

# MicroLC

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# 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 times can also be reduced giving further savings in solvent use or time.

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

## YMC Capillary Column Hardware



All YMC phases are available packed in capillary columns. They are compatible with all NanoLC/ 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  $\mu$ m: 550 bar / 7,975 psi 1.9  $\mu$ m: 600 bar / 8,700 psi. The hardware is pressure rated at 690 bar / 10,000 psi.



1/16" Guard column 5 mm x 300  $\mu m$ 

#### **Guard columns**

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

# YMC Capillary Column Hardware



1/16" Guard column coupled to a 1/16" 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 (bottom), while a stainless steel coupler is provided for 1/32" columns (top). Every coupler can be purchased separately if required.

### **Gluten in Flour and Cookies**

#### **Gluten and food safety**

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. 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.

### **Gluten in Flour and Cookies**

With the help of MicroLC-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.





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, SCIEX, Warrington (UK)

Literature:

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.

### Allergens in Wine

#### Food watch for 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. In addition, a new EU legislation (concerning labelling) 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/Nratio 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.



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

### Allergens in Wine

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, SCIEX, Warrington (UK)

Literature:

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.

Commission Regulation (EU) No 1266/2010 of 22 December 2010 amending Directive 2007/68/EC as regards labelling requirements for wines, 2010.

### Veterinary Drug Residues in Food

#### Veterinary drug residues legislated in the EU

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.



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).

### Veterinary Drug Residues in Food

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, SCIEX, Warrington (UK)

Literature

Commission Regulation (EU) No 37/2010 of 22 December 2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin, 2010.

### **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	T012SP9
YMC-Triart C8, 12 nm, 3 µm	T012S03
YMC-Triart C8, 12 nm, 5 μm	T012S05
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 ProFamily columns

Description	Partial Part No.
YMC-UltraHT <i>Pro</i> C18, 12 nm, 2 µm	AS12S02
YMC-Pack <i>Pro</i> C18, 12 nm, 3 µm	AS12S03
YMC-Pack <i>Pro</i> 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 <i>Pro</i> C8, 12 nm, 3 μm	0S12S03
YMC-Pack <i>Pro</i> C8, 12 nm, 5 μm	0S12S05
YMC-Pack <i>Pro</i> C4, 12 nm, 3 µm	BS12S03
YMC-Pack <i>Pro</i> C4, 12 nm, 5 µm	BS12S05
YMC-Pack <i>Pro</i> C18 RS, 8 nm, 3 μm	RS08S03
YMC-Pack <i>Pro</i> 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) endfitting 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, 12 nm, 5 µm	AA12S05
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	0C20S05
YMC-Pack C8 (Octyl), 30 nm, 5 µm	0C30S05
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 CN (Cyano), 12 nm, 5 μm YMC-Pack CN (Cyano), 30 nm, 5 μm	CN12S05 CN30S05
YMC-Pack Diol-NP, 6 nm, 5 µm YMC-Pack Diol-NP, 12 nm, 5 µm YMC-Pack Diol-NP, 20 nm, 5 µm YMC-Pack Diol-NP, 30 nm, 5 µm	DN06S05 DN12S05 DN20S05 DN30S05
YMC-Pack NH <sub>2</sub> (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 YMC-Pack SIL (Silica), 12 nm, 5 μm YMC-Pack SIL (Silica), 20 nm, 5 μm YMC-Pack SIL (Silica), 30 nm, 5 μm	SL06S05 SL12S05 SL20S05 SL30S05

\*Other YMC phases are available on request

Column ID [µm]	Fitting	Column length				5 mm
	[inch]	50 mm	75 mm	100 mm	150 mm	column] pack of 3
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

### Second part (dimension/hardware) of the part number

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