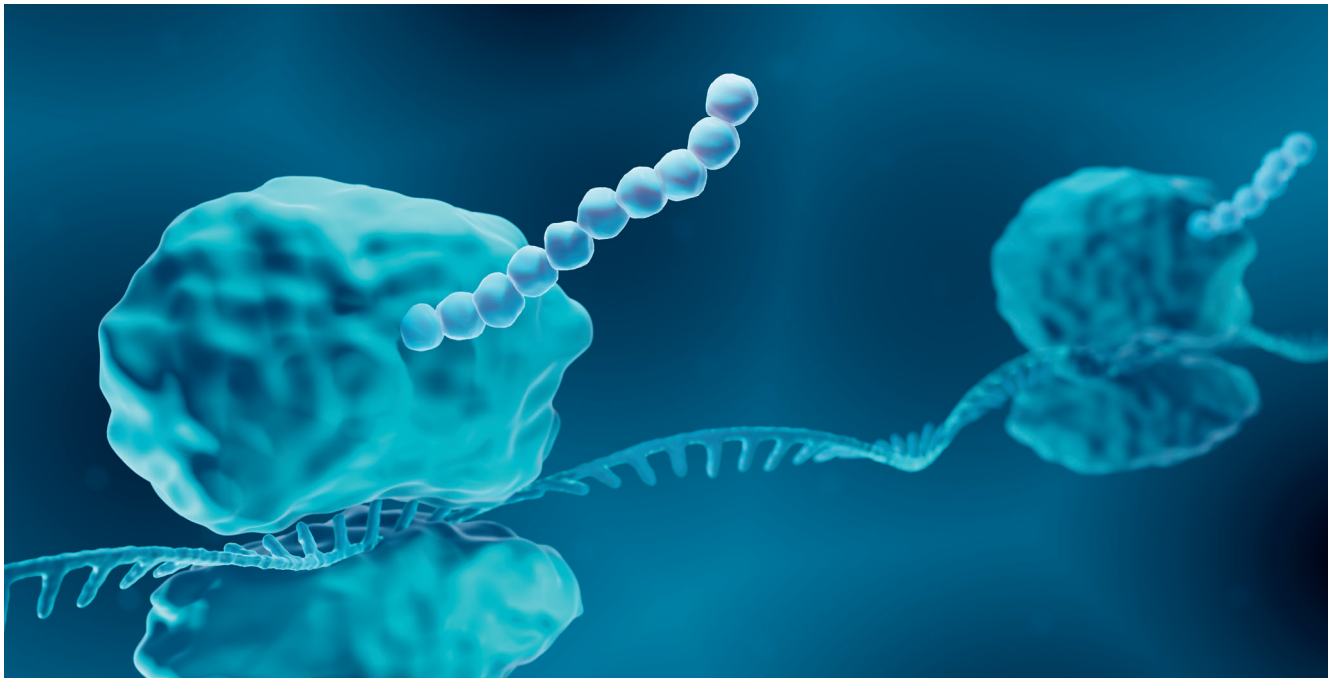
APPLICATION NOTE

High sensitivity peptide analysis using YMC-Triart C18 UHPLC and microLC columns



Peptides fulfill an enormous number of physiological functions, being present in a full dynamic range of biological concentrations (e.g. from mM to aM). Therefore, highly sensitive methods are necessary in proteomics and native peptidomics analysis to characterise even the smallest amounts of these compounds. This application note demonstrates the sensitivity gain which is achieved when using UHPLC columns of different internal diameter, as well as micro-LC approach. For this purpose, a peptide mix (Pierce™ Peptide mix) was analysed using a modified UHPLC system fitted with YMC-Triart C18 columns of different internal diameters: 2.1, 1.0 and 0.5 mm. Figure 1 shows the consequent sensitivity gain achieved with the reduction of column internal diameter. Peak areas achieved with a 0.5 mm ID micro-LC column are up to 3.5 times higher compared to a 1.0 mm ID UHPLC column and even up to 13 times higher compared to a typical UHPLC column with 2.1 mm ID. In addition, the selectivity remains the same. Therefore, the optimum ID can easily be chosen dependent on the available sample amount and chromatographic setup in the lab.

Table 1: Chromatographic conditions.

Columns:	YMC-Triart C18 (1.9 µm, 12 nm) 150 x 2.1 mm ID YMC-Triart C18 (1.9 µm, 12 nm) 150 x 1.0 mm ID YMC-Triart C18 (1.9 µm, 12 nm) 150 x 0.5 mm ID
Part Nos.:	TA12SP9-15Q1PT TA12SP9-1501WT TA12SP9-15J0AU
Eluent:	Water + 0.1% formic acid Acetonitrile + 0.1% formic acid
Gradient:	10–100%B (0–18 min)
Flow rate:	350 µL/min for 2.1 mm ID 80 µL/min for 1.0 mm ID 20 µL/min for 0.5 mm ID
Temperature:	30 °C
Detection:	ESI positive
Injection:	1 µL (500 fmol) 0.5 µL (plasma)
Sample:	Pierce™ Peptide mix (ThermoFisher) Pooled human plasma (protein precipitated) in 40% methanol
System:	Modified Waters I-class UPLC

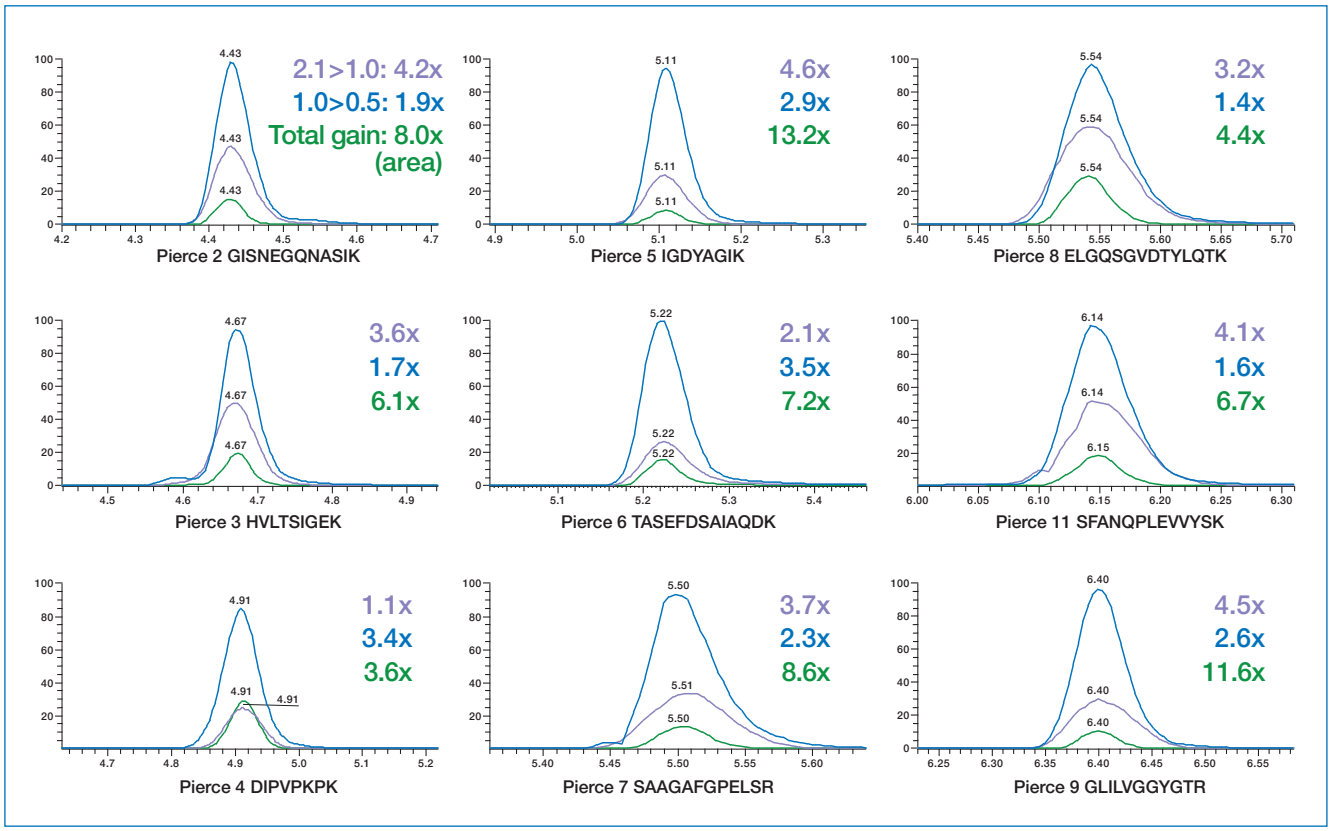


Figure 1: TIC chromatograms of 9 different Pierce™ peptides using 3 YMC-Triart C18 columns: a 2.1 mm ID UHPLC column (green), a 1.0 mm ID UHPLC column (violet) and a 0.5 mm ID microLC column (blue) [1].

This increased sensitivity by the use of a microLC column is especially useful for untargeted profiling with limited sample amounts such as in human plasma (see Figure 2). In the example below, 63% more metabolites were detected than with the classical setup (not shown).

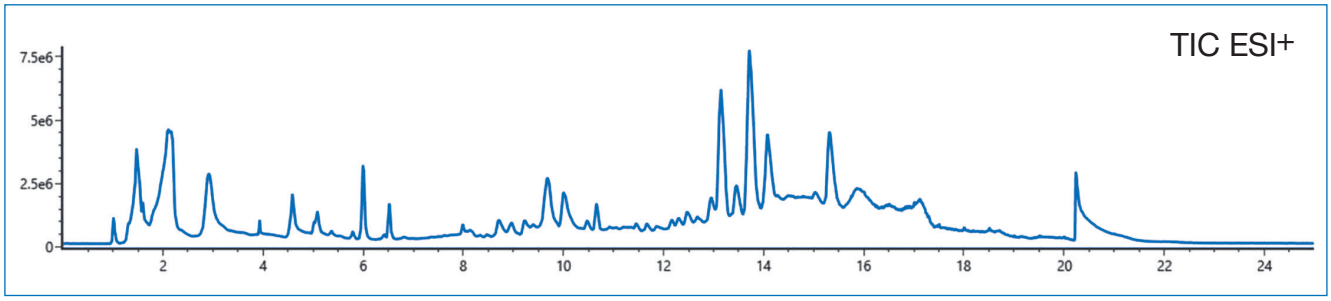


Figure 2: Untargeted screening of a protein precipitated plasma sample using a 0.5 mm ID YMC-Triart C18 microLC column [1].

Literature

[1] Sergey Girel, Víctor González-Ruiz, Serge Rudaz, Operating regular LC in microflow mode to enhance sensitivity and metabolome coverage, Poster (SGMS annual meeting), 2022

**Application data by courtesy of Sergey Girel, Institute of Pharmaceutical Sciences of Western Switzerland (University of Geneva), Geneva, Switzerland.*