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Evaluation of two-dimensional gas chromatography–time-of-flight mass spectrometry for the determination of multiple pesticide residues in fruit

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Abstract

In recent years, comprehensive two-dimensional gas chromatography (GC × GC) has attained increasing attention for its outstanding separation potential and capability to solve demanding analytical tasks. Trace level analysis of pesticides residues in complex food matrices represents such a demanding task. For some commodities, such as baby food, the requirements on method detection limits are very strict and the unambiguous confirmation of the pesticide presence based on mass spectrometric detection is required. In this work, GC × GC coupled to time-of-flight mass spectrometry (TOF MS) has been evaluated for the determination of pesticides residues in fruit samples. Twenty modern pesticides with a broad range of physico-chemical properties were analysed in apple and peach samples. It has been demonstrated that the application of comprehensive two-dimensional gas chromatography brings distinct advantages such as enhanced separation of target pesticides from matrix co-extracts as well as their improved detectability. The limits of detection of the pesticides comprised in the study (determined at $S/N = 5$) ranged from 0.2 to 30 pg, injected with the exception of the last eluted deltamethrin, for which 100 pg could be detected. When compared to one-dimentional GC–TOF MS analysis under essentially the same conditions the detectability enhancement was 1.5–50-fold. Full mass spectral information by time-of-flight mass spectrometry and the deconvolution capability of the dedicated software allowed for reliable identification of most pesticides at levels below 0.01 mg/kg (<10 pg injected) in fruit. Performance characteristics of the GC × GC–TOF MS method, such as linearity of calibration curves, repeatability of (summed) peak areas, as well as repeatability of first and second dimension retention times, were shown to fully satisfy the requirements for trace level analysis of the pesticide residues in food.

Keywords: Food analysis; Pesticides

1. Introduction

Gas chromatographic multi-residue analysis of pesticides in food represents a challenging analytical task, since multiple target analytes have to be determined within one run in samples containing large amounts of co-extracted matrix components. Conventional GC multi-residue methods involve element specific detectors such as NPD, FPD or ECD and the results obtained by these techniques are confirmed by gas chromatography–mass spectrometry. However,

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recently GC–MS has increasingly becoming a primary tool for the determination of pesticides residues in food. According to European Commission guidelines [1], confirmation of analyte identity by SIM or full MS methods has to be performed by the detection of at least four characteristic ions matching the relative abundance criteria. However, GC–MS determination/confirmation of pesticides can be complicated by the interference of matrix components, co-eluting with the analytes of interest. Especially troublesome are those analytes possessing low and hence unspecific *m/z* value ions in their mass spectra. Conventional GC–MS methods may, therefore, fail to determine and confirm these analytes at sufficiently low concentration levels. This problem becomes critical if a low regulation limit is set for the particular commodity, e.g. baby food, MRL = 0.01 mg/kg.

One of the approaches to overcome this problem is to improve the gas chromatographic separation. Recently introduced technique, the comprehensive two-dimensional gas chromatography (GC × GC) brings the separation potential superior to any conventional gas chromatographic separation. GC × GC invented by Liu and Philips in 1991 [2] is an entirely new form of two-dimensional gas chromatography [3]. The principles and instrumentation of comprehensive two-dimensional gas chromatography have been recently reviewed in several papers [4–8]. In GC × GC, two columns of different selectivity are serially coupled via a modulation device, which cuts small portions (typically 2–10 s) of the effluent from the first column, refocuses them and samples onto the second column. Each pulse generates its own very fast chromatogram. A suitable computer programme has to be used to generate a two-dimensional chromatogram. For visualizing the GC × GC data, contour plots, representing the "bird's-eye view" of the chromatogram, are often used. In GC × GC, every compound in the sample mixture is subjected to two independent separation mechanisms, therefore, the technique has enormous potential to resolve very complex mixtures. Application potential in petrochemical, essential oil, food and environmental analysis has been demonstrated by many authors [8].

Since GC × GC produces very narrow chromatographic peaks (typically 50–600 ms at the baseline), the detection system applied has to be fast enough to provide sufficient density of data points per chromatographic peak. Therefore, until recently detection in GC × GC was limited to the use of fast analogue detectors such as FID or ECD. However, the commercialisation of TOF MS instruments providing very fast acquisition rates has considerably enlarged the application potential of GC × GC technique. Very promising results of the coupling of GC × GC with a TOF MS for the analysis of petrochemical samples [9], essential oils [10], cigarette smoke [11] and trace-level determination of pesticides in vegetables [12] have been reported. Very recently, the first fully-integrated GC × GC–TOF MS instrument has been introduced [13]. This system uses a robust dual-stage jet cryogenic modulator and the integrated software enables to fully exploit the capabilities of this powerful technique.

In our study, the LECO Pegasus 4D GC × GC–TOF MS system was evaluated for the analysis of pesticides residues in fruit matrices. Special focus was laid on the potential of the technique to reliably identify the pesticides at levels ≤0.01 mg/kg, which is the maximum residue limit for pesticide residues in baby/infant food. Also, we aimed to evaluate the quantitative performance characteristics of a developed GC × GC–TOF-MS method.

2. Experimental

2.1. Instrumentation

GC × GC–TOF MS instrument Pegasus 4D from LECO, Co. (USA) consisted of Agilent 6890N gas chromatograph with split–splitless injector, 7683 Series autosampler and time-of-flight mass spectrometer LECO Pegasus III (10 ml/min pumping capacity).

Inside the GC oven a dual-stage jet modulator and the secondary oven were mounted. Resistively heated air was used as a medium for hot jets, while cold jets were supplied by gaseous nitrogen, secondary cooled by liquid nitrogen. Instrumental parameters of a GC × GC–TOF MS method were as mentioned below.

2.1.1. GC × GC–TOF MS analysis

2.1.1.1. Gas chromatography. Primary column DB-XLB (30 m × 0.25 mm × 0.25 μm) and secondary column DB-17 (1 m × 0.1 mm × 0.1 μm), both from Agilent, USA; oven temperature program: 70 °C for

1.1 min, 20 °C/min to 300 °C, 20–300 °C for 10 min, secondary oven was held 5 °C above the main oven; helium flow: 1.2 ml/min; injection mode: pulsed splitless; 50 psi at 1.1 min; injection temperature: 250 °C; injection volume: 1 μl; modulation time: 2 s (hot pulse 0.4 s); modulation temperature offset: 30 °C.

2.1.1.2. Mass spectrometric detection. Acquisition rate: 250 Hz; mass range: 45–400 amu; ion source temperature: 220 °C; transfer line temperature: 280 °C; detector voltage: −1800 V.

2.1.2. GC–TOF MS analysis

For the purpose of comparison, samples were measured also by one-dimensional GC–TOF MS method, under essentially identical conditions.

2.1.2.1. Gas chromatography. Primary column DB-XLB (30 m × 0.25 mm × 0.25 μm), Agilent, USA; oven temperature program: 70 °C for 1.1 min, 20 °C/min to 300 °C, 20–300 °C for 10 min; helium flow: 1.2 ml/min; injection mode: pulsed splitless; 50 psi at 1.1 min; injection temperature: 250 °C; injection volume: 1 μl.

2.1.2.2. Mass spectrometric detection. Acquisition rate: 5 Hz; mass range: 45–400 amu; ion source temperature: 220 °C; transfer line temperature: 280 °C; detector voltage: −1800 V.

Total analysis time was 29.1 min for both GC × GC–TOF MS and GC–TOF MS methods. ChromaTOF software (LECO, Co.) was used for the processing of collected data.

2.2. Test samples

The test mixture comprised of 20 pesticides possessing a wide range of physico-chemical properties (see Table 1). The calibration mixtures were prepared by diluting the stock standard solution into ethyl acetate. The concentration of pesticides in individual standard solutions were approximately 1000, 500, 100, 50, 25, 10 and 5 ng/ml.

The purified apple and peach samples were prepared using the following procedure: 25 g of homogenized blank sample were mixed with 100 ml of ethyl acetate. After addition of 75 g of Na_2SO_4, the sample was blended using Turrax tissumizer for 2 min. The mixture was filtered under vacuum and the filtrate was evaporated and made up to 50 ml with cyclohexane to obtain the ratio cyclohexane–ethyl acetate (1:1 (v/v)). Two milliliter of crude extract were purified by high-performance gel permeation chromatography (HPGPC) using a PL gel column (600 mm × 7.5 mm, 50 Å) and cyclohexane–ethyl acetate (1:1 (v/v)) at a flow of 1 ml/min as a mobile phase. The eluate fraction of 14.5–31 ml was evaporated, the residual solvent was blown down under the stream of nitrogen and the content was redissolved in 1 ml of standard pesticides at 50 or 10 ng/ml to prepare spiked matrix samples at 50 and 10 ng/ml, respectively. Blank sample was prepared in a similar way by dissolving the evaporated GPC fraction in ethyl acetate.

3. Results and discussion

Fig. 1(A) and (B) show contour plots obtained by the analysis of peach and apple blank samples. By mass spectral comparison to a library, the major co-extracts were identified as succinic acid in peach and malic and fumaric acids in apple. Fig. 1(C) shows the contour plot of the pesticides standard at a concentration of 1 μg/ml. For the identification of individual pesticides in this figure see Table 1.

3.1. Improved chromatographic separation

The application of GC × GC in pesticide analysis resulted in improved chromatographic resolution both in terms of: (i) separation of individual pesticides from each other; and (ii) separation of pesticides from matrix components.

An example is given in Figs. 2 and 3 for the separation of the pesticides heptachlor, carbaryl and chlorothalonil. Chlorothalonil was not included in the spiking mixture; however, it was present in the peach sample as a contaminant. In 1D analysis, chlorothalonil coelutes with both heptachlor and carbaryl (see Fig. 2(A)). By using the deconvolution feature of the ChromaTOF software it was possible to obtain a pure mass spectrum and identify heptachlor (see Fig. 2(B)). However, in the case of chlorothalonil and carbaryl, complete overlap existed between the two peaks. Therefore, the deconvolution algorithm

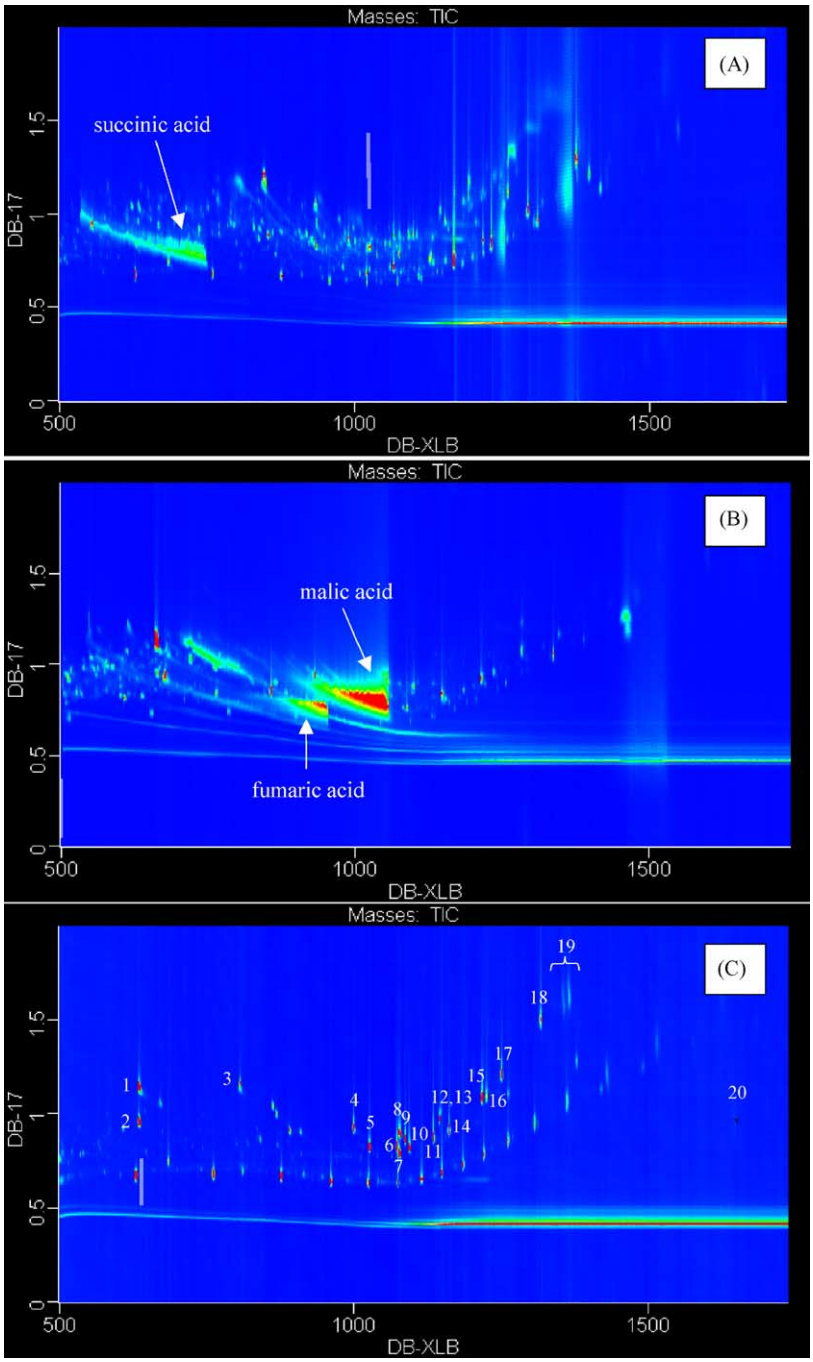


Fig. 1. Contour plots obtained by analysis of pesticide samples: (A) blank peach sample (1 g/ml GPC purified extract in ethyl acetate), 1 μl injected; (B) blank apple sample (1 g/ml GPC purified extract in ethyl acetate), 1 μl injected; (C) standard of pesticides in ethyl acetate, 1 μg/ml each pesticide, 1 μl injected.

Table 1
Limits of detection of pesticides in peach (extrapolated for signal-to-noise ratio = 5), estimated for a spiked peach sample at 50 and 10 ng/ml for GC–TOF MS and GC × GC–TOF MS techniques, respectively

	Pesticide	*m/z*	1D		2D		Detection enhancement factor[c]
			S/N	Limit of detection[a] (ng/ml–μg/kg of peach)	S/N[b]	Limit of detection[a] (ng/ml–μg/kg of peach)	
1	Methamidophos	94	44	6	28	2	3
2	Dichlorvos	109	24	10	26	0.2	50
3	Acephate	136	25	10	30	1.5	6
4	Dimethoate	125	62	4	40	1.5	3
5	Lindane	181	17	15	67	0.8	20
6	Pirimiphos-Me	290	25	10	23	2	5
7	Heptachlor	272	30	9	45	1	8
8	Carbaryl	144	37	7	78	0.6	11
9	Methiocarb	168	35	7	34	1.5	5
10	Chlorpyrifos	197	47	5	29	1.7	3
11	Procymidone	96	17	15	71	0.7	20
12	Thiabendazole	202	39	6	15	3.5	2
13	Captan	149	6	42	8	7	6
14	Endosulfan I	241	14	19	11	4.5	4
15	Endosulfan II	241	10	24	5	10	2
16	Propargite	173	13	19	7	7	3
17	Endosulfan-SO_4	272	19	13	7	8	2
18	Phosalone	182	18	14	8	6	2
19	Permethrin I[d]	183	7	37	8	30	1
19	Permethrin Ii[d]	183	11	23	25	10	2.5
20	Deltamethrin[e]	181	4	140	5	100	1.5

[a] Extrapolated to signal-to-noise (S/N) = 5.
[b] The highest (base) modulated peak has been considered for the calculations.
[c] As a ratio of LOD for 1D GC–TOF MS analysis to LOD of GC × GC–TOF MS analysis.
[d] Estimated from the analysis of peach matrix-matched standard at 50 μg/ml for both techniques.
[e] Estimated from the analysis of peach matrix-matched standard at 100 μg/ml for both techniques.

failed and only one peak with a mixed mass spectrum was identified (see Fig. 2(C)). By the application of GC × GC carbaryl is completely separated from the other two compounds and pure mass spectra with high match values can be obtained for all three pesticides (see Fig. 3).

Regarding the separation of pesticides from co-extracts, application of a "boiling-point" separation on a 1D DB–XLB column followed by polarity separation on 2D DB-17 column led to significant improvement in resolution when compared to the 1D setup. The benefit of GC × GC was obvious, especially for the early eluting analytes, where the risk of matrix interference is most significant. Fig. 4 shows an example of dichlorvos in an apple sample at 10 ng/ml. The most abundant ions in the mass spectrum of dichlorvos are *m/z* 79, 109 and 185. Mass 109, being the base peak in the mass spectrum, is commonly used for quantification. However, in this particular case, in conventional 1D GC separation a matrix compound interferes with dichlorvos at *m/z* 79 and 109 (see Fig. 4(A)). In this situation, neither effective deconvolution nor identification of dichlorvos based on the ratios of the three qualifier ions (as is commonly used with classic GC–MS method) is achievable. When applying GC × GC separation, the matrix compound was fully separated from dichlorvos based on differing retention behaviour on a DB-17 column (see Fig. 4(B)). In this case, a pure mass spectrum could be taken from the separated peak, which resulted in unambiguous identification of dichlorvos with a library search reverse factor of 940 (see Fig. 4(C)). The interfering matrix component was identified as 5-(hydroxymethyl)-2-furancarboxaldehyde (see

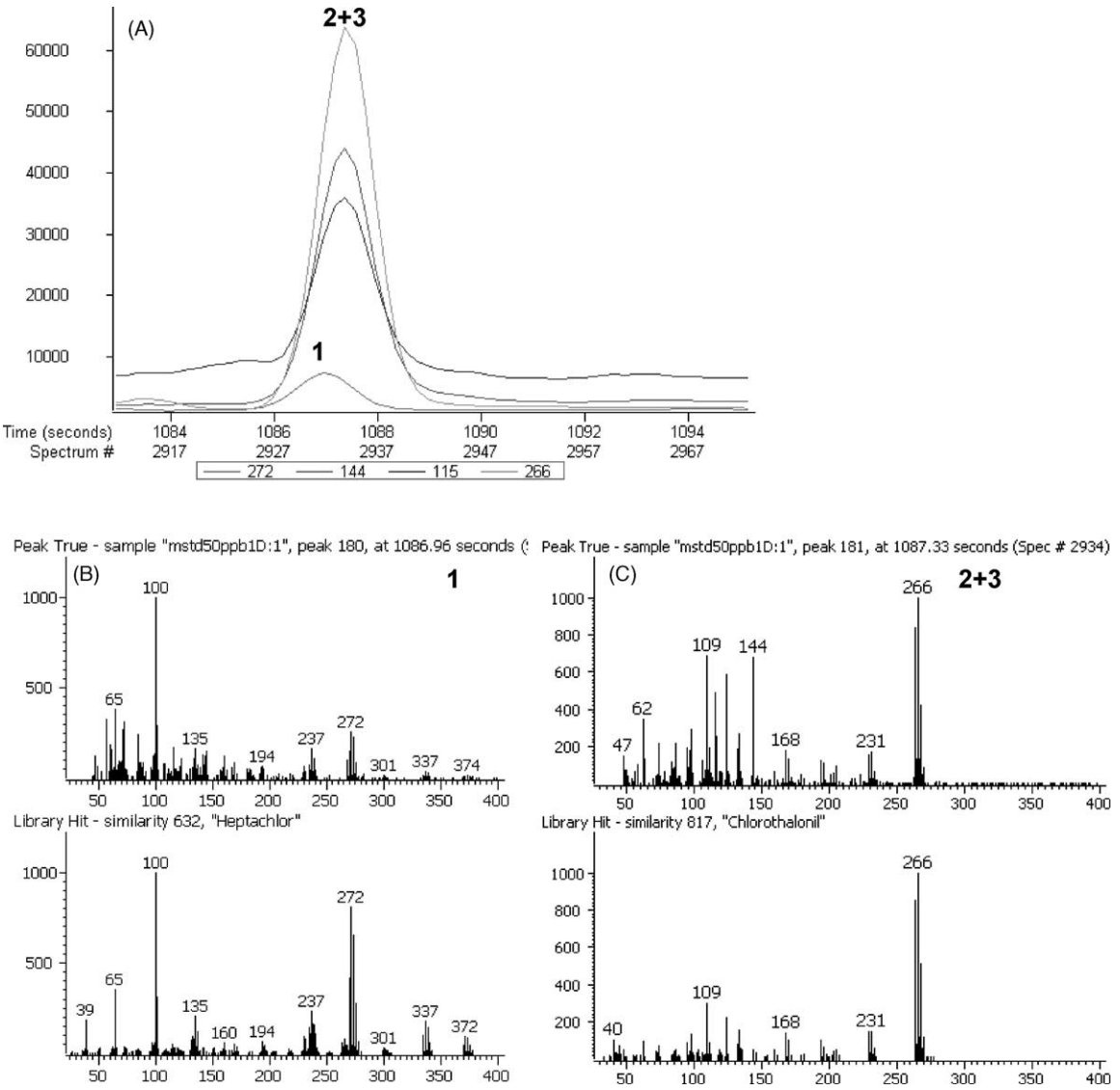


Fig. 2. (A) Chromatogram obtained by 1D GC–TOF MS analysis of GPC purified peach sample spiked with pesticides at 50 ng/ml each: (1) heptachlor: $m/z = 272$, (2) carbaryl: $m/z = 144$ and 115; (3) chlorothalonil: $m/z = 266$; (B) deconvoluted mass spectrum of heptachlor, despite co-elution with two other pesticides, a pure mass spectrum could be obtained by deconvolution; (C) mass spectrum of co-eluted peaks of carbaryl and chlorothalonil, since these two peaks completely overlap, mass spectra could not be "purified" by deconvolution.

Fig. 5), most probably originating from non-enzymic browning reactions of fruit sugars.

Although dichlorvos is shown as a typical example here, the separation from co-extracts was improved generally for most pesticides. In many cases, this fact resulted in a considerable decreasing of detection limits as discussed in Section 3.3.

3.2. Enhancement of signal-to-noise ratio by GC × GC

In GC × GC separation, the peaks of analytes are considerably narrower compared to conventional GC. This is a result of cryofocusing in the modulator region and subsequent very fast separation on a second

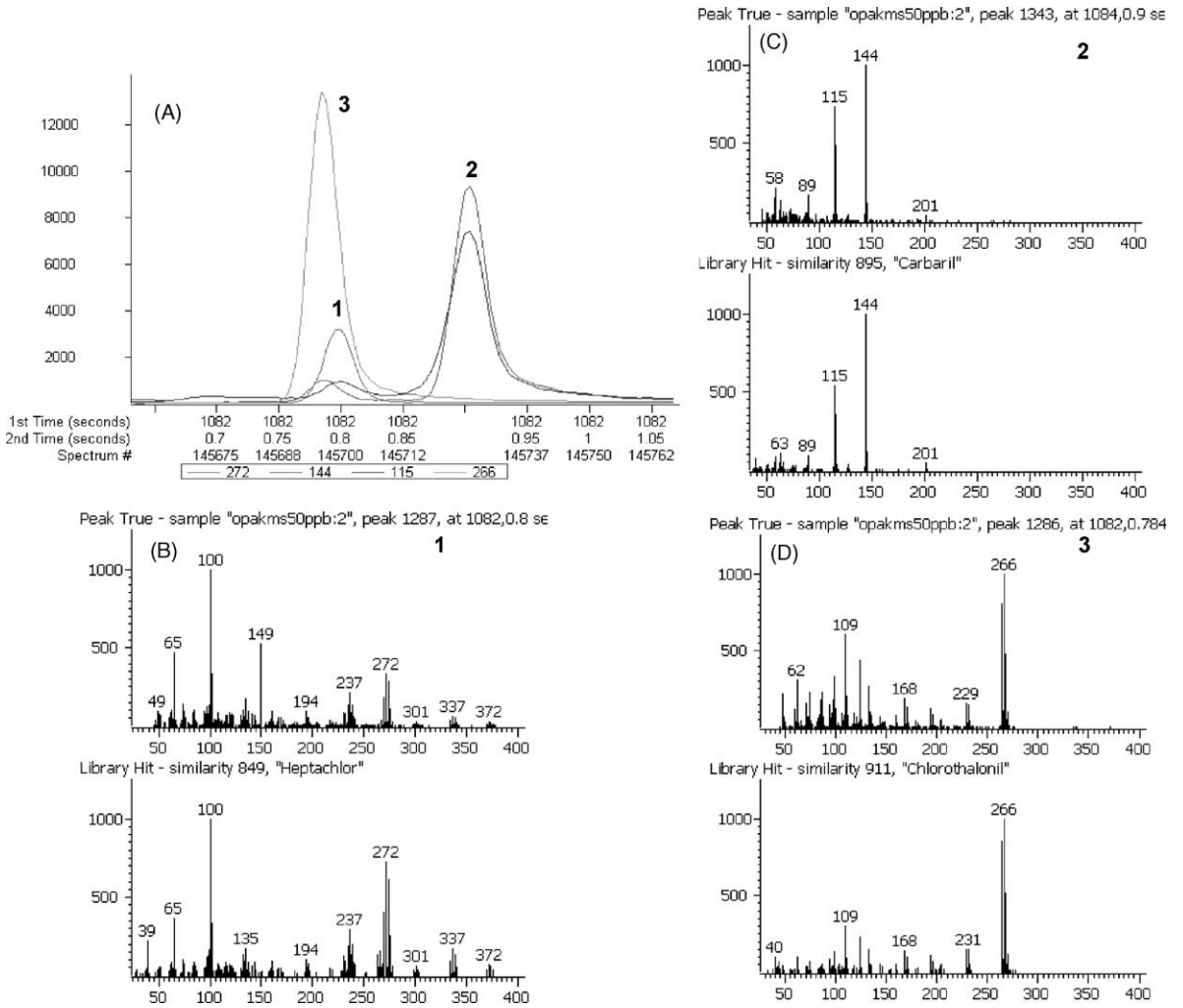
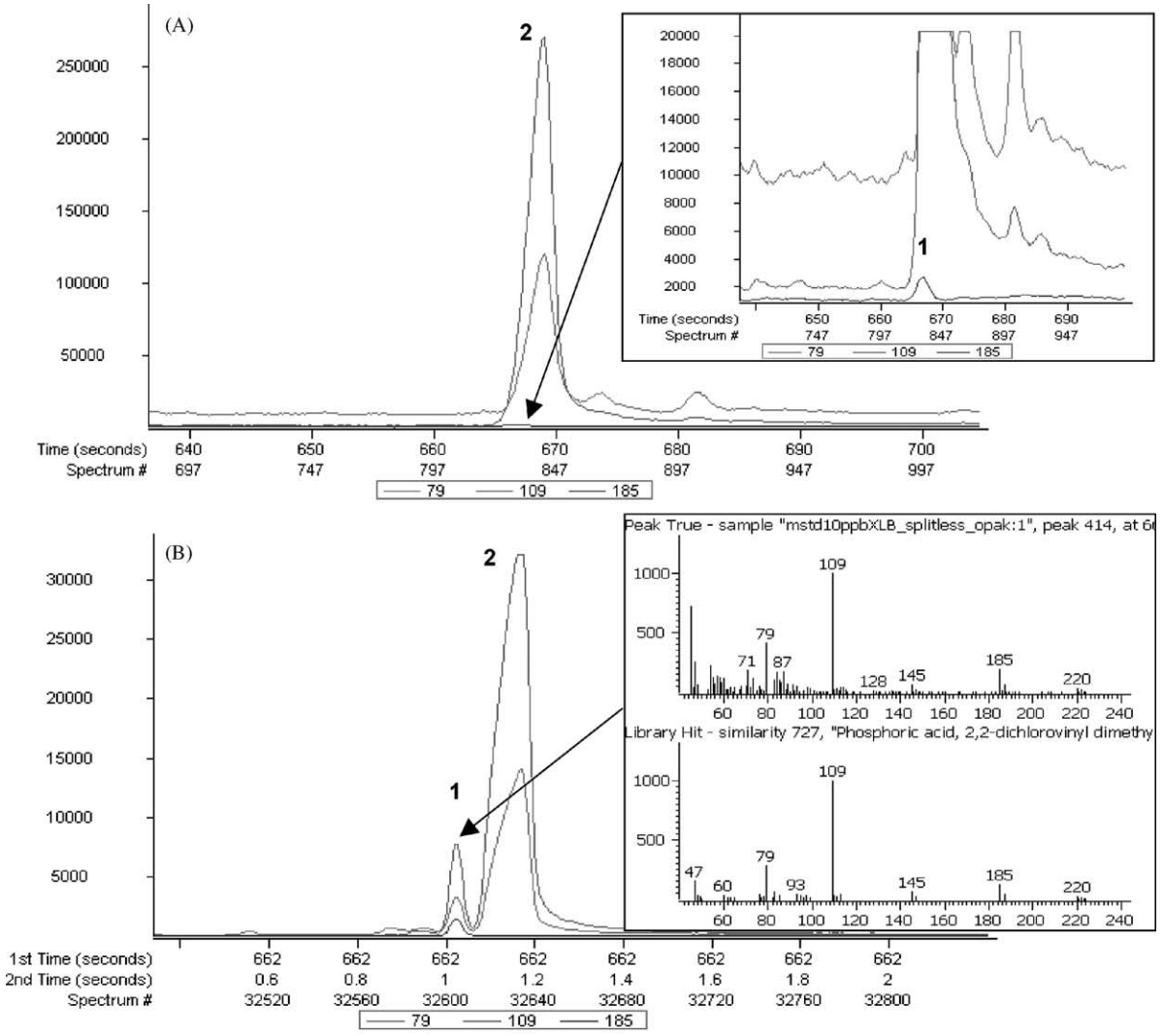


Fig. 3. (A) Chromatogram (selected modulation segment) obtained by GC × GC–TOF MS analysis of GPC purified peach sample spiked with pesticides at 50 ng/ml each: (1) heptachlor, $m/z = 272$; (2) carbaryl, $m/z = 144$, 115; (3) chlorothalonil, $m/z = 266$. Deconvoluted mass spectrum of: (B) heptachlor; (C) carbaryl; and (D) chlorothalonil.

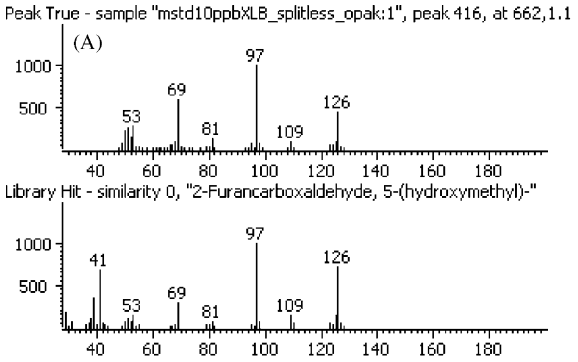
column. However, the mass of one analyte is subdivided into several modulation segments. It is, therefore, obvious that the longer the modulation period and the lower the resulting number of segments are created from a first dimension peak, the better the limits of detection obtained by GC × GC. On the other hand, the separation already achieved in the first dimension should be preserved and, therefore, some minimum number of modulation per peak has to be made. As a rule of thumb, it is often stated that a first dimension peak should be modulated into at least four segments to maintain the first dimension separation [8]. This approach assumes that the peaks of components (partially) separated in the first dimension have Gaussian shapes and comparable widths. However, the analyses of the apple and peach samples have shown that the most "troublesome" co-extracts often elute from the first column as broad asymmetric bands and completely overlap the analytes of interest. In such a case, the requirement for 4–5 modulation per first dimension peak is not fully justified. For this reason, in the presented experiment, the number of mod-



(C)

Hit	Name	Reverse	CAS
1	Phosphoric acid, 2,2-dichlorovinyl dimethyl ester	940	62-73-7
2	Phosphoric acid, 2,2-dichlorovinyl dimethyl ester	729	62-73-7
3	Phosphoric acid, 2,2-dichlorovinyl dimethyl ester	707	62-73-7
4	Phosphoric acid, 2,2-dichlorovinyl dimethyl ester	704	62-73-7
5	Phosphoric acid, 2,2-dichlorovinyl dimethyl ester	700	62-73-7
6	Naled	870	300-76-5

Fig. 4. Separation of: (1) dichlorvos from matrix co-extract, (2) 5-(hydroxymethyl)-2-furancarboxaldehyde, spiked apple sample at 10 ng/ml (10 pg injected). Plotted are three most abundant ions in the mass spectrum of dichlorvos (79, 109, and 185). Chromatogram from: (A) GC–TOF MS analysis of zoomed section shows the peak of dichlorvos at mass 185, at masses 79 and 109 matrix interference is recorded; (B) GC × GC–TOF MS analysis, base modulation, matrix interference has been resolved on secondary DB-17 column; (C) hit table for the peak of dichlorvos obtained by GC × GC–TOF MS analysis.



(B)

Hit	Name	Reverse	CAS
1	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	857	67-47-0
2	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	856	67-47-0
3	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	838	67-47-0
4	1H-Pyrazole, 4,5-dihydro-3-methyl-1-propyl-	776	26964-49-8
5	4-Hexen-3-one, 4,5-dimethyl-	688	17325-90-5
6	4-Hepten-3-one, 4-methyl-	687	22319-31-9

(C)

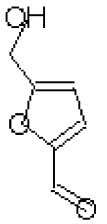


Fig. 5. Library search results for the peak of matrix interference (peak 2) obtained by GC × GC–TOF MS analysis: (A) measured mass spectrum (upper), NIST library mass spectrum (bottom); (B) hit table; (C) structure.

ulation per peak was set to 2–3 to obtain maximum detectability of pesticides. In Table 2, signal-to-noise ratios are calculated for both one-dimensional (1D) and two-dimensional (2D) analyses for two pesticides, pirimiphos-Me and lindane, in a solvent standard at 50 μg/ml. It should be noted that 1D and 2D analyses were performed to obtain a comparable number of spectra acquired per peak (ca. 20). From this table it is clear that with the number of 2–3 modulations per first dimension peak, considerable enhancement of signal-to-noise ratio occurs (ca. 5–10×), although peak area remains practically constant. The extent of this enhancement is, however, dependent on the phase of modulation [14] and the resulting ratios of intensity of secondary peaks. In the example shown here, pirimiphos-Me has been modulated into two secondary peaks of comparable heights, whereas the lindane peak has been modulated three times, forming the main peak that is much more intensive than the other two peaks (see Fig. 6). Therefore, in the latter case more S/N enhancement by GC × GC could be achieved.

Table 2
S/N enhancement for pirimiphos-Me and lindane by GC × GC[a]

Pesticide	1D analysis			2D analysis			Enhancement factor
	Peak: (1) height, (2) area, (3) width[b]	Peak-to-peak noise	S/N	Peak: (1) height[c], (2) area[d], (3) width[b]	Peak-to-peak noise	S/N	
Pirimiphos-Me ($m/z = 290$), modulation: two peaks of similar intensity	(1) 5600, (2) 30000, (3) 3.5 s	300	19	(1) 2200, (2) 29000, (3) 0.1 s	25	88	5
Lindane ($m/z = 181$), modulation: three peaks, central one much higher than the other two	(1) 14000, (2) 83000, (3) 4 s	300	47	(1) 12000, (2) 76000, (3) 0.1 s	30	400	9

[a] Calculated for standard in ethyl acetate at 50 ng/ml.
[b] Width at baseline.
[c] Height of most intense modulated peak.
[d] Summed area of all modulated peaks.

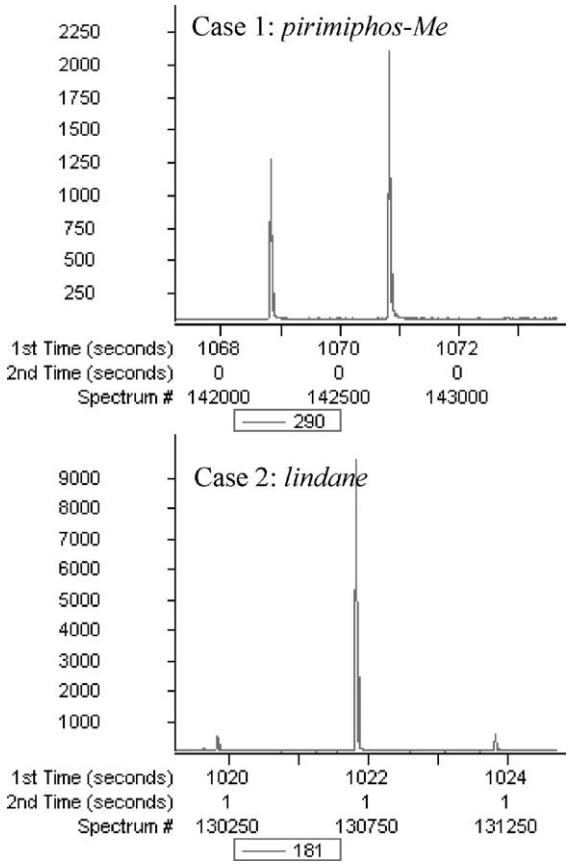


Fig. 6. Example of analytes modulated with different modulation period, GC × GC–TOF MS analysis of standard 50 ng/ml in ethyl acetate.

3.3. Performance characteristics of the GC × GC–TOF MS method

3.3.1. Limits of detection

In Table 1, limits of detection of target pesticides in peach matrix are shown. They were estimated from GC–TOF MS and GC × GC–TOF MS analyses of spiked peach extract at 50 and 10 ng/ml, respectively, by extrapolation to signal-to-noise ratio of 5. The detection limits of 1D and 2D techniques were evaluated using comparable number of data point per chromatographic peak. 1D analysis provided peaks of pesticides broad 3–9 s at the baseline. At 5 Hz, acquisition rate this resulted in 15–45 spectra per peak for individual analytes. For 2D analysis, peaks with widths 90–300 ms were typically obtained, giving 23–75 spectra per peak at an acquisition rate of 250 Hz.

From Table 1, it is obvious that by GC × GC–TOF MS the achieved limits of detection are considerably lower than those obtained by 1D GC–TOF MS analysis (by factor 1.50–50). Detection enhancement observed is a result of two above-discussed effects, i.e. the narrowing of peak bands and better separation from co-extracts by GC × GC. Relatively great variability in the detection enhancement was observed among particular pesticides, depending on their boiling points (typically the enhancement in detectability was less pronounced for higher boiling components), relative ratios of modulated peaks as well as particular coelutions with matrix component.

By the application of GC × GC–TOF MS technique, the limits of detection of most pesticides were well below 10 ng/ml (10 pg injected). These results are especially important for pesticides known for poor detectability by conventional GC–MS. As an example may serve the relatively polar organophosphorous pesticides methamidophos and acephate. These compounds have very low, hence, unspecific qualifier *m/z* in their mass spectra and, therefore, GC–MS determination is often impaired by matrix interference. Moreover, peaks of these compounds typically tail (in the first dimension) due to adverse injector phenomena. In Figs. 7 and 8, determination of methamidophos and acephate is documented at the concentration level 10 ng/ml (corresponds to 0.01 mg/kg of matrix). It is obvious that even at this low level the above pesticides can be reliably determined in fruit matrix and unambiguously identified by means of their full mass spectra. In addition, they are eluted as sharp and symmetrical peaks from the secondary column.

Relatively poor detectability was obtained in the case of deltamethrin. Because of high retention time, the elution zone of this compound is broad in both first and second dimension. This leads to two effects: (i) since the first dimension width of deltamethrin peak is ca. 12 s, it is modulated into 6–7 segments instead of 2–3 as for most other pesticides. This naturally leads to worsened detectability of this compound; (ii) since the second dimension peak of deltamethrin is 500 ms wide, the number of mass spectra acquired for this peak is ca. 125 (at 250 Hz). This “oversampling“ again leads to low signal-to-noise for this compound. The above-mentioned problem could be most

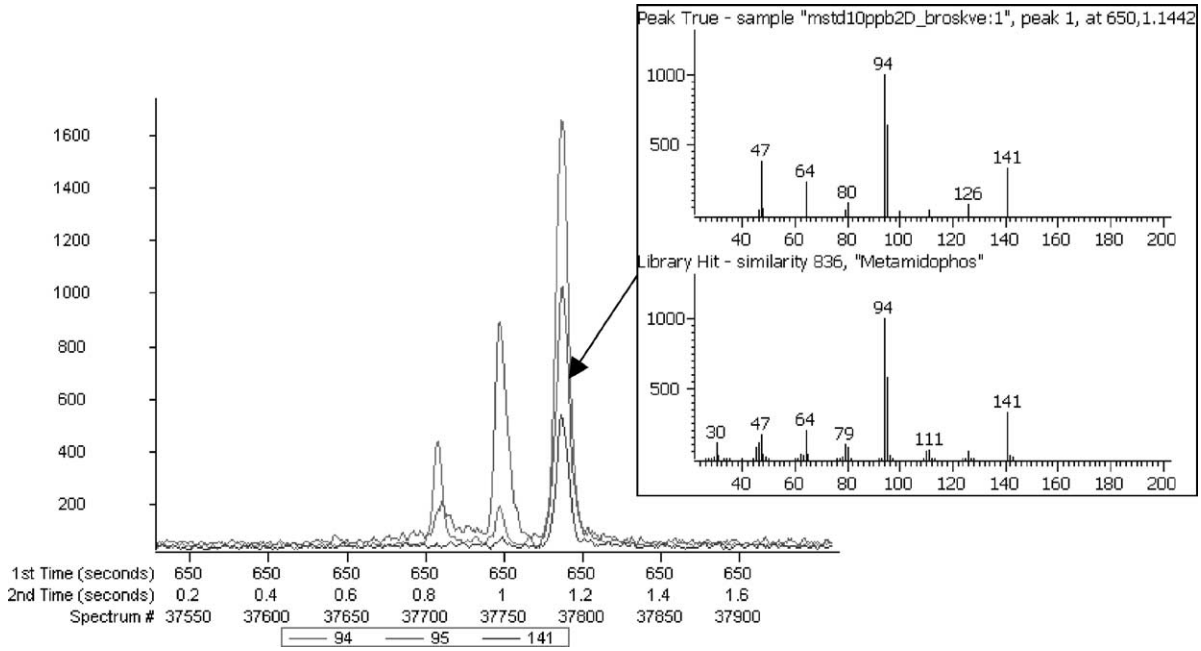


Fig. 7. Methamidophos in GPC purified peach extract (1 g/ml) spiked at 10 ng/ml (10 pg injected). Plotted are the three most abundant ions in the mass spectrum of methamidophos (94, 95, and 141). The peak was identified by library search with reverse factor 841.

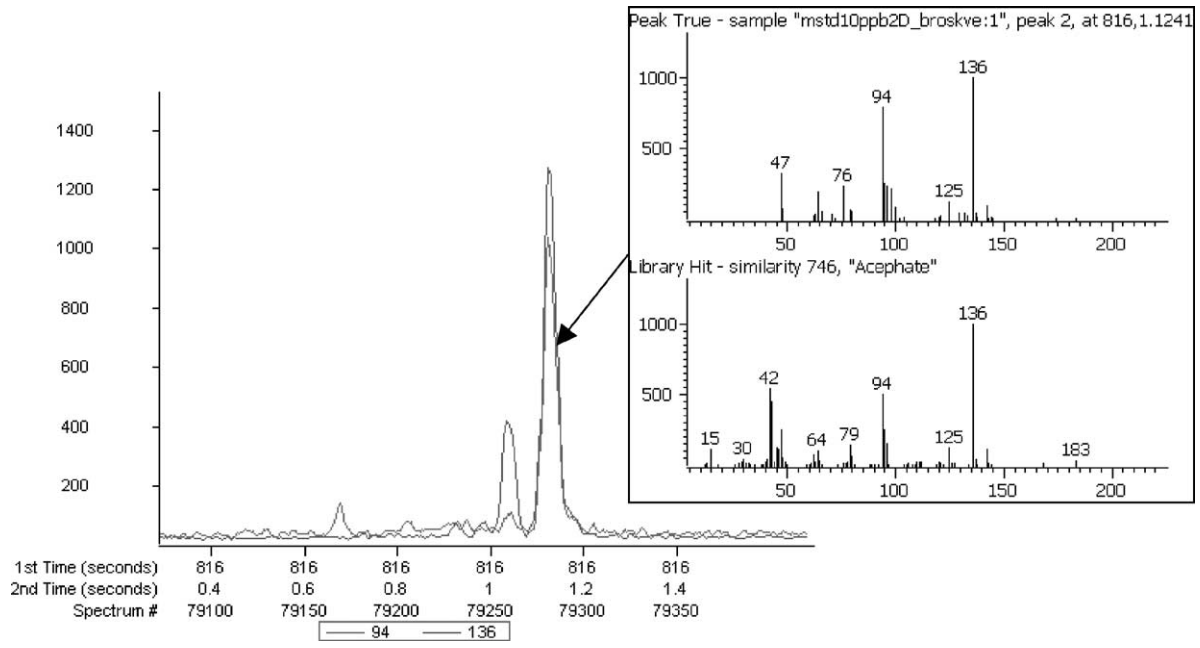


Fig. 8. Acephate in GPC purified peach extract (1 g/ml) spiked at 10 ng/ml (10 pg injected). Plotted are the two most abundant ions in the mass spectrum of acephate (94 and 136). The peak was identified by library search with reverse factor 809.

Table 3
Repeatability of peak areas, first and second dimension retention times, calculated from five repetitive injections of spiked peach sample at 50 ng/ml

Pesticide	*m/z*	R.S.D. of peak area[a] (%)	First dimension				Second dimension				Correlation coefficient (R^2)	
			t_{R1}[b] (s)	Peak width[c] (s)	S.D. of t_{R1}[b](s)	R.S.D. of t_{R1}[b] (%)	t_{R2}[b] (s)	Peak width[d] (s)	S.D. of t_{R2}[b](ms)	R.S.D. of t_{R2}[b] (%)	Conc. range 5–500 ng/ml	Conc. range 5–1000 ng/ml
Methamidophos	94	6.55	644	10	3.6	0.56	1.152	0.129	8.9	0.78	0.9996	0.9953
Lindane	181	5.92	1028	4	4	0.39	0.828	0.110	3.2	0.39	0.9994	0.9990
Chlorpyrifos	197	3.73	1098	4	3.6	0.33	0.828	0.160	2.8	0.34	0.9994	0.9998
Captan	149	5.04	1148	4	4	0.35	0.984	0.120	2.8	0.29	0.9990	0.9996
Phosalone	182	4.17	1321	4	3.6	0.28	1.502	0.270	6.7	0.45	0.9982	0.9968

[a] Summed area of all modulated peaks.
[b] For the most abundant modulated peak.
[c] As "number of modulations per peak × modulation time".
[d] At the baseline.

probably solved by using higher upper temperature in the oven programme (and hence compression of both 1D and 2D chromatographic peaks), however, this was not possible in this study due to limited temperature stability of DB-17 column used in the second dimension (maximum 300 °C). Possibly, also the modulation conditions (hot-pulse time) could affect the signal of this compound. In further experiments, some approaches to address this issue will be tested.

3.3.2. Repeatability of responses

Response repeatability was determined as relative standard deviation (R.S.D.) from five repetitive analyses of matrix-matched standard of a peach at 50 ng/ml (corresponds to 50 μg/kg of peaches, 50 pg of pesticides injected), see Table 3. R.S.D.s of summed areas of modulated peaks of current analyte ranged from 4.2 to 6.6% for these pesticides. Pesticides shown in this table were selected to cover the wide range of physico-chemical properties, such as volatility or polarity. Therefore, the data obtained in this study can be generalized to some extent.

3.3.3. Linearity of calibration

In GC × GC, quantification is performed by summing peak areas of all modulated peaks corresponding to the particular analyte. Very good linearity has been achieved in the concentration range 5–500 ng/ml (R^2 = 0.9982–0.9996). Including also the standard 1000 ng/ml in the calibration resulted in slightly worse correlation factors (R^2 = 0.9968–0.9998), see Table 3.

3.3.4. Repeatability of retention times

In Table 3, repeatability of retention times in both first and second dimension is demonstrated. Both first and second dimension retention time was considered for the apex of most intense modulated peak. Despite the process of modulation is relatively complex, from this table it can be seen that excellent repeatability of retention times was achieved. In the first dimension, the standard deviation (S.D.) of retention time (retention time of the most intense modulated peak) was ca. 4 s for all selected pesticides, which corresponds to two modulation cycles. The second dimension retention time standard deviation for peaks 90–160 ms broad at the baseline was in range of 0.0028–0.0089 s, which corresponds to 0.29–0.78%.

As regards literature data, Dallüge et al. [12] reported 0.11–0.16 s standard deviation of second dimension retention time for peaks of pesticides 1–2.9 s wide ($R.S.D.$ = 5–11%, n = 5) with the use of longitudinal modulation cryogenic system (LMCS). Shellie et al. [15] reported average run-to-run retention times repeatability in second dimension 0.74% (n = 6) when using LMCS system. With thermal sweeper modulator standard deviations of the second dimension retention times of PCBs relative to an internal standard 0.07 s (n = 8) were reported by de Geus et al. [16].

In the study presented here, very good retention time repeatability comparable to data of Shellie et al. [15] has been observed. This is a result of very narrow peak widths produced by dual stage jet modulator as well as precise modulation timing provided by the integrated GC × GC–TOF MS system.

Regarding the linearity and peak area repeatability data, they are in good agreement with the results reported by Dallüge et al. [12].

4. Conclusions

In this study, GC × GC–TOF MS has been demonstrated as a powerful tool for solving the problems with reliable confirmation of pesticide residues at very low concentration levels as required for the analysis of some types of samples such as baby food.

By the application of GC × GC, considerably improved separation from matrix co-extracts has been achieved. This benefit was obvious especially for the early eluting pesticides, for which the risk of matrix interference is most pronounced. The most troublesome matrix interferences observed in apple and peach were eluted as very broad asymmetric peaks completely overlapping the peaks of pesticides in the first dimension “boiling-point” separation. However, thanks to differing retention of these co-extracts on a DB-17 column, these co-extracts were in most cases efficiently separated in the second dimension.

Applying a 2 s modulation cycle, 2–3 modulations per peak were obtained for most pesticides studied. Under these conditions, the enhancement in detectability was 1.5–50-fold when compared to one-dimensional GC–TOF MS analysis. The limits of detection for most pesticides were well below 10 ng/ml and the reliable confirmation of analyte

identity was possible at 10 ng/ml level even for typically troublesome pesticides such as polar organophosphorus pesticides, methamidophos or acephate. The only compound for which relatively poor detectability has been achieved was the last eluted pesticide, deltamethrin. This was due to very broad elution zones of the deltamethrin peak in both dimensions, resulting in a high number of modulated peaks as well as too many data points acquired per (second dimension) peak at the given MS acquisition rate.

For the selected pesticides, quantitative parameters of GC × GC–TOF MS analysis were assessed. The repeatability (as R.S.D.) of peak areas obtained from five consecutive analyses of GPC purified peach extract spiked at 50 ng/ml ranged from 3.7 to 6.6%. The repeatability of retention time as R.S.D. ranged from 0.28 to 0.56% and 0.29 to 0.78% in the first and second dimensions, respectively. Good linearity (R^2 = 0.9982–0.9996) was achieved in the concentration range of 5–500 ng/ml for standards in ethyl acetate.

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