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Twin comprehensive two-dimensional gas chromatographic system: concept and applications

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Abstract

A twin GC × GC system has been designed which enables the analysis of a sample by means of two different and independent column combinations simultaneously. Both combinations are incorporated in the same oven, using the same temperature programme, and are fed using a 50:50 column-entrance-split. It is demonstrated that, employing combinations of a conventional non-polar × polar and a reversed-type polar × non-polar column set, the information content is as high, and the analytical performance is as good as when using two separate GC × GC systems. That is, there is an appreciable gain of time and a reduction of costs without any loss of quality. The general usefulness of performing, and comparing two mutually different GC × GC runs is further illustrated with FAMEs in olive oil, and pollutants in a sediment sample.

Keywords: Comprehensive two-dimensional gas chromatography; Twin system; Orthogonality; Column combinations; Food extracts

1. Introduction

In the food industry but also in other disciplines, highly complex extracts have to be analysed in order to determine traces of (semi-) volatiles. They are often present at the low-ng/g level, especially those responsible for the odour. One-dimensional capillary gas chromatography (1D-GC) generally does not provide sufficient separation for a complete qualitative, let alone, quantitative, analysis — not even when identification/confirmation techniques such as those based on mass spectrometry (MS) are used. Even after careful sample preparation, such extracts often contain high concentrations of matrix constituents that can easily obscure the analytes of interest. Experience shows that this is frequently true, even though complicated sample preparation techniques such as solvent-assisted flavour evaporation (SAFE), various high-vacuum distillation (HVD) methods, steam distillation and/or fractionation are used in order to create sufficient separation of the analytes of interest from the matrix [1].

To solve problems such as the above, comprehensive two-dimensional gas chromatography (GC × GC) is an extremely useful technique, since it enhances separation of analytes of interest from each other and, more importantly, from the matrix background. In the past few years, GC × GC has been shown to provide the capability to considerably improve the analysis of complex samples [2–4]. However, as demonstrated in recent studies on food analysis, where sample extracts contain many types of semi- and highly polar classes of compounds such as aldehydes, ketones, lactones, acids and alcohols, both orthogonal and non-orthogonal approaches have to be used [5–7]: depending on the polarity of the analytes of interest, either one or the other technique may well fail to give a good-quality GC × GC result. In a previous study the two approaches were used with marked success [6]. Next to better overall chromatographic behaviour and separation of the polar and non-polar analytes, interesting ordered structures, which play such a prominent role in group-type identification, were observed in both instances.

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However, for acquiring the desired information on a variety of sample constituents the use of two separate GC × GC systems is required. We then concluded that combining the two approaches in one system is highly desirable and might improve sample throughput considerably. With such a 'twin' GC × GC system, one may expect that the desired information on all the sample constituents of interest can be obtained in a single GC-run, i.e. in a relatively short time and at acceptable costs. The aim of this study is to show the potential of such a system for the trace-level determination of flavour compounds in food extracts and of pollutants in sediment.

2. Experimental

2.1. Analytes and samples

Three standard mixtures were used in the present study. Mixture 1 (for compound names, see Table 1 below) contained *n*-alkanes in hexane. For quantitative aspect a diesel oil sample was obtained from a local service station. Mixture 2, containing 40 flavour compounds found to be responsible for the odour of olive oil, was dissolved in freshly distilled methyl acetate [6]. All 95–99% pure standards were from the Unilever Research Laboratory, which also provided various olive oil samples and extracts in diethyl ether. High-vacuum degassing (HVD) extraction was used to isolate the volatile flavour compounds from these extracts (see [6]). Mixture 3 containing 37 fatty acid methyl esters (FAMEs), with different chain lengths and number of double bonds, was purchased from Supelco (Supelco Park, PA, USA). In this work, the fatty acids will be designated as $C_{a:bnc}$, where *a* is the number of carbon atoms, *b* the number of double bonds and *c* the position of the first double bond beginning at the methyl terminal group. A sediment sample was taken from a bay in the Southeast of Spain on the Mediterranean coast.

Methylation of fatty acids was done using a 5 mM solution of trimethylsulphonium hydroxide (TMSH) from Fluka (Buchs, Switzerland) in methanol. Equal volumes of reagent and sample were mixed and the mixture was held at room temperature for 30 min. Under these mild conditions, *trans*-esterification of esters is minimal [8].

2.2. Twin GC × GC system

The twin GC × GC system consists of a Hewlett-Packard HP 6890 (Agilent Technologies, Palo Alto, CA, USA) gas chromatograph with a split/splitless injector, a short 20 cm × 0.53 mm i.d. retention gap, a glass Y-shaped press-fit connector and two GC × GC column combinations set up in parallel, with one being orthogonal and the other non-orthogonal. The first column set was a CPSil 8 (Varian-Chrompack, Middelburg, The Netherlands; 10 m × 0.25 mm i.d. × 0.25 μm d_f) × BP20 Wax (SGE Europe, Milton Keynes, UK; 1 m × 0.1 mm i.d. × 0.1 μm d_f) combination, and the second one a BP20 Wax (25 m × 0.32 mm i.d. × 0.25 μm d_f) × BPX35 (SGE Europe; 1 m × 0.1 mm i.d. × 0.1 μm d_f) combination. The second-dimension columns were connected to two FIDs which can produce a digital signal at a rate of 200 Hz and were monitored

Table 1
Determination of *n*-alkanes and flavour compounds with twin GC × GC system using orthogonal (A) and non-orthogonal (B) column combinations[a]

Analyte	R.S.D. (%) (n = 5)				Peak areas (%) for			
	A		B		Standards (R.S.D. (%), n = 5)		Sample (R.S.D. (%), n = 3)	
	^1t_R	^2t_R	^1t_R	^2t_R	A	B	A	B
n-Alkanes								
C_{14}	0.1	0.6	0.3	1.5	14.6 (7)	14.7 (6)	20.3 (7)	20.5 (7)
C_{15}	0.1	0.7	0.2	1.7	14.4 (5)	14.6 (5)	18.9 (6)	18.8 (7)
C_{17}	0.1	0.5	0.2	1.2	14.4 (6)	14.3 (4)	20.3 (6)	20.5 (6)
C_{18}	0.1	0.2	0.3	1.3	14.5 (6)	14.4 (7)	13.6 (7)	13.6 (8)
C_{19}	0.1	0.3	0.3	1.1	14.1 (4)	14.2 (7)	12.3 (5)	12.3 (9)
C_{20}	0.1	0.2	0.3	1.2	14.0 (4)	13.8 (4)	7.6 (5)	7.5 (6)
C_{21}	0.1	0.2	0.2	1.4	13.9 (5)	14.0 (6)	7.0 (6)	6.9 (7)
Flavour compounds								
Octanal	0.1	0.6	0.2	0.5	6.5 (3)	6.4 (6)	6.5 (4)	6.5 (7)
Nonanal	0.1	0.7	0.2	0.6	7.3 (2)	7.2 (7)	25.0 (3)	24.9 (7)
trans-2-Octenal	0.2	0.5	0.1	0.4	11.8 (3)	11.8 (4)	4.7 (3)	4.7 (6)
trans-2-Decenal	0.2	0.2	0.1	0.5	7.0 (4)	7.0 (4)	9.1 (5)	9.1 (5)
trans,*trans*-2,4-Nonadienal	0.1	0.3	0.1	0.5	7.5 (4)	7.5 (3)	4.7 (4)	4.7 (4)
trans,*trans*-2,4-Decadienal	0.1	0.2	0.1	0.6	7.3 (5)	7.4 (5)	7.8 (6)	7.9 (5)
Acetic acid-*cis*-3-hexenyl ester	0.1	0.2	0.2	0.8	12.4 (2)	12.5 (7)	6.3 (4)	6.4 (6)
Cyclohexylacetic acid ethyl ester	0.1	0.2	0.1	0.6	10.3 (3)	10.3 (6)	5.8 (3)	5.8 (7)
1-Octen-3-one	0.2	0.3	0.1	0.4	15.0 (4)	15.0 (3)	15.9 (5)	15.8 (4)
3-Octen-2-one	0.1	0.4	0.1	0.3	14.8 (3)	14.8 (3)	14.2 (4)	14.1 (5)

R.S.D. (%) in brackets.
[a] *n*-Alkanes in diesel oil; flavour compounds in olive oil extract.

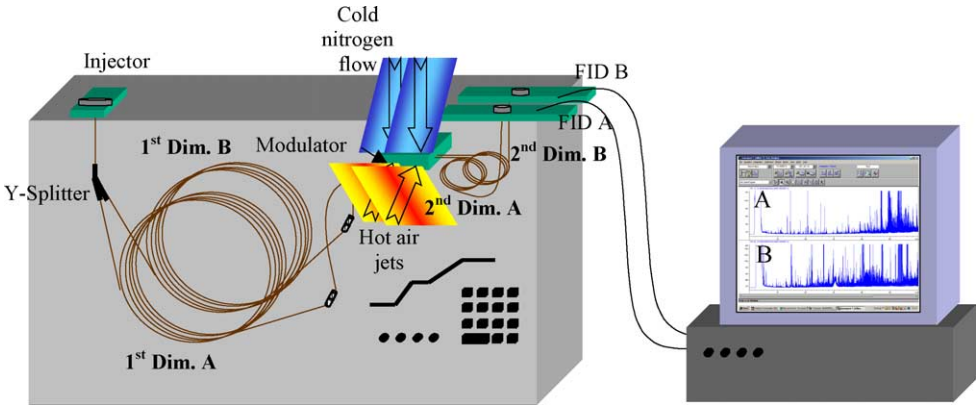

Fig. 1. Schematic of the twin GC × GC system with its two column sets, A and B.

by the same computer using HP Chemstation software (Agilent). One four-jet two-stage modulation system (KT2001) [9] was used to simultaneously modulate on both column sets. The two second-dimension columns are led through the modulation chamber using two-hole ferrules and a short section of each of the columns are placed in parallel close to each other inside the modulator. A schematic of the twin GC × GC system is shown in Fig. 1.

The carrier gas was helium (99.999% purity; Hoekloos, Schiedam, The Netherlands). The temperature of the two GC column sets, which were housed in the same oven, was programmed from 50 °C (2 min hold) to 250 °C (10 min hold) at 5 °C/min. The modulation time was 4 s; the modulator temperature was kept 100 °C below the oven temperature.

For data transformation and visualization two additional programmes were used, a programme to convert the raw data into a two-dimensional array (software provided by Prof. Ph.J. Marriott, Melbourne, Australia) and a programme to generate contour plots from this array ("Transform", part of Noesys software package; Research Systems International, Crowthorne, UK).

3. Results and discussion

3.1. Twin system performance

In order to fully profit from the advantages provided by GC × GC, it has recently been recommended to use both orthogonal and non-orthogonal systems because – contrary to earlier belief – next to improved separation and enhanced detectability, structured chromatograms can also be obtained in the latter case [5,6]. Furthermore, to improve sample throughput, it will be advantageous if both approaches can be integrated in one GC system. Therefore, a twin system was built as described in Section 2.2 and shown in Fig. 1.

The twin system was tested using flavour compounds in olive oil extracts. This is a relevant sample type which has complex, but known composition. Compared to a previous study [6], where the orthogonal and non-orthogonal approaches were used separately, similar chromatograms were obtained, and their valuable complementarity was again observed. As an example, the GC × GC chromatograms of the flavour compounds with the less well-known non-orthogonal approach are displayed in Fig. 2. They compare very well

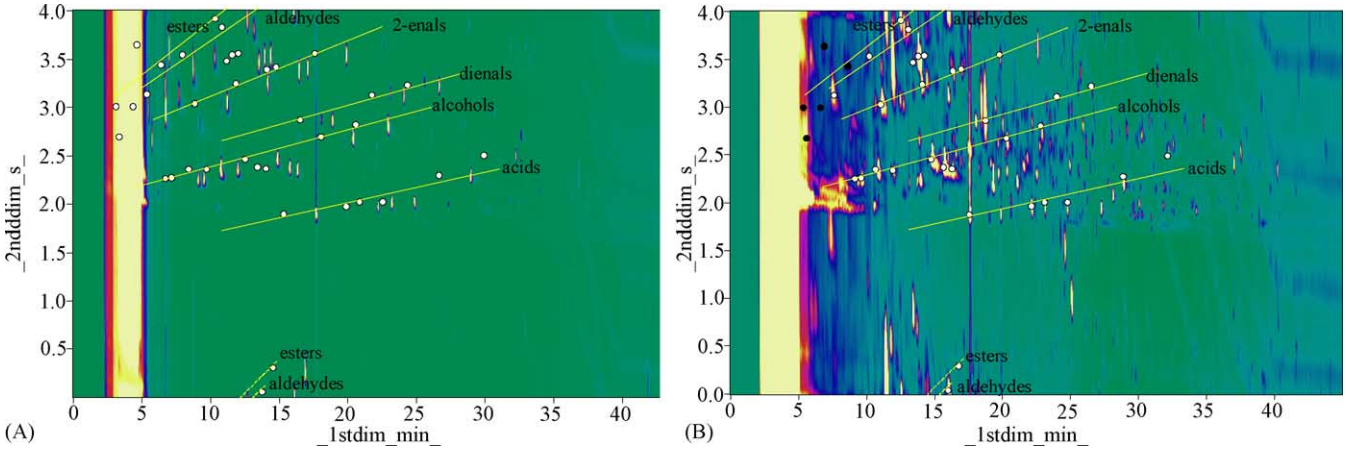

Fig. 2. Non-orthogonal GC × GC-FID chromatograms of 37 olive oil flavour compounds (A) and an olive oil extract (B). White and black spots correspond to non-problematic and problematic flavour compounds, respectively (see text for details). For experimental conditions, see text.

with Figs. 2 and 5 of ref. [6]. The main benefit derived from the non-orthogonal run is the excellent separation of polar analytes such as alcohols and acids. Their tailing and wrap-around phenomena, typically observed in orthogonal GC × GC and extremely problematic not only in crowded regions, but also in non-complex chromatograms [6], has completely disappeared. The fact that, mainly non-polar, analytes eluting close to the solvent peak may cause detection problems in the non-orthogonal approach (Fig. 2B), is not important. These compounds are detected quite easily in the orthogonal run (data not shown). In other words, these preliminary experiments show that, in the twin system, sample throughput can be two-fold increased without any loss of information.

In order to explore the potential and practical usefulness of the twin system in more detail, parameters such as repeatability of retention times and peak areas, which are important for both qualitative and quantitative analysis, were studied. Repeatabilities in both the orthogonal and non-orthogonal systems were calculated as the relative standard deviations (R.S.D.s) of five consecutive injections of the standard mixtures 1 and 2. Results for selected analytes are summarized in Table 1. For all compounds, the R.S.D.s were found to be below 0.3 and 1.7% ($n = 5$) for the first- and second-dimension retention times, respectively.

Table 1 also includes data on peak area measurements. With both standards as well as real-life samples the relative areas of the selected compounds are equal. This indicates that the split at the junction point feeding both column sets does not change during temperature programming. The R.S.D.s for the peak area measurements were also satisfactory: they were below 9% for all compounds. It should be noted that, despite the relative complexity of the twin system, the present results are similar to those reported previously for the two separately used approaches [6]. Similar results were observed for all other analytes. The small differences of the peak areas in the orthogonal versus the non-orthogonal analyses are probably due to the automated integration process. Finally, the split between the two column sets (cf. Fig. 1) is close to 50:50. This is according to expectations because (i) the splitter was designed to affect a 50:50 split, and (ii) the short piece of column (20 cm) between the injector and the splitter will have only minimal influence.

The results presented here allows the conclusion that the twin system can be used for analyte determination and char-

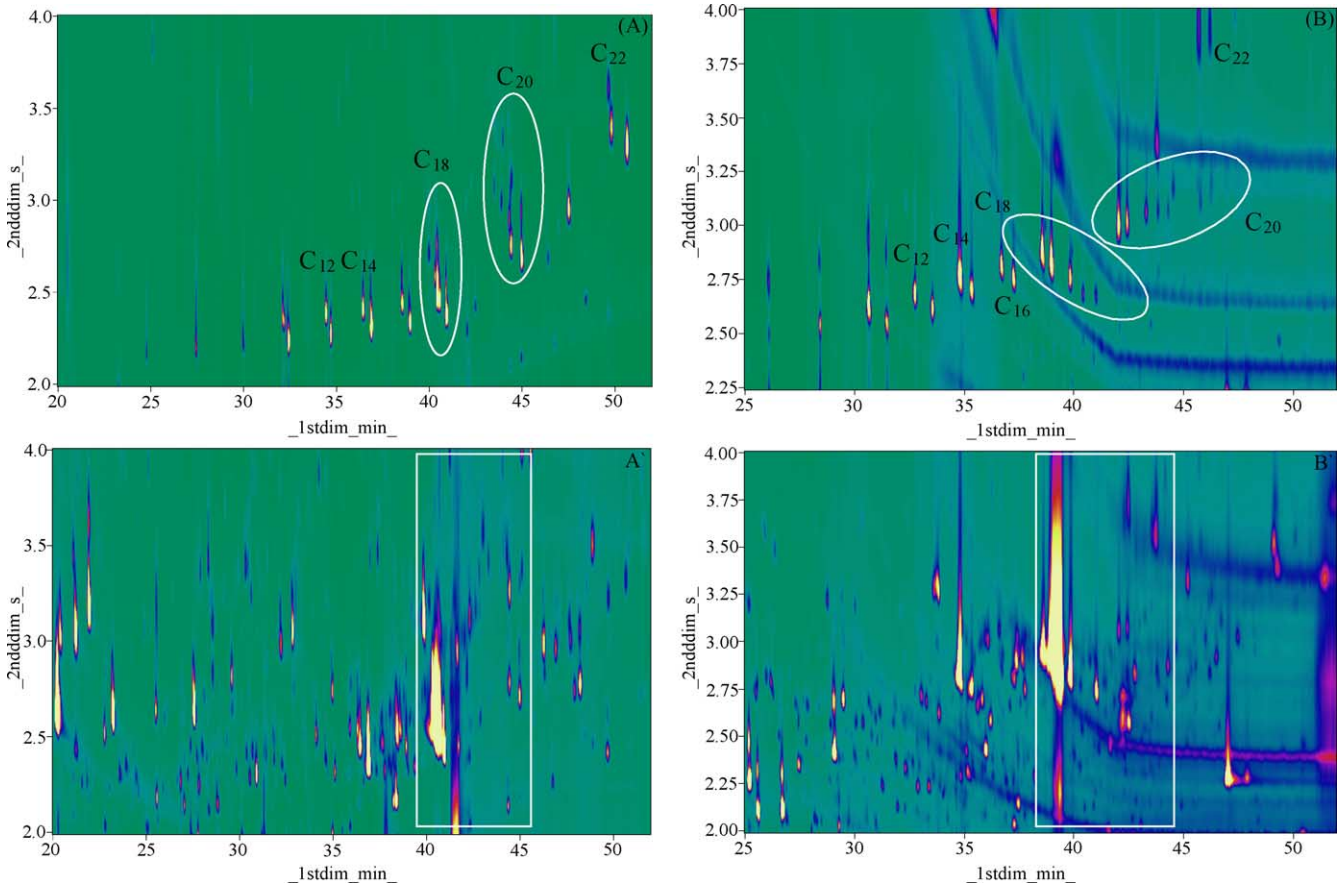

Fig. 3. Twin GC × GC-FID chromatograms of 37 FAME compounds (A, B: orthogonal and non-orthogonal, respectively) and a methylated olive oil extract (A′, B′: orthogonal and non-orthogonal, respectively). The marked C_{18}–C_{20} FAME regions are shown in more detail in Fig. 4. Note that the C_{20} FAs elute in the isothermal part of the chromatogram.

acterisation of complex mixtures. These aspects will be discussed in the next section.

3.2. Applications

3.2.1. FAMEs in olive oil

Next to the fundamental role they play regarding maintaining good health, fatty acids (FA) are very important in olive oil analysis as they are used to detect oil adulterations, i.e., to identify the cultivar and its origin [10]. A large number of FAs, with different chain lengths and different degrees of unsaturation, are present in olive oil. As is usual, we analysed them as FAMEs after methylation of an olive oil extract. In these samples, FAs with even carbon numbers are dominant, while odd-numbered FAs are present as minor components. Because of this, and also because of the general complexity of the FA profile in oils, the odd-numbered FAs often remain undetected in 1D-GC. Actually, even in GC × GC analysis, rather serious problems are encountered [11]. Consequently, it is of interest to use a twin GC × GC system with its two mutually different, but simultaneously run, chromatograms as final outcome. As an example, Fig. 3 shows 'the twin chromatograms' of the FAME standards and of an olive oil extract. Next to the considerably improved overall separation due to the use of a comprehensive 2D-separation technique, the differently ordered structures are clearly visible.

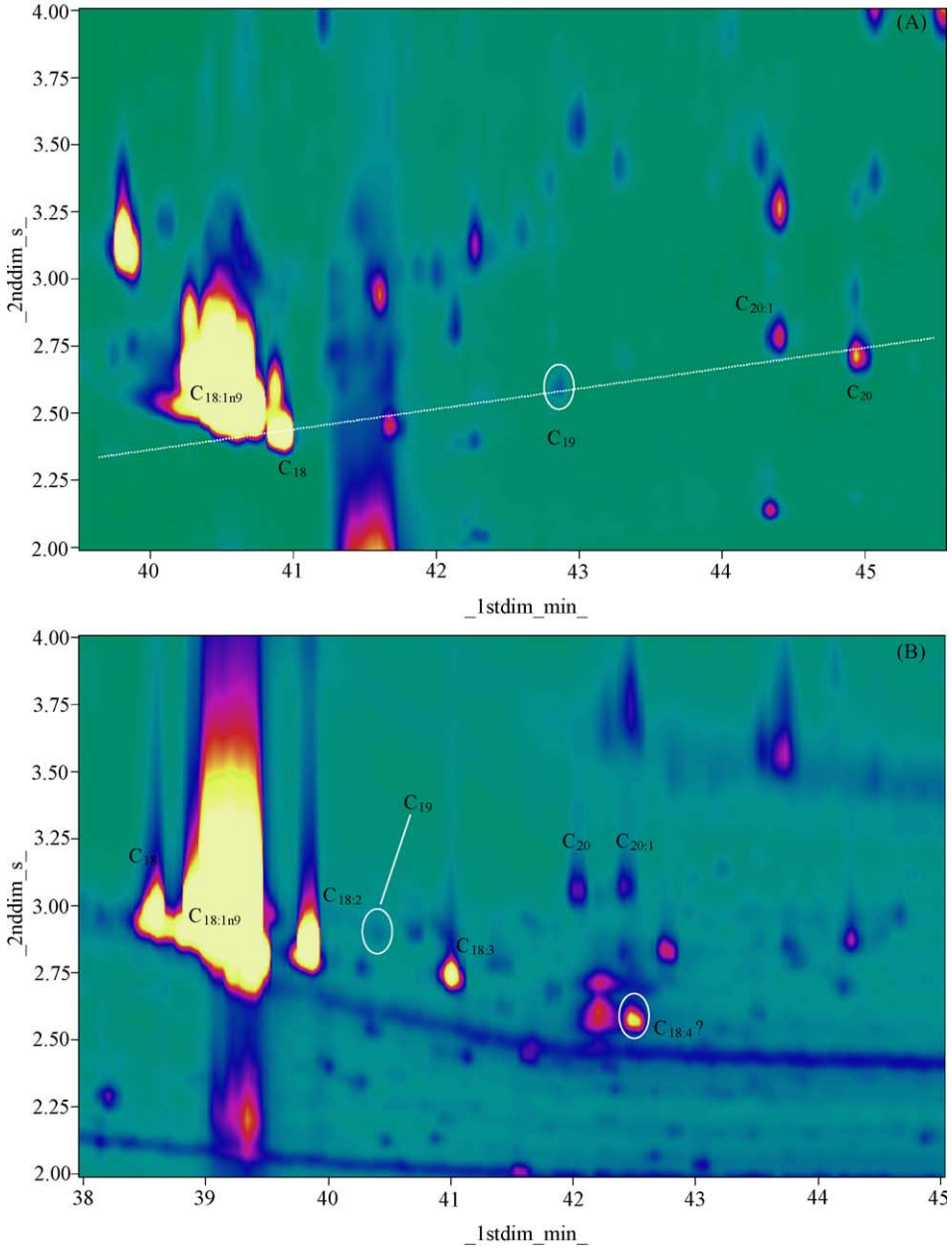

Fig. 4. Details of C_{18}–C_{20} regions of the olive oil extract marked in Fig. 3: A, orthogonal; B, non-orthogonal. Note that, for reasons of convenience, the rectangle dimensions differ from those in Fig. 3.

As regards the general separation, some further improvement would have been possible by optimising the temperature programme. This rather time-consuming operation was not performed because the main goal was to demonstrate the applicability of the system.

In the present instance, the orthogonal separation appears to be superior to its non-orthogonal counterpart. However, also here there is valuable information that can be derived from the non-orthogonal approach. As an example, Fig. 4 gives blow-ups of the C_{18}–C_{20} areas of the olive oil extract marked in Fig. 3; note that the rectangle dimensions (marked areas) in the former figure differ from those in the latter one. Quantitatively, the C_{18} fatty acids are the main constituents of this sample, with an over 85% contribution on the basis of FID-based peak area measurements. The most abundant single constituent is $C_{18:1n9}$ (>75%) which is, therefore, overloading both dimensions of both GC × GC sets. As a consequence, the $C_{18:1n9}$ peak obscures minor peaks, including target compounds such as $C_{18:2}$ and $C_{18:3}$ which virtually co-elute in the first-dimension column of the orthogonal approach (Fig. 4A). With the twin system, this problem is easily solved in the alternative run: now, they are nicely separated in the first dimension and display fairly satisfactory peak shapes (Fig. 4B). It is even possible to provisionally identify $C_{18:4}$. Rather concentrated extracts had to be injected to enable the detection of the minor

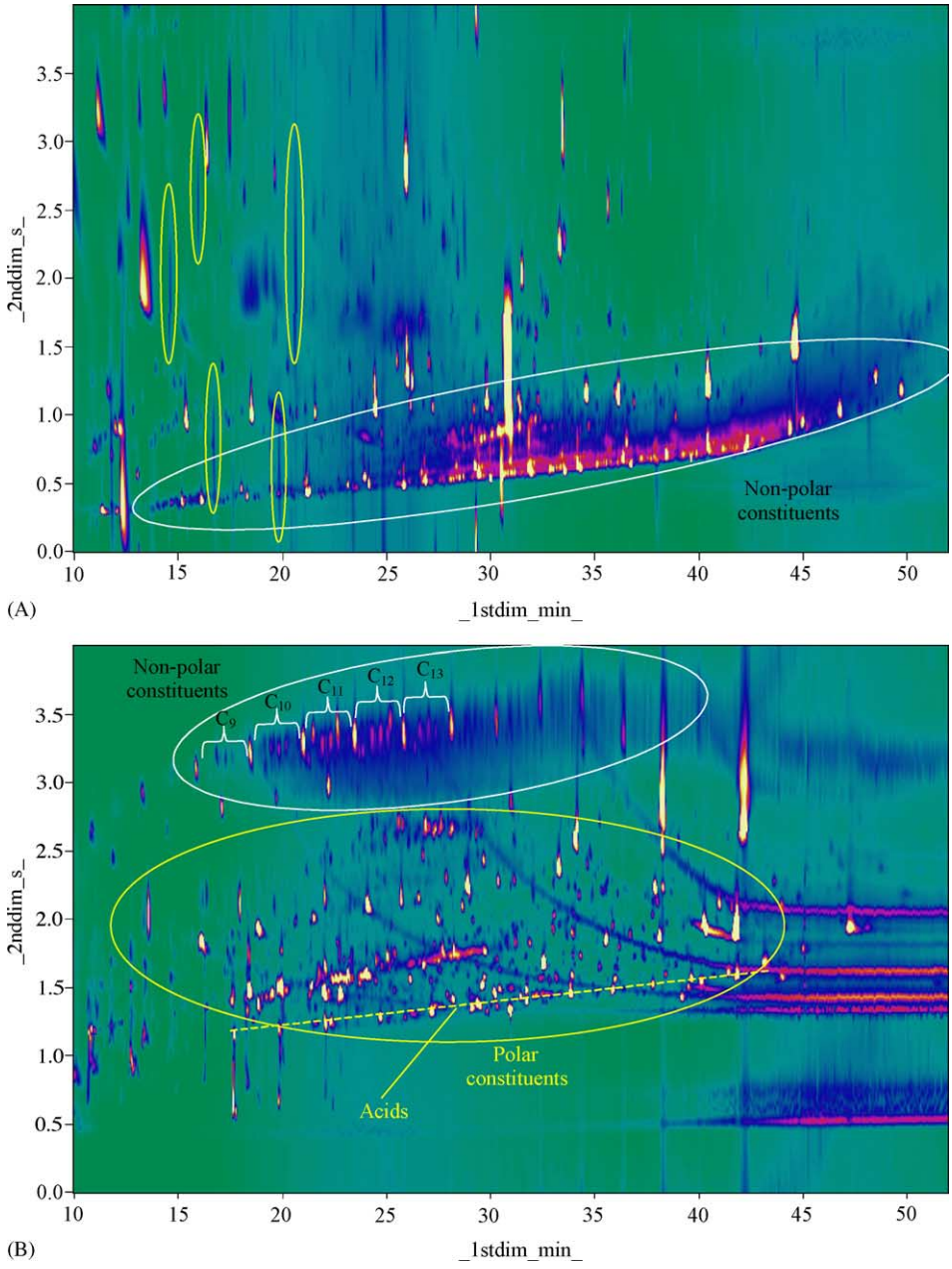

Fig. 5. Twin GC × GC-FID chromatogram of a marine sediment extract: A, orthogonal; B, non-orthogonal. For more details, see text.

FAMEs. One beneficial result of this procedure is the detection of the faint spot at $^1t_R/^2t_R$ of 42.9 min/2.6 s in Fig. 4A. Interpretation on the basis of retention times and ordered structures allows its provisional identification as C_{19}, a fatty acid not included in several recent studies [7,12]. By using proper shading techniques (cf. Jens et al.), the C_{19} spot could also be observed in the non-orthogonal GC × GC chromatogram (Fig. 4B). Further confirmation will require the use of mass spectrometric detection and/or a standard becoming available.

Finally, one should note that the stability of the stationary phases is also important: column bleed of the first column will also be modulated and may cause system peaks in the second-dimension chromatograms [6,13], and co-elution with target compounds will adversely affect their detectability. Polar stationary phases such as, e.g., Carbowax suffer more from this problem than non-polar ones such as, e.g., CPSil 8. If these phases are used in the first-dimension column, as is the case in the non-orthogonal approach, bands of modulated bleed material may show in the contour plots. This is clearly seen in Figs. 3 and 4; the Carbowax column of the non-orthogonal approach starts to bleed at a temperature of about 200 °C (Figs. 3B, B′, and 4B), while such bleeding does not occur in the case of CPSil 8 (Figs. 3A, A′, and 4A). Fortunately, this did not cause any real problems in the present study.

3.2.2. Sediment analysis

Sediment samples can be quite complex, especially if taken from a bay in which much shipping activity exists — and even more if in addition, wastewater treatment plants discharge into it. In such a case, a large number of pollutants widely different in polarity and chemical nature, can be expected to be present [14]. As an example, Fig. 5 shows twin GC × GC-FID chromatograms of such a marine sediment extract. The constituents of the extract are seen to be separated into two main groups. One group, with mainly non-polar analytes which are circled in Fig. 5, contains linear alkylbenzenes (LABs). In the orthogonal run, they are found at ^2t_R of ca. 0.5 s and show severe overlap. As a consequence, their ordered structure is obscured and cannot be seen in the contour plot. In the non-orthogonal run, however, the LAB are grouped at 3.0–3.5 s second-dimension retention times, and their ordered structure is clearly visible. As an illustration, the C_9–C_{13} LAB homologous series is indicated in Fig. 5B.

In both Fig. 5A and B, the more polar compounds take up the larger part of the GC × GC plane. While they are rather scattered (at ^1t_R = 15–20 min) in the orthogonal run, as exemplified by the linear carboxylic acids marked out in Fig. 5A, with wrap-around phenomena causing overlap and adding to identification problems — they are nicely grouped at ^2t_R = 1.0–2.5 s, without any wrap-around, in the non-orthogonal approach of Fig. 5B. The dashed line indicates the small and intense spots of the acids: contrary to what is observed in Fig. 5A, neither qualitative nor quantitative analysis will now cause any real problems.

4. Conclusions

It has previously been demonstrated that performing a complementary set of analyses on both a conventional (non-polar × polar) and a reversed (polar × non-polar) column combination yields higher-quality results than a single GC × GC analysis. Especially for, samples containing polar analytes such an approach yields more information for the entire sample. With the twin GC × GC system presented in this study, these complementary sets of results can be acquired simultaneously — without any loss of quality compared with the earlier technique of using two separate instruments. In other words, there is a considerable time gain and, thus, an increased sample throughput. The various examples shown in the introductory [6] as well as the present paper moreover demonstrate that the ‘twin approach’ is valuable for many different classes of compounds and/or sample types.

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