Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1067 (2005) 245–254
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Comprehensive two-dimensional gas chromatography coupled to a rapid-scanning quadrupole mass spectrometer: principles and applications

Mohamed Adahchour*, Menno Brandt, Hans-Ulrich Baier[1], René J.J. Vreuls, A. Max Batenburg[2], Udo A. Th. Brinkman

Department of Analytical Chemistry and Applied Spectroscopy, Vrije Universiteit, de Boelelaan 1083, 1081 HV Amsterdam, The Netherlands

Available online 6 November 2004

Abstract

The principles, practicability and potential of comprehensive two-dimensional (2D) gas chromatography coupled to a rapid-scanning quadrupole mass spectrometer (GC × GC–qMS) for the analysis of complex flavour mixtures in food, allergens in fragrances and polychlorinated biphenyls (PCBs) were studied. With a scan speed of 10,000 amu/s, monitoring over a mass range of up to 200 atomic mass unit (amu) can be achieved at an acquisition frequency of 33 Hz. Extending this mass range and/or increasing the data acquisition frequency results in a loss of spectral quality. Optimal parameter settings allow, next to unambiguous identification/confirmation of target compounds on the basis of high-quality mass spectra, fully satisfactory quantification (three to four modulations per peak) with linear calibration plots and detection limits in the low-pg level. The potential of time-scheduled data acquisition to increase the effective mass range within one GC × GC run was also explored. The analyses, with baseline separation of the flavours, allergens and PCB target compounds, took less than 30 min.

Keywords: Gas chromatography comprehensive two-dimensional; Detection, GC; Quadrupole mass spectrometer; Flavours; Allergens; Polychlorinated biphenyls

1. Introduction

Comprehensive two-dimensional (2D) gas chromatography (GC × GC) is gaining wide interest and increased acceptance due to its ability to separate and identify analytes in complex samples [1–3]. Impressive results have been obtained in terms of separation efficiency and, also, compound classification based on the presence of ordered structures in GC × GC chromatograms [4,5]. For GC × GC, next to column selection and modulation, detection is also an important consideration. The very fast separation in the short and narrow two-dimension column results in peak widths of, typically, 80–600 ms at the baseline, and in some cases widths as low as 45 ms have been reported [6]. In order to ensure a proper monitoring of these narrow peaks, fast detectors, with a small internal volume and a short detector rise time and a high data acquisition rate, are required. Until recently, the flame ionisation detector (FID) with its high acquisition rate of 50–200 Hz dominated the detection field in GC × GC. Today, micro electron-capture detectors (μECD) with, typically, a 50 Hz sampling rate, are recognised as valuable alternatives, specifically for the many applications dealing with polychlorinated target analytes [4,7].

However, the above detectors do not provide structural information. In conventional GC, mass spectrometry (MS) is nowadays widely used for detection and identification purposes in development as well as in routine laboratories. The main mass analysers are the ion-trap, the sector, the quadrupole (qMS) and the time-of-flight mass spectrometers (ToF MS). There are large mutual differences as regards acquisition rates, detection limits, resolution and quality of the mass spectra obtained. At present, of the four

* Corresponding author. Tel.: +31 20 444 7525; fax: +31 20 444 7543.
E-mail address: m.adahchour@few.vu.nl (M. Adahchour).
[1] Shimadzu Deutschland, Albert-Hahn Straβe 6-10, 47269 Duisburg, Germany.
[2] Unilever Research Laboratory Vlaardingen, Olivier van Noortlaan 120, 3133 AT Vlaardingen, The Netherlands.

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.09.094

types of commercially available mass spectrometers, only a ToF MS can acquire the fifty or more mass spectra per second that are required for the proper monitoring of GC × GC chromatograms and, more importantly, for quantification. Admittedly, the extremely popular benchtop qMS systems are much less expensive and more user-friendly, and several authors have reported their use with GC × GC under appropriate operating conditions [8–12]. However, in these studies it was difficult to perform quantification due to the slow quadrupole duty cycle and the need to scan individual masses in the scan range, which results in slow data acquisition.

In the present investigation, the performance of a modern qMS system for use in GC × GC was studied for both qualitative and quantitative purposes. These modern quadrupole instruments have scan speeds of up to 10,000 amu/s and can also meet the 50 Hz requirement, provided proper mass range settings are selected. The principles of coupling a qMS to a GC × GC system are discussed in terms of data acquisition rate, mass range and mass spectral quality. In addition, three different application areas—flavours in food, allergens in perfumes and PCB analysis—were selected to evaluate these principles, i.e. to study the capabilities of the GC × GC–qMS system for the identification and determination of target compounds in complex samples.

2. Experimental

2.1. Analytes and samples

Two standard mixtures were used in the present study. Mixture 1 containing 25 allergen compounds (for compound names, see Table 1), was dissolved in isooctane and standard solutions were prepared over the concentration range 2–50 mg/L. Perfume samples were diluted 1:1000 (v/v) with isooctane prior to injection. Mixture 2 containing 25 flavour compounds (for compound names, see Table 1) found to be responsible for the odour of olive oil samples (see Section 3.4.1), was dissolved in freshly distilled methyl acetate. All 95–99% pure standards were from the Unilever Research Laboratory, which also provided various olive oil extracts in diethyl ether.

High-vacuum degassing (HVD), which is a suitable technique to isolate flavour compounds from fat or oily matrices under mild conditions, was used to isolate the volatile flavours from the extract [13]. The olive oil samples were subjected to HVD at room temperature 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 diethyl ether or pentane. In order to avoid losses of volatiles, 1 μL of each final extract was injected in the GC system without any pre-concentration.

Table 1
Retention time data of the flavour and allergen standard compounds

Flavours	Quantification mass (amu)	^1t_R (min)	^2t_R (s)	Allergens	No.	Quantification mass (amu)	^1t_R (min)	^2t_R (s)
2-Methyl-1-butanol	70	6.0	1.39	Benzyl alcohol	1	108	8.5	3.17
Ethyl isobutyrate	116	6.2	0.76	Limonene	2	68	8.7	0.64
Butanoic acid	60	6.7	5.15[a]	Linalool	3	93	9.8	0.92
Hexanal	72	6.7	1.33	Methyl 2-octynoate	4	95	11.1	1.07
Ethyl butyrate	88	6.8	0.88	Citronellol	5	69	11.7	0.93
trans-2-Hexenal	98	7.8	1.79	Citral (neral)	6	69	11.8	1.18
Isovaleric acid	60	7.9	4.82[a]	Geraniol	7	69	12.1	2.45
Ethyl-2-methylbutyrate	102	7.9	0.82	Cinnamic aldehyde	8	131	12.1	1.06
trans-2-Hexenol	82	8.4	2.00	Citral (geranial)	9	69	12.2	1.13
1-Hexanol	69	8.6	1.67	Anisyl alcohol	10	108	12.4	3.26
Pentanoic acid	60	8.8	5.33[a]	Hydroxycitronellal	11	59	12.4	1.83
Heptanal	70	9.0	1.18	Cinnamyl alcohol	12	92	12.7	2.82
trans-2-Heptenal	83	10.5	1.70	Eugenol	13	164	13.6	1.64
1-Octen-3-one	70	11.2	1.24	Coumarine	14	146	14.5	3.72
Octanal	84	11.9	1.09	Isoeugenol	15	164	14.8	1.86
trans-2,4-Heptadienal	81	11.9	2.52	α-Isomethylionone	16	135	15.5	0.81
Hexylacetate	84	12.3	0.85	Lilial	17	189	15.9	0.92
3-Octen-2-one	111	12.9	1.58	Amyl cinnamic aldehyde	18	202	17.5	0.92
trans-2-Octenal	70	13.5	1.58	Amyl cinnamic alcohol	19	133	18.0	1.13
1-Octanol	70	14.4	1.33	Farnesol 1	20	81	18.2	0.74
Nonanal	98	14.9	0.97	Farnesol 2	21	93	18.4	0.77
β-Phenylethyl alcohol	122	15.4	0.85	Hexyl cinnamic aldehyde	22	216	18.7	0.92
Ethyl cyclohexanoate	101	15.9	0.88	Benzyl benzoate	23	105	18.8	1.35
trans-2-Nonenal	83	16.6	1.39	Benzyl salicylate	24	91	20.0	1.26
1-Nonanol	70	17.4	1.12	Benzyl cinnamate	25	131	22.2	1.43

For GC×GC conditions, see Section 2.
[a] Compounds showing wrap-around.

2.2. GC × GC–qMS system

GC × GC–qMS was performed on a Shimadzu Model 2010 gas chromatograph equipped with an AOC-20s autosampler, an AOC-20i autoinjector and a QP2010 quadrupole mass spectrometer (Shimadzu Beneluxs Hertogenbosch, The Netherlands). GCMS solution software (Shimadzu) was used to control the GC–MS instruments and to acquire data. Unless stated otherwise, the qMS was operated at a storage rate of 33 Hz using a mass range of *m/z* 50–245 and a scan speed of 10,000 amu/s. One microliter injections were done in the splitless (1 min) mode at 250 °C. The interface and ion source temperatures were 250 and 230 °C, respectively. The ionisation energy was 70 eV and the multiplier was set at 1.5 kV. The solvent delay was 5 min.

A 27 m × 0.32 mm i.d. × 0.32 μm BP1 film (100% methylpolysiloxane) column (SGE Europe, Milton Keynes, UK) was used as the one-dimension column, and a 1 m × 0.1 mm i.d. × 0.1 μm VF-23 ms film (high cyano-containing polymer, with absolute cyano content 70–90%) column (Varian-Chrompack, Middelburg, The Netherlands) as the two-dimension column. The two columns were connected with a press-fit connector.

Unless stated otherwise, the following conditions were used: the carrier gas was helium (99.999% purity; Hoekloos, Schiedam, The Netherlands) at a constant pressure of 100 kPa; the temperature of the two GC columns, which were housed in the same oven, was programmed from 40 to 140 °C at 5 °C/min and from 140 to 280 °C (1 min hold) at 35 °C/min for flavour analysis, from 50 (1 min hold) to 280 °C (1 min hold) at 10 °C/min for allergen analysis, and from 60 (1 min hold) to 210 °C at 30 °C/min and at 5 °C/min to 290 °C (10 min) for PCB analysis.

Cryogenic modulation was performed using a laboratory-made CO_2-cooled dual-jet modulator [14]. Cooling was effected through the Joule–Thompson effect of expanding liquid CO_2 (technical grade; HoekLoos). Briefly, two short sections of the two-dimension column are directly and alternately cooled in order to trap and focus each subsequent first-column effluent fraction which is, next, remobilized by the heat from the surrounding oven air. A modulation time of 4 s was used in all analyses and initiated by the GCMS solution-programmed external events which, via the electronic controller, also starts the modulator operation. The modulator temperature was kept about 100 °C below the oven temperature.

For data transformation and visualization three additional programmes were used, a programme to convert the raw MS data into an ASCI file (Shimadzu), a programme to convert this ASCI file 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 × GC separation

GC × GC–qMS analyses were performed on a non-polar × polar BP1 × VF-23 ms column combination system. The chromatographic conditions were optimised to enable analysis in less than 30 min. All target compounds listed in Table 1, which presents their retention data, were baseline separated in the 2D separation space. Separation of all compounds present in the mixtures was achieved after a limited number of trial runs (two to three trials), which is in marked contrast with the time-consuming—and less satisfactory—1D-GC operation [15,16]. Rather than a tedious 1D optimisation to separate, for example *trans*-2,4-heptadienal and *cis*-3-hexenylacetate (five other pairs in both mixtures showed coelution), the selection of a 30 min gradient and the present column combination give the same optimal, i.e. baseline separation results. The purpose of the present paper is not to discuss the optimisation procedure but, rather, to highlight the benefits of GC × GC–qMS for the trace-level analysis of flavour and allergen compounds. Briefly, the two columns and the modulation time were selected to give good separation in both dimensions with minimum (or no) wrap-around phenomena.

All compounds eluted within 25 min from the one-dimension column and within 4 s from the two-dimension column, except the three acids marked by an asterisk in Table 1. Using, as an alternative, a polyethylene glycol (Carbowax) stationary phase in the two dimension caused excessive retention – not only for the acids but also for other (classes of) polar compounds in the two mixtures, which is due to their strong interaction. Wrap-around, difficult detectability and, hence, poor quantification are the consequences of this excessive retention [15,17]. In addition, since the Carbowax phase can be used only up to a temperature of 250–260 °C, the application range is rather limited.

3.2. qMS detection in GC × GC

Because of the very narrow peaks generated in GC × GC, the detector requirements are pushed to their limits. Therefore, examination of the scanning capabilities of a mass spectrometer is necessary to assess the feasibility of obtaining adequate mass spectral data. The data acquisition rate, which is critical in ensuring data integrity, should be sufficiently high to: (i) accurately reconstruct the chromatogram from the consecutively recorded spectra; and (ii) avoid/minimise changes of the sample concentration in the ion source during the time needed to acquire a spectrum: there should be no mass-spectral skewing, but the relative peak intensities in a given spectrum should be constant. The higher the acquisition frequency, the greater the number of data points that is available to define the chromatographic profile, and the better the accuracy and precision of quantitative analyses. There are rather striking discrepancies in the literature concerning the number of points actually needed to define a chromato-

Table 2
Data points per peak (dppp) and R.S.D. (%; $n = 6$) of peak area obtained at four different data acquisition rates (scan/s; Hz)

Analyte		50 (Hz)		33 (Hz)		25 (Hz)		20 (Hz)	
Name	$W_{6\sigma}$ (ms)[a]	dppp	R.S.D.	dppp	R.S.D.	dppp	R.S.D.	dppp	R.S.D.
Limonene	200	10	3	7	3	5	7	4	10
Linalool	270	13	4	9	3	7	5	5	9
Hydroxycitronellal	280	14	3	9	3	7	4	5	12
3-Octen-2-one	360	18	3	12	4	9	4	7	5
Coumarine	450	22	3	15	3	11	3	9	3

[a] Peak width measured in chromatograms.

graphic peak [19]. To quote a recent communication [18]: on the opinions of different authors, 15–20 or as few as 3–4 points are said to be required or claimed to meet quantitation needs [20–24]. Other issues further complicate this situation. For instance, it is not always clear if full width at half maximum (FWHM) or full peak width at baseline ($W_b = 6\sigma$) are used in the discussions, and if the baseline points at the beginning and end of the peak should be included or not.

Careful selection of the experimental conditions is necessary to achieve satisfactory qualitative and quantitative results. The evaluation presented below will therefore highlight the interdependence of scan speed, data acquisition frequency and mass range, and their effect on number of data points across a peak, spectral quality and analyte detectability.

3.2.1. Required data acquisition rate

3.2.1.1. Required number of data points per peak and mass range. The influence of the data acquisition rate on the precision of peak-area measurements was experimentally determined by injecting the standard solutions (see Section 2 and Table 1) and varying the data acquisition rate between 20 and 50 Hz. For these experiments, the mass range selected, for example, 33 Hz was *m*/*z* 50–245, which covers the quantification masses of all analytes in the two mixtures (cf. Table 1). In addition, the qMS was operated at its maximum scan speed of 10,000 amu/s. The interscan dead time was 10.4 ms.

Since two-dimension peak widths increase with retention time (isothermal chromatograms), varying the frequency over the 20–50 Hz range corresponded with generating 4–10 scans across the two-dimension peak of limonene—the least retained compound in the two mixtures (see Table 1)—while for coumarine, the one of the most retained compound, there were 9–22 scans (Table 2). It should be noted that one of the baseline points is not included in the resulting rounded number. In our opinion it is advisable to count only the points that show up above the baseline, because they actually define the peak profile. Similar results as presented in Table 2 were also obtained for all other test analytes of Table 1. That is, if seven or more data points are recorded across a peak, the relative standard deviation (R.S.D.) of the peak-area measurements becomes 5% or less. The relevant results which are included in Table 2 agree with earlier observations [23]. The effect of too low a number of data points on peak-area R.S.D.s is demonstrated for limonene, linalool and hydroxycitronellal at 20 Hz and for limonene at 25 Hz. For the present study, this clearly indicates that a data acquisition rate of over 25 Hz is required to calculate peak areas with a fully satisfactory precision.

An alternative way to express the acquisition potential of the rapid-scanning qMS, is by studying the beneficial effect of a reduced scan range on the number of data points generated across a peak. Table 3 shows some examples of the interdependence of minimum $W_{6\sigma}$, maximum mass range and available scan time for various acquisition rates. For a brief explanation, when using an acquisition rate of 33 Hz, the qMS can be operated over a scan range of 195 amu, which is sufficient to determine target analytes of the present study, and for many other—though, of course, not for all (see Section 3.3)—routine analyses. In this case, the smallest $W_{6\sigma}$ that is allowed is about 200 ms. This is not an obstacle for the present study, as indicated by the limonene data in Table 2. If a wider mass range is required—e.g. for identification purposes—an acquisition rate of 25 or 20 Hz has to be selected. This is, indeed, possible, but—as was outlined above—peak-area R.S.D.s rapidly become too large, and quantification will be adversely affected. On the other hand, if much faster, i.e. narrower ($W_{6\sigma}$ ca. 150–200 ms) peaks have to be determined, a rate of 50 Hz is required but, then, the mass range does not exceed 100 amu.

The selection of the data acquisition rate also affects analyte detectability. Comparison of analyses run, under otherwise the same conditions, at 50 and 33 Hz showed a general loss of analyte detectability (i.e. reduction of S/N ratios) over the whole volatility range. However, the loss was rather limited, i.e. 10–20%. Finally, it should be emphasised that, if mass ranges were selected wider than permitted for a specific acquisition rate according to Tables 2 and 3, the whole

Table 3
Interdependence of peak width, mass range and scan time for different acquisition rates[a]

Parameter	50 (Hz)	33 (Hz)	25 (Hz)	20 (Hz)
Minimum $W_{6\sigma}$ (ms)[b]	140	210	280	350
Maximum mass range (amu)	95	195	295	395
Scan time (ms)[c]	9.6	19.6	29.6	39.6

[a] Scan speed, 10,000 amu/s: reset time, 10.4 ms.
[b] Peak width required for 7 dppp.
[c] Scan time = total cycle time − reset time; e.g. 50 Hz, the cycle time is 20 ms—with a reset time of 10.4 ms, the available scan time then is 9.6 ms.

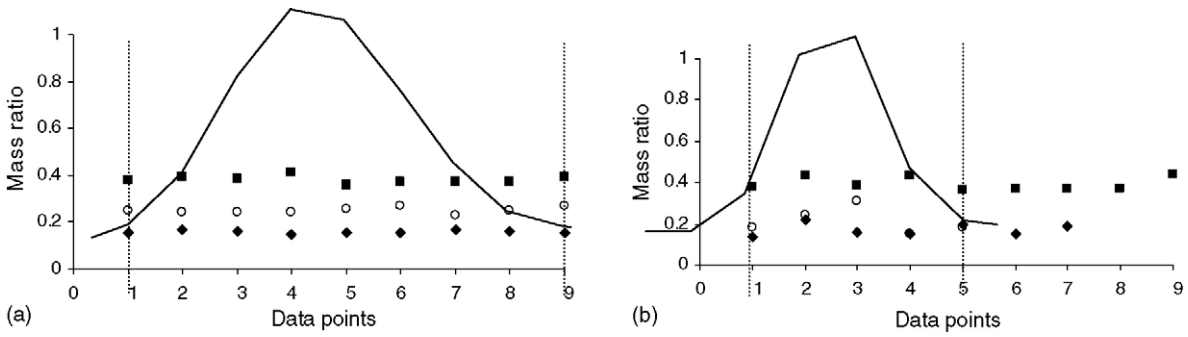

Fig. 1. Mass ratios of three *m*/*z* pairs, (◯) 136/121, (■) 146/89 and (♦) 126/111 in the spectra of linalool, coumarine and 3-octen-2-one, respectively; peaks acquired at data acquisition rates of: (a) 33 Hz and (b) 20 Hz. The peak profile of linalool is shown in both cases; the dashed lines indicate the start and end of the peaks.

data storage process started to malfunction and mass spectral quality was essentially lost. Spectra could not be retrieved nor recognised at all.

3.2.1.2. Quality of the mass spectra. As a final check of the performance of the qMS, the quality of the mass spectra was studied. It is well known that scanning of fast-eluting peaks can cause skewing of the mass spectra. Skewing can be adequately described [25] by plotting the ratios of the masses of several abundant ions in a spectrum. Fig. 1 shows data for three analytes also included in Table 2, viz. linalool (*m*/*z* pair 136/121), 3-octen-2-one (*m*/*z* pair 126/111) and coumarine (*m*/*z* pair 146/89); all spectra recorded during elution of the peaks at 33 and 20 Hz were considered. For further illustration, the peak profiles of linalool recorded at 20 and 33 Hz (5 and 9 data points, respectively) are included in the figure.

Fig. 1a clearly illustrates that when 33 Hz is used, the mass ratios are essentially constant in all three instances: the calculated R.S.D.s of 6, 5 and 4% for linalool, 3-octen-2-one and coumarine, respectively, are fully acceptable. Similar results were obtained for all other analytes in the two standard mixtures: the R.S.D.s were less than 7%, even for the narrowest peaks, which have the highest values.

For the most retained analyte, coumarine, with a baseline peak width of about 450 ms, enough data points per peak are acquired even at 20 Hz (Fig. 1b), and the slightly higher R.S.D. of 7%, is still satisfactory. For linalool, however, the least retained analyte with a peak width of about 270 ms, only five data points were recorded at 20 Hz; the mass ratio started to vary considerably, as indicated by an R.S.D. of 32%. An R.S.D. of 17% was obtained for 3-octen-2-one, which has an intermediate two-dimension retention time. In other words, under the present, rather conventional, GC × GC conditions, 20 Hz causes some mass spectral distortion for the narrowest peaks, but distortion is essentially absent if a properly increased acquisition rate of 33 Hz is used. Indeed, when the experimental mass spectra recorded at 33 Hz were compared with those in the NIST library, similarity indices typically ranged from 94% at low peak abundances to 98–99% at peak

Table 4
Analytical data of selected analytes[a]

Compound	Quantification mass (amu)	Detection limit (pg)	R^2	R.S.D. (%; $n = 6$) of peak area
Flavours				
3-Octen-2-one	111	4	0.9978	6
trans-2,4-Heptadienal	81	10	0.9992	4
Ethyl-2-methylbutyrate	102	1	0.9957	6
Nonanal	98	4	0.9981	5
trans-2-Nonenal	83	20	0.9951	7
Allergens				
Linalool	93	3	0.9987	6
Anisyl alcohol	108	10	0.9978	7
Eugenol	164	6	0.9995	4
α-Isomethylionone	135	2	0.9991	5
Benzyl benzoate	105	5	0.9989	6
CBs				
CB 79	292	1.5	0.9978	7
CB 104	326	1.5	0.9988	6
CB 192	394	2.0	0.9996	5

[a] Acquisition rate, 33 Hz; six concentrations in 0.05–5 ng/μL (flavours), 2–50 ng/μL (allergens) or 0.01–1 ng/μL (CBs) range ($n = 2$); mass range, 50–245 amu for flavours and allergens and 50 amu for each window for CBs; see text for more details.

maxima even without background correction. This demonstrates that the rapid-scanning qMS can be used to sample the rapidly changing analyte concentrations of narrow peaks as encountered in GC × GC.

3.2.2. Limits of detection and linearity

Table 4 summarises relevant data on analyte detectability and linearity for a selected number of analytes in the three standard mixtures. Calibration plots were constructed over the 0.05–5, 2–50 and 0.01–1 ng/μL ranges for flavours, allergens and CBs, respectively. The plots showed good linearity for all analytes with regression coefficients of at least 0.995 (6 data points; $n = 2$). The detection limits (S/N = 3) of 1–20 pg for the flavours, 2–10 pg for the allergens and 1–2 pg for the CBs are fully satisfactory. The same is true for the R.S.D. values of 4–7% ($n = 6$) calculated for peak area measurements, specifically because one should consider that, for these calculations, 2–5 two-dimension peak areas have to be summed (also see [26]).

The retention times were found to be very stable with R.S.D.s at or below 0.1% ($n = 6$) in the one dimension and 0.5% ($n = 6$) in the two dimension, respectively. The early eluting compounds showed the highest R.S.D.s.

3.3. Applications

3.3.1. Flavour compounds in olive oil

In flavour analysis, aroma-active compounds (key flavour compounds) are usually present in (very) low concentrations in complex matrices. In such a situation, both powerful separation and detection techniques are needed [27]. As a typical example, Fig. 2 illustrates the determination of 3-octen-2-one in an extract of olive oil. It is obvious that the extract contains a number of matrix components at high concentrations: intense peaks and spots show up in Fig. 2a and b which display the full-scan 1D-GC–qMS and GC × GC–qMS chromatograms, respectively. In contrast with GC × GC, in 1D-GC these matrix compounds completely obscure the analytes of interest. For example, 3-octen-2-one has exactly the same retention time as benzyl alcohol when separated on the BP1 column (Fig. 2d) and could not be identified by 1D-GC–qMS. Even after baseline subtraction no clean mass spectra could be obtained and the alcohol was the compound actually identified (Fig. 2g and g'). The fully satisfactory outcome after GC × GC is demonstrated in Fig. 2c, e and f, which show baseline separation of 3-octen-2-one not only from the major peak (benzyl alcohol) but also from other minor peaks. Furthermore, 3-octen-2-one shows a good peak shape and there is a sufficient number of data points (12 data points). The mass spectrum was indeed of good quality and 3-octen-2-one could be identified with a similarity match factor of 98% (Fig. 2h and h'). Similarly, good results were obtained for the other key flavours in the mixture.

Finally, the separation created by GC × GC combined with the satisfactory detection potential of rapid-scanning qMS enabled reliable quantification. With *m*/*z* 111 as quantification mass for 3-octen-2-one, a peak area versus concentration plot was constructed for the GC × GC analysis. The calibration plot was linear, as indicated in Table 4. In the sample extract of Fig. 2, 3-octen-2-one was found to be present at 11 ng/g.

3.3.2. Allergens in fragrances

Another interesting example is the determination of allergens in fragrances, which are very complex mixtures. Frequently, separation of all target compounds cannot be achieved by 1D-GC, and laborious sample-treatment procedures are needed even with MS detection. The separation of the fragrance ingredient α-isomethylionone from α- and β-ionone is a well-known example [15,16]. Fig. 3 shows the contour plot of the separation of our allergen standard mixture. All peaks are sufficiently separated from each other (cf. Table 1) and a major part of the 2D plane is used for separation. Moreover, the experimental conditions selected for this analysis ensured that all analytes elute during their own modulation cycle (which is the preferred outcome in terms of GC × GC efficiency) and that their identification yielded no problems. The insert shows a blow-up of the region of the GC × GC chromatogram of a perfume sample, in which α-isomethylionone (no. 16) and lilial (no. 17) elute. Even in this much more complex situation, the problem of co-elution is solved for both target compounds. Consequently, quantification and identification should meet with no difficulties—a sheer impossibility in 1D-GC, as the same insert indicates, unless the target analyte has a unique mass which will enable quantification. Giving emphasis to identification, the bottom part of Fig. 3 demonstrates that, due to the improved separation, high-quality mass spectra are obtained for both α-isomethylionone (A versus library spectrum in B) and lilial (C versus library spectrum in D). In the present set-up, the spot eluting just above α-isomethylionone could be identified as α-ionone, based on the spectrum obtained (base peak, *m*/*z* 177) and the correct mass ratios of *m*/*z* 177/192, 177/159 and 177/149.

3.3.3. Polychlorinated biphenyls (PCBs)

In earlier sections, it has been demonstrated that, under properly selected conditions, mass ranges of some 200 amu can be handled with the present instrumental set-up. While this certainly is enough for most applications, exceptions do occur. A good example is the analysis of the mixture of all 209 CB congeners with their *m*/*z* 188–494 mass range. One can then use the 'group-option' available in the Shimadzu software and optimise the time schedule for each of the *m*/*z* windows. When using this option, no problems were encountered as regards mass spectra quality even during switching from one window to the next. Obviously, the present qMS switches very fast. Taking into account the roof-tile structure of the CBs in the 2D plane [4,28]—i.e. the ordering of the individual congeners along separate parallel lines for each number of chlorine substituents—one can limit the mass range of each window to 50–100 amu; the desired 33–50 Hz acquisi-

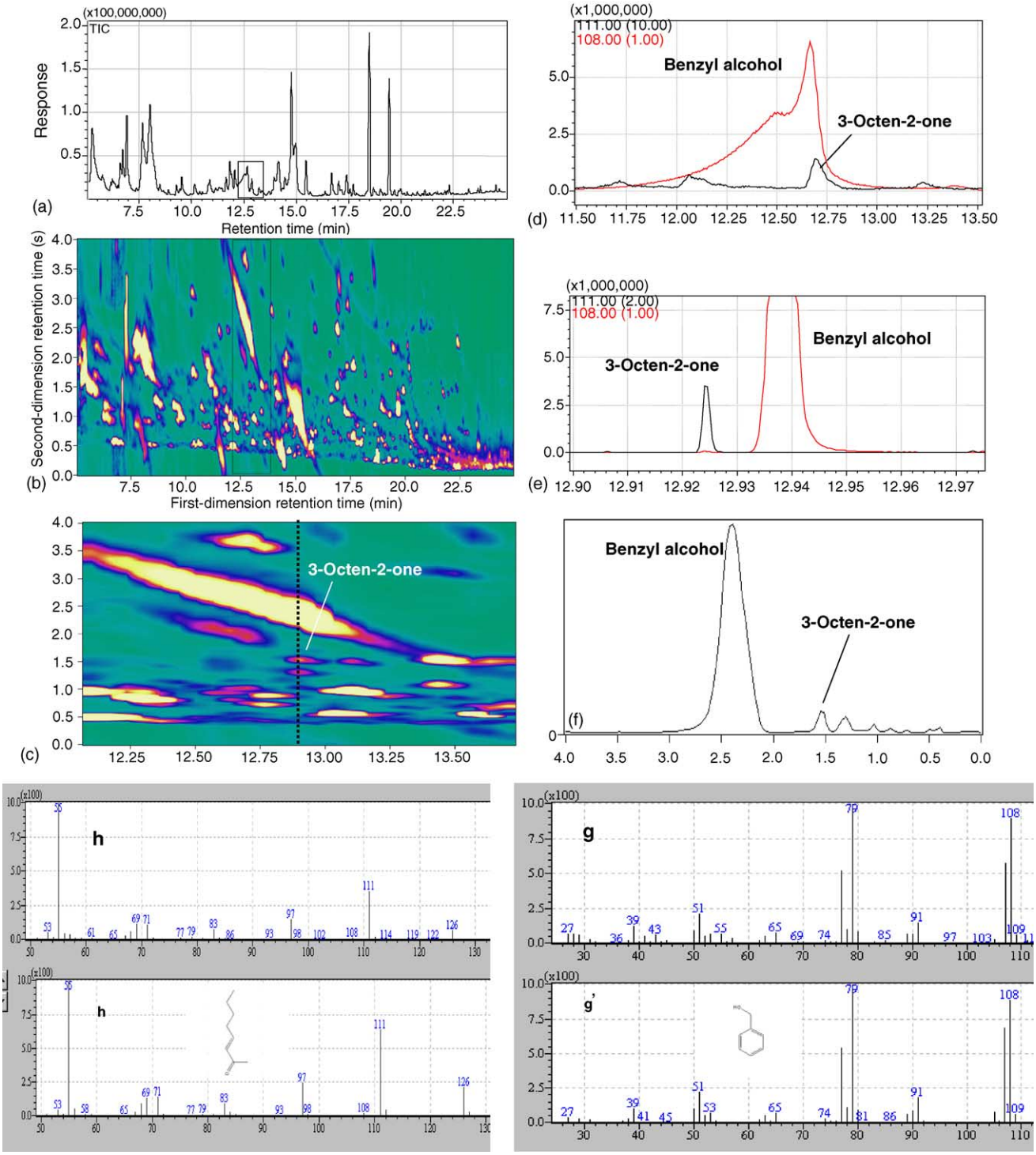

Fig. 2. (a) 1D-GC–qMS and (b) GC × GC–qMS TIC chromatograms (*m/z* 50–245) of an olive oil extract. (c) Blow-up of area marked in (b). (d and e) *m/z* 108 and 111 traces of the marked area in (a) without and with modulation, respectively. (f) Two-dimension chromatogram across the dotted line in (c). (g and h) Mass spectra of 3-octen-2-one from (a) and (b), respectively; (g') and (h') are the corresponding library spectra. Conditions: 1D-GC on BP1 column; GC × GC on BP1 × VF-23 ms columns. For further details, see text.

tion rate is then easily achieved. This mass range had to be selected to provide enough mass information for the peaks eluting in each window. From the seven time-scheduled windows required, the results for the hexa-plus hepta-CB window is showed in Fig. 4. The top frame shows the raw chromatographic trace (three to four modulations per peak), with three hexa- and seven hepta-substituted congeners. The marked area is shown in more detail in the lower frame. The mass spectra of the hepta-CB included in Fig. 4 clearly indicate that, even with the limited mass range, the information is

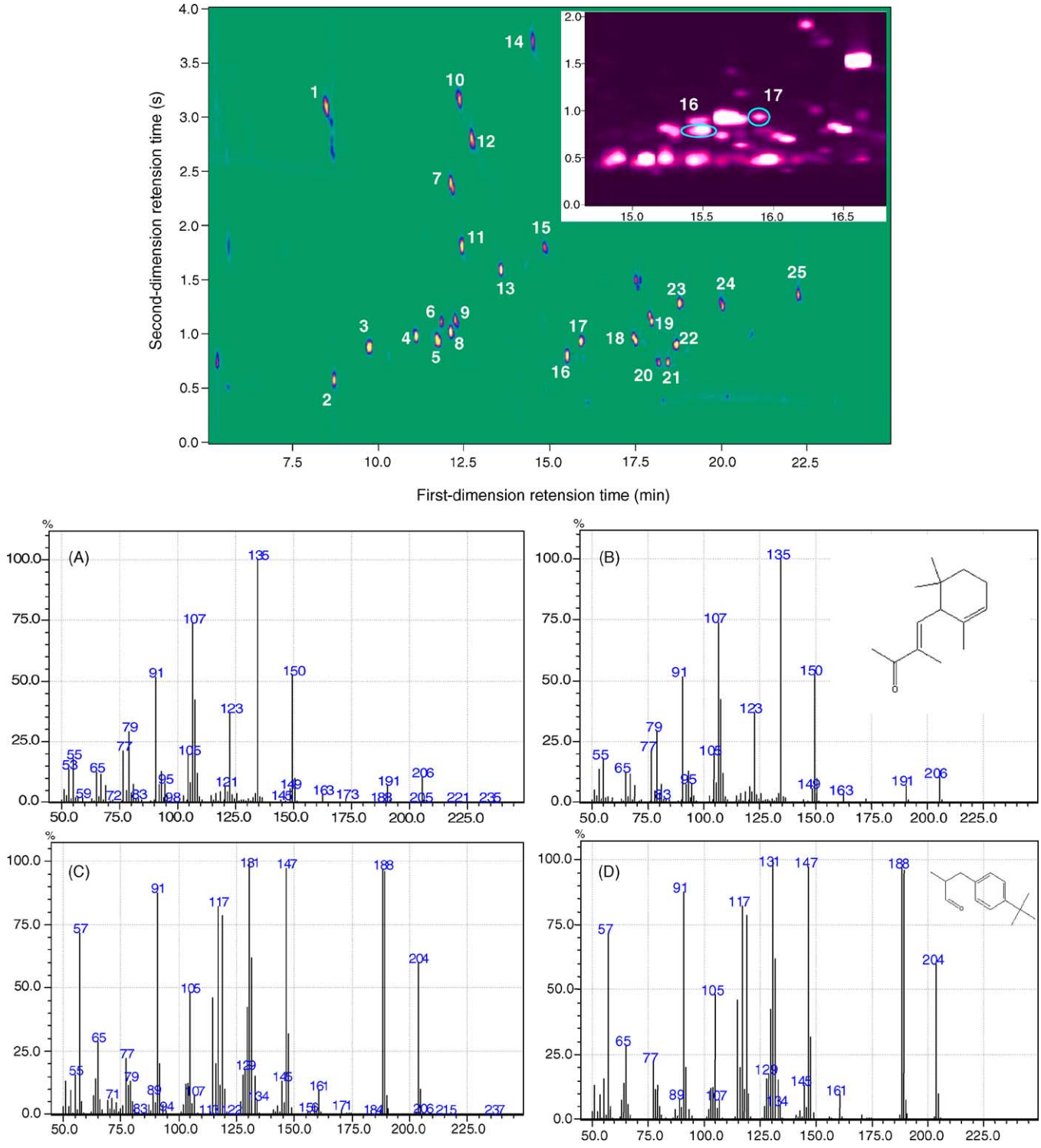

Fig. 3. GC × GC–qMS TIC chromatogram (*m/z* 50–245) of the allergen standard mixture (for peak designations, see Table 1). The insert shows part of the GC × GC–qMS TIC chromatogram of a perfume sample. Mass spectra (compared to the NIST) demonstrate identification of the circled α-isomethylionone (no. 16: mass spectrum, A; library spectrum, B) and lilial (no. 17: mass spectrum, C; library spectrum, D).

still sufficient to obtained 98–99% similarity factor. Similar results were obtained for all other CBs studied.

In a brief study on analyte detectability, detection limits (LODs) of 1–2 pg injected mass were obtained for three selected congeners (cf. Table 4) under the 50 amu-plus-33 Hz conditions. This is a fully acceptable result compared with the LODs of 0.1–0.5 pg reported by Focant et al. [28] when using a LECO ToF MS. It may be interesting to add that, if in our system the mass range was extended to about 300 amu to cover all congeners (25 Hz

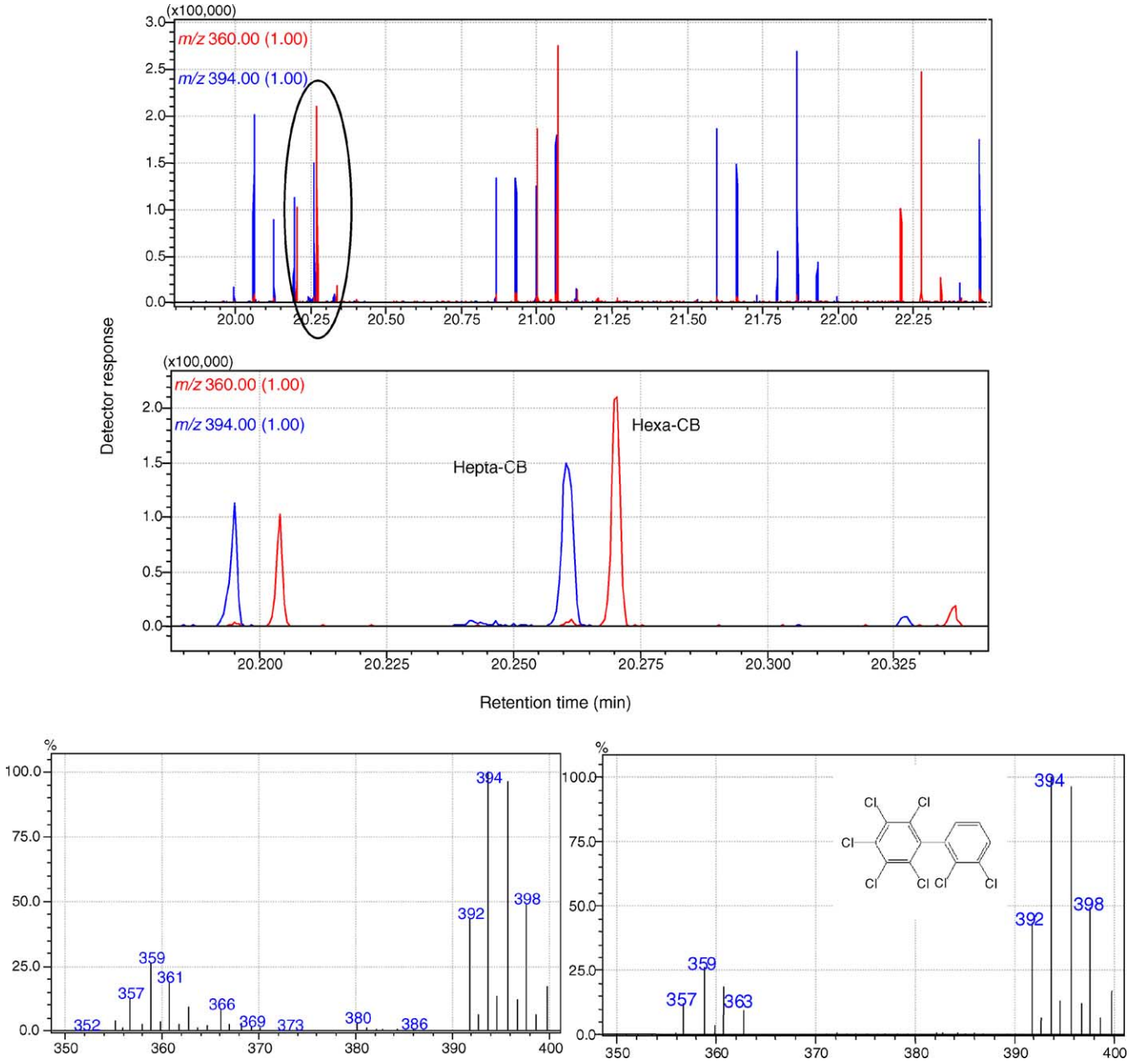

Fig. 4. GC × GC–qMS ion traces (*m*/*z* 360 and 394) of PCBs eluting in hexa plus hepta window (top) with blow-up of indicated peaks in lower trace. Mass spectrum of the hepta-CB (A) and NIST library spectrum (B) (bottom).

acquisition rate), the LODs of the three test CBs increased to 3–5 pg.

4. Conclusions

GC × GC coupled to a rapid-scanning qMS with scan speeds of up to 10,000 amu/s is a powerful separation and identification technique for the analysis of many complex samples. A systematic study of the relevant parameters of the mass spectrometer shows that—with an acquisition rate of 33 Hz and a mass range of up to 200 amu—a sufficient number of data points per peak can be obtained (dppp ≥ 7) to enable identification on the basis of high-quality mass spectra, with proper linearity and quantification. LODs were in the low-pg range for a wide variety of test compounds. If a very large mass range has to be covered in a specific GC × GC run, a time-scheduled option with mass windows on the order of 50–100 amu can be used without a significant loss of analytical performance.

When comparing the present rapid-scanning qMS and a ToF MS, one main difference that should be cited is that the latter instrument always operates in the scan mode, i.e. provides full mass spectra. One may therefore conclude that for

the many applications with a fairly limited (100–200 amu) mass range, a rapid-scanning quadrupole mass spectrometer become a most useful alternative to high-speed ToF MS instruments. However, whenever complex samples containing target analytes with a broad mass range have to be studied or searching for unknowns is an aspect of interest, a much more expensive but also more powerful time-of-flight machine should be selected.

Acknowledgement

This project was financed by the European Union, Measurements and Testing (Growth Programme) no. G6RD-CT2001-00572.

References

[1] J. Dallüge, J. Beens, U.A.Th. Brinkman, J. Chromatogr. A 1000 (2003) 69.
[2] P.J. Marriott, P. Haglund, R.C.Y. Ong, Clin. Chim. Acta 328 (2003) 1.
[3] J.B. Phillips, J. Beens, J. Chromatogr. A 856 (1999) 331.
[4] P. Korytár, P.E.G. Leonards, J. de Boer, U.A.Th. Brinkman, J. Chromatogr. A 958 (2002) 203.
[5] L. Mondello, A. Casilli, P.Q. Tranchida, P. Dugo, G. Dugo, J. Chromatogr. A 1019 (2003) 187.
[6] M. Adahchour, A. Taşöz, J. Beens, R.J.J. Vreuls, A.M. Batenburg, U.A.Th. Brinkman, J. Sep. Sci. 26 (2003) 753.
[7] E.M. Kristenson, P. Korytár, C. Danielsson, M. Kallio, M. Brandt, J. Mäkelä, R.J.J. Vreuls, J. Beens, U.A.Th. Brinkman, J. Chromatogr. A 1019 (2003) 65.
[8] G.S. Frysinger, R.B. Gaines, J. High Resolut. Chromatogr. 22 (1999) 251.
[9] R.A. Shellie, P.J. Marriott, C.W. Huie, J. Sep. Sci. 26 (2003) 1185.
[10] R.A. Shellie, P.J. Marriott, Analyst 128 (2003) 879.
[11] M. Kallio, T. Hyötyläinen, M. Lehtonen, M. Jussila, K. Hartonen, M. Shimmo, M.-L. Riekkola, J. Chromatogr. A 1019 (2003) 251.
[12] C. Debonneville, A. Chaintreau, J. Chromatogr. A 1027 (2004) 109.
[13] U. Krings, D.S. Banavara, R.G. Berger, Eur. Food Res. Technol. 217 (2003) 70.
[14] J. Beens, M. Adahchour, R.J.J. Vreuls, K. van Altena, U.A.Th. Brinkman, J. Chromatogr. A 919 (2001) 127.
[15] R.A. Shellie, P.J. Marriott, A. Chaintreau, Flavour Fragr. J. 19 (2004) 91.
[16] A. Chaintreau, D. Joulain, C. Marin, C.O. Schmidt, M. Vey, J. Agric. Food Chem. 51 (2003) 6398.
[17] M. Adahchour, J. Beens, R.J.J. Vreuls, A.M. Batenburg, U.A.Th. Brinkman, J. Chromatogr. A 1054 (2004) 47.
[18] K. Mastovska, J. Hajslova, S.J. Lehotay, J. Chromatogr. A 1054 (2004) 335.
[19] K. Maštovská, S.J. Lehotay, J. Chromatogr. A 1000 (2003) 153.
[20] M.M. van Deursen, J. Beens, H.-G. Janssen, P.A. Leclercq, C.A. Cramers, J. Chromatogr. A 878 (2000) 205.
[21] J.W. Cochran, J. Chromatogr. Sci. 40 (2002) 254.
[22] J.F. Holland, C.G. Enke, J. Allison, J.T. Stults, J.D. Pinkstone, B. Newcome, Anal. Chem. 55 (1983) 997A.
[23] J. Dallüge, R.J.J. Vreuls, D.J. van Iperen, M. van Rijn, U.A.Th. Brinkman, J. Sep. Sci. 25 (2002) 608.
[24] A. Amirav, H. Jing, Anal. Chem. 67 (1995) 3305.
[25] R.J.J. Vreuls, J. Dallüge, U.A.Th. Brinkman, J. Microcol. Sep. 11 (1999) 663.
[26] J. Dallüge, R.J.J. Vreuls, J. Beens, U.A.Th. Brinkman, J. Sep. Sci. 25 (2002) 201.
[27] M. Adahchour, L.L.P. van Stee, J. Beens, R.J.J. Vreuls, A.M. Batenburg, U.A.Th. Brinkman, J. Chromatogr. A 1019 (2003) 157.
[28] J-.F. Focant, A. Sjödin, D.G. Patterson, J. Chromatogr. A 1040 (2004) 227.