Short Note No. 805/2023

Separation of the triterpene amyrin by SFC (Supercritical Fluid Chromatography)

Purification method of α- / β-amyrin separation using Sepiatec SFC instrument with ELSD coupling

1. Introduction

Amyrin belongs to the natural substances from the triterpenes. They have a double bond and are structured as pentacyclic triterpene alcohol. Due to its chemical structure, amyrin shows insufficient visibility in UV/VIS detection.

A method for separating α-amyrin and β-amyrin using the Sepiatec SFC instrument from BUCHI is presented here. The SFC instrument was coupled with an evaporative light scattering detector (ELSD). To increase productivity, the stacked injections mode was used.

2. Experimental

Set-up: Sepiatec SFC instrument; prep HPLC column Reprosher C30 10 um 100 x 10 mm

Mobile Phase: A = carbon dioxide; B = methanol

Mobile Phase condition: 85 % solvent A and 15 % solvent B; isocratic run

Samples: 25 mg/mL amyrin solution in methanol

Stacked injections: 11 x 100 uL with a stack time of 1.4 min

Collection: 3 peak-based fractions

Separation: The Reprosher C30 10 um 100 x 10 mm was conditioned for 5 min at a flow rate of 30 mL/min with 85 / 15 % carbon dioxide/methanol. The samples were injected automatically using the sample loop and the run was started.

Every 1.4 min the sample was injected again. The back pressure regulator was set at 150 bar and the column oven was heated to 40 °C.

3. Results and discussion

Figure 1 (a) shows the chromatogram of the α-amyrin and β-amyrin separation using stacked injections. Since both substances are not baseline separated, three fractions were collected. The middle fraction was re-injected to increase the yield.
The use of the stationary phase consisting of C30 provides a sufficiently high retardation for the separation. The isocratic separation conditions and rapid elution enable a short stack time of 1.4 min and injections in close sequence by using stacked injections. Compared to RP-LC (Reversed Phase Liquid Chromatography), the use of 85 % carbon dioxide significantly reduces solvent consumption.

4. Conclusion

α-amyrin and β-amyrin can be efficiently separated using prep SFC. The ELSD coupling enables detection and consecutive fractionation with high productivity.

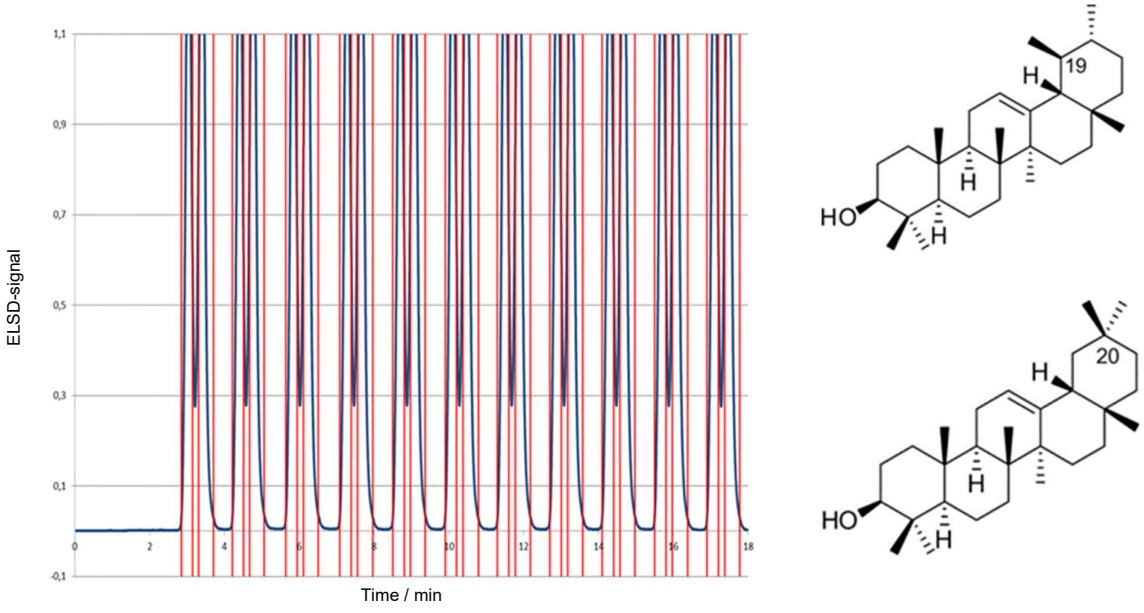

Fig. 1: left: stacked injections chromatogram of the separation of amyrin; right: structure of α-amyrin (above) and β-amyrin (below)