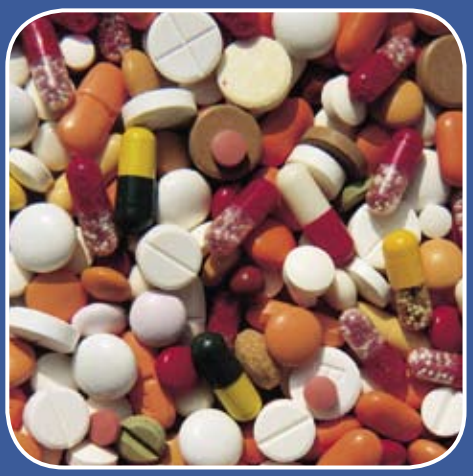
YMC EUROPE GMBH

Application Guide



Ultra Fast HPLC

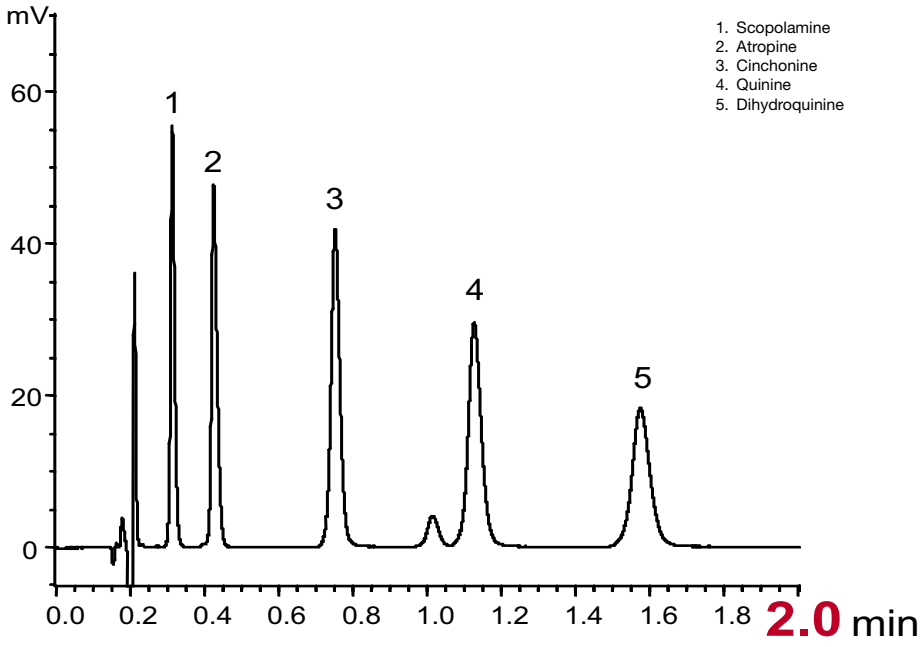
HPLC Columns for Ultra Fast LC

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 5800 psi (400 bar, 40 MPa). 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 µm or sub-2 µm 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 (2µm, 12nm) 50 x 2.0mm ID



Part No.:	AS12S020502WT
Eluent:	20 mM CH_3COOH-CH_3COONH_4 (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:	4350 psi (292 bar, 29.2 MPa)

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:

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

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

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

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

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

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

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

Where L_{HPLC} and L_{Fast} are the lengths of the HPLC and Ultra Fast LC columns, and t_{gHPLC} and t_{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:

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

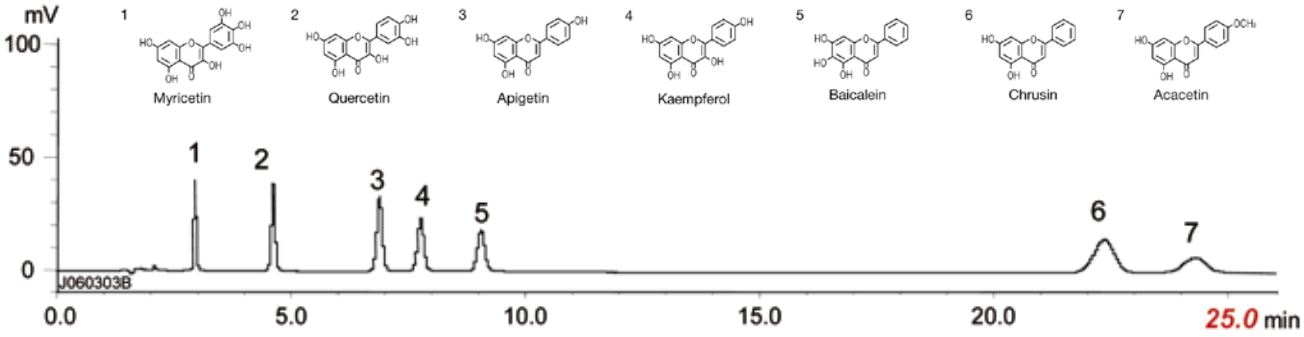
Where F_{Fast} and t_{gFast} are the flow rate and gradient time of the geometrically scaled values (typically 650 µl/min for small molecules on a 2.1 mm id column) and F_3 and t_{g3} are the optimised values.

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.

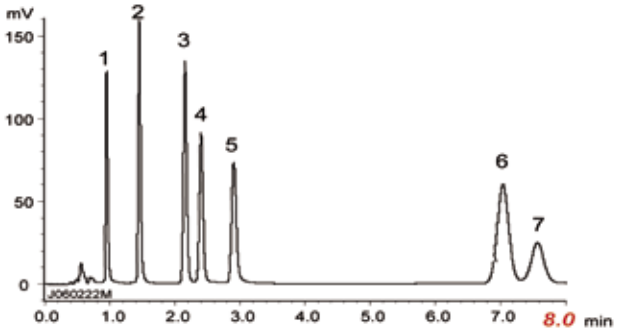
The conventional HPLC is carried out on 150 x 4.6 mm ID packed with 5 µm. The last eluting peak appears after 25 minutes.

Conventional LC method



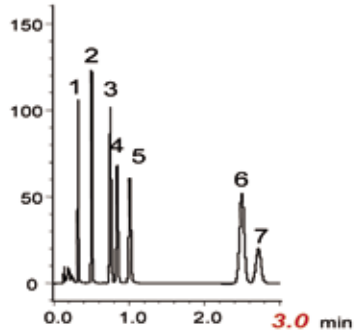
Column:	YMC-Pack *Pro* C18 (5 µm, 12 nm) 150 x 4.6 mm ID
Part No.:	AS12S051546WT
Flow rate:	1.0 ml/min
Injection:	5 µl
Eluent:	acetonitrile/water/formic acid (35/65/0.1)
Temperature:	40 °C
Detection:	UV at 260 nm
Sample:	0.05 mg/ml

Ultra-fast LC method



Column:	YMC UltraHT *Pro* C18 (2 µm, 12 nm) 50 x 2.0 mm ID
Part No.:	AS12S020502WT
Flow rate:	0.2 ml/min (1.06 mm/sec)
Injection:	1 µl
Eluent:	acetonitrile/water/formic acid (35/65/0.1)
Temperature:	40 °C
Detection:	UV at 260 nm

Ultra-fast LC method with increased flow



Column:	YMC UltraHT *Pro* C18 (2 µm, 12 nm) 50 x 2.0 mm ID
Part No.:	AS12S020502WT
Flow rate:	0.6 ml/min (3.18 mm/sec)
Injection:	1 µl
Eluent:	acetonitrile/water/formic acid (35/65/0.1)
Temperature:	40 °C
Detection:	UV at 260 nm

Conclusion

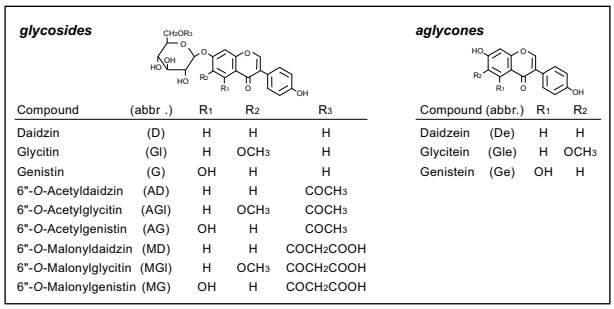
The method transfer from a classical HPLC method to an ultra-fast HPLC method for the analysis of 7 flavonoids has been demonstrated. The reduction of column length and increased efficiency of a 2 µm YMC-Pack *Pro* C18 column makes it possible to reduce the analysis time by a factor of almost 10, with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

* Application data by courtesy YMC Co., Ltd.

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

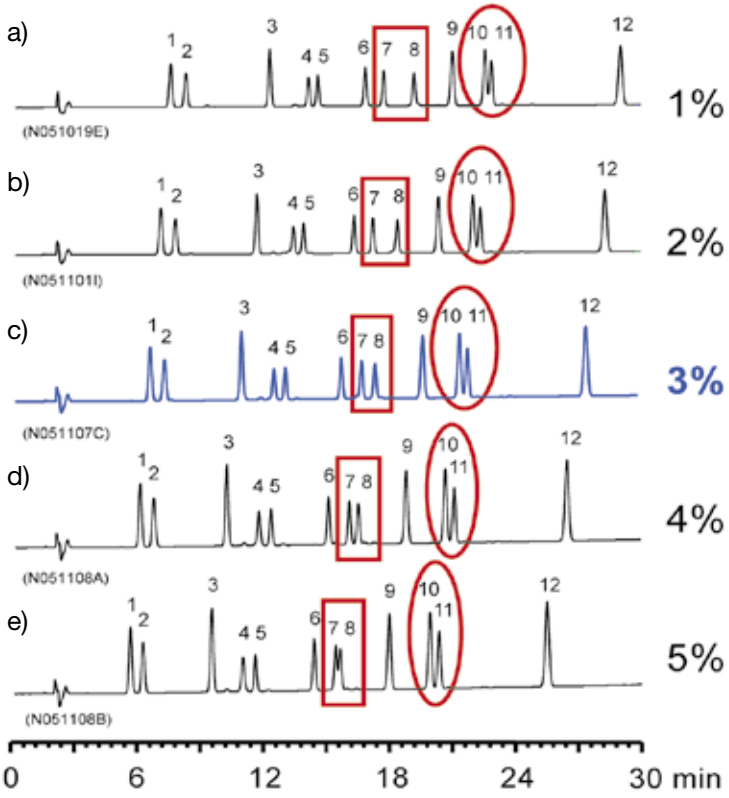


Compound	(abbr .)	R_1	R_2	R_3
Daidzin	(D)	H	H	H
Glycitin	(Gl)	H	OCH_3	H
Genistin	(G)	OH	H	H
6"-O-Acetyldaidzin	(AD)	H	H	$COCH_3$
6"-O-Acetylglycitin	(AGl)	H	OCH_3	$COCH_3$
6"-O-Acetylgenistin	(AG)	OH	H	$COCH_3$
6"-O-Malonyldaidzin	(MD)	H	H	$COCH_2COOH$
6"-O-Malonylglycitin	(MGl)	H	OCH_3	$COCH_2COOH$
6"-O-Malonylgenistin	(MG)	OH	H	$COCH_2COOH$

Compound	(abbr.)	R_1	R_2
Daidzein	(De)	H	H
Glycitein	(Gle)	H	OCH_3
Genistein	(Ge)	OH	H

Figure 1

Influence of acetic acid concentration on soy isoflavone separation



acetic acid concentration	Resolution (Rs) peak 7, 8	peak 10, 11
1%	5.82	1.04
2%	4.55	1.22
3%	**2.51**	**1.30**
4%	1.67	1.47
5%	*n.c.*	1.51

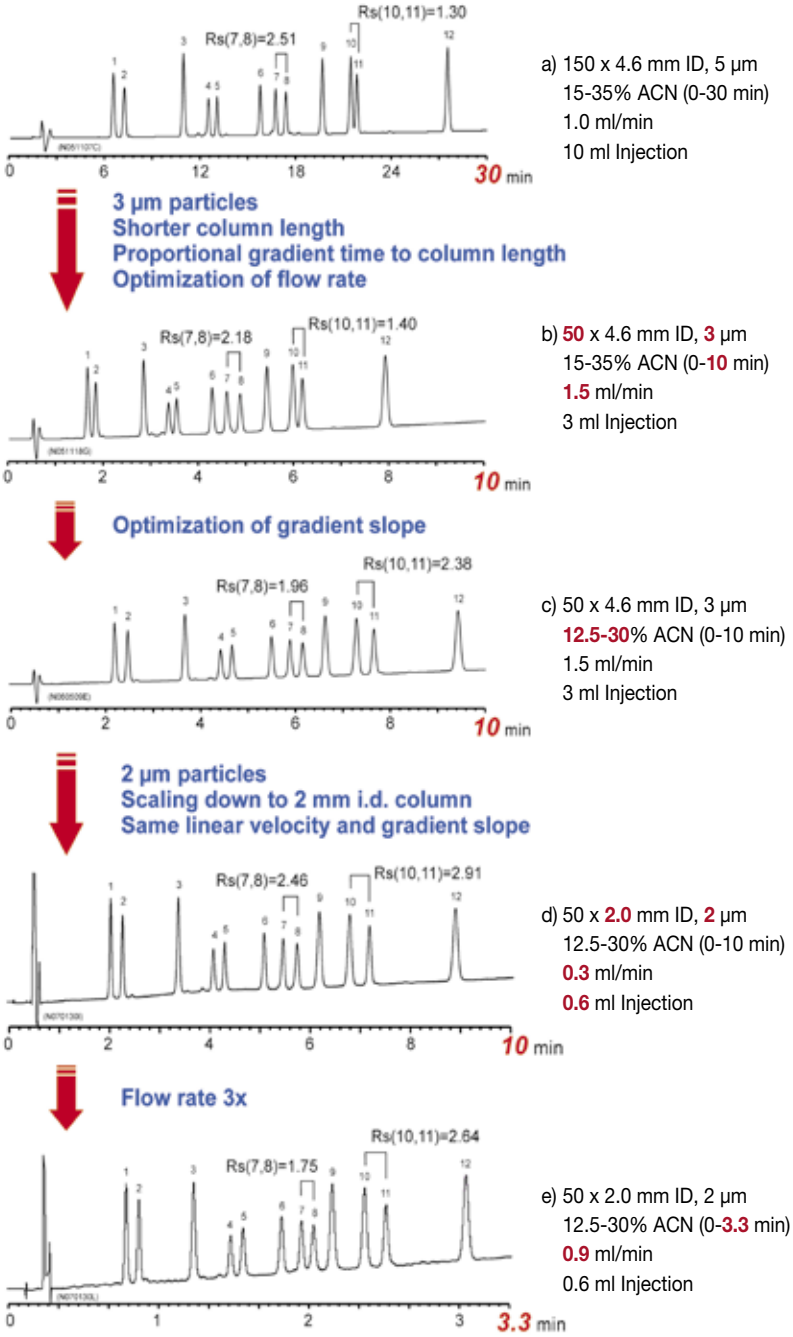
1. D
2. Gl
3. G
4. MD
5. MGl
6. AD
7. AGl
8. MG
9. De
10. Gle
11. AG
12. Ge

Column:	YMC Hydrosphere C18 (5 µm, 12 nm) 150 x 4.6 mm ID
Part No.:	HS12S051546WT
Flow rate:	1.0 ml/min
Temperature:	35 °C
Detection:	UV at 254 nm
Injection:	10 µl (0.01 mg/ml)
Eluent:	A) water/acetic acid B) acetonitrile/acetic acid
Gradient:	15-35% B (0-30 min)

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 (Glyciteine and 6˝-O-acetylgenistine) 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˝-OMalonylgenistine) 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



a) 150 x 4.6 mm ID, 5 µm
15-35% ACN (0-30 min)
1.0 ml/min
10 ml Injection

b) **50** x 4.6 mm ID, **3** µm
15-35% ACN (0-**10** min)
1.5 ml/min
3 ml Injection

c) 50 x 4.6 mm ID, 3 µm
12.5-30% ACN (0-10 min)
1.5 ml/min
3 ml Injection

d) 50 x **2.0** mm ID, **2** µm
12.5-30% ACN (0-10 min)
0.3 ml/min
0.6 ml Injection

e) 50 x 2.0 mm ID, 2 µm
12.5-30% ACN (0-**3.3** min)
0.9 ml/min
0.6 ml Injection

Figure 3

The analysis time of 30 min could be reduced substantially by conventional means of reducing particle size and column dimension (3 µm, 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 µm 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	7. AGI
2. GI	8. MG
3. G	9. De
4. MD	10. GIe
5. MGI	11. AG
6. AD	12. Ge

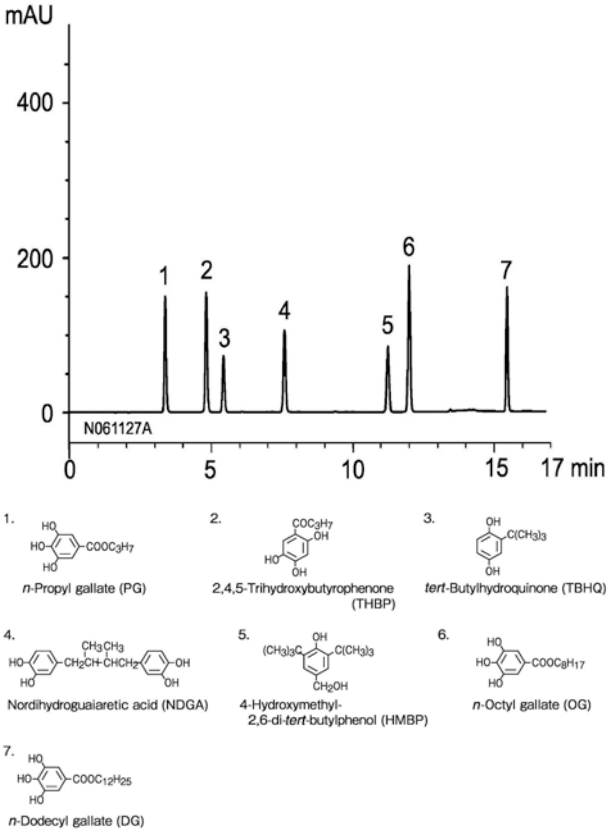
Column:	Hydrosphere C18 (12 nm)
Temp.:	35 °C
Detection:	UV at 254 nm
Eluent:	A) water/acetic acid (100/3)
	B) acetonitrile/acetic acid (100/3)

Conclusion

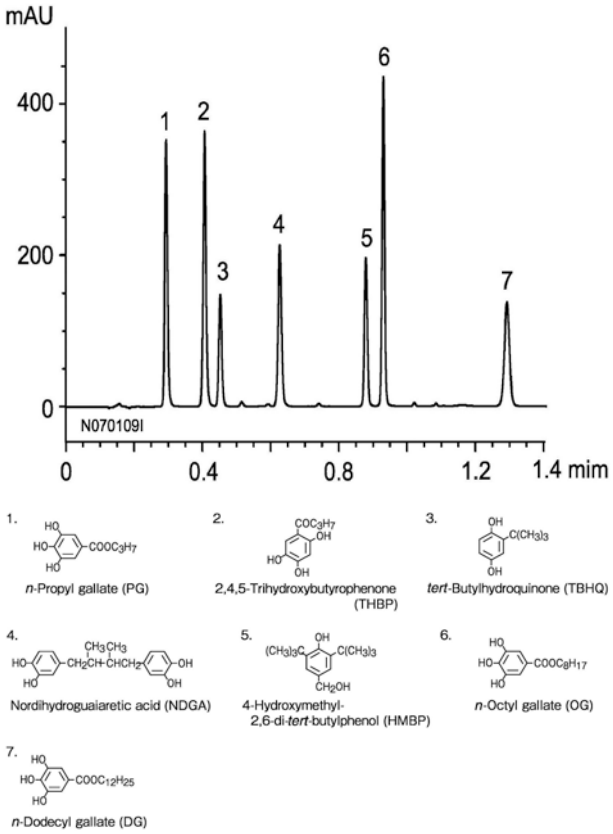
The aim of this study was the development of an ultra-fast method for the determination of isoflavonoids in soy-containing foods. The method transfer from conventional to ultra-fast HPLC systems was successful when using YMC UltraHT Hydrosphere C18 with 2 µm particle size.

* Application data by courtesy YMC Co., Ltd.

Conventional and Ultra-fast LC method for antioxidants



Column:	YMC-Pack *Pro* C18 (5 µm, 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 µl
Temperature:	30 °C
Detection:	UV at 280 nm
Sample:	0.05 ~ 0.3 mg/ml



Column:	YMC-UltraHT *Pro* C18 (2 µm, 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 µl
Temperature:	30 °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 µm 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

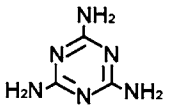
Transfer of this conventional method to an ultra-fast method resulted in solvent and time savings of about 90% with total retention of the resolution of the different antioxidants.

Melamine in pet-food

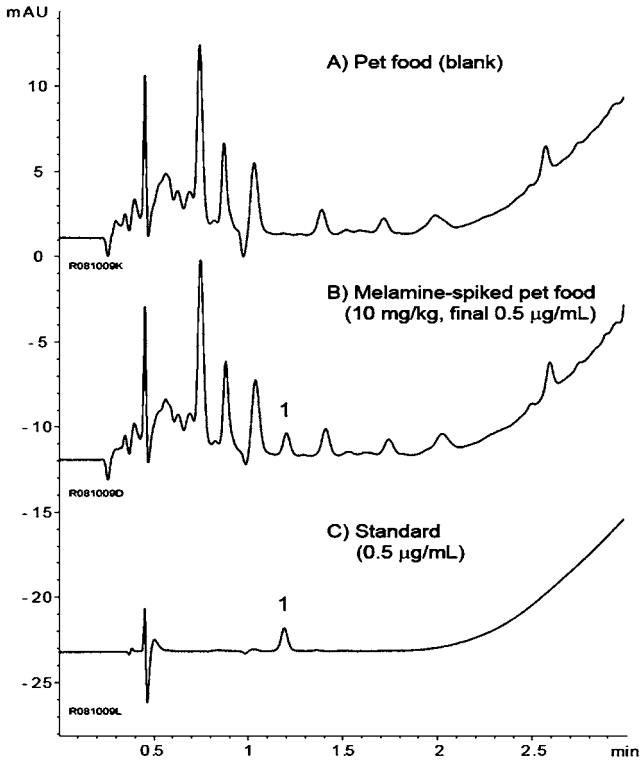
Sample preparation method

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

1

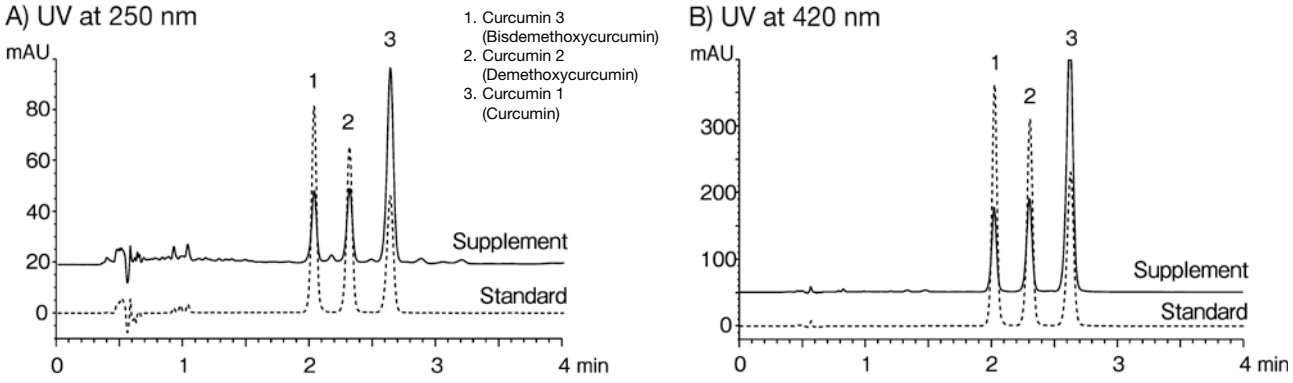


Melamine



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

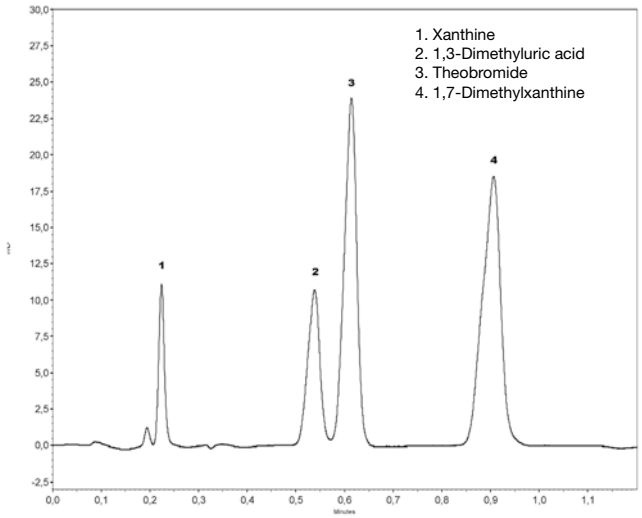


Column:	YMC-UltraHT *Pro* C18 (2 µm, 12 nm) 100 x 2.0 mm ID
Part No.:	AS12S021002WT
Eluent:	acetonitrile/water/formic acid (50/50/0.1)
Flow rate:	0.4 ml/min
Temperature:	40 °C
Detection:	A) UV at 250 nm, B) UV at 420 nm
Injection:	Standard 1 µl (0.1 mg/ml)
	Supplement 1 µl (6.9 mg/ml)
Sample:	methanol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

* Application data by courtesy YMC Co., Ltd.

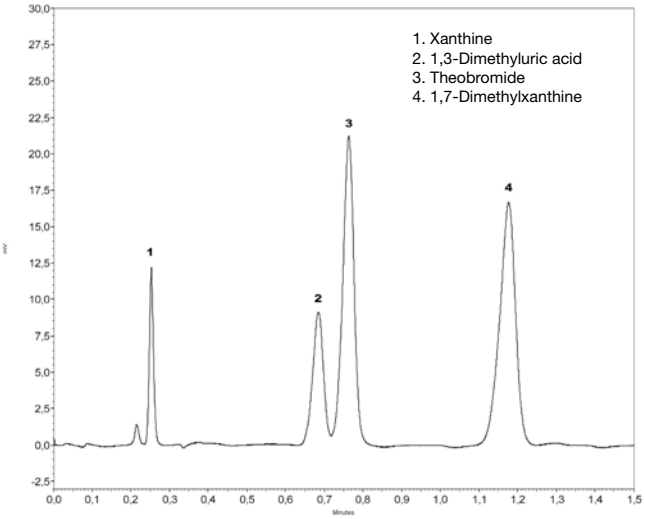
Selected separations of food and beverages

Separation of caffeine metabolites using acetonitrile eluent



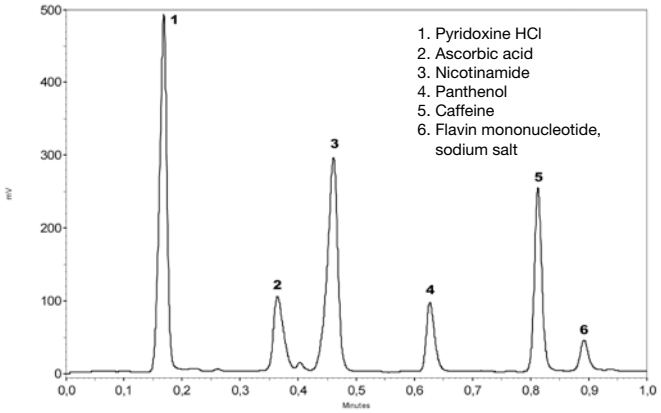
Column:	YMC-UltraHT Hydrosphere C18 (2 µm, 12 nm) 50 x 2.0 mm ID
Part No.:	HS12S020502WT
Mobile Phase:	acetonitrile / acetic acid (pH 2.5) (06/94)
Flow rate:	0.82 ml/min
Detection:	254 nm
Pressure:	420 bar
Injection:	1 µL
Temperature:	40 °C

Separation of caffeine metabolites using methanol eluent



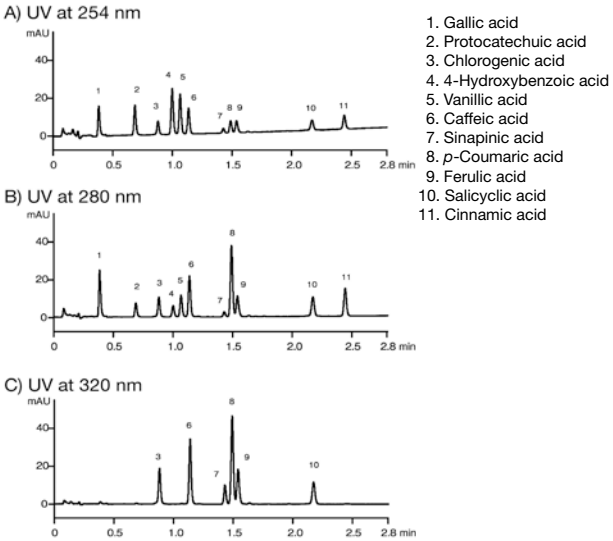
Column:	YMC-UltraHT Hydrosphere C18 (2 µm, 12 nm) 50 x 2.0 mm ID
Part No.:	HS12S020502WT
Mobile Phase:	methanol / acetic acid (pH 2.5) (08/92)
Flow rate:	0.82 ml/min
Detection:	254 nm
Pressure:	465 bar
Injection:	1 µL
Temperature:	40 °C

Separation of water-soluble vitamins



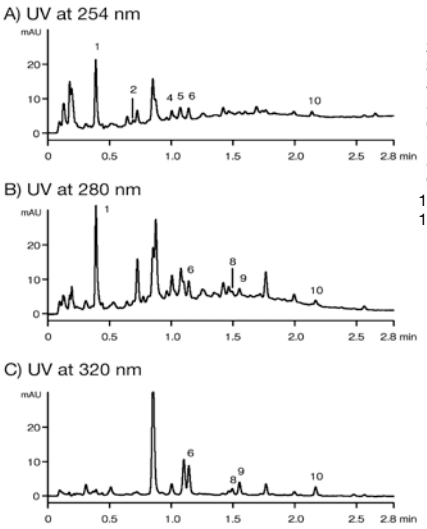
Column:	YMC-UltraHT Hydrosphere C18 (12 nm, 2 µm) 50 x 2.0 mm ID		
Part No.:	HS12S020502WT		
Mobile Phase:	A: acetic acid (pH 3.5)		
	B: acetonitrile / acetic acid (pH 3.5) (20/80)		
Gradient:	Time	A (in %)	B (in %)
	0.0	10	90
	0.2	0	100
	1.0	0	100
	1,2	10	90
	2.0	10	90
Flow rate:	0.76 ml/min		
Detection:	210 nm		
Pressure:	450 bar		
Injection:	1 µL		
Temperature:	40 °C		

Aromatic carboxylic acids in foods



Column:	YMC-UltraHT *Pro* C18 (2 µm,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 °C
Injection:	2 µl (2.530 µg/ml)

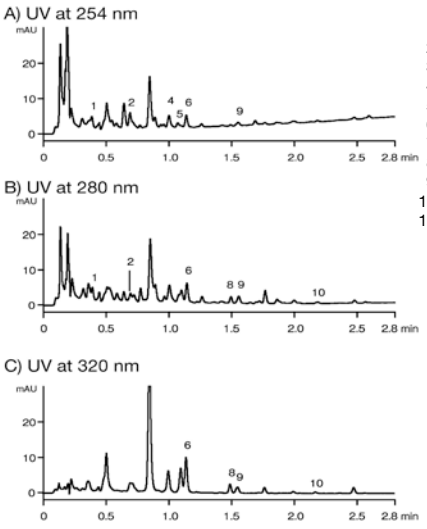
Aromatic carboxylic acids in red wine



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. Salicyclic acid
11. Cinnamic acid

Column:	YMC-UltraHT *Pro* C18 (2 µm, 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 µl
Sample:	5 times dilution of a red wine with water

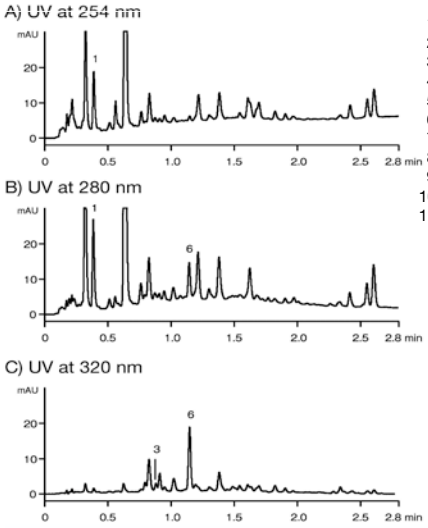
Aromatic carboxylic acids in white wine



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. Salicyclic acid
11. Cinnamic acid

Column:	YMC-UltraHT *Pro* C18 (2 µm, 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 µl
Sample:	2 times dilution of a white wine with water

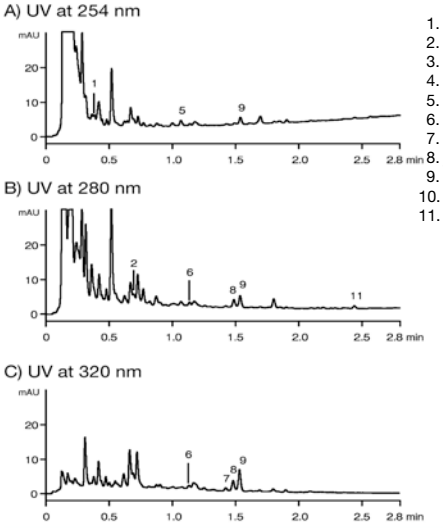
Aromatic carboxylic acids in a tea extract



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. Salicyclic acid
11. Cinnamic acid

Column:	YMC-UltraHT *Pro* C18 (2 µm, 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 µl
Sample:	hot water extract of tea leaves (5 mg/ml)

Aromatic carboxylic acids in a beer

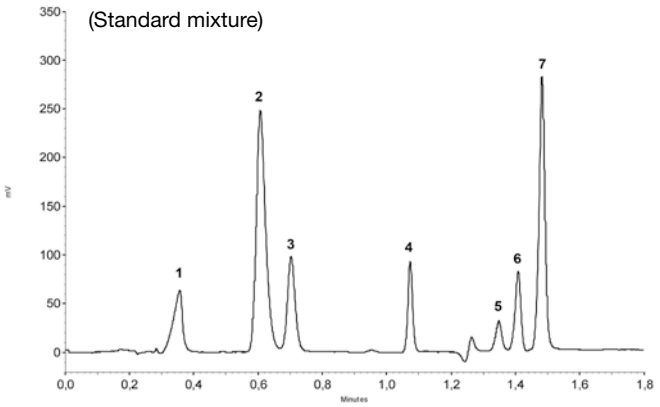


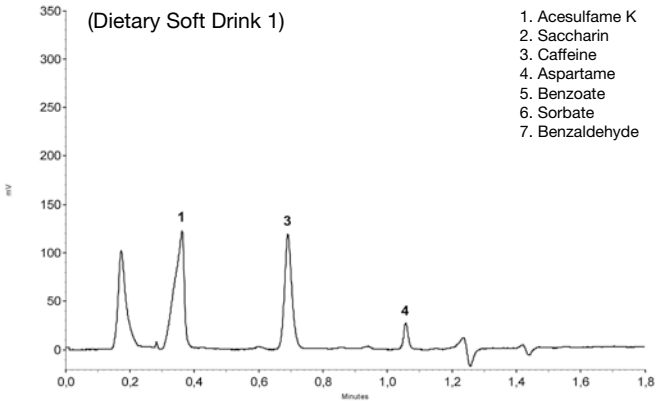
1. Gallic acid
2. Protocatechuic acid
3. Chlorogenic acid
4. 4-Hydroxybenzoic acid
5. Vanillic acid
6. Caffeic acid
7. Sinapinic acid
8. *p*-Coumaric acid
9. Ferulic acid
10. Salicyclic acid
11. Cinnamic acid

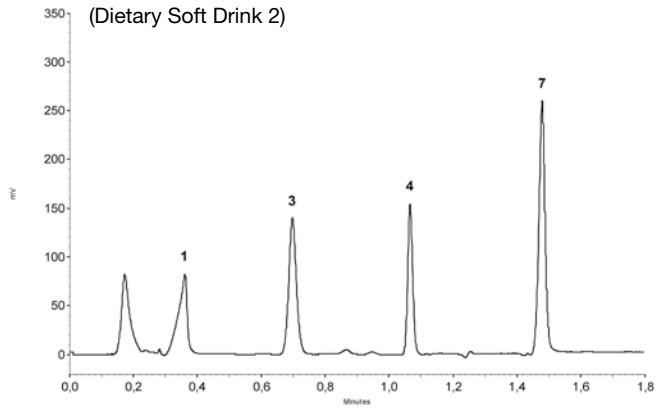
Column:	YMC-UltraHT *Pro* C18 (2 µm, 12 nm) 50 x 2.0 mm ID
Part No.:	AS12S020502WT
Eluent:	A) water/HCOOH (200/0.4) B) acetonitrile/methanol/THF/water/HCOOH (43.5/43.5/13/100/0.4)
Gradient:	20-100%B (0-2.8 min)
Flow rate:	0.5 ml/min
Temperature:	40 °C
Injection:	2 µl
Sample:	2 times dilution of a beer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

* Application data by courtesy YMC Co., Ltd.

Separation of 6 key compounds in soft drinks

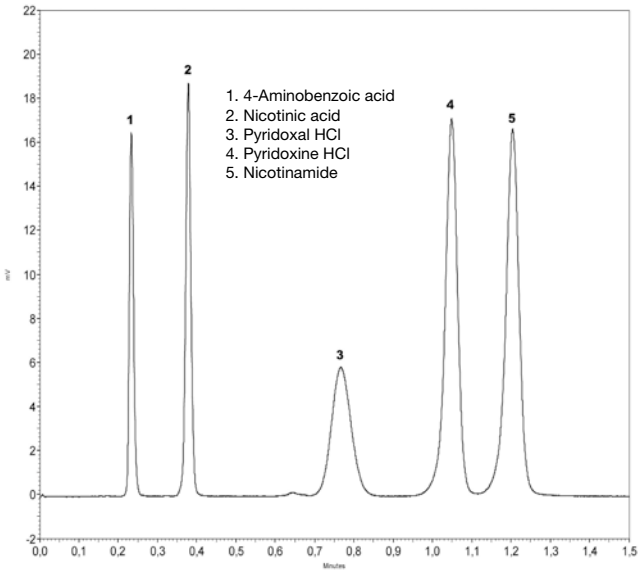






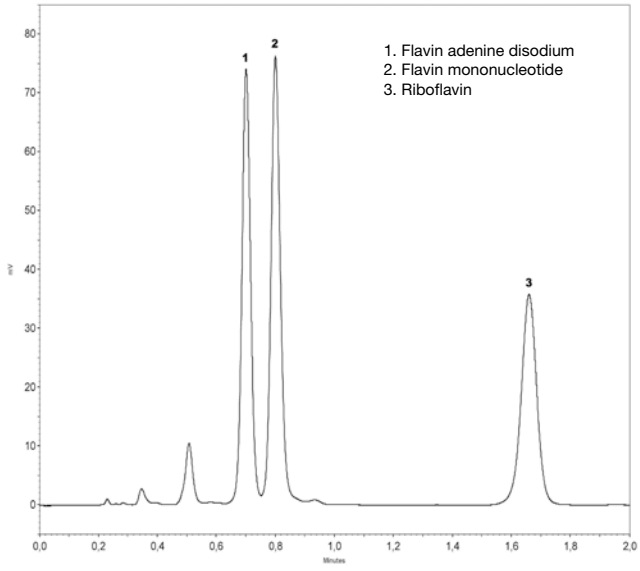
Column:	YMC-UltraHT Hydrosphere C18 (2 µm, 12 nm) 50 x 2.0 mm ID
Part No.:	HS12S020502WT
Mobile Phase:	A: Acetonitrile / MeOH / THF (20/60/20) B: TFA (pH 2.15)
Gradient:	Time A (in %) B (in %) 0 10 90 0.05 10 90 0.55 25 75 0.60 54 46 2.0 54 46 2.5 10 90
Flow rate:	0.68 ml/min
Detection:	254 nm
Pressure:	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:	10 mM NH_4CH_3COO
Flow rate:	0.8 ml/min
Detection:	UV at 254 nm
Pressure:	390 bar
Injection:	1 µl
Temperature:	40 °C

Riboflavin and related compounds

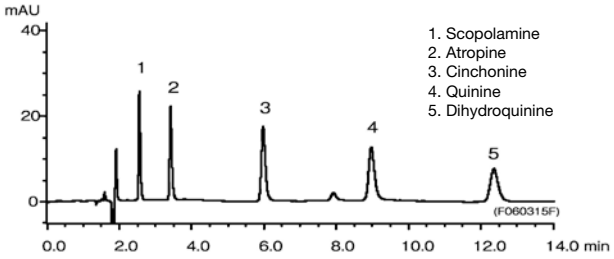


Column:	YMC-UltraHT Hydrosphere C18 (2 µm, 12 nm) 50 x 2.0 mm ID
Part No.:	HS12S020502WT
Mobile Phase:	MeOH / 50 mM NH_4CH_3COO (25/75)
Flow rate:	0.55 ml/min
Detection:	UV at 260nm
Pressure:	400 bar
Injection:	1 µl
Temperature:	40 °C

Alkaloids

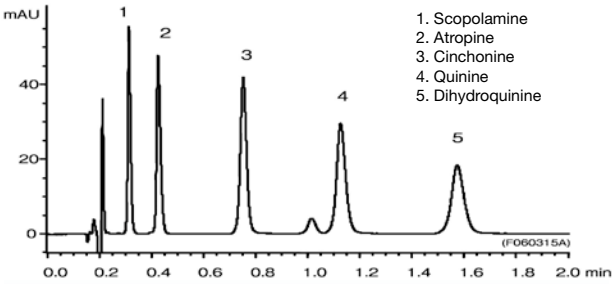
The term “alkaloid” is used to describe any nitrogen-containing base, but this classification is too general for the wide variety of compound which occurs. True alkaloids are naturally occurring chemical compounds containing at least one basic nitrogen atom in a ring system derived from amino acids (except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



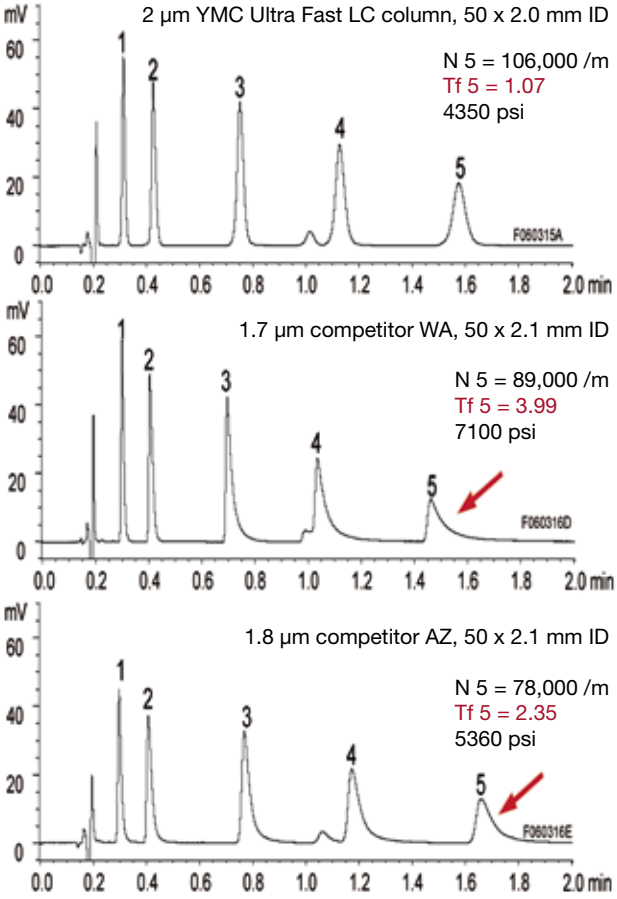
Column:	YMC-Pack *Pro* C18 (5 µm, 12 nm) 150 x 4.6 mm ID
Part No.:	AS12S051546WT
Flow rate:	1.0 ml/min
Eluent	20 mM CH_3COOH-CH_3COONH_4 (pH 4.9)/ acetonitrile (80/20)
Temperature:	40 °C
Detection:	UV at 220 nm
Injection:	5 µl
Sample:	0.02 ~ 0.1 mg/ml

Ultra-fast LC method



Column:	YMC UltraHT *Pro* C18 (2 µm, 12 nm) 50 x 2.0 mm ID
Part No.:	AS12S020502WT
Flow rate:	0.6 ml/min
Eluent:	20 mM CH_3COOH-CH_3COONH_4 (pH 4.9)/ acetonitrile (80/20)
Temperature:	40 °C
Detection:	UV at 220 nm
Injection:	1 µl
Sample:	0.02 ~ 0.1 mg/ml

Comparison of YMC-UltraHT with alternative sub-2-µm products



Eluent:	20 mM CH_3COOH-CH_3COONH_4 (pH 4.9) / acetonitrile (80/20)
Flow rate:	0.6 ml/min
Temperature:	40 °C
Detection:	UV at 220 nm

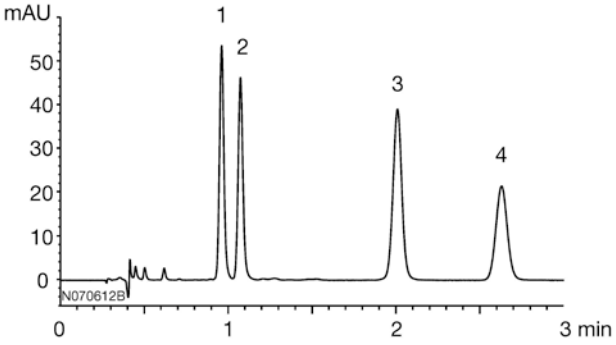
Conclusion

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 µm 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.

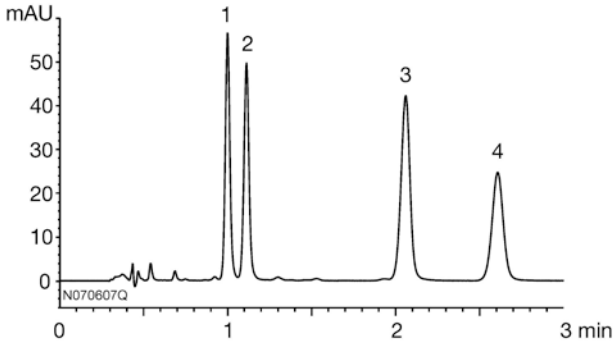
* Application data by courtesy YMC Co., Ltd.

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



1. Cephalexin

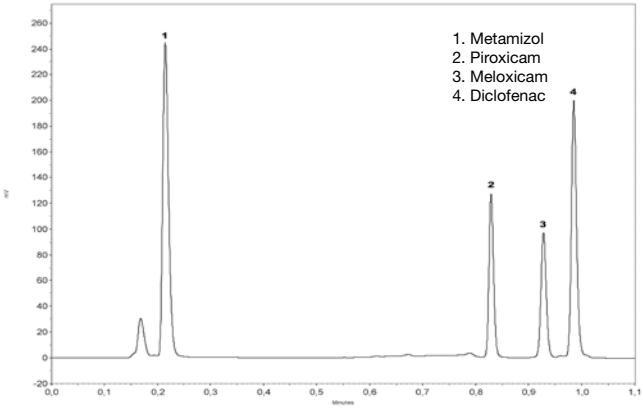
2. Cefaclor

3. Cephaloglycin

4. Cephaloridine

Column: YMC-UltraHT *Pro* C18 (2 µm, 12 nm)
Eluent: acetonitrile/20 mM KH_2PO_4 (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)

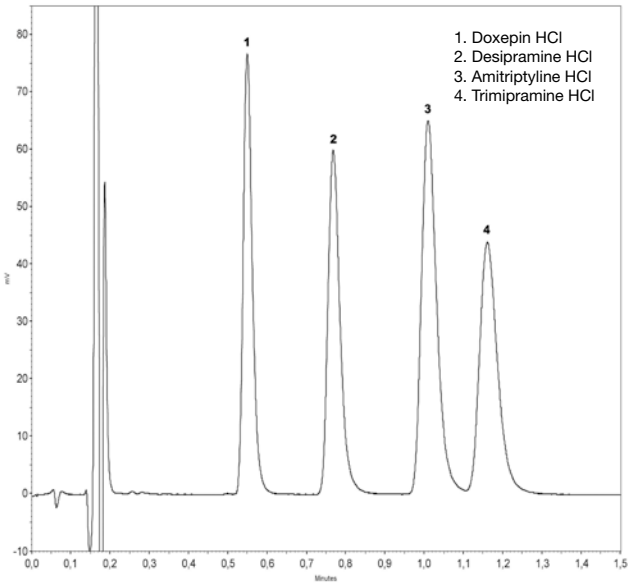
Column: YMC-UltraHT *Pro* C18 (2 µm, 12 nm) 50 x 2.0 mm ID
Part No.: AS12S020502WT
Mobile Phase: A: acetonitrile
B: formic acid (0,15%)

Gradient:

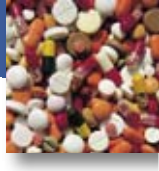
Time	A (in %)	B (in %)
0.0	20	80
0.1	20	80
0.4	90	10
1.2	90	10

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

Separation of tricyclic antidepressants

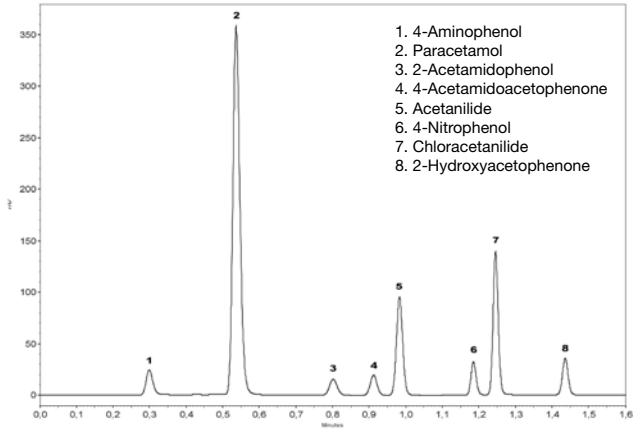


Column: YMC-UltraHT *Pro* C18 (2 µm, 12 nm) 50 x 2.0 mm ID
Part No.: AS12S020502WT
Mobile Phase: ACN / 20mM NH_4CH_3COO+CH_3COOH (pH 4.6) (35/65)
Flow rate: 0.7 ml/min
Detection: UV at 215 nm
Pressure: 440 bar
Injection: 1 µl
Temperature: 40 °C



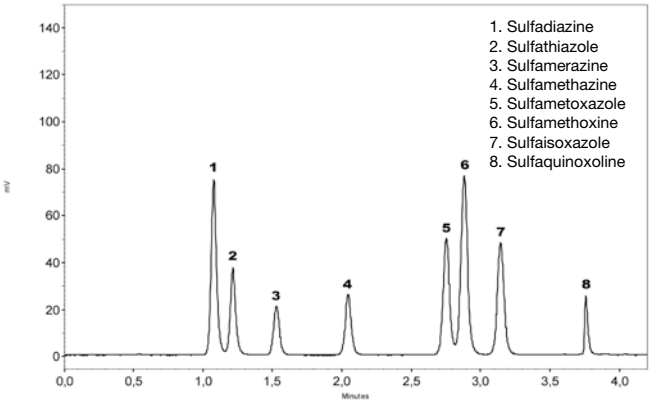
Selected separations of pharmaceuticals

Separation of paracetamol and impurities



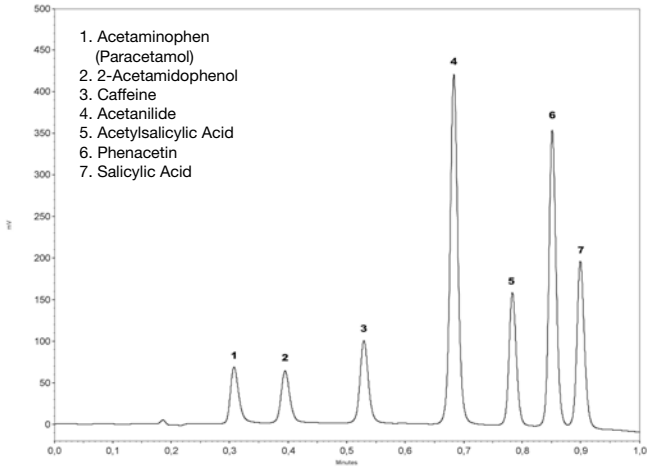
Column:	YMC-UltraHT *Pro* C18 (2 µm, 12 nm) 75 x 2.0 mm ID		
Part No.:	AS12S02L502WT		
Mobile Phase:	A: acetonitrile		
	B: formic acid (pH 2.8)		
Gradient:	Time	A (in %)	B (in %)
	0.0	42	58
	0.1	42	58
	0.5	80	20
	1.5	80	20
Flow rate:	0.5 ml/min		
Detection:	UV at 254 nm		
Pressure:	420 bar		
Injection:	1 µl		
Temperature:	40 °C		

Separation of sulfa drugs



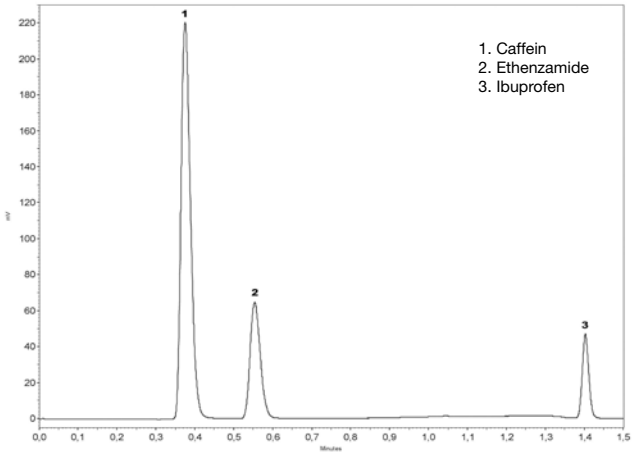
Column:	YMC-UltraHT Hydrosphere C18 (2 µm,12 nm) 75 x 2.0 mm ID		
Part No.:	HS12S02L502WT		
Mobile Phase:	A: methanol		
	B: formic acid (pH 2.5)		
Gradient:	Time	A (in %)	B (in %)
	0.0	18	82
	0.1	18	82
	2.8	42	56
	2.9	100	0
	4.0	100	0
Flow rate:	0.45 ml/min		
Detection:	UV at 280 nm		
Pressure:	460 bar		
Injection:	1 µl		
Temperature:	40 °C		

Separation of seven analgenics



Column:	YMC-UltraHT Hydrosphere C18 (2 µm, 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 µl		
Temperature:	40 °C		

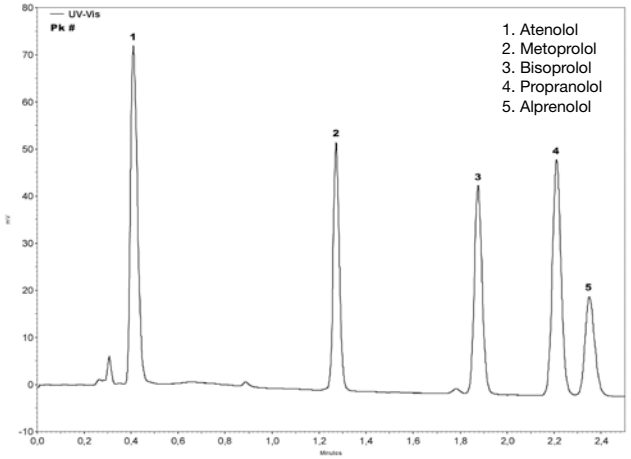
Separation of pain killing drugs



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 (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 µl		
Temperature:	40 °C		

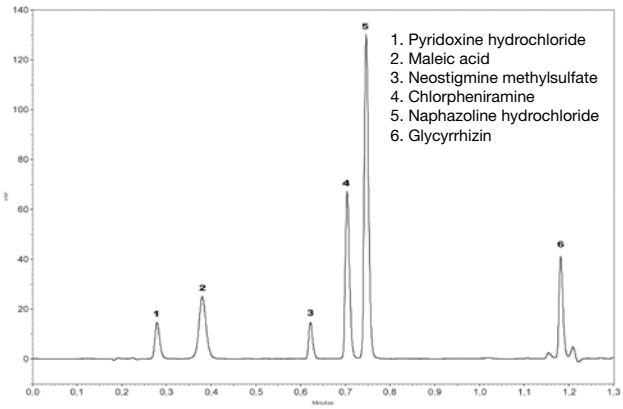
* Application data by courtesy YMC Co., Ltd.

Separation of beta-blockers



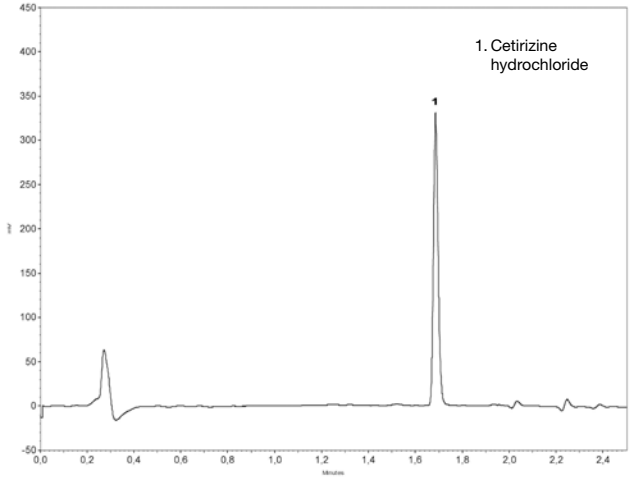
Column:	YMC-UltraHT *Pro* C18 (2 µm, 12 nm) 50 x 2.0 mm ID		
Part No.:	AS12S020502WT		
Mobile Phase:	A: methanol; B: trifluoroacetic acid (0.05%)		
Gradient:	Time	A (in %)	B (in %)
	0.0	30	70
	0.1	30	70
	0.2	55	45
	2.5	55	45
	3.0	30	70
Flow rate:	0.5 ml/min		
Detection:	UV at 254 nm		
Pressure:	390 bar		
Injection:	1 µl		
Temperature:	40 °C		

Ingredients in an eye drop formulation



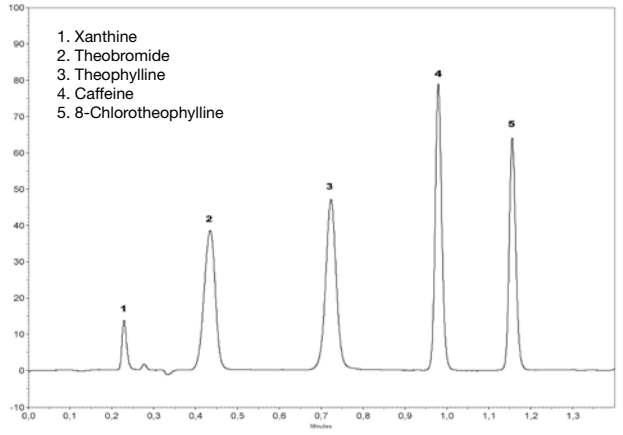
Column:	YMC-UltraHT Hydrosphere C18 (2 µm, 12 nm) 50 x 2.0 mm ID		
Column:	HS12S020502WT		
Mobile Phase:	A: acetonitrile; B: 0.05% trifluoroacetic acid		
Gradient:	Time	A (in %)	B (in %)
	0.0	10	90
	0.1	10	90
	0.3	50	50
	0.8	50	50
	0.9	90	10
	1.3	90	10
Flow rate:	0.85 ml/min		
Detection:	UV at 265 nm		
Pressure:	390 bar		
Injection:	1 µl		
Temperature:	40 °C		

Analysis of Zyrtec® – Gradient Method



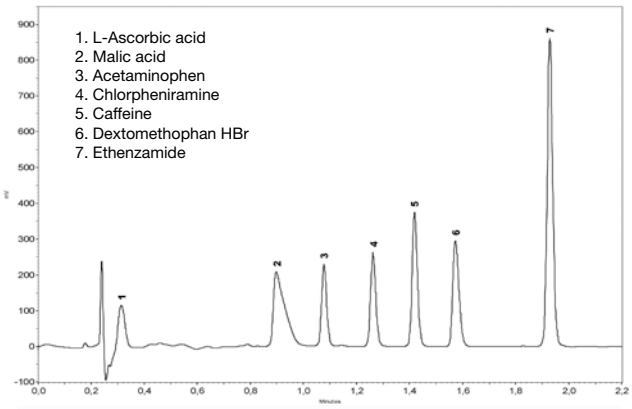
Column:	YMC-UltraHT *Pro* C18 (2 µm, 12 nm) 50 x 2.0 mm ID		
Oder-No.:	AS12S020502WT		
Mobile Phase:	A: methanol; B: formic acid (pH 2.8)		
Gradient:	Time	A (in %)	B (in %)
	0.0	30	70
	0.2	30	70
	0.7	90	10
	2.0	90	10
Flow rate:	0.5 ml/min		
Detection:	UV at 230 nm		
Pressure:	380 bar		
Injection:	1 µl		
Temperature:	40 °C		

Separation of xanthines



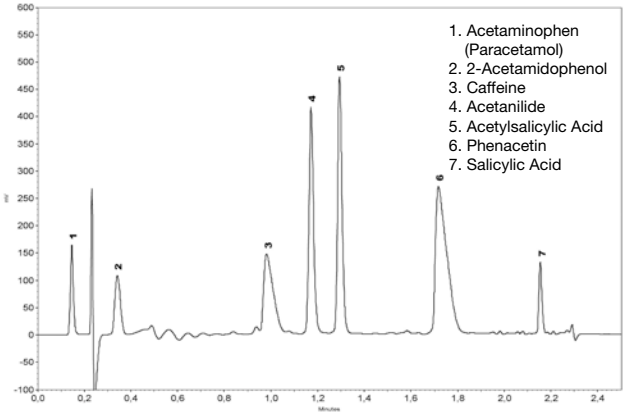
Column:	YMC-UltraHT Hydrosphere C18 (2 µm, 12 nm) 50 x 2.0 mm ID		
Part No.:	HS12S020502WT		
Mobile Phase:	A: methanol		
	B: formic acid (pH 2.5)		
Gradient:	Time	A (in %)	B (in %)
	0.0	20	80
	0.4	20	80
	0.7	70	30
	1.3	70	30
Flow rate:	0.72 ml/min		
Detection:	UV at 254 nm		
Pressure:	470 bar		
Injection:	1 µl		
Temp:	40 °C		

Ingredients of a cough cold medication



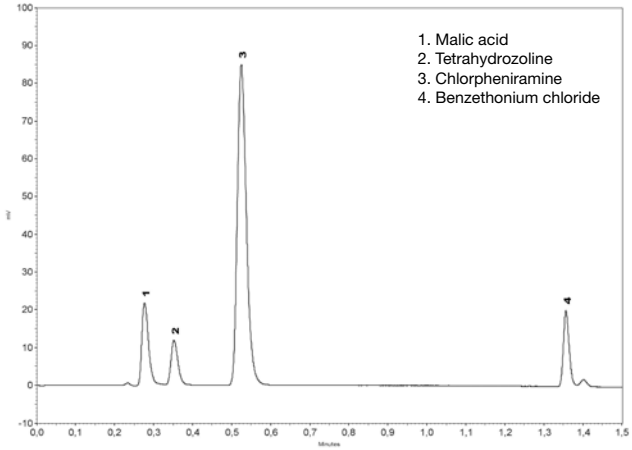
Column:	YMC-UltraHT Hydrosphere C18 (2 µm, 12 nm) 50 x 2.0 mm ID		
Part No.:	HS12S020502WT		
Mobile Phase:	A: methanol + 0.15% formic acid		
	B: formic acid (0,15%)		
Gradient:	Time	A (in %)	B (in %)
	0.0	10	90
	0.6	55	45
	1.2	55	45
	2.0	90	10
	2.2	90	10
Flow rate:	0.65 ml/min		
Detection:	UV at 230 nm		
Pressure:	400 bar		
Injection:	1 µl		
Temperature:	40 °C		

Ingredients of a cough cold medication



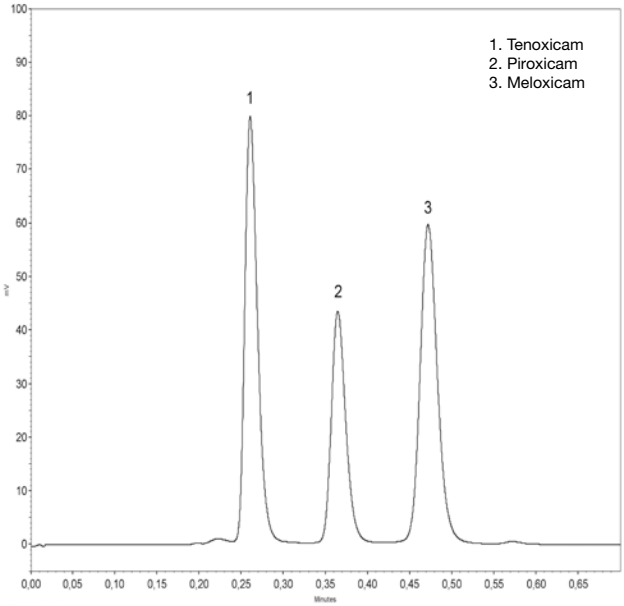
Column:	YMC-UltraHT Hydrosphere C18 (2 µm, 12 nm) 50 x 2.0 mm ID		
Part No.:	HS12S020502WT		
Mobile Phase:	A: methanol + 0.15% formic acid		
	B: formic acid (0,15%)		
Gradient:	Time	A (in %)	B (in %)
	0.0	0	100
	0.6	70	30
	1.2	70	30
	1.5	100	0
	2.5	100	0
Flow rate:	0.65 ml/min		
Detection:	UV at 230 nm		
Pressure:	400 bar		
Injection:	1 µl		
Temperature:	40 °C		

Ingredients of a nasal spray



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

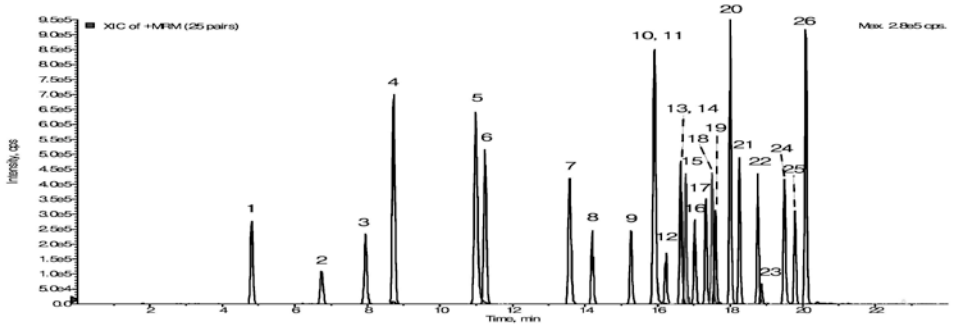


Column:	YMC-UltraHT *Pro* C18 (2 µm, 12 nm) 50 x 2.0 mm ID
Part No.:	AS12S020502WT
Mobile Phase:	methanol / formic acid (0.15%) (75/25)
Flow rate:	0.7 ml / min
Detection:	UV at 260 nm
Pressure:	460 bar
Injection:	1 µL
Temperatur:	40 °C

* Application data by courtesy YMC Co., Ltd.

Pesticides

Simultaneous separation of pesticides by LC/MS



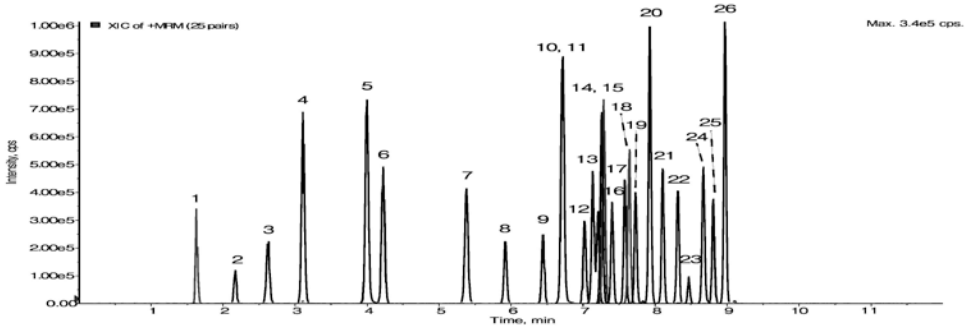
1. Thiamethoxam (Q1/Q3 : 292.3/211.3)
2. Clothianidin (Q1/Q3 : 250.3/169.1)
3. Chloridazon (Q1/Q3 : 222.3/77.0)
4. Thiacloprid (Q1/Q3 : 253.3/126.0)
5. Thiabendazole (Q1/Q3 : 202.3/175.2)
6. Azamethiphos (Q1/Q3 : 325.2/183.0)
7. Dimethirimol (Q1/Q3 : 210.4/71.1)
8. Isoxaflutole (Q1/Q3 : 360.2/251.1)
9. Pyriftalid (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-Pack *Pro* C18 (3 µm, 12 nm) 150 × 2.0 mm ID
Part No.: AS12S031502WT
Eluent: A) 5 mM CH_3COONH_4 in water
B) 5 mM CH3COONH4 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 µl (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. Thiacloprid (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. Pyriftalid (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 µm, 12 nm) 75 × 2.0 mm ID
Part No.: AS12S02L502WT
Eluent: A) 5 mM CH_3COONH_4 in water
B) 5 mM CH_3COONH_4 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

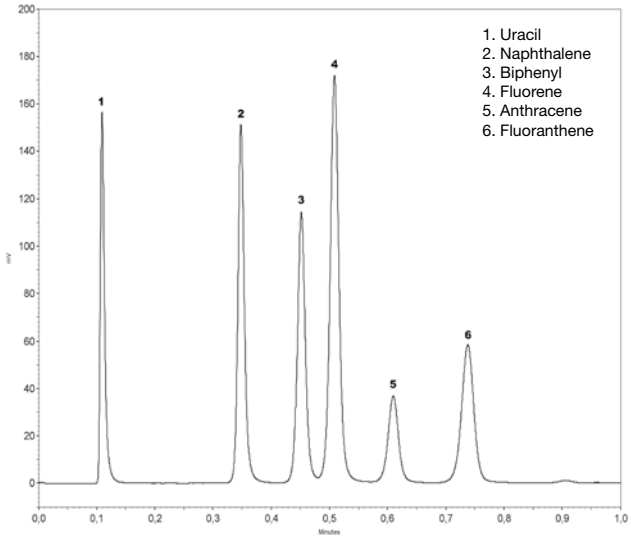
Flow rate: 0.4 ml/min
Temperature: ambient
Detection: API5000, ESI, Positive, MRM
Injection: 3 µl (10 ng/ml)
Sample: Pesticide Mixture Standard Solution PL-7-2, manufactured by Wako Pure Chemical Industries, Ltd.

Conclusion

This example again shows the simple method transfer from a conventional HPLC method developed on YMC-Pack *Pro* C18 3 µm to an ultra-fast LC application. The use of the new YMC UltraHT *Pro* C18, with its particle size of 2 µm enables downscaling without any further method development.
The combination of small particles and short column lengths results in shorter retention times without compromising resolution.

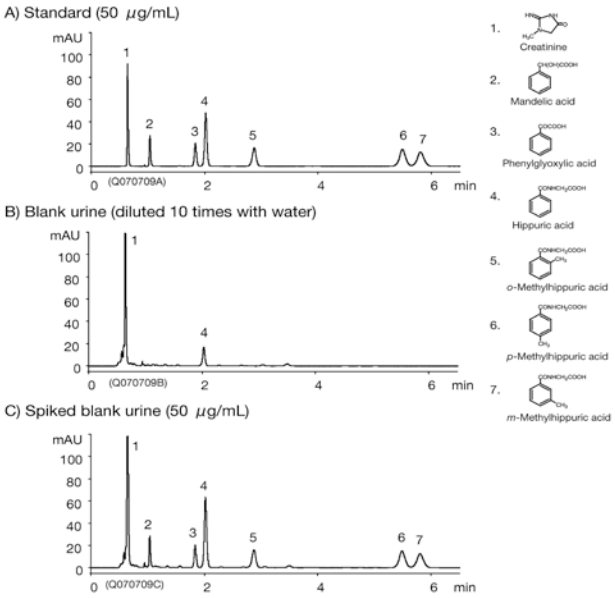
Selected environmental separations

Separation of Uracil and 5 Hydrocarbons with YMC Hydrosphere C18



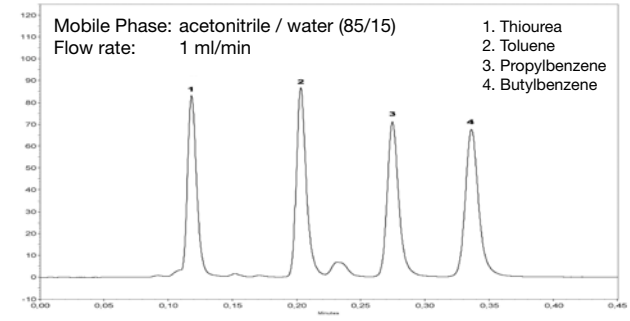
Column:	YMC-UltraHT Hydrosphere C18 (2 µm, 12 nm) 50 x 2.0 mm ID
Part No.:	HS12S020502WT
Mobile Phase:	acetonitrile / water (70/30)
Flow rate:	1.2 ml/min
Detection:	UV at 270 nm
Pressure:	410 bar
Injection:	0.8 µl
Temperature:	40 °C

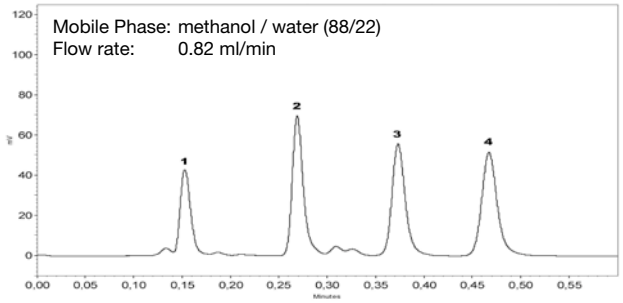
Metabolites of organic solvents in human urine



Column:	YMC-UltraHT Hydrosphere C18 (2 µm, 12 nm) 100 x 2.0 mm ID
Part No.:	HS12S021002WT
Eluent:	20 mM CH_3COONH_4/2-propanol (97/3)
Flow rate:	0.4 ml/min
Temperature:	35 °C
Detection:	UV at 225 nm
Injection:	1 µl

Thiourea & 3 alkylbenzenes





Column:	YMC-UltraHT *Pro* C18 (2 µm, 12 nm) 50 x 2.0 mm ID
Part No.:	AS12S020502WT
Detection:	254 nm
Injection:	1 µl
Temperature:	40 °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 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).

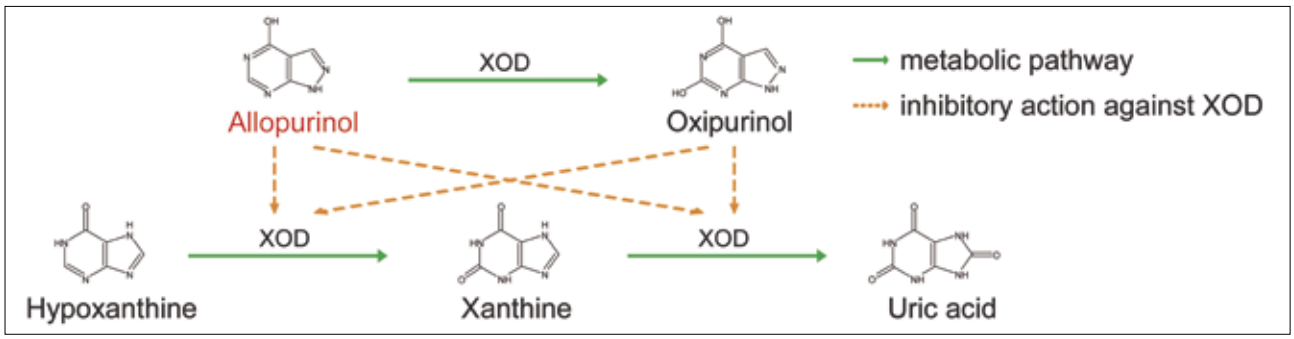


Figure 1: Xanthine oxidase inhibitor and related metabolites

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.

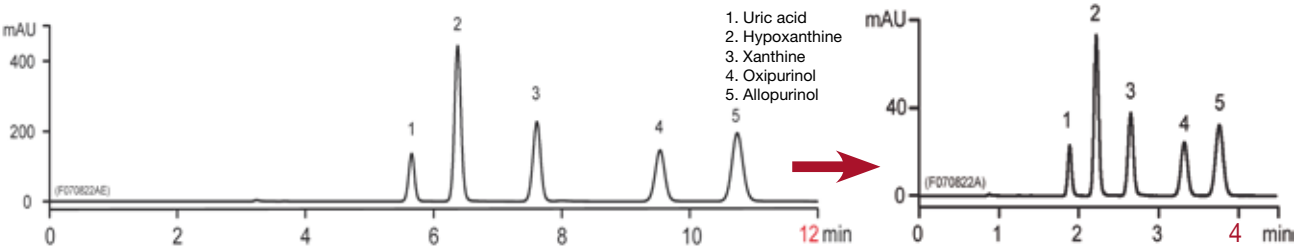
Results

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



Column:	YMC Hydrosphere C18 (5 µm, 12 nm) 150 x 4.6 mm ID
Part No.:	HS12S0521546WT
Eluent:	10 mM CH_3COOH-CH_3COONH_4 (pH 4.5)
Flow rate:	1.0 ml/min
Temperature:	37 °C
Detection:	UV at 254 nm
Injection:	7.5 µl
Sample:	standard (100 µg/ml)

Column:	YMC-UltraHT Hydrosphere C18 (2 µm, 12 nm) 50 x 2.0 mm ID
Part No.:	HS12S020502WT
Eluent:	10 mM CH_3COOH-CH_3COONH_4 (pH 4.5)
Flow rate:	0.2 ml/min
Temperature:	37 °C
Detection:	UV at 254 nm
Injection:	0.5 µl
Sample:	standard (100 µg/ml)

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).

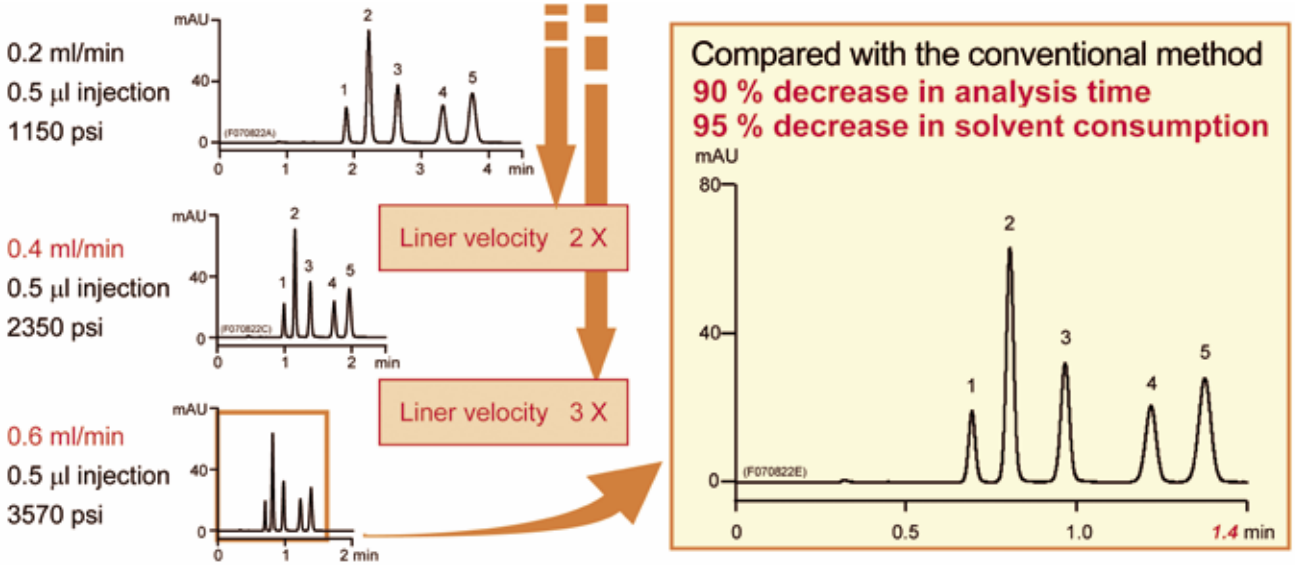
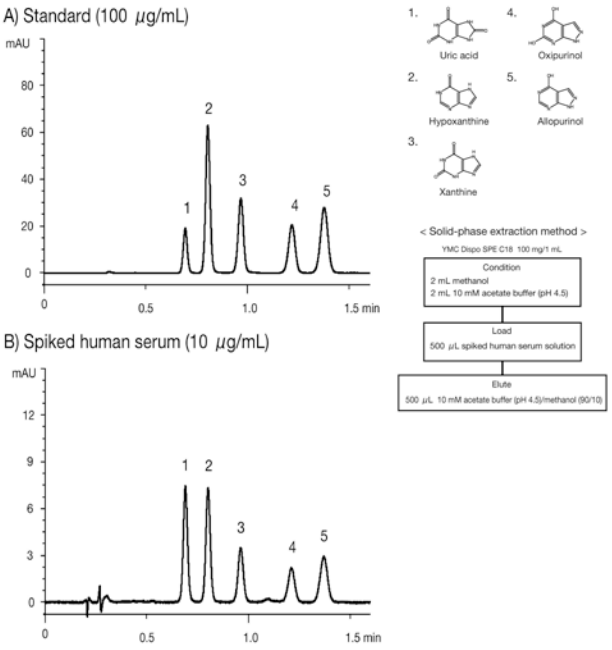


Figure 3: Ultra-fast LC optimisation

Xanthine oxidase inhibitor and metabolites in human serum



Column:	YMC-UltraHT Hydrosphere C18 (2 µm, 12 nm) 50 x 2.0 mm ID
Part No.:	HS12S020502WT
Eluent:	10 mM CH_3COOH-CH_3COONH_4 (pH 4.5)
Flow rate:	0.6 ml/min
Temperature:	37 °C
Detection:	UV at 254 nm
Injection:	0.5 µl

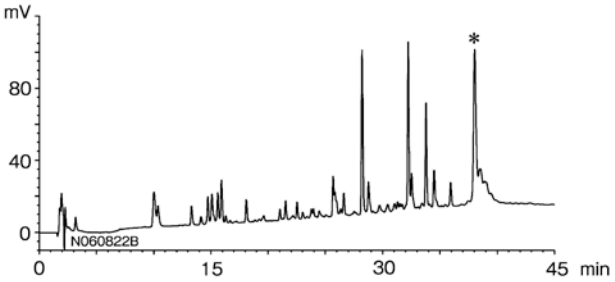
* Application data by courtesy YMC Co., Ltd.

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-µm 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 µm and 2 µm

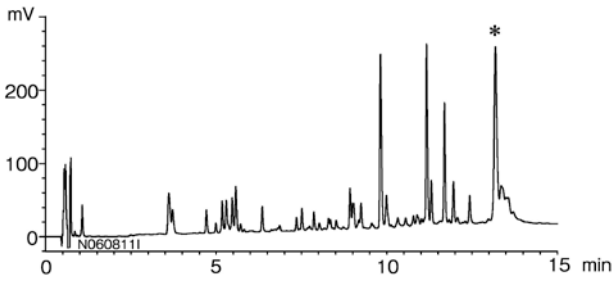


*undigested Cytochrome *c*

Column:	YMC-UltraHT *Pro* C18 (5 µm, 12 nm) 150 × 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 µl
Sample:	Tryptic digest of Cytochrome c



*undigested Cytochrome *c*

Column:	YMC-UltraHT *Pro* C18 (2 µm, 12 nm) 50 × 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 µl
Sample:	Tryptic digest of Cytochrome c

Ordering Information Ultra Fast LC

YMC-UltraHT *Pro* C18, 12 nm, 2 µm

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

YMC-UltraHT Hydrosphere C18, 12 nm, 2 µm

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

Substance Index

* Application data by courtesy YMC Co., Ltd.

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