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Modelling of RP-HPLC Retention Times for Sucrose Capric Acid Monoester Regioisomers

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Introduction

Enzymatic synthesis of sugar fatty acid monoesters produces positional isomers called regioisomers. The position of substitution is determined by the regioselectivity of the enzyme. The maximum number of monoester regioisomers is the number of hydroxyl groups in the sugar molecule. Sucrose (see Figure 1) has eight hydroxyl substituents and can form up to eight monoester regioisomers.

The traditional gradient profile for reversed-phase high-pressure liquid chromatography (RP-HPLC) is an increasing concentration of organic solvent with elution time. For the analysis of sucrose fatty acid monoesters, it has been demonstrated that alternative elution profiles for acetonitrile (CH_3CN) in water (H_2O) can improve the separation of the monoester regioisomers.
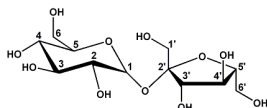

Figure 1: Haworth perspective formula of sucrose, with carbon atom numbering.

Aim

The present study of the chromatographic analysis of sucrose monocaprate regioisomers using RP-HPLC was aimed at building a multivariate model of retention times as a function of elution parameters. Analysis of the model could identify the important main effects of the elution parameters and important interaction effects between these.

Method

A commercial sample of sucrose monocaprate (Sigma, ~95 %) was analysed on an HPLC-system (HP 1100) with charged aerosol detector (ESA Corona CAD). The stationary phase was a C18 column (Waters Symmetry, 5 μm, 4.6x250mm, with guard column) and gradients of acetonitrile (CH_3CN) in water (H_2O) were used as mobile phase. The detector signal was recorded using HP Chemstation software. Chromatograms with elution profiles were generated in MATLAB.

The study was conducted using experimental design. The chosen elution profiles consisted of isocratic sections defined by five elution parameters (variables). Variable ranges were determined from previous work and isocratic elutions at various concentrations of acetonitrile. The variables are summarised in Table 1 and illustrated in Figure 1. The Unscrambler X software was used to generate a face-centred central composite (FCC) experimental design and to analyse and model the data by principal component analysis (PCA) and partial least squares regression (PLS).

Table 1: Experimental variables

| Variable	Short Name	Description	Range
A	Initial concentration	Initial concentration of acetonitrile	35-45 %
B	Duration of A	Duration of initial concentration	2-6 min.
C	Middle concentration	Mid-section concentration of acetonitrile	20-40 %
D	Duration of C	Duration of mid-section concentration	1-5 min.
E	Final concentration	Final concentration of acetonitrile	35-55 %
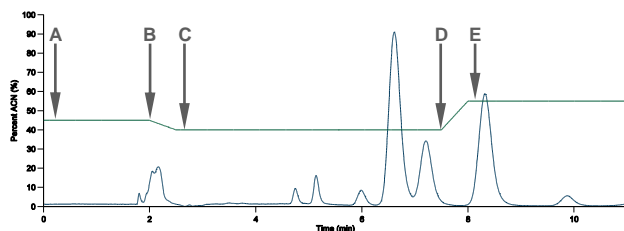
Figure 2: Chromatogram and elution profile for sucrose monocaprate analysis, with illustration of experimental variables
Variable values: A) 45 %, B) 2 min., C) 40 %, D) 5 min., E) 55 %.
Sucrose monocaprate regioisomers are eluted from 4.5 minutes onwards.

Results

As a result of the experiments performed as part of the design, the separation of sucrose monocaprate regioisomers has been improved sufficiently to achieve peaks for all eight possible regioisomers (see Figure 2). Five of the peaks show baseline separation, while the remaining group of three peaks (labelled 4) have resolutions (R_s) no higher than 1.0. About 30 % of the different experiments conducted show peaks for all eight regioisomers.
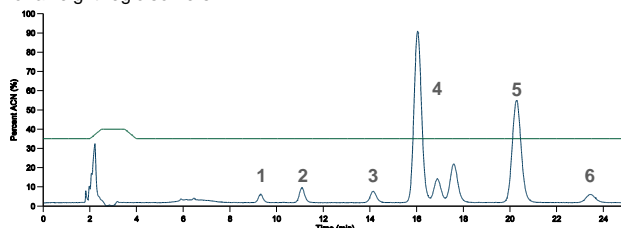

Figure 3: Chromatogram and elution profile for sucrose monocaprate analysis showing eight regioisomer peaks.
Variable values: A) 35 %, B) 2 min., C) 40 %, D) 1 min., E) 35 %.
Peak identification: 1) 3'-O-; 2) 2-O-; 3) 4-O-; 4) unresolved 6-O-, 3-O- & 1'-O-; 5) 6'-O-; 6) 4'-O-

Analysis of the response data (retention times) using PCA revealed some distinct trends connected to regioisomer peak 5 (RP5). The samples with unresolved RP5 is characterised by having high values for either or both of the variables A and E. For variable E the middle value also led to unresolved RP5. These results suggest that variable A should have its range adjusted lower, while variable E could be changed to a constant at the low value.

Table 2 summarises the results from PLS-analysis of the data in terms of importance of effects in the model of the chromatographic elution. The initial concentration of acetonitrile (variable A) clearly is the most important variable, as shown by its high significance individually, as a quadratic, and as part of interactions with the other concentration variables. Overall, the other concentration variables (C, E) also appear more important than the time variables (B, D). An interesting result is that variable D appears insignificant individually and as part of interactions, which means that for the data analysed, the effects of variations in D are indistinguishable from the experimental error (see Table 2).

Table 2: Significance of main effects, variable interactions and quadratic terms
Based on estimated p-values from DOE PLS-analysis, averaged across responses:
+++ p<0.005, ++ p<0.01, + p<=0.05, 0 p>0.05.
Experimental error (6 replicates): 2.4 % relative standard deviation.

Term	Significance	Term	Significance	Term	Significance
A	+++	A*B	0	A*B*C	+
B	0	A*C	+++	A*B*D	0
C	+	A*D	0	A*B*E	+
D	0	A*E	+++	A*C*D	0
E	+	B*C	0	A*C*E	+++
A*A	+++	B*D	0	A*D*E	0
B*B	0	B*E	0	B*C*D	0
C*C	+	C*D	0	B*C*E	0
D*D	0	C*E	+++	B*D*E	0
E*E	+	D*E	0	C*D*E	0

Conclusions

- Separation of all eight sucrose monocaprate esters is achievable.
- The initial concentration of acetonitrile is the most important elution parameter for separation of sugar fatty acid monoester regioisomers in the type of elution profiles applied here.
- Experimental design and multivariate data analysis are appropriate tools for the study and optimization of HPLC-analysis.