Customer Experience:

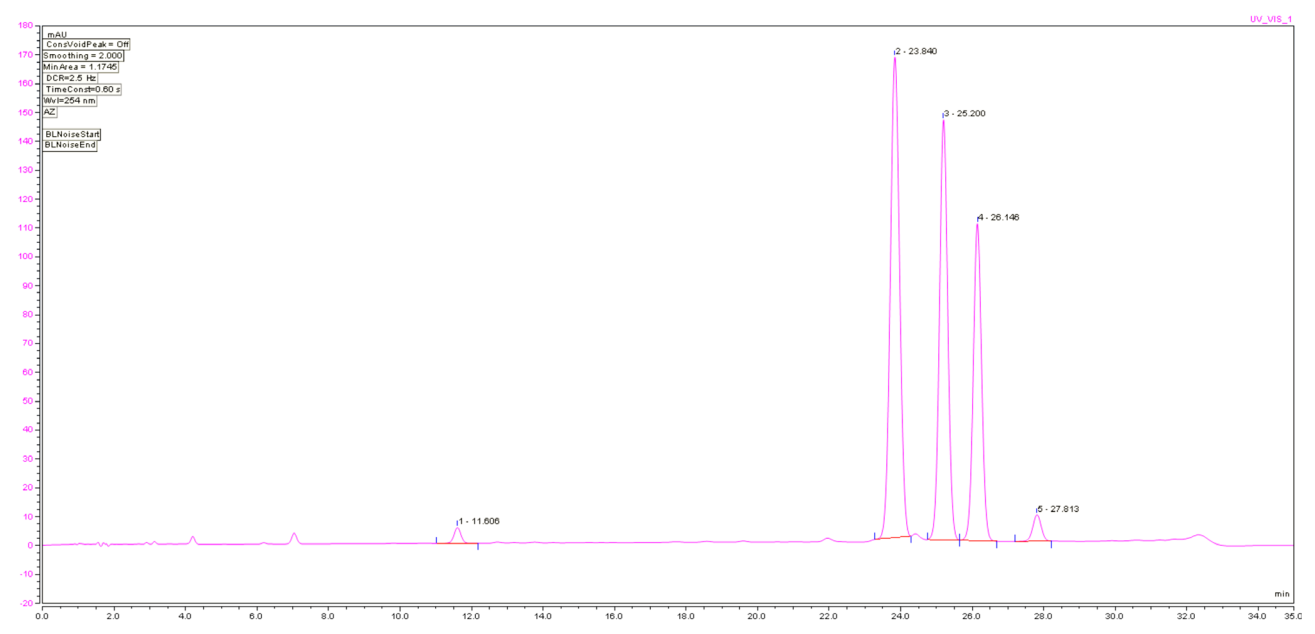
Purification of 4 Isomers Using YMC-Actus Triart C18

Novalix in France developed a method for the purification of four isomers. Stationary phases from different manufacturers were tested, but YMC-Triart C18 was chosen due to its ideal selectivity for this separation task and its easy scalability.

The scale-up from analytical to semi-preparative scale could be performed smoothly as the semi-preparative YMC-Actus column hardware allow high performance purifications with high flow rates that are required as a result of the linear scale-up.

Step 1: Development of the Analytical Method

In a first step, the analytical method had to be developed. Excellent resolution and sharp peaks were obtained by choosing a 150 x 4.6 mm ID YMC-Triart C18 column combined with a gradient of ammonium bicarbonate and a mixture of acetonitrile and methanol in equal parts which could not be obtained using the competitor phase (data not shown).

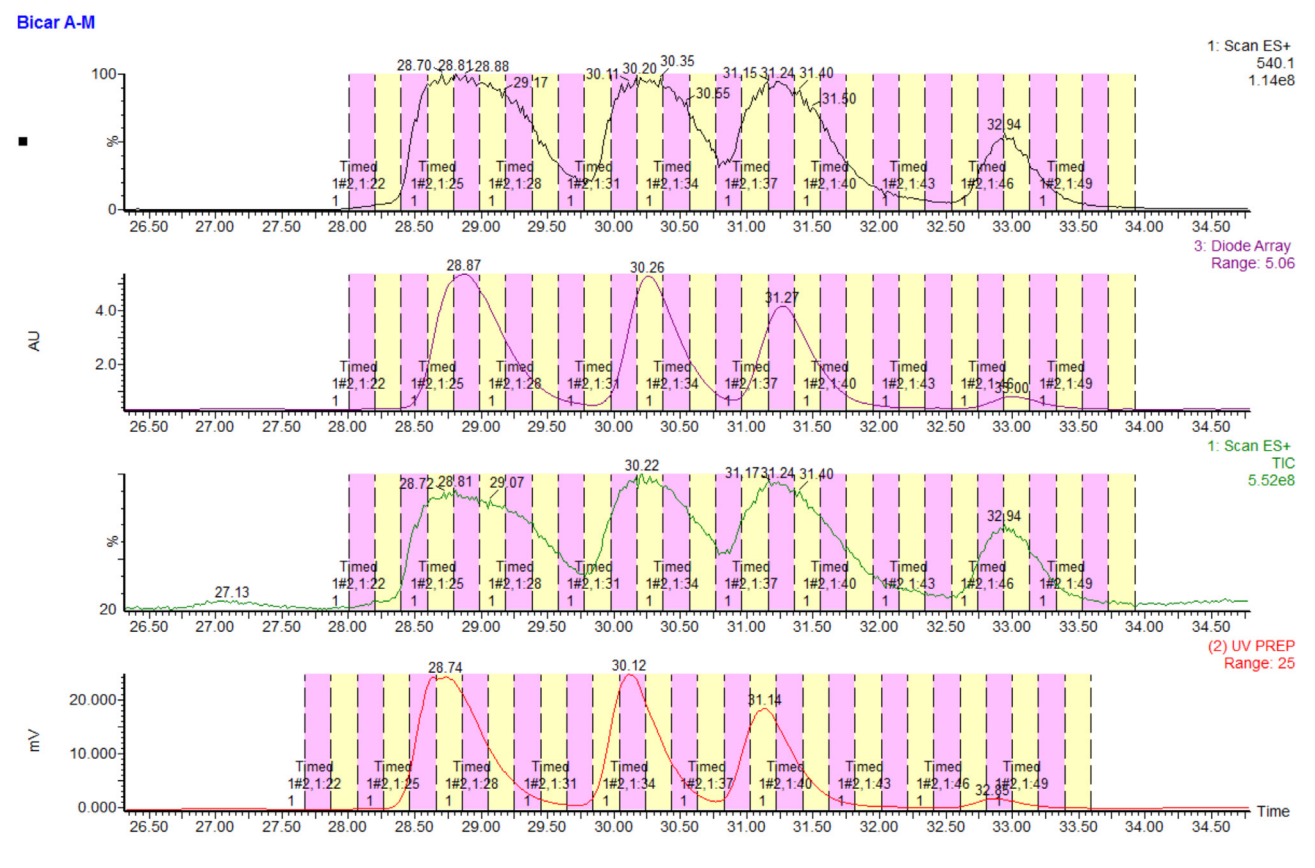


Column:	YMC-Triart C18 (5 µm, 12 nm) 150 x 4.6 mm ID
Part No.:	TA12S05-1546PTH
Eluent:	A) 0.2% NH_4HCO_3 pH 7.9
	B) acetonitrile/methanol (50/50)
Gradient:	75–95%B (0–37.5 min)
Flow rate:	1.0 mL/min
Detection:	UV at 254 nm

Figure 1: Separation of four isomers using a YMC-Triart C18 column and final analytical conditions.

Step 2: Scale-Up for Purification

In a next step, the analysis was scaled-up to a semi-preparative scale in order to purify the isomers. A 30 mm ID YMC-Actus Triart C18 column of the same length was chosen for the purification. All method parameters could be adapted according to the scale-up factor of 42.5 resulting in the linear scale-up from 4.6 to 30 mm ID. The high resolution turned out to be extremely beneficial as the isomers could easily be fractionated.

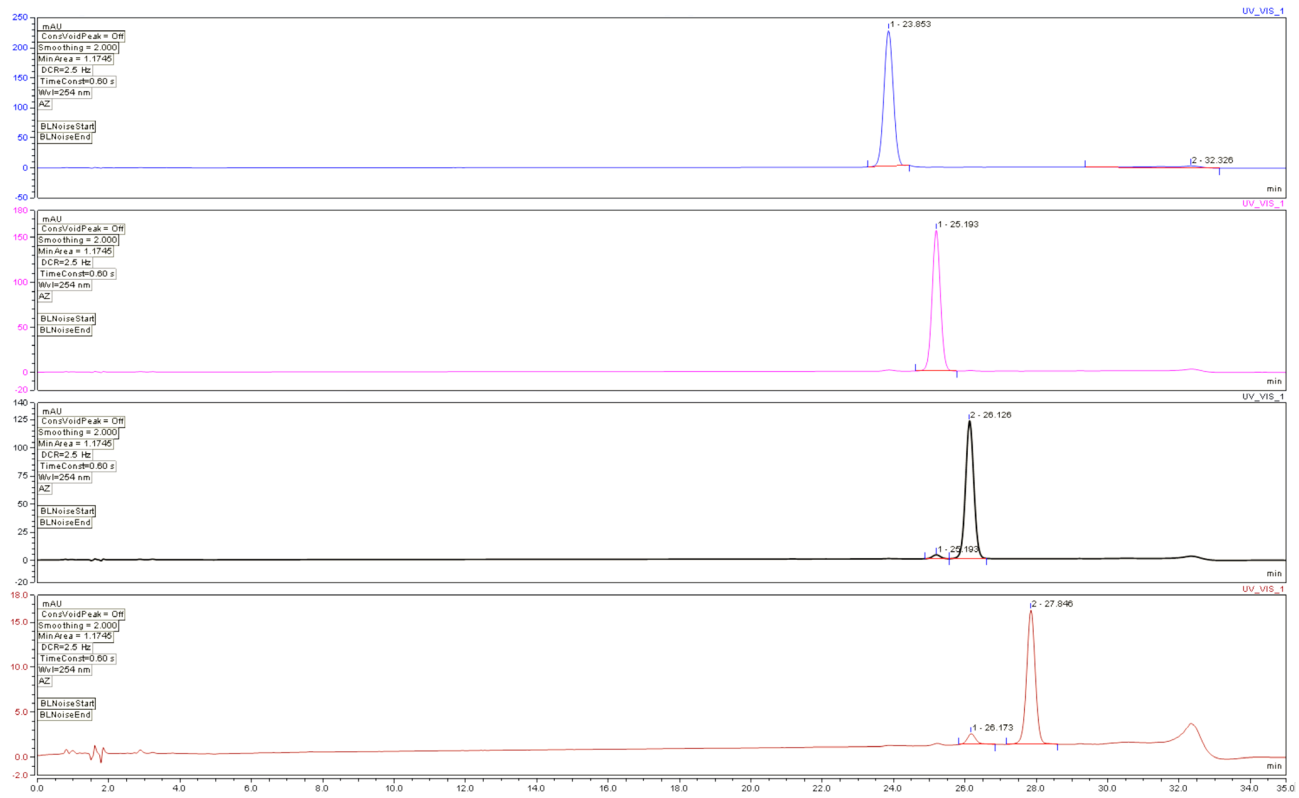


Column:	YMC-Triart C18 (5 µm, 12 nm) 150 x 30 mm ID
Part No.:	TA12S05-1530WX
Eluent:	A) 0.2% NH_4HCO_3 pH 7.9
	B) acetonitrile/methanol (50/50)
Gradient:	75–95%B (0–39.8 min)
Flow rate:	40.0 mL/min

Figure 2: Purification of four isomers using different detectors.

Step 3: Analysis of Fractions

In order to verify the purity of the fractionated samples, all fractions were injected onto the analytical column in a final analysis using the analytical chromatographic conditions. Fractions with the highest purity were obtained using the semi-preparative separation using YMC-Actus Triart C18.



Column:	YMC-Triart C18 (5 µm, 12 nm) 150 x 4.6 mm ID
Part No.:	TA12S05-1546PTH
Eluent:	A) 0.2% NH_4HCO_3 pH 7.9
	B) acetonitrile/methanol (50/50)
Gradient:	75–95%B (0–37.5 min)
Flow rate:	1.0 mL/min
Detection:	UV at 254 nm

Figure 3: Analysis of fractionated samples.

Application data by courtesy of Cyril Henry, Novalix, France.