

YMC ProFamily



- YMC-Pack Pro C18, Pro C8, Pro C4 based on ultra high purity silica
- YMC-Pack Pro C18 RS; as a high carbon load version (C= 22%)
- Hydrosphere C18 for stability in aqueous mobile phases
- every packed column supplied with:
 - lot certificate on physicochemical properties of bare silica, minimal metal traces, bonding and chromatographic performance
 - test chromatogram showing high performance plate count together with tight specification on peak symmetry



	Pro C18	Pro C8	Pro C4	Pro C18 RS	Hydrosphere C18
Particle size / μm	3; 5	3; 5	3; 5	3; 5	3; 5
Pore size / nm	12	12	12	8	12
Surface area / m^2g^{-1}	340	340	340	510	340
Carbon content / %	17	11	8	22	12
pH range	2 - 8	2 - 8	2 - 8	1 - 10	2 - 8
Specification metal content	Actual*	Actual*	Actual*	Actual*	Actual*
Al / ppm < 10	0.3	0.2	0.6	0.3	0.7
Fe / ppm < 10	2.8	2.5	2.9	0.1	1.2
Na / ppm < 10	0.3	1.4	1.0	1.3	0.7
Ti / ppm < 0.5	0.1	0.1	0.1	0.1	0.1

see pages 28-31 see pages 32-33 see pages 34-37

see pages 38-43

see pages 44-49

* Randomly selected lots

General

One of the main challenges in RP-HPLC is the quantitation of ionisable compounds including drugs, degradation products, etc. For this purpose symmetrical, sharp peaks are required to provide highest resolution and reliable integration. The stationary phases of the YMC ProFamily fulfil these demands making them an excellent choice for the pharmaceutical and biotechnology industries. This product line consists of the three octadecyl-phases YMC-Pack Pro C18 RS, YMC-Pack Pro C18 and Hydrosphere C18 together with the octyl- and butyl-phase YMC-Pack Pro C8 and YMC-Pack Pro C4.

Properties

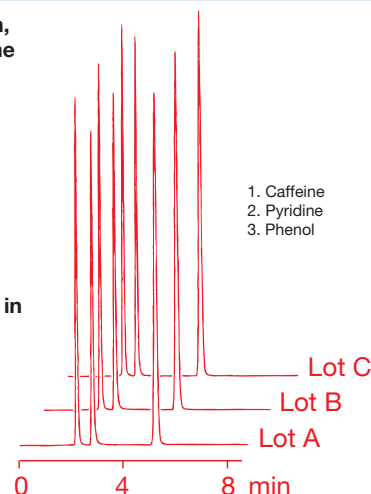
The base material for the stationary phases of the ProFamily is silica, which is covered with silanol groups on the surface. In the derivatisation step, these groups are reacted with, for example, octadecylsilanes to give C₁₈ reversed-phases. To minimize unwanted interactions between residual silanols and the sample molecules that are responsible for asymmetric peaks, YMC utilizes a proprietary, highly effective endcapping process.

In addition, silica with an extremely low metal content is used for the phases of the ProFamily to suppress polar interactions, as metal impurities influence silanol groups to become more acidic. The table above illustrates that the total metal content is lower than 10 ppm for those elements reported.



Lot-to-lot reproducibility for Pro C18, 5 μm

In this comparison, phenol and pyridine were selected to show the peak performance for acidic and basic compounds, respectively. The low metal content and the proprietary endcapping result in a very good peak performance from lot-to-lot-to-lot.

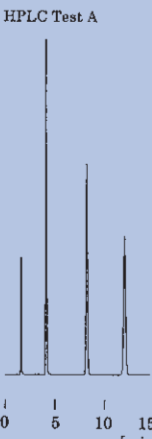



YMC ProFamily

Individual Lot Test

INSPECTION REPORT

Pro C18 S-5 lot # 5491

		Specification	Result
Base silicagel material			
Average particle size(d50)	[μ m]	4.5~5.0	4.7
Average pore size	[nm]	12.0~13.0	12.2
Surface area	[m ² /g]	320~350	341
Pore volume	[mL/g]	1.03~1.09	1.04
Atomic emission			
Al	[ppm]	<10	0.3
Fe	[ppm]	<10	0.7
Na	[ppm]	<10	0.3
Ti	[ppm]	<0.5	0.1
Carbon content			
	[%]	15.5~17.0	16.0
HPLC Test A			
k'(Ethylbenzene)/k'(n-Propylbenzene)		0.629~0.653	0.633
k'(4-n-Butoxybenzoic acid)/k'(n-Propylbenzene)		0.238~0.263	0.240
HPLC Test B			
k'(Pyridine)/k'(Phenol)		0.313~0.385	0.329
k'(Caffeine)/k'(Phenol)		0.168~0.194	0.182
Chromatograms			
HPLC Test A	HPLC Test B	Test A Conditions Column size: 150×4.6mm I.D. Eluent: 20mM KH ₂ PO ₄ -H ₃ PO ₄ (pH3.5)/acetonitrile (40/60, v/v) Flow rate: 1.0 mL/min Detection: UV at 254nm Temperature: 37 °C Sample: 1.Uracil 2.4-n-Butoxybenzoic acid 3.Ethylbenzene 4.n-Propylbenzene	
		Test B Conditions Column size: 150×4.6mm I.D. Eluent: acetonitrile/water (30/70, v/v) Flow rate: 1.0 mL/min Detection: UV at 254nm Temperature: 37 °C Sample: 1.Caffeine 2.Pyridine 3.Phenol	

Approved : Quality Control Dept.

A. Murada Date: Oct.-31-2002

YMC Co. Ltd.
Kyoto, JapanTightly specified
physical parameters of
silica support

Minimal metal content

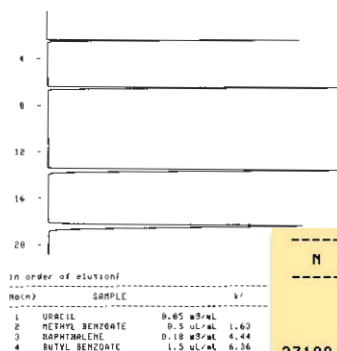
Bonding efficiency

Highly specified
reproducibility for
representative
chromatographic
properties

Individual Column Test

NAME: PARTICLE : YMC-Pack Pro C18, S-5, μ m, 120A
 PRODUCT CODE : AS1200-25000T, AS-360
 SIZE, SER.NO. : 250 x 4.6 mm I.D., No. 912520473 (0)

ELUENT : ACETONITRILE:WATER (60/40)
 FLOW RATE : 1.0 mL/min
 PRESSURE : 10 MPa (102 Kgf/cm²)
 TEMPERATURE : AMBIENT
 DETECTION : UV at 278 nm, 0.32 AUFS
 INJECTION VOLUME : 5 μ L
 CHART SPEED : 5 mm/min

Indicates the efficiency
of the column retention
characteristics and
symmetry of the test peaks

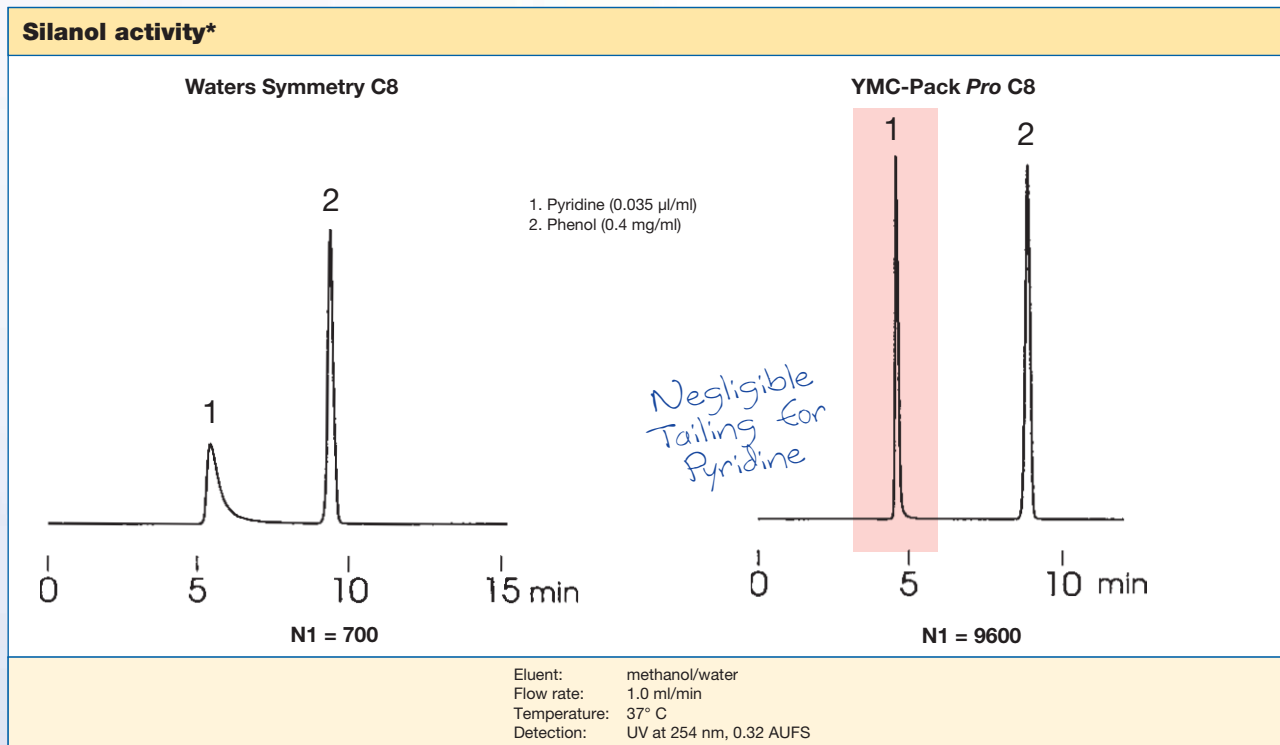
To give our customers an insight into the strict criteria with regard to the silica base, the derivatised final product and the reproducible chromatographic behaviour, each column of the ProFamily is supplied with a lot inspection report and an individual column test chromatogram. The first report illustrates the narrow window for physical parameters such as particle size distribution or surface area and the reproducibility of chemical properties. The test chromatogram illustrates the efficiency of the column with a guaranteed minimum performance of 100,000 theoretical plates for 150 and 250 x 4.6 mm i.d. and an asymmetry of 0.90 to 1.15 (at 10% peak height for 5 μ m particle size).

Guarantee 25000 ≤ N ≤ 32000 , 0.90 ≤ As ≤ 1.15

YMC ProFamily

The following test for silanol activity is used for the whole *ProFamily* and is demonstrated here using *Pro C8* as an example. The use of pyridine and phenol, one basic and one acidic substance, under unbuffered HPLC conditions is an ideal way to “measure” the silanol activity of a stationary phase. A good peak symmetry for both substances indicates a low silanol activity due to an extensive endcapping process. The resulting symmetrical peak shape is important to achieve separations of acidic and basic substances, even if they are part of quite complex sample mixtures.

The comparison shows that the *ProFamily* gives very symmetrical peak shapes, which is not easily achievable for other competitive products.

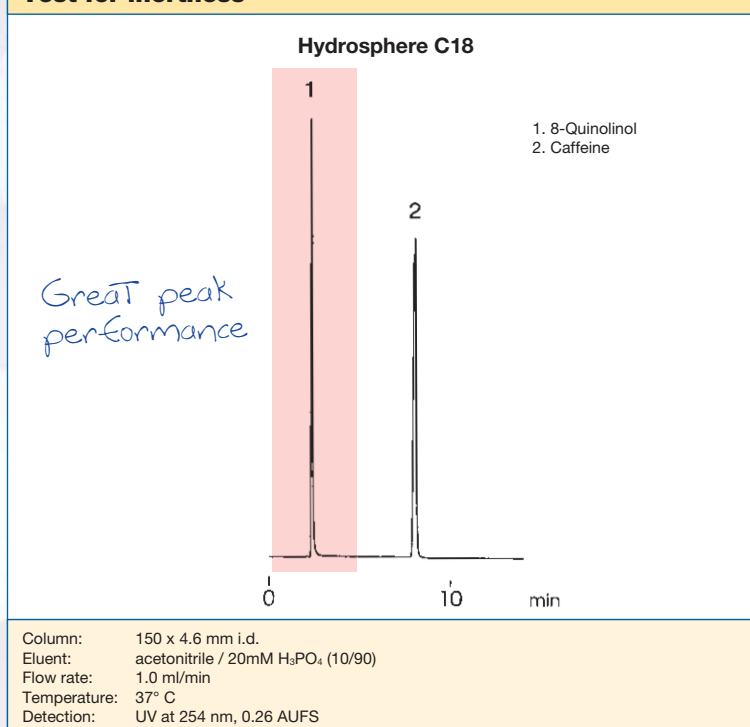


* By courtesy of YMC Co., Ltd.

One reason for a compound showing peak tailing has been described as the interaction of basic compounds and residual silanol groups, the so called residual silanol activity.

Another reason for peak tailing is the metal content of the silica support. The effect can easily be demonstrated by using chelating or metal complexing compounds. The test described here for inertness again demonstrates the very low metal content of the *ProFamily* members where the metal complexing agent 8-quinolinol is used with Hydrosphere C18 as an example.

Test for inertness



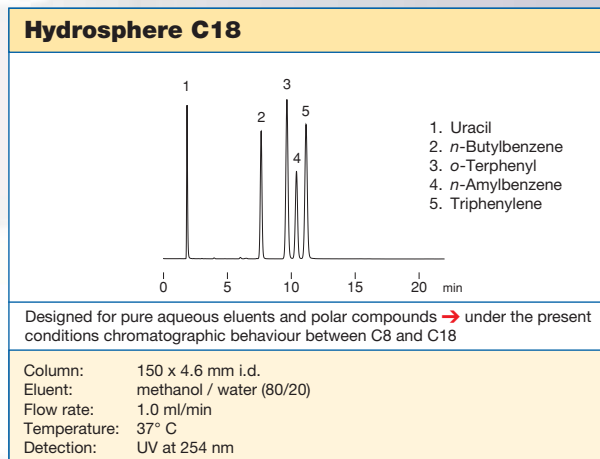
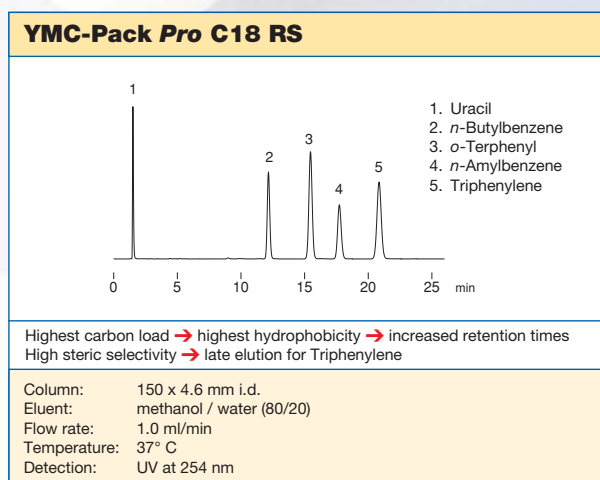
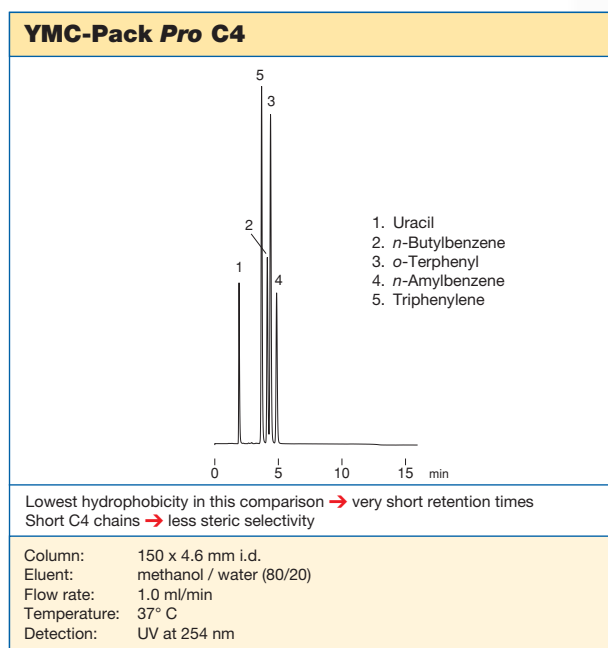
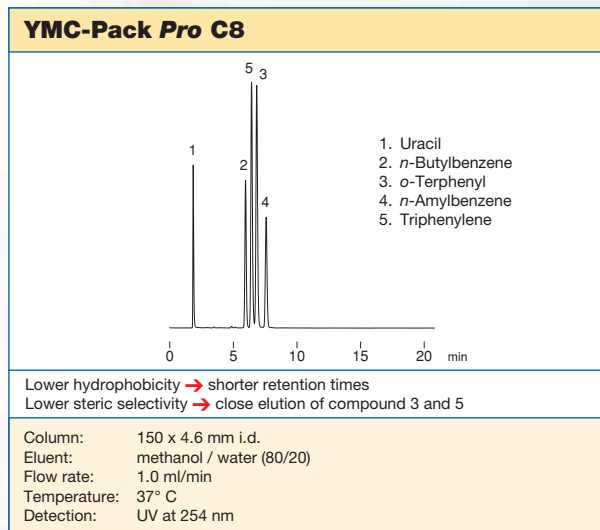
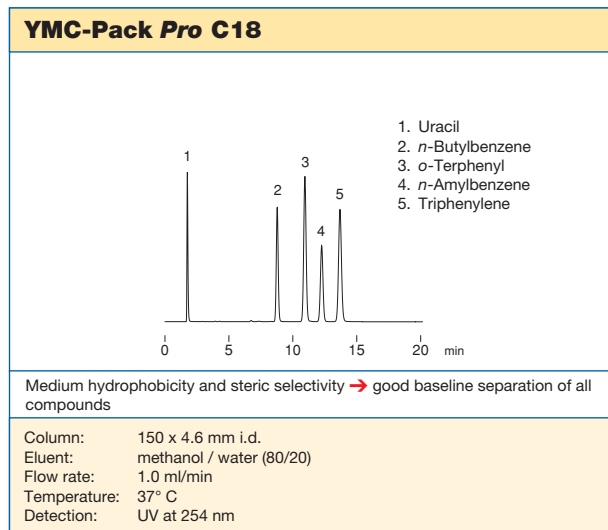
These two tests are combined in the HPLC Test B, described in the Inspection Report (see page 23) to continuously monitor the quality of each individual lot.

YMC ProFamily

Hydrophobicity and steric selectivity

This comparison shows the different properties of the *ProFamily* members giving a good indication on their potential for method development.

The compounds 1. uracil (dead volume marker) 2. *n*-butylbenzene 3. *o*-terphenyl 4. *n*-amylbenzene and 5. triphenylene are used to determine the hydrophobicity (2. and 4.) and the steric selectivity (3. and 5.) of each *ProFamily* member under unbuffered chromatographic conditions.



The whole *ProFamily* covers a large area of hydrophobicity and steric selectivity, as presented in this comparison, which offers the opportunity to accomplish optimisation of chromatographic methods even for complicated separation problems.

For more applications please refer to our "Application Data Collections" or contact us directly.

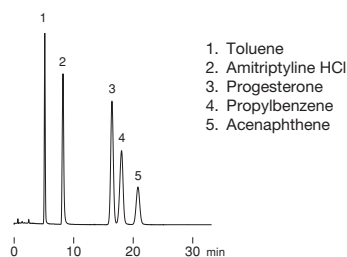
YMC ProFamily

Hydrophobicity and hydrogen bonding capacity in combination with basic substances

In order to compare the hydrophobicity and hydrogen bonding capacity of each *ProFamily* member toluene, propylbenzene and acenaphthene were selected as substances having different hydrophobicity whilst progesterone and amitriptyline were selected to determine the individual behaviour towards substances with different polarities. In addition, amitriptyline is a good indicator for the peak performance while separating basic compounds.

Firstly, all *ProFamily* members give, under these isocratic conditions, excellent symmetrical peak shapes for all compounds. Secondly, each individual member shows not only different retention times, but different elution orders for the tested substances as well, which will be discussed in the figure below.

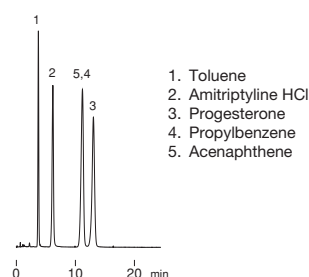
YMC-Pack Pro C18



Elution order of all compounds due to their polarity/hydrophobicity as expected
Amitriptyline (here protonated) → relatively early elution

Column: 50 x 2.0 mm i.d.
Eluent: 10 mM CH₃COONH₄ / methanol (45/55)
Flow rate: 0.2 ml/min
Temperature: 37° C
Detection: UV at 254 nm

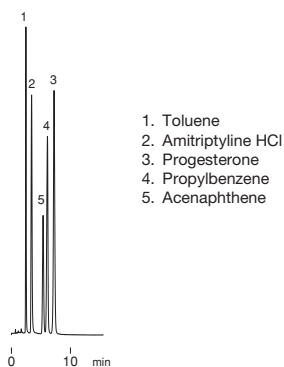
YMC-Pack Pro C8



Lower hydrophobicity of *Pro C8* → change of elution order of peak 3 and 5

Column: 50 x 2.0 mm i.d.
Eluent: 10 mM CH₃COONH₄ / methanol (45/55)
Flow rate: 0.2 ml/min
Temperature: 37° C
Detection: UV at 254 nm

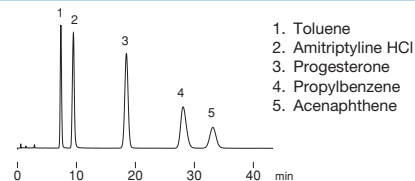
YMC-Pack Pro C4



Lowest hydrophobicity/high hydrogen bonding capacity → still all compounds baseline separated → shortest retention

Column: 50 x 2.0 mm i.d.
Eluent: 10 mM CH₃COONH₄ / methanol (45/55)
Flow rate: 0.2 ml/min
Temperature: 37° C
Detection: UV at 254 nm

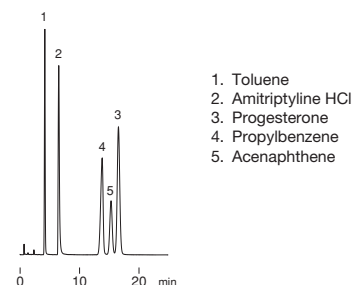
YMC-Pack Pro C18 RS



Higher carbon load, higher hydrophobicity → not surprisingly, increased retention times for hydrophobic compounds in comparison to YMC-Pack *Pro C18*

Column: 50 x 2.0 mm i.d.
Eluent: 10 mM CH₃COONH₄ / methanol (45/55)
Flow rate: 0.2 ml/min
Temperature: 37° C
Detection: UV at 254 nm

Hydrosphere C18



Relatively low hydrophobicity/high hydrogen bonding capacity, especially designed for polar substances → under these conditions a C8/C18 selectivity is obtained

Column: 50 x 2.0 mm i.d.
Eluent: 10 mM CH₃COONH₄ / methanol (45/55)
Flow rate: 0.2 ml/min
Temperature: 37° C
Detection: UV at 254 nm

This second comparison of the *ProFamily* members' characteristics with regard to hydrophobicity and hydrogen bonding capacity approves their suitability for a variety of chemically completely different substances under completion of the endusers' highly demanding requirements.

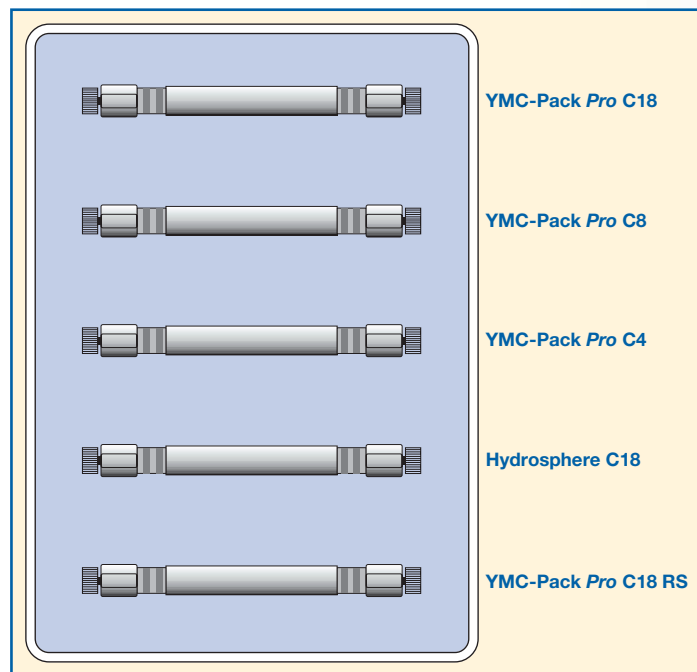
YMC ProFamily

Efficient method development with the ProFamily

The *ProFamily* is far more than just a range of selectivities with graduated values in respect to hydrophobicity. It represents a series of top of the range stationary phases within the YMC portfolio with individual separation properties. The comparisons on the previous two pages show that all *ProFamily* members can be used for virtually the complete field of chromatographic problems, namely the separation of polar, basic, acidic, steric demanding or hydrophobic compounds.

To utilise this applicability, a method development kit consisting of all five *ProFamily* members represents a meaningful tool to start method development for fast, robust and effective HPLC methods. With short columns of 50 or 33 mm length, smallest possible particle sizes and column internal diameters of 1.0, 2.1, 3.0 or 4.0 mm, this product meets all demands for a quick, highly efficient and significant selectivity screening using YMC's latest innovative phases.

Their previously demonstrated benefits, their variety of chromatographic features and their ability of easily solving various chromatographic problems, open a large application area.



YMC-Pack Pro C18



- specifically designed for pharmaceutical and biotechnical R&D
- extreme narrow specifications
- high lot-to-lot reproducibility
- high column-to-column reproducibility
- ideal for basic, acidic and polar compounds



YMC-Pack Pro C18	Specification
Particle size / μm	3; 5
Pore size / nm	12
Surface area / m^2g^{-1}	340
Carbon content / %	17
Recommended pH range	2 - 8

General

In 1996, YMC introduced the first member of the *ProFamily*, YMC-Pack Pro C18, into the market. Since then, YMC-Pack Pro C18 has proven to be one of the first choices for a wide range of HPLC applications in pharmaceutical and biotechnological research and production, where efficiency and reliability are highly appreciated.

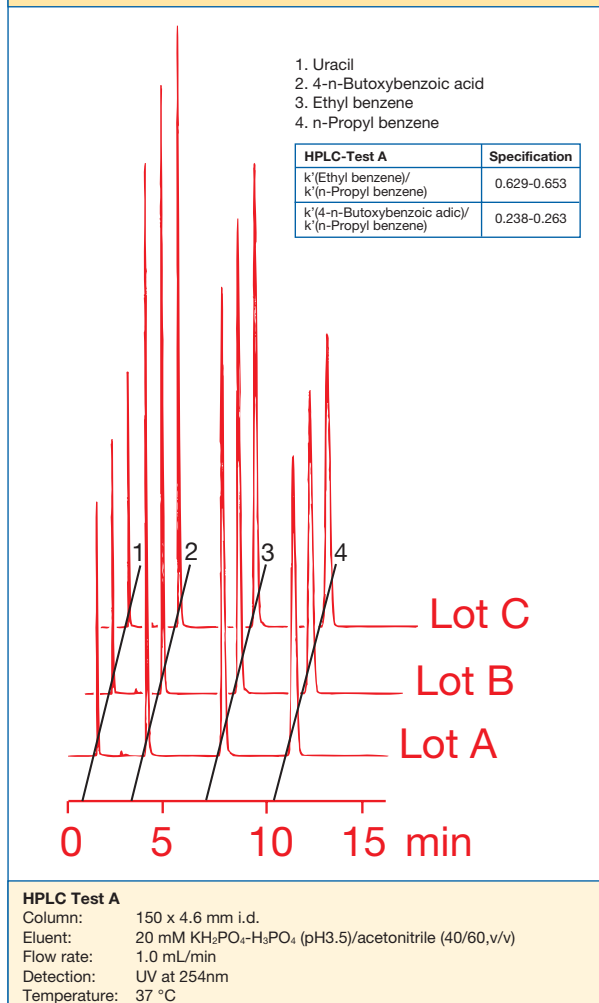
The main reasons for this success are, besides the very good performance in HPLC separations, the high lot-to-lot and column-to-column reproducibility which are essential for chromatography in general. The achievement of this superior performance is not as trivial as first impressions might give. To ensure high quality continuously, a consistent and stringent controlling system is required. The figure on the right demonstrates the high lot-to-lot reproducibility for three lots selected at random. The stringent specifications applied as HPLC Test A, and the actual results obtained from the particular lot, are displayed on each lot inspection report, which is supplied with each individual column.

The retention of compounds ethylbenzene and butoxybenzoic acid relative to propylbenzene is used to monitor the hydrophobicity of each individual lot.



YMC-Pack Pro C18 is also available in pre-packed preparative columns with 11 or 16 μm particle size, respectively.

Lot-to-lot reproducibility of YMC-Pack Pro C18



YMC-Pack Pro C18

High lot-to-lot reproducibility is the essential basis for high performance HPLC columns. A high column performance itself can only be achieved when the columns are well packed and continuously give high numbers of theoretical plates and good asymmetry factors. The figure below clearly demonstrates the high level of reproducible quality from column to column to column.

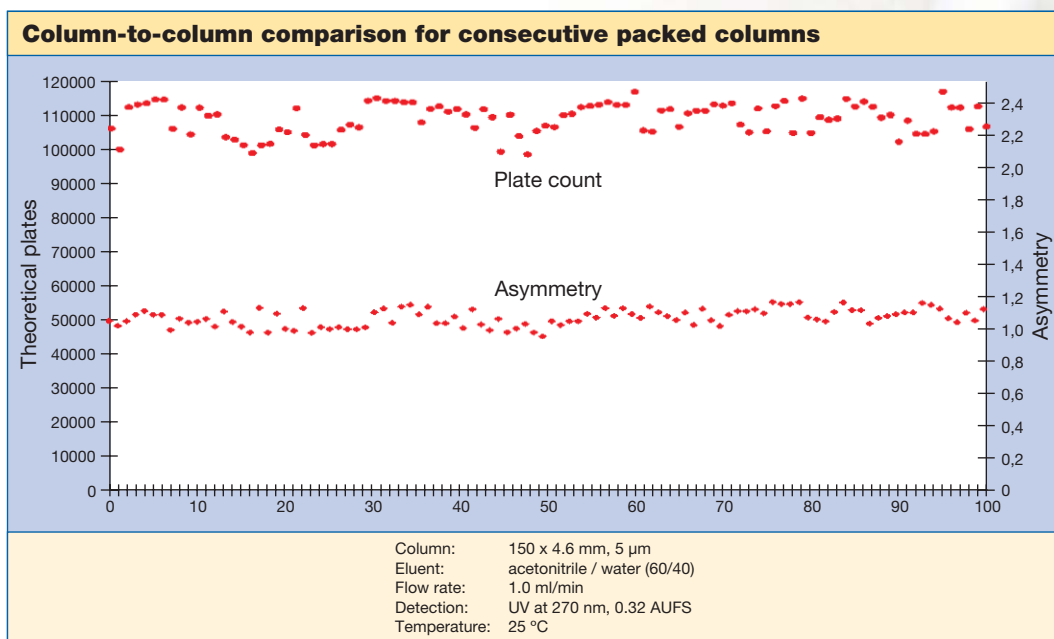
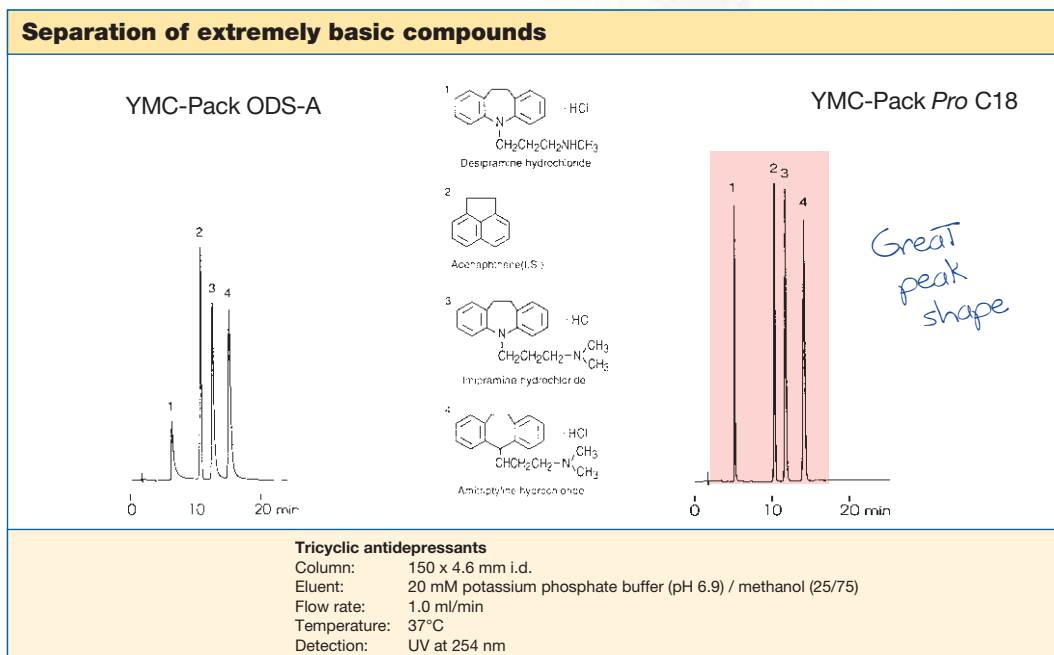


Plate count and asymmetry were calculated for butyl benzoate.

Properties

YMC-Pack Pro C18 is based on an ultra pure silica support, which is used for the whole ProFamily. Due to a proprietary endcapping process especially designed for this type of silica, YMC-Pack Pro C18 is perfectly suitable for the separation of acidic and basic molecules. The inertness of the silica makes it an excellent choice for the analysis of drugs or metabolites, compounds that are susceptible to polar interactions with residual silanol groups and metal impurities as demonstrated in the following comparison. The extreme basic substances are selected to prove the very good performance of YMC-Pack Pro C18 in regard to their separation and the peak performance that cannot be achieved with classical materials.

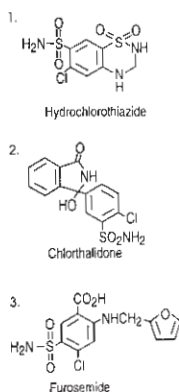
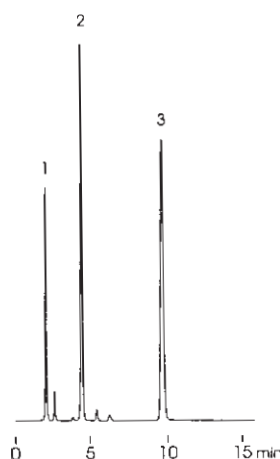


YMC-Pack Pro C18

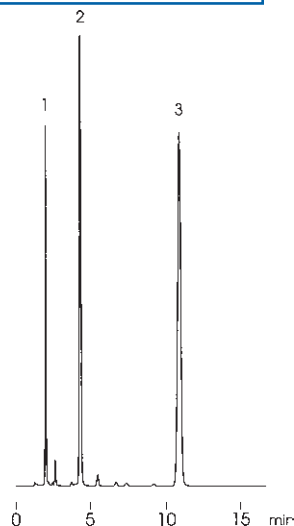
An additional benefit of this phase is the possibility to replace additives, such as phosphate buffers, by volatile acids or bases which are required for LC-MS detection, where the eluent has to be vaporized in the interface. The two figures below demonstrate the ease of transfer of a standard HPLC method into an LC-MS compatible HPLC method without the loss of performance or any changes in the separation itself. Even the request for fast, short and narrow LC-MS columns can be fulfilled with YMC-Pack Pro C18, which gives a wide range of opportunities for LC-MS method development.

LC-MS method transfer on YMC-Pack Pro C18

20 mM Phosphate Buffer

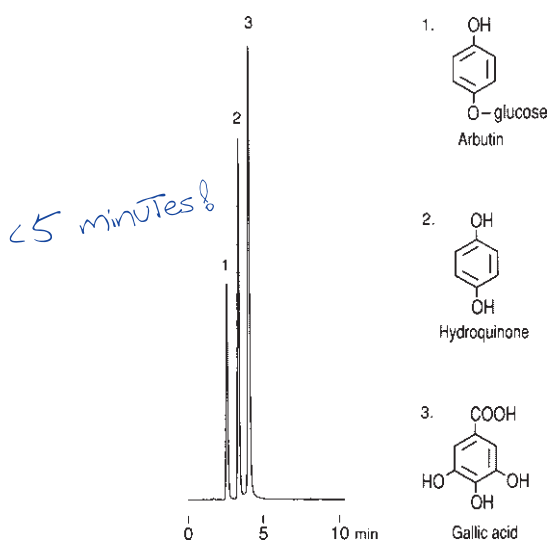


1% Acetic Acid



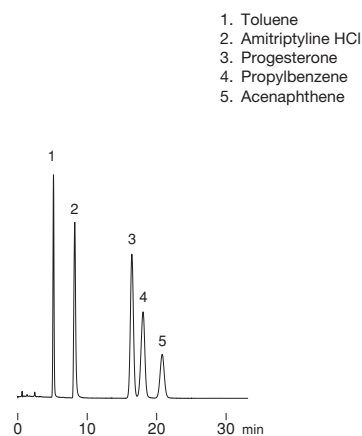
Column: YMC-Pack Pro C18 (5 μ m, 12 nm, 150 x 2.0 mm)
 Eluent: 20 mM $\text{NH}_4\text{H}_2\text{PO}_4$ - H_3PO_4 (pH 3.2) or 1% acetic acid / methanol (60/40)
 Flow rate: 0.2 ml/min
 Temperature: 37°C
 Pressure: 12.0 MPa
 Detection: UV at 280 nm, 0.16 AUFS
 Injection: 3 μ l (0.015 - 0.2 mg/ml)

Fast LC-MS separations



Column: YMC-Pack Pro C18 (3 μ m, 12 nm, 50 x 2.0 mm)
 Eluent: 10 mM $\text{CH}_3\text{COONH}_4$ / methanol (45/55)
 Flow rate: 0.2 ml/min
 Temperature: 37°C
 Detection: UV at 254 nm

Baseline separation of compounds with different polarity

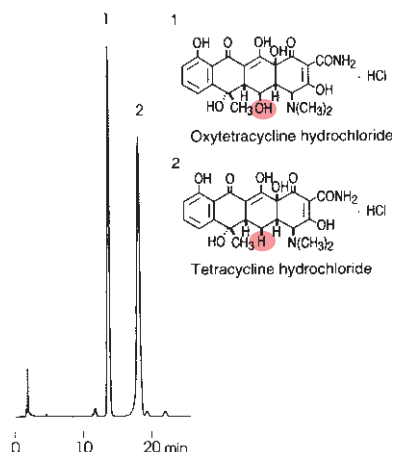


Column: YMC-Pack Pro C18 (3 μ m, 12 nm, 75 x 2.0 mm)
 Eluent: methanol / water / TFA (5/95/0.5)
 Flow: 0.2 ml/min
 Detection: UV at 280 nm, 0.16 AUFS
 Temperature: 30°C
 Injection: 1 μ l (0.2 - 0.5 mg/ml)

YMC-Pack Pro C18

This small collection of applications can only give a brief insight into the multiple applications for *Pro C18*.

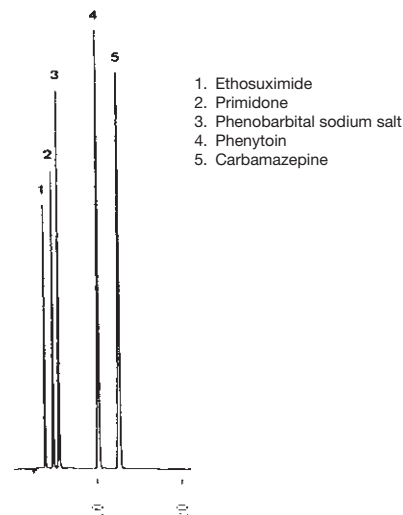
Separation of related tetracycline antibiotics



Tetracycline antibiotics

Column: YMC-Pack Pro C18 (5 μ m, 12 nm, 150 x 4.6 mm)
 Eluent: acetonitrile / 10mM oxalic acid (10/90)
 Flow: 1.0 ml/min
 Detection: UV at 280 nm, 0.13 AUFS
 Temperature: 37 °C
 Injection: 6 μ l (0.2 mg/ml)

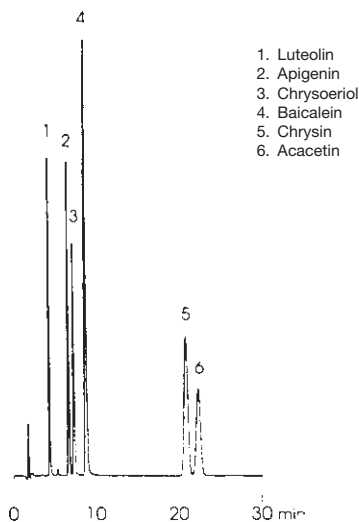
Flavonoids



Antiepileptics

Column: YMC-Pack Pro C18 (5 μ m, 12 nm, 150 x 4.6 mm)
 Eluent: 50 mM $\text{NH}_4\text{H}_2\text{PO}_4$ - $(\text{NH}_4)_2\text{HPO}_4$ (pH 6.7) / methanol (50/50)
 Flow: 0.7 ml/min
 Detection: UV at 215 nm, 0.32 AUFS
 Temperature: 30 °C
 Injection: 8 μ l (0.035 ~ 0.7 mg/ml)

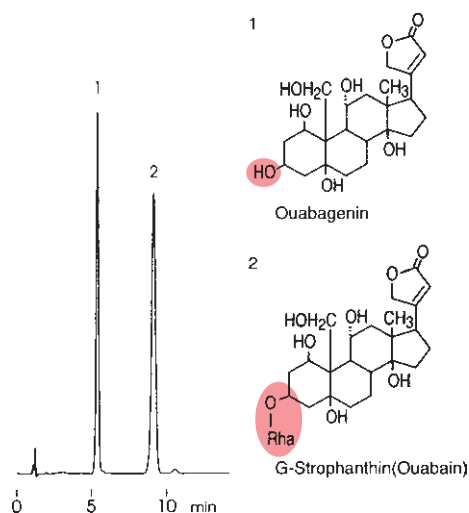
Antiepileptics



Flavonoids

Column: YMC-Pack Pro C18 (5 μ m, 12 nm, 150 x 4.6 mm)
 Eluent: acetonitrile / 10 mM H_3PO_4 (35/65)
 Flow: 1.0 ml/min
 Detection: UV at 280 nm, 0.13 AUFS
 Temperature: 37 °C
 Injection: 5 μ l (0.05 mg/ml)

Separation of related cardiac glycosides



Cardiac glycosides

Column: YMC-Pack Pro C18 (5 μ m, 12 nm, 75 x 4.6 mm)
 Eluent: acetonitrile / water (10/90)
 Flow: 1.0 ml/min
 Detection: UV at 220 nm, 0.26 AUFS
 Temperature: 37 °C
 Injection: 5 μ l (0.2, 0.4 mg/ml)

For more applications please refer to our "Application Data Collections" or contact us directly.

Column care

YMC Pack *Pro C18* is stable towards hydrolysis between pH 2-8. Remove acid and buffer salts before storage. Store the column in methanol / water = 70/30.

For detailed information please refer to the "Column Care and Use Instructions" which are shipped with each analytical column. For detailed ordering information on YMC-Pack *Pro C18* please refer to page 36/37.



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地址：北京回龙观龙冠大厦 719 室

电话：010-51528296/97/98

Email: sales@herbs-extract.com

邮编：102208

传真：010-51528299

<http://www.herbs-extract.com>