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Two-dimensional fatty acid retention indices

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Abstract

A two-dimensional retention index system for fatty acid methyl esters (2D-FARI) is proposed. The system is based on the application of different temperature and pressure programs on a single capillary column. A calibration sample is analysed and the retention data is calibrated against a set of reference 2D-FARI values. The calibration models are then applied to predict the 2D-FARI values for compounds that are not present in the calibration sample. The two dimensions in the retention index system lead to increased selectivity and a reduced risk of retention index overlap between different compounds. The 2D-FARI system is also more robust towards differences in stationary phase properties than ordinary retention indices and is therefore convenient for comparison of retention data acquired on different columns, or at different times at the same column.
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1. Introduction

In gas chromatography, various types of retention indices are widely applied to characterize the analysed compounds. The general principle is that retention indices are defined for a set of analysed reference compounds (usually a series of homologs) and the mathematical relationship between retention times and retention index is established. The retention indices for compounds not belonging to the reference series are then calculated from this relationship. While Kovats' indices [1] are dominating as general purpose system, other systems have been developed for specific compound classes and purposes. A review of alternatives to Kovats' indices can be found elsewhere [2].

For the analysis of fatty acids as their methyl esters (FAME), equivalent chain lengths (ECL) [3,4] is usually the preferred system. The saturated unbranched FAMEs are used as calibration series and the ECL value is by definition set equal to the number of carbons in the fatty acid carbon chain.

The ECL concept was originally developed for isothermal chromatography, where a linear relationship between carbon numbers and $\log(t'_R)$ was observed. The relationship between ECL value and $\log(t'_R)$ was found by linear interpolation between two consecutive members of the saturated reference series [5,6], or by linear regression over the complete series. Later, it has been found that there are deviations from linearity, and higher order regressions have been applied to give increased precision [7,8]. Polynomial regressions have also been applied to establish direct relationships between retention time and ECL values in temperature-programmed gas chromatography [9–11].

ECL values are considered to be characteristic for a certain compound analysed on a certain stationary phase. However, analytical conditions, such as temperature [12] and the conditions of the stationary phase will also have some influence. Temperatures have a significant effect on the ECL values, especially on the polar cyanopropyl columns [5,6,13]. Also the carrier gas flow will influence the ECL values in temperature-programmed chromatography. The dependence of ECL values on analytical conditions limits the feasibility of these indices for identification of unknown compounds,

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and for comparisons from lab to lab, or on the same column over time.

There are also other drawbacks of the ECL system. Even though the ECL value for a specific compound is characteristic, it is not unique, because several compounds may have the same ECL value. Especially on polar columns, where there is large degree of overlap in retention times between fatty acids of different chain lengths, the ECL value gives no information about the structure of the compound. More unique retention data, and information about the structure, can be achieved from two- (or higher) dimensional data by comparing retention indices from several columns with different polarity [14,15]. It has also been shown that similar information can be achieved by comparing ECL values obtained by varying the temperature conditions on the same column [11,12].

The purpose of this work has been to develop a retention index system that copes with the drawbacks of the present ECL system. The proposed concept provides two-dimensional retention data from the use of only one column. It is robust towards changes in column properties and different analytical conditions, and it can be applied with temperature-programmed chromatography. The principles are outlined in the following section.

2. Outline of the method

In a recent paper it was shown that multivariate analysis can be used to project ECL data from different temperature and pressure programs onto two-dimensional maps where the fatty acids are distributed according to the chain length and number of double bonds [11]. A problem with these maps, and with the use of retention indices on polar stationary phases in general, is that the values tend to drift with small variations in chromatographic properties, such as column ageing. In this work, the drift problem is solved by the use of a calibration sample with common saturated and unsaturated fatty acids, and a set of two-dimensional target values for each of the compounds in the calibration sample.

Each time the calibration sample is analysed, the ECL-data is aligned to the target values by multivariate regression. Because the compounds in the calibration sample are aligned to the same spots on the two-dimensional map, the regression model will project any compound that is not present in the calibration sample to a certain point in the retention index map that is only dependent on fatty acid structure, and not on the chromatographic conditions. The coordinates of the position in the map is the two-dimensional fatty acid retention indices (2D-FARI) for the given compound. It is important that the calibration sample span a large variation in fatty acid structure. The calibration sample used contains fatty acids with 8–28 carbons and 0–6 double bonds. It is also important that the different temperature and pressure programs applied will induce significant differences in the ECL values of the polar fatty acids. Five programs suitable for this pur-

pose have been found previously [11].The target values can be any values that span the variation in the structure of the analysed compounds and that can be accurately explained by the dataset consisting of ECL values acquired on the different temperature and pressure programs. These two requirements are met by applying the ECL values as independent variables (X-variables) in two multivariate regressions with the fatty acid chain length and number of double bonds as dependent variables (Y-variables). The predicted chain length and predicted number of double bonds are then used as target values that define the two fatty acid retention indices, referred to as $FARI_A$ and $FARI_B$, respectively. It is important to note that the target values used further is not the true chain length and the true number of double bonds, but the *predicted* chain length and the *predicted* number of double bonds from these regression models. This ensures that the target values can be accurately explained from the dataset of ECL values. The calculations of the target values are only performed once and the same set of values are used as targets in future application of the method.

3. Materials and methods

3.1. Instrumentation

All analyses were performed on a HP-5890 GC equipped with split/splitless injector, electronic pressure control (EPC) [16], HP-7673A autosampler, and HP-5972 MS detector. The system was equipped with G1034C MS Chemstation software. BPX-70, $L = 70$ m, i.d. = 0.25 mm, $d_f = 0.25$ μm (SGE, Ringwood Australia) and SP-2560, $L = 100$ m, i.d. = 0.25 mm, $d_f = 0.20$ μm (Supelco, Bellefonte, PA, USA) were used as analytical columns. Helium, 99.996% was used as carrier gas. Two BPX-70 columns with different degree of ageing were applied. Column 1 was a new column, while Column 2 had been used for approximately 18 months and had lower polarity as a result of ageing.

3.2. GC parameters

Programs with linear temperature gradients were applied. To induce changes in ECL values, three levels of starting temperature, temperature gradients and column flow were used. The samples were injected at an oven temperature of 60 °C that was hold for 4 min. The temperature was increased by 30 °C/min to start temperature A, followed by a gradient of B °C/min until the final compound was eluted. The injector pressure was increased with oven temperature to give a constant velocity of C (cm/s). The levels of the parameters A, B and C are given in Table 1. The samples (0.5 μL) were injected in splitless mode. The split valve was opened after 4 min. Injector temperature was 250 °C and MS transfer line temperature was 270 °C.

The mass detector was used in selected ion monitoring mode, and the ions m/z 55, 74, 79, 80, 91, and 93 were

Table 1
Levels of start temperature (A), temperature gradient (B), and column flow (C) applied in the GC programs

Program	Column	A: start temperature (°C)	B: temperature, gradient (°C/min)	C: flow[a] (pressure[b]) (cm/s, kPa)	ECL 22:6 $n-3$
1	BPX-70	160	2.0	26 (125)	25.06[c]; 24.98[d]
2	BPX-70	160	4.0	18 (55)	25.42[c]; 25.32[d]
3	BPX-70	175	3.0	22 (90)	25.29[c]; 25.21[d]
4	BPX-70	190	2.0	26 (125)	25.22[c]; 25.15[d]
5	BPX-70	190	4.0	18 (55)	25.52[c]; 25.43[d]
6	BPX-70	145	1.5	26 (125)	24.94[c]
7	BPX-70	145	4.0	18 (55)	25.39[c]
8	SP-2560	145	2.5[e]	18 (159)	26.84
9	SP-2560	145	1.5	24 (246)	26.46
10	SP-2560	145	1.0	24 (246)	26.28

More details about the chromatographic conditions are given in Section 3.

[a] Estimated by Chemstation software.

[b] Pressure at 60 °C, increased with temperature to keep constant carrier gas velocity.

[c] Column 1, new.

[d] Column 2, old.

[e] 5 min isothermal after 255 °C.

recorded at a frequency of 3.5 scans per second. The combination of these ions has proved to be suitable for fatty acid identification [17].

3.3. Samples

The calibration sample was GLC-461 FAME reference mixture (Nu-Chek Prep, Elysian, MN, USA) spiked with additional reference compounds: 19:0, 21:0, 25:0, 26:0, 27:0, 28:0 and 22:3 $n-3$. Other samples were silver ion HPLC fractions of FAME from various marine sources [17], and additional FAME reference compounds: 18:1 $n-12$, 18:1 $n-7$, 19:1 $n-9$, 19:2 $n-6$, 20:1 $n-15$, trans 16:1 $n-7$ and all-trans 18:2 $n-6$. The analysed samples were spiked with a mixture of saturated FAMEs from 12:0 to 28:0 (not

including 23:0). All reference compounds were purchased from Nu-Chek Prep.

3.4. ECL-regressions

The peak apex was used to determine the retention time and the unbranched saturated fatty acids from 8:0 to 28:0 (not including 23:0) were used as references. The relation between retention time and retention index (RI) was determined by a stepwise procedure using local second order regressions. The independent variable (X-variable), t_x is the scan numbers (or retention time) of the reference compounds, and the dependent variable (Y-variable) is the retention indices, RI, defined for the corresponding compounds. For any interval between two reference compounds (n and $n+1$) the relationship be-
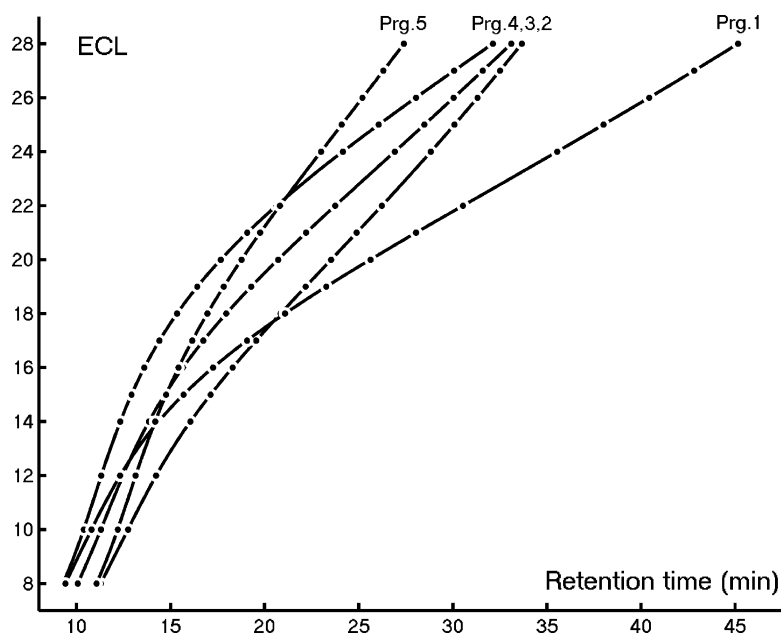

Fig. 1. Equivalent chain length (ECL) vs. retention time for GC Programs 1–5 (Table 1).

tween retention index and retention time is calculated as follows. A polynomial regression, $f_1(t_x)$, is fitted to the three reference compounds $n-1$, n, and $n+1$. A second polynomial regression $f_2(t_x)$, is fitted to the three reference compounds n, $n+1$, and $n+2$. The range between n and $n+1$ is covered by both polynomials, and for any retention time in this interval, the corresponding retention index is determined by weighting the two functions as follows:

$$\text{RI} = (1-w) \times f_1(t_x) + w \times f_2(t_x), \quad w = \frac{t_x - t_n}{t_{n+1} - t_n} \quad (1)$$

The routine is then repeated for the next interval by increasing n by one. This procedure can only calculate ECL values for retention between the second and the second last reference compound. ECL values outside this area were calculated by application of the first or last polynomial of the series. These regressions will give a smooth curve passing through all the regression points of the standard series. The regressions for the Programs 1–5 are shown in Fig. 1.

3.5. Curve resolution

With GC–MS of FAMEs self-modelling curve resolution can be applied to achieve more precise retention times for any overlapping peaks, even of samples with very similar spectra [18–21]. Peak overlaps were resolved by the modified Borgen method [22] combined with alternating regression [23].

3.6. Software

Curve resolution, determination of retention times and calculation of ECL values were performed in an in-house written program running under Matlab 6.5 (Mathworks, Natick, MA, USA). Principal component analysis (PCA), principal component regression (PCR) and partial least squares (PLS) regression were performed in Unscrambler 7.5 (CAMO, Oslo, Norway).

4. Results

4.1. Calculation of target values

Programs 1–5 (Table 1) has been shown previously to give variation in ECL data suitable for identification purposes [11]. These programs (standard programs) were applied on a previously unused BPX-70 column (Column 1) to calculate the target 2D-FARI values for the calibration sample.

The ECL values of the 37 fatty acids in the calibration sample analysed by the five programs were organized in a matrix with the fatty acid as objects and the GC programs as variables. PCA on the dataset gave the scores plotted in Fig. 2a. This procedure is identical to the method used previously for identification of fatty acid methyl esters [11]. The FAMEs are distributed according to the chain length and the number of double bonds. However, the values on the axes

Table 2
Target 2D-FARI values for the FAMEs in the calibration sample

Number	FAME	FARI$_A$	FARI$_B$
1	8:0	7.972	0.054
2	10:0	9.981	0.038
3	12:0	11.990	0.023
4	14:0	14.000	0.007
5	14:1 $n-5$	14.227	0.729
6	15:0	15.004	−0.001
7	16:0	16.009	−0.009
8	16:1 $n-7$	15.987	0.943
9	17:0	17.013	−0.017
10	17:1 $n-7$	16.935	1.044
11	18:0	18.018	−0.025
12	18:1 $n-9$	17.804	1.135
13	18:2 $n-6$	18.005	2.017
14	18:3 $n-6$	18.037	2.794
15	18:3 $n-3$	18.359	2.864
16	19:0	19.023	−0.033
17	20:0	20.027	−0.041
18	20:1 $n-9$	19.751	1.229
19	20:2 $n-6$	19.942	2.154
20	20:3 $n-6$	19.870	3.123
21	20:3 $n-3$	20.336	2.938
22	20:4 $n-6$	19.764	3.889
23	20:5 $n-3$	20.119	4.794
24	21:0	21.032	−0.049
25	22:0	22.036	−0.057
26	22:1 $n-9$	21.746	1.242
27	22:2 $n-6$	21.936	2.188
28	22:3 $n-3$	22.272	3.102
29	22:4 $n-6$	21.703	4.222
30	22:5 $n-3$	22.062	5.151
31	22:6 $n-3$	22.016	5.730
32	24:0	24.046	−0.073
33	24:1 $n-9$	23.750	1.266
34	25:0	25.050	−0.081
35	26:0	26.055	−0.089
36	27:0	27.059	−0.097
37	28:0	28.064	−0.105

have no chemical meaning. The axes can be assigned more meaningful values by regression on the score values with the chain length and number of double bonds as targets. Two-component principal component regression models gave the predicted values plotted in Fig. 2b.

The predicted values from the PCR models were used as targets for the other regression models. The values are given in Table 2 and are by definition the 2D-FARI values for the components in the calibration sample.

4.2. Column differences

The reference mixture and various samples were analysed by the standard programs (Programs 1–5) on two different BPX-70 columns. Column 1 was identical to the column used for calculation of the reference values, while Column 2 had been used for approximately 18 months and had lower polarity as a consequence of column ageing. A comparison of the two columns is shown in Fig. 3, and the ECL of 22:6 $n-3$ for all programs is also shown in Table 1. It can be seen that the
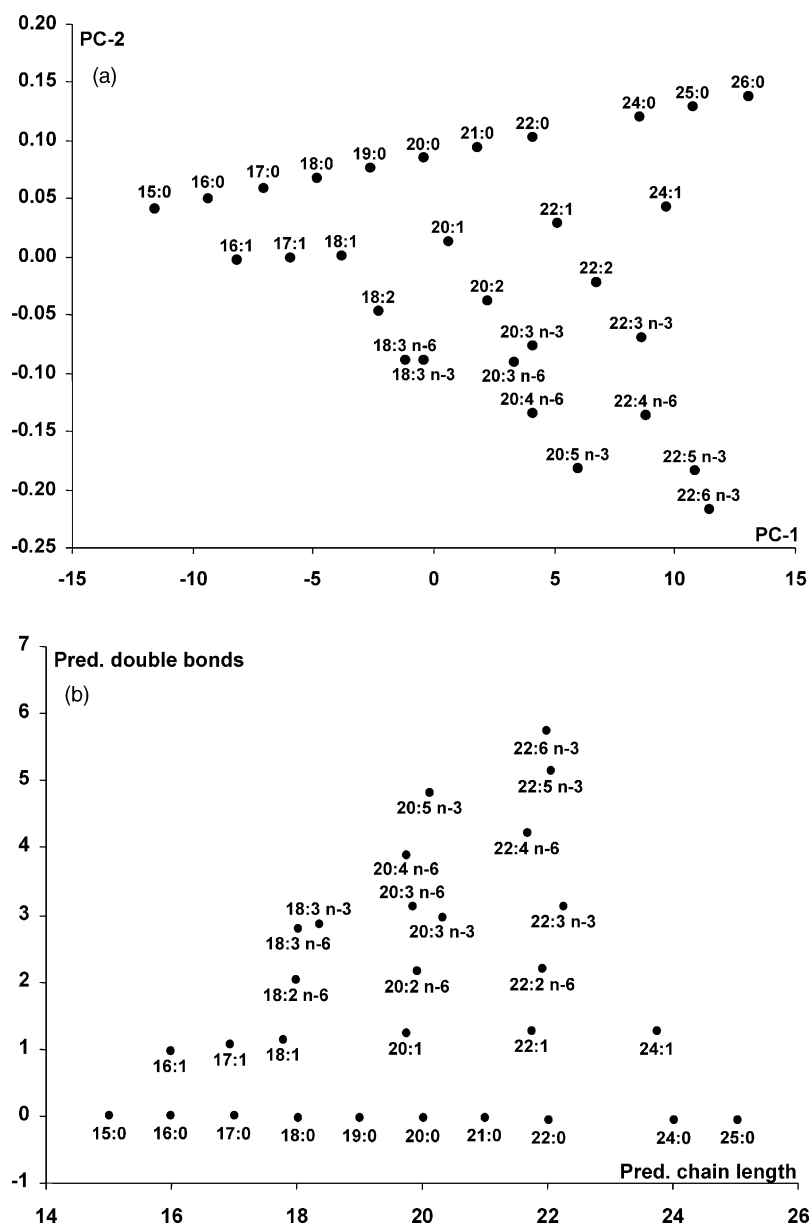
Fig. 2. (a) Score plot after PCA of the ECL values of the reference sample analysed by Programs 1–5 (Table 1). Explained variance PC1, PC2: 99.991, 0.009%. (b) Predicted chain length and predicted number of double bonds after PCR with the ECL values of the reference sample analysed by Programs 1–5 as independent variables (X-variables).

difference between the two columns is significant. The difference in ECL for 22:6 $n-3$ is 0.08 for Program 1 and 0.09 for Program 5. The plot also illustrates the large difference in ECL values that can be found for different temperature programs. The difference between the two programs was 0.46 ECL units for 22:6 $n-3$.

4.3. Evaluation of different combinations of programs and columns

The ability to predict the 2D-FARI values was evaluated for different column and program combinations. The dataset used for Column 1 is not identical to the dataset used to cal-

culate the target 2D-FARI values in Table 2, but was acquired under similar conditions (a few weeks later). Cross-validated principal component regression was used for prediction of the 2D-FARI values. Standard error of prediction (SEP) was used to measure the error. SEP is the standard deviation of the prediction error [17].

With cross-validated regression, each sample (fatty acid) is left out of the calibration set, and the model is calibrated on the remaining samples. Then the Y-value (target) for the excluded sample is predicted from the model. The process is repeated for every sample in the calibration set. Thus, every sample is predicted from a data set where it is not present. The error estimate from a cross-validated regression is therefore
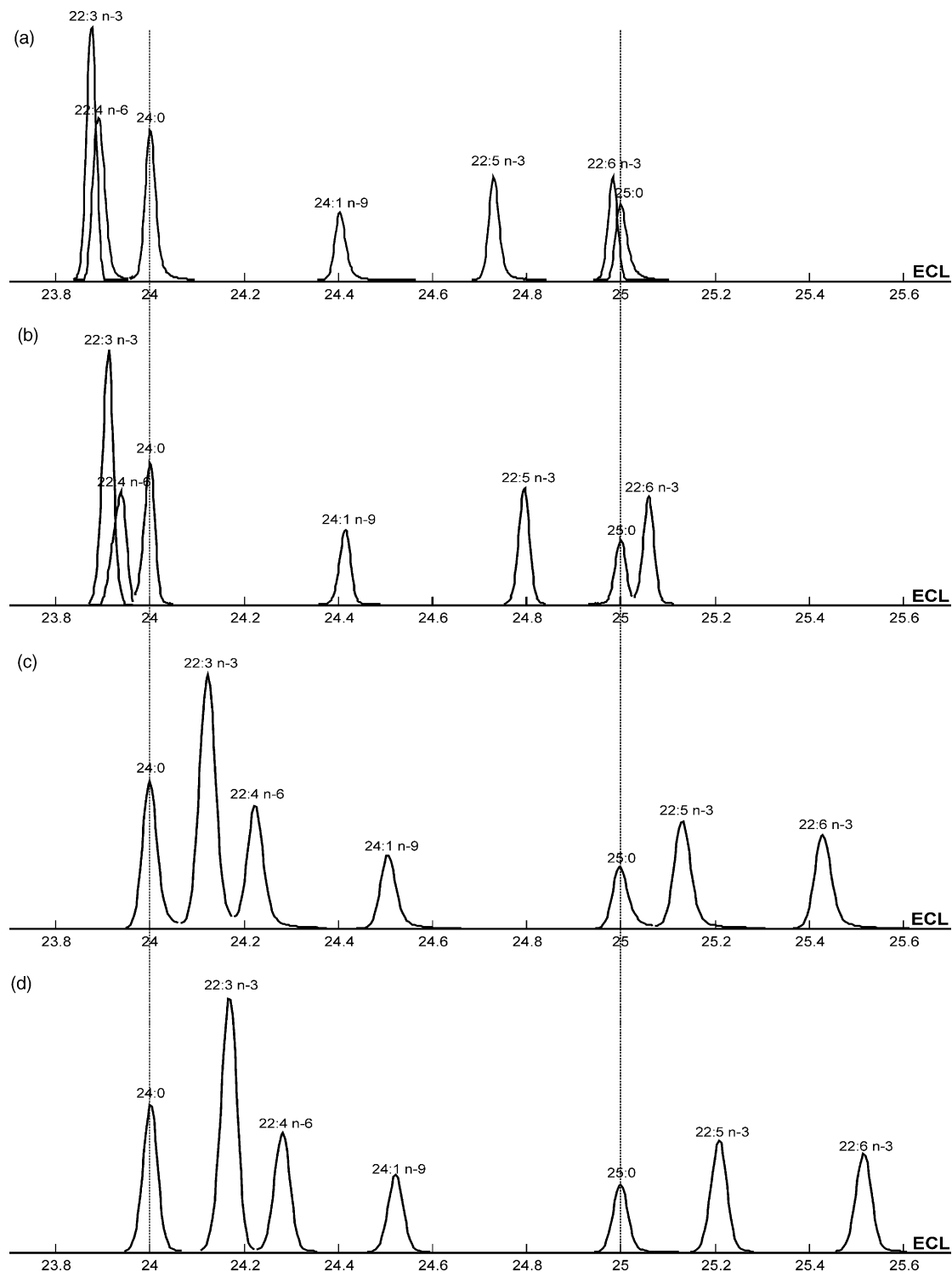
Fig. 3. Chromatograms (ECL scale) of fatty acids eluting in the ECL-region 23.8–25.6: (a) Program 1, old column; (b) Program 1, new column; (c) Program 5, old column; (d) Program 5, new column. Overlapping peaks, 22:3 $n-3$/22:4 $n-6$ and 22:6 $n-3$/25:0 were resolved by curve resolution as described in Section 3.

expected to give a good estimate of the error of any sample that is not present in the calibration set. SEP of the various models is given in Table 3. There was only a small difference in SEP for models based on ECL data for Programs 1–5 acquired on the two BPX-70 columns. SEP was smallest

on Column 1, which was expected because the target values (Table 2) were calculated from ECL data acquired on the same column. SEP for FARI$_A$ was 0.014 and 0.019, and SEP for FARI$_B$ was 0.025 and 0.029 for Column 1 and 2, respectively; 22:6 $n-3$ is the most polar fatty acid in the dataset

Table 3
Standard error of prediction (SEP) for the prediction of 2D-FARI values (Table 2) for the compounds in the calibration sample

Column	Program combinations[a]										SEP		II
	1[b]	2[b]	3[b]	4[b]	5[b]	6	7	8	9	10	FARI$_A$	FARI$_B$	PC[c]
BPX-70, 1	x	x	x	x	x						0.014	0.025	2
BPX-70, 2	x	x	x	x	x						0.019	0.029	2
BPX-70, 1		x	x	x	x						0.029	0.049	3
BPX-70, 2		x	x	x	x						0.043	0.071	3
BPX-70, 1	x		x	x	x						0.015	0.027	3
BPX-70, 2	x		x	x	x						0.020	0.034	3
BPX-70, 1	x	x		x	x						0.014	0.025	2
BPX-70, 2	x	x		x	x						0.018	0.029	2
BPX-70, 1	x	x	x		x						0.015	0.026	3
BPX-70, 2	x	x	x		x						0.017	0.029	3
BPX-70, 1	x	x	x	x							0.013	0.022	3
BPX-70, 2	x	x	x	x							0.021	0.040	3
BPX-70, 1	x		x		x						0.055	0.090	2
BPX-70, 2	x		x		x						0.061	0.096	2
BPX-70, 1	x				x						0.056	0.090	2
BPX-70, 2	x				x						0.057	0.099	2
BPX-70, 1						x	x				0.138	0.054	2
SP-2560								x	x	x	0.067	0.105	3
SP-2560								x		x	0.111	0.144	2

Combinations of ECL values acquired by different programs are used as independent variables (*X*-variables).

[a] The GC programs applied in the PCR model of 2D-FARI values. Programs are listed in Table 1.

[b] Standard set used to acquire the data for calculation of the calibration 2D-FARI values given in Table 2.

[c] Number of principal components included in the PCR models. The number of PCs that gave minimum SEP (or no further decrease) was selected. In all cases, the optimal numbers were equal for prediction of index 1 and 2.

and therefore most susceptible to drift in the ECL values. The difference in ECL values for 22:6 $n - 3$ acquired on the two columns ranged from 0.07 to 0.09 units when Programs 1–5 were considered. The 2D-FARI values for 22:6 $n - 3$ was 21.991; 5.781 (FARI$_A$; FARI$_B$) on Column 1 and 22.003; 5.757 on Column 2. This is a difference of 0.012 for FARI$_A$ and 0.002 for FARI$_B$. The ECL values and FARI$_A$ are on a scale with the same range (8.0–28.0). The lower difference in the indices therefore illustrates the improved robustness of the 2D-FARI values compared to ECL values.

In some cases, an accurate ECL value may not be obtained for a compound of interest because of peak overlap in some of the standard programs. The accuracy of predicting 2D-FARI values from subsets of the five standard programs were therefore investigated. Exclusion of Program 1 led to an increase in SEP of 2–2.5 times, while the SEP was approximately the same when any of the other programs was excluded. However, the optimal number of principal components in the regression models increased from two to three, except when Program 3 was excluded. Program 3 is in the centre of the variation space spanned by the other programs [11] and may therefore not provide any unique information when the other four programs are included. Both columns showed the same patterns regarding increase in SEP and optimal number of principal components in the models.

The accuracy of models with only the two most extreme programs (1 and 5) and the extreme plus the centre (1, 3 and 5) were also tested. Exclusion of more than one program led to a significant increase in SEP for both indices, but SEP was still below 0.1. In many practical situations, this accuracy may be

sufficient, e.g. to exclude alternatives for a tentative identification of an unknown compound. However, reference data for future use should be acquired by four or five programs. The accuracy for the model based on the two extremes was approximately the same as for the models based on the two extremes plus the centre point.

Another combination of two programs was also evaluated. Programs 6 and 7 were not included among the programs used for calculation of the values in Table 2. They differ from the standard programs by having no variation in the start temperature Table 1 column 1), which was lower than used in the standard programs (Programs 1–5). The temperature gradient for Program 6 was also weaker than the weakest gradient applied in the standard programs. SEP for FARI$_A$ (0.138) was higher than for the combination of Programs 1 and 5 (0.056, Column 1), while the SEP for FARI$_B$ (0.054) was lower than for the combination of 1 and 5 (0.090, Column 1). This illustrates that the temperature and pressure programs applied may not necessarily be among the five standard programs as long as sufficient shifts in retention indices are achieved.

It was also tested if ECL data from a more polar cyanopropyl column, SP-2560, could be used to predict the 2D-FARI values for the BPX-70 column. Only three programs were applied on the SP-2560. The span in ECL values between the two most extreme programs was slightly higher than for the BPX-70 columns, Table 1). The SEP was 0.067 for FARI$_A$ and 0.105 for FARI$_B$, which is not much higher than for the models based on three programs on BPX-70. The design of the SP-2560 programs is different than for the BPX-70 programs. There is no variation in the start temperature
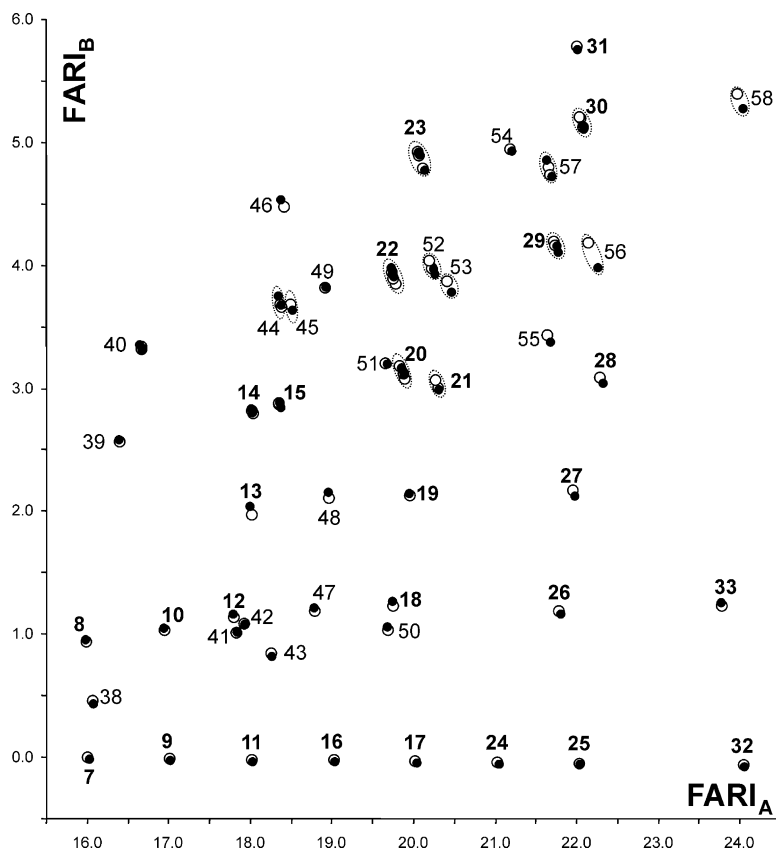
Fig. 4. 2D-FARI map of compounds analysed on a new (open circles) and old (closed circles) BPX-70 column. Numbers refer to identifications given in Tables 2 and 4. Fatty acids present in the calibration sample are shown in bold face.

(Table 1, Column A) for the three SP-2560 programs. It has not been tested if a full set of programs similar to the ones used for BPX-70 will provide more accurate predictions.

Other multivariate regression methods than PCR can also be applied for calculation of the 2D-FARI values. A widely applied regression method in chemistry is partial least squares regression (PLS). Both the PLS-1 and PLS-2 algorithm [24] were tested. The choice of regression method had no significant influence on the results.

4.4. Example of application

The reference mixture and several samples containing various fatty acids with 1–5 double bonds were analysed by Programs 1–5 on the two different BPX-70 columns. The compounds that were not commercial reference compounds have been previously identified by mass spectrometry [17]. The ECL data for the calibration sample was used to build PCR models for prediction of the 2D-FARI values in Table 2. The models were then applied to predict 2D-FARI values for the compounds in the remaining samples. The predicted values are plotted in the retention index map in Fig. 4. The calculated 2D-FARI values for compounds not present in the calibration set are given in Table 4. Some of the compounds were present in more than one sample, and the variation between calculated values for the same compound is clearly

Table 4
Average calculated 2D-FARI values for samples not present in the calibration sample

Number	FAME	$FARI_A$	$FARI_B$	n
38	16:1 $n-7$ t	16.066	0.451	2
39	16:3 $n-4$	16.384	2.580	2
40	16:4 $n-1$	16.658	3.334	4
41	18:1 $n-12$	17.831	1.019	2
42	18:1 $n-7$	17.928	1.086	2
43	18:2 $n-6$ tt	18.253	0.839	2
44	18:4 $n-3$	18.363	3.696	6
45	18:4 $n-1$	18.502	3.664	4
46	18:5 $n-1$	18.386	4.508	2
47	19:1 $n-9$	18.776	1.206	2
48	19:2 $n-6$	18.953	2.134	2
49	19:4 $n-3$	18.915	3.823	2
50	20:1 $n-15$	19.683	1.052	2
51	20:3 NMI[a]	19.659	3.205	2
52	20:4 $n-3$	20.226	3.985	4
53	20:4 $n-1$	20.431	3.833	2
54	21:5 $n-3$	21.182	4.942	2
55	22:3 NMI[b]	21.657	3.407	2
56	22:4 $n-3$	22.188	4.087	2
57	22:5 $n-6$	21.660	4.781	4
58	24:5 $n-3$	24.002	5.338	2

[a] Non-methylene interrupted double bonds, tentatively identified as 5, 11, 14–20:3.

[b] Non-methylene interrupted double bonds, tentatively identified as 7, 13, 16–22:3.

larger than indicated by the SEP for the calibration. The main reason for the larger deviation is probably that the compounds were present in different samples, and that a sample-to-sample variation in chromatographic properties was not present in the calibrations. Because of large differences in concentrations in the samples, some peaks were also slightly skewed because of column overload. In some samples column overload was also observed for the saturated FAME series added for ECL calibration.

Even with these inaccuracies caused by non-ideal chromatographic conditions there is no overlap between any of the analysed compounds. There is also good agreement between values acquired on the old and the new BPX-70 column, even though there is a considerable difference in the ECL values achieved on the two columns.

When rounded to the nearest integer, the 2D-FARI values may be used as estimates for the chain length and number of double bonds in *cis*-isomers. However, the $n-1$ series for C16 and C18 fatty acids is not accurately predicted. The accuracy for these isomers could possibly be improved by including similar fatty acids in the calibration set. However, these isomers are not commercially available, and are therefore not suitable for use in the calibration sample.

5. Conclusions

A two-dimensional retention index system for fatty acid methyl esters (2D-FARI) has been proposed. The 2D-FARI system is more robust towards differences in stationary phase properties than ECL values and is therefore convenient for comparison of retention data acquired on different columns or at different times at the same column. The two dimensions in the retention index system lead to increased selectivity and a reduced risk of retention index overlap between different compounds. The system is based on application of temperature-programmed chromatography and a fatty acid calibration sample analysed on a single capillary column.

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