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Rapid multi-residue method for the determination of azinphos methyl, bromopropylate, chlorpyrifos, dimethoate, parathion methyl and phosalone in apricots and peaches by using negative chemical ionization ion trap technology

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Abstract

A rapid, selective and sensitive multi-residue method for the determination of six common pesticides in stone fruit samples is described. The proposed method involves the extraction of the pesticides with the use of acetone solvent followed by liquid–liquid partition with a mixture of dichloromethane and light petroleum (40–60 °C) and subsequent determination by a gas chromatographic–mass spectrometry system using ion trap technology in negative ion chemical ionization mode. The average percent recoveries of bromopropylate and phosalone in the concentration range 0.2–2.0 mg/kg were 97.3±6.7 to 120±1.0%, while the recoveries of chlorpyrifos and parathion methyl examined in the concentration range 0.02–0.2 mg/kg were 95.5±7.5 to 145±3.6%, the recoveries of azinphos methyl in the range 0.05–0.5 mg/kg were 74.8±29.6 to 96.5±13% and those of dimethoate in the range 0.1–1.0 mg/kg were 73.1±5.7 to 92.8±2.8% for $n=3$ for all the above pesticides. The high mean recovery (145%) for chlorpyrifos is attributed to a matrix enhancement effect. The limits of quantitation in apricots were 0.01 mg/kg for chlorpyrifos, 0.02 mg/kg for dimethoate and parathion methyl, 0.05 mg/kg for azinphos methyl and phosalone and 0.1 mg/kg for bromopropylate. The usefulness of tandem mass spectrometry for confirmation purposes was also examined. The method was applied successfully to the determination of the target pesticides in 32 samples of stone fruits (apricots and peaches).

Keywords: Fruits; Food analysis; Matrix effects; Pesticides

1. Introduction

In the last few years several multi-residue methods have been reported that allow the identification and quantification of one or more classes of pesticides in fruits and vegetables. Mass spectrometric techniques are often the most practical and least equivocal approach to analyte confirmation [1,2]. The main disadvantage of the commonly used electron impact (EI) technique combined with a quadrupole analyzer is the relatively poor sensitivity for many pesticides in the full-scan mode. Using the single ion recording (SIR) technique, for better sensitivity, it is possible

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to lose all valuable information for confirmation purposes [3,4]. The introduction of the ion trap (IT) detector enhances the limits of detection (LODs), but the presence of matrix co-extractives in the sample still interferes with the results of the analysis [5], leading to false-positive and/or false-negative detection [2]. The negative chemical ionization (NCI) technique is recognized for the improved selectivity and sensitivity that can be achieved in the detection of electron-captive compounds, i.e. compounds with sufficient electron affinity [6–9]. With this technique, usually a few ions of high abundance are observed in the relevant mass spectrum and this enhances analyte detectability.

The NCI technique in pesticide residue analysis has mostly been used for environmental samples [9–14]. Only a few studies have been published on the use of this technique with samples of plant origin [15,16]. Detection limits with NCI-MS methods are usually two orders of magnitude lower than the corresponding EI-MS or positive chemical ionization MS (PCI-MS) methods [17,18].

In this work a multi-residue method is presented which applies a simple treatment of samples before obtaining the final solution for injection. A gas chromatography (GC)–NCI-IT-MS system, for quantification and confirmation of five organophosphorus insecticides (azinphos methyl, chlorpyrifos, dimethoate, parathion methyl, phosalone) and one acaricide (bromopropylate), was used. The method was applied to the analysis of apricot and peach samples within a pesticide monitoring programme in Greece. The six compounds studied are mainly used for the protection of stone fruits.

2. Materials and methods

2.1. Chemicals (solvents, analytical standards)

All solvents used were pesticide residue analysis grade and were obtained from LabScan (Ireland).

Pesticide standards of dimethoate (98.5%), parathion methyl (98.5%), and bromopropylate (99.5%) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Chlorpyrifos methyl (99.8%), azinphos methyl (99.2%) and phosalone (99.5%) were obtained from Dow AgroSciences (Greece), Bayer (Greece) and Rhone-Poulenc (Greece), respectively. The standards were used for the preparation of stock standard solutions for each pesticide at 1000 μg/mL in acetone and stored at −20 °C. Working standard mixture solutions for spiking purposes were prepared by appropriate dilution of stock solutions in acetone. Calibration standards (at six levels) were prepared in extracts of apricots, previously analysed twice for the absence of compounds interfering with the analytes, as follows [16]: a 500 μL aliquot of the final concentrated extract was evaporated to dryness under a gentle stream of nitrogen and the residue was redissolved in 500 μL of 2,2,4-trimethylpentane–toluene (90:10) containing a mixture of the pesticides under study.

Table 1 shows the pesticides studied and pertinent

Table 1
Target compounds and relevant information

Compound	Number of electronegative groups	MRLs of EU in stone fruit (mg/kg)	Quantitation ions (m/z) in NCI mode and relative abundance
Azinphos methyl	–P ester	0.5	157 (100)
Bromopropylate	–Br(2)	2.0	79 (100), 81 (99), 366 (8), 368 (18), 370 (7)
Chlorpyrifos ethyl	–Cl(3), –P ester	0.2	313 (100), 314 (30), 315 (66), 212 (34), 169 (32)
Dimethoate	–P ester	1.0	157 (100)
Parathion methyl	$-NO_2$, –P ester	0.2	154 (100), 263 (21)
Phosalone	–Cl, –P ester	2.0	185 (100), 186 (8), 187 (10)

information such as the electronegative groups, the permitted European Union (EU) maximum residue levels (MRLs) and the characteristic ions in NCI mode for quantitation and confirmation purposes.

2.2. Instrument

A Thermo Quest TRACE 2000 gas chromatograph coupled to a GCQ (Thermo Quest, Austin, TX, USA) ion trap mass spectrometer was used. The GC was equipped with a split/splitless injector operated in the splitless mode and an autosampler AS-2000. The analytical column used was a 30 m×0.25 mm I.D., 0.25 μm film thickness, Rtx-5 ms (Restek, Bellefonte, PA, USA) coated with a 5% diphenyl–95% dimethylsiloxane stationary phase. The temperature programme consisted of 1.0 min hold at 50 °C, ramp at 30 °C/min to 180 °C, 1.5 °C/min to 260 °C, 30 °C/min to 300 °C and a final hold for 5 min. The injector was operated at 210 °C with a split flow of 50 mL/min and a splitless time of 0.75 min. The helium carrier gas flow was 1 mL/min.

2.2.1. Operating conditions for IT-MS

The ion source was operated in the CI mode with methane as reagent gas. The source temperature was set at 200 °C, pressure at $1.2 \cdot 10^{-4}$ Torr (1 Torr= 133.322 Pa), and transfer line temperature at 275 °C. The system was tuned in the negative ion chemical ionization mode with heptacosafluorotributylamine-$(C_4F_9)_3N$ (FC-43, ULTRA Scientific, North Kingstown, USA) with the electron multiplier set at 1375 V and trap offset at 7 V.

2.2.2. Instrumental parameters for MS–MS experiments

The phosalone ion with m/z 185, which is attributed to $-S(P=S)(OCH_2CH_3)_2$ [9], was chosen as precursor ion for MS–MS experiments. The product ions for confirmation purposes were the ions with m/z values of 111, 157 and 185. Three parameters are involved in MS–MS experiments. (a) The ''q value'', which refers to the main radiofrequency (RF) voltage that is used during mass analyzer collision-induced dissociation. The default value of 0.225 worked perfectly for this compound and the precursor ion. (b) The collision energy, V, also referred to as the resonance excitation RF voltage that is applied to the endcap electrodes causing the production of daughter ions from the precursor ion. Three values of this parameter were examined (0.50, 0.60 and 0.75 V). (c) Excitation time (all measurements were carried out at 15 ms).

2.3. Extraction procedure

The extraction procedure [19] was very simple. From the homogenized sample of stone fruit, an aliquot of 15 g was weighed into a 250 mL PTFE centrifuge bottle (Nalgene, Rochester, NY, USA) and extracted with 30 mL of acetone for 30 s with an Ultra-Turrax T25 (IKA, Germany) at 8000 rpm. A 60 mL volume of dichloromethane–light petroleum (1:1) was added and the mixture was extracted for a further 30 s. The mixture was then centrifuged at 4000 rpm for 5 min and the supernatant liquid filtered through filter paper. An aliquot of 25 ml was concentrated to dryness in a water bath at 60 °C. The residue was redissolved in 5 ml of a mixture of 2,2,4-trimethylpentane–toluene (90:10). Then 1 μL of this solution was injected onto the GC–MS system.

2.4. Preparation of fortified samples

Fruits from untreated apricot trees were used as control samples and for the fortification experiments. These fruits were homogenized and analyzed in duplicate and then 15 g sub-samples were kept frozen until spiking. Each was spiked at three different levels of each pesticide, using each time the appropriate standard mixture solutions of the six pesticides in the study, which were prepared after dilution of the stock solutions in acetone solvent. Spiking samples were left to stand for 3 h before analysis to allow pesticide absorption onto the matrix.

3. Results and discussion

During a preliminary study we found that pesticide residues studied in relevant fruit extracts presented a

strong matrix effect. Accordingly, it was decided to use fruit extracts for the preparation of the calibration standards. Several clean-up processes were tried. They were found to be time-consuming and labor-intensive accompanied by loss of analytes, while matrix effects were still present. Clean-up procedures alone are not sufficient to prevent matrix enhancement for all organophosphates [20]. Fruit extracts prepared under the extraction procedure were used for the preparation of working analytical standards. Matrix effects are more prominent and intense in GC–IT-MS than in GC with classical pesticide-specific detection methods, electron-capture (ECD) and nitrogen–phosphorous (NPD) [21]. Schenck and Lehotay [22] also found in their work that GC–IT-MS was more sensitive to matrix enhancement than GC–flame photometric detection (GC–FPD).

Calibration curves were constructed for each compound using six different concentration levels. Correlation coefficients were greater than 0.961. Recovery and precision data of the proposed method are given in Table 2 for three concentration levels, while a typical chromatogram of a mixture of the six pesticides is shown in Fig. 1. Recoveries were between 73.1 and 124.6% (with the exception of one extreme value of 145%). Overall recoveries ranged between 82.03 and 114.36% (without taking into account the extreme 145% recovery value for chlorpyrifos at the 0.02 mg/kg fortification level), indicating good precision. Relative standard deviations (RSDs) were between 0.4 and 15.1 (with the exception of one extreme value of 29.6% for the 0.5 mg/kg fortification level of azinphos methyl). These results are satisfactory for residue analysis [1].

Table 2
Recovery and precision data ($n=3$) of the compounds in apricots

Compound	Fortification level (mg/kg)	Mean recovery (%)	RSD (%)
Azinphos methyl	0.05	96.5	13.2
	0.20	74.8	18.8
	0.50	74.8	29.6
Bromopropylate	0.20	106	2.94
	0.80	97.3	6.72
	2.0	97.5	8.93
Chlorpyrifos	0.02	145	3.57
	0.08	120	0.44
	0.20	95.5	7.46
Dimethoate	0.10	92.8	1.96
	0.40	73.1	5.71
	1.0	74.7	3.83
Parathion methyl	0.02	125	1.02
	0.08	98.1	3.58
	0.20	95.7	3.04
Phosalone	0.20	121	1.04
	0.80	118	10.9
	2.0	105	15.1

The extreme value of 145% for chlorpyrifos can be attributed to a matrix enhancement effect. Matrix-induced chromatographic response enhancement is a phenomenon that causes excessively high recovery results for some pesticides in food [23]. To our knowledge this phenomenon in the area of pesticide residue analysis was reported for the first time by Luke et al. in 1981 [24] for many polar pesticides. The matrix-induced effect is influenced by many factors such as pesticide character, matrix type, state of GC system and analyte/matrix concentration [25]. Erney et al. studied the appropriate matrix concentration that is required for optimal transfer of analyte from the injector to the column [26]. The high mean recovery of 145% for chlorpyrifos at the lowest fortification level, 0.02 mg/kg, can be attributed to the low ratio of analyte concentration in relation to the matrix concentration. The matrix concentration was the same in all solutions, calibration standards, fortified samples and real samples, but the analyte concentrations were different. The mean recoveries for chlorpyrifos at higher fortification levels decline in relation to the lowest fortification level (120% at 0.08 mg/kg and 95.5% at 0.2 mg/kg). The same behavior can be observed for parathion methyl, phosalone and bromopropylate, i.e. the mean recovery value declines from lower to higher fortification levels. Podhorniak et al. [20] proposed that acceptable recoveries range from 50 to 150% for low concentration levels (0.001–0.030 mg/kg) and, according to this, the value of 145% for chlorpyrifos is acceptable.

Matrix-induced peak enhancement remains a major problem in pesticide residue analysis. It is attributed either to co-extractives competing for the active sites in the injection port, ‘‘protecting’’ the analytes from adsorption [22,26,27], or for the column’s active sites [28], especially active sites

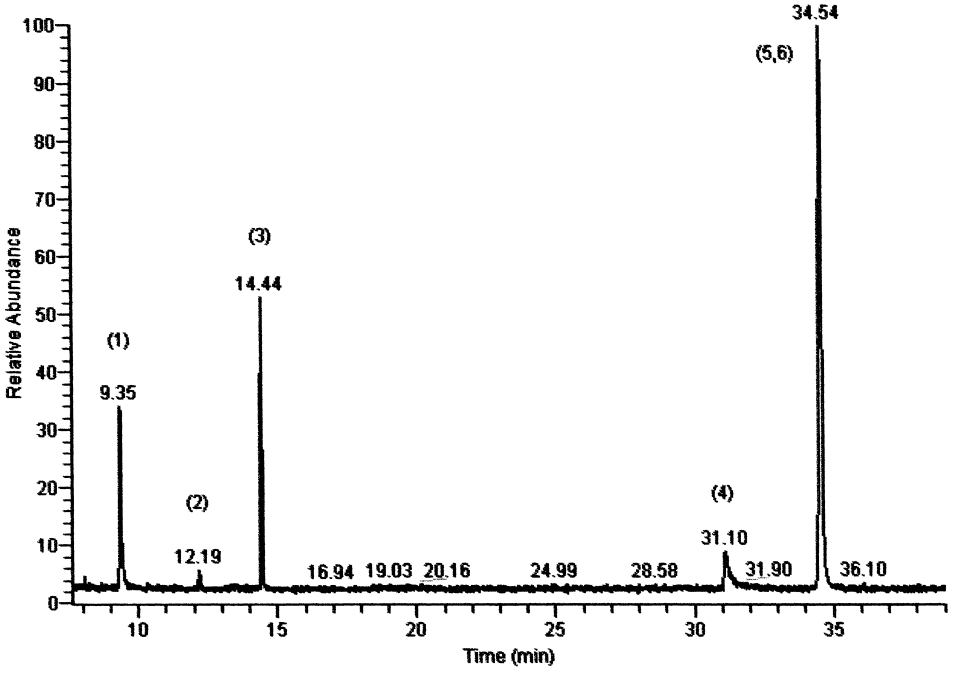

Fig. 1. Chromatogram of a mixture of the six pesticides for the quantitation ions selected (from Table 1). Peaks: 1 = dimethoate (m/z 157) 0.07 ng, 2 = parathion methyl (m/z 154, 263) 0.014 ng, 3 = chlorpyriphos (m/z 169, 212, 313–315) 0.014 ng, 4 = bromopropylate (m/z 79, 81, 366–370) 0.14 ng, 5 = azinphos methyl (m/z 157) 0.035 ng, 6 = phosalone (m/z 185–187) 0.14 ng.

which may actually be present on the head of the column [20]. Several authors have proposed a variety of solutions for this problem; all solutions reduced the matrix enhancement effect, but did not eliminate it. The first proposal is the use of standards prepared in blank matrix extract to compensate for the matrix-induced effects for quantitation, obtaining in this case more accurate results, but this solution is a compromise [25–27,29]. The second approach is using clean-up procedures alone or in combination with other techniques. More rigorous clean-up or the use of a GC column with fewer active sites [28], using clean-up with different solid-phase extraction (SPE) cartridges [22] or a daily column-cutting procedure (after each set of 10 to 12 samples) in combination with SPE extract clean-up and pulsed flame photometric detection (P-FPD) [20] are some of the proposals. In spite of using these clean-up methods the problems remain. Even with the use of three different combined SPE cartridges the enhancement factor remained >20% for certain pesticides despite the more extensive clean-up [22]. A reduction of matrix effects was obtained by pressure pulsed splitless injection and by using larger sample injection volumes up to 4 μL [30]. A different approach, proposed by Gonzalez et al. [31], applied correction functions to the obtained data. Correction functions were obtained and validated over a period of 4 months.

A conservative estimate of the method's detection limit is the product of the worst-case standard deviation at the lowest validation level with the Student t-value [32]. At the 99% confidence level and for two degrees of freedom (three replicates) this t-value is 6.965. Thus the detection limits (mg/kg) using this approach were 0.04 for azinphos methyl and bromopropylate, 0.007 for chlorpyrifos, 0.01 for dimethoate, 0.002 for parathion methyl and 0.02 for phosalone. However, the actual method detection limits according to the lowest calibration level are higher than the theoretical values.

Determinations at the level of 0.02 mg/kg for parathion methyl, 0.05 mg/kg for azinphos methyl and dimethoate, 0.01 mg/kg for chlorpyrifos and

0.14 mg/kg for bromopropylate and phosalone were readily achieved. The use of the latter value as the limit of determination for phosalone and bromopropylate is not a problem, due to the existence of high MRLs for these pesticides in apricots and peaches.

Selected values of the collision energy gave MS–MS spectra with three ions with a relative abundance >10%, 0.5 V [185(100), 157(20), 111(44)], 0.60 V [185(56), 157(36), 111(100)] and 0.75 V [185(10), 157(47), 111(100)], suitable for confirmation purposes [3,4]. Three different concentrations of phosalone, 0.1, 1 and 2 ng/μL, were used to obtain the relative abundances of the selected ions. The RSDs of the relative abundances were very low (<2%) for all combinations of concentration and collision energy values used (Table 3). This indicates that the use of the MS–MS technique for confirmation purposes together with the retention time is an appropriate approach.

The method was applied to the analysis of 32 samples of apricots and peaches. The apricot samples originated from Corinth and Nauplion in the Peloponnese and the peach samples were from the area of Veria in North Greece. No residues of the target pesticides were detected in 22 of the samples. Chlorpyrifos was detected in one apricot and five peach samples at concentrations from 0.01 to 0.06 mg/kg. Parathion methyl was detected in four peach samples from 0.02 to 0.07 mg/kg. The pesticides detected in one sample only were bromopropylate (0.36 mg/kg), phosalone (0.14 mg/kg) and azinphos methyl (0.05 mg/kg).

Table 3
Relative abundances of the MS–MS product ions of m/z 185 of phosalone (excitation $q = 0.225$, excitation time 15 ms)

Excitation voltage (V)	Amount (ng)	Relative abundances of ions		
		111	157	185
0.50	0.1	45	20	100
	1.0	46	20	100
	2.0	46	26	100
0.60	0.1	100	36	56
0.75	0.1	100	45	9.3
	1.0	100	49	10
	2.0	100	49	9.2

In conclusion, the proposed multi-residue method presents several advantages. The sample pretreatment is simple and quick without a clean-up step. The proposed GC–NCI-MS analytical method combined with the identification power of MS–MS achieves low detection limits and confirmation of the presence of target compounds sometimes unavailable with EI quadrupole instruments.

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