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Comparison of gas chromatography-based approaches after fast miniaturised sample preparation for the monitoring of selected pesticide classes in fruits

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ABSTRACT

The feasibility of a miniaturised generic sample preparation method based on matrix solid-phase dispersion for the determination of three relevant classes of pesticides (organophosphorus pesticides, triazines and pyrethroids) in selected fruits, i.e. orange, apple, pear and grape, have been demonstrated. Satisfactory results were obtained with gas chromatography coupled to mass spectrometry with recoveries of 78–113% in orange, 62–102% in grape, 71–116% in apple and 91–110% in pear, and reproducibilities in general below 20%. The feasibility of simultaneous separation of the three families of pesticides by comprehensive two-dimensional gas chromatography with micro-electro-capture detector was also evaluated. Columns with different polarity and selectivity, including ZB-5, HT-8 and DB-17, were assayed as first dimension and combined with columns of increasing polarity in the second dimension, i.e. HT-8, BPX-50 and Supelcowax-10. The best results for real-life samples after treatment by the proposed miniaturised method were achieved with ZB-5 × BPX-50 column combination. The low limits of detection achieved with this technique (in general, below 0.56 μg/kg) proved its suitability for accurate monitoring of the pesticides classes included in the study at the maximum residue levels set in the European Union.

1. Introduction

Most analytical procedures dealing with the determination of pesticides in fruit are protocols involving separate treatments for exhaustive extraction of a relatively large amount of sample (ca. 2–100 g) and subsequent purification and concentration of the extracts before conventional chromatographic analysis. This results in very selective but time consuming and expensive protocols not really suitable for routine analysis. Matrix-solid-phase dispersion (MSPD) can be regarded as a valuable alternative to these classical multi-step sample preparation procedures allowing the extraction and (preliminary) clean-up to be carried out in a single step [1].

Comprehensive two-dimensional gas chromatography (GC × GC) is a powerful separation technique in which two gas capillary columns with different separation mechanism are coupled via an interface called *modulator*. The main features of GC × GC, the influence of the experimental parameters in the final peak capacity and separation power as well as the main advantages of GC × GC as compared to other multidimensional chromatographic separation techniques for different application fields have been discussed in a number of recent reviews [2–4].

Up to now, the high peak capacity and distinctly superior separation power of GC × GC has been used to unravel classes of compounds in complex samples such as aromas, essential oils, petroleum mixtures [4], or to identify individual components within families of persistent pollutants with a large number of isomers [4], including polychlorinated biphenyls [5–7], polychlorinated dibenzo-*p*-dioxins and furans [6,8,9], polybrominated diphenyl ethers [6,9] and Polychloronaphthalenes [9]. However, to date, the feasibility of GC × GC for pesticides analysis has scarcely been investigated [10–14] and, to the best of our knowledge, no research on group-type separation of close related families of these pollutants has been carried out.

The present study focuses on this latter topic and evaluates the relative merits of several column combinations for the simultaneous screening of selected environmentally relevant classes of pesticides, triazines, organophosphorus pesticides (OPPs) and pyrethroids, in different types of fruits. The feasibility of using GC × GC in combination with a miniaturised generic MSPD-based sample preparation method for the fast monitoring of pesticides in real (i.e. non-spiked) samples has been evaluated. The advantages and shortcomings of this type of approach as compared to more conventional instrumental analysis procedures based on gas chromatography–quadrupole mass spectrometry (GC–qMS) have been evaluated and discussed.

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2. Materials and methods

2.1. Chemicals and reagents

Triazines (atraton, simazine, prometon, atrazine, propazine, terbuthylazine, simetryn, ametryne, prometryne and terbutryne) were purchased as pesticide mixture-619 (500 ng/μL each component) from Chem Service (West Chester, PA, USA). OPPs (dichlorvos, mevinphos, dimethoate, diazinon, disulfoton, bromophos-methyl, bromophos-ethyl, paraoxon-ethyl, parathion-methyl, parathion-ethyl, malathion, chlorpyrifos-ethyl, fenthion, chlorfenvinphos, and ethion) were purchased from Dr. Ehrenstorfer (Augsburg, Germany) as Pesticides-Mix 235 (200 ng/μL each component). Cypermethrin (technical), deltamethrin, fenvalerate and permethrin (1000 ng/μL each) and fenpropathrin (500 ng/μL) were obtained from Dr. Ehrenstorfer as Pesticide-Mix 195. Additionally, α-cypermethrin and cyfluthrin were obtained from Sigma–Aldrich (St. Louise, MO, USA) as Pestanal pyrethroid standard mixture (1000 ng/μL each component). Working standard solutions were prepared from these commercial mixtures in isooctane at concentrations between 100 and 1000 pg/μL. These solutions were used for further dilution and, when required, spiking of the samples. All solutions were stored at 4 °C.

All solvents were of trace analysis grade and purchased from Merck (Darmstadt, Germany) and Scharlau Chemie (Barcelona, Spain). Water purified with a Milli-Q system (Millipore, Bedford, MA, USA) was used in all experiments. Reverse phase octasilyl derivatised silica (C8, endcapped, 50 μm) was purchased form IST (Mid Glamorgan, UK).

Orange, grape, pear and apple samples were purchased from supermarkets in Madrid. Representative portions of the selected fruit, around 5 g, were cut into small pieces, homogenised in a Sorvall Omni-mixer (DuPont Instruments, Wilmington, DE, USA) and kept in a freezer at −20 °C until used. For practical reasons [15], the whole fruit was analysed in the case of grapes, while only the peel was used for all other fruits investigated.

2.2. MSPD method

Sample preparation was based on a miniaturised MSPD procedure previously validated for a limited number of OPPs and permethrin [15]. However, because of the several new compounds included in the present study as compared to the original one, and the different amount of sample involved in the experiments, i.e. 100 mg *vs.* 25 mg of [15], preliminary experiments were conducted to optimise the MSPD analytical procedure. In these experiments, orange samples spiked at the 0.5 μg/g level were used. The fruit samples were spiked before sample treatment. Briefly, the procedure consisted on the dispersion of 500 mg of the untreated peel on 500 mg of C8 by gently blending of the mixture for a few minutes in a glass mortar using a pestle. 200-mg of the resulting dry-powder-like homogeneous mixture were packed on a 3 mL solid-phase extraction (SPE) barrel (J.T. Baker, Deventer, The Netherlands). The SPE cartridge was then placed in a SPE-12G (J.T. Baker) apparatus attached to a water aspirator via a pressure-metering valve, washed with 15 mL of Milli-Q water and extracted with 700 μL of ethyl acetate. This MSPD method was subsequently evaluated for pear and grape. For apple, a similar procedure was followed but no clean-up with water was required. In all instances, the extract was collected in a vial, concentrated under a gentle nitrogen stream, reconstituted in isooctane and directly subjected to GC–qMS analysis. No extra concentration was required before GC × GC analysis. GC–qMS was used for the analysis of the fruit extracts during MSPD method optimisation.

Procedural blanks were regularly analysed to check for contamination throughout the analytical procedure showing no presence of the analytes of interest. All MSPD experiments were carried out in quadruplicate and each extract was injected once.

2.3. GC–qMS

Pesticide determination in the concentrated MSPD extracts was performed by GC–qMS (HP 6890N, Agilent Technologies, Palo Alto, USA; MSD, HP 5973 Network). Extracts were manually injected in the splitless mode (1 μL; 250 °C; splitless time, 1 min) in a ZB-5 column (5% phenyl 95% methylpolysiloxane; Zebron, Torrance, CA, USA) for separation and, because of the intended comparison, the rest of the chromatographic parameters were kept as similar as possible to those used for GC × GC analysis. That is, the column temperature was programmed from 60 °C (2 min) to 140 °C (1 min) at 12 °C/min, and then to 280 °C (30 min) at 6 °C/min. Helium (99.999%, Praxair, Madrid, Spain) was used as carrier gas (constant flow, 0.8 mL/min). The temperature of the transfer line and the ion source were set at 250 and 270 °C, respectively. Data were acquired in the scan mode for triazines and OPPs (m/z range, 55–550), and in the selected ion monitoring (SIM) mode for pyrethroids (Table 1). Confirmation criteria for the detection and quantification of the studied pesticides included (i) simultaneous detection of the three selected most abundant and selective ions produced for a given compound by electron ionization (EI) at 70 eV at the corresponding retention time ±1 s with signal-to-noise (S/N) ratio ≥3 for each, and (ii) maintenance of the two most abundant ions ratio within ±15% of the theoretical value. For triazines and OPPs, comparison with the NIST MS library for additional confirmation was also possible.

2.4. GC × GC–μECD

Pesticide determination in the non-concentrated MSPD extracts was performed by GC × GC on an Agilent 6890N equipped with a micro-electron-capture detector (μECD) and the KT2003 cryogenic loop modulator (Zoex, Lincoln, NE, USA). Liquid nitrogen was used to create the cold jet, while the temperature of the hot jet heater was kept 80 °C over the main oven temperature program. The secondary oven holding the second dimension column was programmed to track the main oven. Helium was used as carrier gas in the constant flow mode. The μECD was maintained at 300 °C throughout the study and nitrogen was used as make-up gas at a flow rate of 150 mL/min. In all instances, the modulation period was set at 4 s with a 200 ms hot jet pulse duration. Injections were performed in the splitless mode (1 μL; splitless time, 0.75 min) at 250 °C. Data acquisition rate was set at 50 Hz.

The column combinations assayed for GC × GC were selected on the basis of data previously reported in the literature [10,12–14] our experience, and the required orthogonality of the GC × GC separations. Three GC columns were tested as first dimension (typical dimensions, 30 m × 0.25 mm, 0.25 μm film thickness): ZB-5, HT-8 (5% phenyl 92% polysiloxane-carborane; SGE, Darmstadt, Germany) and DB-17 (50% phenyl 50% methylpolysiloxane; J&W Scientific, USA). As second dimension, HT-8, BPX-50 (50% phenyl 50% polysilphenilene siloxane; SGE) and polyethylene glycol type (Supelcowax-10, SW; Supelco, Bellefonte, PA, USA) columns were assayed (dimensions, 0.8 m × 0.10 mm, 0.10 μm film thickness). The deactivated fused silica column (1.5 m × 0.10 mm) used as modulator loop was purchased from Supelco and connected via mini press-fits (Techrom, Purmerend, The Netherlands) to the chromatographic columns.

ChemStation software was used for acquiring the raw data, which were further exported as comma separated values to the GC Image v1.4 program (University of Nebraska, Lincoln, USA) for further data analysis. The target pesticides were identified matching a template generated from standard solutions containing the stud-

Table 1
Retention time on a ZB-5 under the experimental conditions used, *m/z* ions selected for quantification (bold font) and identification of the analytes investigated by GC–qMS, and recoveries (%) and repeatability (as RSD, $n = 4$) obtained for the studied pesticides using the optimised MSPD method proposed. Spiking level, 0.5 μg/g.

Pesticide class	Analytes	Retention time (min)	(*m/z*) ion	Recovery, % (RSD, %)			
				Orange	Pear	Grape	Apple
OPPs	Dichlorvos	9.90	**109**/185	121 (20)	103 (3)	ND[a]	97 (10)
	Mevinphos	12.93	109/**127**	46 (8)	102 (4)	62 (18)	67 (16)
	Dimethoate	18.26	**125**/143	95 (8)	103 (12)	29 (7)	109 (7)
	Diazinon	19.52	152/**179**	107 (5)	102 (4)	80 (14)	108 (15)
	Disulfoton	19.65	125/**153**	102 (10)	92 (15)	80 (17)	71 (9)
	Parathion-Me	21.05	**125**/263	113 (17)	93 (15)	88 (8)	121 (10)
	Paraoxon-Et	21.47	**109**/149	104 (22)	99 (5)	81 (7)	109 (8)
	Malathion	22.29	125/**173**	95 (5)	96 (15)	78 (6)	97 (11)
	Chlorpyrifos-Et	22.56	125/**278**	103 (9)	92 (3)	49 (4)	89 (8)
	Fenthion	22.63	197/**314**	103 (18)	65 (13)	76 (8)	96 (19)
	Parathion-Et	22.65	**139**/291	104 (10)	91 (12)	83 (6)	83 (18)
	Bromophos-Me	23.18	**125**/331	104 (22)	92 (6)	81 (7)	104 (22)
	Chlorfenvinphos	23.94	**267**/323	106 (15)	107 (10)	74 (1)	93 (30)
	Bromophos-Et	24.55	**303**/331	103 (15)	110 (10)	68 (17)	96 (23)
	Ethion	27.01	153/**231**	102 (18)	99 (2)	64 (3)	111 (21)
Triazines	Atraton	18.26	**196**/211	114 (21)	117 (15)	52 (3)	73 (13)
	Simazine	18.43	186/**201**	98 (16)	105 (9)	97 (4)	121 (3)
	Prometon	18.47	**210**/225	110 (17)	101 (1)	59 (8)	88 (13)
	Atrazine	18.62	**200**/215	111 (18)	109 (10)	88 (3)	93 (22)
	Propazine	18.77	**214**/229	122 (19)	105 (3)	94 (4)	92 (21)
	Terbuthylazine	19.11	**214**/229	108 (7)	106 (13)	80 (6)	100 (20)
	Symetryn	21.16	198/**213**	111 (8)	97 (9)	63 (1)	75 (13)
	Ametryne	21.33	212/**227**	103 (5)	91 (10)	66 (3)	87 (18)
	Prometryne	21.45	226/**241**	107 (4)	102 (11)	69 (5)	94 (10)
	Terbutryne	21.86	226/**241**	111 (10)	99 (14)	63 (2)	96 (6)
Pyrethroids	Fenpropathrin	29.75	**181**/265	89 (13)	102 (1)	98 (10)	52 (16)
	Permethrin (I)	32.38	163/**183**	94 (4)	98 (4)	89 (5)	75 (10)
	Permethrin (II)	32.47	163/**183**	98 (7)	96 (2)	93 (14)	94 (12)
	Cyfluthrin (I)	33.31	**163**/165	100 (5)	95 (11)	ND	100 (16)
	Cyfluthrin (II)	33.48	**163**/165	96 (4)	96 (1)	102 (9)	92 (13)
	Cyfluthrin (III)	33.62	**163**/165	90 (3)	94 (3)	74 (21)	100 (10)
	Cyfluthrin (IV)	33.69	**163**/165	75 (15)	94 (5)	67 (14)	89 (13)
	Cypermethrin (I)	33.86	163/**181**	78 (8)	93 (2)	84 (6)	86 (10)
	Cypermethrin (II)	34.03	163/**181**	103 (5)	106 (16)	85 (1)	89 (3)
	Cypermethrin (III + IV)	34.21	163/**181**	102 (1)	100 (4)	93 (5)	94 (13)
	Fenvalerate (I)	35.75	**125**/167	103 (11)	97 (5)	92 (7)	71 (18)
	Fenvalerate (II)	36.20	**125**/167	100 (15)	103 (5)	92 (26)	ND
	Deltamethrin	37.56	**181**/253	99 (9)	109 (6)	99 (26)	89 (13)

[a] ND, Not Determined.

ied analytes and analysed under identical experimental conditions with the corresponding column set.

3. Results and discussion

3.1. Optimisation of the MSPD method

On the basis of results previously obtained for selected OPPs and permethrin [15], C8-bonded silica and ethyl acetate were tentatively chosen as sorbent for MSPD of the investigated fruits and as extraction solvent. However, due to (i) the new analytes included in the present study as compared to the previous work (i.e. nine new OPPs—dichlorvos, mevinphos, dimethoate, disulfoton, bromophos-ethyl, paraoxon-ethyl, parathion-ethyl, chlorfenvinphos, and ethion, five pyrethroids—cypermethrin, deltamethrin, fenpropathrin, fenvalatate and cyfluthrin, and a new class of pesticides, triazines, including ten compounds); and (ii) the different sample size involved in the experiments (100 mg *vs.* 25 mg in [15]), preliminary experiments were carried out to re-optimise the original experimental conditions and ensure proper analyte extraction and clean-up.

First of all, possible breakthrough of the most polar pesticides during the clean-up step was investigated. Spiked orange samples were used in these experiments, which consisted on the collection of consecutive fractions of the washing solvent, Milli-Q water, and their subsequent liquid–liquid extraction with ethyl acetate by 5 min manual shaking. None of the target compounds were detected in the washing solvent when 15 mL of Milli-Q water at 0.5 mL/min were used for clean-up of the MSPD mixture. Therefore, this purification procedure was adopted for subsequent experiments.

Regarding the extraction step, using 100 mg of sample and a 1:1 (w/w) C8:fruit ratio, 700 μL of ethyl acetate at ca. 0.2 mL/min flow rate were found to suffice for quantitative extraction of the investigated analytes provided that the cartridge was dried for 20 min under suction to ensure complete water removal before elution. As previously mentioned, no further treatment of the collected extracts was carried out, apart from concentration to a final volume of 100 μL before GC–qMS analysis to reach adequate limits of detection (LODs) for pesticides in fruit samples in the scan mode with 1 μL injection.

In agreement with previous observations [15–17], a severe matrix effect, resulting in both under- and over-estimation of the concentrations, was observed during GC–qMS analysis for a number of pesticides belonging to the three classes investigated. This problem was solved by using matrix-matched calibration curves (five data points in the 0.025–0.50 μg/mL range), but even in this careful selection of the ions used for quantification and identification of each analyte was mandatory to prevent interference from coextracted matrix components (Table 1). Finally, two separate injections per fruit extract, one in the scan mode for OPPs and

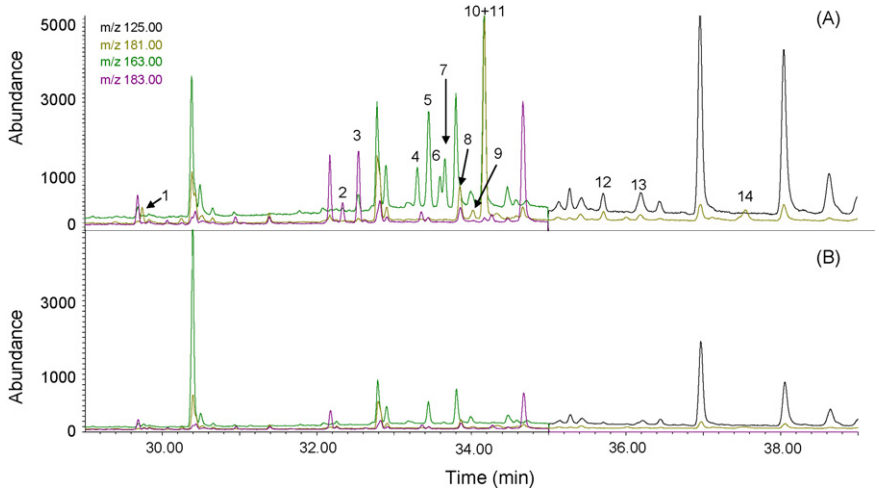

Fig. 1. Typical GC–qMS (SIM) reconstructed fragmentograms obtained for (A) an orange sample spiked with a standard mixture containing the pyrethroids investigated (spiking level, 0.5 mg/kg) and prepared using the developed MSPD method and (B) the same non-spiked orange sample extract. Peak numbering: (1) fenpropathrin, (2) permethrin (I), (3) permethrin (II), (4) cyfluthrin (I), (5) cyfluthrin (II), (6) cyfluthrin (III), (7) cyfluthrin (IV), (8) cypermethrin (I), (9) cypermethrin (II), (10) cypermethrin (III), (11) cypermethrin (IV), (12) fenvalerate (I), (13) fenvalerate (II), and (14) deltamethrin.

triazines, and another one in the SIM mode for pyrethroids, were carried out. The MSPD method was optimised for orange, and latter on used without modification for the determination of the target pesticides in pear, grape and apple (for this matrix, neither washing nor drying of the cartridge were necessary).

Recovery data obtained for the various tested fruits are summarised in Table 1. For orange, satisfactory recoveries in the 95–113% range were obtained for OPPs, with the only exceptions of dichlorvos (121%) and mevinphos. In general, similar recovery ranges were obtained for triazines (98–111%) and pyrethroids (recoveries above 75%) in this fruit. Satisfactory results were also obtained for pear for the three families of pesticides, i.e. recoveries in the range 91–110% for OPPs, 91–117% for triazines, and 93–109% for pyrethroids. The only relevant exception was fenthion (65%), a trend previously observed [15]. The recoveries for grape fall within short ranges (in general, 62–88% for OPPs, 63–97% for triazines, and 67–102% for pyrethroids), which can be considered as acceptable because of the general low relative standard deviations (RSDs) obtained for a large majority of the tested compounds (below the 20% recommended in current legislations for multiresidual analysis [18]). Dichlorvos and cyfluthrin (I) were not analysed in grape because of severe interference from matrix components. Finally, good results were also obtained for apple (range 71–121% for OPPs, 73–121% for triazines, and 71–110% for pyrethroids). Although the elimination of the washing step during the treatment of this particular matrix could be responsible for the poorer precision obtained for this fruit compared to other tested fruits, the collected extracts were clean enough to allow accurate GC–qMS determination of the target compounds and no additional interfering peaks showed up, except for fenvalerate (II). The overall results obtained in this study are similar to [19,20] or better than [21] those previously reported by other authors after MSPD of larger sample amounts (typically, above 500 mg) with much larger extraction solvent volumes (10 mL) than those used in the present work. More importantly, irrespective of the fruit type and pesticide class considered and with only a few exceptions, the obtained recoveries lay in the 70–120% interval typically accepted for multiresidual analysis [18].

As an illustration of the results obtained in this part of the study, Fig. 1, shows the typical reconstructed GC–qMS fragmentograms obtained for the isomers of the studied pyrethroids in a spiked and a non-spiked orange extract. The clean fragmentograms prove the selectivity of the complete analytical procedure developed.

As an example of the typical results obtained in this part of the study, Table 2 summarises the LODs for the studied pesticides as determined in real-life oranges (LOD = 3 × S/N ratio). OPPs and triazines were analysed in the scan mode while SIM was used for

Table 2
Comparison of LODs (μg/kg) calculated for orange after miniaturised sample preparation with GC–qMS (scan mode for OPPs and triazines, and SIM mode for pyrethroids) and GC × GC–μECD.

Pesticide class	Analyte	GC–qMS	GC × GC–μECD
OPPs	Dichlorvos	83	0.046
	Mevinphos	35	0.071
	Dimethoate	100	0.065
	Diazinon	94	0.12
	Disulfoton	150	1.10
	Parathion-Me	86	0.015
	Paraoxon-Et	250	0.085
	Malathion	47	0.020
	Chlorpyrifos-Et	15	0.005
	Fenthion	69	0.56
	Parathion-Et	200	ND[a]
	Bromophos-Me	86	0.018
	Chlorfenvinphos	86	1.0
	Bromophos-Et	47	1.2
	Ethion	46	0.060
Triazines	Atraton	83	0.054
	Simazine	113	0.29
	Prometon	63	0.27
	Atrazine	47	0.33
	Propazine	63	0.44
	Terbuthylazine	31	1.9
	Symetryn	41	0.029
	Ametryne	56	0.045
	Prometryne	43	0.015
	Terbutryne	73	0.045
Pyrethroids	Fenpropathrin	14	0.069
	Permethrin (I)	27	2.1
	Permethrin (II)	60	3.6
	Cyfluthrin (I)	15	0.22
	Cyfluthrin (II)	17	0.37
	Cyfluthrin (III)	18	0.90
	Cyfluthrin (IV)	18	0.86
	Cypermethrin (I)	9	0.076
	Cypermethrin (II)	17	0.15
	Cypermethrin (III + IV)	9	0.088
	Fenvalatate (I)	24	0.088
	Fenvalatate (II)	ND	0.20
	Deltamethrin	71	0.27

[a] Not determined.

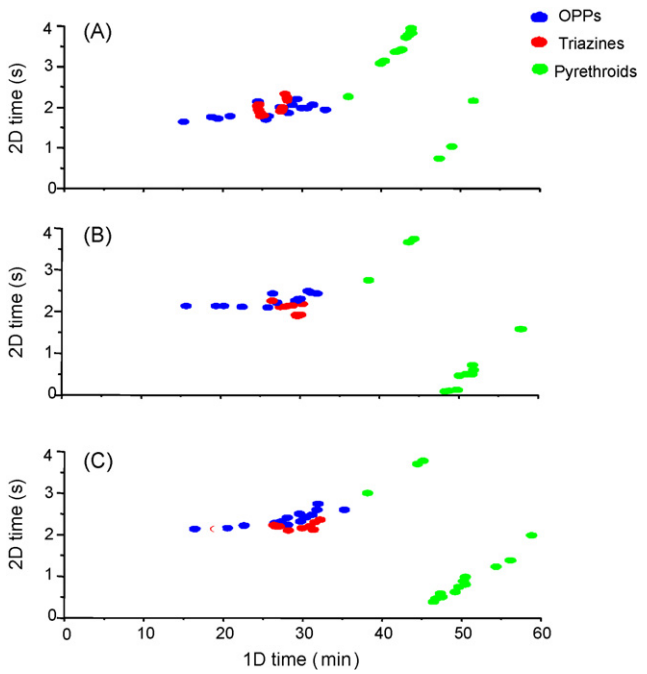

Fig. 2. Typical reconstructed contour plots obtained by GC × GC–μECD of the target pesticides in three selected column sets: (A) ZB-5 × BPX-50, (B) DB-17 × HT-8 and (C) HT-8 × BPX-50.

pyrethroids. With only a few exceptions, and in agreement with that observed for pear and grape, LODs were in the 10–100 μg/kg range, i.e. laid in the range of those previously found for close related matrices using MSPD combined with either GC–qMS [15,22] or liquid chromatography-mass spectrometry [23,24] for final determination. They are lower than or similar to the maximum residue levels (MRLs) set in current European Union (EU) legislations for these fruits [25–27]. The slightly higher LODs obtained for apple (although still below 150 μg/kg for a large majority of the analytes) could be associated to the elimination of the washing step for this particular matrix.

3.2. GC × GC–μECD pesticide group analysis

As previously indicated, the assayed column combinations were selected on the basis of data previously reported [10,12–14] our experience and, when possible, the desirable orthogonality of the GC × GC separations. The temperature program, which was kept similar for all investigated column combinations, was set such that provided an adequate separation of the target compounds in the first dimension. All investigated analytes eluted during the slow temperature program ramp in order to maximise their separation on the second dimension from the coextracted matrix components. This resulted in total analysis times ranging from 45 to 62 min, depending on the maximum working temperature of the GC column phases involved in the experiment. Although no offset of temperature relative to the primary oven was applied to the second dimension column, for all assayed column sets, wrap-around was only observed for the less volatile pyrethroids studied. No coelution between compounds issued from different modulations cycles was observed and the separation profile was conserved in all instances (also for sample extracts, as will be shown below).

Six column combinations with different polarities were tested. In this part of the study, standard mixtures containing the target pesticides were separately and jointly analysed. Firstly, an apolar phase, ZB-5, was combined with phases of increasing polarity,

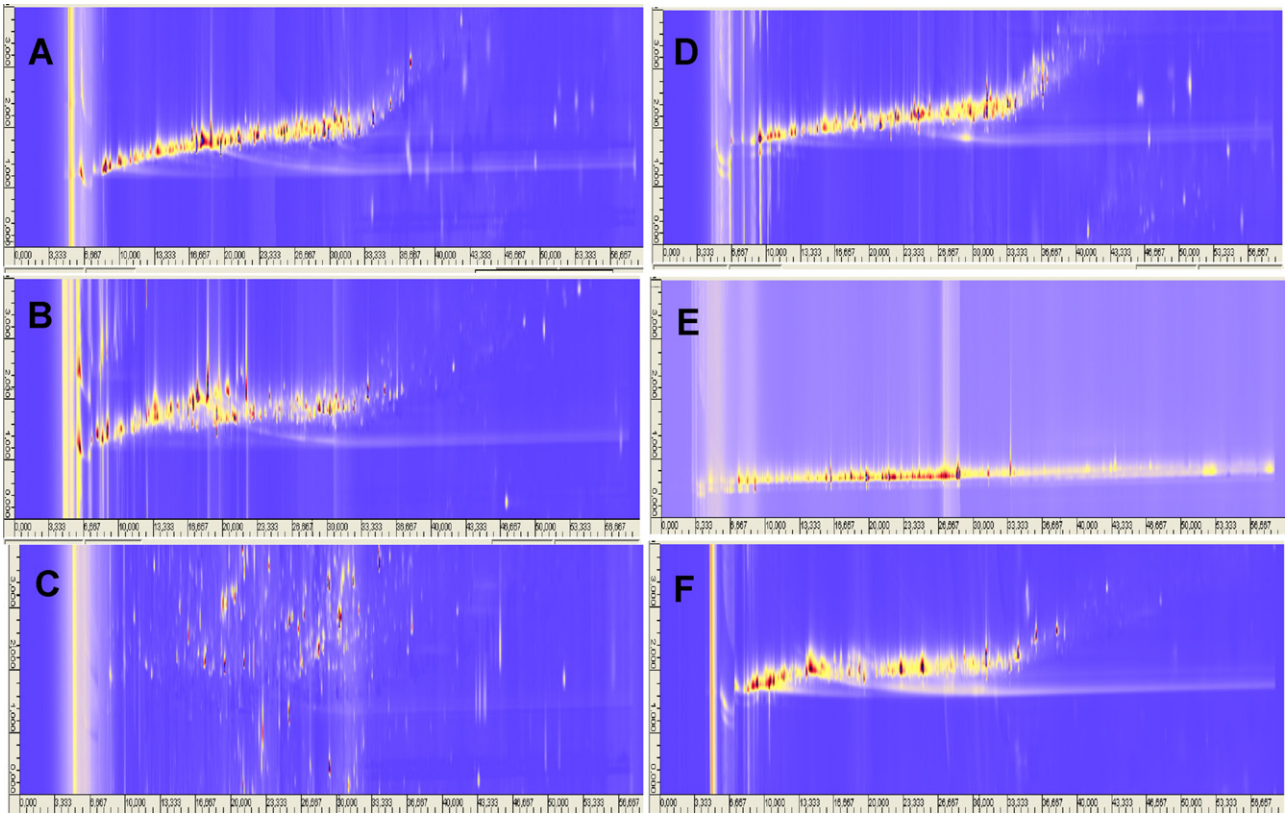

Fig. 3. Analysis of a non-spiked orange extract by GC × GC–μECD using (A) ZB-5 × HT-8, (B) ZB-5 × BPX-50, (C) ZB-5 × SW10, (D) DB-17 × HT-8, (E) DB-17 × BPX-50 and (F) HT-8 × BPX-50 as column combinations.

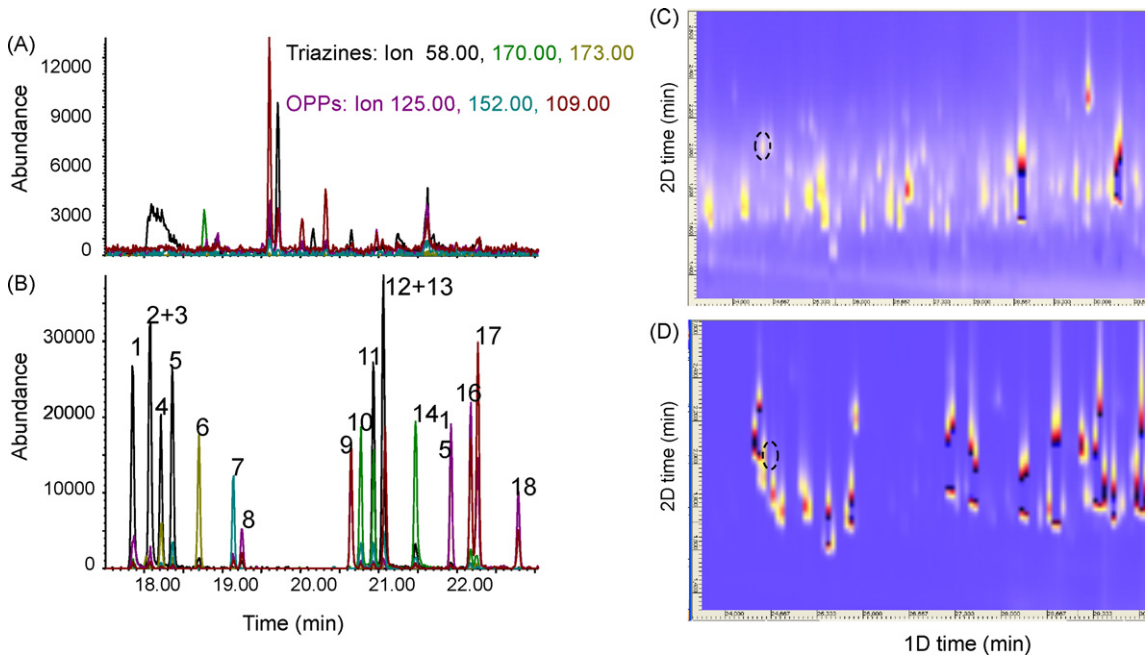

Fig. 4. Zoom of a selected area (17.5–23.0 min) of the typical chromatograms obtained by GC–qMS (scan) using a ZB5 columns for (A) a non-spiked grape and (B) a mixture of pesticides at the 0.5 mg/kg level, and by GC × GC–μECD using ZB5 × BPX50 as column combination for (C) the same grape extract and (D) mixture of pesticides. The position of atraton has been indicated with a cycle in (C) and (D). Peak numbering: (1) atratron, (2) simazine, (3) prometron, (4) atrazine, (5) propazine, (6) terbuthylazine, (7) diazinon, (8) disulfoton, (9) parathion-methyl, (10) simetryn, (11) ametryn, (12) prometryn, (13) paraoxon-ethyl, (14) terbutryne, (15) malathion, (16) chlorpyrifos-ethyl, (17) fenthion, and (18) bromophos-methyl.

i.e. BPX-50 and SW, and of different selectivity, i.e. HT-8. Severe degradation of some of the investigated OPPs and pyrethroids was observed on the second dimension with ZB-5 × SW under the experimental working conditions proposed preventing from further evaluation of this polar stationary phase in other column sets (see Fig. 3 below). Secondly, a more polar phase, DB-17, was tested as first dimension in combination with HT-8 and BPX-50 as second dimension columns. The last evaluated column set was HT-8 × BPX-50.

As an example of the typical results obtained in this part of the study, Fig. 2, shows the reconstructed contour plots of the target analytes in three selected column sets, i.e. ZB-5 × BPX-50, DB-17 × HT-8 and HT-8 × BPX-50. As expected, in all instances, pyrethroids eluted in the final part of the chromatogram, completely separated form the other pesticides studied, and with adequate separation among the isomers (when applicable). Apart form the quoted above degradation observed for some of these compounds in the SW phase, good peak shapes were obtained for pyrethroids with all other column combinations tested. Although OPPs and triazines distribution into the GC × GC retention plane differed depending on the column combination used, satisfactory separations, both between and within group, were observed for these pesticides with all column combinations assayed under the experimental conditions finally proposed, with the only somehow expected exception of DB-17 × BPX-50 (see below). This group-type separation was particularly evident for DB-17 × HT-8 and HT-8 × BPX-50 (Fig. 2B and C, respectively) probably because of the higher orthogonality provided by these column sets. No degradation (apart from that previously indicated for some particular OPPs in SW) became apparent for OPPs and triazines from the contour plots obtained with the several column sets tested. Only a small tailing in the anyway good shaped peaks was observed for selected OPPs when using BPX-50 as second dimension (see Fig. 3 below), an observation that agrees with results reported by other authors for OPPs [10–12] and other pesticides [13] when using this semi-polar phase as second dimension.

Despite the promising group-type separations achieved with DB-17 × HT-8 and HT-8 × BPX-50 when analysing standard mixtures of the three classes of pesticides studied, all six previously mentioned column combinations were also evaluated for real fruit extracts analysis because (i) all of them provided a satisfactory separation among the individual pesticides studied, and (ii) to prevent false positive with this type of detector, the separation of the target analytes from other coextracted matrix components should also be considered.

3.3. Application to real-life samples

Fig. 3 shows the contour plots obtained for a non-spiked orange sample prepared using the previously optimised miniaturised MSPD method with the six column combinations investigated. The results obtained for this real-life extract confirmed previous observations with standard solutions for the DB-17 × BPX-50 column combination, i.e. no separation was observed in the second dimension (Fig. 3E). They also proved that the use of the polar SW phase resulted in a severe degradation of some of the coextracted matrix components, which hamper proper identification of the analytes in real extracts (Fig. 3C). While a satisfactory resolution of the target compounds from matrix components was achieved with ZB-5 × BPX-50 (Fig. 3B), other column combinations involving DB-17 (Fig. 3D) and HT-8 (Fig. 3F) as first dimension did not provide such a separation and consequently were not further considered in this study. (Lack of separation among the test pesticides and the coextracted material with these latter column sets was confirmed by the analysis of orange extracts spiked with the studied OPPs and triazines under similar conditions; data not shown).

As a demonstration of the suitability of the methodologies proposed for the fast monitoring of the pesticides classes investigated in fruits, Fig. 4 shows the reconstructed GC–qMS fragmentogram and an expansion of the corresponding ZB-5 × BPX-50 contour plot area obtained for a grape treated according to the miniaturised sample preparation procedure proposed, i.e. 100 mg of sample washed with water and extracted with 700 μL of ethyl acetate were

either concentrated to a final volume of 100 μL before GC–qMS analysis, or directly analysed by GC × GC–μECD. Fig. 4C demonstrates the separation power provided by the GC × GC technique and that, despite the use of a non-selective detector, fast unambiguous determination of the target compounds in these complex extracts was possible by simple matching of the templates constructed from the corresponding standard mixtures contour plots. An extra benefit associated to the use of GC × GC is the sensitivity enhancement achieved through the modulation process [3]. As an example, Table 2 summarises the GC × GC LODs estimated for the orange extract. (These LODs have been estimated as the concentration corresponding to three times the average signal-to-noise ratio experimentally determined, on the base of peak volumes, in the vicinity to the corresponding pesticide for an orange extract spiked close to background level.) As expected, the response varied widely depending on the number of electrophilic atoms present in the molecule but, with only a few exceptions, LODs below 0.56 μg/kg were obtained even if no further concentration of the collected extract was carried out. Because of the satisfactory separation achieved with ZB-5 × BPX-50 among the test compounds and the matrix components for the four fruit types evaluated, rather similar GC × GC–μECD LODs were estimated for all matrices. These LODs were much lower than the MRLs currently legislated [25–27], which proves the feasibility of the proposed methodology of miniaturised generic sample preparation combined with GC × GC–μECD for fast screening of the target compounds in these kinds of samples. As an illustration of this statement, Fig. 4C shows that while atraton was unambiguously detected in a non-spiked grape by GC × GC–μECD, signals close to the LOD (i.e. 0.043 mg/kg) that compromise its accurate identification were found by GC–qMS (scan). Obviously, the sensitivity of the latter procedure could easily be improved by operating the system in the SIM mode or by injecting a larger amount of the final extract, however, both approaches at the price of larger analysis times and the use of more sophisticated instrumentation, i.e. higher cost. None of the pesticides in the test set were detected in the other fruit extracts investigated.

4. Conclusions

A miniaturised MSPD-based method involving a small amount of sample (i.e. 100 mg) and minimum solvent consumption (700 μL of ethyl acetate) has been developed and successfully applied to the analysis of selected OPPs, triazines and pyrethroids in fruits in combination with either GC–qMS or GC × GC–μECD. The enhanced sensitivity and separation among the target compounds and the coextracted matrix components provided GC × GC–μECD allowed accurate determination of the analytes at levels far below the MRLs set in current EU legislations even if no further concentration of the collected extract was carried out. These results made the MSPD-based plus GC × GC–μECD method proposed in this study to be consider a particularly interesting analytical approach in monitoring studies in which a larger number of samples of different nature are usually involved, but for which in many instances a quick yes/no answer is enough although peremptory.

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