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Comprehensive two-dimensional gas chromatography of complex samples by using a 'reversed-type' column combination: application to food analysis

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Abstract

The practicability and potential of a non-orthogonal approach in comprehensive two-dimensional gas chromatography (GC × GC) were studied and compared to those of the orthogonal approach for two different complex matrices, and using conventional flame ionisation (FID) and time-of-flight mass spectrometry (ToF MS) detection. The separation of a diesel oil showed that the non-orthogonal approach also provides interesting, but completely reversed, ordered structures. For the more extensively studied flavour analysis in food samples, improved peak shapes and, also, different types of ordered structures and retention behaviour, and improved detectability for polar compounds make the two approaches complementary to each other. As a consequence, identification and/or determination of targets and/or unknowns can be performed more reliably. Analytical performance (close to three-order linearity; LODs, 2–30 pg injected in most cases; R.S.D.s, 1–6% (n = 6)) was fully satisfactory.

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

1. Introduction

Comprehensive two-dimensional gas chromatography (GC × GC) is currently receiving widespread attention for the analysis of complex samples [1–5]. This is due to the fact that in GC × GC very high peak capacities can be obtained. Peak capacity is an important measure of a separation, and peak capacity in GC × GC, ${}^{2D}n$, is generally considered to be the arithmetic product of the peak capacities obtained in the two individual dimensions, ${}^{1}n$ and ${}^{2}n$:

$${}^{2D}n = {}^{1}n \times {}^{2}n.$$

However, this is only an approximation, because retention correlation across the dimensions will reduce the peak capacity. In practice, the actual peak capacity is less than the calculated because a truly orthogonal separation is seldom obtained. The concept of orthogonality in 2D separations has not been precisely defined, but it is generally understood that a separation is truly orthogonal if the constituent dimensions operate independently and synentropy across the dimensions is zero. Minimizing synentropy is important in multidimensional separations because the larger it becomes, the larger becomes the part of the separation space that is unoccupied or even inaccessible. In such a case, sample constituents tend to cluster along a diagonal in the 2D plane: the separation is closely similar to that in the first dimension, with the second dimension having a negligible contribution. A detailed discussion of separation orthogonality in GC × GC was reported by Venkatramani et al. [6], while Liu and Patterson [7] developed procedures to estimate the degree of orthogonality and practical peak capacity in comprehensive two-dimensional separations.

From the very beginning, the analysis of petrochemical products was frequently used to demonstrate the practicability and usefulness of GC × GC. With (non-polar) × (po-

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lar) column combinations, orthogonality was essentially ensured. Under these conditions, highly efficient separations of the many thousands of individual – and often isomeric – compounds were achieved and, even more importantly, group-type separations could be easily recognised because of the ordered structures of the GC × GC chromatograms [8]. Because of the success of the approach, the same type of column combination – i.e., with a non-polar first-dimension column – has been used in some 80% of all presently available studies, irrespective of the application area of interest.

In a recent study on food analysis [9], where sample extracts will contain many types of semi- and highly polar classes of compounds such as aldehydes, ketones, lactones, acids and alcohols, the above approach was also used with marked success. However, when samples were really complex, the overall 2D separation became rather unsatisfactory and much of the desired information on flavour components could not be retrieved. As we then concluded, a drastically different approach is obviously required to solve such problems. One way to go would be to use a polar column in the first dimension, and a non- or, at least, less polar one in the second dimension. With such a reverse combination, one may expect that better overall chromatographic behaviour, and separation, of the polar sample constituents can be achieved. The present paper explores this option in some detail, since it is of course also important to maintain the separation of the non-polar sample constituents and – if at all possible – still to find ordered structures, which play such a prominent role in group-type identification (cf. above).

2. Experimental

2.1. Analytes and samples

Three standard mixtures were used in the present study. Mixture 1 (for compound names, see Table 2) containing 16 flavour compounds, which represent classes of compounds often encountered in food samples, was dissolved in freshly distilled methyl acetate (J.T. Baker, Deventer, The Netherlands). The mixture contained *n*-propylcyclohexane, naphthalene and benzothiophene as additional reference compounds because their retention data for various column sets are known. Mixture 2 (for compound names, see Table 2) containing 13 flavour compounds found to be responsible for the odour of vanilla samples (see Section 3.2.1) was dissolved in freshly distilled pentane. Mixture 3 (for compound names, see Table 5) containing 40 flavour compounds found to be responsible for the odour of olive oil samples (see Section 3.2.2) was dissolved in freshly distilled methyl acetate. All 95–99% pure standards, except *n*-propylcyclohexane, naphthalene and benzothiophene, were from the Unilever Research Laboratory, which also provided various vanilla extracts in ethanol or pentane, and olive oil extracts in diethyl ether. High-vacuum degassing (HVD) extraction was used to isolate the volatile flavour compounds from these extracts (see Section 2.2).

A diesel oil sample was obtained from a local service station.

2.2. Sample preparation

HVD is a suitable technique to isolate flavour compounds from fat or oily matrices under mild conditions [10]. The commercial vanilla extracts, dissolved in ethanol or ethanol and propylene glycol (PG), were diluted with water (1:2, w/w) and extracted with myglyol 812 CONDEA (1:1, w/w), a mixture of pure triglycerides made of medium-length fatty acids (MCT). After centrifugation, the oily phases were subjected to HVD for extraction at room temperature and under high vacuum (1.6×10^{-6} mbar). After 5 h of extraction, the solid material trapped by means of liquid nitrogen (−185 °C) was dissolved in 2 mL of ethanol or pentane. Similar to vanilla extracts, but without pre-extraction with myglyol 812, olive oil samples were subjected directly to HVD. In order to avoid losses of volatiles, 1 μL of each final extract was injected in the GC system without any pre-concentration.

2.3. GC × GC systems

The following conditions were used for the GC × GC system. The gas chromatograph was a Hewlett-Packard HP 6890 (Agilent Technologies, Palo Alto, CA, USA) instrument with a split/splitless injector and a flame ionisation detector (FID) capable of producing a digital signal at a rate of 200 Hz. HP Chemstation software (Agilent) was used to control the GC instruments and to acquire data.

The GC × GC–ToF MS system consisted of a HP 6890 (Agilent Technologies) gas chromatograph equipped with an Optic 2 programmable injector with a multicapillary liner (ATAS, Veldhoven, The Netherlands). The detector was a Pegasus II time-of-flight mass spectrometer (LECO, St. Joseph, MI, USA). The ToF MS was operated at a storage rate of 50 Hz, using a mass range of *m*/*z* 40–400 and a multi-channel plate voltage of −1800 V.

The GC columns used in the first and second dimensions are listed in Table 1. The columns were connected with a press-fit connector (Varian universal quick seal, Varian-Chrompack, Middelburg, The Netherlands).

Unless stated otherwise, the carrier gas was helium (99.999% purity; Hoekloos, Schiedam, The Netherlands) at a constant flow of 1.3 mL/min. The temperature of the two GC columns, which were housed in the same oven, was programmed from 40 °C (2 min hold) to 240 °C (3 min hold) at 5 °C/min.

For both systems, thermal modulation was performed with a home-made CO_2-cooled dual-jet modulator [11]. Cooling was effected through the Joule–Thompson effect of expanding liquid CO_2 (technical grade; HoekLoos). Briefly, two short sections of the second-dimension column are directly and alternately cooled in order to trap and focus each subse-

Table 1
Survey of first- and second-dimension GC columns used

Commercial code	Stationary phase	Temperature limit[a] (°C)	Dimensions[b]	Producer
First-dimension columns				
DB-1	100% methylpolysiloxane	−60 to 325/350	30 m × 0.25 mm × 0.25 μm	J&W Scientific
BP1	100% methylpolysiloxane	−60 to 340/360	30 m × 0.25 mm × 0.25 μm	SGE International
BP1	100% methylpolysiloxane	−60 to 340/360	15 m × 0.25 mm × 0.25 μm	SGE International
CP-Wax 58 (FFAP) CB	Polyethylene glycol	35 to 250/270	25 m × 0.32 mm × 0.30 μm	Chrompack
BP21 (FFAP)	Polyethylene glycol (TPA treated)	35 to 240/250	30 m × 0.25 mm × 0.25 μm	SGE International
Second-dimension columns				
CP-Sil 8 CB	5% phenyl 95% dimethylpolysiloxane	−60 to 330/350	1 m × 0.10 mm × 0.12 μm	Chrompack
BPX-35	35% phenyl polysilphenylene-siloxane	0 to 360/370	1 m × 0.10 mm × 0.10 μm	SGE International
BPX-50	50% phenyl polysilphenylene-siloxane	0 to 360/370	1 m × 0.10 mm × 0.10 μm	SGE International
BPX-70	70% cyanopropyl polysilphenylene-siloxane	50 to 250/260	1 m × 0.10 mm × 0.10 μm	SGE International
BP20	Polyethylene glycol	50 to 250/260	1 m × 0.10 mm × 0.10 μm	SGE International

[a] Maximum isothermal temperature/maximum programmed temperature.
[b] For the second-dimension columns only effective lengths are reported.

quent fraction, which is, next, remobilised by the heat from the surrounding oven air. The modulation time was in the range of 2–8 s; the modulator temperature was kept about 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 Dr. 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. GC column selection, orthogonality and structured chromatograms

3.1.1. Diesel oil

In order to explore the potential of reversed instead of conventional GC × GC – i.e., of using a polar × less/non-polar instead of a non-polar × polar column combinations – in terms of (i) separation efficiency and (ii) creating, or maintaining, ordered structures, a first study was made with diesel oil. This has the combined advantages of being a relevant sample type while it has a complex, but known composition.

Illustrative GC × GC–FID chromatograms for two column sets are shown in Fig. 1. The conventional separation, in this instance generated by using a DB-1 × BP20 combination and shown in the chromatogram in Fig. 1A, shows the well-known profile with a clearly ordered structure – as is indicated for three main classes of compounds. A second-dimension oven was needed and set 20 °C higher than the main oven in order to keep the most polar compounds eluting within the selected 8-s modulation-time window. The non-orthogonal approach, shown in the chromatogram in Fig. 1B and C for a BP21 × BPX-35 combination, gives a completely reversed order of separation of these alkanes, mono-aromatics and di-aromatics. A first item of interest is that the di-aromatics have distinctly shorter retention times now in the second dimension, while the opposite is true for the alkanes (and any cycloalkanes) present. The latter actually display a two-fold wrap around. This can be explained as follows. When a polar Carbowax column (BP21 or CP-Wax 58, which gave similar results) is used in the first dimension, non-polar compounds will elute at rather low temperatures. At these low temperatures, these compounds will exhibit quite high retention times on the relatively low-polar BPX-35 second-dimension column. On the other hand, more polar compounds such as the di-aromatics will have relatively high elution temperatures on the first column and, consequently, low second-dimension retention times. More importantly, and rather surprisingly, the reverse-type chromatogram also shows that the various classes of compounds display very clearly ordered structures, while they are packed tighter together than in the conventional column set-up. This is, in fact, an advantage when group-type determination is desired or a larger number of classes of analytes has to be distinguished. It should be added that, under properly optimised experimental conditions – primarily temperature control – not only can the alkanes be separated from the cycloalkanes, but a detailed separation within each class in the form of sub-classes can also be obtained (Fig. 1C and inserts of B). The results presented in Fig. 1B were obtained by using the second oven (40 °C higher temperature), but those in Fig. 1C without the second oven. This indicates that, when a more detailed separation is required within or between groups of analytes that are present in a complex matrix, such a reversed set-up can provide a satisfactory solution to the problem.

The main conclusion of this first test is that, next to the orthogonal approach, the non-orthogonal one shows most interesting results, especially as regards the well ordered, but completely reversed, structures of different classes of compounds. In order to fully profit from these advantages in the case of flavour analysis – where high(er) polarity plays a more dominant role – a systematic comparison of different column sets is needed. This is done in the next sections.

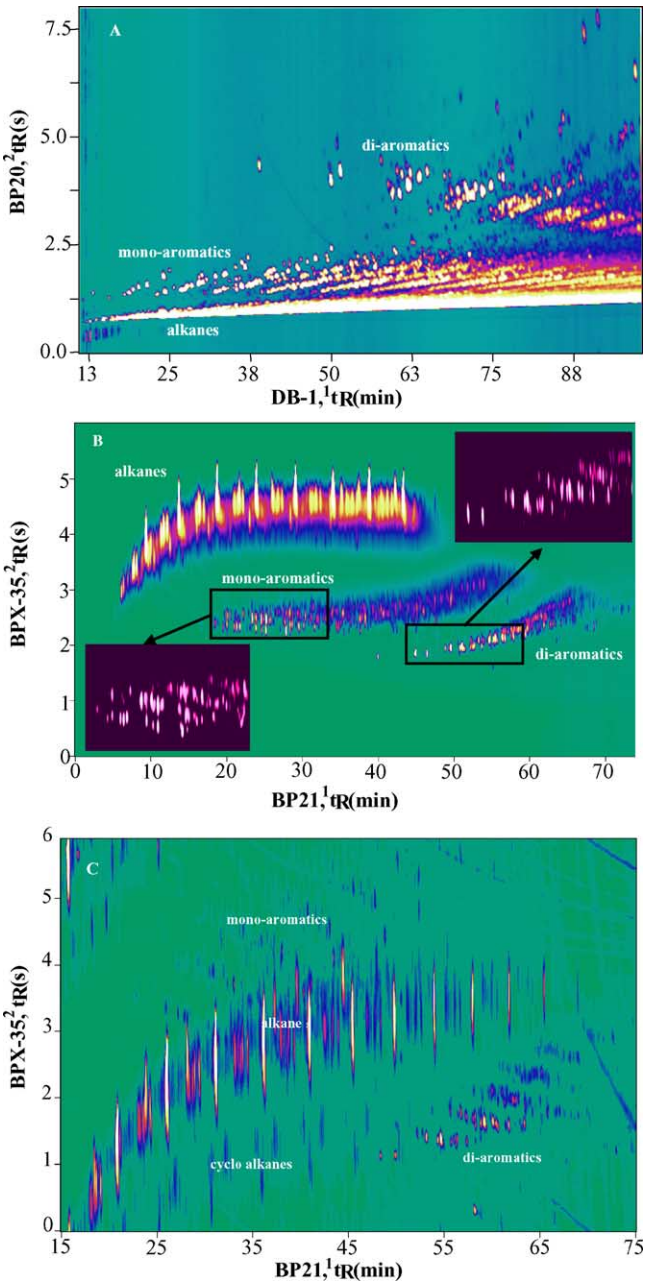

Fig. 1. GC × GC–FID chromatograms of a diesel oil sample analysed on a DB-1 × BP20 (A) and a BP21 × BPX-35 (B and C) column set. The two columns were, temperature-programmed independently (20 and 40 °C temperature difference in A and B, respectively) and in the same oven in C. For details, see text.

3.1.2. Flavour compounds

For a systematic study of conventional and reverse column combinations, the former was exemplified by using a DB-1 first-dimension, with BPX-50 and -70 second-dimension columns. In the present study, various alternative column combinations appeared to be more relevant. A polar stationary phase in the first dimension (that is, a polyethylene glycol stationary phase, CP-Wax 58 or BP21) was combined with four second-dimension columns with increasing polarity: CP-Sil 8, BPX-35, BPX-50 and the very polar BPX-70 (cf. Table 1). As an example, relevant data for selected analytes in test mixtures 1 and 2 are given in Table 2. Since CP-Wax 58 and BP21 give closely similar results as first-dimension columns, only the results for the former are shown.

As regards the DB-1-based orthogonal column sets, as is to be expected, especially the polar compounds are retained much more strongly on the 70% cyanopropyl (BPX-70) than the 50% phenyl (BPX-50) polysilphenylene-siloxane column. With the former combination, wrap-around – i.e., retention times higher than the selected 6-s modulation time – was observed, as is shown by the second-dimension retention times of 11–20 s for six test analytes. When a Carbowax (polyethylene glycol) was used instead of BPX-70, or BPX-35 instead of BPX-50, similar results were obtained (data not shown).

With the above non-polar × polar column combinations, the entire 2D separation plane is occupied. However, in practice, a second oven is needed to reduce the retention on the second column and avoid wrap-around since, in complex matrices, this phenomenon frequently causes co-elution of the relatively broad wrap-around peaks with peaks eluting in their own modulation cycle. Furthermore, to analyse highly polar compounds such as, e.g., carboxylic acids, a suitable first-dimension column has to be selected. From 1D-GC analyses that have been reported in the literature for a wide variety of samples, the retention behaviour of numerous polar compounds is well known. This information indicates that rather polar columns should be used in the first dimension. In flavour analysis, Carbowax-based columns are commonly used [12]. Separation will now depend on both the volatility and polarity of the analytes. In other words, irrespective of the nature of the second-dimension column, the GC × GC separations will never be truly orthogonal – which is, anyway, not the main goal in GC × GC, as was already mentioned in Section 1.

As is illustrated in Table 2, the separation of the majority of the polar compounds, including acids such as butanoic, pentanoic, hexanoic and 2-methylbutanoic acid, is completed within 2 s on three of the second-dimension columns selected, viz. CP-Sil 8, BPX-35 and BPX-50. The only exceptions were hexanal on two, and δ-octalactone on all three columns. Rather similar separations were generated on the three columns with, however, slightly more resolution in the case of BPX-35. Almost the entire 2D plane is occupied although the systems are not orthogonal. The advantage of the fast separation on the second column is that, with these reverse column sets, close to optimal separation (in terms of flow and temperature-programming conditions) can be performed in the first dimension, while still having the required four modulations across a first-dimension peak. Such performance is seldom found when using conventional orthogonal set-ups – actually, only if a 50-μm i.d. column is used in the second dimension [13].

The fourth column set, CP-Wax 58 × BPX-70, does not give the desired separation in the second dimension. All compounds of interest show up as a nearly horizontal band in the

Table 2
Second-dimension retention times (s) of selected standards on six column sets using GC × GC–FID[a]

Compound	DB-1 ×		CP-Wax 58 ×			
	BPX-70	BPX-50	CP-Sil 8	BPX-35	BPX-50	BPX-70
Butanoic acid	–[b]	–[b]	0.7	1.2	1.0	0.7
2-Methylbutanoic acid	–[b]	1.4	0.5	0.5	0.5	0.5
Pentanoic acid	–[b]	1.5	0.5	0.5	0.5	0.4
Hexanoic acid	–[b]	1.5	0.5	0.5	0.5	0.4
Hexanal	9.0	3.1	4.1	3.2	1.2	0.5
Methional	18.3	3.1	0.6	1.1	0.9	0.5
2-Furfurylthiol	6.8	3.1	0.7	1.0	0.8	0.5
n-Propylcyclohexane	1.6	4.2	1.1	1.7	1.4	0.6
Dimethyltrisulphide	5.8	5.1	1.3	1.7	1.3	0.7
1-Octen-3-one	4.5	2.7	0.9	1.1	0.9	0.5
1-Octen-3-ol	4.6	2.7	1.1	1.6	1.2	0.6
Trimepyrazine	4.5	3.8	1.3	0.8	1.0	0.6
Furaneol	19.3	5.9	1.0	0.6	0.9	0.6
Sotolon	11.6	4.2	0.5	0.8	0.7	0.6
Maltol	5.8	5.0	0.8	1.4	1.2	0.6
2-Methoxyphenol (guaiacol)	11.9	2.7	1.1	1.3	1.0	0.7
Linalool	12.9	4.2	1.3	1.9	1.4	0.5
trans-2-Nonenal	4.6	2.7	0.9	1.5	1.2	0.6
Naphthalene	5.4	3.2	0.8	1.4	1.2	0.6
Benzothiophene	5.4	3.8	0.7	1.3	2.0	0.5
δ-Octalactone	15.6	5.1	6.4	5.7	5.3	0.5

[a] Condition: temperature programme, 40 °C (2 min), at 5 °C/min to 240 °C (3 min); constant flow of 1.3 mL/min for all column sets.
[b] (–) Not possible to recognise peak parameters.

GC × GC plane at around 0.4–0.7 s: since the polarity of both columns is nearly the same and the elution temperature of a compound from the first-dimension column determines its retention behaviour on the second column, the second dimension has no real added value. The 2D separation is, in fact, a 1D-GC analysis.

For a further study of ordered structures – but now, for polar compounds – a mixture containing 40 flavours was selected, found to be responsible for the odour of olive oil (see Section 3.2.2). This mixture contains at least six classes of analytes. The same column combinations were used as for the diesel oil analysis, viz. DB-1 × BP20 and BP21 × BPX-35. The results are shown in Fig. 2. As expected, the orthogonal approach provides ordered structures for homologous series such as, e.g., aldehydes, 2-enals, alcohols and dienals, but not for the group of acids. Neither the individual acids nor the corresponding ordered structure could be recognised because of the very high retention of these compounds on the polyethylene glycol phase, which causes serious peak broadening and wrap-around. In the inserts of Fig. 2 this is illustrated for isovaleric acid and, also, for three alcohols, 1-hexanol, 3-*cis*-hexenol and 2-*trans*-hexenol. The alcohols show severe tailing on the DB-1 stationary phase, which is most likely due to interactions of their hydroxy groups and active sites present in the capillary column wall. Such phenomena easily cause serious identification and quantification problems, not only in 1D-GC but also in GC × GC, because of co-elution and peak overlap.

On the other hand, with the non-orthogonal approach, there is a very clear ordered (but completely reversed) structure of the various classes of analytes – now including the acids, which show up with the lowest retention time on the second column. In addition, all analytes display a good peak shape, as is exemplified by the top inserts of Fig. 2. Another important advantage is the short modulation time of 2–4 s instead of 4–8 s that can be used without any wrap-around occurring. Again, this also means that the first-dimension separation can be used under near-optimum flow conditions without compromising the required four modulations across a peak.

In summary, provided column sets in GC × GC are selected to maximise the use of separation space for the sample components, both the conventional orthogonal approach and the reverse, non-orthogonal approach proposed here provide highly efficient separations and interesting (although different) ordered structures. For a specific problem, the preferred choice will primarily depend on the nature, i.e., polarity, of the main analytes of interest. However, next to the separation of the target analytes from each other and their ordered structures, also their separation from the matrix is important. This aspect is studied below.

3.2. Real-life applications

3.2.1. Vanilla extracts

While with petroleum analysis group-type separation generally is the main point of attention, in flavour analysis a large number of individual components – and, especially, those that contribute to the odour – are of interest. Furthermore, due to the complexity of flavour mixtures it is often relatively difficult to detect ordered structures. The most important goal then

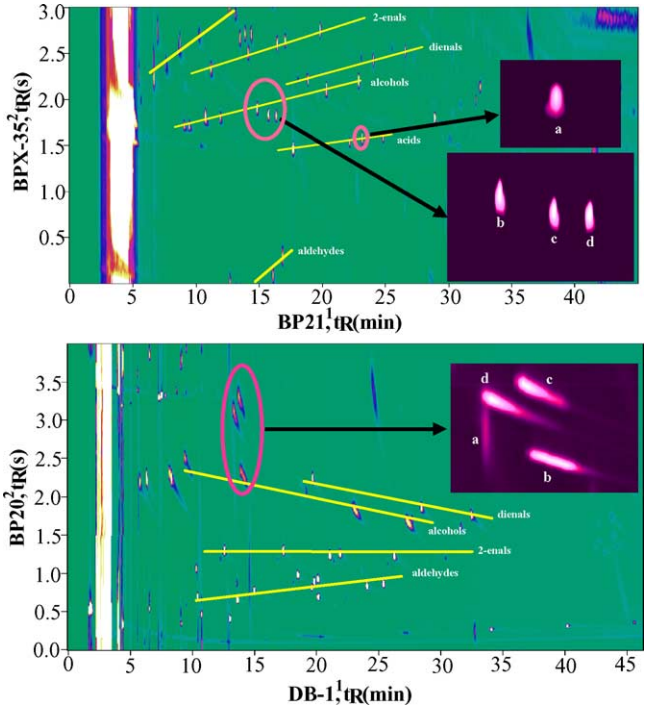

Fig. 2. GC × GC–FID analysis of 40 olive oil flavours on two column sets: (top) BP21 × BX-35 and (bottom) DB-1 × BP20. The inserts show (a) 3-methylbutanoic acid and three alcohols, (b) 1-hexanol, (c) *cis*-3-hexenol and (d) *trans*-2-hexenol.

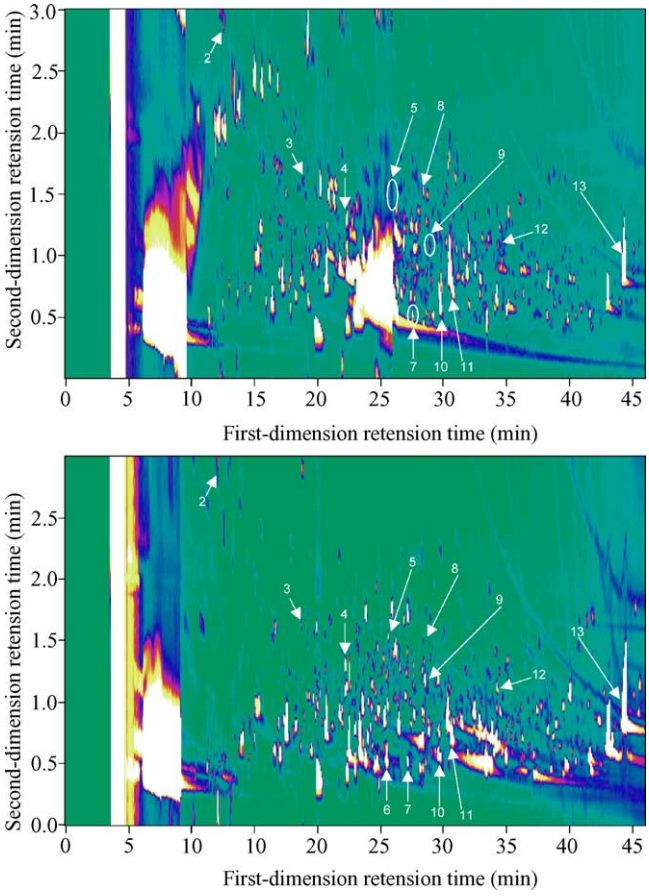

Fig. 3. GC × GC–FID chromatograms of the two vanilla extracts obtained with the non-orthogonal approach: (top) vanilla dissolved in ethanol and propylene glycol; (bottom) vanilla dissolved in ethanol. For peak assignment, see Table 3.

is to achieve the required separation of the analytes of interest from each other and from the matrix. As an illustration, two different vanilla extracts were analysed and characterised on the reverse (BP21 × BPX-35) column combination discussed above. Fig. 3 shows that this combination – compared to the orthogonal approach [9] – provides the overall selectivity required to separate most of the sample constituents from each other. However, it is also clear that propylene glycol – which is an essential solvent in the preparation of specific vanilla extracts – elutes in a critical region of the chromatogram, viz. at around 25 min in the first dimension, and shows up as a highly overloaded peak with a very intense and strong tail in both dimensions. Consequently, all information in this region is destroyed and the detection of trace-level analytes –

Table 3
GC × GC–ToF MS vs. 1D-GC–ToF MS of flavour standards in vanilla extracts[a,b]

No.	Compound	Extract 1[a]		Extract 2[a]	
		1D-GC (s/r)[b]	GC × GC (s/r)[b]	1D-GC (s/r)[b]	GC × GC (s/r)[b]
1	Dimethyldisulphide	nf	765/798	nf	745/773
2	Limonene	700/860	927/937	910/910	925/925
3	3-Octen-2-one	690/729	758/835	nf	720/793
4	Linalool	nf	819/859	nf	859/922
5	Benzoic acid ethyl ester	671/899	926/956	738/866	912/930
6	2-Methylbutanoic acid	764/818	815/826	818/818	859/879
7	Pentanoic acid	776/805	916/920	861/879	937/939
8	2-Phenylethyl acetate	732/798	891/912	nf	800/886
9	Nerol	nf	857/895	898/916	911/926
10	Hexanoic acid	840/849	921/934	912/922	916/922
11	2-Methoxyphenol	939/943	917/926	926/928	940/942
12	4-Methoxybenzaldehyde	919/938	938/944	853/906	947/953
13	Vanillin	935/942	938/943	907/911	940/944

[a] Extract dissolved in (1) ethanol and propylene glycol; (2) in ethanol.
[b] s/r, similarity/reverse; nf, not found by LECO software (after data processing).

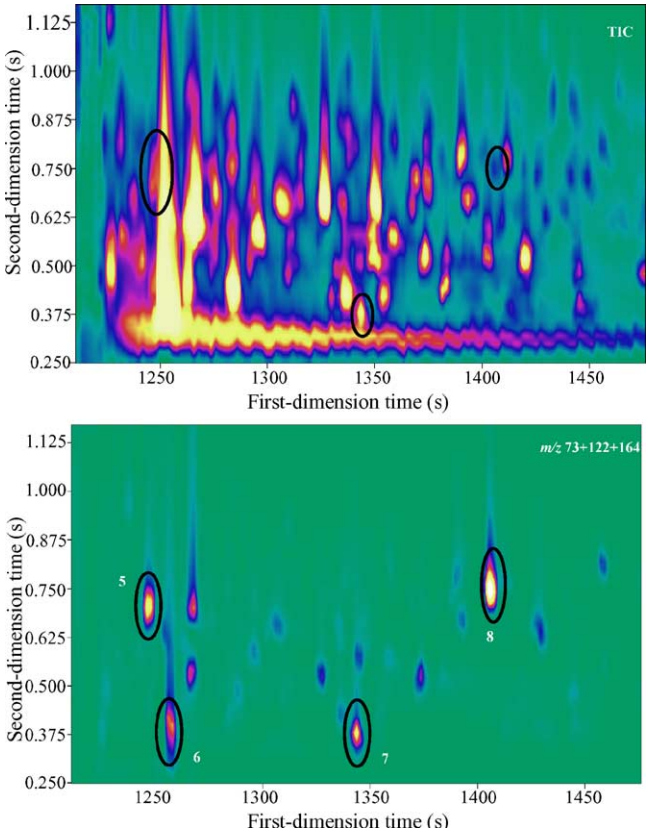

Fig. 4. Detail of GC × GC–ToF MS chromatogram of the critical propylene glycol region: (top) total ion chromatogram and (bottom) combined reconstructed ion chromatograms of *m/z* 73 (×0.2), 122 and 164 (×10). For peak designation, see Fig. 3 and Table 3.

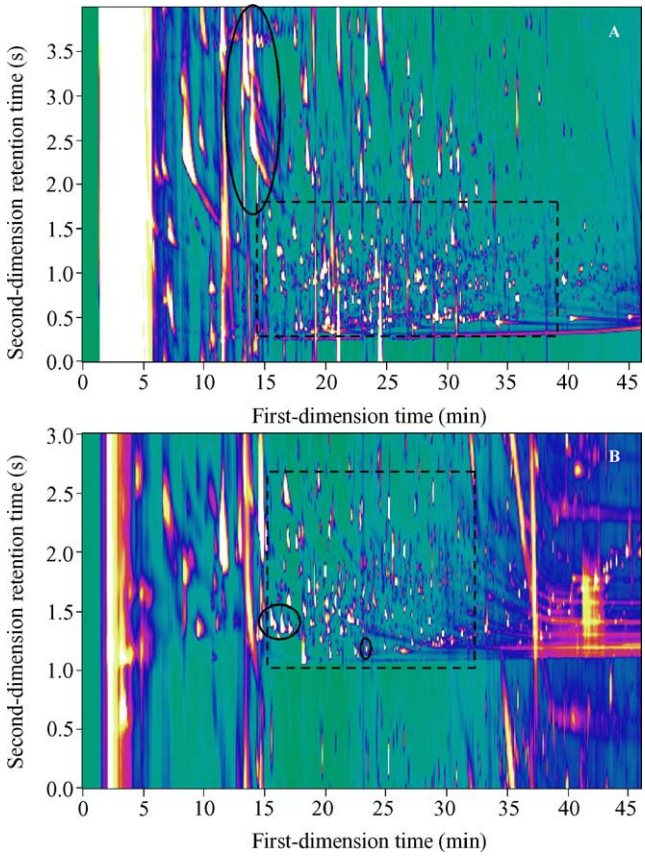

Fig. 5. GC × GC–FID chromatograms of an olive oil extract obtained with (A) orthogonal and (B) non-orthogonal approach. The circled spots show 3-methylbutanoic acid and the three alcohols, 1-hexanol, *cis*-3-hexenol and *trans*-2-hexenol. The zones marked by dashed lines delineate mainly non-polar analytes in (A) and polar analytes in (B). For details, see text.

such as, in this case, the targets benzoic acid ethyl ester, 2-methylbutanoic acid, pentanoic acid and 2-phenylethyl acetate – becomes extremely problematic.

The above problem was solved by using highly selective ToF MS, instead of traditional FID, detection. The results of this approach for the target compounds are summarised in Table 3, where two aspects are highlighted. One is the necessity to use GC × GC instead of 1D-GC analysis even if ToF MS detection is used: with the latter technique, several analytes cannot be identified at all, and for most of the early eluting targets, the similarity/reverse values found are distinctly inferior to those recorded in the case of GC × GC (cf. [9,14–17]). The other aspect is illustrated in Fig. 4, which

Table 4
Analytical performance data for selected standards in GC × GC–FID on CP-Wax 58 × BPX-35

No.	Compound	R.S.D. (%) (n = 6)			r^{2a}	LOD (pg)
		^1t_R	^2t_R	Area		
1	Dimethyldisulphide	0.2	1.4	4	0.9995	25
2	Limonene	0.2	1.4	3	0.9996	20
3	3-Octen-2-one	0.2	1.3	2	0.9993	10
4	Linalool	0.2	1.6	2	0.9997	5
5	Benzoic acid ethyl ester	0.2	1.6	2	0.9998	10
6	2-Methylbutanoic acid	0.2	0.8	2	0.9996	5
7	Pentanoic acid	0.1	0.3	2	0.9993	5
8	2-Phenylethyl acetate	0.1	0.7	2	0.9997	5
9	Nerol	0.2	0.5	2	0.9995	5
10	Hexanoic acid	0.1	0.3	2	0.9996	5
11	2-Methoxyphenol	0.1	0.5	1	0.9998	5
12	4-Methoxybenzaldehyde	0.1	0.8	2	0.9996	5
13	Vanillin	0.1	0.3	2	0.9989	5

[a] Linear model; five calibration levels in duplicate (range, 0.01–5 ng/μL).

displays the excellent resolution obtained for the four problematic target analytes in the propylene glycol region referred to above: combining the traces of *m*/*z* 73, 122 and 164 gives the desired result.

3.2.2. Olive oil extracts

For the olive oil extracts, the complementarity of the orthogonal and non-orthogonal approaches mentioned earlier, was found to be important. Fig. 5 shows two pertinent GC × GC chromatograms. The orthogonal approach yields better results for relatively non-polar analytes. These are retained less strongly than other sample constituents in the second dimension and show up as a band between 0.3 and 1.5 s in the chromatogram. In most instances, this creates an efficient separation from the polar matrix. However, as Fig. 5A shows that co-elution with polar compounds showing wrap-around, is a distinct drawback (see, e.g., the elongated spots eluting in the 20–25 min first-dimension time window). The non-orthogonal approach, on the other hand, is more suitable for more polar analytes, as was already noted when discussing Fig. 2. They are retained relatively strongly on the first column, which causes, in most instances, separation from the non-polar sample constituents (see Fig. 5B, zone with 17–32 min first-dimension, and 1.0–2.5 s second-dimension retention times). Compared with the orthogonal approach, there is now no problem of wrap-around (with, now, the non-polar analytes) and the peak shapes of the polar analytes are fully satisfactory. As an illustration, the zones containing the three alcohols of Fig. 2 are indicated in both GC × GC planes.

Finally, to demonstrate that no really satisfactory solution analyte to identification can be found by means of 1D-GC–ToF MS, 14 (out of 40) compounds that could not be found by the LECO software after 1D-GC–ToF MS are marked in Table 5. GC × GC was used, all compounds were identified with similarly high similarity/reverse values as given earlier in Table 3.

3.3. Analytical performance

The results presented in the previous sections allow us to conclude that the non-orthogonal approach is a powerful alternative GC × GC strategy, specifically for real-life applications in which polar analytes play a predominant role. The BP21 × BPX-35 combination was, therefore, also used to assess the potential of GC × GC for quantitative and routine analysis. To this end, relevant performance data were determined (Tables 4 and 5). The repeatability of the retention times of the analytes as well as their peak areas was calculated as the relative standard deviations (R.S.D.s) of six consecutive injections of the standard mixtures 2 and 3. The mean R.S.D.s for all compounds were found to be lower than 0.2 and 1.6% for the first- and second-dimension retention times, respectively. The R.S.D.s for the peak area measurements were also satisfactory; they were lower than 5% for all compounds. Linearities, expressed as the correlation coefficients of linear regression, r^2, were calculated from measurements

Table 5
Analytical performance data for selected standards in GC × GC–FID on CP-Wax 58 × BPX-35

Compound	R.S.D. (%) (n = 6)			r^{2a}	LOD (pg)
	^1t_R	^2t_R	Area		
2-Methylbutanal	0.1	1.1	5	0.9980	30
2-Methylpropanoic acid ethyl ester	0.1	1.6	5	0.9988	25
3-Pentanone	0.1	0.8	4	0.9988	10
1-Penten-3-one	0.1	1.2	4	0.9989	5
Butanoic acid ethyl ester	0.1	0.4	5	0.9990	15
2-Methylbutanoic acid ethyl ester	0.1	0.1	4	0.9994	20
Hexanal	0.1	0.9	4	0.9995	25
Ethylbenzene	0.1	0.4	3	0.9997	20
1-Butanol	**0.2**	**0.7**	**1**	**0.9999**	**10**
1-Penten-3-ol	0.2	0.7	1	0.9998	5
***cis*-3-Hexenal**	**0.1**	**1.1**	**3**	**0.9996**	**10**
Heptanal	0.1	0.4	3	0.9997	15
2-Methyl-1-butanol	0.2	0.5	2	0.9998	5
3-Methyl-1-butanol	0.2	0.8	2	0.9998	5
***trans*-2-Hexenal**	**0.1**	**1.2**	**3**	**0.9995**	**10**
1-Pentanol	0.2	1.1	2	0.9999	5
Acetic acid hexyl ester	0.1	0.8	3	0.9996	15
Octanal	0.1	0.5	3	0.9994	10
1-Octen-3-one	**0.1**	**0.6**	**1**	**0.9997**	**10**
Acetic acid-*cis*-3-hexenyl ester	0.1	0.5	3	0.9995	10
trans-2-Heptenal	0.1	0.5	2	0.9997	5
Acetic acid-*trans*-2-hexenyl ester	0.1	0.8	4	0.9995	10
1-Hexanol	0.2	0.5	2	0.9998	5
***cis*-3-Hexenol**	**0.1**	**1.2**	**2**	**0.9999**	**5**
Nonanal	**0.1**	**0.5**	**3**	**0.9996**	**10**
***trans*-2-Hexenol**	**0.1**	**0.9**	**1**	**0.9998**	**5**
Cyclohexylacetic acid ethyl ester	**0.1**	**0.4**	**5**	**0.9995**	**10**
3-Octen-2-one	**0.1**	**0.3**	**2**	**0.9997**	**5**
***trans*-2-Octenal**	**0.1**	**0.4**	**3**	**0.9996**	**5**
Acetic acid	0.2	0.7	3	0.9980	5
trans,*trans*-2,4-Heptadienal	0.1	0.7	1	0.9997	5
trans-2-Nonenal	0.1	0.5	2	0.9996	5
1-Octanol	0.1	0.9	1	0.9999	5
Butanoic acid	0.1	0.2	1	0.9998	5
1-Nonanol	**0.1**	**0.6**	**2**	**0.9998**	**5**
3-Methylbutanoic acid	**0.1**	**0.3**	**1**	**0.9998**	**5**
***trans*,*trans*-2,4-Nonadienal**	**0.1**	**0.5**	**2**	**0.9997**	**5**
Pentanoic acid	**0.1**	**0.2**	**1**	**0.9998**	**5**
trans,*trans*-2,4-Decadienal	0.1	0.6	2	0.9997	5
1-Phenyl-2-ethanol	0.1	0.7	1	0.9997	10

Bold: compounds not found by LECO software after 1D-GC–ToF MS and data processing (see text for details).

[a] Linear model; five calibration levels in duplicate (range, 0.005–5 ng/μL).

of five solutions in the 0.005–5 ng/μL concentration range. Tables 4 and 5 show that the results are gratifying, with all correlation coefficients being higher than 0.999 (except for four analytes in mixture 3: 0.9980–0.9989). The limits of detection (LODs) were calculated at a signal-to-noise ratio of 3:1 using GC × GC–FID in the non-orthogonal mode. As the tabulated data show, LODs of 5–30 pg can typically be expected for all flavour compounds. It should be added that LODs in GC × GC strongly depend on the number of modulations, analyte polarity and, of course, the column combination used [1]. Since the present system was optimised for polar analytes, the best results were obtained for these compounds.

4. Conclusions

In comprehensive two-dimensional GC, interesting separations are obtained not only under orthogonal, but also under non-orthogonal conditions – i.e., with polar × less/non-polar column combinations. This was demonstrated by analysing two different types of complex matrices. For diesel oil, the non-orthogonal approach also shows very clear ordered – but completely reversed – structures of alkanes, mono-aromatics and di-aromatics. Compared to the orthogonal approach, the groups are packed tighter together – which is an advantage when group-type determination is desired. With complex flavour mixtures in food, the non-orthogonal approach effected a considerable improvement of the peak shape of polar compounds such as aliphatic acids and alcohols. This also improved the, reverse-type, ordered structure of the chromatograms. The complementarity of the conventional and reverse GC × GC data can be considered a substantial help in the determination and identification of targets and/or unknowns in future studies.

More specifically, the present results and the fully satisfactory analytical performance data (linearity, repeatability, detectability) make reverse-type GC × GC with FID and/or ToF MS detection, a promising and flexible technique for the (screening) analysis of flavours and other classes of (semi-) polar compounds. Improving the performance of the technique by combining the reverse and conventional approaches in a single GC × GC system will be one of our next goals.

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