IONIC LIQUID PHASES WITH COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY OF FATTY ACID METHYL ESTERS USING SOLID-STATE MODULATION

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Abstract

New generation inert ionic liquid (iIL) GC columns IL60i, IL76i and IL111i, comprising phosphonium or imidazolium cationic species, were investigated for separation of fatty acid methyl esters (FAME). In general, the iIL phases provide comparable retention times to their corresponding conventional columns, with only minor selectivity differences. The average tailing factors and peak widths were noticeably improved (reduced) for IL60i and IL76i, while they were slightly improved for IL111i. Inert IL phase columns were coupled with conventional IL columns in comprehensive two-dimensional GC (GC×GC) with a solid state modulator which offers variable modulation temperature (T_M), programmable T_M during analysis, and trapping stationary phase material during the trap/release (modulation) process, independent on oven T and column sets. Although IL phases are classified as polar, relative polarity of the two phases comprising individual GC×GC column sets permit combination of less-polar IL/polar IL and polar IL/less-polar IL column sets; it was observed that a polar/less-polar column set provided better separation of FAME. A higher first dimension (^{1}D) phase polarity combined with a lower ^{2}D phase polarity, for instance ^{1}D IL111i with ^{2}D IL59 gave the best result; the greater difference in ^{1}D / ^{2}D phase polarity results in increasing occupancy of peak area in the 2D space. The IL111i / IL59 column set was selected for analysis of fatty acids in fat and oil products (butter, margarine, fish oil and canola oil). Compared with the conventional IL111, IL111i showed reduced column bleed which makes this more suited to GC×GC analysis of FAME. The proposed method offers a fast profiling approach with good repeatability of analysis of FAME.

Introduction

In recent years, commercial ionic liquid (IL) phases have been available as alternative to traditional phases. They are highly polar and provide different selectivity (such as hydrogen bond acidity) to classical phases, can be applied to both polar and non-polar compounds, and offer different elution patterns in gas chromatography (GC) [1-3]. Commercial IL columns have been applied to various classes of compounds such as polychlorinated biphenyls, pesticides, sulfur- or nitrogen-containing compounds, essential oils, and fatty acid methyl esters (FAME) [2, 4]. Among these IL columns, SLB-IL111 (1,5-di(2,3-dimethylimidazolium)pentane bis(trifluoromethylsulfonyl)imide) is an attractive highly polar phase offering improved selectivity towards FAME with double bonds [5-7]. Compared with poly(siloxane) and poly(ethylene glycol) stationary phases, highly polar phases such SLB-IL100 and SLB-IL111 provide improved separation of FAME in the C16, C18 and C20 regions and conjugated linoleic acid isomer regions [6, 8, 9].

IL columns have been used in one-dimensional (1D) GC for analysis of FAME in various samples such as edible oils [10, 11], milk fats [9, 12], marine oils [13-15], or food samples [8]. Also, IL columns have been successfully used in advanced separations such as multidimensional GC (MDGC) and comprehensive 2D GC (GC×GC). Improved separation of FAMEs was reported by using two coupled IL columns [16-18] or IL columns combined with other phases [19, 20]. Compared with 1D GC, GC×GC is superior in terms of overall separation and increased peak capacity, and provides improved detection limit as a result of the cryogenic refocusing effect, along with reduced 'chemical background' from interfering peaks. The technique conventionally requires use of two columns providing different selectivity connected sequentially, with a modulator located between these columns that offers an effective modulation process [21]. A recently reported solid state modulator provides Peltier cooling between the columns, with the modulation region external to the

oven. A modulation column is oscillated longitudinally within the modulator according to the desired modulation period (P_M) [22].

With their useful selectivity differences, IL phases have been used to provide greater separation in MDGC and for selectivity tuning [4, 23]. Although the selectivity properties of IL columns are recognised, peak tailing for alcohols and polar compounds on imidazolium-based ILs containing trifluoromethanesulfonate, hexafluorophosphate, and bis(trifluoromethylsulfonyl)imide anions were reported [24, 25]. In some cases, undesired peak tailing of FAME on IL columns were observed [26, 12] which can reduce separation performance and further influence characterisation of natural products. The effects of choice of IL stationary phases on coffee volatiles with respect of reduced recovery was recently reported [27].

In 2016, a new generation of inert IL (iIL) phases as capillary GC column coatings was launched, including SLB-IL60i, SLB-IL76i, and SLB-IL111i. The iIL were prepared with modified surface treatment to improve surface inactivation, in order to reduce adsorption towards polar or active compounds. The performance of iIL phases for separation of essential oils and fragrances were reported by Cagliero *et al* [28]. Their inertness improved peak symmetry, and the study concluded that the new iIL phases somewhat altered selectivity compared to the earlier version IL phases. The inert SLB-IL111i phase was applied to analysis of FAME in fish oil and flaxseed oil [13]. It is of interest to apply the new iIL phases to GC×GC analysis.

In this study, the performance of several inert and conventional IL columns was evaluated for separation of FAME in 1DGC, in particular to assess elution temperature trends that will be important retention information for subsequent GC×GC studies. Both inert and conventional IL column combinations were then used in GC×GC, applied to analysis of FAME in commercial fats and oils.

Materials and methods

Chemicals and Materials

Chromatography grade dichloromethane (DCM), methanol and toluene, and analytical grade sodium hydroxide (NaOH), acetic acid and anhydrous sodium sulfate were purchased from Merck (Darmstadt, Germany). A mixture of 37 FAME standards (product no. 47885-U) and nonadecanoic acid methyl ester were obtained from Supelco (Sigma-Aldrich, Bellefonte, PA). The 37 component FAME standard was diluted to 5 mg mL^{-1} with DCM prior to the GC or GC×GC analysis. Commercial fish oil, canola oil, butter and margarine were purchased from a local supermarket in Australia.

Preparation of FAME samples

Derivatisation of commercial oils was performed according to modification of the method reported by Kittirattanapiboon and Krisnangkura [29]. In brief, sample (10 mg) and nonadecanoic acid methyl ester (0.4 mg, as an internal standard) were mixed with 3 mL of toluene in a screw-capped glass tube. 5% w/v methanolic NaOH (1 mL) was added to the above solution. The mixture was vortexed for 3 min then glacial acetic acid (1 mL) was added. The toluene phase was washed several times with distilled water. The toluene phase was then collected, dried over anhydrous sodium sulfate and diluted by two-fold before analysis with GC×GC.

One-dimensional GC

Gas chromatographic analysis was performed on an Agilent 6850 GC (Mulgrave, Australia) equipped with a flame-ionisation detector (FID). The ionic liquid columns (30 m × 0.25 mm i.d. × 0.20 µm d_f) of two generations, conventional IL phases (SLB-IL60, SLB-IL76 and SLB-IL111) and iIL phases (SLB-IL60i, SLB-IL76i and SLB-IL111i) were provided by Supelco. Injector and detector temperatures were set at 250 °C. Samples (1 µL) were injected

in split mode using a 500:1 split ratio. Hydrogen (99.999%) was used as carrier gas at a constant flow rate of 1.5 mL min^{-1}. Isothermal separations of the standard 37 FAME were performed over the temperature range of 150 - 230 °C.

Comprehensive two-dimensional gas chromatography (GC×GC)

All GC×GC analyses were performed on an Agilent 7890A GC (Agilent Technologies, Mulgrave, Australia) equipped with a FID and SSM1800 Solid State Modulator (J&X Technologies Co. Ltd., Shanghai, China). Each ^{1}D iIL column was separately combined with each of SLB-IL59, SLB-IL60, SLB- IL76 and SLB-IL111 as ^{2}D column (0.825 m × 0.1 mm i.d. × 0.08 µm d_f). GC conditions were chosen according to Nolvachai *et al.* [16]. Hydrogen was used as carrier gas with a constant flow rate of 1.5 mL $min^{-1}$and a split ratio of 20:1. Injector and detector temperatures were 250 and 260 °C, respectively. The temperature program was initially held at 100 °C for 1 min, then increased at a rate of 8 °C min^{-1} to 230 °C (held for 5 min).

The modulation period (P_M) was 6 s and the Peltier trapping temperature was set at -50 °C. Desorption and delay times were 1 s and 3 s, respectively. Temperatures of the inlet and outlet zones of the trapping column inside the modulator were set to be the same as the oven temperature. Canvas version w1.0.13.2 (J&X Technologies, Shanghai, China) was used for data analysis and generation of 2D plots.

Retention time normalisation and calculation of orthogonality

Analyte retention time values were normalised according to Eq. (1)

$$ {}^{i}t_{\mathrm{R,normalised}} = \frac{{}^{i}t_{\mathrm{R}} - {}^{i}t_{\mathrm{R,min}}}{{}^{i}t_{\mathrm{R,max}} - {}^{i}t_{\mathrm{R,min}}} \qquad (1) $$

where $t_{R,normalised}$ is the normalised retention time of each peak. t_R is the analyte retention time. $t_{R,min}$ and $t_{R,max}$ are retention times of the earliest and latest eluted analytes respectively, in the separation space. The superscript i is the index for the column dimension i = 1 or 2. The

$t_{R,normalised}$ data were then used to calculate orthogonality (O) for each separation. O is a scale describing analyte peak distribution in 2D chromatograms. The approach for the O calculation was adapted from Zeng *et al.* [30]. Briefly after normalisation, correlation of distribution pattern of 2D peaks (C_{peak}) can be calculated from R^2 of the correlation of $^1t_{R,normalised}$ and $^2t_{R,normalised}$ as

$$C_{peak} = 1 - R^2 \quad (2)$$

Note that correlation between retentions on the two dimensions can be either linear or nonlinear depending on the elution temperature on each ^{2}D column (governed by the type of ^{1}D column). In this study, correlation linearity can be derived from the elution of pattern saturated FAME. For example, a linear equation could be effectively used for the saturated FAME correlation using IL111i×IL60 (**Fig. 5B**), but is not sufficient to explain the elution pattern (curving down and up) of saturated FAME on IL60i×IL60 (**Fig. 6A**). A polynomial of degree 3 was applied in the latter case.

The total number of bins in the normalised chromatogram ($bins_{total}$), being the same as total number of the analytes of interest) and the number of bins occupied by analyte peaks ($\Sigma bins$) were counted. The bin coverage ratio (C_{pert}) was calculated according to eq 3.

$$C_{pert} = \frac{\sum bins}{0.63 \times bins_{total}} \quad (3)$$

O can then be calculated from the product of C_{peak} and C_{pert} as

$$O = C_{pert} \times C_{peak} \quad (4)$$

Results and discussion

Isothermal 1DGC analysis of FAMEs on the inert IL columns

Elution patterns of FAMEs on the two generations of IL columns are shown in Fig. 1. The elution orders of FAMEs on the inert and the conventional IL columns were apparently equivalent. However, all iIL columns (IL60i, IL76i and IL111i) exhibited slightly better resolution of FAME than their corresponding conventional columns. The IL60i showed superior capability to separate *cis*/*trans* isomers of C18:1 and C20:2, C20:4 and C20:3n6. The compounds C20:0; C20:1 and C23:0; C22:2 and C24:1; C22:6 were seen to co-elute on the IL76 column but they were adequately separated using IL76i; baseline separation of C24:1 and C22:6 was noted. IL111i shows better separation of C22:1 and C23:0 than on IL111. Their separation patterns are in agreement with previous studies [1, 26]. Fig. 2 presents retention factors of selected FAME on inert and conventional IL columns. Both saturated and unsaturated FAME retentions were slightly greater retained on the inert columns, except for the IL76/IL76i column. The reason for greater retention on the iIL columns may be caused by differences in film thickness, but they were of the same nominal thickness. In practice, identical retention factors of the two generations of IL columns having the same phase might be unexpected due to their different surface treatments.

The inert surface effects of iIL phases on peak tailing of FAME were investigated. The parameters selected for FAME evaluation was peak asymmetry, given by the tailing factor (T_f), which was calculated at 5% of peak height, and peak width at half height (w_h). The C18:3n3 was chosen as a representative FAME to observe the w_h and T_f values, which is well-separated on all the IL columns in this study. The observation temperatures were 150 ºC and 180 ºC. Table 1 shows that the average tailing factor was markedly improved for IL60i and IL76i compared with their comparative IL60 and IL76 columns. Both IL111i and IL111 provided the same average tailing factor. These results agreed with the data from Cagliero *et al.* [28], where the tailing factor was noticeably improved on IL60i and IL76i, while it was slightly improved for IL111i. Thus iIL columns generally show improved peak shapes, whilst

providing almost the same selectivity towards FAME separation. However whilst of the similar selectivity, the narrower peak widths and resulting improved resolution are noted.

GC×GC analysis of FAME on the inert IL columns

Even though one-dimensional GC is widely accepted for FAME separation, some degree of co-elution of saturated and unsaturated FAME in complex samples is a general expectation. A classical approach is to use very long columns (e.g. 100 or 200 m) in order to improve separations [31]. GC×GC is an alternative method to overcome this problem. The new iIL columns were applied here to investigate GC×GC separation.

^{1}D iIL columns were coupled with different ^{2}D IL columns (IL59, IL60, IL76 and IL111); the use of two different and disparate stationary phases normally of different polarity is required to provide some measure of orthogonality in 2D space. Although all IL phases are classified as polar, their polarities are significantly different [26]. Separation of FAMEs using different coupled IL column sets has been reported [17]. Fig. 3-5, examine iIL phases IL60i, then IL76i and IL111i respectively as ^{1}D, coupled with various ^{2}D regular IL phases. In general, IL60i and IL76i do not exhibit good spread of FAME in the 2D space, even when using the IL111 phase as ^{2}D. This corresponds to a less polar / more polar combination. Thus the large polarity difference in the case of IL60i /IL111 does not provide good orthogonality. In contrast, in previous work a low polarity / high polarity apolar polysiloxane / wax combination does give good FAME isomer 2D separation. The best option appeared to be a high polarity ^{1}D / low polarity ^{2}D phase combination. Part of this interpretation appears to be related to the observation of the decreasing elution temperature (T_e) of FAME as the ^{1}D polarity increases. If T_e is high, the effect of the polar ^{2}D phase has less of an effect in retaining analyte due to reduced retention factor at high temperature [32].

As shown in Fig. 5, when highly polar IL111i was used as the ^{1}D column, the separation of FAME was progressively improved as ^{2}D IL polarity decreases. According to a

previous report [3], IL59 and IL60 columns are the least polar IL phases, of similar polarity to polyethylene glycol (wax) stationary phase. They comprise the same stationary phase but IL60 is a modified version. As a result, similar results were obtained with IL59 and IL60 as the ^{2}D columns here.

The IL111i/IL59 column set exhibits good separation of FAMEs including C15:0/C14:1; C16:0/C15:1; C18:0/C17:1; C20:0/C18:2c and C18:2t; C21:0/C20:1 and C18:3n6; and C22:0/C20:2. All these respective solutes tend to co-elute in the ^{1}D separation. As a result, the most polar iIL with least-polar IL column set provided the best separation here, *e.g.* of complex geometrical and positional isomers of unsaturated FAME [33].

Fig. 5c and 5d show GC×GC results of IL111i and IL111 as the ^{1}D column. Their performance was comparable for the separation of FAME. Furthermore, the inert IL111i column clearly demonstrated lower column bleed than IL111. This may allow increased column lifetime as well as improved signal-to-noise for analysis of FAME at high temperature in routine analysis.

Peak broadening was observed for higher molar mass FAME (C22:0 to C24:0). This is probably due to poor 'modulation' performance, i.e. the remobilisation process to effectively transfer modulated analytes to the ^{2}D column. In order to check this, increasing the temperature in the modulation zone did not improve modulated peak widths, so the effect is not clear. The modulation column segment passing through the Peltier device comprises a specially-coated capillary column that is proprietary, so its performance and function in the modulation process is uncertain.

In general, GC×GC employing the same phase in each of the ^{1}D and ^{2}D dimensions is considered not advised, due to correlated retentions – i.e. lack of phase polarity difference. The primary consideration is whether there is some change in retention mechanism with temperature as the GC×GC experiment proceeds. Thus in the case of the IL111 phase, it is

possible that the single phase can provide 2D separation in GC×GC (1-phase GC×GC) [16], surmised to have a tendency to increase orthogonality when the thermal and surface sensitivity of the phase changes. The IL111 had the greatest sensitivity as temperature varies [16] and hence may show adequate separation of FAME. As displayed in Fig. 6, IL60i/IL60, IL76i/IL76 and IL111i/IL111 were tested for 2D separation of FAME, and the latter confirmed the unusual nature of the IL111 phase supporting the claim that a column with high and variable thermal sensitivity can provide greater separation of FAME using 1-phase GC×GC.

Analysis of FAME from butter, margarine, fish oil and canola oil samples

The IL111i×IL59 column set was applied for analysis of FAME in derivatised fat and oil product samples using the standard analysis conditions. FAME from fish oil, butter, margarine, and canola oil were selected as the samples to investigate their GC×GC profiles (Fig. 7 and Fig. 8). Their fatty acid compositions determined in this work, are given in Table 2.

This present study reports fatty acid composition including minor short chain FAME. Fatty acid profiles for butter, margarine and canola oil are generally in agreement with previous reports, although there is no certainty that data for same sample type is indicated [34-36]. Margarine shows a similar fatty acid profile to olive oil since it was olive oil light margarine which is expected to be similar to blended olive oil. The majority of polyunsaturated fatty acid (PUFA) in a fish oil capsule was eicosapentaenoic acid (EPA, C20:5). In addition to EPA, docosahexaenoic acid (DHA, C22:6) was the most abundant PUFA together with other fatty acids such as palmitic and oleic acids. Clearly the fatty acid composition of the fish oil capsule was different from the previous study, most likely due to a different fish species source [37].

A representative sample from fish oil was evaluated for repeatability of the modulated data. Fig. 7 shows good presentation of the GC×GC data in 2D space. The lower 1D modulated chromatogram has sharp symmetrical peaks, which are better displayed in the three replicate expanded 7.7-8.5 min traces to the right. The C16:1 component is seen to be marginally overloaded on the ^{1}D column, due to the pattern of the modulated peaks (*). At least 3 other components are seen here (given by Δ, □, and ○ markers), which can be seen as feint contours in the 2D plot. The method has good repeatability of retentions; FAME retention times in the fish oil sample were calculated from three replicate injections for C16 and C18 components. Repeatability was checked through the relative standard deviation (%RSD) of retention times in ^{1}D and ^{2}D as shown in Table 3. It might seem unusual to have a $^{1}t_{R}$ uncertainty of 0.00% RSD, suggesting that there is absolute precision of retention on the first column, but this is an artefact of the modulation process and the precise time for a particular modulated peak, and it is more realistic to consider the reproducibility of the modulated peak distribution [38, 39]. If a single modulated peak is measured, its total retention is largely controlled by the very precise timing of the modulation process. When compared with the total retention of the analyte, this gives a diminishing small %RSD. The variability of $^{2}t_{R}$ was from 0.46 - 0.78%, which is of the order of ± 0.02 s. The overall impression is that the solid state modulator provides good reliability of modulation.

Conclusions

Application of iIL columns for improved peak shapes (reduced peak width and tailing) for FAME analysis was demonstrated. Two generations of IL columns present comparable retentions and elution patterns with apparently almost equivalent selectivity for FAME compounds. When used in the GC×GC experiment, a ^{1}D IL111i phase showed less column bleeding, and when combined with an IL59 phase column, proved to be a good choice for GC×GC analysis of FAME. The proposed method offers an effective profiling

approach with good repeatability for analysis of FAME in a variety of food and related samples as demonstrated here for fats and oil products.

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Conflict of interest: The authors declare that they have no conflict of interest.

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List of Figure Captions:

Fig. 1 Separation of 37 FAMEs mix on the inert and conventional IL columns using isothermal temperature at 170 °C.

Fig. 2 Comparison between retention factors of selected FAMEs from the inert (●) and conventional (○) IL columns in the range of 150 – 230 °C. The columns were (a) IL60/IL60i, (b) IL76/IL76i and (c) IL111/IL111i

Fig. 3 Contour plots of 37 FAMEs mix obtained on inert IL60i as ^{1}D column combined with (a) IL111, (b) IL76, and (c) IL59 as ^{2}D columns.

Fig. 4 Contour plots of 37 FAMEs mix obtained on inert IL76i as ^{1}D column combined with (a) IL111, (b) IL60, and (c) IL59 as ^{2}D columns.

Fig. 5 Contour plots of 37 FAMEs mix obtained on inert IL111i as ^{1}D column combined with (a) IL76, (b) IL60, and (c) IL59 as ^{2}D column. The conventional IL111/IL59 column set (d) was used for comparison.

Fig. 6 Contour plots of 37 FAMEs mix obtained on the same phase type used for ^{1}D and ^{2}D columns. The column sets were (a) IL60i/IL60, (b) IL76i/IL76 and (c) IL111i/IL111.

Fig. 7 2D contour plot of fish oil sample on the IL111i/IL59 column set. The raw modulated chromatogram is shown below the 2D plot. The repeatability of analysis is presented by three replicate injections over the range 7.7-8.5 min, with the modulated peaks corresponding to the major C16:1 component asterisked (*), and minor components shown as Δ, □, and ○.

Fig. 8 Contour plots of (a) butter, (b) margarine, and (c) canola oil on the IL111i/IL59 column set. C19:0 is added to the samples as an internal standard.

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Table 1 Peak widths at half height (w_h) and tailing factors (T_f) of C18:3n3 analysed on inert and conventional IL columns, at 150 and 180 °C.

Table 2 Fatty acid composition of the selected fat and oil samples determined in the present work based on relative peak area response

Table 3 Repeatability of first dimension ($^{1}t_R$) and second dimension ($^{2}t_R$) retention times of selected FAME compounds obtained from three replicate analyses of fish oil.

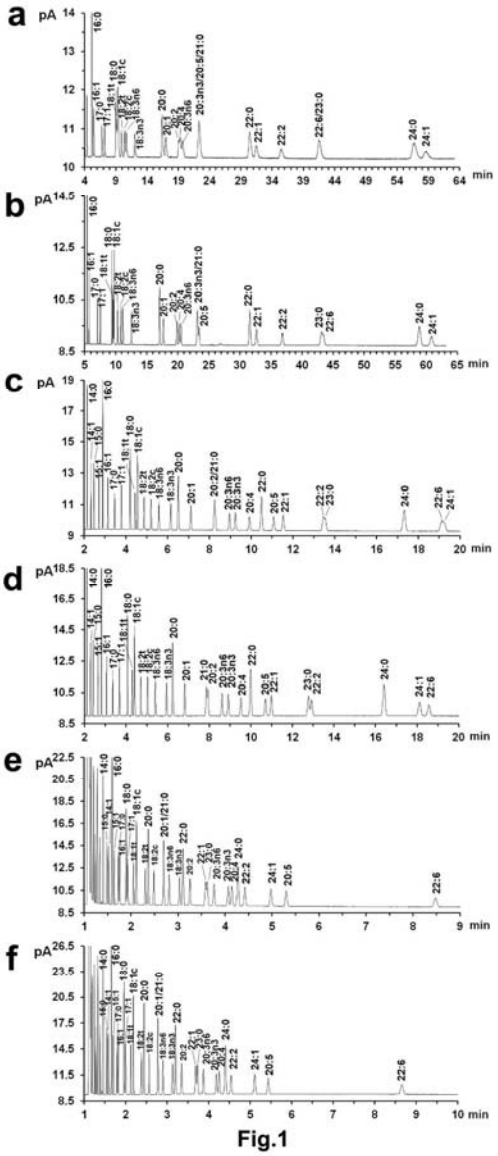

Fig.1

Separation of 37 FAMEs mix on the inert and conventional IL columns using isothermal temperature at 170 °C.

165x380mm (300 x 300 DPI)

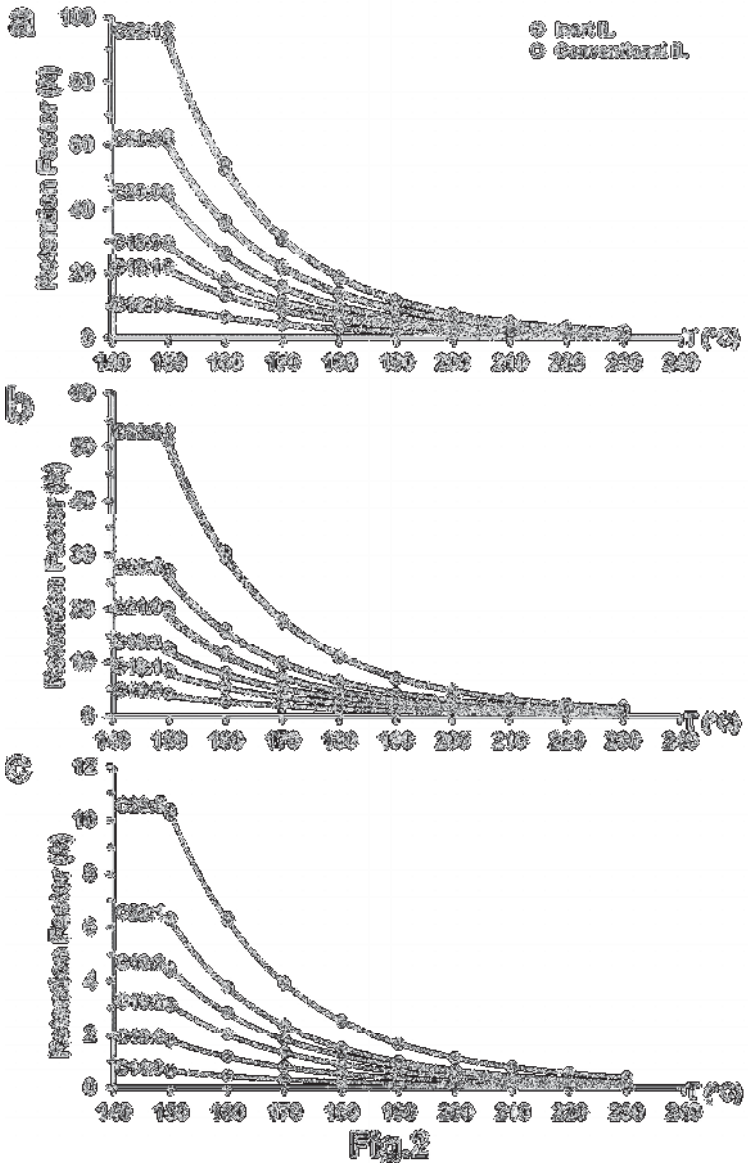

Fig.2

Comparison between retention factors of selected FAMEs from the inert (●) and conventional (○) IL columns in the range of 150 – 230 °C. The columns were (a) IL60/IL60i, (b) IL76/IL76i and (c) IL111/IL111i

107x167mm (300 x 300 DPI)

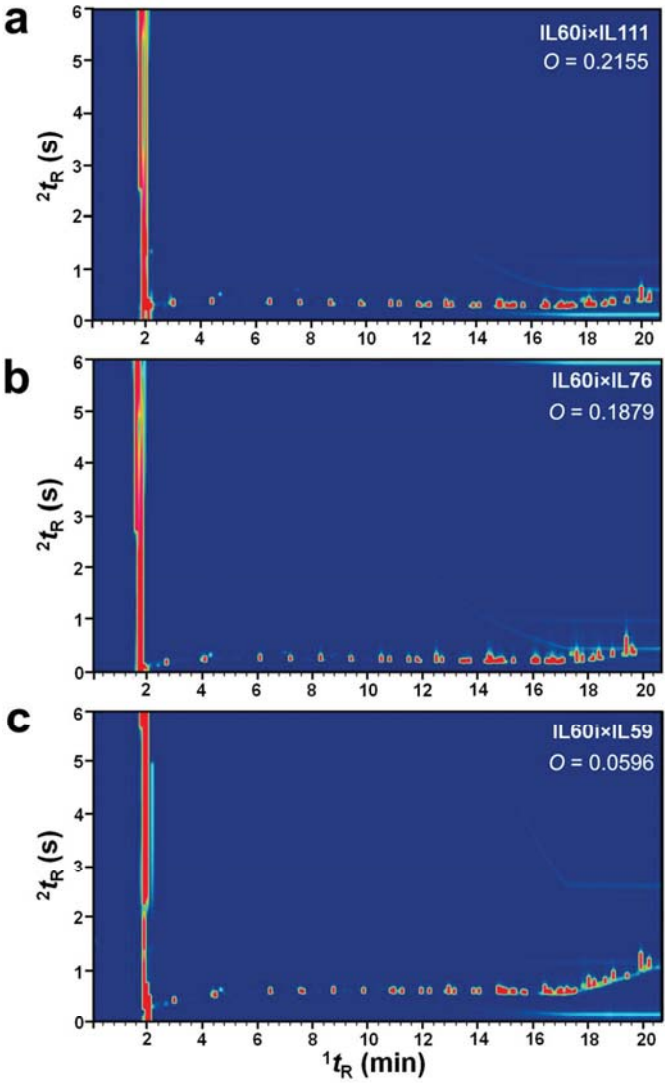

Fig.3

Contour plots of 37 FAMEs mix obtained on inert IL60i as ^{1}D column combined with (a) IL111, (b) IL76, and (c) IL59 as 2D columns.

106x187mm (300 x 300 DPI)

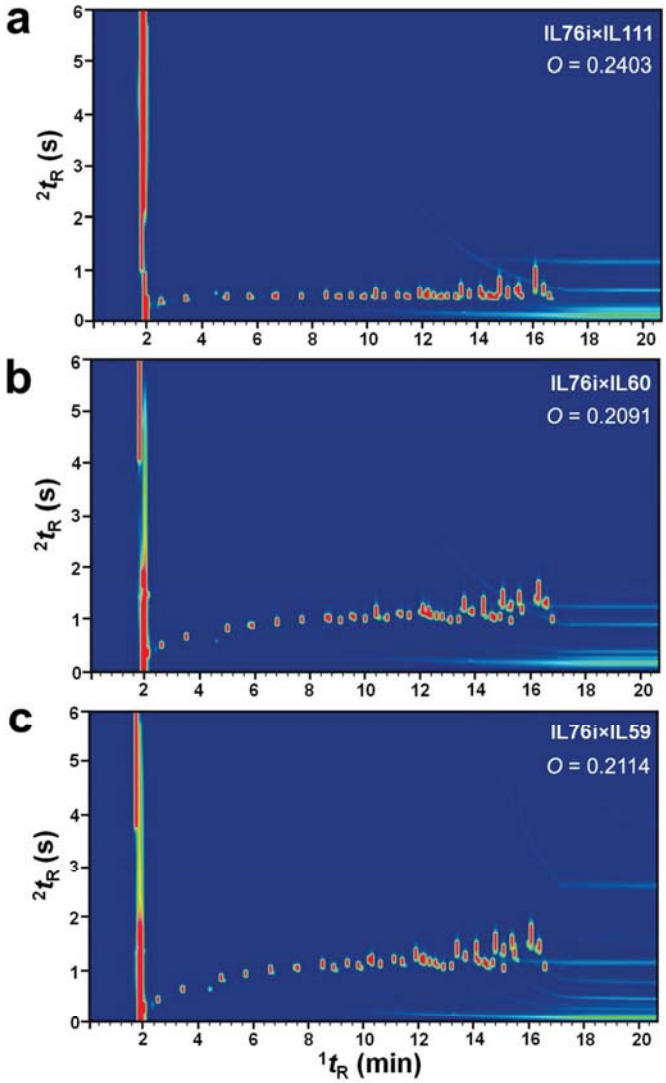

Fig.4

Contour plots of 37 FAMEs mix obtained on inert IL76i as ^{1}D column combined with (a) IL111, (b) IL60, and (c) IL59 as ^{2}D columns.

106x187mm (300 x 300 DPI)

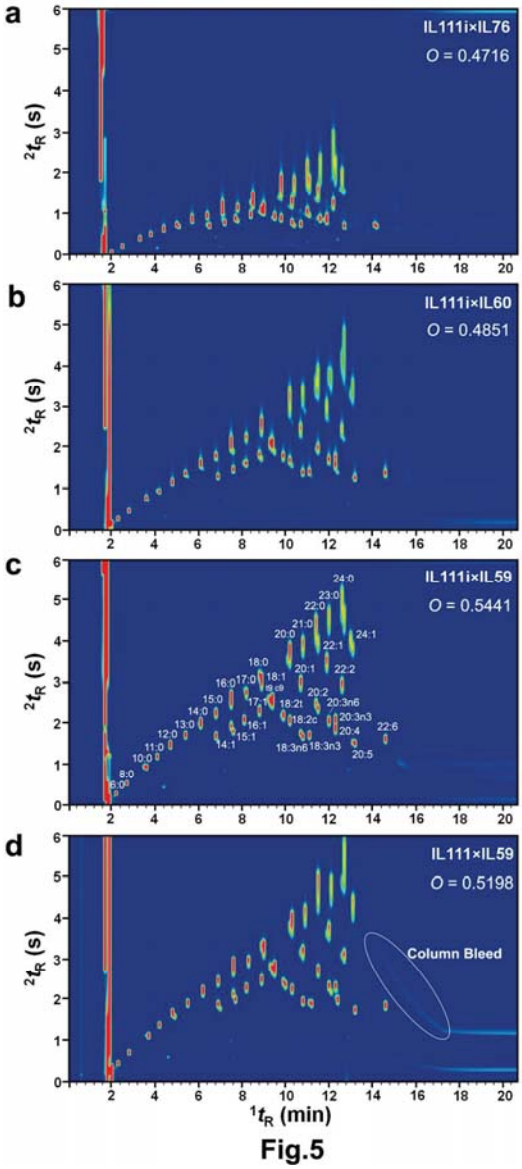

Fig.5

Contour plots of 37 FAMEs mix obtained on inert IL111i as [1]D column combined with (a) IL76, (b) IL60, and (c) IL59 as [2]D column. The conventional IL111/IL59 column set (d) was used for comparison

134x304mm (300 x 300 DPI)

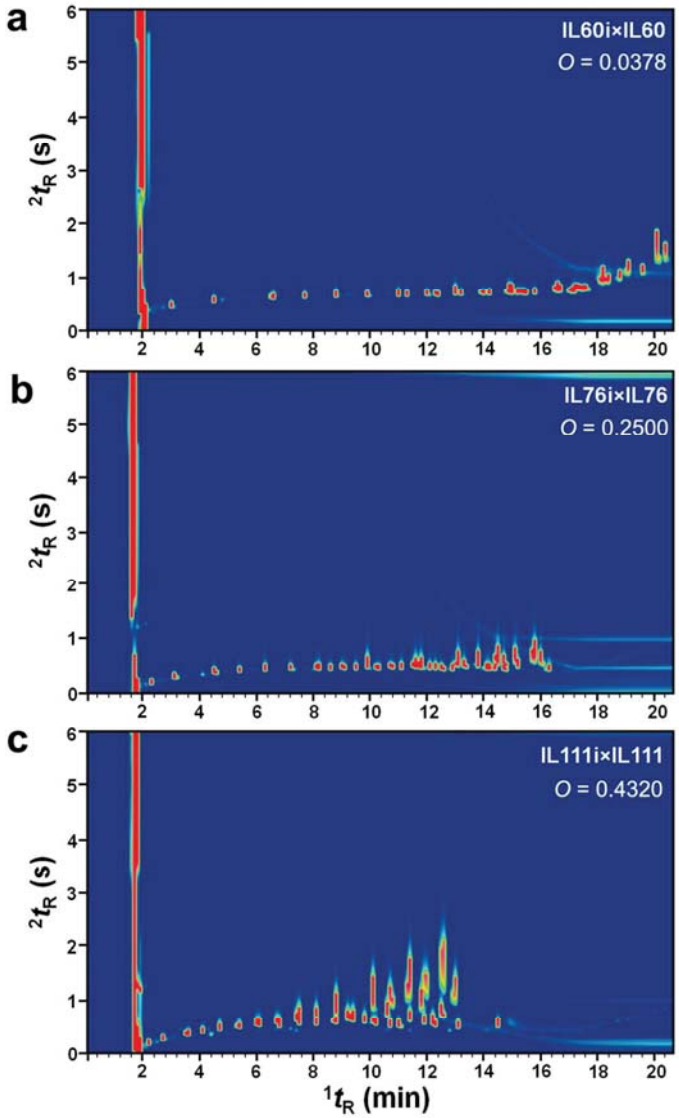

Fig.6

Contour plots of 37 FAMEs mix obtained on the same phase type used for ^{1}D and ^{2}D columns. The column sets were (a) IL60i/IL60, (b) IL76i/IL76 and (c) IL111i/IL111

102x177mm (300 x 300 DPI)

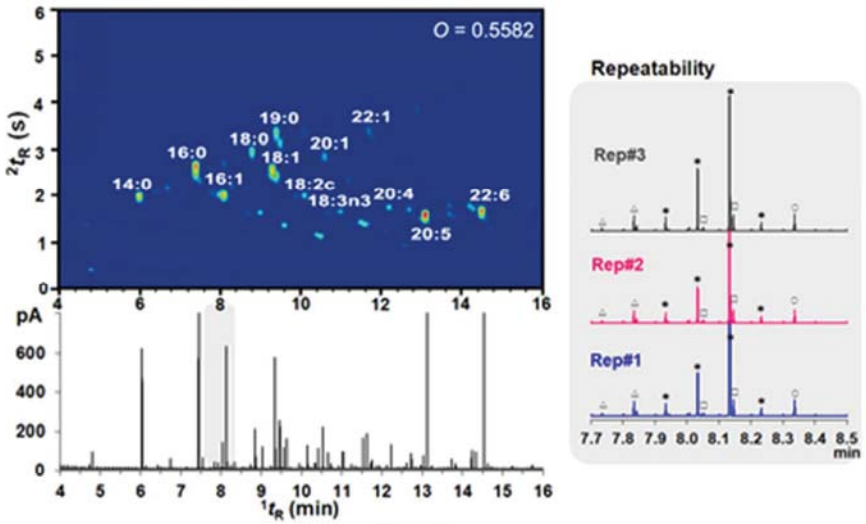

Fig.7

2D contour plot of fish oil sample on the IL111i/IL59 column set. The raw modulated chromatogram is shown below the 2D plot. The repeatability of analysis is presented by three replicate injections over the range 7.7-8.5 min, with the modulated peaks corresponding to the major C16:1 component asterisked (*), and minor components shown as ∆, □, and ○.

39x24mm (300 x 300 DPI)

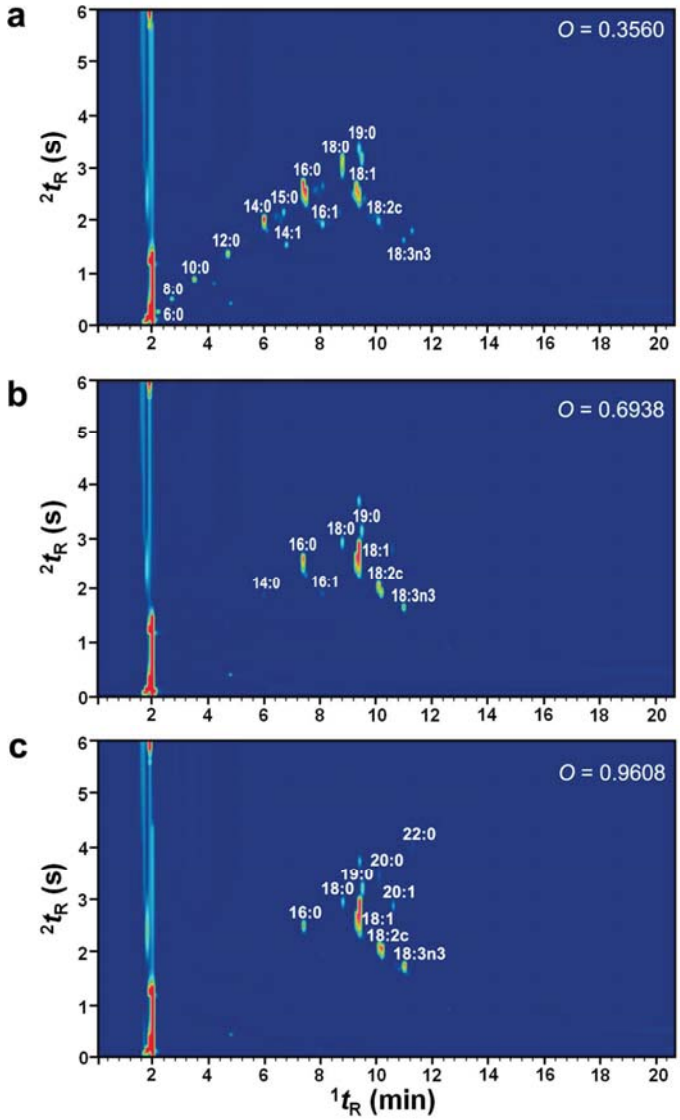

Fig.8

Contour plots of (a) butter, (b) margarine, and (c) canola oil on the IL111i/IL59 column set. C19:0 is added to the samples as an internal standard

104x181mm (300 x 300 DPI)

Table 1 Peak widths at half height (w_h) and tailing factors (T_f) of C18:3n3 analysed on inert and conventional IL columns, at 150 and 180 ºC.

Column		T = 150 ºC			T = 180 ºC		
		t_R (min)	w_h (min)	T_f [a]	t_R (min)	w_h (min)	T_f
SLB-IL60	IL	30.927	0.431	1.65	8.037	0.099	1.52
	iIL	32.137	0.222	1.22	8.405	0.057	1.14
SLB-IL76	IL	14.313	0.105	1.18	4.353	0.030	1.26
	iIL	13.821	0.101	1.15	4.238	0.030	1.10
SLB-IL111	IL	6.138	0.050	1.10	2.339	0.019	1.01
	iIL	6.265	0.049	1.10	2.411	0.019	1.01

[a] Peak asymmetry according to tailing factors are calculated at 5% peak height.

Table 2 Fatty acid composition of the selected fat and oil samples determined in the present work based on relative peak area response.

Fatty acids	Composition of fatty acid (% w/w of total fatty acid) [a]							
	Butter		Margarine		Canola oil		Fish oil	
	Present study	Literature [34]	Present study	Literature [35]	Present study	Literature [36]	Present study	Literature [37]
Saturated								
C6:0	1.8	2.1						
C8:0	1.2	1.2					0.8	
C10:0	2.8	2.8					0.6	
C12:0	3.6	3.2		0.5				
C14:0	12.1	10.9	0.3	0.7			7.8	
C15:0	1.3	1.5						
C16:0	34.1	26.6	16.2	13.9	4.2	4.3	19.5	0.6
C18:0	10.7	10.8	2.7	6.6	1.8	1.7	3.8	0.9
C20:0		0.4			0.6			
C22:0		0.2			0.2			
Unsaturated								
C14:1	1.3	0.4						
C16:1	1.7		0.4	<1.5			10.9	
C18:1	26.2	24.8	64.2	72.4	64.2	60.5	15.6	2.6
C18:2c	2.2	2.1	13.5	3.5	19.1	20.8	1.4	0.4
C18:3n3	0.7	0.3	2.8		8.7	10.1	1.0	0.4
C20:1		0.1			1.1	1.3	1.4	6.9
C20:4		0.1					1.4	1.6
C20:5		0.1					21.1	42.0
C22:1		0.1				0.5	0.9	0.9
C22:6		0.1					13.7	21.0
Total [b]	84.7		87.1		*		67.7	

[a] Data for fatty acid composition in various literature studies is reported; these are only indicative, and may not represent the exactly same sample type.

[b] Total fatty acid composition (g/100g sample)

* Total fatty acid composition is more than 99% in pure canola oil

Table 3 Repeatability of first dimension (^1t_R) and second dimension (^2t_R) retention times of selected FAME compounds obtained from three replicate analyses of fish oil.

FAME	^1t_R (ave) (min)	^2t_R (ave) (s)	RSD (%)	
			^1D	^2D
C16:0	7.400	2.653	0.00	0.78
C16:1	8.100	2.027	0.00	0.62
C18:0	8.800	2.973	0.00	0.78
C18:1	9.300	2.590	0.00	0.77
C18:2c	10.100	2.000	0.00	0.50
C18:3n3	11.000	1.648	0.00	0.46