

Purification method of stevioside in stevia officinalis extract using Sepiatec SFC instrument.

### 1. Introduction

Stevioside (structural formula see Fig 1 (b)) belongs to the steviol glycosides, which are responsible for the sweetness of the stevia plant. The sweetening power of stevia is many times higher than the sweetness of cane sugar and is therefore a sugar alternative, which has been approved in the EU since 2011 as food additive E 960. The stevia plant itself is not yet approved as a food.

A method for separating stevioside from a stevia extract using the Sepiatec SFC instrument from BUCHI is presented here. The separation is carried out using the food-compatible solvents carbon dioxide, ethanol, and water as an additive.

### 2. Experimental

**Set-up:** Sepiatec SFC-50 instrument; prep HPLC column Nucleodur Si 5  $\mu$ m 250 x 4.0 mm

**Mobile Phase:** A = carbon dioxide (100 %); B = ethanol / water (95 / 5 %)

**Mobile Phase condition:**

0-2 min: 95 % A / 5 % B

2-25 min: 5-35 % B

25-31 min: 35 % B

**Samples:** 200 mg/mL extract of stevia officinalis in ethanol

**Separation:** Nucleodur Si 5  $\mu$ m 250 x 4.0 mm was conditioned for 5 min at a flow rate of 4 mL/min

with 95/5 % supercritical carbon dioxide and a 95/5 % mixture of ethanol/water. The samples were injected automatically using the sample loop and the run was started (run time = 31 min). The UV detection wavelength was set at 210 nm, 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 stevia extract. The extraction of the stevia plant with ethanol has dissolved many components, as can be seen in the chromatogram. Stevioside as a polar molecule interacts strongly with the polar stationary phase (Silica) of the column. Consequently, stevioside elutes when the polarity of the mobile phase has been strongly increased by the gradient. Figure 1 (b) suggests a very high purity. The stevioside fraction also has the highest sweetness and implies separation from rebaudioside A, which is another steviol glycoside present in stevia.

The purification of food substances is preferably carried out with ethanol. Solvents typical for RP-LC such as acetonitrile and methanol are not food-compatible. The addition of water as an additive improves the peak shape of polar analytes due to the increase in polarity of the mobile phase.

### 4. Conclusion

Stevioside can be efficiently separated using prep SFC from stevia extract. The use of food-compatible solvents enables the purification of food extracts by using SFC.

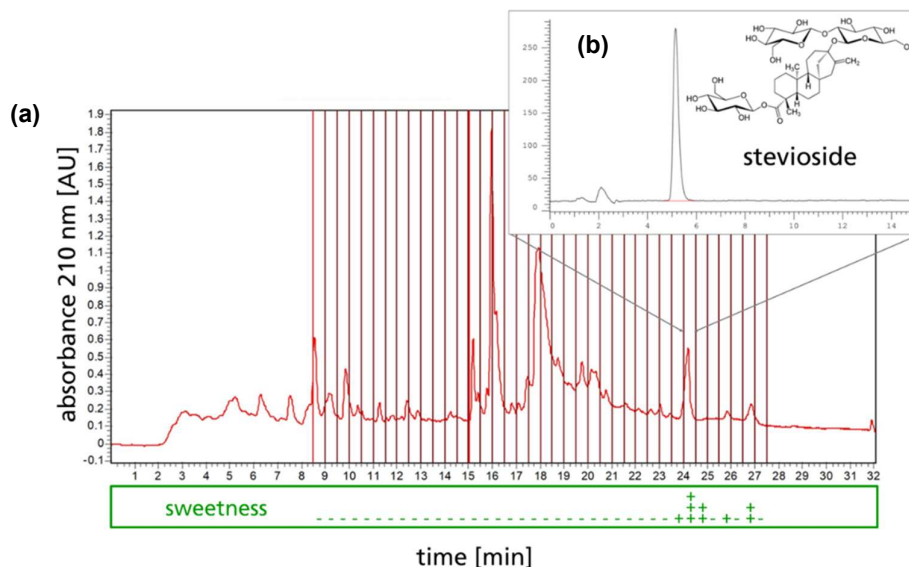


Fig. 1: (a) purification of stevia officinalis extract and (b) purity analysis of fraction 24