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Technical Report

Analysis of Perfume Allergens by using Comprehensive 2D GC and a Rapid-Scanning Quadrupole Mass Spectrometer

Luigi Mondello[1]

Abstract:

Comprehensive GC (GC×GC) falls into the category of multidimensional techniques, which achieve high separation power using two different type of columns. Since GC×GC requires the fast detector, more attention was devoted to TOF as its detector. The present study has confirmed the validity of the novel fast quadrupole MS system for reliable quantification purposes in GC×GC by the analysis of allergens in a commercial fragrance.

Keywords: Allergens, Perfume, GC×GC, Comprehensive GC, Quadrupole mass spectrometer

1. Introduction

Comprehensive gas chromatography, namely GC×GC, is the last destination reached by separation science. It falls into the category of multidimensional techniques, mainly due to the association of two different mechanisms of separation. Compared to the more known MDGC (Multidimensional Gas Chromatography), GC×GC is more "comprehensive" toward the separation process involving sample analytes; in other words, this means that in GC×GC, every portion of the eluate coming from the primary (1D) column undergoes a further 2D separation, instead of MDGC, where the most common method utilized, "heart-cutting"[1–4], transfers only selected portions of eluate from the first to the second dimension. Subjecting the entire sample to a double separation process becomes a necessary conditions to achieve a GC×GC separation. Also, analytes separated in the first column must remain separated when passing to the second column. These two analytical phenomena, that make GC×GC a unique separation technique, can be achieved through the "core" of all GC×GC instrumental set-ups: the modulator, which acts as a living interface between the two columns or dimensions of separation.

Basically, a comprehensive GC apparatus exploits two different stationary phases (the most common set is non-polar, with conventional dimensions, and a polar, characterized by "fast" features), located in the same or in separate GC ovens. The modulator is placed between the 1D column exit and the 2D column inlet and its functions are to trap, isolate, focus and reinject the bands of 1D eluate in the 2D column. Samples are normally injected at the head of 1D column, they undergo separation, then, by means of the modulator, are diverted to the second dimension, where analytes undergo further separation; finally, they reach the detector, located at the exit of the 2D column.

The exhaustive transfer of a primary dimension eluting peak into the secondary column can be achieved with an appropriate modulation time, which is the time employed by the modulator for sampling (trapping and releasing) 1D peaks. Commonly, the modulation time, being in the order of seconds, is not sufficient to transfer an entire peak, but more reasonably slices of it, generating a series of 2D retention times. Such a separation mechanism adds a new dimension to the visual information that can be obtained by the analyst: the GC×GC chromatogram.

Compared to the conventional GC plots, in comprehensive GC the chromatogram is built up no longer on two (retention time vs. signal), but on three axes, adding an extra dimension consisting of 2D retention times[5]. Therefore, the look of GC×GC chromatograms appears completely different from conventional GC profiles, showing a bidimensional plane where analyte spots are scattered about.

Separations occurring in the second dimension are really fast (generally 4–8 s), therefore making the use of very fast detectors necessary. Modulated peaks are typically 100–600 ms wide at the baseline. The history of GC×GC detectors was initially dominated by the FID; however, the necessity of structural information led to the use of MS instruments also in comprehensive GC. Quadrupole MS systems were then quite slow, and therefore more attention was devoted to the faster TOF-MS instruments; for example, the latter were utilized in 83% of the published work in the 2006–2009 period. However, this doesn't imply a lack of capability of quadrupole systems for GC×GC separations or the absolute predominance and excellence of TOF-MS systems over quadrupole MS. It must be specified that even TOF-MS instruments suffer from some limits: high data acquisition frequency instruments lack resolution, *viceversa*, high mass accuracy instruments lack acquisition speed. Furthermore, before the introduction into the market of very fast scanning qMS instrumentation (today available), some successful alternative attempts have been made,

1 Department of Pharmaceutical Science and Health Products, University of Messina

such as using a restricted mass range (e.g. 50–245 *m/z*), enabling the generation of 33 spectra/s[6], or using the ECNI (electron-capture negative ionization, Perkin Elmer) mode with a 300 amu mass range[7]. Both cases can be considered as "rapid scanning" methods.

Recently, Shimadzu has introduced a fast-scanning qMS system (*GCMS-QP2010 Ultra*) capable of operating at a scan speed of 20,000 amu/s. This instrumentation has been evaluated by G. Purcaro *et al*. in 2010, in an application on perfume allergens[8]. The latter have become a matter of great concern in the last years, with the dramatic increase of cases of contact allergy due to the use of cosmetic products. Because of such an occurrence, the EU has issued a series of regulations, one in particular being the EU.

Directive 2003/15/EC that fixes specific limits for these substances in different types of cosmetic products[9]. In their investigation, G. Purcaro *et al*. exploited a particular column set, consisting of apolar and ionic liquid stationary phases. Ionic liquids (ILs) fall into a class of organic non-molecular solvents, generally consisting of an organic cation containing nitrogen or phosphorous (*e.g.* phosphonium) counterbalanced by an anion of either organic or non organic nature.

2. Experimental

2-1. Samples and sample preparation procedures

A commercial fragrance was purchased in Messina (Italy) and was injected neat, without any sample preparation. A solution of twenty-four allergens was supplied by Sigma-Aldrich (Milan, Italy), along with 1,4-dibromobenzene and 4,4'-dibromobiphenyl, used as internal standards. Six working solutions were prepared in methanol for calibration procedures.

2-2. Configuration of the instrument

GC×GC-qMS analyses were carried out on a Shimadzu *GC×GCMS-QP2010 Ultra* system. The GC was equipped with a split/splitless injector, an SLB-5ms column (30 m × 0.25 mm ID × 0.25 mm film thickness), which was connected through a 1.4 m × 0.25 mm ID uncoated capillary segment (double loop) to the custom-made secondary column SLB-IL59 (1.0 m × 0.1 mm ID × 0.08 mm film thickness), all provided by Supelco. A loop-type modulator was used (Zoex, Houston, TX). The software packages utilized for data handling were *GCMSsolution* (Shimadzu) and ChromSquare (Shimadzu Europa GmbH, Germany), while the mass spectral database utilized was the *FFNSC 2* (Shimadzu).

2-3. Method parameters

GC oven temperature program was from 50°C to 260°C at 5°C/min. Injection temperature was 250°C. Sample volume was 1.0 mL, injected in the split mode (1:10). Carrier gas (He) was delivered at an initial pressure of 140.0 kPa (constant linear velocity mode). Modulation period was 4.5 s and the hot pulse (325°C) duration was 375 ms.

Mass spectrometric parameters were as follows: full scan mode; scan speed of 20,000 amu/s; mass range of 40–330 *m/z*. Interface and ion source temperatures: 250°C and 200°C, respectively.

The standard mixture (about 10 mg/L for each compound) was tested twice at a 33 and 25 Hz acquisition rate. Six-point calibration curves were constructed, with 2 replicates for each point.

3. Results and Discussion

Fig. 1 shows the GC×GC-qMS chromatogram obtained for the commercial fragrance analyzed. Twelve allergens were determined, all of them reported on the label, therefore at concentrations higher than those prescribed by the EU regulations for such products. Table 1 reports the quantitative results obtained from the calibration curves together with method repeatability results.

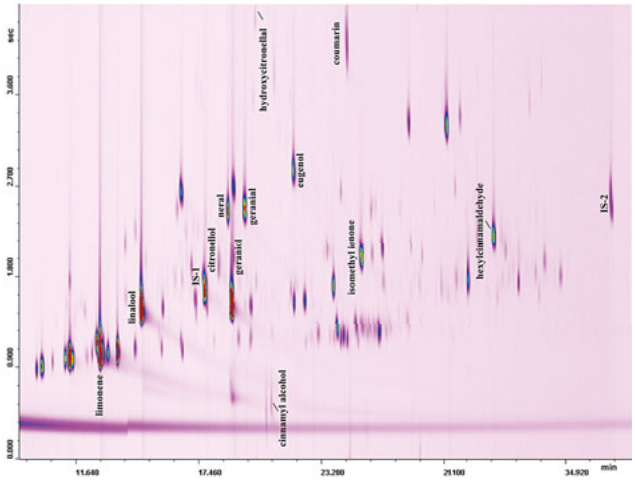

Fig. 1 GC×GC-qMS chromatogram of a commercial fragrance
The compounds denominated are allergens. IS-1 and IS-2 are the internal standards.
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Table 1 Allergens identified and quantified in a commercial fragrance (n = 3)[a].
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Compound	Concn (mg/L)	CV (%) (n=3)	1D t_R (min)	SD	2D t_R (min)	SD
limonene	4102.7	8.1	12.797	0.000	2.826	0.012
linalool	2721.1	5.3	14.755	0.001	3.288	0.047
citronellol	73.3	7.5	18.437	0.002	3.683	0.081
citral (neral)	455.1	3.0	18.895	0.001	4.232	0.065
geraniol	56.1	3.9	19.163	0.044	3.777	0.035
citral (geranial)	519.3	3.6	19.671	0.043	4.266	0.042
hydroxycitronellal	12.5	7.7	20.202	0.001	1.607	0.053
cinnamyl alcohol	54.8	11.4	20.889	0.002	2.304	0.071
eugenol	301.9	11.3	22.052	0.001	0.127	0.058
coumarin	187.9	1.7	24.622	0.001	1.319	0.046
isomethylionone	506.9	1.8	25.213	0.044	3.770	0.046
hexylcinnamaldehyde	462.1	2.3	31.492	0.001	3.984	0.042

[a] Retention times in the first and second dimensions (1D t_R and 2D t_R) are reported, along with the standard deviation (SD)

The chromatographic conditions were tuned in order to obtain optimal conditions. An acquisition frequency of 50 Hz was obtained without the need for a restricted mass range or use of the SIM operational mode: scan speed was 20,000 amu/s, interscan delay was no more than 5 ms and mass range was 40–330 *m/z*. Four compounds were chosen, and here reported in Table 2, for assessing the extent of peak reconstruction. As can be seen from the data reported in Table 2, it is possible to have more than 10 data points/peak when operating at 25 Hz only if the peak width is above 400 ms, condition that increases the number of data points/peak to more than 20 when operating at 50 Hz. Additional information reported in the table is relative to the intensity ratio between the target ion (T) and the qualifier ions (Q1 and Q2). Fig. 3 shows three plots, obtained at three different acquisition frequencies, relative to four parameters, namely the similarity match (MS%), the T/Q1 ratio, the T/Q2 ratio, the Q2/LM ratio (where LM is the lowest mass ion). The four peaks analyzed for their spectral quality showed constant MS% values at each data point and comparable T/Q ratios (limited peak skewing) under the different conditions investigated.

Table 2 Target Ion (T) and Qualifier Ions (Q1 and Q2) evaluated for each compound and Peak width at the baseline and at half-height and number of data points acquired for the main modulated peak of linalool, eugenol, lilial and benzyl salicylate

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					50 Hz				33 Hz				25 Hz			
compd	T	Q1	Q2	LM	peak width (ms)	no. of points	half-peak (ms)	no. of points	peak width (ms)	no. of points	half-peak (ms)	no. of points	peak width (ms)	no. of points	half-peak (ms)	no. of points
linalool	93	71	121	55	360	18	180	9	360	12	180	6	360	9	180	4
eugenol	164	103	149	55	480	24	240	12	480	16	240	8	480	12	240	6
lilial	189	147	204	57	420	21	190	10	420	14	180	6	360	9	180	5
benzyl salicylate	91	228	65		480	24	240	12	480	16	240	8	480	12	240	6

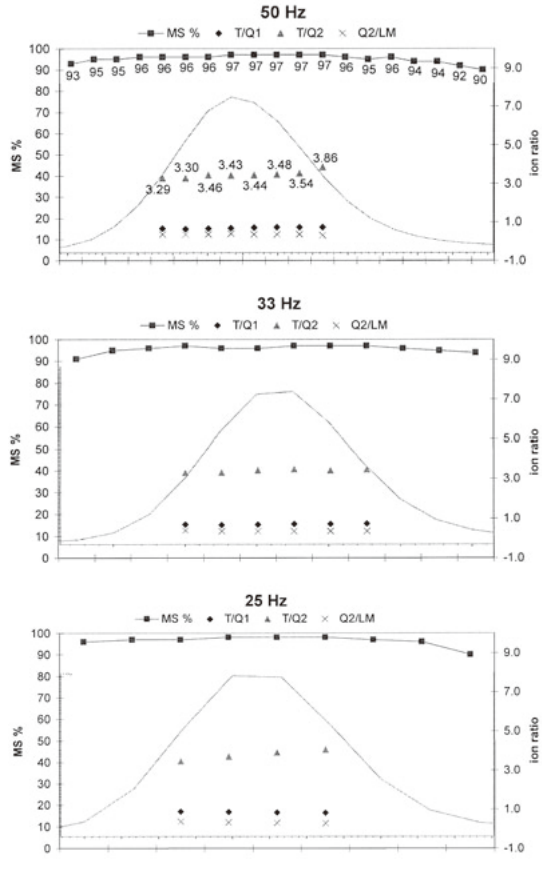

Fig. 3 Spectral quality evaluation of linalool at 25, 33 and 50 Hz in terms of mass spectrum similarity (MS%) at each data point acquired and ion ratios across the width at half-height: T, 93 *m/z*; Q1, 71 *m/z*; Q2, 121 *m/z*; LM, 55 *m/z*. In the 50 Hz graph, MS and T/Q2 values at each data point acquired are shown.

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4. Conclusion

The present study has confirmed the validity of the novel quadrupole MS system for reliable quantification purposes in GC×GC. Satisfactory peak reconstruction was obtained with more than 15 data points/peak, demonstrating that the qMS system developed is a new and alternative option to the predominant use of TOF-MS instruments. It has been clearly demonstrated that even an acquisition frequency of 33 Hz can be sufficient for the achievement of proper peak reconstruction.

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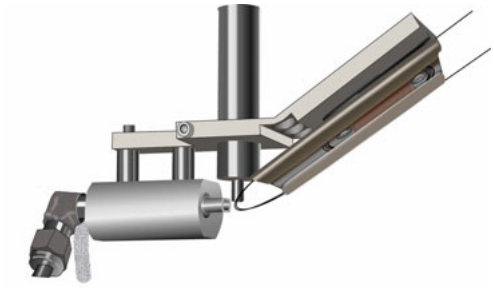
[9] Directive 2003/15/EC of the European Parliament and of the Council of 27 February 2003.

(This article is extracted from "GC×GC Handbook" written by Luigi Mondello.)

Shimadzu GC×GC-QP System

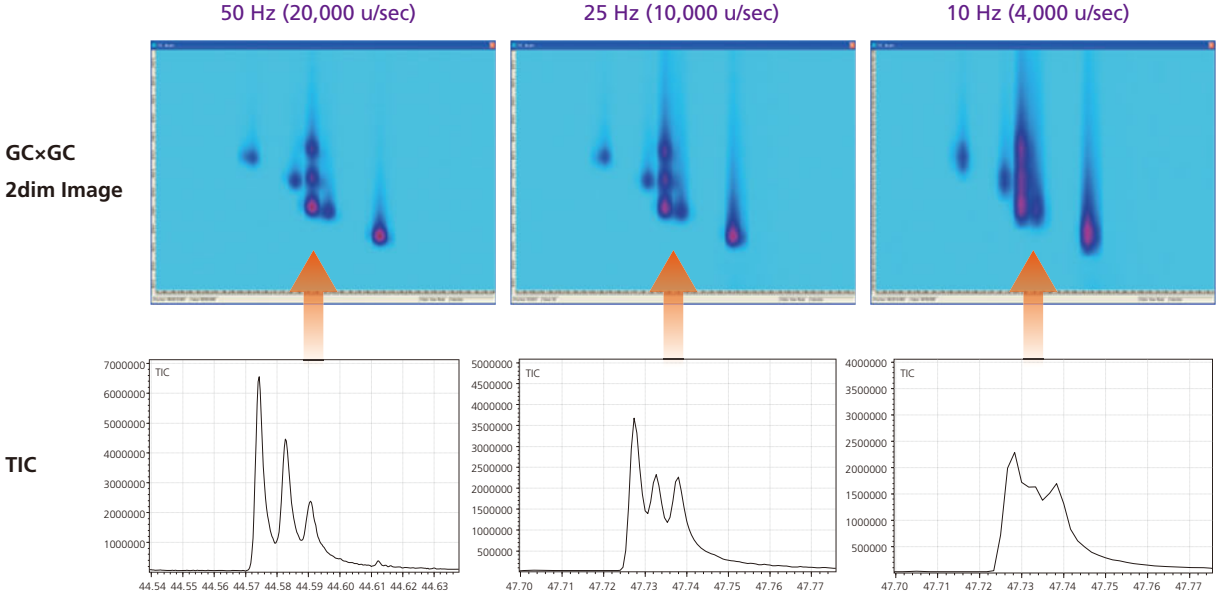


Zoex ZX1 2stage thermal modulator providing excellent modulation.

Shimadzu GCMS-QP2010 Ultra makes possible to obtain the data with high scan speed up to 20,000 u/sec.

The 2-dim chromatograms of fatty acids and scan speed

The high scan speed of GCMS-QP2010 Ultra has the potential of increasing the separation power of the second dimension, that promotes applicability of high sensitive, user friendly and economical quadrupole mass spectrometer to GC×GC-MS analysis.



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