Step-Down Gradient Improves the Chromatographic Separation of Sucrose Monocaprate Regioisomers

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Publication date:
2011

Document Version
Early version, also known as pre-print

Link to publication from Aalborg University

Citation for published version (APA):
Introduction
Sugar fatty acid esters have a broad range of industrial applications and are used in the food, cosmetic and pharmaceutical industries. These compounds can be synthesized by conventional chemical processes or by enzymatic methods in organic solvents. Their physical and chemical properties depend on fatty acid chain length and both position and degree of esterification. The sugar monoesters are the most important types of these compounds because of higher solubility in water compared to the corresponding diesters.

In analysing sugar fatty acid monoester syntheses, it is necessary to achieve separation of the regioisomers. Ritthitham et al. (2009) previously demonstrated that when using reversed-phase high-pressure liquid chromatography (RP-HPLC), a step down in the concentration of acetonitrile in the eluent, below the initial concentration, improved the separation of sucrose fatty acid monoesters significantly. They also provided the elution order for seven regioisomers of sucrose monopcaprate. Sucrose, shown in Figure 1, has eight hydroxyl substituents, and can form up to eight monoester regioisomers.

Aim
The aim of the present study was to improve the chromatographic separation of sucrose monopcaprate regioisomers using reversed-phase high-pressure liquid chromatography.

Method
A commercial sample of sucrose monopcaprate (>95 %) was analysed on an HPLC-system (HP Series 1100) with evaporative light-scattering detector (Alltech ELSD 800). The stationary phase was a C18 column (Waters Symmetry, 5 µm, 4,6x250mm) and gradients of acetonitrile (CH3CN) in water (H2O) were used as mobile phase.

Results
The present investigation of sucrose monopcaprate regioisomers analysed using RP-HPLC, a systematic study of the separation effects was performed. Three elution factors important for separation were identified:

A) Initial concentration of CH3CN in the eluent mixture
B) Duration of the initial isocratic elution
C) Step-down concentration of CH3CN in the eluent mixture

In one experiment elution factor C was varied while all other parameters were kept constant, while the effects of variation of elution factors A and B was investigated separately.

Figures 2 and 3 show that elution factor C has a significant effect on the separation of the sucrose monopcaprate regioisomers. In figure 2, a change in elution factor C from 30 % to 33 % between elution programmes 1 and 2 resulted in retention times for individual regioisomers being reduced with up to 21 % (peak 4). In addition, the band broadening of individual regioisomers – expressed as the peak height (or height equivalent to a theoretical plate) – was reduced by a factor of up to about 3.5, or increased by a factor of up to about 7.9, with this change in elution factor C (peaks 4 and 5, respectively).

Similar results were observed for the change in elution factor C between elution programmes 3 and 4, shown in figure 3.

The results also indicate how the other elution factors affect retention times. Elution factor A has an influence on the retention times of all the sugar monoester peaks. While the effect of elution factor C appear to be dependent on an interaction between elution factors B and C, as the change in elution factor B from 3.5 to 3 minutes significantly reduced the impact of changes in elution factor C on peaks 1-3 (compare figures 2 and 3).

Conclusions
A step-down gradient can significantly improve the separation of sucrose monopcaprate regioisomers in reversed-phase high-pressure liquid chromatography analysis.

The improvement achieved in the separation of sucrose monopcaprate regioisomers in terms of retention times was in the area of 400 %.