Improving the UHPLC separation of fatty acid methyl ester isomers by column coupling

The separation of very similar substances such as isomers has proved as challenging. (U)HPLC phases with a high steric selectivity are required for such analyses. In this application note the fatty acid methyl esters methyl linolenate and methyl γ-linolenate are separated using YMC-Triart C18 ExRS. Due to its exceptional high hydrophobicity and steric selectivity, YMC-Triart C18 ExRS is especially suitable for the analysis of these hydrophobic isomers.

When using typical UHPLC dimensions (150 x 3 mm ID) both compounds could not be fully separated, even though an optimised mobile phase consisting of 30% water/acetonitrile (50/50) and 70% tetrahydrofuran was used. To further improve and achieve baseline separation an extended separation length of 250 mm is required. As this length is usually not available for UHPLC columns a coupling of two standard columns can easily solve this issue. By coupling of 150 mm and 100 mm length columns the resolution could be improved from 1.1 to 1.6 (see fig. 2).

To avoid unnecessary band broadening and extra column volume a short, dead volume free connector with a very low integral volume, such as the MarvelX™ universal coupler (70 mm x 125 µm ID), should be used (see fig. 1).

Chromatographic conditions

Columns:	YMC-Triart C18 ExRS (1.9 µm, 8 nm) 150 x 3 mm ID + 100 x 3 mm ID
Coupler:	MarvelX™ stainless steel column coupler (70 mm x 125 µm ID)
Part numbers:	TAR08SP9-1503PT + TAR08SP9-1003PT
	UPFS-YM6125070
Eluent:	Water + acetonitrile (50/50) / tetrahydrofuran (70/30)
Flow rate:	0.3 mL/min
Detection:	UV at 210 nm
Temperature:	35 °C
Injection:	1 µL

Figure 1: Coupling of two YMC-Triart C18 ExRS UHPLC columns using the dead volume free MarvelX™ connector.

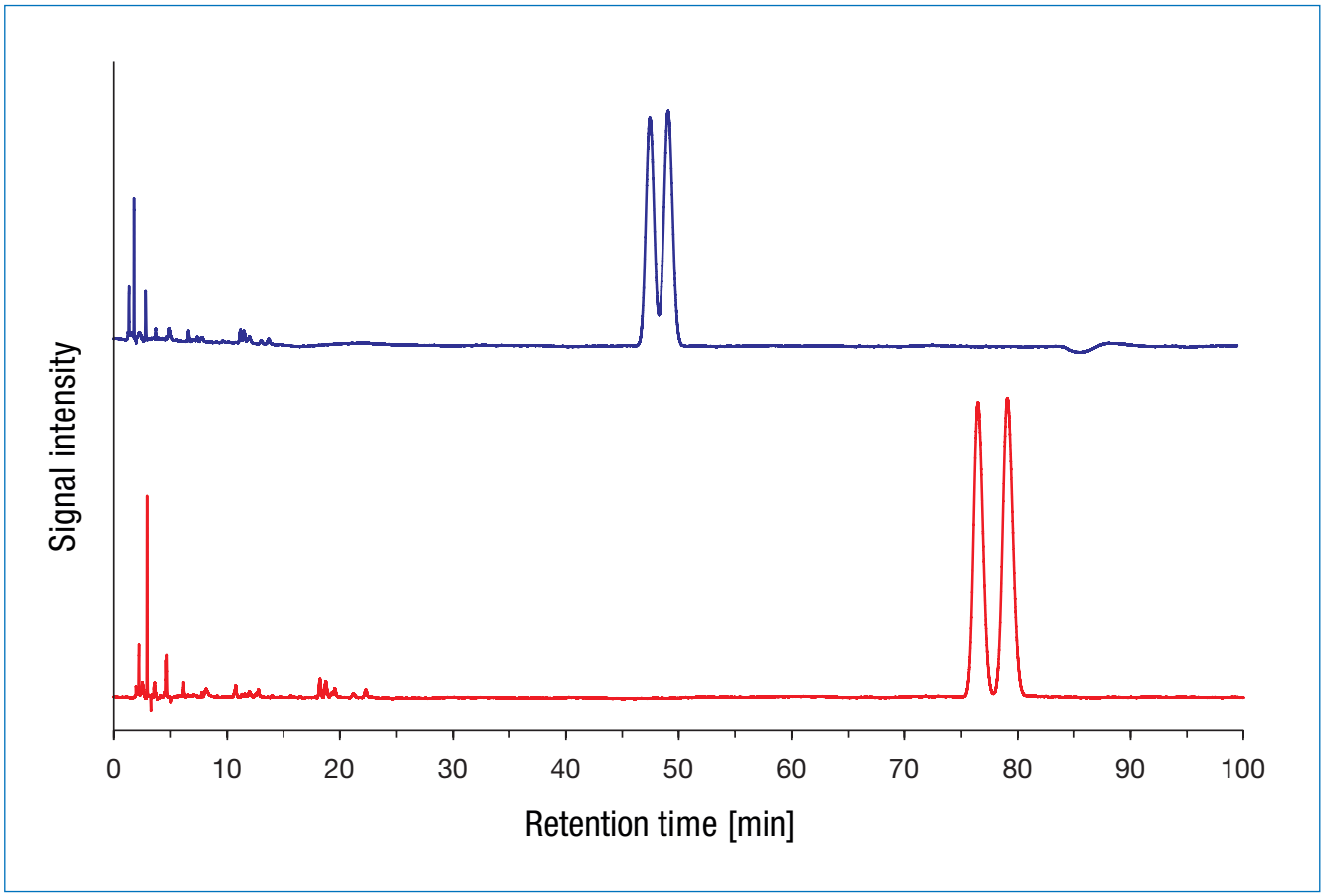

Figure 2: Analysis of methyl linolenate isomers using a single YMC-Triart C18 ExRS column (blue, Rs = 1.1 at 455 bar) and two coupled columns (red, Rs = 1.6 at 714 bar).