub> - H<sub>3</sub>PO<sub>4</sub> (pH 7.5)  
Gradient:

Time	A (in %)	B (in %)
0.0	48	52
0.2	48	52
1.8	95	05
2.5	95	05

Flow rate: 0.5 ml/min  
Detection: UV at 270 nm  
Pressure: 470 bar  
Injection: 1 μl  
Temperature: 40 °C

**Ingredients of several antihistaminic drugs**

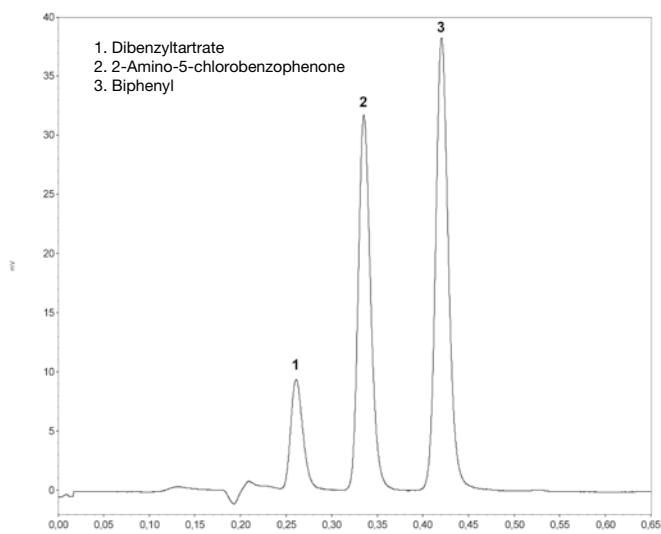


Column: YMC-UltraHT Pro C18 (12 nm, 2 μm)  
75 x 2.0 mm ID  
Part No.: AS12S02L502WT  
Mobile Phase: A: acetonitrile; B: 0.02M Na<sub>2</sub>HPO<sub>4</sub> - H<sub>3</sub>PO<sub>4</sub> (pH 2.5)  
Gradient:

Time	A (in %)	B (in %)
0.0	15	85
0.6	72	28
1.8	95	5
1.9	95	5

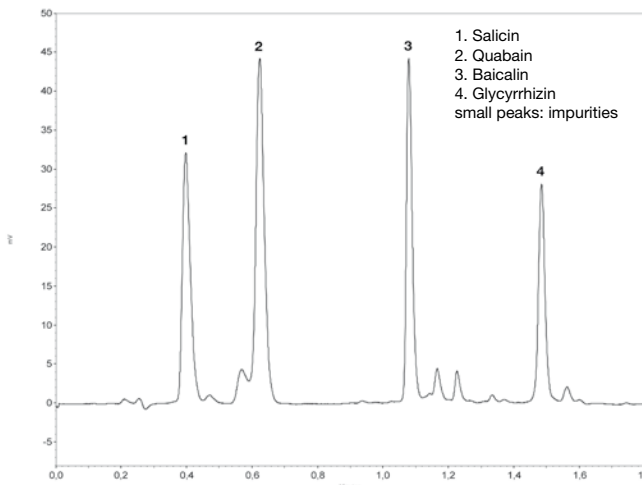
Flow rate: 0.5 ml/min  
Detection: UV at 220 nm  
Pressure: 460 bar  
Injection: 1 μl  
Temperature: 40 °C

### Separation of Dibenzyltartrate, 2-Amino-5-chlorobenzophenone, Biphenyl



Column: YMC-UltraHT Hydrosphere C18 (12 nm, 2 µm)  
50 x 2.0 mm ID  
Part No.: HS12S020502WT  
Mobile Phase: methanol / water (85/15)  
Flow rate: 0.7 ml/min  
Detection: 254 nm  
Pressure: 400 bar  
Injection: 1 µl  
Temperature: 40 °C

### Glycosides

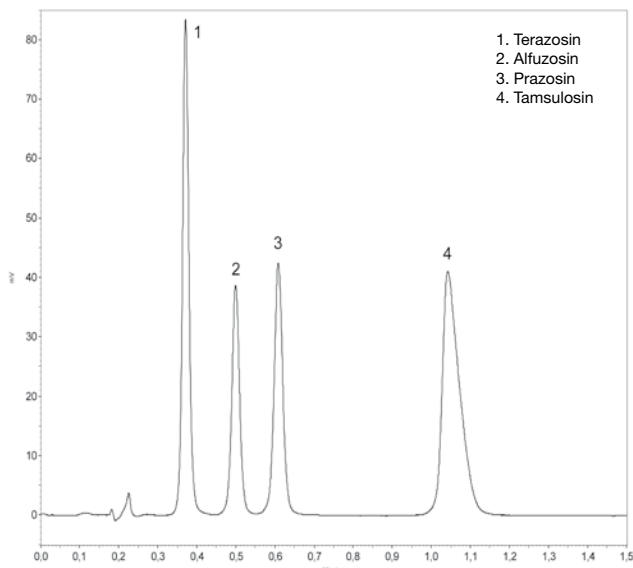


Column: YMC-UltraHT Pro C18 (12 nm, 2 µm) 50 x 2.0 mm ID  
Part No.: AS12S020502WT  
Mobile Phase: A: methanol; B: formic acid (0.15%/pH 2.5)  
Gradient: 

Time	A (in %)	B (in %)
0	35	65
0.5	95	5
1.8	95	5

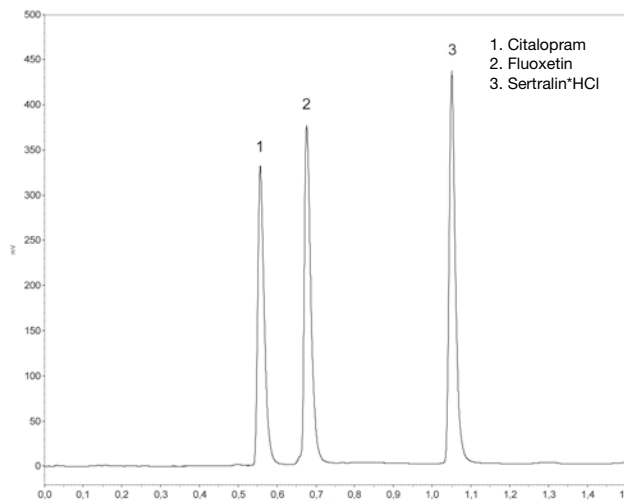
  
Flow rate: 0.5 ml/min  
Detection: 250 nm  
Pressure: 400 bar  
Injection: 1 µl  
Temperature: 40 °C

### Separation of Alpha-1 blockers



Column: YMC-UltraHT Hydrosphere C18 (12 nm, 2 µm)  
50 x 2.0 mm ID  
Part No.: HS12S020502WT  
Mobile Phase: acetonitrile / 0,05% TFA pH 2.15 (25/75)  
Flow rate: 0.7 ml/min  
Detection: UV at 240 nm  
Pressure: 400 bar  
Injection: 1 µl  
Temperature: 40 °C

### Antidepressives



Column: YMC-UltraHT Pro C18 (12 nm, 2 µm) 50 x 2.0 mm ID  
Part No.: AS12S020502WT  
Mobile Phase: A: acetonitrile; B: 20mM Na<sub>2</sub>HPO<sub>4</sub> + H<sub>3</sub>PO<sub>4</sub> (pH 7.0)  
Gradient: 

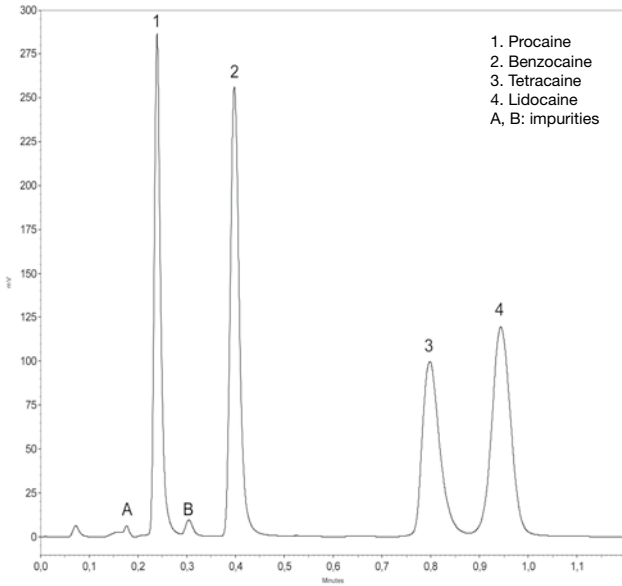
Time	A (in %)	B (in %)
0	40	60
0.8	90	10
1.3	90	10

  
Flow rate: 0.7 ml/min  
Detection: 230 nm  
Pressure: 400 bar  
Injection: 1 µl  
Temperature: 40 °C



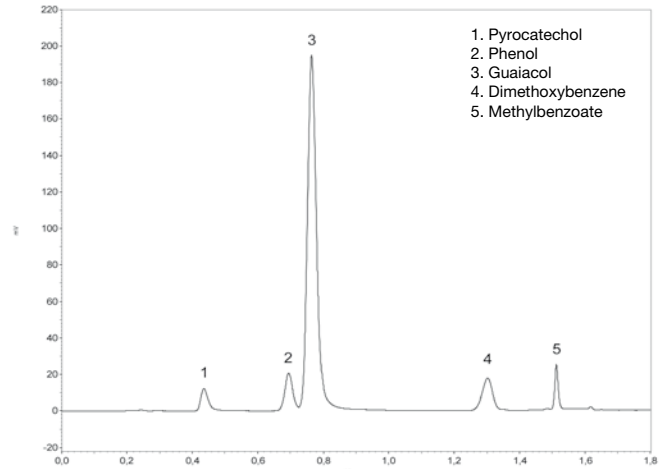


**Local anesthetics**



Column: YMC-UltraHT Pro C18 (12 nm, 2 µm) 50 x 2.0 mm ID  
 Part No.: AS12S020502WT  
 Mobile Phase: acetonitrile / 20mM Na<sub>2</sub>HPO<sub>4</sub> + H<sub>3</sub>PO<sub>4</sub>, pH 7,0 (48/52)  
 Flow rate: 0.75 ml/min  
 Detection: 220 nm  
 Pressure: 390 bar  
 Injection: 1 µl  
 Temperature: 40°C

**Guaiaicol and impurities**

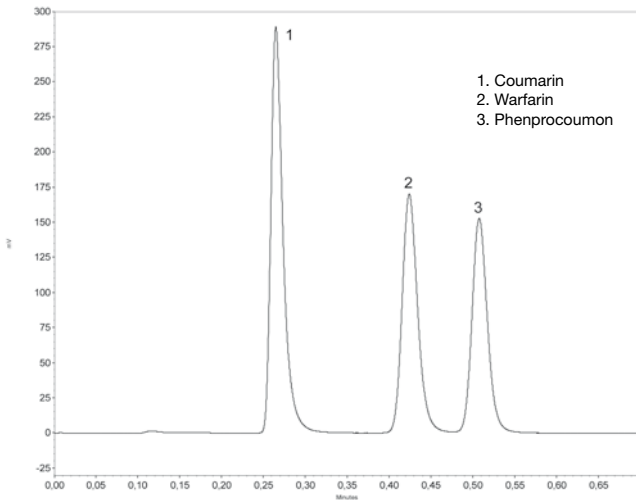


Column: YMC-UltraHT Pro C18 (12 nm, 2 µm) 50 x 2.0 mm ID  
 Part No.: AS12S020502WT  
 Mobile Phase: A: acetonitrile; B: water  
 Gradient: 

Time	A (in %)	B (in %)
0	35	65
0.8	35	65
1.0	95	05
1.6	95	05

  
 Flow rate: 0.5 ml/min  
 Detection: 254 nm  
 Pressure: 445 bar  
 Injection: 1 µl  
 Temperature: 40 °C

**Coumarins**

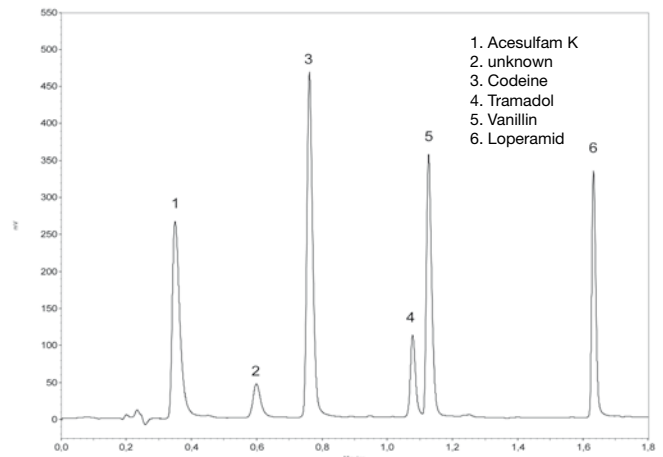


Column: YMC-UltraHT Pro C18 (12 nm, 2 µm) 50 x 2.0 mm ID  
 Part No.: AS12S020502WT  
 Mobile Phase: A: acetonitrile; B: 0.2% formic acid (pH 2.5)  
 Gradient: 

Time	A (in %)	B (in %)
0	60	40
0.5	80	20
0.7	80	20

  
 Flow rate: 0.75 ml/min  
 Detection: 260 nm  
 Pressure: 390 bar  
 Injection: 1 µl  
 Temperature: 40 °C

**Ingredients in opioid pharmaceuticals**

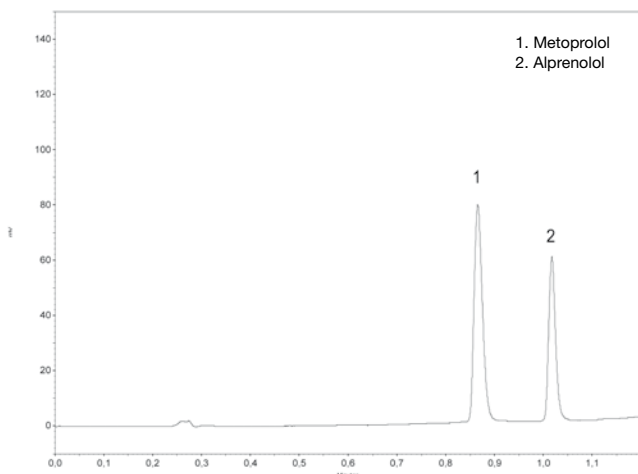


Column: YMC-UltraHT Pro C18 (12 nm, 2 µm) 50 x 2.0 mm ID  
 Part No.: AS12S020502WT  
 Mobile Phase: A: acetonitrile + 0,05% TFA (pH 2.15)  
 B: water + 0,05% TFA (pH 2.15)  
 Gradient: 

Time	A (in %)	B (in %)
0	12	88
0.1	12	88
1.5	90	10
1.8	90	10

  
 Flow rate: 0.6 ml/min  
 Detection: 210 nm  
 Pressure: 390 bar  
 Injection: 1 µl  
 Temperature: 40 °C

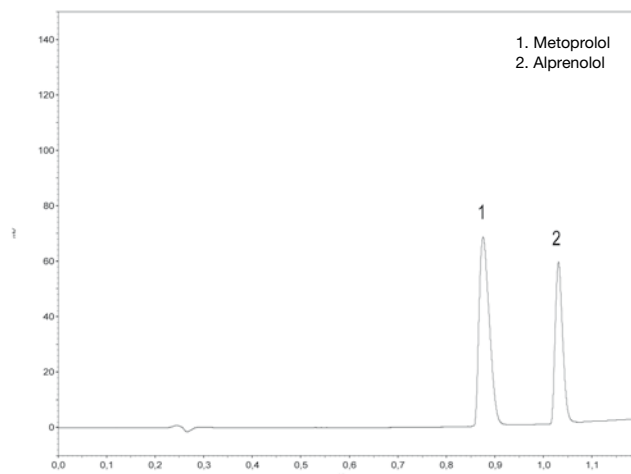
\* Application data by courtesy YMC Co., Ltd.

**Metoprolol and Alprenolol**

Column: YMC-UltraHT Hydrosphere C18 (12 nm, 2 µm)  
50 x 2.0 mm ID  
Part No.: HS12S020502WT  
Mobile Phase: A: water + 0,1% formic acid  
B: acetonitrile + 0,1% formic acid  
Gradient: 

Time	A (in %)	B (in %)
0	95	05
1.2	0	100

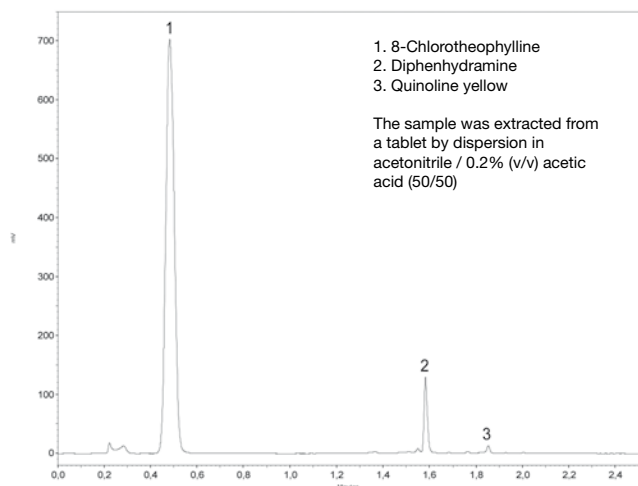
  
Flow rate: 0.6 ml/min  
Detection: 254 nm  
Pressure: 350 bar  
Injection: 1 µl  
Temperature: 25 °C

**Metoprolol and Alprenolol**

Column: YMC-UltraHT Pro C18 (12 nm, 2 µm)  
50 x 2.0 mm ID  
Part No.: AS12S020502WT  
Mobile Phase: A: water + 0,1% formic acid  
B: acetonitrile + 0,1% formic acid  
Gradient: 

Time	A (in %)	B (in %)
0	95	05
1.2	0	100

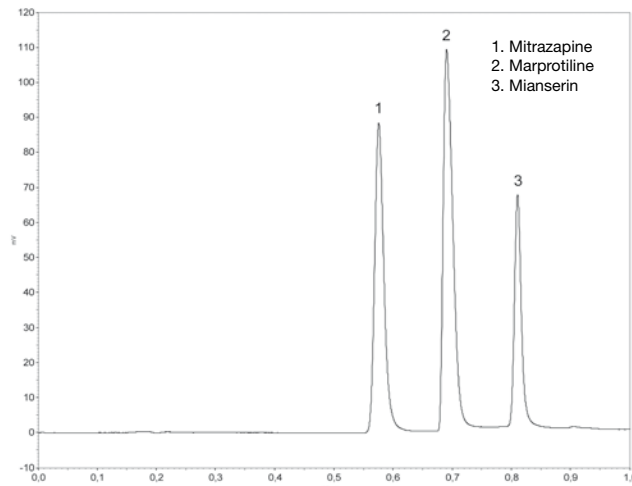
  
Flow rate: 0.6 ml/min  
Detection: 254 nm  
Pressure: 350 bar  
Injection: 1 µl  
Temperature: 25 °C

**Analysis of the antiemetic Vomex A®**

Column: YMC-UltraHT Pro C18 (12 nm, 2 µm) 50 x 2.0 mm ID  
Part No.: AS12S020502WT  
Mobile Phase: A: acetonitrile; B: 20mM NH<sub>4</sub> Ac (pH 6.8)  
Gradient: 

Time	A (in %)	B (in %)
0	10	90
0.5	10	90
1.5	90	10
2.2	90	10

  
Flow rate: 0.7 ml/min  
Detection: 260 nm  
Pressure: 420 bar  
Injection: 0.5 µl  
Temperature: 40 °C

**Tetracyclic antidepressants**

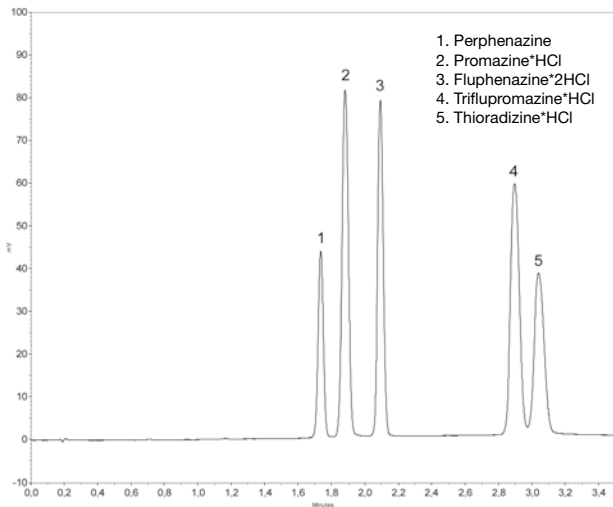
Column: YMC-UltraHT Pro C18 (12 nm, 2 µm) 50 x 2.0 mm ID  
Part No.: AS12S020502WT  
Mobile Phase: A: acetonitrile; B: 20mM NH<sub>4</sub>Ac + acetic acid (pH 5,4)  
Gradient: 

Time	A (in %)	B (in %)
0	32	68
0.1	32	68
0.5	98	02
0.9	98	02

  
Flow rate: 0.7 ml/min  
Detection: 254 nm  
Pressure: 420 bar  
Injection: 1 µl  
Temperature: 40 °C



**Phenothiazines**

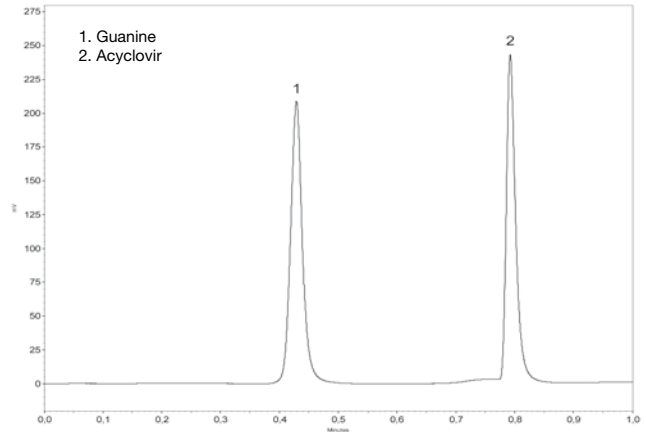


Column: YMC-UltraHT Pro C18 (12 nm, 2 µm) 50 x 2.0 mm ID  
 Part No.: AS12S020502WT  
 Mobile Phase: A: acetonitrile + 0.05% TFA; B: 0.05% TFA  
 Gradient: 

Time	A (in %)	B (in %)
0	25	75
2.0	40	60
3.3	40	60

  
 Flow rate: 0.7 ml/min  
 Detection: 254 nm  
 Pressure: 440 bar  
 Injection: 1 µl  
 Temperature: 40 °C

**Acyclovir from its major impurity Guanine**



Column: YMC-UltraHT Hydrosphere (12 nm, 2 µm)  
 75 x 2.0 mm ID  
 Part No.: HS12S02L502WT  
 Mobile Phase: A: acetonitrile + 0.1% formic acid (v/v)  
 B: 0.1% formic acid (v/v)  
 Gradient: 

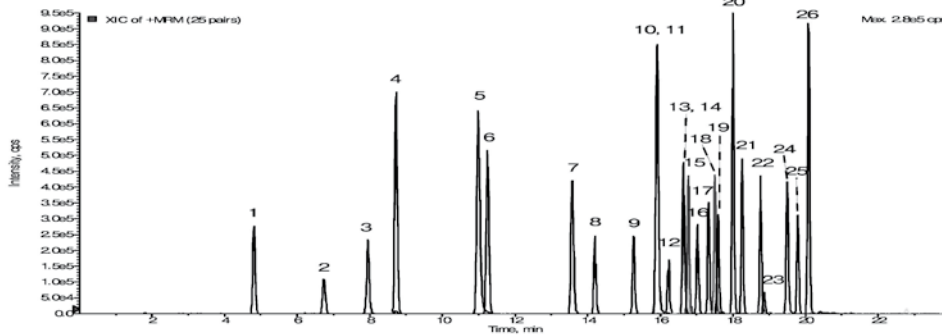
Time	A (in %)	B (in %)
0	01	99
0.1	01	99
0.2	50	50
1.0	50	50

  
 Flow rate: 0.55 ml/min  
 Detection: 254 nm  
 Pressure: 450 bar  
 Injection: 1 µl  
 Temperature: 40 °C

\* Application data by courtesy YMC Co., Ltd.

# Pesticides

## Simultaneous separation of pesticides by LC/MS



Column: YMC-Pack Pro C18 (3  $\mu$ m, 12 nm) 150  $\times$  2.0 mm ID  
 Part No.: AS12S031502WT  
 Eluent: A: 5mM CH<sub>3</sub>COONH<sub>4</sub> in water; B: 5mM CH<sub>3</sub>COONH<sub>4</sub> in methanol  
 Gradient:

Time	A (in %)	B (in %)
0.0	85	15
1.0	60	40
3.5	60	40
6.0	50	50
8.0	45	55
17.5	5	95
22.0	5	95

Flow rate: 0.2 ml/min

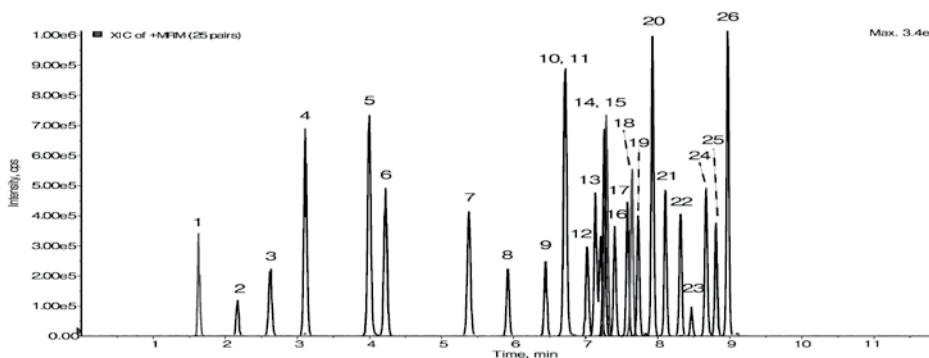
Temperature: ambient

Detection: API5000, ESI, Positive, MRM

Injection: 3  $\mu$ l (10 ng/ml)

Sample: Pesticide Mixture Standard Solution PL-7-2, manufactured by Wako Pure Chemical Industries, Ltd.

1. Thiamethoxam (Q1/Q3 : 292.3/211.3)
2. Clothianidin (Q1/Q3 : 250.3/169.1)
3. Chloridazon (Q1/Q3 : 222.3/77.0)
4. Thiachlorid (Q1/Q3 : 253.3/126.0)
5. Thiabendazole (Q1/Q3 : 202.3/175.2)
6. Azamethiphos (Q1/Q3 : 325.2/183.0)
7. Dimethirimol (Q1/Q3 : 210.4/71.1)
8. Isoxaflutole (Q1/Q3 : 360.2/251.1)
9. Pyrifthalid (Q1/Q3 : 319.3/139.1)
10. (E)-Ferimzone (Q1/Q3 : 255.4/91.1)
11. (Z)-Ferimzone (Q1/Q3 : 255.4/91.1)
12. Methoxyfenozide (Q1/Q3 : 369.4/149.3)
13. Iprovalicarb (Q1/Q3 : 321.4/119.3)
14. Chromafenozide (Q1/Q3 : 395.4/175.1)
15. Butafenacil (Q1/Q3 : 492.1/331.1)
16. Simeconazole (Q1/Q3 : 249.3/70.1)
17. Cyazofamid (Q1/Q3 : 325.2/108.0)
18. Naproanilide (Q1/Q3 : 292.3/171.3)
19. Fenoxycarb (Q1/Q3 : 302.3/88.1)
20. Anilofos (Q1/Q3 : 368.2/199.1)
21. Cyflufenamid (Q1/Q3 : 431.3/295.2)
22. Pyrazolynate (Q1/Q3 : 439.1/91.0)
23. Indoxacarb (Q1/Q3 : 528.1/203.2)
24. Benzofenap (Q1/Q3 : 431.2/105.1)
25. Furathiocarb (Q1/Q3 : 383.3/195.2)
26. Cloquintocet-mexyl (Q1/Q3 : 336.3/238.2)



Column: YMC-UltraHT Pro C18 (2  $\mu$ m, 12 nm) 75  $\times$  2.0 mm ID  
 Part No.: AS12S02L502WT  
 Eluent: A: 5mM CH<sub>3</sub>COONH<sub>4</sub> in water; B: 5mM CH<sub>3</sub>COONH<sub>4</sub> in methanol  
 Gradient:

Time	A (in %)	B (in %)
0.0	85	15
0.50	60	40
1.75	60	40
3.0	50	50
4.0	45	55
8.75	5	95
11.0	5	95

Flow rate: 0.4 ml/min

Temperature: ambient

Detection: API5000, ESI, Positive, MRM

Injection: 3  $\mu$ l (10 ng/ml)

Sample: Pesticide Mixture Standard Solution PL-7-2, manufactured by Wako Pure Chemical Industries, Ltd.

1. Thiamethoxam (Q1/Q3 : 292.3/211.3)
2. Clothianidin (Q1/Q3 : 250.3/169.1)
3. Chloridazon (Q1/Q3 : 222.3/77.0)
4. Thiachlorid (Q1/Q3 : 253.3/126.0)
5. Thiabendazole (Q1/Q3 : 202.3/175.2)
6. Azamethiphos (Q1/Q3 : 325.2/183.0)
7. Dimethirimol (Q1/Q3 : 210.4/71.1)
8. Isoxaflutole (Q1/Q3 : 360.2/251.1)
9. Pyrifthalid (Q1/Q3 : 319.3/139.1)
10. (E)-Ferimzone (Q1/Q3 : 255.4/91.1)
11. (Z)-Ferimzone (Q1/Q3 : 255.4/91.1)
12. Methoxyfenozide (Q1/Q3 : 369.4/149.3)
13. Iprovalicarb (Q1/Q3 : 321.4/119.3)
14. Chromafenozide (Q1/Q3 : 395.4/175.1)
15. Butafenacil (Q1/Q3 : 492.1/331.1)
16. Simeconazole (Q1/Q3 : 249.3/70.1)
17. Cyazofamid (Q1/Q3 : 325.2/108.0)
18. Naproanilide (Q1/Q3 : 292.3/171.3)
19. Fenoxycarb (Q1/Q3 : 302.3/88.1)
20. Anilofos (Q1/Q3 : 368.2/199.1)
21. Cyflufenamid (Q1/Q3 : 431.3/295.2)
22. Pyrazolynate (Q1/Q3 : 439.1/91.0)
23. Indoxacarb (Q1/Q3 : 528.1/203.2)
24. Benzofenap (Q1/Q3 : 431.2/105.1)
25. Furathiocarb (Q1/Q3 : 383.3/195.2)
26. Cloquintocet-mexyl (Q1/Q3 : 336.3/238.2)

### Conclusion

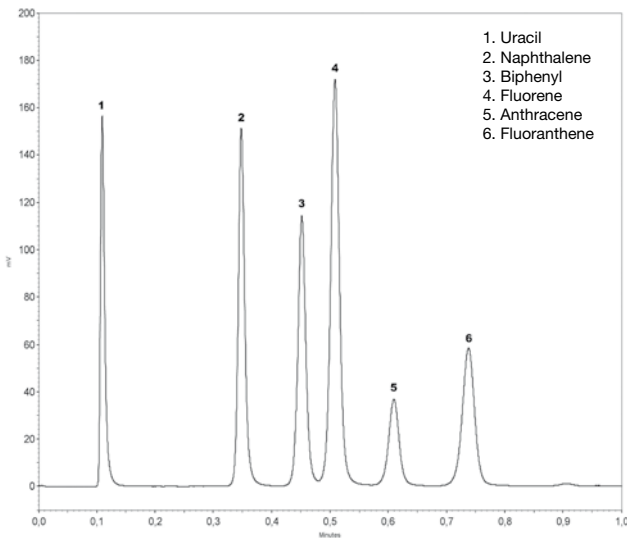
This example again shows the simple method transfer from a conventional HPLC method developed on YMC-Pack Pro C18 3  $\mu$ m to an ultra-fast LC application. The use of the new YMC UltraHT Pro C18, with its particle size of 2  $\mu$ m enables downscaling without any further method development.

The combination of small particles and short column lengths results in shorter retention times without compromising resolution.



# Selected environmental separations

## Separation of Uracil and 5 Hydrocarbons with YMC Hydrosphere C18

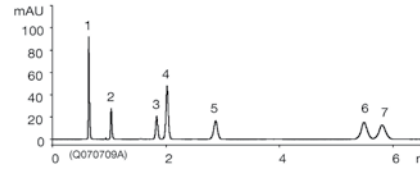


1. Uracil
2. Naphthalene
3. Biphenyl
4. Fluorene
5. Anthracene
6. Fluoranthene

Column: YMC-UltraHT Hydrosphere C18 (2  $\mu$ m, 12 nm)  
 50 x 2.0 mm ID  
 Part No.: HS12S020502WT  
 Mobile Phase: acetonitrile / water (70/30)  
 Flow rate: 1.2 ml/min  
 Detection: UV at 270 nm  
 Pressure: 410 bar  
 Injection: 0.8  $\mu$ l  
 Temperature: 40  $^{\circ}$ C

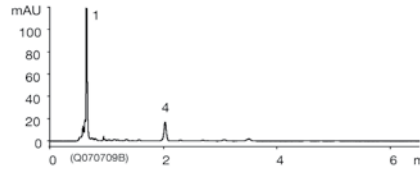
## Metabolites of organic solvents in human urine

A) Standard (50  $\mu$ g/mL)

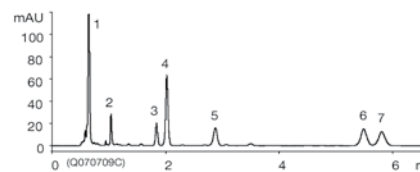


1. C1=NC2=C(N1)C(=O)N(C2)O Creatinine
2. O=C(O)c1ccccc1 Mandelic acid
3. O=C(O)c1ccc(cc1)C(=O)O Phenylglyoxylic acid
4. O=C(O)c1ccc(cc1)C(=O)O Hippuric acid
5. O=C(O)c1ccc(cc1)C(=O)O o-Methylhippuric acid
6. O=C(O)c1ccc(cc1)C(=O)O p-Methylhippuric acid
7. O=C(O)c1ccc(cc1)C(=O)O m-Methylhippuric acid

B) Blank urine (diluted 10 times with water)

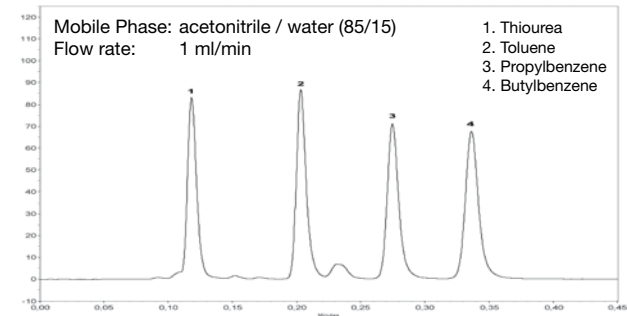


C) Spiked blank urine (50  $\mu$ g/mL)

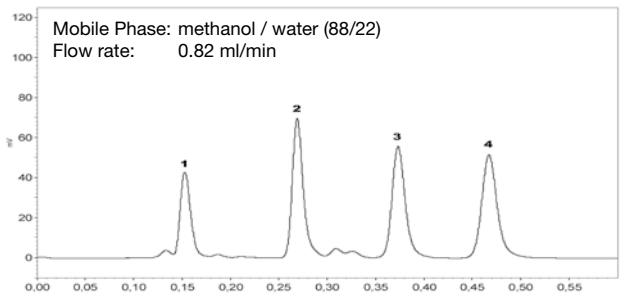


Column: YMC-UltraHT Hydrosphere C18 (2  $\mu$ m, 12 nm)  
 100 x 2.0 mm ID  
 Part No.: HS12S021002WT  
 Eluent: 20mM  $\text{CH}_3\text{COONH}_4$  / 2-propanol (97/3)  
 Flow rate: 0.4 ml/min  
 Temperature: 35  $^{\circ}$ C  
 Detection: UV at 225 nm  
 Injection: 1  $\mu$ l

## Thiourea & 3 alkylbenzenes



1. Thiourea
2. Toluene
3. Propylbenzene
4. Butylbenzene



Column: YMC-UltraHT Pro C18 (2  $\mu$ m, 12 nm) 50 x 2.0 mm ID  
 Part No.: AS12S020502WT  
 Detection: 254 nm  
 Injection: 1  $\mu$ l  
 Temperature: 40  $^{\circ}$ C

\* Application data by courtesy YMC Co., Ltd.

# Xanthine oxidase

In addition to diabetes and hypertension, gout has become one of the diseases of civilisation that is probably due to dietary habits. The symptoms have been known for many hundreds of years and most of the mechanisms have been elucidated. Gout is caused by an increase of uric acid (hyperuricemia)

in the blood stream. This results in the crystallisation of uric acid and monosodium urate in the joints, tendons and surrounding tissue, causing swelling and severe pain. The reason for the presence of uric acid in our metabolism is connected to the purine degradation process, which ends at uric acid (see fig. 1).

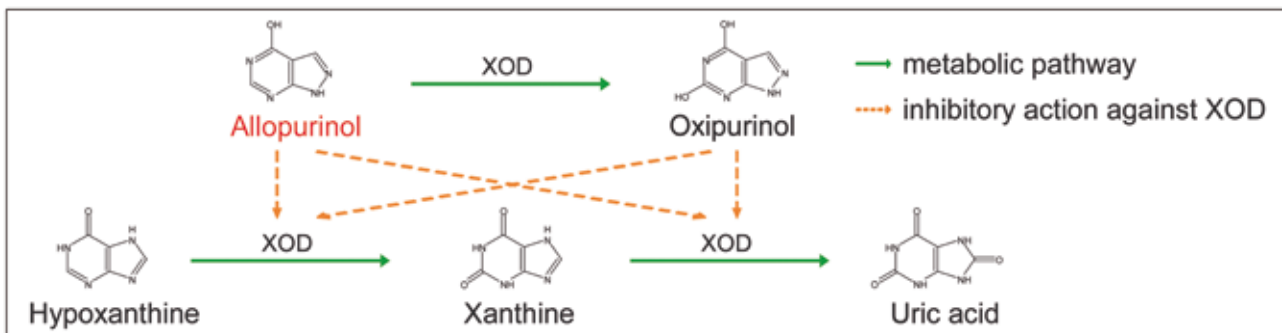


Figure 1: Xanthine oxidase inhibitor and related metabolites

One of the degradation mechanisms is controlled by the enzyme xanthine oxidase, which converts hypoxanthine via xanthine to uric acid. Allopurinol, a structural isomer of hypoxanthine which is also a xanthine oxidase inhibitor is often administered to avoid an uric acid excess or pain in passing urine (see fig. 1).

Therefore, it is most important to have an analytical method, which monitors all of these related metabolites.

## Results

The isocratic method below has been successfully developed using Hydrosphere C18. The application enables a baseline separation of all five metabolites (see fig. 2).

This method is useful for analysing xanthine oxidase metabolites and monitoring xanthine oxidase inhibitors and their related metabolites.

## Method Transfer

For transfer the above application developed for conventional LC systems onto ultra-fast LC systems, YMC-UltraHT Hydrosphere C18 is the column of choice. As this is the same sorbent, with the

same degree of selectivity is provided, the method transfer is very easy. The separation remains exactly the same but takes only 4 minutes instead of 12 minutes (see fig. 2).

## Method transfer from conventional LC to ultra-fast LC

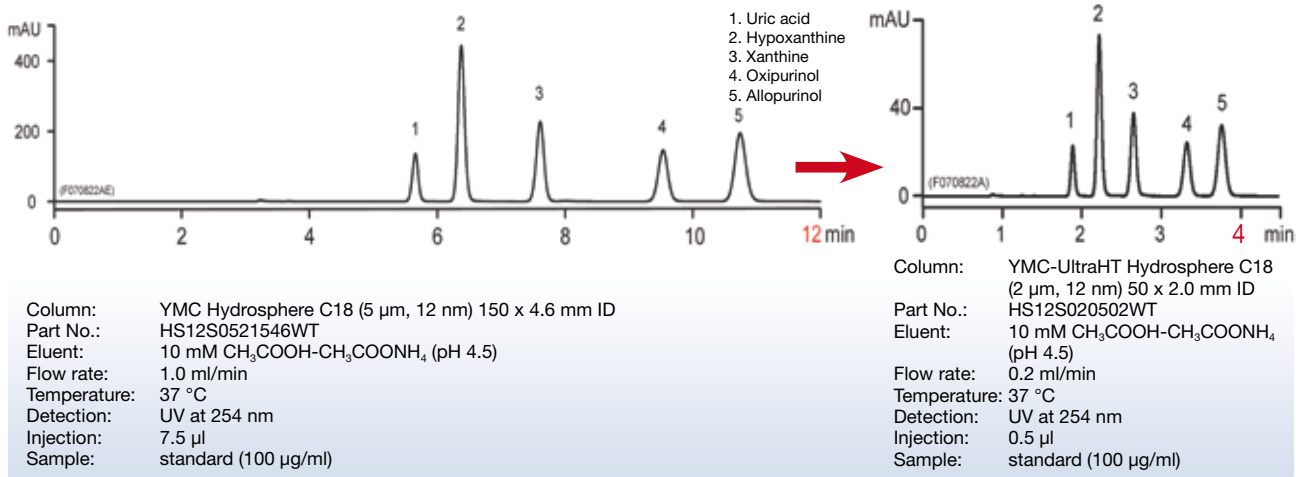


Figure 2





In order to optimise the application further, the flow rate can be increased by the factor 3. A further

retention time reduction can be achieved without compromising the resolution (see fig. 3).

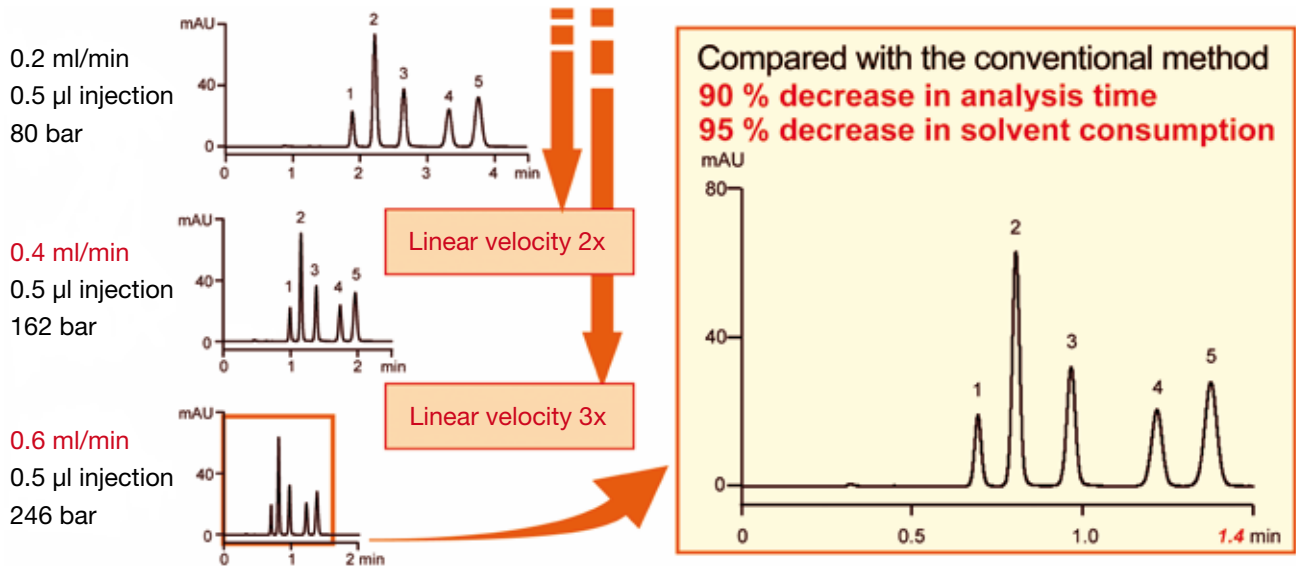
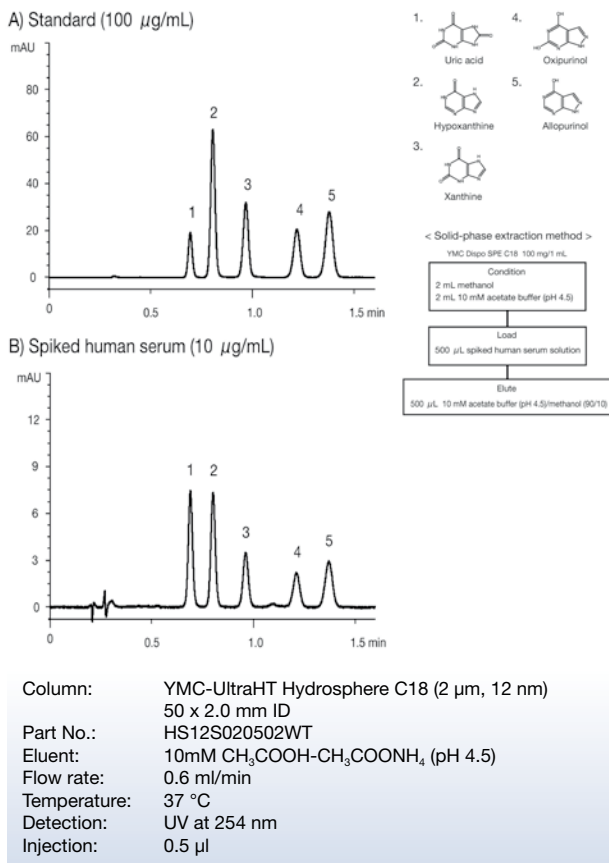


Figure 3: Ultra-fast LC optimisation

### Xanthine oxidase inhibitor and metabolites in human serum



\* Application data by courtesy YMC Co., Ltd.



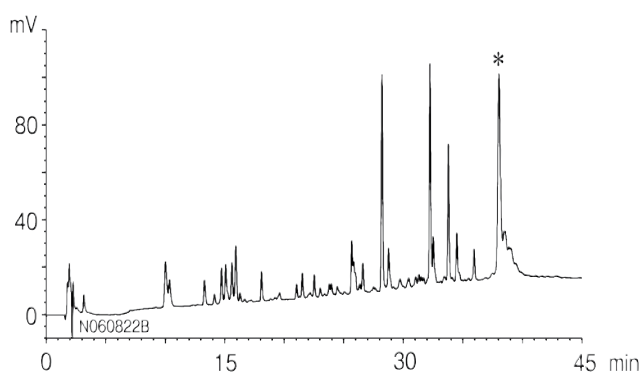
# Peptide mapping

In order to succeed in developing protein-based drugs, a fast and easy detection of alteration within the protein structure is necessary. In many cases, peptide mapping is the method of choice, where proteins are analysed after digestion. The small peptide units are examined using LC-MS. It is equally important to employ a powerful MS instrument together with a highly efficient high resolving column, as a result of, not only the large number of peptide subunit produced, but also the wide variety of modified structures which can occur.

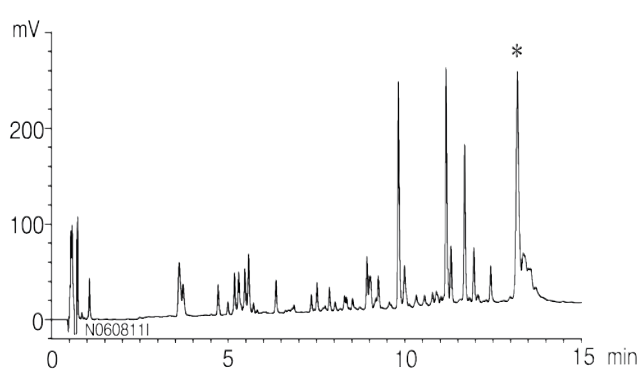
All HPLC separations have been performed on a Jasco X-LC system. The gradient was formed from acetonitrile and water with TFA as an ion pair agent. The gradient conditions are described in figure 1. The conventional HPLC method was carried out using a 150 x 4.6 mm ID column packed with 5- $\mu$ m YMC-Pack Pro C18.

The conventional HPLC method described above was easily transferred to an ultra-fast method using a short YMC UltraHT Pro C18, in order to lower solvent consumption.

## Peptide mapping - excellent reproducibility between 5 $\mu$ m and 2 $\mu$ m



\*undigested Cytochrome c



\*undigested Cytochrome c

Column: YMC-UltraHT Pro C18 (5  $\mu$ m, 12 nm) 150 x 2.0 mm ID  
 Part No.: AS12S051502QT  
 Eluent: A) acetonitrile / water / trifluoroacetic acid (10/90/0.1)  
 B) acetonitrile / water / trifluoroacetic acid (35/65/0.1)  
 Gradient: 

Time	A (in %)	B (in %)
0	100	0
5	100	0
40	0	100
45	0	100

  
 Flow rate: 0.2 ml/min  
 Temperature: 37 °C  
 Detection: UV at 220 nm  
 Injection: 1  $\mu$ l  
 Sample: Tryptic digest of Cytochrome c

Column: YMC-UltraHT Pro C18 (2  $\mu$ m, 12 nm) 50 x 2.0 mm ID  
 Part No.: AS12S020502WT  
 Eluent: A) acetonitrile / water / trifluoroacetic acid (10/90/0.1)  
 B) acetonitrile / water / trifluoroacetic acid (35/65/0.1)  
 Gradient: 

Time	A (in %)	B (in %)
0	100	0
1.65	100	0
13.35	0	100
15.00	0	100

  
 Flow rate: 0.2 ml/min  
 Temperature: 37 °C  
 Detection: UV at 220 nm  
 Injection: 1  $\mu$ l  
 Sample: Tryptic digest of Cytochrome c

# Ordering Information Ultra Fast LC

## YMC-UltraHT Pro C18, 12 nm, 2 µm

Column ID	Column length (mm)				
	30	50	75	100	150
2.0 mm	AS12S020302WT	AS12S020502WT	AS12S02L502WT	AS12S021002WT	AS12S021502WT
3.0 mm		AS12S020503WT	AS12S02L503WT	AS12S021003WT	AS12S021503WT

## YMC-UltraHT Hydrosphere C18, 12 nm, 2 µm

Column ID	Column length (mm)				
	30	50	75	100	150
2.0 mm	HS12S020302WT	HS12S020502WT	HS12S02L502WT	HS12S021002WT	HS12S021502WT
3.0 mm		HS12S020503WT	HS12S02L503WT	HS12S021003WT	HS12S021503WT

# Substance Index

Acacetin	5	Dihydroquinone	2,14	4-Nitrophenol	16
Acesulfame K	12,21	Diltiazem	19	Nordihydroguaiaretic acid (NDGA)	8
4-Acetamidoacetophenone	16	Dimethirimol	24	Norfloxacin	19
2-Acetamidophenol	16	Dimethoxybenzene	21	n-Octyl gallate (OG)	8
Acetaminophen (Paracetamol)	16,18	Dimetindene	19	Ofloxacin	19
Acetaminophenol	18	1,3-Dimethyluric acid	10	Oxipurinol	26,27
4-Aminophenol	16	1,7-Dimethylxanthine	10	Panthenol	10
Acetanilide	16,18	Diphenhydramine	22	Paracetamol	16
6'-O-Acetylaidzin	6,7	Doxepin HCl	15	Phenacetin	16,18
6'-O-Acetylgenistin	6,7	(E)-Ferimzone	24	Phenol	21
6'-O-Acetylglycitin	6,7	Ethenzamide	16,18	Phenprocoumon	21
Acetylsalicylic acid	16,18	Ethylbenzoat	13	Phenylbenzoat	13
Acyclovir	23	Fenoxycarb	24	Phenylglyoxylic acid	25
Alfuzosin	20	(Z)-Ferimzone	24	Perphenazine	23
Allopurinol	26,27	Ferulic acid	10,11,19	Piroxicam	15,18
Alprenolol	17,22	Flavin adenine disodium	12	Prazosin	20
2-Amino-5-chlorobenzophenone	20	Flavin mononucleotide, sodium salt	10	Procaine	21
4-Aminobenzoic acid	12	Flavin mononucleotide	12	Promazine*HCl	23
Amitriptyline HCl	15	Fluoranthene	25	Propranolol	17
Anilofos	24	Fluorene	25	Propylbenzene	25
Anthracene	25	Fluoxetin	20	Propylbenzoat	13
Apigetin	5	Fluphenazine*2HCl	23	n-Propylgallate (PG)	8
Ascorbic acid	10	Furathiocarb	24	Protocatechuic acid	10,11
L-Ascorbic acid	18	Furosemide	18	Pyrazolynate	24
Aspartam	12	Gallic acid	10,11,13	Pyridoxal HCl	12
Atenolol	17	Genistein	6,7	Pyridoxine HCl / Pyridoxine hydrochloride	10,12,17
Atropine	2,14	Genistin	6,7	Pyrifitalid	24
Azamehiphos	24	Gentisic acid	13	Pyrocatechol	21
Baicalin	5	Glycitein	6,7	Quabain	20
Baicalin	20	Glycyrrhizin	17,20	Quercetin	5
Benzaldehyde	12	Glyzitin	6,7	Quinine	2,14
Benzethonium chloride	18	Guaiaacol	21	Quinoline yellow	22
Benzoate	12	Guanine	23	Ranitidine	19
Benzocaine	21	Hippuric acid	25	Riboflavin	12
Benzoferap	24	Hydrochlorothiazide	18	Saccharin	12
Benzoic acid	13	2-Hydroxyacetophenone	16	Salicin	20
Benzylbenzoat	13	4-Hydroxybenzoic acid	10,11	Salicylic acid	10,11,13,16,19
Biphenyl	25	4-Hydroxymethyl-2,6-di-tert-butylphenol (HMBP)	8	Scopolamine	2,14
Bisdemethoxycurcumin	9	Hypoxanthine	26,27	Sertralin*HCl	20
Bisoprolol	17	Ibuprofen	16	Simeconazole	24
Butafenacil	24	Indoxacarb	24	Sinapinic acid	10,11
Butylbenzene	25	Iprovalicarb	24	Sorbate	12
Butylbenzoat	13	Iso-Ferulic acid	19	Sparfloxacin	19
tert-Butylhydroquinone (TBHQ)	8	Isoxalflutole	24	Sulfaamthoxine	16
Caffeic acid	10,11,19	Kaempferol	5	Sulfadiazine	16
Caffeine	10,12,16,17,18	Lidocaine	21	Sulfaisoxazole	16
Cefaclor	15	Loperamid	21	Sulfamerazine	16
Cephalexin	15	Maleic acid	17,19	Sulfamethazine	16
Cephaloglycin	15	Malic acid	18	Sulfametoxazole	16
Cephaloridine	15	6'-O-Malonyldaidzin	6,7	Sulfaquinoxoline	16
Cetirizine hydrochloride	17,19	6'-O-Malonylgenistin	6,7	Sulfathiazole	16
Chloracetanilide	16	6'-O-Malonylglycitin	6,7	Tamsulosin	20
Chloridazon	24	Mandelic acid	25	Tenoxicam	18
Chlorogenic acid	10,11	Marprotiline	22	Terazosin	20
8-Chlorotheophylline	17,22	Meclozine	19	Tetracaine	21
Chlorpheniramine	17,18	Melamine	9	Tetrahydrozoline	18
Chromafenozide	24	Meloxicam	15,18	Theobromide	10,17
Chrusin	5	Metamizol	15	Theophylline	17
Cinchonine	2,14	Methoxyfenozide	24	Thiabendazole	24
Cinnamic acid	10,11	Methylbenzoate	13,21	Thiacloprid	24
Citalopram	20	Methylparabene	19	Thiamethoxam	24
Cloquintocet-mexyl	24	m-Methylhippuric acid	25	Thioradizine*HCl	23
Clothianidin	24	o-Methylhippuric acid	25	Thiourea	25
p-Coumaric acid	10,11	p-Methylhippuric acid	25	Toluene	25
Creatinine	25	Metoprolol	17,22	Tramadol	21
Codeine	21	Mianserin	22	Triflupromanzine*HCl	23
Coumarin	21	Mitrazapine	22	2,4,5-Trihydroxybutyrophenone (THBP)	8
Curcumin	9	Myricetin	5	Trimipramine HCl	15
Cyazofamid	24	Nalidixic acid	19	Uracil	25
Cyflufenamid	24	Naphazoline hydrochloride	17	Uric acid	26,27
Cytochrome c	28	Naphthalene	25	Vanillic acid	10,11
Daidzein	6,7	Naproanilide	24	Vanillin	21
Daidzin	6,7	n-Dodecyl gallate (DG)	8	Verapamil*HCl	19
Demethoxycurcumin	9	Neostigmine methylsulfate	17	Warfarin	21
Desipramine HCl	15	Nicotinamide	10,12	Xanthine	10,17,26,27
Dextomethophan HBr	18	Nicotinic acid	12	Xipamide	18
Dibenzyltartrate	20	Nifedipine	19		
Diclofenac	15	Nitrendipine	19		

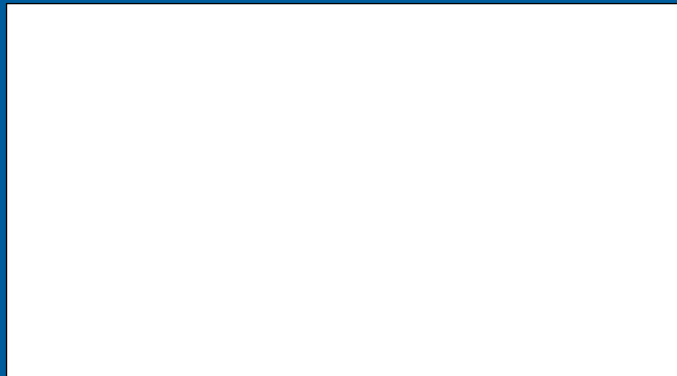
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