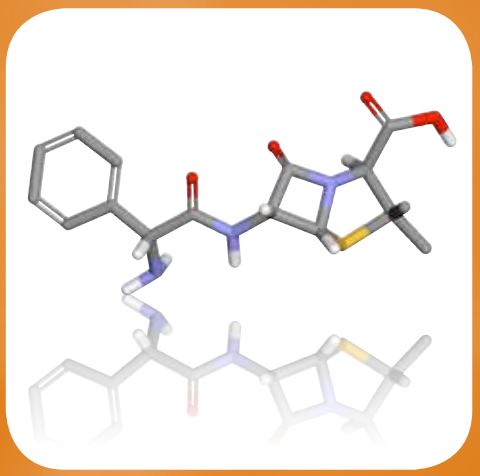
YMC EUROPE GMBH

MicroLC



MicroLC

CapillaryLC

NanoLC

Introduction

Miniaturisation of liquid chromatography in combination with mass spectrometry has several advantages including improvements in sensitivity, especially at low concentration levels and dramatically reduced solvent consumption, compared to conventional HPLC or UHPLC. With further method optimisation, run time can also be reduced and therefore more solvent or time can be saved.

To meet the requirements of MicroLC/capillaryLC/NanoLC YMC offers capillary columns especially designed to use with the corresponding chromatography systems.

- ✔ **all YMC-phases available**
- ✔ **compatible with Micro-/NanoLC/MS systems**
- ✔ **extremely low sample volumes and flow rates**
- ✔ **phases for RP, NP, and HILIC**
- ✔ **1.9, 2, 3, and 5 µm particles**
- ✔ **lengths from 50 to 150 mm**
- ✔ **ID from 75 to 500 µm**
- ✔ **guard columns, usable for trapping/desalting**

Contents

YMC capillary columns for MicroLC/NanoLC

		PRODUCT	PHASE (silica-based unless stated)	END-CAPPED	USP CLASS NO.	PARTICLE SIZE (µm)
Reversed Phase	C30	Carotenoid	proprietary polymeric bonding chemistry	—	—	3, 5
	C18	Triart C18 ExRS	organic/inorganic hybrid particle	yes	L1	1.9, 3, 5
		Triart C18	pH stable and robust hybrid particle	yes	L1	1.9, 3, 5
		Pro C18	very low residual non-specific interactions	yes	L1	3, 5
		UltraHT	2 µm *Pro* C18 for fast and ultra fast separations			2
		Pro C18 RS	high carbon load with polymeric bonding C18	yes	L1	3, 5
		Hydrosphere C18	can be used in 100% aqueous eluent	yes	L1	3, 5
		UltraHT	2 µm Hydrosphere C18 for fast and ultra fast separations			2
		ODS-A	one of the YMC's international bestsellers	yes	L1	3, 5
		ODS-AM	high performance C18 column for validated methods operation	yes	L1	3, 5
		ODS-AQ	"hydrophilic" endcapping, for 100% aqueous eluent systems	yes	L1	3, 5
		J'sphere	C18-family with differently controlled hydrophobicity for method development	yes	L1	4
		ODS-AL	traditional C18 for "mixed mode" separations	no	L1	5
		Polymer C18	polymethacrylate matrix, wide pH applicability	—	—	6
	C8	Triart C8	pH stable and robust hybrid particle	yes	L7	1.9, 3, 5
		Pro C8	C8, with very low residual non-specific interactions	yes	L7	3, 5
		C_8 (Octyl)	traditional C8	yes	L7	3, 5
		YMCbasic	monomeric bonded chains of C8 and smaller	—	L7	3, 5
		Ph (Phenyl)	monomeric bonded phenyl	yes	L11	3, 5
		Triart Phenyl	polymeric bonding phenyl butyl	yes	L11	1.9, 3, 5
		Triart PFP	polymeric bonding PFP propyl	no	L43	1.9, 3, 5
	C4	*Pro* C4	C4, with very low residual non-specific interactions	yes	L26	3, 5
		C_4 (Butyl)	traditional C4	yes	L26	3, 5
		Protein RP	high stability, good recovery rates	yes	L26	5
		YMC-PAH	proprietary bonding chemistry	—	—	3, 5
		TMS (C1)	trimethyl silane	—	L13	3, 5
NormalPhase/HILIC		PVA-SIL	polyvinyl alcohol bonded on silica support	—	L24	5
		Polyamine II (PBMN)	mixed secondary and tertiary amino derivative	—	—	5
		NH_2 (Amino)	primary amino derivate	—	L8	5
		CN (Cyano)	useful for SFC applications	yes	L10	3, 5
		Triart-Diol HILIC	versatile HILIC column	—	L20	1.9, 3, 5
		Diol (DN)	versatile alternative to silica for normal phase separations	—	L20	5
		SIL (Silica)	ultra high purity, high mechanical stability	—	L3	3, 5

Capillary columns for MicroLC/NanoLC

PORE SIZE (nm)	CARBON LOAD (%C)	pH	TYPICAL APPLICATIONS
proprietary	proprietary	2.0-7.5	isomeric carotenes, retinols, steroids, fat-soluble vitamins
8	25	1.0-12.0	stereoisomers and hydrophobic analytes
12	20	1.0-12.0	acidic, neutral, basic compounds, ”versatile“ stationary phase
12	16	2.0-8.0	antioxidants, metabolites
8	22	1.0-10.0	acidic and basic compounds
12	12	2.0-8.0	strong polar compounds, water-soluble vitamins
12, 20, 30	17, 12, 7	2.0-7.5	general purpose phase
12	17	2.0-7.5	purines, phenols, PTC-amino acids, angiotensins, alkaloids
12, 20	14, 10	2.0-7.5	strong polar compounds
8	22, 14, 9 (JH, JM, JL)	1.0-9.0 (JH) 2.0-7.5 (JM+JL)	positional isomers, complexing agents, pharmaceuticals
12	17	2.0-7.5	tocopherols, fat-soluble vitamins, disinfectants
proprietary	10	2.0-13.0	phenols, anilines, quaternary amines
12	17	1.0-12.0	acidic, neutral, basic and chelating compounds, metabolites, ”versatile“ stationary phase
12	10	2.0-7.5	acidic, neutral, basic and chelating compounds, drugs and metabolites
12, 20, 30	10, 7, 4	2.0-7.5	proteins and peptides, estrogens, general purpose phase
20	7	2.0-7.5	basic molecules w/o modifiers, anilines, alkaloids, antidepressants
12, 30	9, 3	2.0-7.5	phenols, fullerenes, sweeteners
12	17	1-10	pharmaceuticals, sweeteners
12	15	1-8	halogenated and polar compounds
12	7	2.0-7.5	polar acidic, neutral, basic and chelating compounds, polar peptides
12, 20, 30	7, 5, 3	2.0-7.5	biological separations, polar compounds
20	4	1.5-7.5	proteins, peptides
proprietary	proprietary	2.0-8.0	polyaromatic hydrocarbons
12	4, 3	2.0-7.5	water-soluble vitamins
12	—	2.0-9.5	proteins, phospholipids, retinoids, lipids
12	—	2.0-7.5	malto-oligosaccharides, tocopherols, nucleotides, sugars
12	—	2.0-7.5	sugars, nucleotides, water-soluble vitamins
12, 30	7, 3	2.0-7.5	proteins, steroids, catechols
12	—	2.0-10.0	peptides, proteins, malto-oligosacchardes
6, 12	—	2.0-7.5	peptides, proteins, malto-oligosacchardes
6, 12	—	2.0-7.5	small oganic molecules, fat-soluble vitamins, tocopherols

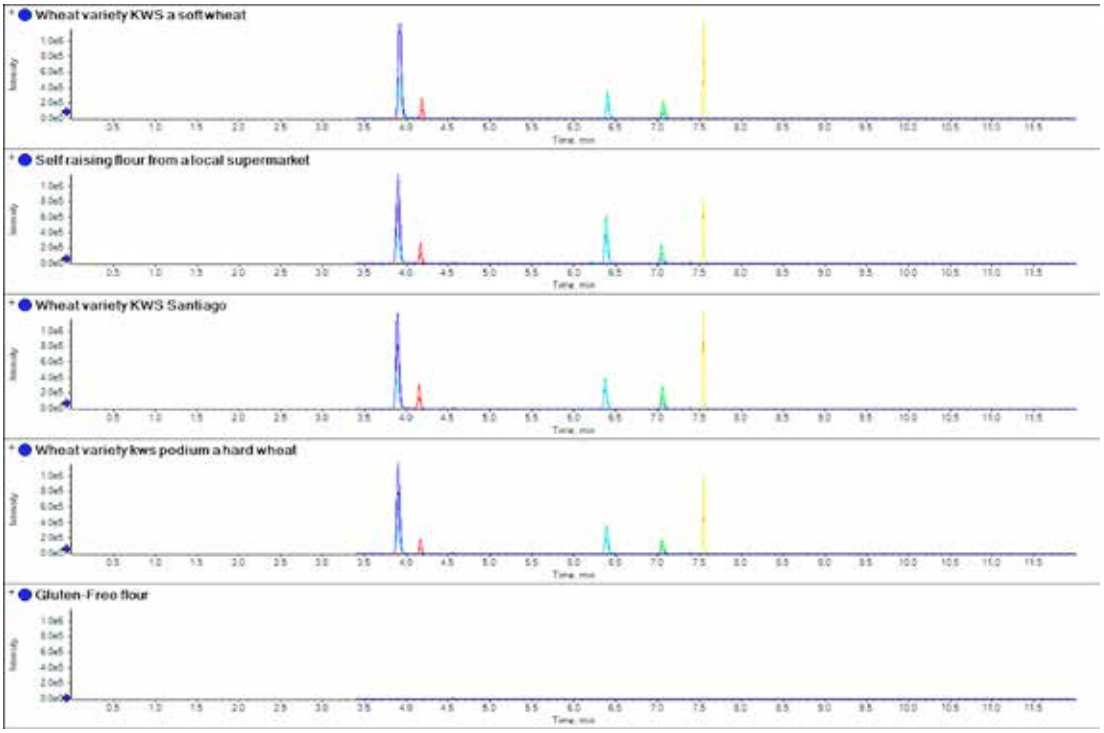
Gluten in flour and cookies

Gluten can cause allergic responses and even celiac disease if an intolerance occurs. The intolerance level is often depending on the gluten variety, which is relevant to the use of oats having low effect on celiac suffers.

So far, ELISA based on R5 antibody detection is used [1]. This assay can detect the presence of barley, rye and wheat, but cannot differentiate between them. It is not sensitive to oats. Further, it has the disadvantage of giving false positive or negative results due to either unspecific binding to the protein region or changes in the protein structure by processing.

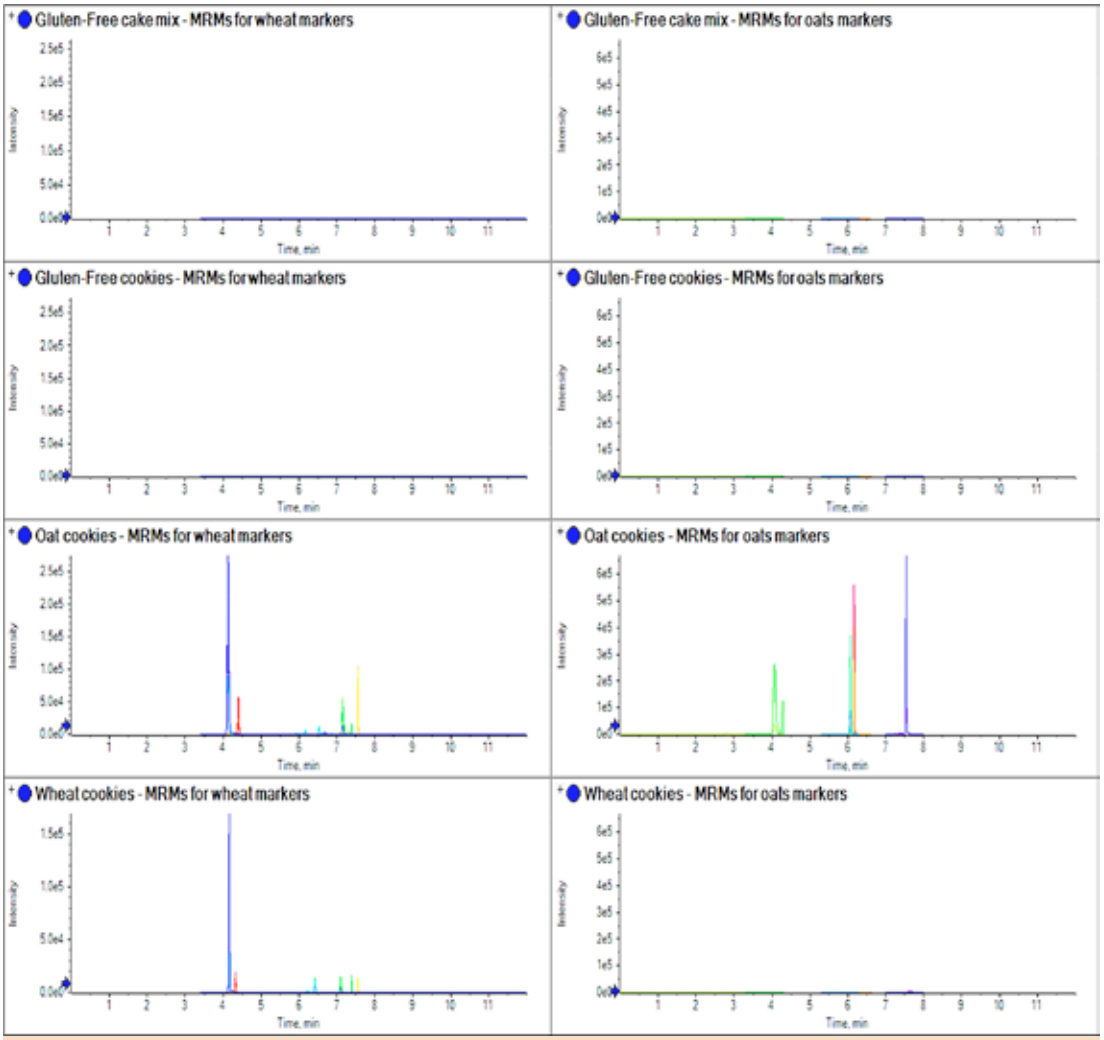
MicroLC-MS/MS using YMC-Triart C18 capillary columns can not only detect gluten markers in processed food, but it can also distinguish between varieties.

Here, five different flour samples including a gluten-free and a supermarket self raising flour were analysed for wheat peptide markers. In all the flours except the gluten-free one, wheat peptide markers can be found.



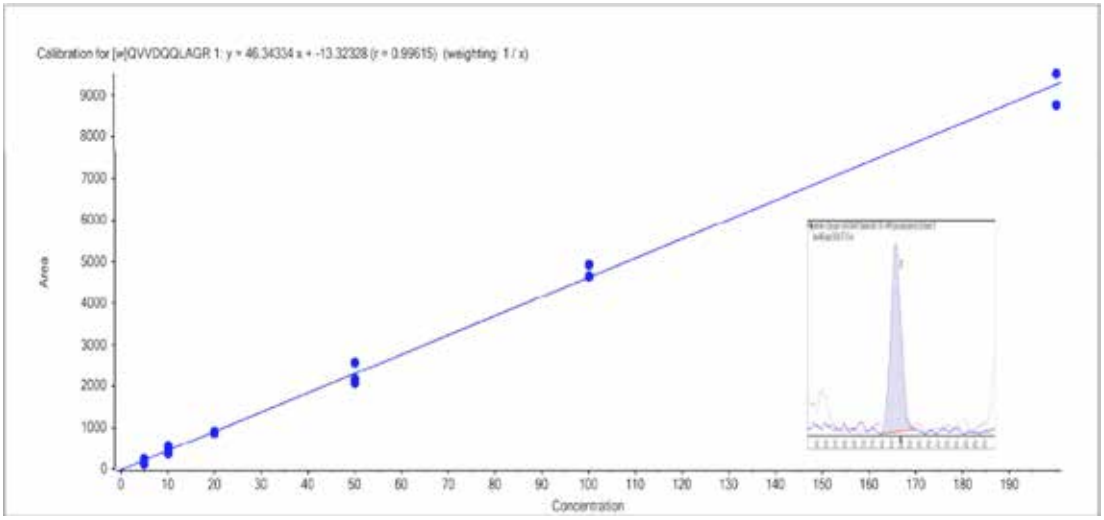
The comparison of separate extracts of several samples of wheat obtained from single variety grain samples, as well as a sample of gluten-free flour and the self raising flour obtained from a local supermarket.

With the help of mircoLC-MS/MS it is further possible to detect markers in processed food and also distinguish between varieties. In the oat cookies, wheat and oats markers were detected while in the wheat cookies only wheat peptide markers were found. The gluten-free products were actually gluten free, as no markers where detectable.



Column:	YMC-Triart C18, 12 nm, 3 µm, 100 x 0.5 mm ID, 1/32" OD	Flow:	25 µl/min (MicroLC)
Part No.:	TA12S03-10J0RU	Injection:	10 µl
LC-System:	Eksigent ekspert MicroLC 200	Eluent:	A: H_2O + 0.1% FAC
MS/MS-System:	AB SCIEX 5500 QTRAP, ESI		B: acetonitrile + 0.1% FAC
Temperature:	40°C	Gradient:	Time 0 – 1 min 6 min 8 min 9 min 9.2 min 12 min
			% B 5 25 95 95 5 5

MicroLC applications



The calibration line obtained from the spiking of gliadin, a specific wheat protein, into gluten-free wheat from the range of 5–200 ppm for wheat peptide 3. Inlayed in the calibration line is the chromatogram for the 10 ppm spike of gliadin into gluten-free flour.

By courtesy of: Stephen Lock, AB SCIEX, Warrington (UK)

Allergens in wine

In response to a wine survey, where casein was found in trace amounts (<2 ppm), the European Food Safety Authority (EFSA) concluded, in 2011, that wine fined with casein, caseinate or milk products can cause adverse reactions in sensitive individuals [2]. In addition, a new EU legislation (concerning labelling) [3] pointed out that, if fining reagents such as casein, egg ovalbumin, etc are used in processing, methods for detection of these products in wine are needed.

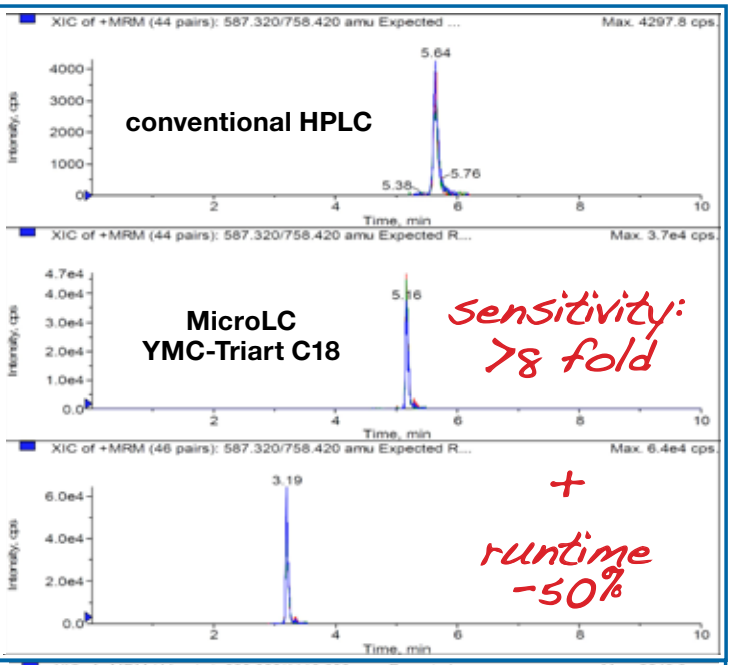
With this MicroLC method using a YMC-Triart C18 capillary column various milk and egg markers can be detected simultaneously in white wine. Due to a detection limit below 100 ppb, the requirements of detecting trace amounts are met.

The initial results for column selection show a typical sensitivity increase of between 4 and 12 fold in S/N-ratio when switching from high to micro flow. The results clearly demonstrate

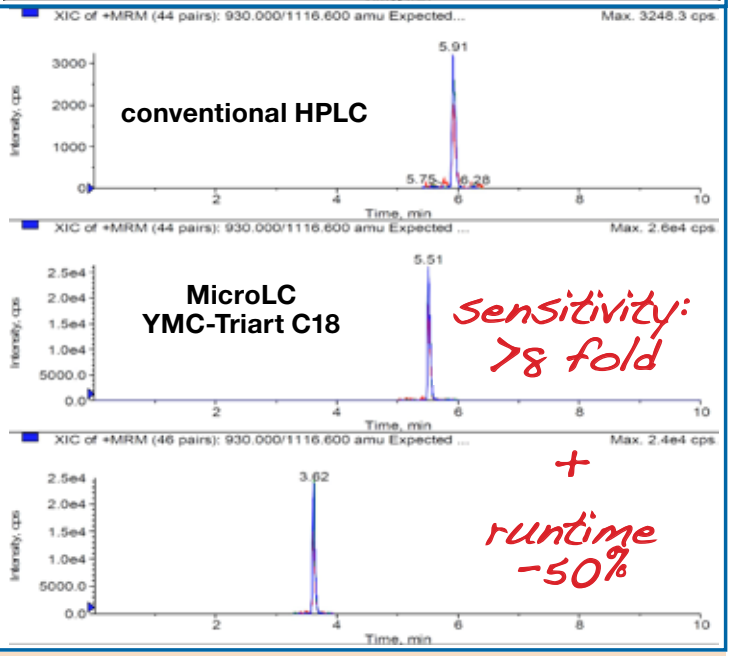
an improvement in sensitivity when moving to MicroLC which is not lost when the analysis time is further shortened to a runtime of 5.5 min to speed up the analysis. In addition, the reduced analysis time also reduces solvent consumption through the use of MicroLC.

MicroLC applications

Casein peptide



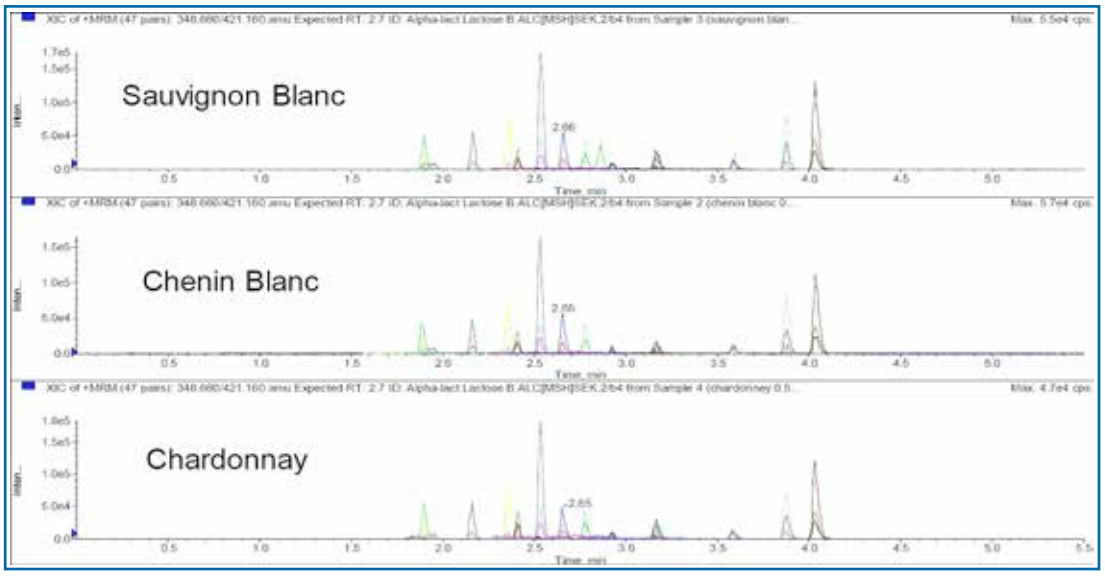
Egg peptide



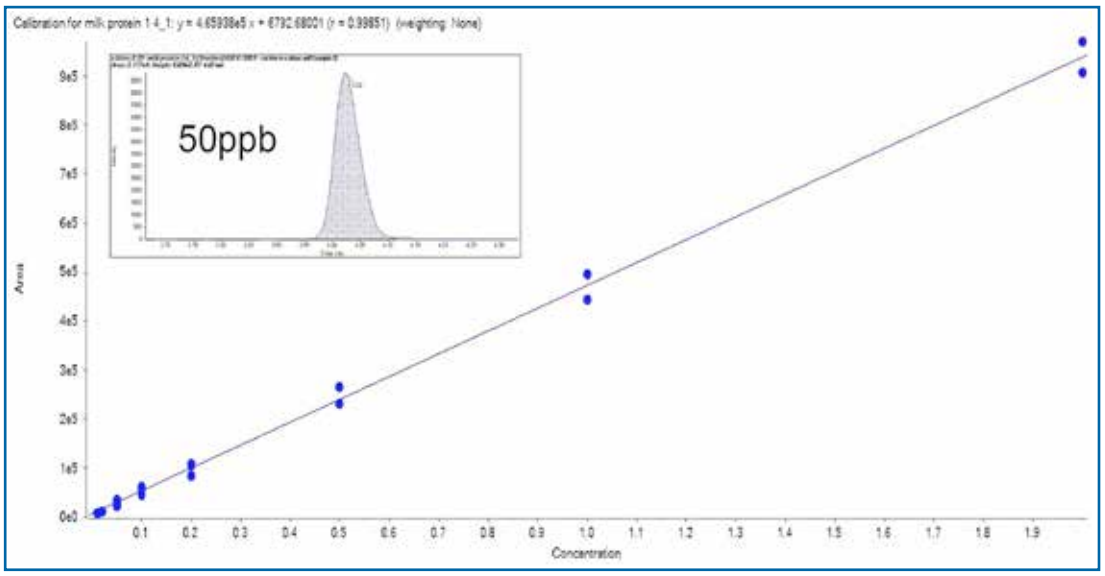
Columns:	**YMC-Triart C18**, 12 nm, 3 µm, 50 x 0.5 mm ID, 1/32" OD (MicroLC) Kinetex XB-C18, 10 nm, 2.6 µm, 50 x 2.1 mm ID (HPLC)		Flow:	300 µl/min (HPLC) 25 µl/min (MicroLC)			
Part No.:	TA12S03-05J0RU		Injection:	10 µl			
LC-System:	Shimadzu Nexera XR (HPLC) Eksigent ekspert MicroLC 200		Eluent:	A: H_2O + 0.1% FAC B: acetonitrile + 0.1% FAC			
MS/MS-System:	AB SCIEX 5500 QTRAP, ESI		HPLC Gradient:	Time	0 - 2	8	8.2- 9	9.1 - 10
Temperature:	40°C			% B	2	60	95	2
			MicroLC Gradient:	Time	0 - 0.3	4	4.1- 4.3	4.4 – 5.5
				% B	2	40	95	2

Comparison of HPLC vs. MicroLC using a white wine spiked with 1 ppm casein peptide (top) and an egg peptide (bottom).

The LC-MS/MS approach has the additional advantage of being a potential multi allergen screen where different allergens, such as egg and milk, can be detected by a single method.



On the YMC-Triart C18 capillary column MicroLC-MS/MS analysis was performed. 3 different white wines spiked with 0.5 ppm samples of milk and egg proteins were analysed. Furthermore, it was possible to detect and identify several milk and egg proteins in one run.



A casein peptide is spiked into a Sauvignon Blanc (0.05 – 2 ppm) to demonstrate linearity and sensitivity. Linearity is provided without use of any internal standards. The inset chromatogram for 50 ppb spiked sample demonstrates highest sensitivity.

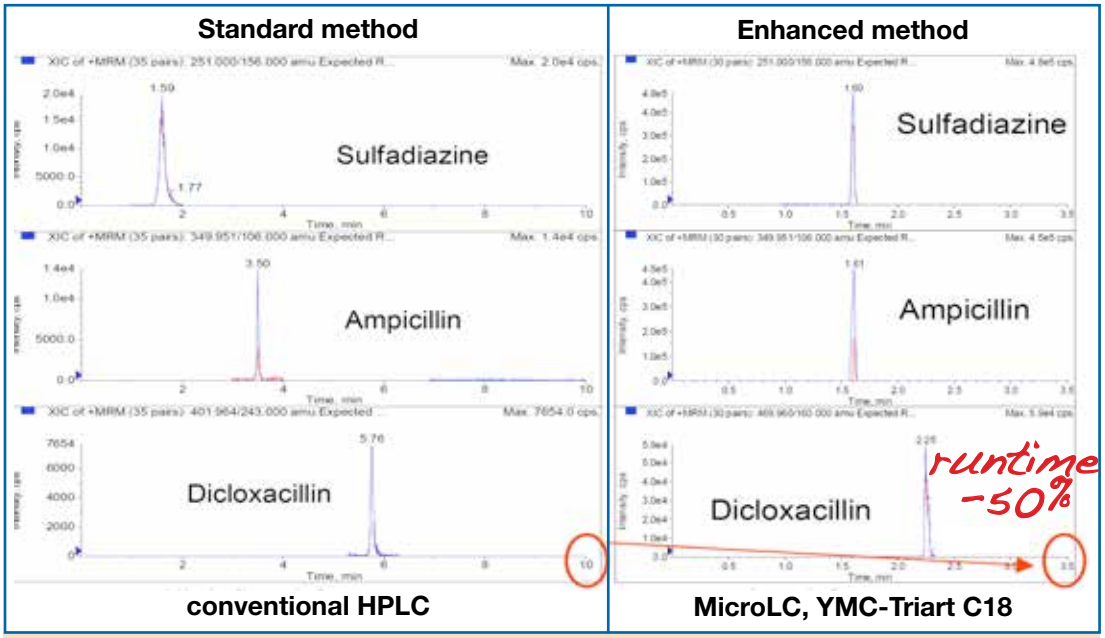
By courtesy of: Stephen Lock, AB SCIEX, Warrington (UK)

Veterinary drug residues in food

The levels and presence of veterinary drug residues in food of animal origin are legislated in the EU with limits often varying with the drug residue [4].

The MicroLC method on a YMC-Triart C18 capillary column easily fulfils the requirements of the current EU legislation. A gain in signal by a factor of more than 8-fold when switching from high to micro flow for some components is observed.

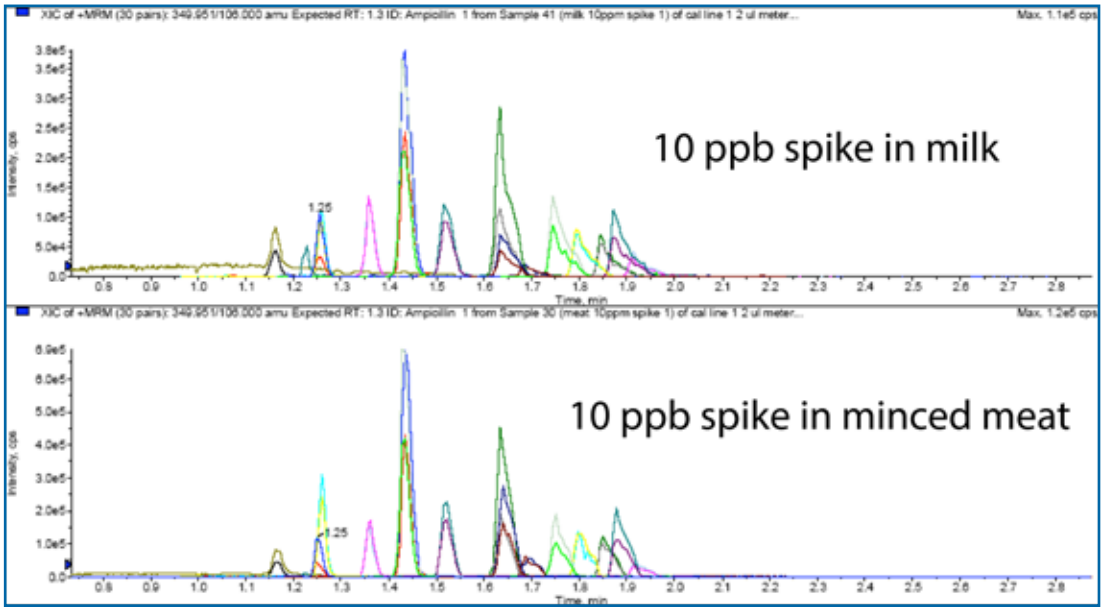
The chromatograms clearly demonstrate an improvement in sensitivity when moving to MicroLC. It is not lost when the analysis time of 10 min is further shortened to a run time of 3.5 min to speed up the analysis. Furthermore, the cut in analyse time provides great potential of cost savings by up to 90% in regards to solvents.



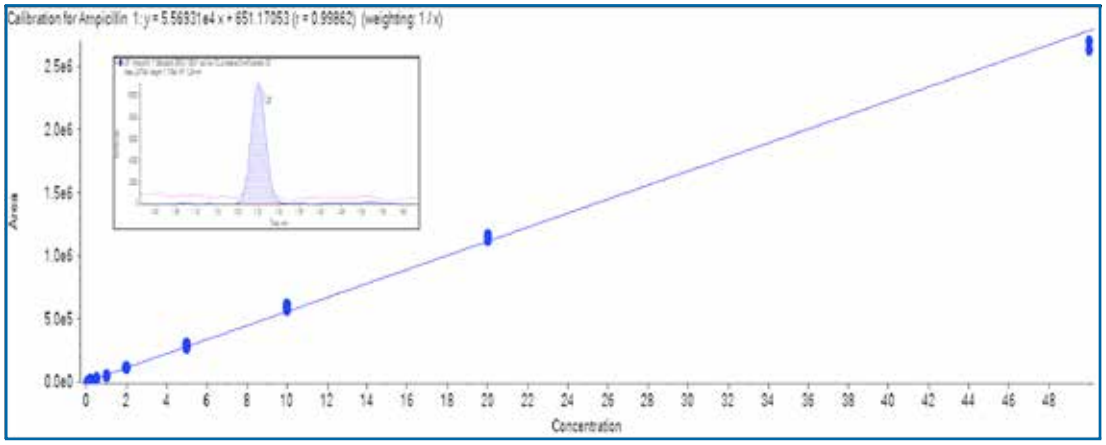
Columns:	**YMC-Triart C18**, 12 nm, 3 µm, 50 x 0.5 mm ID, 1/32" OD (MicroLC) Kinetex XB-C18, 10 nm, 2.6 µm, 50 x 2.1 mm ID (HPLC)	Temperature:	60°C
		Flow:	600 µl/min (HPLC) 30 µl/min (MicroLC)
		Injection:	2 µl
Part No.:	TA12S03-05J0RU	Eluent:	A: H_2O + 0.1% FAC B: acetonitrile + 0.1% FAC
LC-System:	Shimadzu Nexera XR (HPLC) Eksigent ekspert MicroLC 200	Enhanced Gradient:	Time 0 - 0.5 1.7 1.8 - 2.3 2.4 - 3.5 % B 2 65 100 2
MS/MS-System:	AB SCIEX 5500 QTRAP, ESI		

Comparison of 3 different 1 ppb standard solutions separated by a standard HPLC method using a Kinetex C18 column (left) and the MicroLC method using a YMC-Triart C18 capillary column (right).

The MicroLC/MS/MS approach has the additional advantage of being a potential drug residue screen where different residues can be detected by a single method.



In the final analysis a total of 32 multiple reaction monitoring (MRM) transitions were evaluated for 15 veterinary drug residues over a 3.5 minute run time on the YMC-Triart C18 capillary column. Milk and meat samples have been spiked at a 10 ppb level with standard compounds. The recoveries from meat were generally higher and it shows that recoveries are affected by the matrix.



Linearity and sensitivity of this method is demonstrated for Ampicillin from 0.05 – 50 ppb. Linearity is provided without use of any internal standards. The inset chromatogram for a 0.5 ppb spiked sample demonstrates the high level of sensitivity.

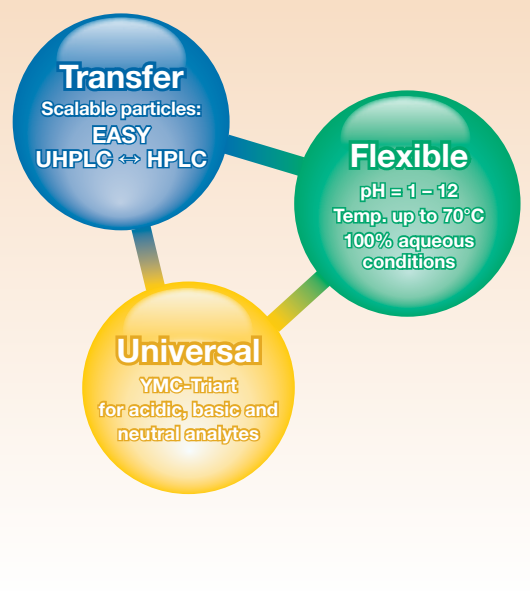
By courtesy of: Stephen Lock, AB SCIEX, Warrington (UK)

pH stable and robust phases: YMC-Triart

YMC-Triart is a material prepared using tightly controlled particle formation technology which has been adapted from micro-reactor technology. This novel production process results in exceptionally narrow particle and pore size distributions.

With YMC-Triart, challenging pH, high temperatures and even the use of 100% aqueous conditions are no longer a limitation to the day-to-day work in laboratories. Most importantly, due to its unique particle composition, balanced hydrophobicity and silanol activity are achieved which makes YMC-Triart a “First Choice” column in method development.

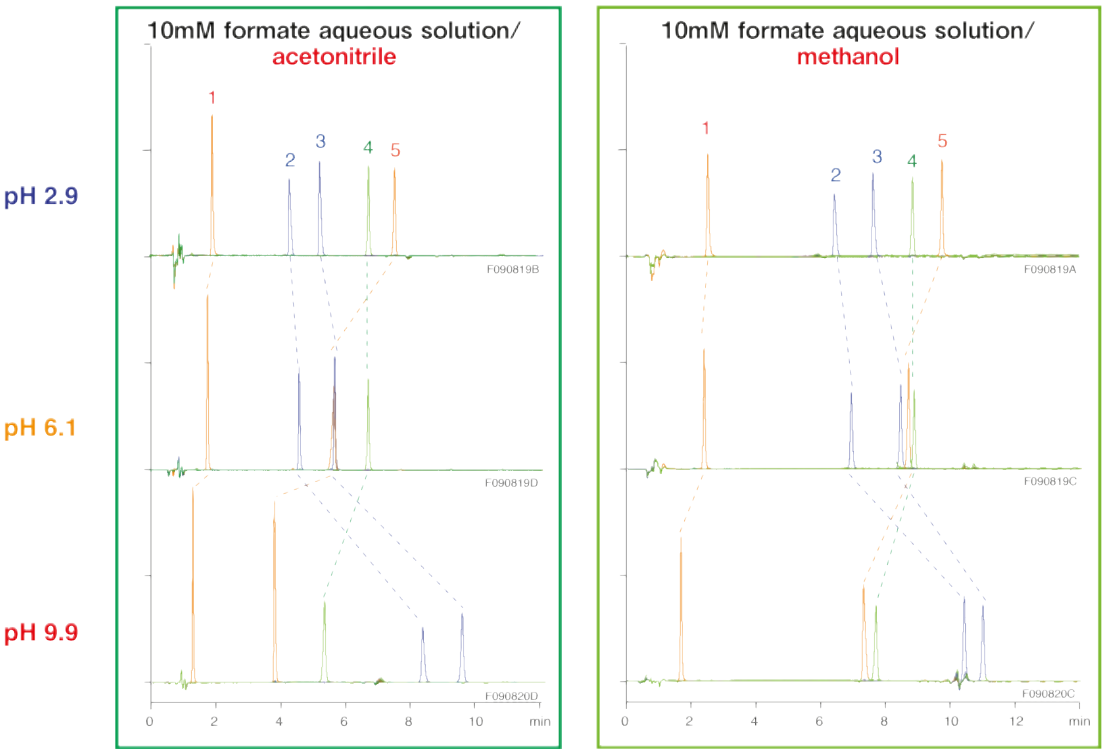
YMC-Triart is the ideal choice for use in capillary columns. The versatile hybrid phases are available with 6 different chemistries and particle sizes of 3, 5 and even 1.9 µm. All specifications are valid for use in capillary columns.

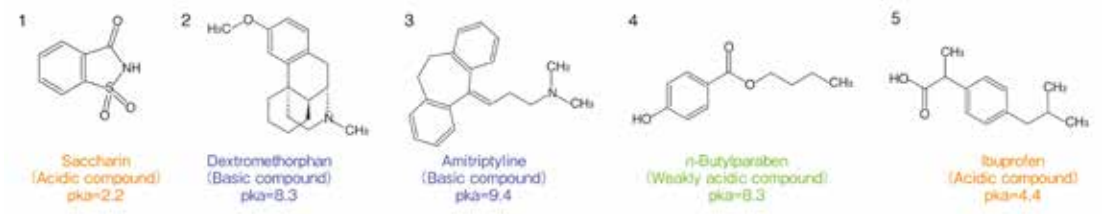


Specifications

	YMC-Triart C18	YMC-Triart C18 ExRS	YMC-Triart C8	YMC-Triart Phenyl	YMC-Triart PFP	YMC-Triart Diol-HILIC
Base	organic/inorganic silica					
Stationary phase	C18 (USP L1)	C18 (USP L1)	C8 (USP L7)	Phenyl (USP L11)	Pentafluoro-phenyl (USP L43)	Diol (USP L20)
Particle size	1.9, 3 and 5 µm					
Pore size	12 nm / 120 Å	8 nm / 80 Å	12 nm / 120 Å	12 nm / 120 Å	12 nm / 120 Å	12 nm / 120 Å
Specific surface	360 m^2/g	430 m^2/g	360 m^2/g	360 m^2/g	360 m^2/g	360 m^2/g
Bonding	polymeric type					
Endcapping	multi-stage hybrid groups	multi-stage hybrid groups	multi-stage hybrid groups	multi-stage hybrid groups	none	none
Carbon load	20%	25%	17%	17%	15%	—
pH range	1 ~ 12	1 ~ 12	1 ~ 12	1 ~ 10	1 ~ 8	2 ~ 10
Temperature range	pH 1-7: 70 °C, pH 7-12: 50 °C	pH 1-7: 70 °C, pH 7-12: 50 °C	pH 1-7: 70 °C, pH 7-12: 50 °C	50°C	50°C	50°C

Flexibility in method development





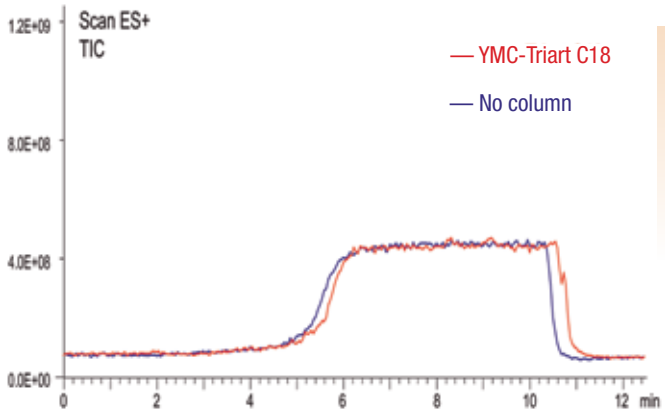
Columns:	YMC-Triart C18, 12 nm, 5 µm, 50 x 2.0 mm ID	Flow rate:	0.2 ml/min
Part No.:	TA12S05-0502WT	Temperature:	25 °C
Eluent:	A) 10 mM HCOOH for pH 2.9 10 mM $HCOONH_4$ for pH 6.1 10 mM $HCOONH_4$-NH_3 for pH 9.9 B) organic solvent	Detection:	UV at 230 nm

On reversed-phase HPLC, pH and organic solvent are the most important factors to control retention and selectivity. Triart C18 delivers symmetrical peak shapes for all types of compounds. Moreover, this feature is independent from mobile phase pH and mobile phase condition. Chromatographers can choose the most optimal condition by combining various mobile phase conditions such as mobile phase pH, and types of organic solvent / buffer system.

* Application data by courtesy YMC Co., Ltd.

pH stable and robust columns

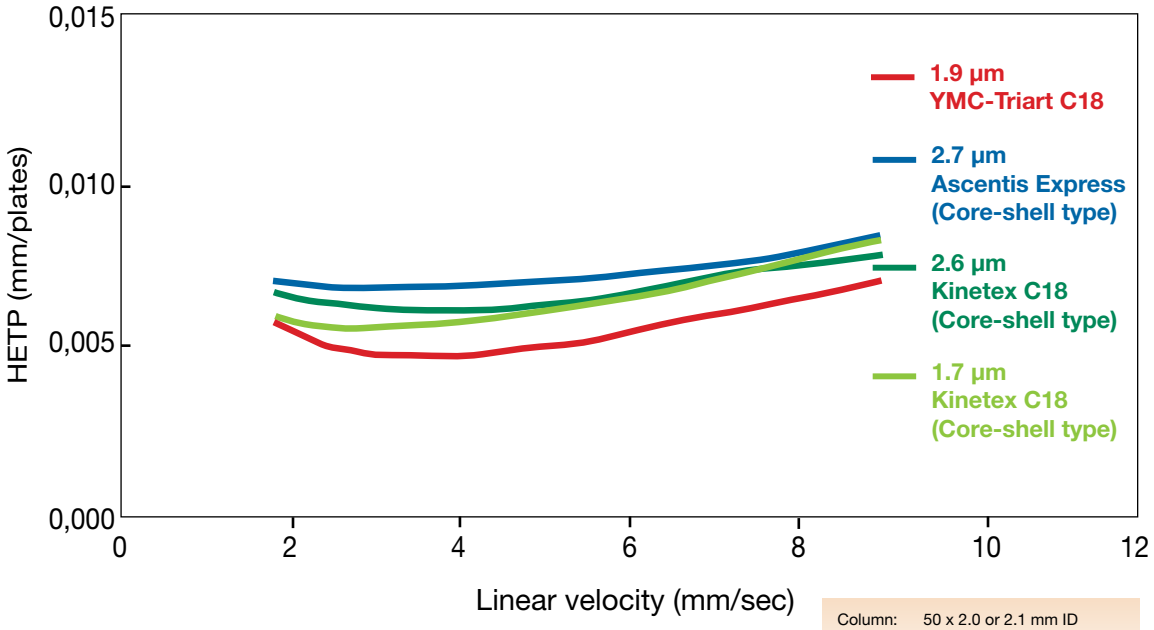
LC/MS compatibility



Column:	5 µm, 50 x 2.0 mm ID
Part-No.:	TA12S05-0502WT
Eluent:	A) water + 0.1% formic acid B) acetonitrile + 0.1% formic acid 5% B (0-1 min), 5-100% B (1-5 min), 100% B (5-10 min), 100-5% B (10-10.1 min), 5% B (10.1-12.5 min)
Flow rate:	0.4 ml/min
Temperature:	40 °C
Detection:	ESI positive, TIC (Mass Range: 50-1000)

Column bleeding, caused by the fragments of stationary phase, is the main reason for background noise and restrictions on detection limits. No bleed is observed in the test of total ion current (TIC) measured by LC/MS with blank or with YMC-Triart C18. So in terms of the signal/noise ratio (S/N ratio), YMC-Triart C18 can be expect to not only reduce the background noise but to also increase the sensitivity of the analysis.

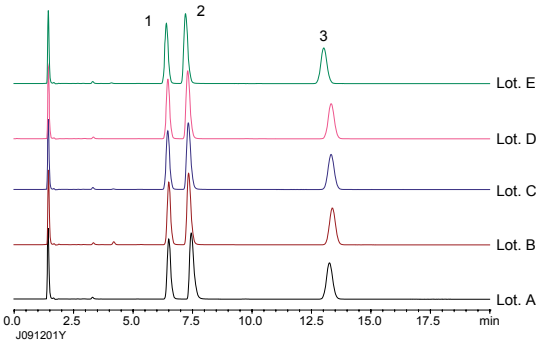
Lower HETP means higher resolution!



Column:	50 x 2.0 or 2.1 mm ID
Eluent:	acetonitrile / water (60/40)
Detection:	UV at 254 nm
Sample:	Butylbenzoate

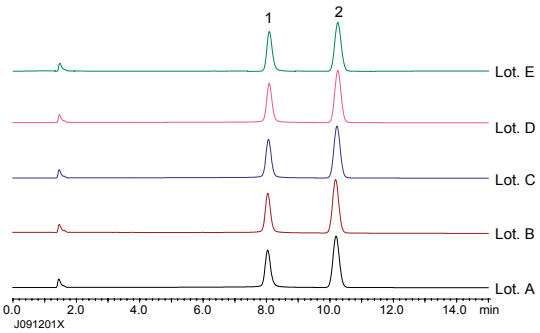
Batch-to-batch reproducibility

Basic compounds



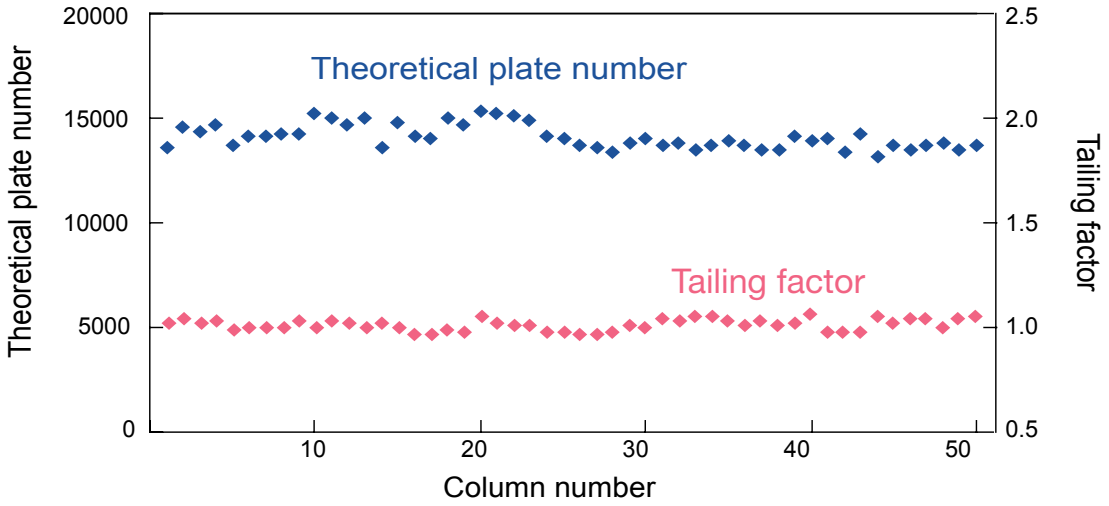
Column: YMC-Triart C18, 5 µm, 150 x 3.0 mm ID
Part-No.: TA12S05-1503WT
Eluent: 20 mM KH_2PO_4 (pH 6.9) / acetonitrile (65/35)
Flow rate: 0.425 ml/min
Temperature: 40 °C
Detection: UV at 235 nm

Coordination compounds



Column: YMC-Triart C18, 5 µm, 150 x 3.0 mm ID
Part-No.: TA12S05-1503WT
Eluent: acetonitrile / 0.1% H_3PO_4 (40/60)
Flow rate: 0.425 ml/min
Temperature: 40 °C
Detection: UV at 254 nm

Excellent reproducibility of YMC-Triart phases is available even for the analysis for basic and coordination compounds which normally exhibit tailing and adsorption effects.



The reproducibility of packed columns is shown in terms of theoretical plate number (N) and tailing factor (Tf). YMC-Triart packed columns exhibit a very narrow range of variation.

Column: YMC-Triart C18, 5 µm, 150 x 4.6 mm ID
Part-No.: TA12S05-1546WT
Eluent: acetonitrile / water (40/60)
Flow rate: 1.0 ml/min
Temperature: ambient
Sample: butyl benzoate

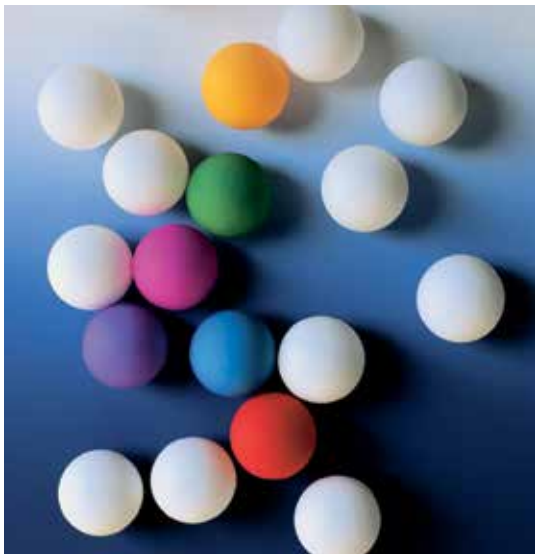
* Application data by courtesy YMC Co., Ltd.

Columns for the pharmaceutical industry: YMC-Pack *Pro*Family

YMC *Pro*Family is based on a silica with an extremely low metal content. Low metal content suppresses polar interactions, as metal impurities cause silanol groups to become more acidic. This ensures YMC *Pro*Family phases provide excellent resolution for a wide range of compounds, especially basic pharmaceuticals.

To minimise the unwanted interactions between residual silanols and the sample molecules that are responsible for asymmetric peaks, YMC utilises a proprietary, highly effective endcapping process.

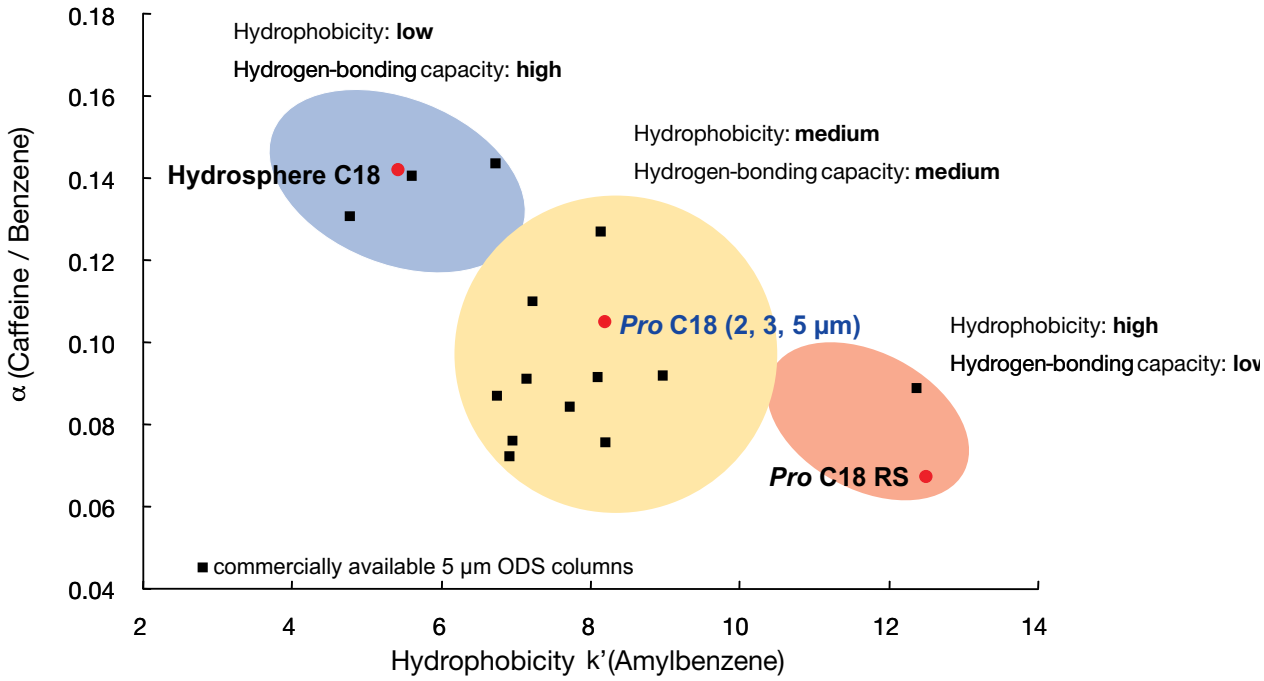
Important for every chromatographer: YMC ensures that the physical properties e.g. particle size, pore size, metal content are extremely reproducible from LOT to LOT.



Specifications

	Pro C18	*Pro* C8	*Pro* C4	Hydrosphere C18	*Pro* C18 RS
Particle size	2; 3; 5 µm	3; 5 µm	3; 5 µm	2; 3; 5 µm	3; 5 µm
Pore size	12 nm / 120 Å				8 nm / 80 Å
Surface area	330 m^2/g				510 m^2/g
Carbon content	16%	10%	7%	12%	22%
pH range	2.0 - 8.0	2.0 - 7.5	2.0 - 7.5	2.0 - 8.0	1.0 - 10.0

Selectivity Chart



*Pro*Family provides excellent coverage of selectivity patterns for pharmaceutical compounds.

* Application data by courtesy YMC Co., Ltd.

Solutions for polar substances

Today, HPLC is a widely used method of analysis for a vast number of compounds, due to its simplicity and reproducibility of the method.

However, a fundamental difficulty exists: the low retention of polar analytes makes HPLC method development more demanding. Nevertheless, modern HPLC offers a range of options to the chromatographer to overcome this problem.

C18 under aqueous conditions

YMC-Triart „AQ“

State of the art phase for polar analytes even under (U)HPLC-conditions with enhanced stability against

- pH 1-12
- temp. up to 70°C
- 100% H_2O

YMC-Pack ODS-AQ

The synonym for stability under aqueous conditions.

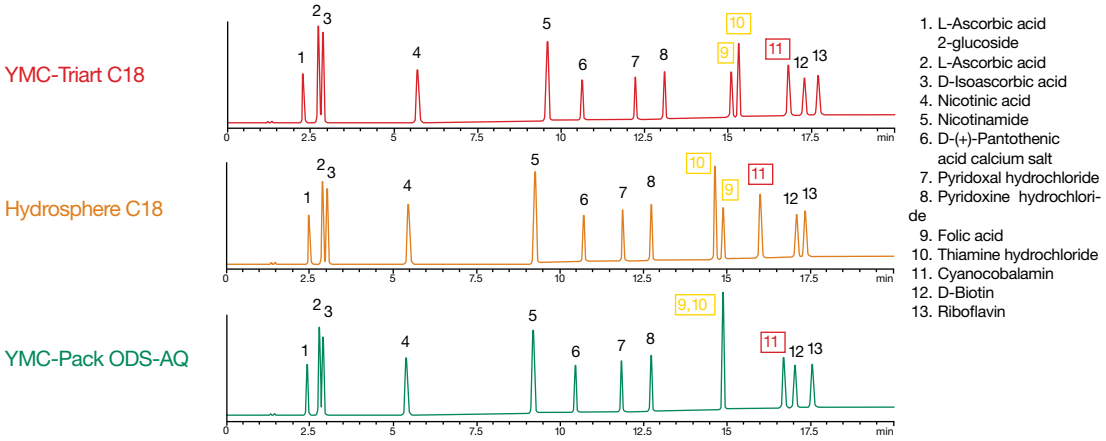
- “hydrophilic” C18
- balanced surface chemistry
- polar recognition
- metabolite recognition

Hydrosphere C18

A new, ultrapure silica base was introduced whilst adapting the surface chemistry to maintain the “AQ”-type properties.

- “hydrophilic” C18 surface for enhanced polar recognition
- stable when used with 100% aqueous eluent
- no need for ion pair reagents
- addition of 2 µm particle size for Fast-LC (YMC-Pack UltraHT)

Retention behaviour



1. L-Ascorbic acid 2-glucoside
2. L-Ascorbic acid
3. D-Isoascorbic acid
4. Nicotinic acid
5. Nicotinamide
6. D-(+)-Pantothenic acid calcium salt
7. Pyridoxal hydrochloride
8. Pyridoxine hydrochloride
9. Folic acid
10. Thiamine hydrochloride
11. Cyanocobalamin
12. D-Biotin
13. Riboflavin

Column: 3 µm, 150 x 3.0 mm ID
Part No.: TA12S03-1503WT
Eluent: A) 20 mM KH_2PO_4-H_3PO_4 (pH 2.8) containing 5 mM $CH_3(CH_2)_5SO_3Na$
B) 20 mM KH_2PO_4-H_3PO_4 (pH 2.8) / acetonitrile (80/20) containing 5 mM $CH_3(CH_2)_5SO_3Na$
Flow rate: 0.425 ml/min
Temperature: 40°C
Detection: 210 nm

Retention behaviour of water-soluble vitamins on three YMC ODS phases which can be used with 100% aqueous mobile phases is compared. The retention times and peak elution order for folic acid (peak 9), thiamine hydrochloride (peak 10) and cyanocobalamin (peak 11) are different for the three phases due to the balance of hydrophobicity and hydrogen bond capacity differing between the three phases.

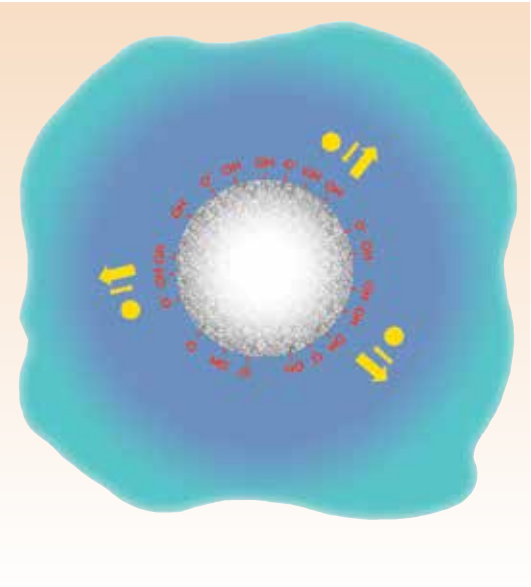
Specifications of AQ-type phases

	YMC-Triart C18 "AQ"	YMC-Pack ODS-AQ	Hydrosphere C18
Particle size	1.9; 3; 5 µm	3; 5 µm	2; 3; 5 µm
Pore size	12 nm / 120 Å	12; 20 nm / 120: 200 Å	12 nm / 120 Å
Carbon content	20%	14; 10%	12%
pH range	1.0 - 12.0	2.0 - 7.5	2.0 - 8.0

* Application data by courtesy YMC Co., Ltd.

HILIC

- retention of extremely polar compounds
- reproducible chromatographic results
- very high LC/MS selectivity



Specifications of HILIC phases

	YMC-Triart Diol-HILIC	YMC-Pack Diol-NP	YMC-Pack Polyamine II	YMC-Pack NH_2 (Amino)
Modification	1,2-Dihydroxypropyl (USP L20)	1,2-Dihydroxypropyl (USP L20)	Sec./tert. amines	Aminopropylsilane (USP L8)
Particle sizes	1.9; 3; 5 µm	5 µm	5 µm	3; 5 µm
Pore size	12 nm / 120 Å	6; 12 nm / 60; 120 Å	12 nm / 120 Å	12 nm / 120 Å
Surface area	360 m^2/g	450; 330 m^2/g	330 m^2/g	330 m^2/g
pH range	2 ~ 10	2 ~ 7.5	2 ~ 7.5	2 ~ 7.5

	YMC-Pack SIL (Silica)	YMC-Pack PVA-Sil	YMC-Pack TMS (C1)	YMC-Pack CN (Cyano)
Modification	Silica (USP L3)	Polyvinyl alcohol (USP L24)	Trimethylsilane (USP L13)	Cyanopropyl (USP L10)
Particle sizes	3; 5 µm	5 µm	3; 5 µm	3; 5 µm
Pore size	6; 12; 20; 30 nm / 60; 120; 200; 300 Å	12 nm / 120 Å	12; 30 nm / 120; 300 Å	12; 30 nm / 120; 300 Å
Surface area	450; 330; 175; 100 m^2/g	330 m^2/g	330; 175 m^2/g	330; 175 m^2/g
pH range	2 ~ 7.5	2 ~ 9.5	2 ~ 7.5	2 ~ 7.5

In the field of biochromatography, phase selection is a key to success!

With the YMC's "Column Selection Tool" for Bio-LC, stationary phase selection is almost too easy. As shown in the table (below), the C18 column with 12 nm pore size is suitable for small peptides up to a MW of 5000. The best efficiency for large peptides or small proteins can be obtained by using a C8 phase characterised by a 20 nm pore size whilst most proteins are eluted effectively on a C4 column with 30 nm porosity.

However, the separation may also be influenced by the hydrophobicity of the peptide/protein and the nature of the column's bonded phase. Therefore, for initial method development, it might be useful, in the first instance, to follow the arrow shown in the table for method optimisation.

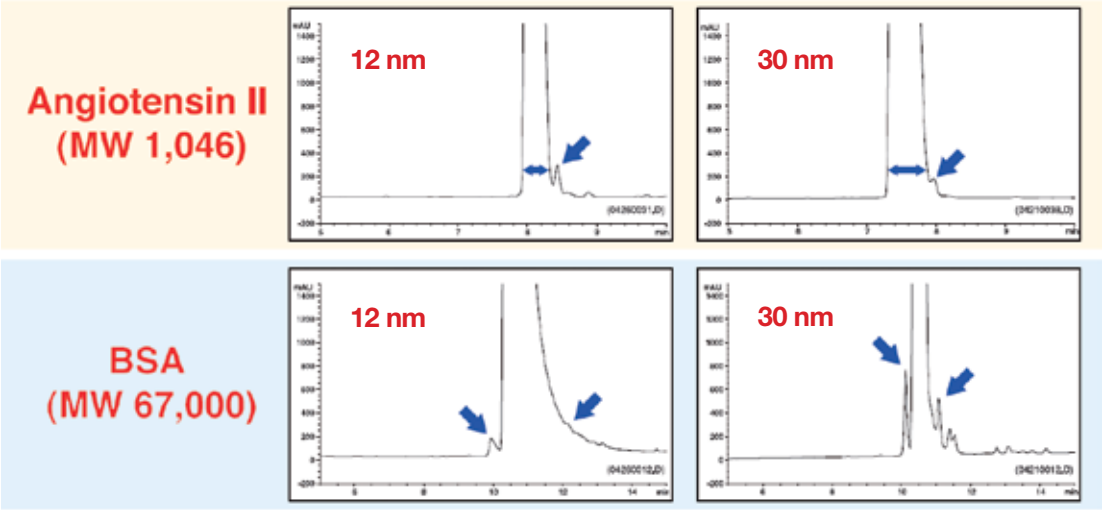
Column selection tool

MW		C18	C8	C4
5000 ↓ 20000 ↓ 100000	12 nm	◎	○	△
	20 nm	○	◎	○
	30 nm	△	○	◎

◎: excellent, ○: good, △: moderate

* Application data by courtesy YMC Co., Ltd.

Comparison of peaks on C4 with 12 nm and 30 nm pore sizes



For smaller peptides a small pore size is more successful. Larger molecules are separated much better with larger pore sizes!

C18-Selectivities for peptides

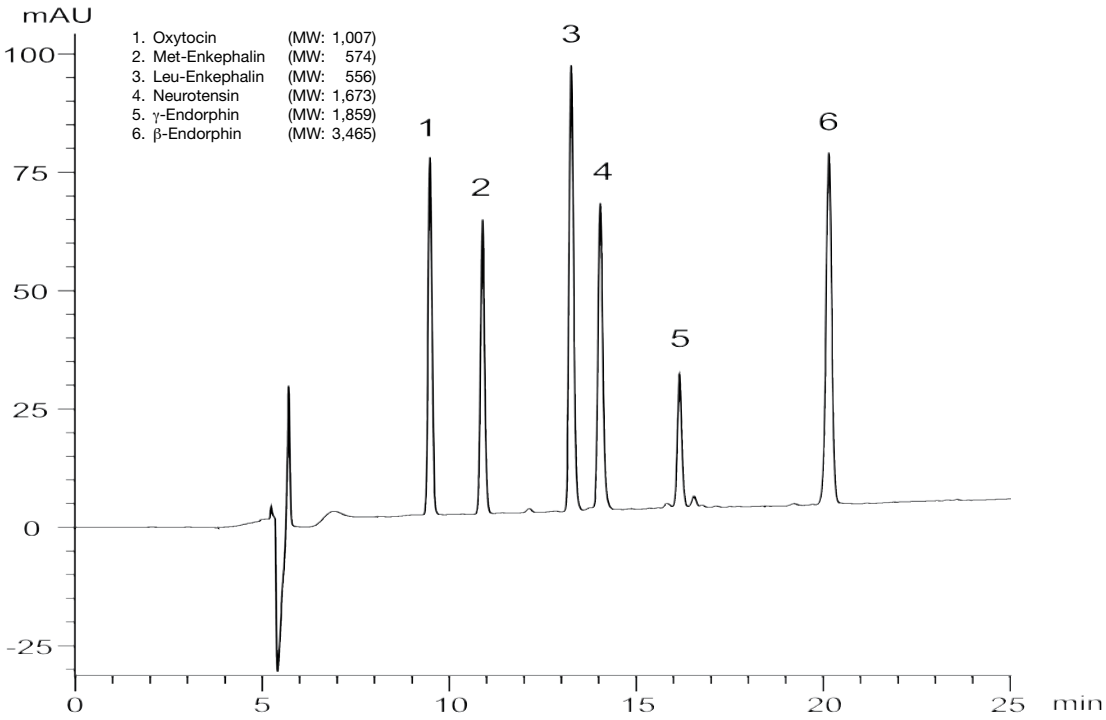
	YMC-Triart C18	YMC-Pack *Pro* C18	YMC-Pack ODS-A	YMC-Pack ODS-AQ	Hydrosphere C18
Particle size	1.9; 3; 5 µm	2; 3; 5 µm	3; 5 µm	3; 5 µm	2; 3; 5 µm
Pore size	12 nm / 120 Å	12 nm / 120 Å	12; 20; 30 nm / 120; 200; 300 Å	12; 20 nm / 120; 200 Å	12 nm / 120 Å
Carbon content	20%	17%	17; 12; 7%	14; 10%	12%
pH range	1.0 - 12.0	2.0 - 8.0	2.0 - 7.5	2.0 - 7.5	2.0 - 8.0

C8- and C4-Selectivities for peptides and proteins

	YMC-Pack C8	YMCbasic	YMC-Pack C4	YMC-Pack Protein RP
Particle size	3; 5 µm	3; 5 µm	3; 5 µm	5 µm
Pore size	12; 20; 30 nm / 120; 200; 300 Å	20 nm / 200 Å	12; 20; 30 nm / 120; 200; 300 Å	20 nm / 200 Å
Carbon content	10; 7; 4%	7%	7; 5; 3%	4%
pH range	2.0 - 7.5	2.0 - 7.5	2.0 - 7.5	1.5 - 7.5

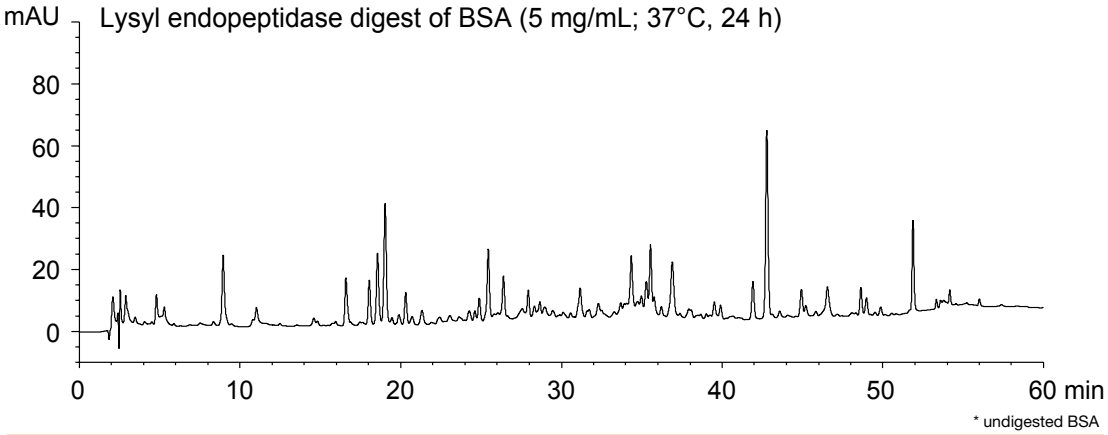
Examples of Peptide and Protein Applications

Peptides



Column:	YMC-Triart C18 (5 µm, 12 nm) 150 x 2.0 mm ID	Flow rate:	0.2 ml/min
Part No.:	TA12S05-1502WT	Temperature:	37 °C
Eluent:	A) water / TFA (100/0.1)	Detection:	UV at 220 nm
	B) acetonitrile / TFA (100/0.1)	Injection:	2 µl (0.075 ≈ 0.25 mg/ml)
Gradient:	20%-45% B (0-25 min)		

Peptide mapping



* undigested BSA

Column:	YMCbasic (5 µm) 150 x 2.0 mm ID	Flow rate:	0.2 ml/min
Part No.:	BA99S05-1502WT	Temperature:	37 °C
Eluent:	A) water / TFA (94/6) B) acetonitrile / TFA (100/0.1)	Detection:	UV at 220 nm
Gradient:	5-35% B (0-50 min), 35-45% B (50-55 min), 45% B (55-60 min)	Injection:	1 µl

Speciality column: YMC Carotenoid (C30)

The YMC Carotenoid (C30) stationary phase provides sufficient phase thickness to enhance interaction with long chained molecules. Therefore, geometric and positional isomers of conjugated double bonding systems are recognised and resolved by the YMC Carotenoid phase.

YMC Carotenoid can also be used for various analytes, including polar carotenes, polar and nonpolar xanthophylls, steroids, retinols and fat-soluble vitamins. The phase is an excellent choice for LC-MS applications.

Specifications

	Specification
Particle Size	3; 5 µm
Pore Size	proprietary
Surface area	proprietary
Carbon content	proprietary
Recommended pH range	2.0 - 7.5

Applications

YMC30 columns are successfully used in the food industry, for the analysis of vitamin formulations, in environmental analysis, and for the control of algal growth. Other potential applications include the separation of prostaglandins and leucotrienes.

Columns for normal phase chromatography

Whilst historically it was the earliest form of HPLC, normal phase analytical separations are currently receiving less attention due to the belief that it is complicated and unpredictable. However, normal phase chromatography is a powerful tool for the separation of positional isomers that are difficult to separate in reversed phase mode. Due to a rigid surface, compared to the more flexible carbon chains of reversed phases, the analytes are influenced by well-defined steric interaction with polar groups.

YMC offers columns packed with non-bonded silica or with silica gel modified with polar groups.

Specifications

	YMC-Pack SIL (Silica)	YMC-Pack PVA-Sil	YMC-Pack CN (Cyano)	YMC-Pack Diol-NP
Modification	Silica (USP L3)	Polyvinyl alcohol (USP L24)	Cyanopropyl (USP L10)	1,2-Dihydroxypropyl (USP L20)
Particle sizes	3; 5 µm	5 µm	3; 5 µm	5 µm
Pore size	6; 12; 20; 30 nm / 60; 120; 200; 300 Å	12 nm / 120 Å	12; 30 nm / 120; 300 Å	6; 12 nm / 60; 120 Å
Surface area	450; 330; 175; 100 m^2/g	330 m^2/g	330; 175 m^2/g	450; 330 m^2/g
pH range	2 ~ 7.5	2 ~ 9.5	2 ~ 7.5	2 ~ 7.5

	YMC-Pack Polyamine II	YMC-Pack NH_2 (Amino)	YMC-Pack TMS (C1)
Modification	Sec./tert. amines	Aminopropylsilane (USP L8)	Trimethylsilane (USP L13)
Particle sizes	5 µm	3; 5 µm	3; 5 µm
Pore size	12 nm / 120 Å	12 nm / 120 Å	12; 30 nm / 120; 300 Å
Surface area	330 m^2/g	330 m^2/g	330; 175 m^2/g
pH range	2 ~ 7.5	2 ~ 7.5	2 ~ 7.5

YMC capillary column hardware

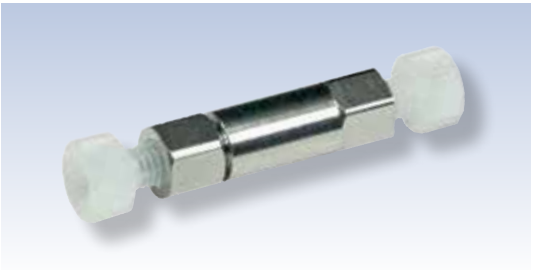
Capillary columns

All YMC phases are available in capillary columns. They are compatible with all Nano-/MicroLC/MS systems. Capillary columns are suitable for extremely low sample volumes and low flow rates. They are available either with 1/16” connections (10-32 thread) or with 1/32” connections (6-40 thread).

Pressure stability

Pressure stability of the phase is dependent on particle size:
2/3 µm: 550 bar / 7,975 psi
1.9 µm: 600 bar / 8,700 psi.

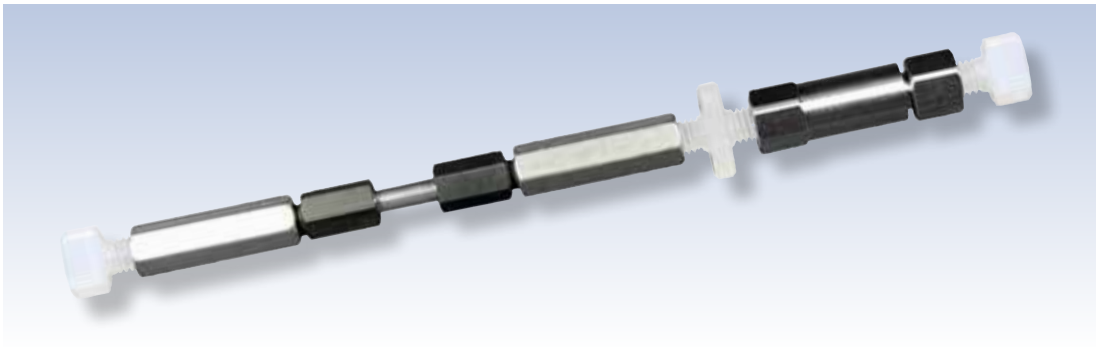
The hardware is pressure rated at
690 bar / 10,000 psi.



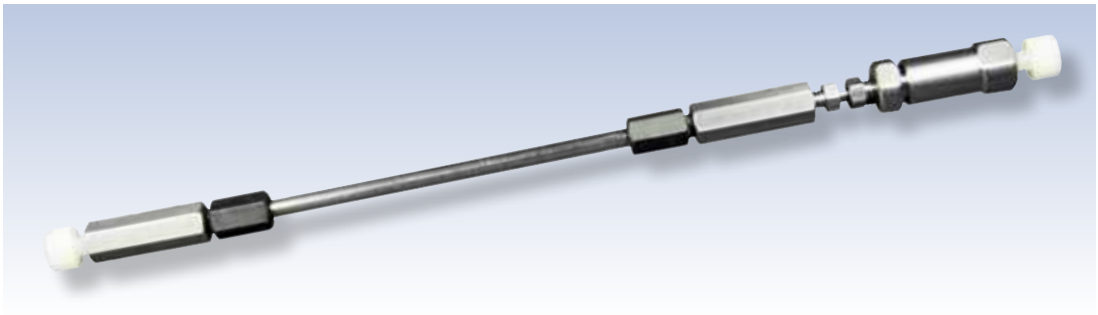
1/16” Guard column 5 mm x 300 µm

Guard columns

Guard columns are recommended for challenging matrices or for the use as trapping columns.

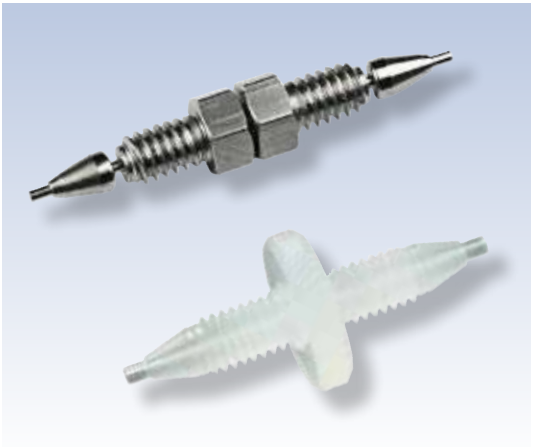


1/16” Guard column coupled to a 1/16” analytical column



1/32” Guard column coupled to a 1/32” analytical column

Guard columns are connected directly to the analytical column using the column couplers supplied.



1/32” (top), 1/16” (bottom)
Column coupler

Column coupler

A column coupler is supplied with every pack of capillary guard cartridges to guarantee the optimum connection with low dead volume. A polymer-based (PCTFE) coupler is provided for 1/16” columns (right), while a stainless steel coupler is provided for 1/32” columns (left). Every coupler can be purchased separately if required.

Dimensions and part numbers

YMC capillary columns are available with 1/16” (10-32 thread) or with 1/32” (6-40 thread) connections. All column part numbers indicate the connection size by use of additional terminal letters:

1/**16**” fittings end with **AU**

1/**32**” fittings end with **RU**

The specific part number for a given column consists of two parts describing chemistry and dimension/hardware details. Both parts of the part number can be taken from the tables below:

First part (chemistry) of the part number

YMC-Triart Columns

Description	Partial Part No.
YMC-Triart C18 ExRS, 8 nm, 1.9 µm	TAR08SP9
YMC-Triart C18 ExRS, 8 nm, 3 µm	TAR08S03
YMC-Triart C18 ExRS, 8 nm, 5 µm	TAR08S05
YMC-Triart C18, 12 nm, 1.9 µm	TA12SP9
YMC-Triart C18, 12 nm, 3 µm	TA12S03
YMC-Triart C18, 12 nm, 5 µm	TA12S05
YMC-Triart C8, 12 nm, 1.9 µm	TO12SP9
YMC-Triart C8, 12 nm, 3 µm	TO12S03
YMC-Triart C8, 12 nm, 5 µm	TO12S05
YMC-Triart Diol-HILIC, 12 nm, 1.9 µm	TDH12SP9
YMC-Triart Diol-HILIC, 12 nm, 3 µm	TDH12S03
YMC-Triart Diol-HILIC, 12 nm, 5 µm	TDH12S05
YMC-Triart PFP, 12 nm, 1.9 µm	TPF12SP9
YMC-Triart PFP, 12 nm, 3 µm	TPF12S03
YMC-Triart PFP, 12 nm, 5 µm	TPF12S05
YMC-Triart Phenyl, 12 nm, 1.9 µm	TPH12SP9
YMC-Triart Phenyl, 12 nm, 3 µm	TPH12S03
YMC-Triart Phenyl, 12 nm, 5 µm	TPH12S05

YMC *Pro* Family columns

Description	Partial Part No.
YMC-UltraHT *Pro* C18, 12 nm, 2 µm	AS12S02
YMC-Pack *Pro* C18, 12 nm, 3 µm	AS12S03
YMC-Pack *Pro* C18, 12 nm, 5 µm	AS12S05
YMC-UltraHT Hydrosphere C18, 12 nm, 2 µm	HS12S02
Hydrosphere C18, 12 nm, 3 µm	HS12S03
Hydrosphere C18, 12 nm, 5 µm	HS12S05
YMC-Pack *Pro* C8, 12 nm, 3 µm	OS12S03
YMC-Pack *Pro* C8, 12 nm, 5 µm	OS12S05
YMC-Pack *Pro* C4, 12 nm, 3 µm	BS12S03
YMC-Pack *Pro* C4, 12 nm, 5 µm	BS12S05
YMC-Pack *Pro* C18 RS, 8 nm, 3 µm	RS08S03
YMC-Pack *Pro* C18 RS, 8 nm, 5 µm	RS08S05

Important Note:

For use with Ekisgent Micro- and NanoLC systems, order columns with 1/32” (6-40 thread) end-fitting and use either, Eksigent 6/40 fitting p/n 5019621 or VALCO p/n ZNF.5FPK.

Dimensions and part numbers

A selection of other YMC columns*

Description	Partial Part No.
YMC-Pack ODS-A, 20 nm, 5 µm	AA20S05
YMC-Pack ODS-A, 30 nm, 5 µm	AA30S05
YMC-Pack ODS-AQ, 12 nm, 5 µm	AQ12S05
YMC-Pack ODS-AQ, 20 nm, 5 µm	AQ20S05
J'sphere H80, 8 nm, 4 µm	JH08S04
J'sphere M80, 8 nm, 4 µm	JM08S04
J'sphere L80, 8 nm, 4 µm	JL08S04
YMC-Pack C8 (Octyl), 20 nm, 5 µm	OC20S05
YMC-Pack C8 (Octyl), 30 nm, 5 µm	OC30S05
YMCbasic, 20 nm, 3 µm	BA99S03
YMCbasic, 20 nm, 5 µm	BA99S05
YMC-Pack C4 (Butyl), 20 nm, 5 µm	BU20S05
YMC-Pack C4 (Butyl), 30 nm, 5 µm	BU30S05
YMC-Pack Ph (Phenyl), 12 nm, 5 µm	PH12S05
YMC-Pack Ph (Phenyl), 30 nm, 5 µm	PH30S05
YMC Carotenoid (C30), 3 µm	CT99S03
YMC Carotenoid (C30), 5 µm	CT99S05

Description	Partial Part No.
YMC-Pack TMS (C1), 12 nm, 5 µm	TM12S05
YMC-Pack TMS (C1), 30 nm, 5 µm	TM30S05
YMC-Pack CN (Cyano), 12 nm, 5 µm	CN12S05
YMC-Pack CN (Cyano), 30 nm, 5 µm	CN30S05
YMC-Pack Diol-NP, 6 nm, 5 µm	DN06S05
YMC-Pack Diol-NP, 12 nm, 5 µm	DN12S05
YMC-Pack Diol-NP, 20 nm, 5 µm	DN20S05
YMC-Pack Diol-NP, 30 nm, 5 µm	DN30S05
YMC-Pack NH_2 (Amino), 12 nm, 5 µm	NH12S05
YMC-Pack Polyamine II, 12 nm, 5 µm	PB12S05
YMC-Pack PVA-Sil, 12 nm, 5 µm	PV12S05
YMC-Pack SIL (Silica), 6 nm, 5 µm	SL06S05
YMC-Pack SIL (Silica), 12 nm, 5 µm	SL12S05
YMC-Pack SIL (Silica), 20 nm, 5 µm	SL20S05
YMC-Pack SIL (Silica), 30 nm, 5 µm	SL30S05
YMC-Pack SIL (Silica), 30 nm, 5 µm	SL30S05

*Other YMC phases are available on request

Second part (dimension/hardware) of the part number

Column ID [µm]	Fitting [inch]	Column length				5 mm [guard column]
		50 mm	75 mm	100 mm	150 mm	
75	1/32	-05E8RU	-L5E8RU	-10E8RU	-15E8RU	—
100	1/32	-05F0RU	-L5F0RU	-10F0RU	-15F0RU	—
300	1/32	-05H0RU	-L5H0RU	-10H0RU	-15H0RU	-E5H0RU
500	1/32	-05J0RU	-L5J0RU	-10J0RU	-15J0RU	-E5J0RU
75	1/16	-05E8AU	-L5E8AU	-10E8AU	-15E8AU	—
100	1/16	-05F0AU	-L5F0AU	-10F0AU	-15F0AU	—
300	1/16	-05H0AU	-L5H0AU	-10H0AU	-15H0AU	-E5H0AU
500	1/16	-05J0AU	-L5J0AU	-10J0AU	-15J0AU	-E5J0AU

Example: Triart C18, 12 nm, 5 µm, 100 mm x 300 µm, 1/16” => TA12S05-10H0AU

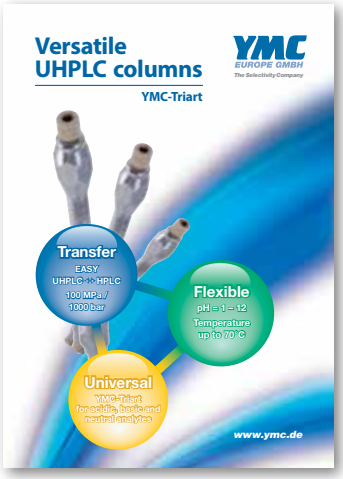
Brochures

Other brochures available

„AQ-Validated“ for stability in 100% aqueous conditions (16 p.)

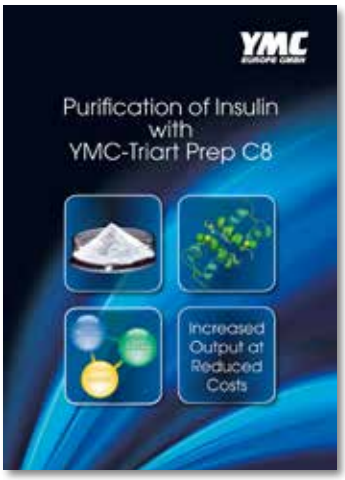
General Brochure (52 p.)

UHPLC Columns (24 p.)



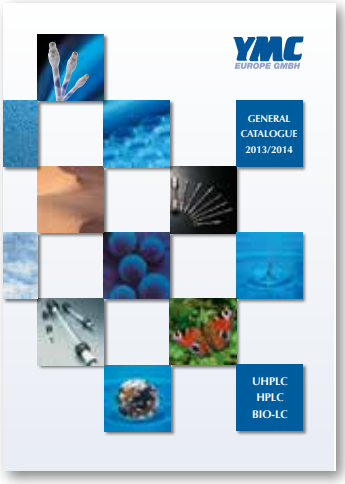
Prep Brochure (12 p.)

Insulin Case Study (16 p.)

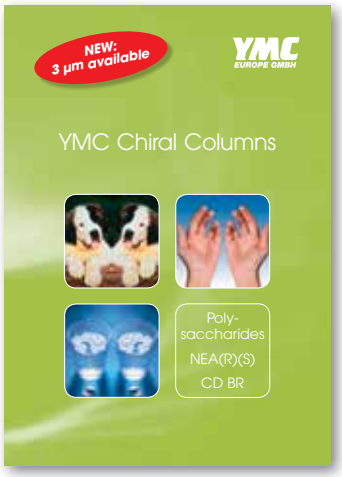


Other brochures available

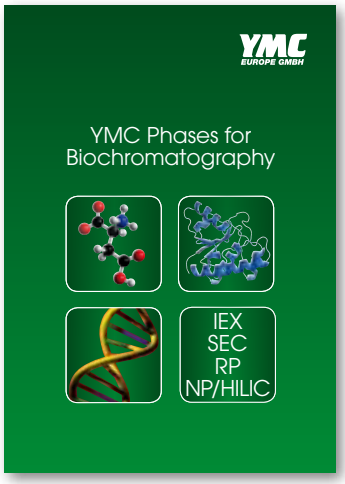
YMC
General Catalogue
UHPLC - HPLC - Bio-LC
(254 p.)



YMC
Chiral Columns
(32 p.)

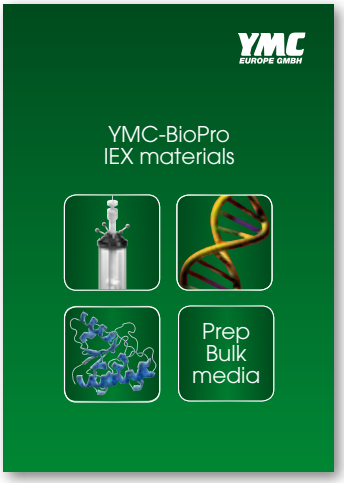


YMC
Phases for
Biochromatography
(64 p.)



„High Grade“ Silica
Bulk Media (36 p.)

IEX Bulk Media
(16 p.)



Literature:

[1] Heick, J.; Fischer, M.; Pöpping, B. First screening method for the simultaneous detection of seven allergens by liquid chromatography mass spectrometry. J. Chromatogr. A 2011, 1218, 938–943.

[2] Scientific Opinion related to a notification from the International Organization of Vine and Wine on casein/caseinate/milk products to be used in the manufacture of wine as clarification processing aids pursuant to Article 6, paragraph 11 of Directive 200/13/EC – for permanent exemption from labelling, EFSA Journal 2011, 9(10), 2384.

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