Regioselective synthesis of sucrose laurate and investigation of antimicrobial properties: Method development for RP-HPLC analysis

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Regioselective synthesis of sucrose laurate and investigation of antimicrobial properties: Method development for RP-HPLC analysis

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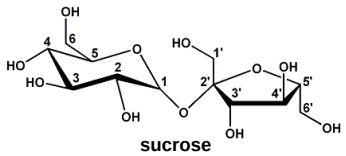
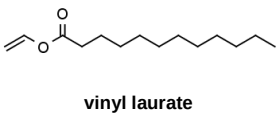
Project objectives and outline

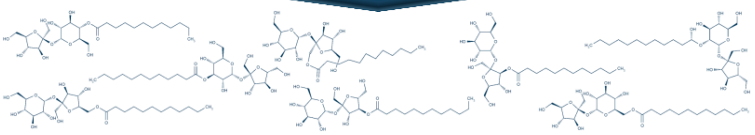


sucrose



vinyl laurate

Regioselective biocatalytic synthesis of sucrose laurate



sucrose laurate regioisomers

Product Analysis
- analytical HPLC

Product Purification
- preparative HPLC
- isolate products in gram amounts

Assay antimicrobial properties
- on isolated regioisomers

Introduction

Sugar fatty acid esters are used as additives in foods, cosmetics, medical supplies and oral-care products, and have been shown to possess antimicrobial properties. This project is focussed on **sucrose laurate** as a representative compound for this class, due to its properties:

- Antimicrobial effects include inhibition of *Bacillus* sp. and *Lactobacillus plantarum* [1]
- Eight possible monoester regioisomers
- Conventional chemical production gives product mixtures, oligoacylation and by-products.

Aim

Develop methods for regioselective biocatalytic synthesis of sucrose laurate regioisomers and investigate the antimicrobial properties of isolated regioisomers.

Outcome

Improved production efficiency can decrease costs and reduce environmental impact.

Increased knowledge of antimicrobial properties can improve control and efficiency in application of these compounds.

Sugar fatty acid esters represent a class of antibiotics that have seen little research and application in clinical context: advancements can lead to new antibiotic drugs.

Analysis Method Development Using Design of Experiments

Reversed-phase HPLC

The RP-HPLC analysis of sucrose caprate was investigated using a design-of-experiments approach. Based on the improved regioisomer separation shown by Ritthitham *et al* [2], from a step down in acetonitrile concentration, below the initial concentration, the elution gradients of acetonitrile (CH_3CN) in water were determined by four variables, as defined in table 1. The variables described variations in elution parameters followed by a constant eluent composition, as illustrated in fig. 1.

Table 1: Experimental variables

Variable	Description	Range
A **(%)**	Initial acetonitrile concentration	30-40
B **(min)**	Duration of initial concentration	2-6
C **(%)**	Mid-section acetonitrile concentration	20-40
D **(min)**	Duration of mid-section concentration	1-5

Separation of sucrose caprate regioisomers

The HPLC analysis resulted in the resolution of the eight possible regioisomers of sucrose caprate, with baseline separation achieved for six regioisomers (fig.1). R_s-values ranged from 1.31 to 6.82. In most practical applications $R_S \geq 1.0$ is considered the level of adequacy for analytical quantification, and all R_s-values were above this level. Regioisomer identification was based on previously obtained results [2, 3].

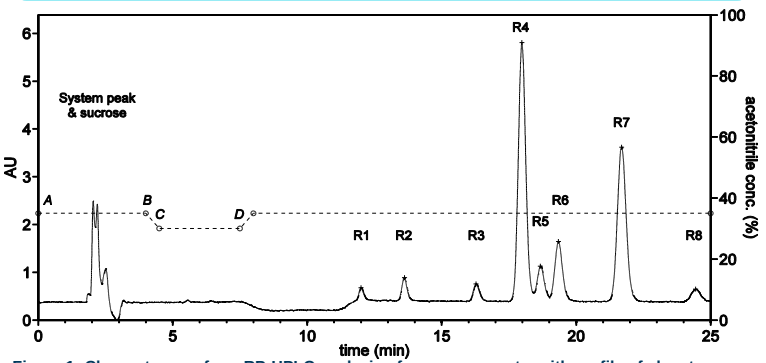


Figure 1: Chromatogram from RP-HPLC analysis of sucrose caprate, with profile of eluent acetonitrile concentration (dotted line).
Elution variables *A* = 35 %, *B* = 4 min, *C* = 30 %, *D* = 3 min. Sucrose caprate regioisomer identification and R_S-values: R1 3'-*O*-, N/A; R2 2-*O*-, 4.85; R3 4-*O*-, 6.82; R4 6-*O*-, 3.59; R5 1'-*O*-, 1.36; R6 3-*O*-, 1.31; R7 6'-*O*-, 4.11; R8 4'-*O*-, 4.30.

Design-of-experiments analysis

The chosen design was a face-centred composite (FCC) optimization design, performed in two replicates and with seven samplings of the centre point. The resulting dataset was analysed using multivariate partial least-squares (PLS) regression, incorporating variable interactions between up to four variables and exponential terms up to exponent 4.The significance of variable effects was evaluated based on the weighted regression coefficients and the results of cross-validation coefficient-variance significance testing.

The significance of the four design variables was shown to be unequal (see table 2). The concentration variables (*A* and *C*) had the most significant effects, as isolated variables, as exponential terms, and as part of the interaction between them (AC). In addition, the interaction between the concentration variables and the duration of the initial concentration (ABC) was highly significant. The time variables were only significant as part of interactions with one or both of the concentration variables.

Table 2: Significant effects in PLS analysis of face-centred composite design, using cross-validation uncertainty testing.
Based on estimated p-values: +++ ($p < 0.005$), ++ ($p < 0.01$), + ($p <= 0.05$), 0 ($p > 0.05$). Effects not included in the table were insignificant ($p > 0.05$). The significance levels for the exponential terms were equal for all exponents in the range $2 \leq x \leq 4$.

	Responses							
Effect	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**
A	+++	+++	+++	+++	+++	+++	+++	+++
C	+++	+++	+++	+++	+++	+++	+++	+++
AC	+++	+++	+++	+++	+++	+++	+++	+++
BC	+	+	+	+	+	+	+	+
ABC	+++	+++	+++	+++	+++	+++	+++	+++
ACD	+	+	+	+	+	+	+	+
ABCD	+	+	+	+	+	+	0	0
A^x	+++	+++	+++	+++	+++	+++	+++	+++
C^x	+++	+++	+++	+++	+++	+++	+++	+++

Conclusion

A method for quantitative RP-HPLC analysis of regioisomers of sucrose caprate was developed. Resolutions above the level of adequacy for quantification, $R_S \geq 1.0$, were achieved for all regioisomers.

Design of experiments and multivariate analysis were shown to be applicable and useful tools for method development in RP-HPLC analysis of sucrose fatty acid esters.

References

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