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Transfer of retention patterns in gas chromatography by means of response surface methodology

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ABSTRACT

Accurate transfer of retention patterns in temperature-programmed gas chromatography is challenging because minor variations in column properties and experimental conditions may have significant impact on the elution patterns. An experimental method for accurate transfer of retention indices is proposed and validated. The methodology is based on response surface methodology and experimental design. The temperature rate and the start temperature of the rate are varied systematically in the region where the optimal conditions are expected to be found. Response surfaces that explain the absolute deviation to the target retention indices are calculated for each compound. These response surfaces are thereafter averaged and the minimum in the average surface is regarded as optimal conditions for reproduction of the retention pattern. The methodology was applied on fatty acid methyl esters using equivalent chain lengths as the retention index system. Two different target patterns were tested on two BPX-70 columns with different dimensions. Validation of the proposed conditions showed that the retention patterns could be reproduced with an error that was only fractions of a peak width.

1. Introduction

Fatty acid methyl esters (FAME) are usually analysed by gas chromatography on medium to highly polar stationary phases. Compared with other common stationary phases many polar phases have properties that are highly temperature dependent [1]. This temperature dependence of the stationary phase properties has advantages and disadvantages. An advantage is that retention patterns can easily be modified by adjusting temperatures and flows, and overlapping peaks can often be resolved by minor adjustments of the chromatographic conditions. However, when optimal conditions have been found, it can be difficult to transfer the retention pattern to other systems. The columns may also change their properties with time, leading to problems with stability.

The retention patterns, *i.e.* the chromatographic selectivity, can be described by retention indices in gas chromatography. Retention indices were first introduced by Kováts [2], using n-alkanes as calibration compounds. Equivalent chain lengths (ECL), where the saturated straight chain fatty acids are used as calibration standards [3,4] is the dominating retention index system in analyses of FAMEs. While the number of carbons in the n-alkane is multiplied by 100 to get the Kováts indices, the number of carbons in the fatty acid part of the saturated FAMEs is used directly on the ECL scale (excluding the carbon from methanol in the methyl ester).

It has been shown previously that response surface methodology can be applied for accurate predictions of ECL values as functions of the applied chromatographic conditions [5]. The purpose of this work has been to develop a suitable strategy based on this principle for accurate transfer of retention patterns between different systems.

Response surface methodology is based on the use of experimental designs. Different experimental designs vary in the number of experiments required, and in the complexity of the mathematical relationships that can be described. In a previous work on a similar system as described here, the carrier gas velocities, the temperature rates, and the start temperatures of the rates were varied [5]. Resolving a common response surface model with three parameters, interactions and quadratic terms will require at least 12 experiments, which may be too many for many practical purposes. It was therefore decided to keep the carrier gas velocities constant near the values that will maximize separation efficiency, and varying the two other parameters. With two parameters, the Doehlert design [6] is the most efficient of common experimental designs in terms of number of experiments that must be conducted to resolve a response surface with interactions and quadratic terms [7]. If a centre point is included the Doehlert design requires seven

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experiments. The applied methodology is based on this design and described in detail in the following theory section.

2. Theory

2.1. Response surface methodology

The purpose of response surface modelling is to establish a multivariate model of several varied parameters (predictor variables) that adequately describe a response in the studied system. It is common to model the response (y) as a function of the predictors (x_i), their interactions ($x_i x_j$) and their quadratic terms (x_i^2). For a system with two varied parameters, the aim is therefore to find the regression coefficients (b) that gives the best possible estimate of y. With two predictor variables the response surface can be estimated by solving the following equation:

$$\hat{y} = b_0 + b_1 x_1 + b_2 x_2 + b_{12} x_1 x_2 + b_{11} x_1^2 + b_{22} x_2^2 \quad (1)$$

Since there are six b coefficients in the equation, at least six experiments must be conducted to solve the equation by regression. To avoid correlation between the predictors the experiments must be conducted according to a proper experimental design. Common experimental designs for response surface methodology based on independent predictors includes the three-level factorial design, the central composite design and the Doehlert design (also called uniform shell design) [8,9]. The Box-Behnken design is also a suitable alternative if there are more than two predictors [8,9].

A two-dimensional Doehlert design is hexagonal, where one of the parameters is varied over three levels and the other over five levels. It is common practice to add a centre point to the design, leading to a total of seven experiments. Further details on properties and chromatographic applications of the designs can be found in recent reviews on the Doehlert design [7,10] and experimental designs in general [8,9].

2.2. Outline of the method

A brief explanation of the principle of the methodology is given below. The purpose is to reproduce a *target* pattern defined by the retention indices of several calibration compounds.

Suitable experimental conditions must first be selected according to the design pattern. It is important that the selected conditions span the region where the optimal conditions for reproducing the target pattern are expected to be. However, spanning a too large experimental region may decrease accuracy around the optimum, and one should also consider whether the experimental conditions lead to reasonable retention times and chromatographic efficiency. Setting up the initial design therefore requires some *a priori* knowledge about the system. In the example shown in Fig. 1, the temperature rate and the start temperature of the rate are varied. It is possible to replace the temperature rate with carrier gas velocity, and in principle the methodology is not limited to two parameters, so all three can be varied. But more parameters require more experiments to calculate the models.

One or more reference samples containing calibration compounds are analyzed at the selected experimental conditions and the retention indices for the compounds are calculated. For each compound, a model explaining the retention index as a result of the varied parameters is thereafter calculated by solving Eq. (1). This gives *individual response surfaces* for each calibration compound similar to the one in Fig. 1a. The target values for the calibration compounds are thereafter subtracted from the corresponding response surfaces, and all values in the resulting response surfaces are converted to absolute values. This gives *individual target surfaces* for each compound similar to Fig. 1b. In the example shown the target value is ECL 22.60. The target surface shows how far

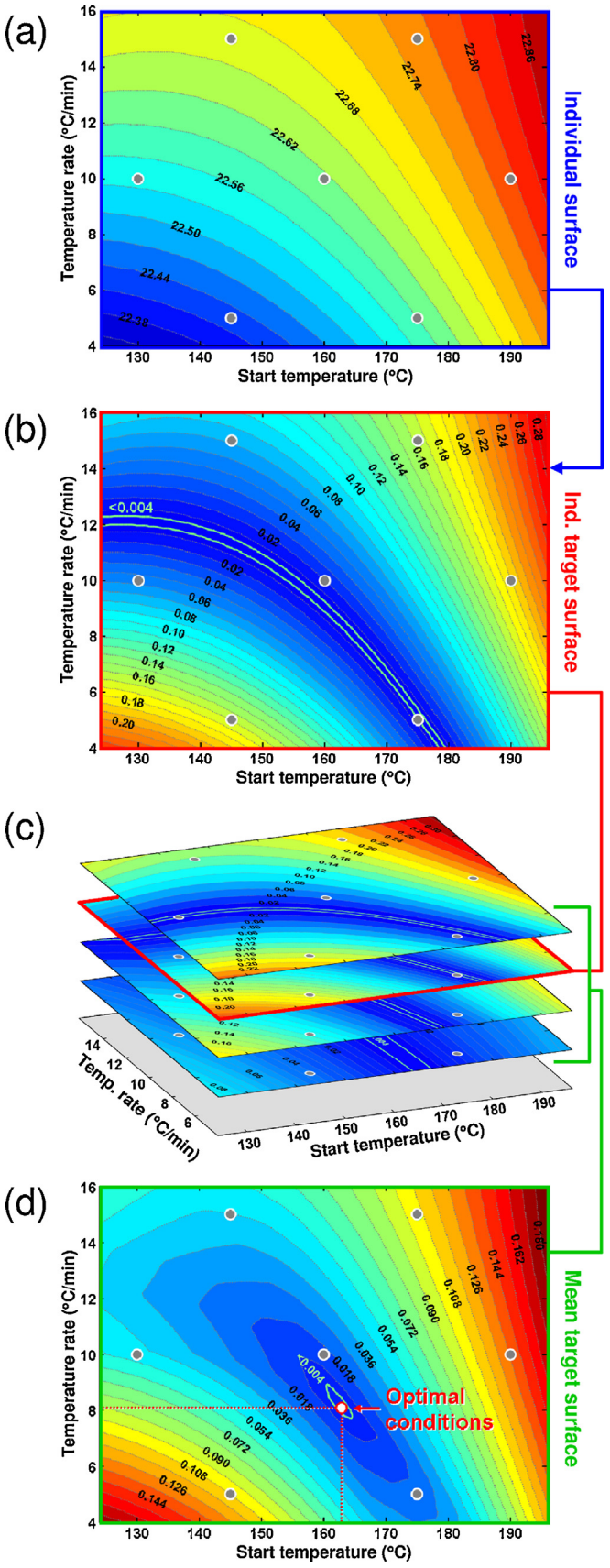

Fig. 1. Illustration of the methodology used in this work from the individual surface of a FAME (a), to the individual target surface (b), the overlay of all individual target surfaces from all FAME in a sample (c) resulting in a mean target surface (d).