YMC ProFamily

Contents

Introduction

YMC *Pro*Family

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 *Pro*Family fulfil these demands making them an excellent choice for the pharmaceutical and biotechnology industries. This product line consists of the three C18-phases: YMC-Pack *Pro* C18 RS, YMC-Pack *Pro* C18 and Hydrosphere C18 together with the C8- and C4-phase: YMC-Pack *Pro* C8 and YMC-Pack *Pro* C4.

YMC ProFamily

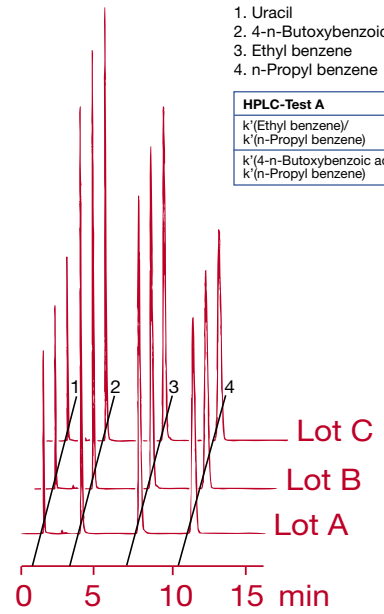
- **YMC-Pack *Pro*Family based on ultra high purity silica**
- **Hydrosphere C18 for stability in aqueous mobile phases**
- **every packed column supplied with:**
  - **lot certificate**
  - **test chromatogram**

	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	2.0 - 8.0	2.0 - 7.5	2.0 - 7.5	1.0 - 10.0	2.0 - 8.0
Metal content	(Randomly selected lots)				
Al / ppm	0.3	0.2	0.6	0.3	0.7
Fe / ppm	2.8	2.5	2.9	0.1	1.2
Na / ppm	0.3	1.4	1.0	1.3	0.7
Ti / ppm	0.1	0.1	0.1	0.1	0.1
	see pages 62-65	see pages 66-67	see pages 68-69	see pages 70-73	see pages 74-77

Properties

Strict quality control is enforced during the manufacturing of the underlying silica, bonding of the stationary phase, end capping and column packing operations to supply high performance columns of high reproducible quality over a long period of time.

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



HPLC-Test A	Specification
k'(Ethyl benzene)/ k'(n-Propyl benzene)	0.629-0.653
k'(4-n-Butoxybenzoic adic)/ k'(n-Propyl benzene)	0.238-0.263

HPLC Test A
Column: 150 x 4.6 mm i.d.
Eluent: 20 mM KH_2PO_4-H_3PO_4 (pH3.5) / acetonitrile (40/60,v/v)
Flow rate: 1.0 mL/min
Detection: UV at 254nm
Temperature: 37 °C

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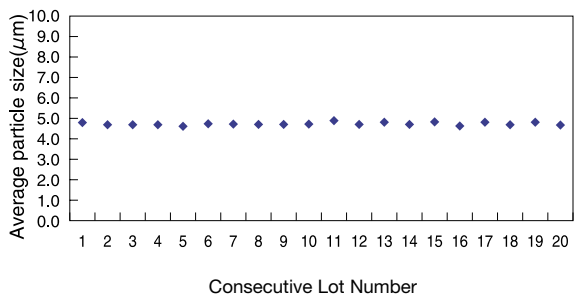
Underlying silica gel support

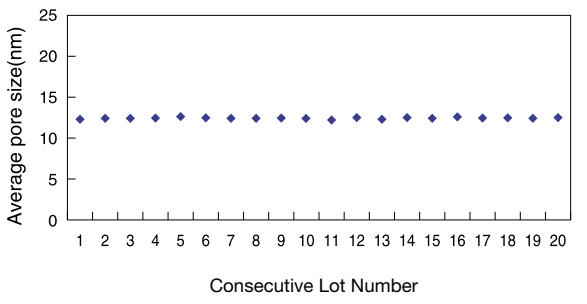
The physical properties of silica gel have a great effect on the selectivity and performance of the bonded packing. For the purpose of supplying columns of stable quality, the physical properties of silica gel used for packing such as particle size, pore size, specific surface area, pore volume and amount of metal contamination have to be strictly controlled.

Physical properties (*Pro* C18, 5 µm, 12 nm)

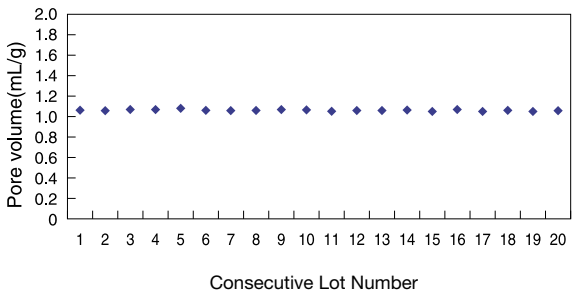
Particle size



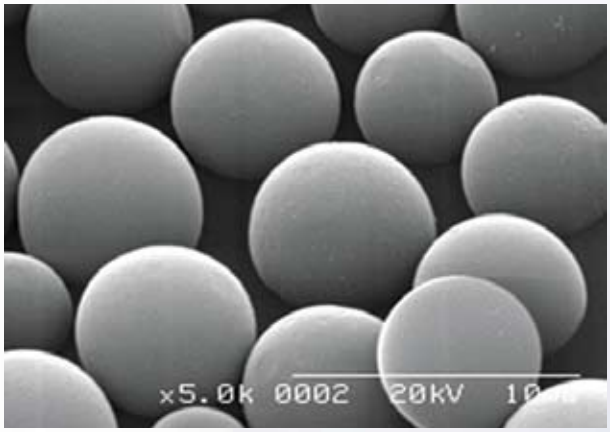
Pore size



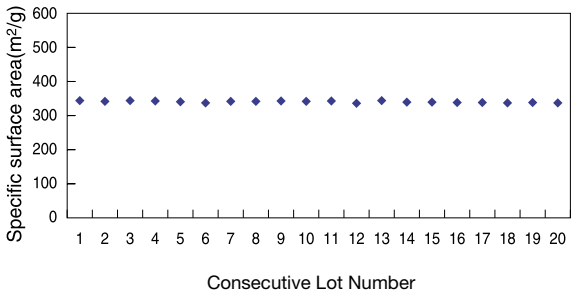
Pore volume



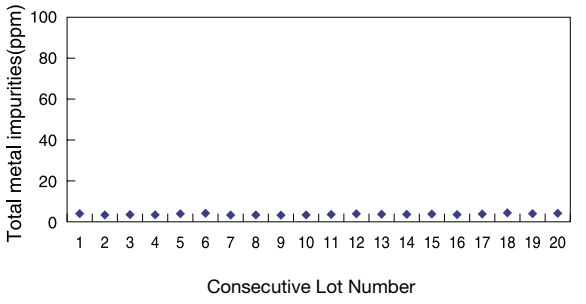
Silica Support Material (5 µm, 12 nm)



Specific surface area



Amount of metal content



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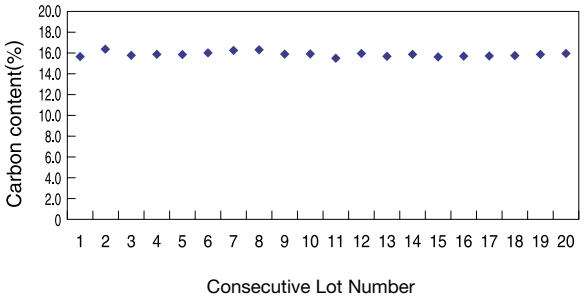
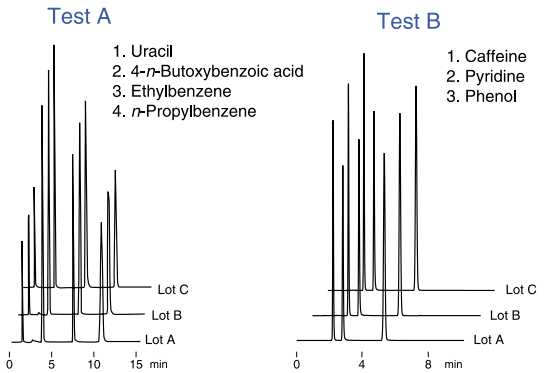
Packing material

Excellent reproducibility of the *Pro* C18 is shown not only in the separation of hydrophobic compounds but also in that of hydrophilic, basic, and acidic compounds.

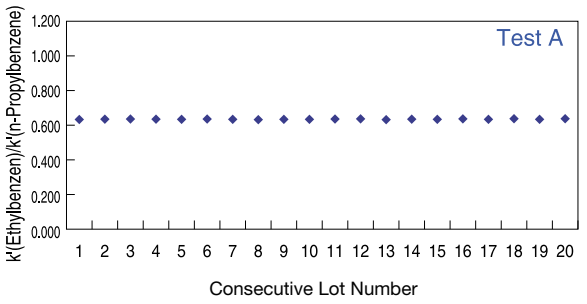
Pro C18 5 µm, Reproducibility between batches

Percent Carbon Content

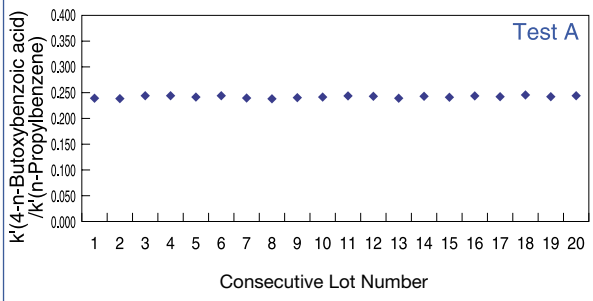




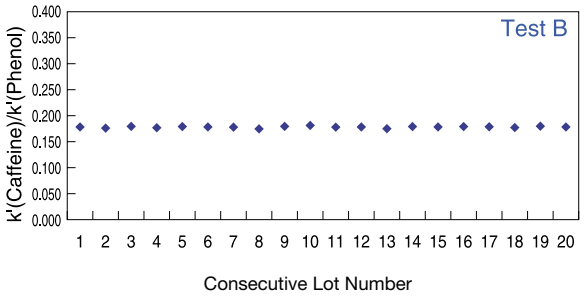
Hydrophobicity



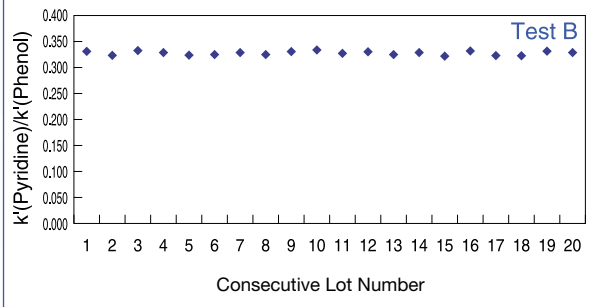
Acidic compounds



Hydrogen bonding ability



Basic compounds



Individual Column Test

To give our customers an insight into the strict criteria with regard to the silica base, the bonded final product and the reproducible chromatographic behaviour, each column of the *Pro*Family 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 µm particle size).

```
////////////////////////////////////////////////////////////////////////
//                YMC HPLC COLUMN INSPECTION REPORT                   //
////////////////////////////////////////////////////////////////////////

NAME, PARTICLE      :  YMC-Pack Pro C18, S-5 µm,12nm          GEL LOT,7602
PRODUCT CODE        :  AS12S05-1546WT, AS-302
SIZE, SER.NO.       :  150 x 4.6 mmI.D., No.0415125458(W)

ELUENT              :  ACETONITRILE/WATER ( 60/40 )
FLOW RATE           :  1  mL/min
PRESSURE            :  6.86  MPa
TEMPERATURE         :  AMBIENT
DETECTION           :  UV at 270 nm,  0.32  AUFS
INJECTION VOLUME    :  5  uL
CHART SPEED         :  5  mm/min
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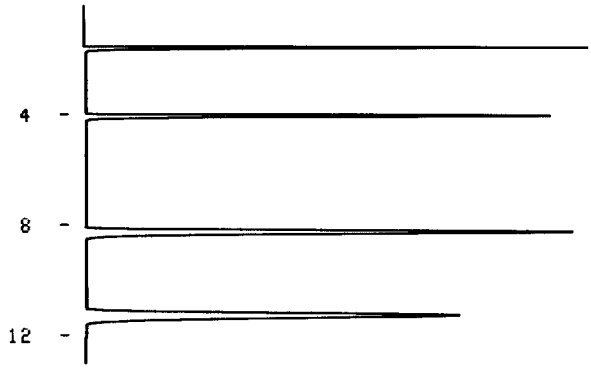


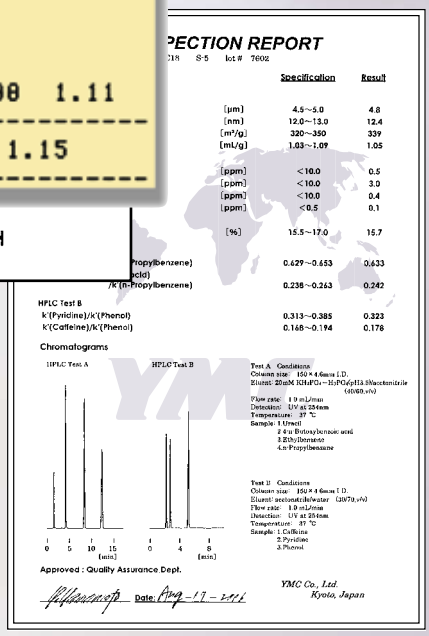
in order of elution;

No(n)	SAMPLE		k'	Alpha	N	As
1	URACIL	0.05 mg/mL				
2	METHYL BENZOATE	0.5 uL/mL	1.6			
3	NAPHTHALENE	0.18 mg/mL	4.34	2.71		
4	BUTYL BENZOATE	1.5 uL/mL	6.31	1.45	17000	1.11

Guarantee 15000 - N - 19000 , 0.90 - As - 1.15

As, asymmetry factor at 10% Peak height
[SYSTEM No. 102] [INSPECTED BY M.BANDO] YMC Co.,Ltd., JAPAN

Individual Lot Test


PECTION REPORT

	Specification	Result
[µm]	4.5~5.0	4.8
[nm]	12.0~13.0	12.4
[m²/g]	320~350	339
[mL/g]	1.03~1.09	1.05
[ppm]	<10.0	0.5
[ppm]	<10.0	3.0
[ppm]	<10.0	0.4
[ppm]	<0.5	0.1
[%]	15.5~17.0	15.7
Propylbenzene)	0.629~0.653	0.633
acid)		
/k'(n-Propylbenzene)	0.238~0.263	0.242
HPLC Test B		
k'(Pyridine)/k'(Phenol)	0.313~0.385	0.323
k'(Caffeine)/k'(Phenol)	0.168~0.194	0.178

Chromatograms

HPLC Test A HPLC Test B

Approved : Quality Assurance Dept.

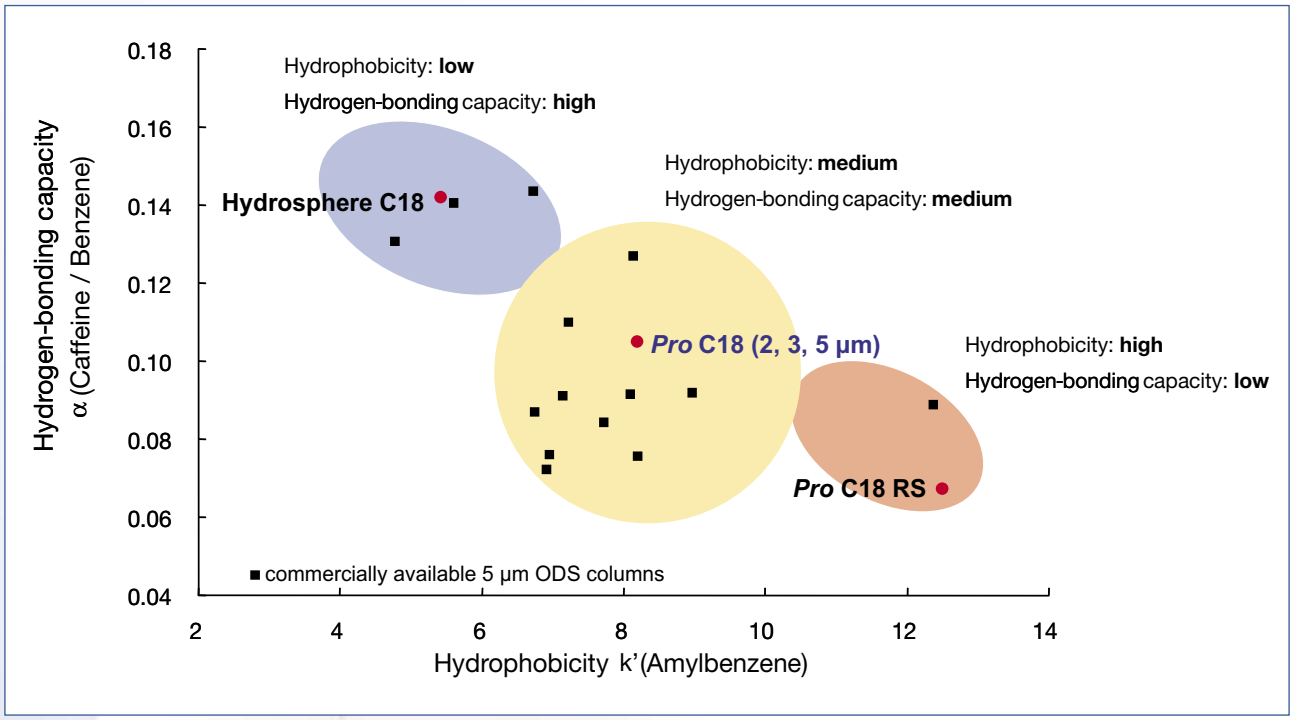
YMC Co., Ltd.
Kyoto, Japan

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

YMC ProFamily

Comparison of separative selectivity

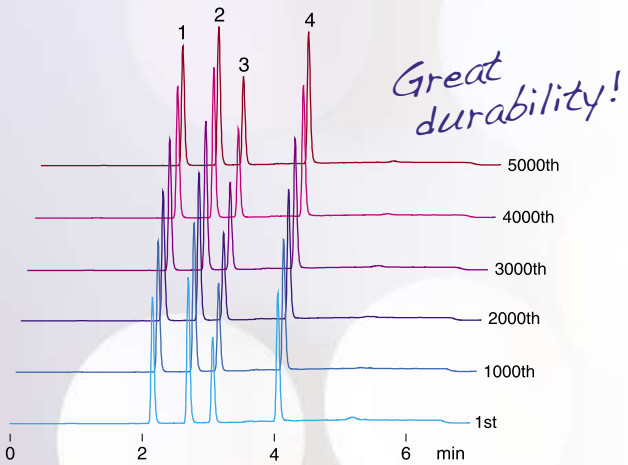
The selectivity characteristics of each column are shown using hydrophobicity and hydrogen-bonding ability as indicators. The *Pro*Family series of ODS phases is designed to make Hydrosphere C18 and *Pro* C18 RS have contrasting separation characteristics, with standard *Pro* C18 in between. Also, *Pro* C8 and C4 have different selectivity from the ODS phases. By choosing one from these 5 types of columns, one can easily optimise the separation of polar and non-polar compounds.



Source: Courtesy of YMC Co., Ltd.

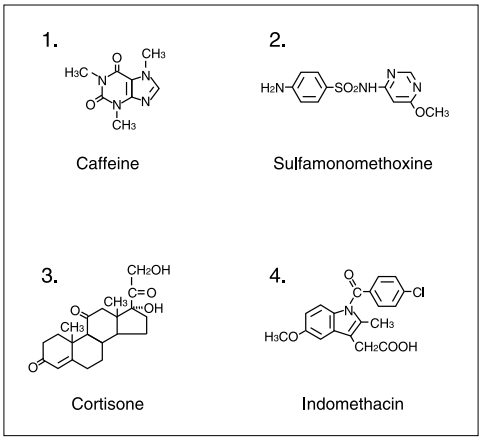
Durability for repetitive analysis

The long-term stability of a *Pro* C18 (3 µm) short column used in repeated analysis is shown below. There is no change found in the separation of all compounds after 5000 injections (8 hours/day for 5 month) during gradient analysis.



	tR(4)	N(4)	Rs(4-3)
1st	4.06	37700	11.86
1000th	4.05	37600	11.85
2000th	4.05	37600	11.84
3000th	4.05	37600	11.84
4000th	4.06	37800	11.84
5000th	4.06	37800	11.86

Column : YMC-Pack Pro C18 (3 µm, 12 nm)
50 x 4.6 mm i.d.
Eluent : A) water / formic acid (100/0.05
B) acetonitrile / formic acid (100/0.05)
10-90% B (0-3 min, linear), 90% B (3-5 min), 10% B (5-9 min)
Flow rate : 1.0 ml/min
Temperature : 37 °C
Detection : UV at 254 nm



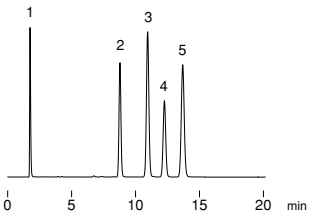
YMC ProFamily

Hydrophobicity and steric selectivity

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

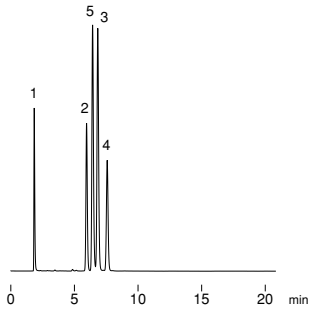
The compounds 1. uracil (dead volume marker) 2. n-butylbenzene 3. o-terphenyl 4. n-amylbenzene and 5. triphenylene are used to determine the hydrophobicity (2. and 4.) and the steric selectivity (3. and. 5.) of each *Pro*Family member under unbuffered chromatographic conditions.

YMC-Pack *Pro* C18



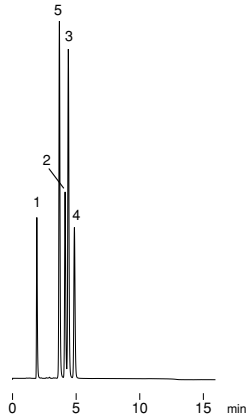
balanced hydrogen bonding, capacity and hydrophobicity

YMC-Pack *Pro* C8



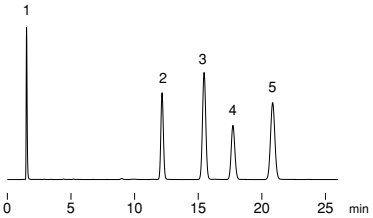
lower hydrophobicity

YMC-Pack *Pro* C4



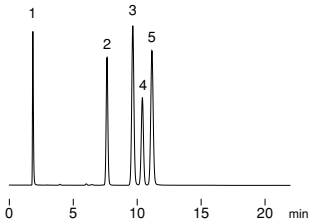
lowest hydrophobicity

YMC-Pack *Pro* C18 RS



highest carbon load

Hydrosphere C18



high polar recognition

Column:	150 x 4.6 mm i.d.	1. Uracil
Eluent:	methanol / water (80/20)	2. *n*-Butylbenzene
Flow rate:	1.0 ml/min	3. *o*-Terphenyl
Temperature:	37° C	4. *n*-Amylbenzene
Detection:	UV at 254 nm	5. Triphenylene

Source: Courtesy of YMC Co., Ltd.

The whole *Pro*Family 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.

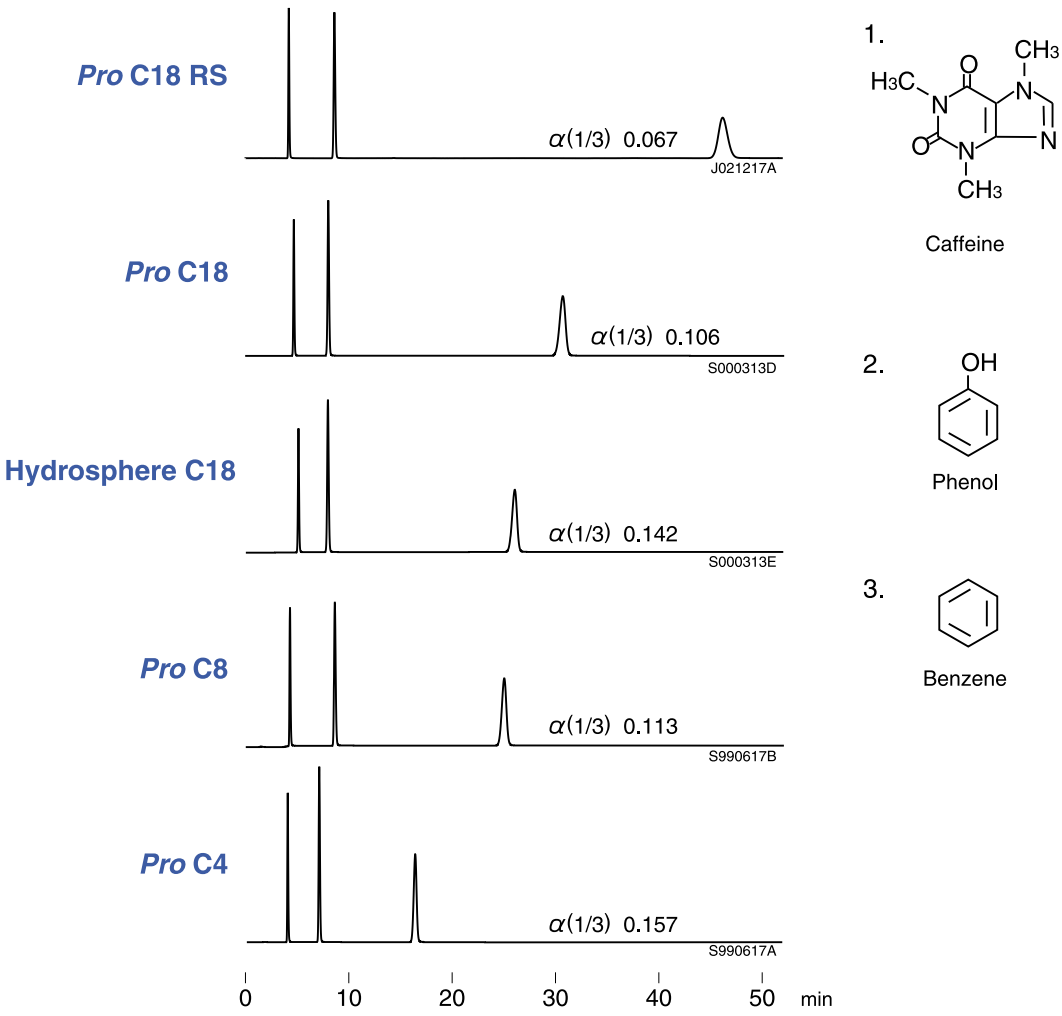
For more applications please refer to our “Application Data Collections” or contact us directly.

YMC ProFamily

Hydrogen bonding capacity

Hydrogen bonding capacity is evaluated by examining the relative retention coefficient as α (caffeine / benzene). Among the *Pro* series both Hydrosphere C18 with low density of C18, and *Pro* C4 with short alkyl chain have high hydrogen-bonding capacity. Benzene with non-polar groups is retained according to hydrophobicity of the packing, while retention of caffeine and phenol (hydrophilic compounds), is greatly affected by hydrogen-bonding capacity, and these packing have similar retention time, but show different selectivity.

Hydrogen bonding capacity*



Column:	150 x 4.6 mm i.d.
Eluent:	methanol / water (30/70)
Flow rate:	1.0 ml/min
Temperature:	37° C
Detection:	UV at 254 nm

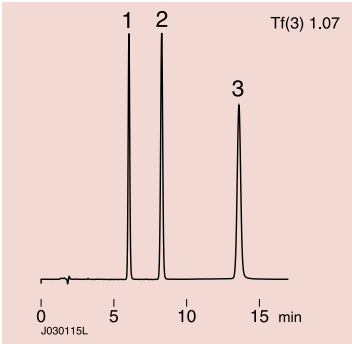
YMC ProFamily

Acidic and basic compounds

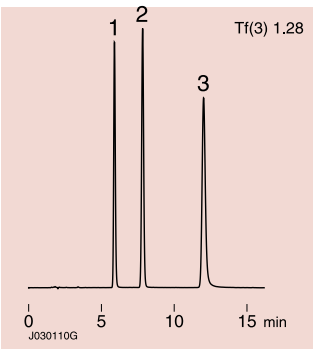
The chromatogram below show the separation of some food additives. Dehydroacetic acid, a compound that can exhibit peak tailing on competitive columns shows excellent peak shape on *Pro*Family columns.

Acidic compounds*

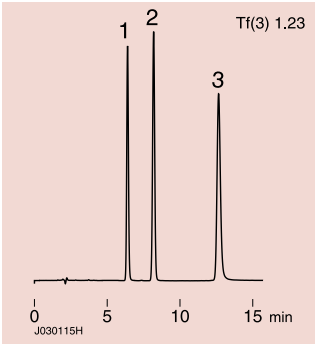
Pro C18 RS



Pro C18



Hydrosphere C18

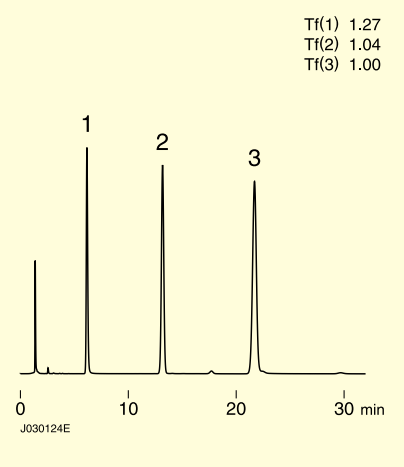


Column: 150 x 4.6 mm i.d.
Eluent: 20 mM CH_3COONa-CH_3COOH (pH 4.4) / acetonitrile (80/20)
Flow rate: 1.0 ml/min
Temperature: 37° C
Detection: UV at 230 nm

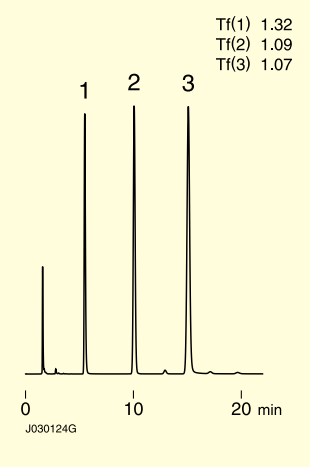
1. p-Hydroxyacetophenone (I.S.)
2. Sorbic acid
3. Dehydroacetic acid

Basic compounds*

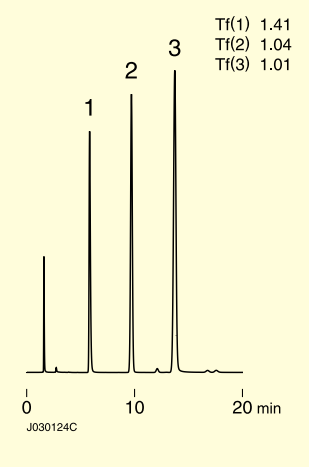
Pro C18 RS

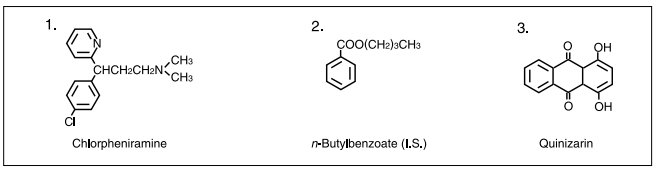


Pro C18



Hydrosphere C18





Column: 150 x 4.6 mm i.d.
Eluent: 20 mM KH_2PO_4-K_2HPO_4 (pH 6.9) / methanol (30/70)
Flow rate: 1.0 ml/min
Temperature: 37° C
Detection: UV at 254 nm

* Source: Courtesy of YMC Co., Ltd.

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	2*; 3*; 5
Pore size / nm	12
Surface area / m^2g^{-1}	340
Carbon content / %	17
Recommended pH range	2.0 - 8.0

* please be referred to page 35 ff for Ultra Fast LC and Fast LC Columns

General

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 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.

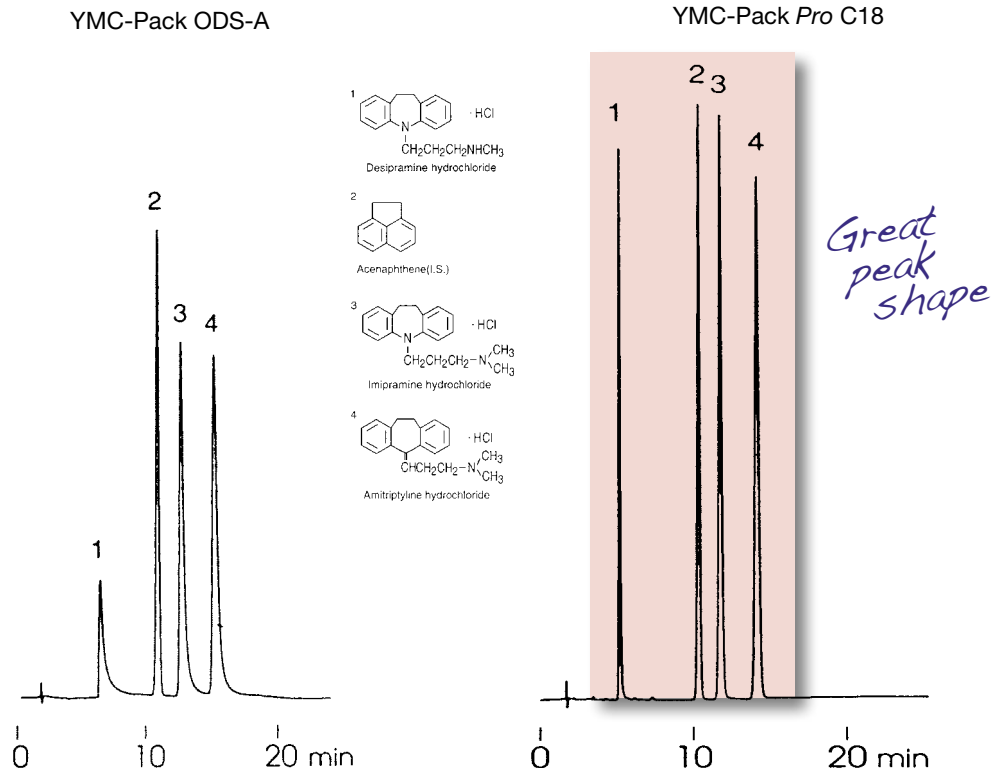
YMC-Pack *Pro* C18 is also available in preparative particle sizes.

YMC-Pack Pro C18

Properties

YMC-Pack *Pro* C18 is based on an ultra pure silica support, which is used for the whole *Pro*Family. 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.

Basic compounds



Tricyclic antidepressants

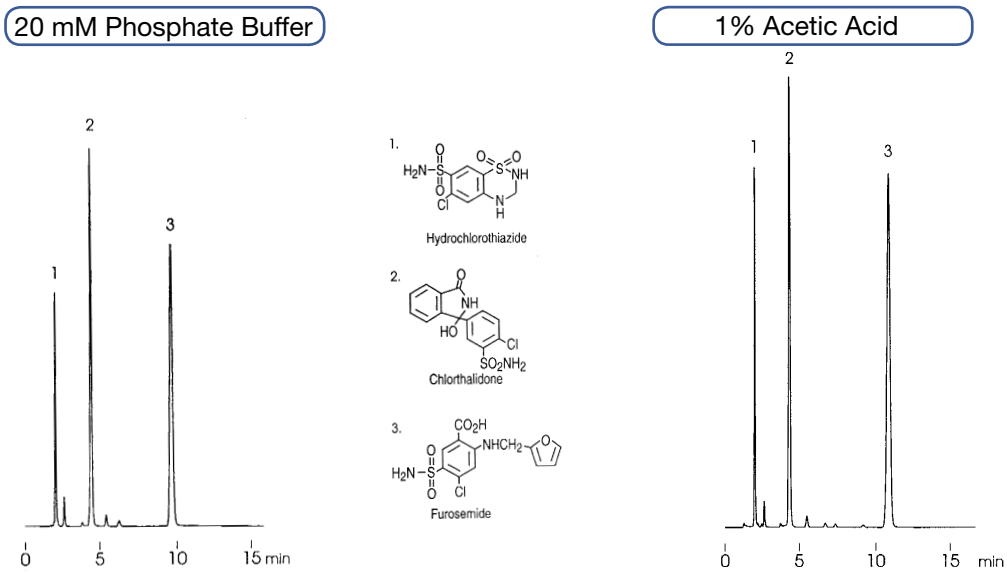
Column:	YMC-Pack *Pro* C18 (5 µm, 12 nm) 150 x 4.6 mm i.d.
Eluent:	20 mM potassium phosphate buffer (pH 6.9) / methanol (25/75)
Flow rate:	1.0 ml/min
Temperature:	37°C
Detection:	UV at 254 nm

YMC-Pack Pro C18

LC-MS Application

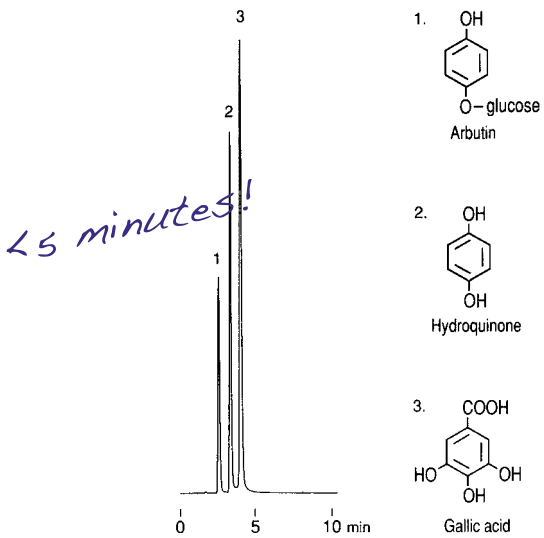
An additional benefit of this phase is the possibility to replace additives, such as phosphate buffers, by volatile acids or bases which are required for LC-MS detection, where the eluent has to be vaporized in the interface. The two figures below demonstrate the ease of transfer of a standard HPLC method 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



Column: YMC-Pack *Pro* C18 (5 µm, 12 nm) 150 x 2.0 mm i.d.
Eluent: 20 mM $NH_4H_2PO_4$-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 µl (0.015 - 0.2 mg/ml)

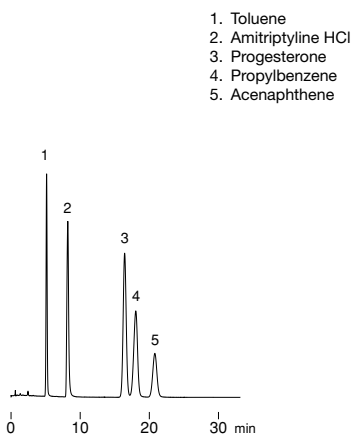
Fast LC-MS separations



< 5 minutes!

Column: YMC-Pack *Pro* C18 (3 µm, 12 nm) 50 x 2.0 mm i.d.
Eluent: 10 mM CH_3COONH_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



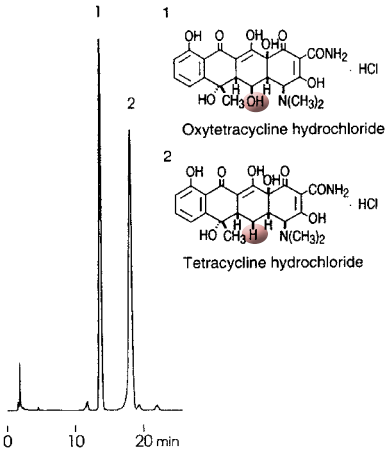
Column: YMC-Pack *Pro* C18 (3 µm, 12 nm) 75 x 2.0 mm i.d.
Eluent: methanol / water / TFA (5/95/0.5)
Flow: 0.2 ml/min
Detection: UV at 280 nm, 0.16 AUFS
Temperature: 30 °C
Injection: 1 µl (0.2 – 0.5 mg/ml)

YMC-Pack Pro C18

Application

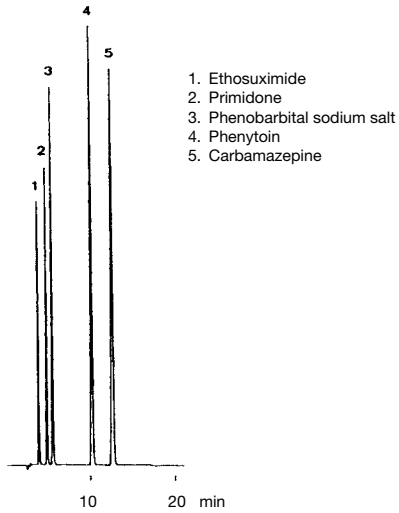
This small collection of applications can only give a brief insight into the multiple applications for *Pro* C18.

Tetracycline antibiotics



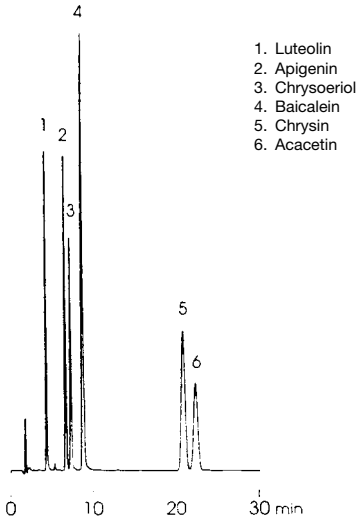
Column:	YMC-Pack *Pro* C18 (5 µm, 12 nm) 150 x 4.6 mm i.d.
Eluent:	acetonitrile / 10 mM oxalic acid (10/90)
Flow:	1.0 ml/min
Detection:	UV at 280 nm, 0.13 AUFS
Temperature:	37 °C
Injection:	6 µl (0.2 mg/ml)

Antiepileptics



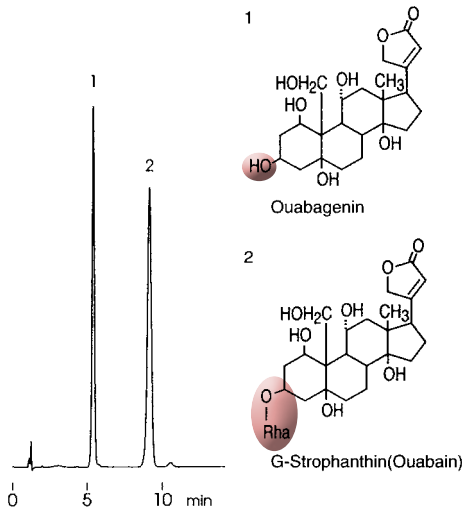
Column:	YMC-Pack *Pro* C18 (5 µm, 12 nm) 150 x 4.6 mm i.d.
Eluent:	50 mM $NH_4H_2PO_4$-$(NH_4)_2HPO_4$ (pH 6.7) / methanol (50/50)
Flow:	0.7 ml/min
Detection:	UV at 215 nm, 0.32 AUFS
Temperature:	30 °C
Injection:	8 µl (0.035 ~ 0.7 mg/ml)

Flavonoids



Column:	YMC-Pack *Pro* C18 (5 µm, 12 nm) 150 x 4.6 mm i.d.
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 µl (0.05 mg/ml)

Cardiac glycosides



Column:	YMC-Pack *Pro* C18 (5 µm, 12 nm) 75 x 4.6 mm i.d.
Eluent:	acetonitrile / water (10/90)
Flow:	1.0 ml/min
Detection:	UV at 220 nm, 0.26 AUFS
Temperature:	37 °C
Injection:	5 µl (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 2.0-8.0. 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.

YMC-Pack Pro C8

OS

L7

LC MS

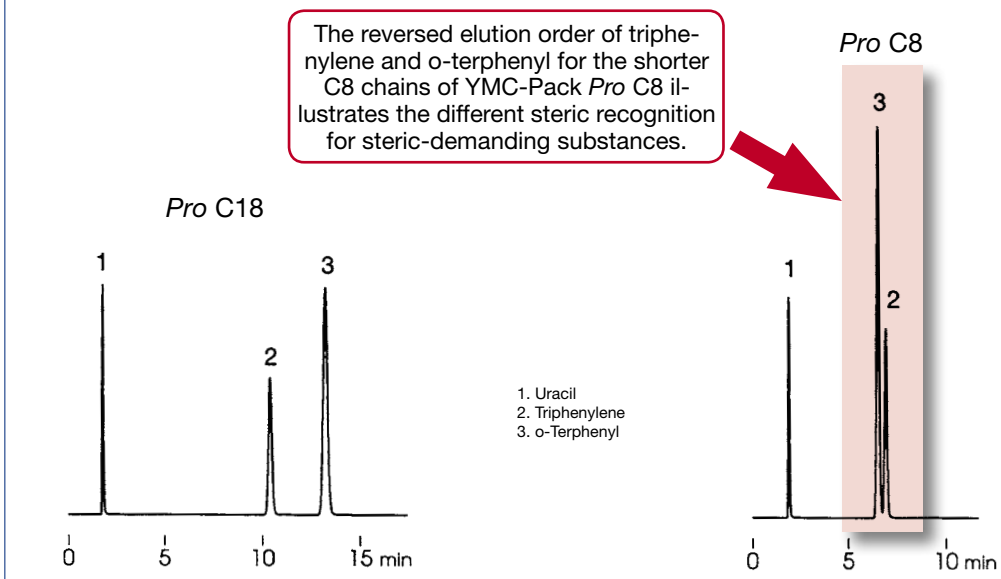
- **extremely broad selectivity pattern**
- **good alternative to C18-phases**
- **suitable for all types of organic molecules, especially basic pharmaceuticals**

YMC-Pack *Pro* C8	Specification
Particle size / µm	3; 5
Pore size / nm	12
Surface area / m^2g^{-1}	340
Carbon content / %	11
Recommended pH range	2.0 - 7.5

General

Within the *Pro*Family, the YMC-Pack *Pro* C8 provides an additional, less hydrophobic stationary phase for all types of compounds, but especially for basic and metal chelating substances. For many applications regarding the separation of peptides, nucleic acids and similar compounds with LC-MS detection, conventional C8-stationary phases require ion pair reagents and ion-suppression to obtain satisfactory separation and low detection limits. In contrast, *Pro* C8 with its ultra pure silica allows the analysis without these modifiers but still generates excellent chromatograms. In addition to the reduced hydrophobicity of YMC-Pack *Pro* C8 compared with YMC-Pack *Pro* C18, the different steric selectivity offers new possibilities in method optimisation as demonstrated in the figure below:

Steric selectivity YMC-Pack *Pro* C18 vs. YMC-Pack *Pro* C8



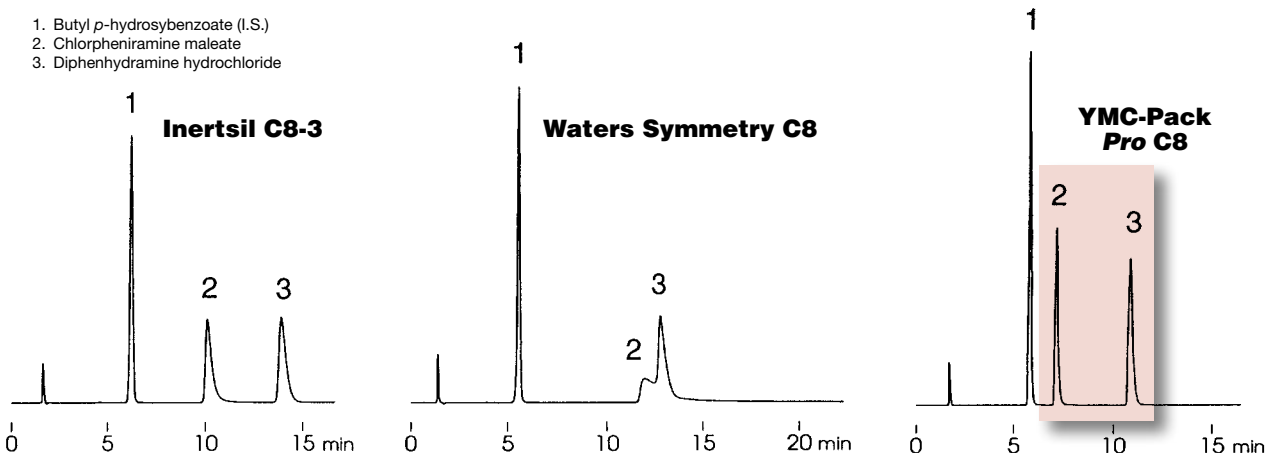
Column: 150 x 4.6 mm i.d.
Eluent: methanol / water (80/20)
Flow rate: 1.0 ml/min
Temperature: 37° C
Detection: UV at 254 nm

Column Kits
MD VAL R&D PS
pages 22 - 31

YMC-Pack Pro C8

YMC-Pack *Pro* C8 is a member of the *Pro*Family and as a result gives excellent peak shapes even for basic substances, due to its low metal content and the endcapping procedure, which is identical to that used for YMC-Pack *Pro* C18. This shall be demonstrated in the comparison below where YMC-Pack *Pro* C8 outperforms competitive state of the art products.

Peak performance of YMC-Pack *Pro* C8 for basic substances*

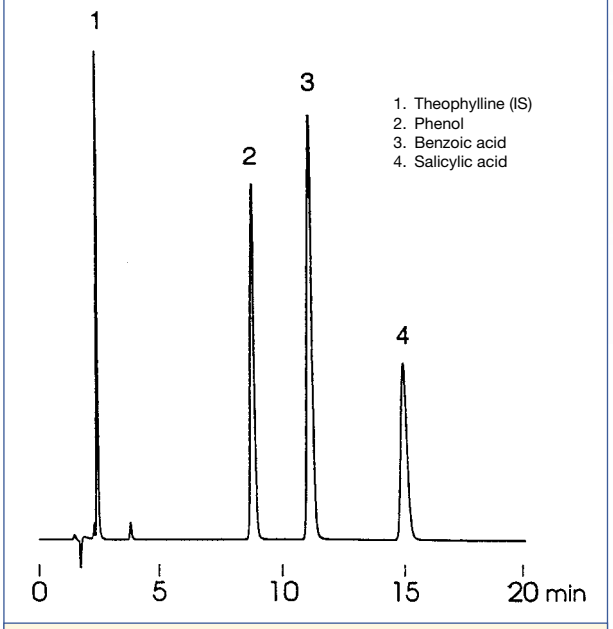


Histamine antagonists

Column:	150 x 4.6 mm i.d.
Eluent:	20 mM KH_2PO_4-K_2HPO_4 (pH 6.9) / methanol (35/65)
Flow rate:	1.0 ml/min
Temperature:	37° C
Detection:	UV at 260 nm, 0.26 AUFS

* By courtesy of YMC Co., Ltd.

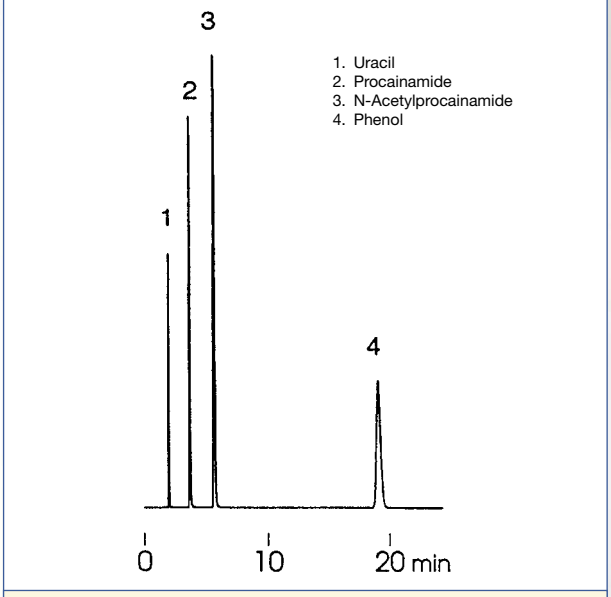
No need for ion pair reagents



Disinfectants

Column:	YMC-Pack *Pro* C18 (5 µm, 12 nm) 150 x 4.6 mm i.d.
Eluent:	acetonitrile / 20 mM H_3PO_4 (20/80)
Flow rate:	1.0 ml/min
Temperature:	37° C
Detection:	UV at 270 nm, 0.32 AUFS

Good peak performance for acidic and basic compounds



Basic compounds

Column:	YMC-Pack *Pro* C18 (5 µm, 12 nm) 150 x 4.6 mm i.d.
Eluent:	20 mM KH_2PO_4-K_2HPO_4 (pH 6.9) / acetonitrile (90/10)
Flow rate:	1.0 ml/min
Temperature:	37° C
Detection:	UV at 254 nm, 0.32 AUFS

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

Column care

YMC-Pack *Pro* C8 is stable towards hydrolysis between pH 2.0-7.5. 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.

YMC-Pack Pro C4

BS

L26

LC MS

- **proprietary endcapping in order to minimize the effect of residual silanols**
- **for polar organic molecules, especially basic pharmaceuticals and peptides**
- **ideal for fast chromatography**

YMC-Pack *Pro* C4	Specification
Particle size / µm	3; 5
Pore size / nm	12
Surface area / m^2g^{-1}	340
Carbon content / %	8
Recommended pH range	2.0 - 7.5

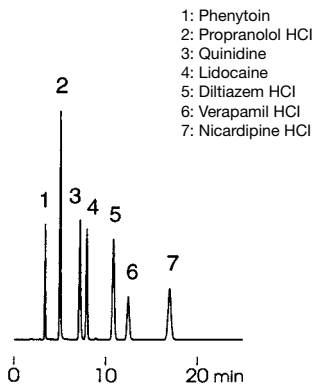
General

More than 80% of the RP-HPLC analyses are accomplished on octyl- or octadecyl-phases. Because of this overwhelming majority, many chromatographers neglect other selectivities that might be better suited to their separation, such as butyl phases. With *Pro* C4, YMC offers a stationary phase based on the well-known ultra pure silica of the *Pro*Family. Compared to a C18-phase with the same eluent, this less hydrophobic material gives shorter retention times for non-polar compounds while the retention time of polar analytes are virtually unaffected. This makes the *Pro* C4 an interesting alternative especially when short analysis times are required.

For this reason, mixtures with a wide range of component polarity are best analysed by short chains, such as YMC-Pack *Pro* C4.

Within the *Pro*Family, YMC-Pack *Pro* C4 is the selectivity of choice to reduce time of analysis in combination with the given advantages of the *Pro*Family, namely the high purity silica support and the low metal content, which result in excellent peak performance as below.

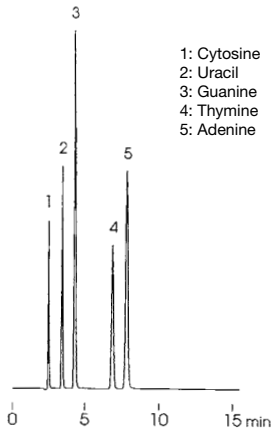
Efficient separation of pharmaceutical drugs



Antiarrhythmic drugs

Column:	YMC-Pack *Pro* C4 (5 µm, 12 nm) 150 x 4.6 mm i.d.
Eluent:	20 mM KH_2PO_4-K_2HPO_4 (pH 6.9) / methanol (40/60)
Flow rate:	1.0 ml/min
Temperature:	37° C
Detection:	UV at 220 nm

Fast separation of nucleic acid bases



Nucleic acid bases

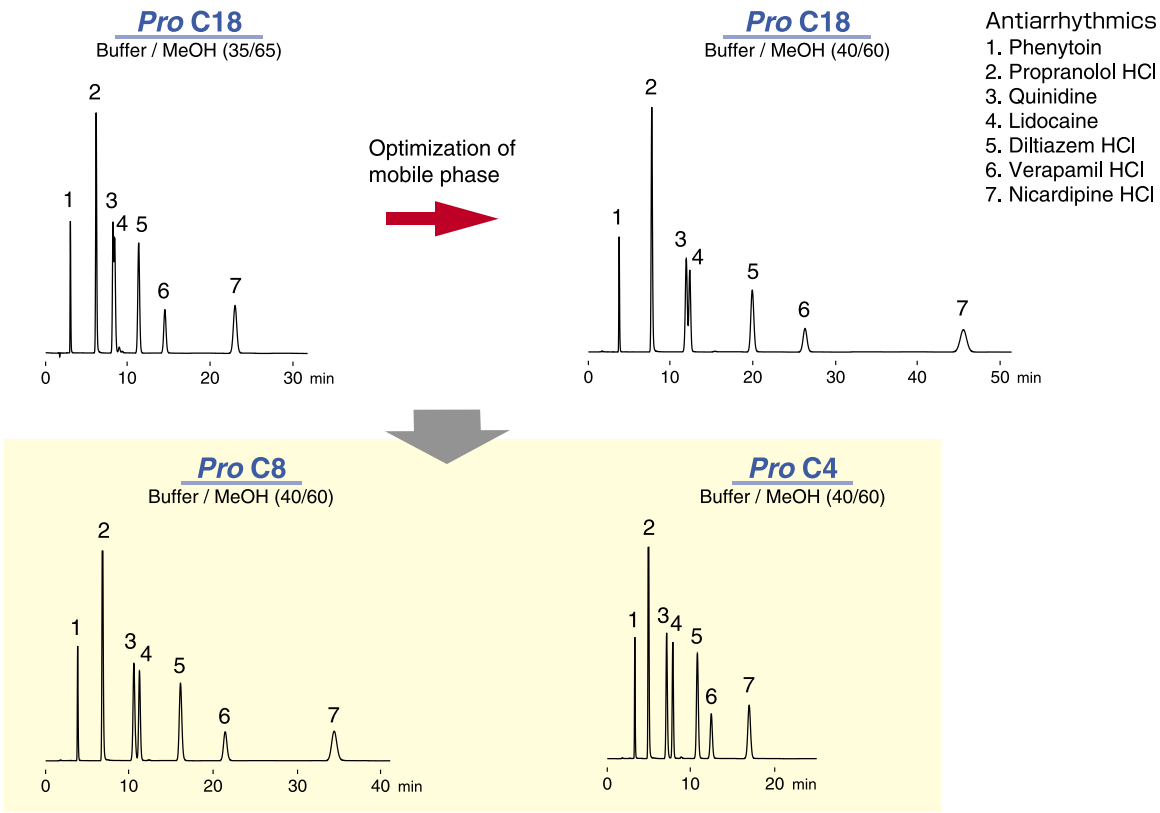
Column:	150 x 4.6 mm i.d.
Eluent:	20 mM KH_2PO_4
Flow rate:	1.0 ml/min
Temperature:	37° C
Detection:	UV at 254 nm

Column Kits
MD VAL R&D PS
pages 22 - 31

YMC-Pack Pro C4

The comparison shown below demonstrates that YMC-Pack *Pro* C4 is the column of choice when fast HPLC is required. There is almost no difference in retention times for the first three compounds whilst Nicardipine HCl elutes faster on YMC-Pack *Pro* C4 due to its lower polarity.

Optimisation of separation using *Pro* C8 and *Pro* C4



Column:	150 x 4.6 mm i.d.
Eluent:	20 mM KH_2PO_4 -K_2HPO_4 (pH 6.9) / methanol
Flow rate:	1.0 ml/min
Temperature:	37° C
Detection:	UV at 220 nm

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

Column care

YMC-Pack *Pro* C4 is stable towards hydrolysis between pH 2.0-7.5. Remove acid and buffer salts before storage. Store the column in methanol / water = 70/30. Clogged inlet frits often can be cleaned by changing the flow direction or replacement. For detailed information please refer to the “Column Care and Use Instructions”, which are shipped with each analytical column.

YMC-Pack Pro C18 RS

- **strongly hydrophobic due to carbon content of 22%**
- **exhibits extraordinary steric selectivity**
- **extended pH and temperature stability**
- **for the separation of hydrophobic, acidic and basic molecules**

YMC-Pack *Pro* C18 RS	Specification
Particle size / µm	3; 5
Pore size / nm	8
Surface area / m^2g^{-1}	510
Carbon content / %	22
Recommended pH range	1.0 - 10.0*

* it is recommended to use at least 10% organic solvent composition near the pH limits and over 50% at pH values above pH 9.0 to perserve column lifetimes

General

YMC-Pack *Pro* C18 RS is the latest *Pro*Family member designed to provide a complementing selectivity pattern resulting from a high carbon load of 22% and a broader pH stability range.

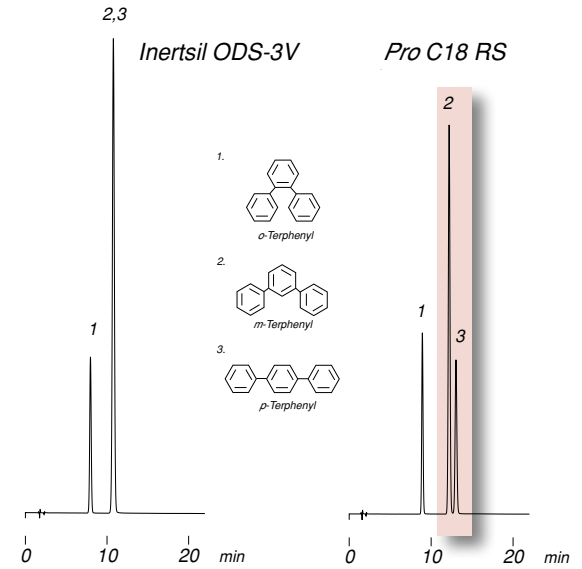
For the separation of large non-polar molecules, highly hydrophobic stationary phases are required. In many cases, it is only with this kind of columns that the chromatographer is able to separate positional isomers that are often by-products in pharmaceutical and other industrial manufacturing processes.

YMC-Pack *Pro* C18 RS fulfils these demands and, thus additionally gives further opportunities in method development due to its extended pH stability.

Properties

The relatively high carbon load of YMC-Pack *Pro* C18 RS with 22% amplifies the selectivity's ability to discriminate between closely related compounds such as positional or steric isomers. A good system to test this steric selectivity is a mixture of *o*-, *m*- and *p*-terphenyl separated under methanol/water conditions. These three compounds differ only in their three-dimensional structure and not in their hydrophobicity or polarity. YMC-Pack *Pro* C18 RS recognizes even slight steric differences as shown in the chromatogram on the right, whilst a more conventional carbon load (15%) C18 chemistry does not.

Steric selectivity*



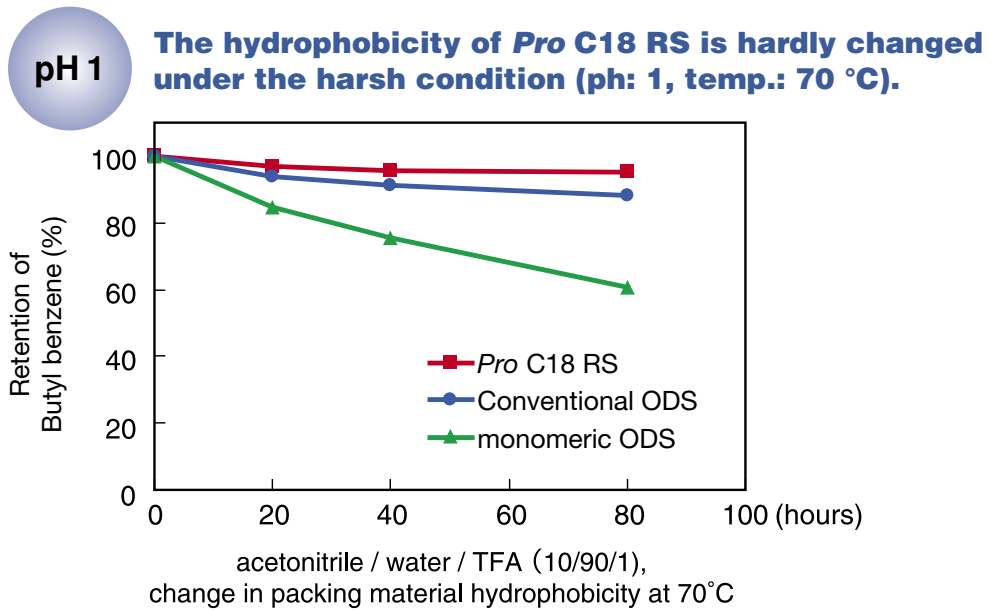
Column:	150 x 4.6 mm i.d.
Eluent:	methanol / water (85/15)
Flow rate:	1.0 ml/min
Temperature:	37° C
Detection:	UV at 254 nm

YMC-Pack Pro C18 RS

For the analysis of ionisable compounds with reversed phase HPLC, it is recommended to adjust the pH of the mobile phase to a value which is two units higher or lower than the pK value of the individual analytes to insure a complete protonation or deprotonation of acids or bases. This guarantees that poor peak symmetry is not based on interactions between e.g. protonated and unprotonated forms of a base.

YMC-Pack *Pro* C18 RS exhibits an extended pH-stability from pH1 to 10. Its long-term stability at pH1 and at pH10, respectively, has been tested under very harsh conditions as shown below.

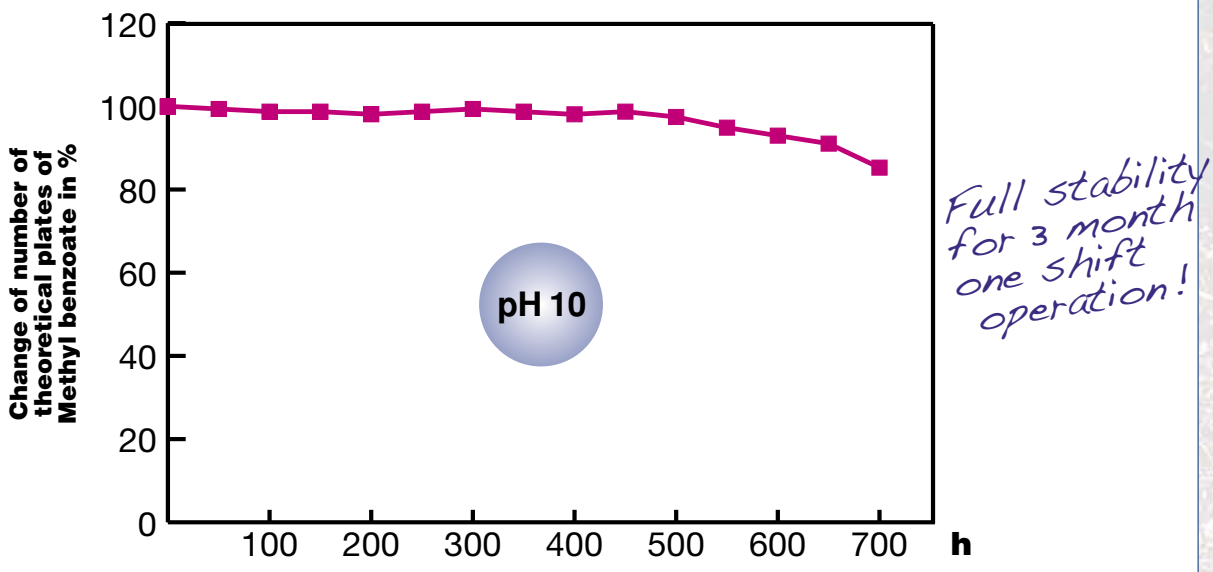
Stability under acidic conditions*



Note: When assessing pH stability data, please take care to certify that complete chromatographic conditions are presented.

Basic eluents may significantly affect silicas and traditional bonding chemistries. Therefore, stability data should be considered only after verifying that the buffer system used maintains the selected pH during preparation and use. Furthermore, it must be verified that the eluent is not recycled, since the "active" basic sites may equilibrate to a saturation level with time, resulting in no further interactions taking place. Consequently, only continuous flow of "fresh" and thoroughly buffered eluent will provide accurate and meaningful performance data.

Stability under basic conditions*



For pH 10, a borate buffer system (20 mM H_3BO_3-NaOH (pH 9.8) / methanol 50/50 at 30°C) was selected to be continuously pumped through the column, while checking the number of theoretical plates for methyl benzoate every 50 hours.

* By courtesy YMC Co., Ltd.

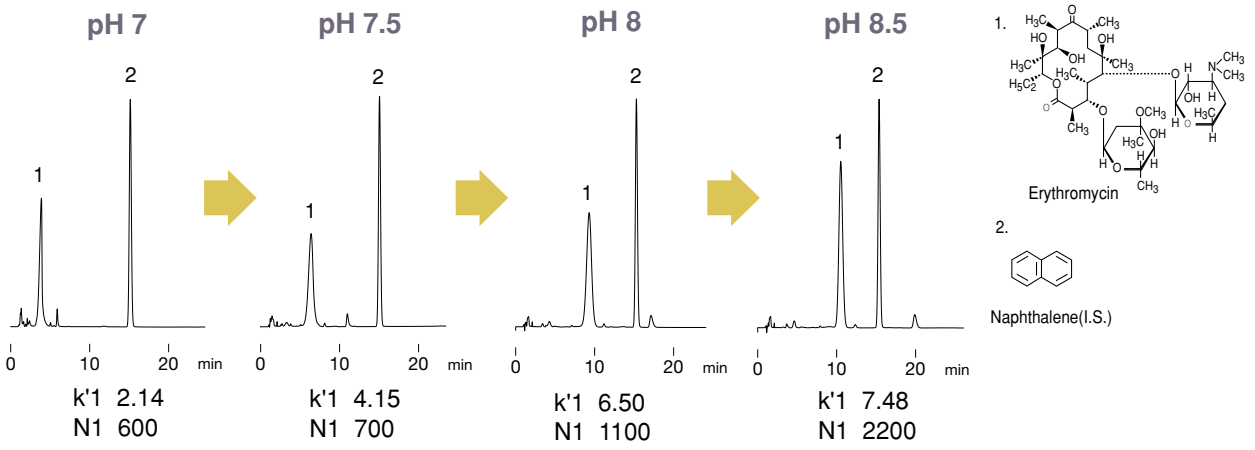
YMC-Pack Pro C18 RS

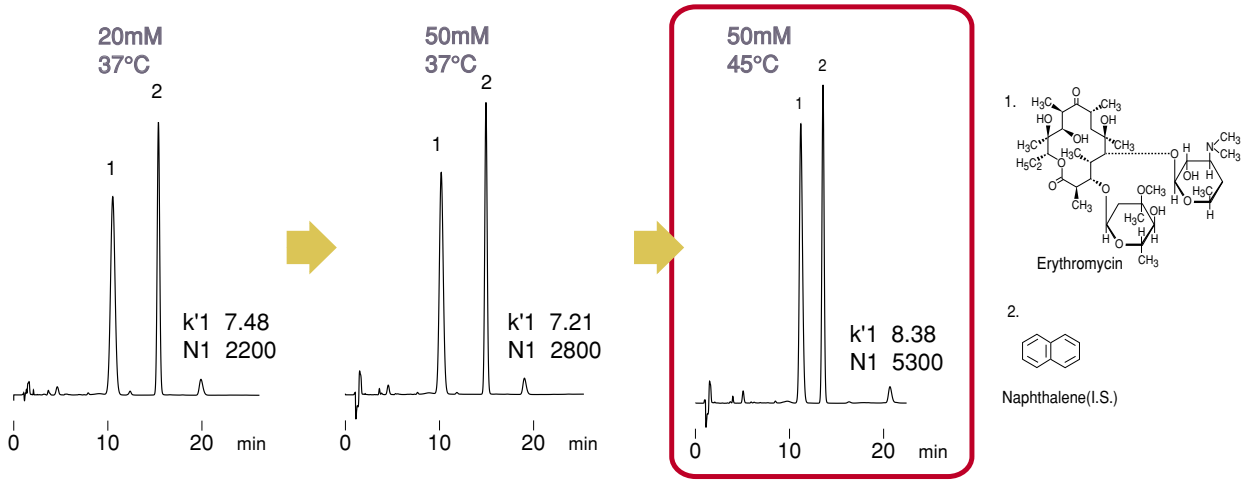
The advantages of this operating stability of YMC-Pack *Pro* C18 RS under harsh pH conditions can be demonstrated with one example.

In this case, the separation of erythromycin is optimised with regards to pH, buffer concentration and temperature. The stepwise enhancement of all three parameters shows a stepwise increase of the number of theoretical plates for erythromycin, while naphthalene is used as an internal standard.

Finally, a pH of 8.5 at 45 °C and 50 mM buffer concentration appears to the best conditions for this separation in terms of producing an efficient peak performance for erythromycin.

Method optimization





Column:	YMC-Pack *Pro* C18 RS (5 µm, 8 nm) 150 x 4.6 mm i.d.
Eluent:	potassium phosphate buffer (pH 8.5) / acetronitrile (50/50)
Flow rate:	1.0 ml/min
Temperature:	37° C
Detection:	UV at 210 nm

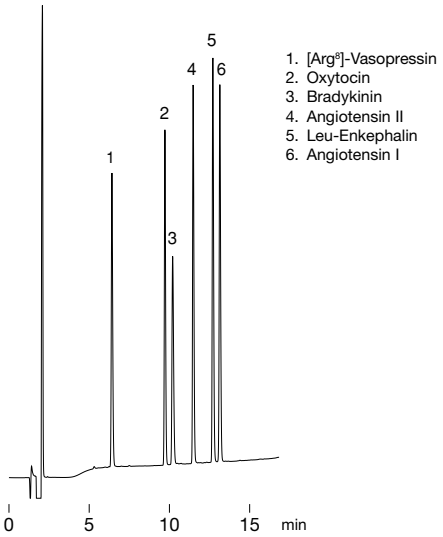
YMC-Pack *Pro* C18 RS:
Ideal for the separation of steric demanding compounds and/or for use under broader pH conditions!

YMC-Pack Pro C18 RS

Applications

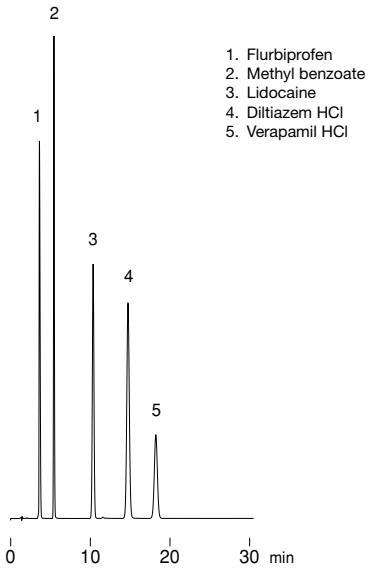
The specific properties of YMC-Pack *Pro* C18 RS make it an excellent choice for the separation of non-polar structurally related analytes. The extended resistance towards acidic and basic conditions not only widens the possibilities in method development but also provides further selectivities for demanding separations such as LC-MS or combinatorial chemistry of: positional isomers, large hydrophobic molecules, basic and acidic compounds, peptides

Separation of peptides under LC-MS compatible conditions



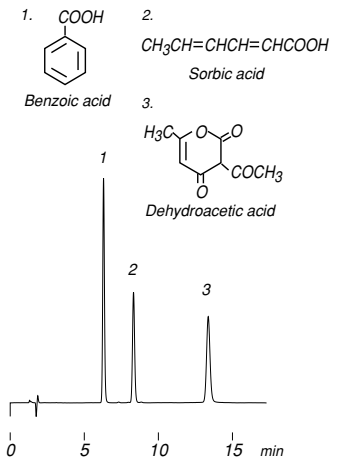
Column:	YMC-Pack *Pro* C18 RS (5 µm, 8 nm) 150 x 4.6 mm i.d.
Eluent:	A: water / TFA (100/0.1) B: acetonitrile / TFA (100/0.1)
Gradient:	15-35% B (0-15 min)
Flow:	1.0 ml/min
Detection:	UV, 220 nm, 0.32 AUFS
Temperature:	37 °C
Injection:	20 µl (0.05 mg/ml)

Separation of acidic and basic drugs under LC-MS compatible conditions



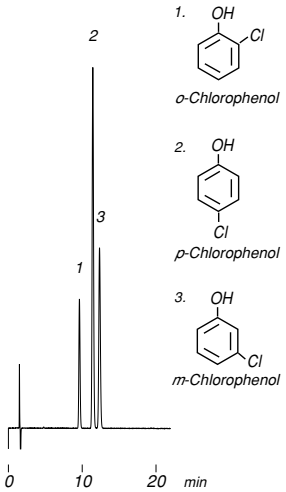
Column:	YMC-Pack *Pro* C18 RS (5 µm, 8 nm) 150 x 4.6 mm i.d.
Eluent:	20 mM CH_3COONH_4-NH_3 (pH 8.9) / methanol (35/65)
Flow:	1.0 ml/min
Detection:	UV, 220 nm, 0.64 AUFS
Temperature:	30 °C
Injection:	10 µl (0.1 mg/ml, 0.036 µl/ml)

Food preservatives



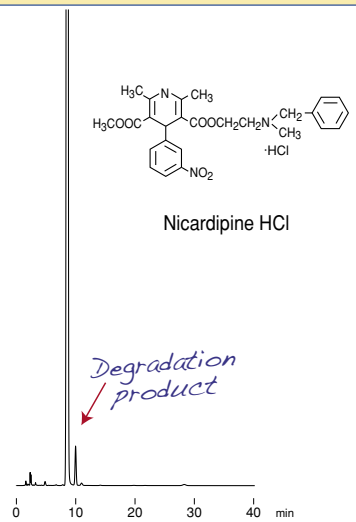
Column:	YMC-Pack *Pro* C18 RS (5 µm, 8 nm) 150 x 4.6 mm i.d.
Eluent:	20 mM CH_3COONa - CH_3COOH (pH 4.3) / acetonitrile (80/20)
Flow:	1.0 ml/min
Detection:	UV at 230 nm, 0.26 AUFS
Temperature:	37 °C
Injection:	10 µl (0.02 mg/ml)

Chlorophenol isomers



Column:	YMC-Pack *Pro* C18 RS (5 µm, 8 nm) 150 x 4.6 mm i.d.
Eluent:	acetonitrile / water / acetic acid (30/70/1)
Flow:	1.0 ml/min
Detection:	UV at 230 nm, 0.32 AUFS
Temperature:	37 °C
Injection:	10 µl (0.1 - 0.2 mg/ml)

Nicardipine hydrochloride



Column:	YMC-Pack *Pro* C18 RS (5 µm, 8 nm) 150 x 4.6 mm i.d.
Eluent:	20 mM KH_2PO_4-K_2HPO_4 (pH 6.9) / methanol (25/75)
Flow:	1.0 ml/min
Detection:	UV at 254 nm, 0.32 AUFS
Temperature:	37 °C
Injection:	10 µl

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

Hydrosphere C18

- **stable under the use of 100% aqueous eluent**
- **"hydrophilic" C18 surface for enhanced polar recognition**
- **no need for ion pair reagents**
- **based on highly inert, ultrapure, pH neutral silica**
- **specifically designed for pharmaceutical and biotechnology R&D**

Hydrosphere C18	Specification
Particle size / µm	2*; 3*; 5
Pore size / nm	12
Surface area / m^2g^{-1}	340
Carbon content / %	12
Recommended pH range	2.0 - 8.0

* please be referred to page 35 ff for Ultra Fast LC and Fast LC Columns

General

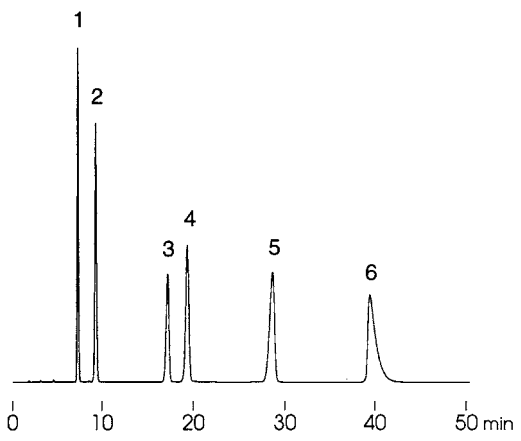
The separation of polar compounds in many cases requires highly aqueous mobile phase conditions to achieve sufficient retention on the stationary phase. Conventional reversed phase selectivities do not give reproducible results under these conditions due mainly to the collapse of the C18 chains, Hydrosphere C18 has been developed, on the ultra pure silica support of the *Pro*Family, as the next generation of speciality phases following the well known YMC-Pack ODS-AQ, which was developed in 1987 and is still a very interesting selectivity option for these purposes.

Hydrosphere C18

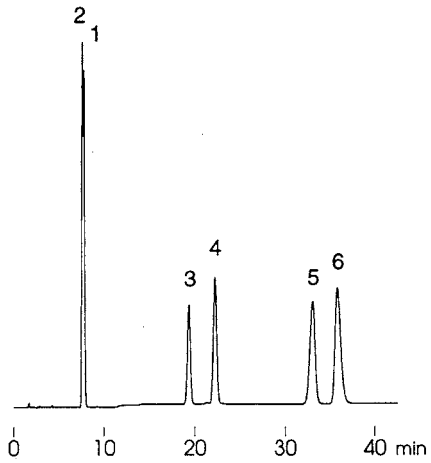
Traditionally, YMC-Pack ODS-AQ was designed for the separation of polar compounds under 100% aqueous conditions and YMC-Pack *Pro* C18 for the separation of acidic and basic substances.

As a consequence, Hydrosphere C18 could be understood as a synergy of the advantages of YMC-Pack ODS-AQ and YMC-Pack *Pro* C18.

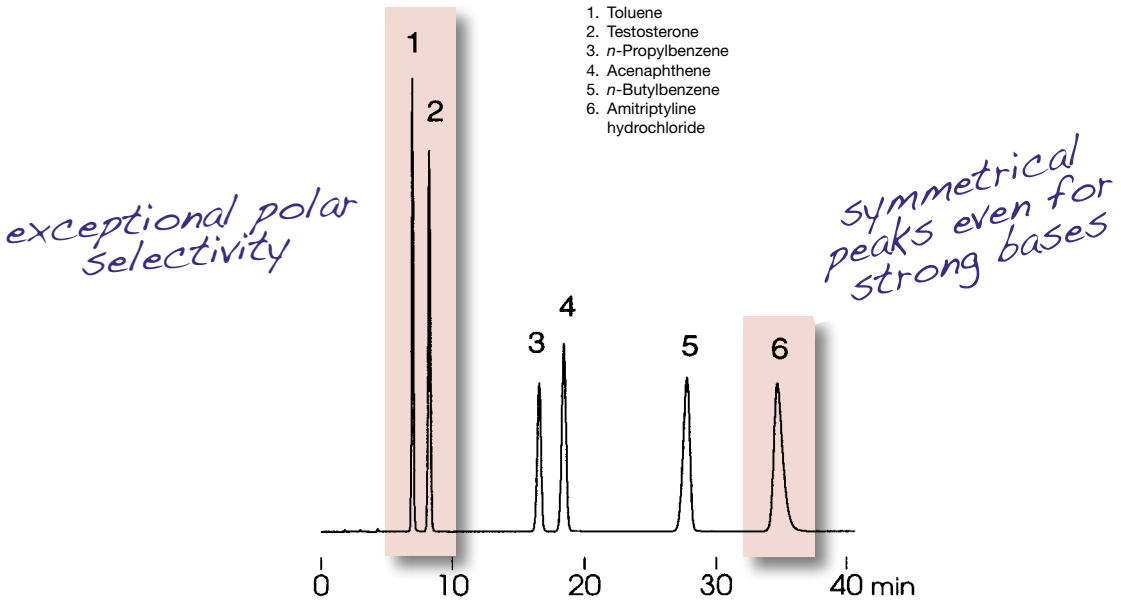
YMC-Pack ODS-AQ



YMC-Pack *Pro* C18



Hydrosphere C18



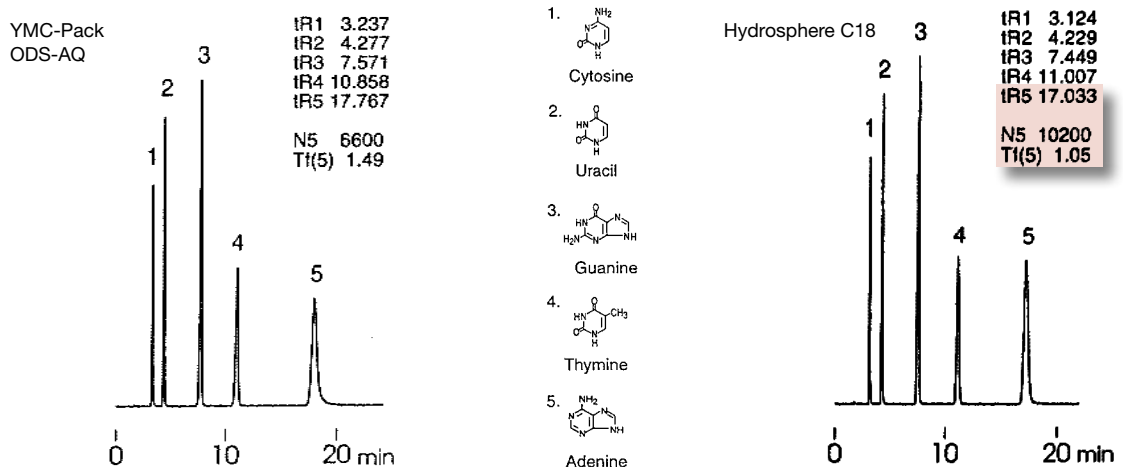
Column:	Hydrosphere C18 (5 µm, 12 nm) 150 x 4.6 mm i.d.
Eluent:	20 mM KH_2PO_4-K_2HPO_4 (pH 6.9) / methanol (35/65)
Flow rate:	1.0 ml/min
Temperature:	37 °C
Detection:	UV at 254 nm

Hydrosphere C18

Properties

In order to compare the differences and similarities of both reversed phases, nucleic acid bases are separated under pure aqueous eluent conditions on both selectivities. Interestingly uracil, normally used as a dead volume marker, can be retained on both phases, whereas adenine elutes later on YMC-Pack ODS-AQ. This peak on Hydrosphere C18 is virtually symmetrical with a tailing factor of 1.05, but on YMC-Pack ODS-AQ it shows a slightly tailing peak (with a tailing factor of 1.49).

Hydrosphere C18 vs. YMC-Pack ODS-AQ



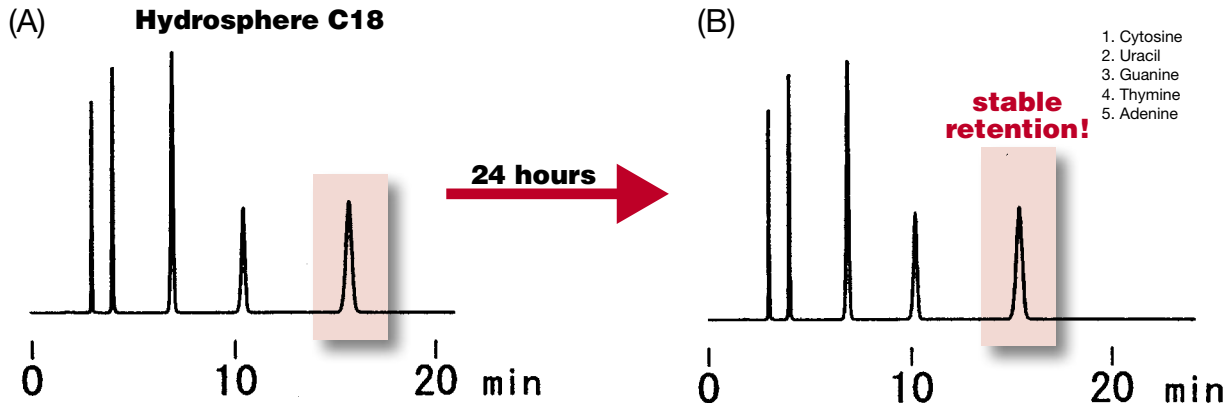
Column:	150 x 4.6 mm
Eluent:	20 mM KH_2PO_4-K_2HPO_4 (pH 6.9)
Flow rate:	1.0 ml/min
Temperature:	37° C
Detection:	UV at 254 nm

As a consequence, both Hydrosphere C18 and YMC-Pack ODS-AQ are the selectivities of choice within the YMC portfolio to separate polar compounds when using 100% aqueous eluent conditions. YMC-Pack ODS-AQ shows stronger retention for more hydrophobic substances, while Hydrosphere C18 is ideal for basic substances.

Both selectivities are a “must” for approaching the separation of polar compounds.

A proprietary derivatisation procedure enables Hydrosphere C18 to be penetrated by water without losing its brush-like chain structure. This model of the so-called phase collapse is one explanation for a phenomenon, where the retention times decrease after a very short time under 100% aqueous eluent on a conventional C18 phase.

Solution: Hydrosphere C18 under 100% aqueous chromatographic conditions*



Column:	Hydrosphere C18 (5µm, 12 nm) 150 x 4.6 mm i.d.
Eluent:	20 mM KH_2PO_4-K_2HPO_2 (pH 6.9)
Flow rate:	1.0 ml/min
Temperature:	37 °C
Detection:	UV at 254 nm

After use, the column was stored overnight without any washing procedure. The next day, the application was continued.

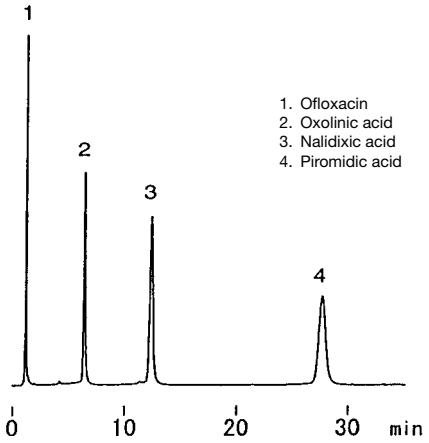
* By courtesy YMC Co., Ltd.

Its “hydrophilic” C18 surface gives Hydrosphere C18 the capability to show stable retention times even after 24 hours under these chromatographic conditions.

Hydrosphere C18

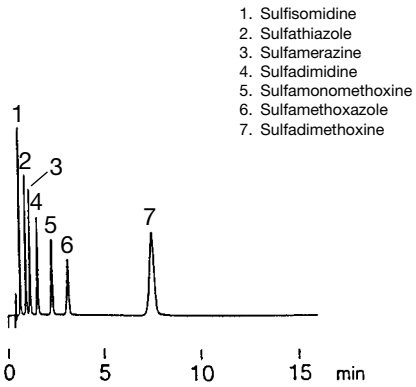
Finally, Hydrosphere C18 is, in common with all *Pro*Family members, applicable to LC-MS methods as shown below. High throughput LC-MS separations with enhanced flow rates are easily achievable with the 3 µm version of Hydrosphere C18.

LC-MS compatibility



Column:	Hydrosphere C18 (3 µm, 12 nm) 100 x 2.0 mm i.d.
Eluent:	10 mM CH_3COOH - CH_3COONH_4 (pH 4.3) / acetonitrile (75/25)
Flow:	0.2 ml/min
Detection:	UV at 260 nm
Temperature:	37 °C

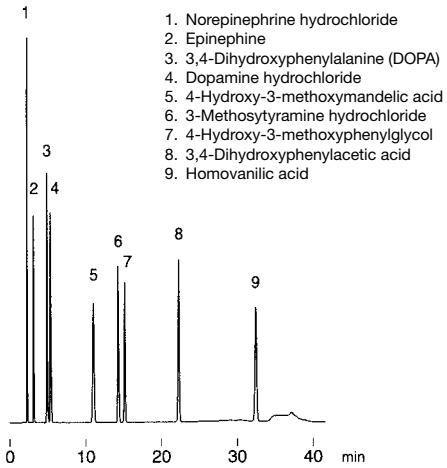
High throughput separation



Column:	Hydrosphere C18 (3 µm, 12 nm) 50 x 3.0 mm i.d.
Eluent:	10 mM CH_3COOH - CH_3COONH_4 (pH 4.6) / acetonitrile (85/15)
Flow:	1.0 ml/min
Detection:	UV at 275 nm
Temperature:	37 °C

Hydrosphere C18 is the column of choice within the YMC portfolio for a wide range of different substances. As an almost "universal" selectivity it can be used under standard reversed phase conditions as well as pure aqueous eluents as demonstrated by this collection of applications.

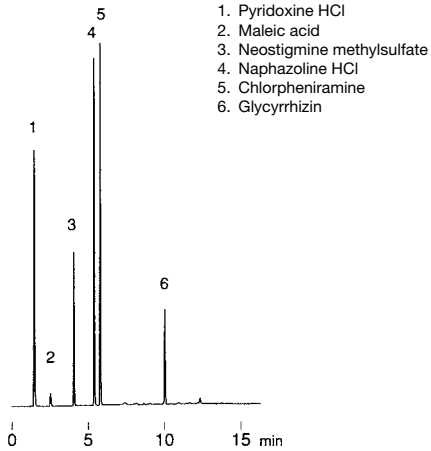
No ion pair reagent required



Catecholamines

Column:	Hydrosphere C18 (5 µm, 12 nm) 150 x 4.6 mm i.d.
Eluent:	A) 50 mM KH_2PO_4 - H_3PO_4 (pH 3.1) B) 50 mM KH_2PO_4 - H_3PO_4 (pH 3.1) / acetonitrile (90/10) 0% B (0-5 min), 0-100% B (5-25 min, linear), 100% B (25-35 min)
Flow:	1.0 ml/min
Detection:	UV at 280 nm, 0.64 AUFS
Temperature:	30 °C
Injection:	10 µl (0.15 - 0.25 mg/ml)

Efficient separation of complex substance mixtures



Ingredients in an eye drop

Column:	Hydrosphere C18 (3 µm, 12 nm) 50 x 4.6 mm i.d.
Eluent:	A) 20 mM KH_2PO_4 - H_3PO_4 (pH 2.5) B) acetonitrile 0-60% B (0-10 min), 60% B (10-15 min)
Flow rate:	1.0 ml/min
Temperature:	37 °C
Detection:	UV at 265 nm, 0.128 AUFS
Injection:	5 µl (0.03 - 0.3 mg/ml)

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

Column care

Hydrosphere C18 is stable towards hydrolysis between pH 2.0-8.0 in up to 100% aqueous systems and a maximum of 50 °C. 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.

Ordering Information

Tested QC passed Guard Cartridges

YMC-Pack *Pro* C18, 12 nm, 3 µm

AS 12 nm 3 µm

Column i.d. (mm)	Column length (mm) 33	50	100	150	250	Guard cartridges with 10 mm length (pack of 5)
2.1	AS12S030302QT	AS12S030502QT	AS12S031002QT	AS12S031502QT	AS12S032502QT	AS12S030102
3.0	AS12S030303QT	AS12S030503QT	AS12S031003QT	AS12S031503QT	AS12S032503QT	AS12S030103
4.0	AS12S030304QT	AS12S030504QT	AS12S031004QT	AS12S031504QT	AS12S032504QT	AS12S030104
4.6	AS12S030346WT	AS12S030546WT	AS12S031046WT	AS12S031546WT	AS12S032546WT	AS12S030104

For other dimensions please refer to page 81

YMC-Pack *Pro* C18, 12 nm, 5 µm

AS 12 nm 5 µm

Column i.d. (mm)	Column length (mm) 33	50	100	150	250	Guard cartridges with 10 mm length (pack of 5)
2.1	AS12S050302QT	AS12S050502QT	AS12S051002QT	AS12S051502QT	AS12S052502QT	AS12S050102
3.0	AS12S050303QT	AS12S050503QT	AS12S051003QT	AS12S051503QT	AS12S052503QT	AS12S050103
4.0	AS12S050304QT	AS12S050504QT	AS12S051004QT	AS12S051504QT	AS12S052504QT	AS12S050104
4.6	AS12S050346WT	AS12S050546WT	AS12S051046WT	AS12S051546WT	AS12S052546WT	AS12S050104

For other dimensions please refer to page 81

YMC-Pack *Pro* C8, 12 nm, 3 µm

OS 12 nm 3 µm

Column i.d. (mm)	Column length (mm) 33	50	100	150	250	Guard cartridges with 10 mm length (pack of 5)
2.1	OS12S030302QT	OS12S030502QT	OS12S031002QT	OS12S031502QT	OS12S032502QT	OS12S030102
3.0	OS12S030303QT	OS12S030503QT	OS12S031003QT	OS12S031503QT	OS12S032503QT	OS12S030103
4.0	OS12S030304QT	OS12S030504QT	OS12S031004QT	OS12S031504QT	OS12S032504QT	OS12S030104
4.6	OS12S030346WT	OS12S030546WT	OS12S031046WT	OS12S031546WT	OS12S032546WT	OS12S030104

For other dimensions please refer to page 81

YMC-Pack *Pro* C8, 12 nm, 5 µm

OS 12 nm 5 µm

Column i.d. (mm)	Column length (mm) 33	50	100	150	250	Guard cartridges with 10 mm length (pack of 5)
2.1	OS12S050302QT	OS12S050502QT	OS12S051002QT	OS12S051502QT	OS12S052502QT	OS12S050102
3.0	OS12S050303QT	OS12S050503QT	OS12S051003QT	OS12S051503QT	OS12S052503QT	OS12S050103
4.0	OS12S050304QT	OS12S050504QT	OS12S051004QT	OS12S051504QT	OS12S052504QT	OS12S050104
4.6	OS12S050346WT	OS12S050546WT	OS12S051046WT	OS12S051546WT	OS12S052546WT	OS12S050104

For other dimensions please refer to page 81

Ordering Information

YMC-Pack *Pro* C4, 12 nm, 3 µm

BS 12 nm 3 µm

Column i.d. (mm)	Column length (mm) 33	50	100	150	250	Guard cartridges with 10 mm length (pack of 5)
2.1	BS12S030302QT	BS12S030502QT	BS12S031002QT	BS12S031502QT	BS12S032502QT	BS12S030102
3.0	BS12S030303QT	BS12S030503QT	BS12S031003QT	BS12S031503QT	BS12S032503QT	BS12S030103
4.0	BS12S030304QT	BS12S030504QT	BS12S031004QT	BS12S031504QT	BS12S032504QT	BS12S030104
4.6	BS12S030346WT	BS12S030546WT	BS12S031046WT	BS12S031546WT	BS12S032546WT	BS12S030104

For other dimensions please refer to page 81

YMC-Pack *Pro* C4, 12 nm, 5 µm

BS 12 nm 5 µm

Column i.d. (mm)	Column length (mm) 33	50	100	150	250	Guard cartridges with 10 mm length (pack of 5)
2.1	BS12S050302QT	BS12S050502QT	BS12S051002QT	BS12S051502QT	BS12S052502QT	BS12S050102
3.0	BS12S050303QT	BS12S050503QT	BS12S051003QT	BS12S051503QT	BS12S052503QT	BS12S050103
4.0	BS12S050304QT	BS12S050504QT	BS12S051004QT	BS12S051504QT	BS12S052504QT	BS12S050104
4.6	BS12S050346WT	BS12S050546WT	BS12S051046WT	BS12S051546WT	BS12S052546WT	BS12S050104

For other dimensions please refer to page 81

YMC-Pack *Pro* C18 RS, 8 nm, 3 µm

RS 8 nm 3 µm

Column i.d. (mm)	Column length (mm) 33	50	100	150	250	Guard cartridges with 10 mm length (pack of 5)
2.1	RS08S030302QT	RS08S030502QT	RS08S031002QT	RS08S031502QT	RS08S032502QT	RS08S030102
3.0	RS08S030303QT	RS08S030503QT	RS08S031003QT	RS08S031503QT	RS08S032503QT	RS08S030103
4.0	RS08S030304QT	RS08S030504QT	RS08S031004QT	RS08S031504QT	RS08S032504QT	RS08S030104
4.6	RS08S030346WT	RS08S030546WT	RS08S031046WT	RS08S031546WT	RS08S032546WT	RS08S030104

For other dimensions please refer to page 81

YMC-Pack *Pro* C18 RS, 8 nm, 5 µm

RS 8 nm 5 µm

Column i.d. (mm)	Column length (mm) 33	50	100	150	250	Guard cartridges with 10 mm length (pack of 5)
2.1	RS08S050302QT	RS08S050502QT	RS08S051002QT	RS08S051502QT	RS08S052502QT	RS08S050102
3.0	RS08S050303QT	RS08S050503QT	RS08S051003QT	RS08S051503QT	RS08S052503QT	RS08S050103
4.0	RS08S050304QT	RS08S050504QT	RS08S051004QT	RS08S051504QT	RS08S052504QT	RS08S050104
4.6	RS08S050346WT	RS08S050546WT	RS08S051046WT	RS08S051546WT	RS08S052546WT	RS08S050104

For other dimensions please refer to page 81

Note: For your first order on guard cartridges, please add one guard cartridge holder per system to your order list:
integral guard cartridge holder for columns with 2.1-4.0 mm i.d. **XPGCS-Q1** or universal guard cartridge holder for columns with 4.6 mm i.d. **XPGCH-Q1**
The holder can continuously be re-used and, thus it is not required for repeat orders.

Ordering Information

Tested QC passed Guard Cartridges

HS 12 nm 3 µm

YMC-Pack Hydrosphere C18, 12 nm, 3 µm

Column i.d. (mm)	Column length (mm) 33	50	100	150	250	Guard cartridges with 10 mm length (pack of 5)
2.1	HS12S030302QT	HS12S030502QT	HS12S031002QT	HS12S031502QT	HS12S032502QT	HS12S030102
3.0	HS12S030303QT	HS12S030503QT	HS12S031003QT	HS12S031503QT	HS12S032503QT	HS12S030103
4.0	HS12S030304QT	HS12S030504QT	HS12S031004QT	HS12S031504QT	HS12S032504QT	HS12S030104
4.6	HS12S030346WT	HS12S030546WT	HS12S031046WT	HS12S031546WT	HS12S032546WT	HS12S030104

For other dimensions please refer to page 81

HS 12 nm 5 µm

YMC-Pack Hydrosphere C18, 12 nm, 5 µm

Column i.d. (mm)	Column length (mm) 33	50	100	150	250	Guard cartridges with 10 mm length (pack of 5)
2.1	HS12S050302QT	HS12S050502QT	HS12S051002QT	HS12S051502QT	HS12S052502QT	HS12S050102
3.0	HS12S050303QT	HS12S050503QT	HS12S051003QT	HS12S051503QT	HS12S052503QT	HS12S050103
4.0	HS12S050304QT	HS12S050504QT	HS12S051004QT	HS12S051504QT	HS12S052504QT	HS12S050104
4.6	HS12S050346WT	HS12S050546WT	HS12S051046WT	HS12S051546WT	HS12S052546WT	HS12S050104

For other dimensions please refer to page 81

Sure-Fit™ Connector

Since different column hardware manufacturers have introduced a variety of connecting port geometries over the years, the Sure-Fit™ Connector represents an ideal tool to avoid dead volumes or leakages, irrespective of the column brand, when connecting different columns to an HPLC system. YMC strongly recommends the use of the Sure-Fit™ Connector due to its internal spring tensioned mechanism, which automatically self-adjusts to any port depth while maintaining constant pressure on the capillary tubing. This guarantees a connection free from any dead volume. Column changeovers become easy, especially as no tools are required. The Sure-Fit™ Connector is fingertight to 6000 psi and is available in a range of capillary tubing i.d. and lengths.

Column i.d.* (mm)	Tubing i.d.* (mm)	Single end unit (incl. 20 mm stainless steel capillary)	Double end unit (incl. 20 mm stainless steel capillary)
1.0-3.0	0.13	SFS2005	SFD2005
3.0-4.6	0.18	SFS2007	SFD2007

* Other versions available on request

Ordering Information

The previous product listing represents commonly used standard column dimension. In order to identify any specific product version and order number, please see the example and the table below.

Full listing of all chemistries and dimensions

Gel Code								Hardware Code					
Chemistry code		Pore size [nm]		Particle shape		Particle size [µm]		Length [mm]		Inner diameter [mm]		Column Type	
YMC30	CT	6	06	spherical	S	3	03	10	01	0.05	E5	Quick Seal	QT
Pro C18	AS	8	08			4	04	20	02	0.075	E8	Cartridge	QC
Pro C18 RS	RS	12	12			5	05	33	03	0.1	F0	Waters type	WT
Hydrosphere C18	HS	20	20			6	06	50	05	0.2	G0		
ODS-A	AA	30	30					75	L5	0.3	H0		
ODS-AM	AM	100	A0			10	11	100	10	0.5	J0		
ODS-AQ	AQ	proprietary	99			15	16	125	R5	0.8	M0		
J'sphere ODS-H80	JH	non-porous	00			20	21	150	15	1.0	01		
J'sphere ODS-M80	JM					50	50	250	25	2.1	02		
J'sphere ODS-L80	JL					75	75			3.0	03		
ODS-AL	AL							300	30	4.0	04		
PAH	YP					63/210	A4						
PolymerC18	PC					150	A5	500	50	4.6	46		
Pro C8	OS							1000	A0	6.0	06		
C8 (Octyl)	OC									8.0	08		
YMCbasic	BA									10	10		
Ph (Phenyl)	PH									20	20		
Pro C4	BS									30	30		
C4 (Butyl)	BU												
Protein-RP	PR									50 (2000 psi)	52		
TMS (C1)	TM									70 (2000 psi)	72		
PVA-Sil	PV									100 (2000 psi)	A2		
Polyamine II	PB									150 (2000 psi)	B2		
NH_2 (Amino)	NH									200 (2000 psi)	C2		
CN (Cyano)	CN												
Diol	DL												
SIL (Silica)	SL												
BioPro-QA	QA												
BioPro-SP	SP												
BioPro-QA-F	QF												
BioPro-SP-F	SF												
Chiral NEA (R)	NR												
Chiral NEA (S)	NS												
Chiral CD BR α	DA												
Chiral CD BR β	DB												
Chiral CD BR γ	DG												
Chiral Prep CD ST	ST												
Chiral Prep CD PM	PM												

Example

Choose your column and fill in the "Gel and Hardware Code" or detailed description (The part number consists of the "Gel Code" and the "Hardware Code").

YMC-Pack ODS-A	12 nm	spherical	3 µm	250 mm	1.0 mm	Quick Seal
AA	**12**	**S**	**03**	**25**	**01**	**QT**

Your column part number: AA12S032501QT (Example)

Please note that combinations of features cannot be selected at random, but only from the possible specifications for a chosen stationary phase. These can be determined from the individual product sections in this catalogue or from our homepage www.ymc.de.

For more details

contact your local distributor or
YMC Europe GmbH, Schöttmannshof 19, D-46539 Dinslaken, Phone: +49 (0) 2064 / 427-0,
Fax +49 (0) 2064 / 427-222, e-mail: info@ymc.de, homepage: www.ymc.de