Comparison of Disposable Pipette Extraction and Dispersive Solid-Phase Extraction in the QuEChERS Method for Analysis of Pesticides in Strawberries

Virgínia C. Fernandes, Valentina F. Domingues, Nuno Mateus and Cristina Delerue-Matos

In this study, we sought to assess the applicability of GC–MS/MS for the identification and quantification of 36 pesticides in strawberry from integrated pest management (IPM) and organic farming (OF). Citrate versions of QuEChERS (quick, easy, cheap, effective, rugged and safe) using dispersive solid-phase extraction (d-SPE) and disposable pipette extraction (DPX) for cleanup were compared for pesticide extraction. For cleanup, a combination of $MgSO_4$, primary secondary amine and C18 was used for both the versions. Significant differences were observed in recovery results between the two sample preparation versions (DPX and d-SPE). Overall, 86% of the pesticides achieved recoveries (three spiking levels 10, 50 and 200 μg/kg) in the range of 70–120%, with <13% RSD. The matrix effects were also evaluated in both the versions and in strawberries from different crop types. Although not evidencing significant differences between the two methodologies were observed, however, the DPX cleanup proved to be a faster technique and easy to execute. The results indicate that QuEChERS with d-SPE and DPX and GC–MS/MS analysis achieved reliable quantification and identification of 36 pesticide residues in strawberries from OF and IPM.

Introduction

Pesticides are powerful chemicals widely used in current agricultural practices around the world, and it is common that pesticides occur in food products. Nevertheless, pesticides continue to be used because they contribute significantly to the control and elimination of various types of pests and thereby improve food production throughout the world. Organic farming (OF) and integrated pest management (IPM) are ecological approaches to agricultural pest control (1). The use of pesticides is prohibited in OF and strictly limited in IPM. IPM is an approach that relies on current, comprehensive information on the life cycles of pests and their interaction with the environment. OF systems rely on prevention rather than cure. However, the frequency of food pesticide contamination is greater than expected, and pesticide control is required. Increasing public concern about health risks from pesticide residues in the diet has led to strict regulation of maximum residue levels (MRLs) in food (2, 3).

There is growing interest in developing simple and reliable methods for the analysis of trace contaminants in fruits and vegetables. Routine and comprehensive testing of multiresidue pesticides is a challenging task because of the presence of large amounts of sample matrix components that occur naturally in food (4).

Many methods have been reported for the extraction of pesticides from fruits and vegetables (5). The liquid–liquid extraction (6), solid-phase extraction (SPE) (7), solid-phase microextraction (8), single drop microextraction (9), stir bar sorptive extraction (10), matrix solid-phase dispersion (11), microwave-assisted extraction (12) and pressurized liquid extraction (13) have been commonly employed for the extraction and cleanup of multiresidue pesticide analysis. In the last decade, special attention has been given to QuEChERS (quick, easy, cheap, effective, rugged and safe) method, which removes sample matrix components from extracts, with most research focused on reducing or eliminating interferences. The QuEChERS method has been developed for the rapid analysis of multiresidue pesticides in fruits and vegetables (14–16). Different versions of QuEChERS based on primary–secondary amine (PSA) or aminopropyl sorbent to bind fatty acid compounds, $MgSO_4$ to remove water and C18 or graphitized carbon black (GCB) are being used to remove sample matrix components (17). The QuEChERS method is usually performed in a "dispersive" manner, where the sorbent is mixed with the sample solution and subsequently separated through centrifugation.

Disposable pipette extraction (DPX) is a new SPE method used to rapidly extract sample solutions. Unlike traditional SPE devices, in DPX, solutions are mixed with the sorbent in a dispersive manner to provide rapid equilibration (18). In this method, the sorbent is contained inside a disposable pipette tip and is thoroughly mixed with sample solution (14). The interferents are concentrated on the sorbent and a clean extract is dispensed, thus reducing the need for solvent evaporation (17). In a previous study, some authors developed a DPX method using an $MgSO_4$, C18, PSA and GCB composition that provided high recoveries of several pesticides in tomato, strawberry, potato, orange and lettuce (19). Another study reported the use of DPX-reverse phase in orange and carrots (17).

The focus of the present research is the development and validation of a multiresidue method for the analysis of pesticides in strawberries from IPM and OF by comparing two QuEChERS versions using DPX and d-SPE followed by GC–MS/MS analysis. Also the aim is to study the different QuEChERS and cleanup compositions. The present research was driven to compare using GC–MS/MS the efficiencies of DPX method and d-SPE method for the extraction of pesticides in strawberries produced by IPM and OF. The strawberries obtained from different agricultural practices are different in their chemical composition (20), so the importance of this study was to evaluate whether the farming practices influence strawberry matrices and the efficiency of the extraction methodology. To the best of our knowledge, this is the first study comparing the pesticide extraction using citrate version of QuEChERS with DPX (without GCB) and d-SPE in strawberries from different agricultural practices. At the same time, it is also intended to study the samples from different cultivation practices.

Material and methods

Reagents

A total of 36 pesticides listed in Table I were included for the analysis. All pesticide standards and the 4,4′-dichlorobenzophenone had purity of ≥95% (typically >99%) and were obtained from Sigma-Aldrich (Steinheim, Germany), Riedel-de Häen (Seelze, Germany) and Chem Service (West Chester, PA, USA). *n*-Hexane, methanol and acetonitrile were of chromatographic grade and were supplied by Merck (Darmstadt, Germany). Stock standard solutions were prepared by dissolving reference standards in *n*-hexane [350 μg/L for organochloride pesticides (OCP)] and methanol (1,000 μg/L for other pesticide classes), and were stored at 4°C. Working pesticide standard mixtures were prepared by diluting stock solutions in *n*-hexane. 4,4′-Dichlorobenzophenone was used as quality control (QC). QuEChERS commercial products were used for sample preparation. The selected citrate version of QuEChERS (Ref EUMIV50CT-VP) and 2-mL dispersive solid-phase extraction (d-SPE) cleanup (Ref CUMPS15C18CT) were obtained from United Chemical Technologies (UCT) (Bristol, PA, USA) and 5-mL DPX tips from DPX Labs (Oakland, FL, USA).

Sampling

OF and IPM strawberries were collected in the first week of May in three consecutive years (2009, 2010 and 2011) from crops in central of Portugal. The OF and IPM fields were 7 km apart on average; the OF was different every year due to crop rotation. One kilogram of different varieties of strawberries, including Siba, Camarosa, Festival and San Andreas, in both types of farming were collected. The samples were chopped and stored frozen at −20°C in a freezer. The samples were analyzed by the procedure described below. Samples checked without any target analytes were used as blank strawberry samples in the preparation of matrix-matched standards and the recovery studies.

Sample preparation

For the initial extraction step, 15 g of chopped strawberries were placed into a 50-mL centrifuge tube and 50 μL of QC solution was added. For recovery studies, the sample was spiked at three levels (10, 50 and 200 μg/kg) for each pesticide. The strawberry sample was left for 30 min at room temperature to let the *n*-hexane evaporate before the addition of 10 mL of acetonitrile. The resulting solution was shaken for 1 min before the addition of 6 g anhydrous magnesium sulfate, 1.5 g sodium chloride, 1.5 g trisodium citrate dihydrate and 0.75 g disodium hydrogenocitrate sesquihydrate. The centrifuge tube was capped and shaken vigorously for 1 min to prevent salt agglomeration before centrifugation at 3,000 rpm for 5 min at room temperature. For cleanup, two types of commercial products were also evaluated for strawberry matrices. UCT provided 2-mL mini-centrifuge tubes for d-SPE and 5-mL tips for DPX. The tests with d-SPE and DPX were performed in the same day.

Dispersive solid-phase extraction

An aliquot of 1.5 mL was sampled from the upper layer of the prepared sample into a 2 mL d-SPE cleanup mini-tube containing 150 mg primary secondary amine (PSA), 150 mg $MgSO_4$ and 50 mg C18, and again vortexed for 1 min and then centrifuged for 5 min at 4,000 rpm at room temperature. From the upper layer of the prepared sample, an aliquot of 500 μL was transferred into a vial and placed in the autosampler of the GC.

DPX tips

For DPX cleanup, 5-mL DPX tips containing 150 mg anhydrous $MgSO_4$, 50 mg PSA and 50 mg C18 was adapted in a syringe. An aliquot of 1.5 mL of upper layer acetonitrile extract obtained from QuEChERS extraction was aspirated into the DPX tip and was transferred into a 15-mL glass vial. The syringe was used to aspirate air into the tip for proper mixing of the sorbents with the acetonitrile strawberry extracts (The procedure was repeated twice times). The strawberry extract was dispensed into the same glass vial. Finally, 500 μL was transferred into a vial and placed in the autosampler of the GC.

GC–MS/MS analysis

The GC–MS analysis in this study was performed on a TRACE GC Ultra gas chromatograph Polaris Q coupled with ion trap mass spectrometer (Thermo Fisher Scientific) operated in the electron impact ionization (EI) at 70 eV controlled by Xcalibur 1.3 software. Injection (1 μL) was conducted by autosampler (AI3000) in combination with a split/splitless mode, and the injector temperature was 240°C. Ultra high-purity helium was used as carrier gas at 1 mL/min (purity >99.999%). The analytes were separated on a ZB-XLB capillary column from Phenomenex® (30 m × 0.25 mm × 0.25 μm). The column oven temperature was programmed as follows: initial temperature 40°C (held for 1 min), increased by 30°C/min to 220°C (held for 5 min), increased by 10°C/min to 250°C and held for 20 min and finally increased again by 5°C/min to 285°C and held for 5 min. The ion source temperature was 250°C, transfer line temperature was 250°C and the electron multiplier was at 1,900 V (autotune to gain of 1×10^7). The pesticides involved in this study were identified using the retention time and by comparing three ions (one target and two qualifiers) with the MS^2-NIST library. Comparing these MS/MS spectra with standard and matrix-matched standard spectra, the MS/MS conditions were fixed for each compound, trying to select the one with highest m/z ratio and abundance as precursor ion (Table I). At the same time, ion trap parameters (excitation voltage, excitation time, isolation time, factor q and isolation mass window) were selected according to a previous optimization work [21]. The limit of detection (LOD) was established by the signal-to-noise ratio equal to 3 in an analysis of each sample, spiked at a lowest concentration, and the limit of quantification (LOQ) was defined by the signal-to-noise ratio equal to 10.

Table I

Validation Parameters: MS Information for the Targeted Pesticides, DPX Matrix-Matched Coefficient of Determination (R^2), LOD and LOQ using DPX and d-SPE Method

Pesticides	Chemical class	Type of pesticides	MW	Precursor ion (*m*/*z*)	Q_1 (*m*/*z*)	Q_2 (*m*/*z*)	R^2 (DPX)	MRL (μg/kg)	LOD[a] (μg/kg) DPX	LOD[a] (μg/kg) d-SPE	LOQ[b] (μg/kg) DPX	LOQb (μg/kg) d-SPE
α-Hexachlorocyclohexane (HCH)	OC	INS	291	183	179	177	0.9972	10	3.6	4.0	12.1	13.6
β-HCH	OC		291	183	179	177	0.9976	10	0.9	2.1	3.0	7.2
δ-HCH	OC		291	183	179	177	0.9979	10	0.8	2.4	2.8	8.0
Hexachlorobenzene (HCB)	OC		285	284	214	249	0.9998	10	1.1	2.0	3.6	12.0
Lindane	OC		291	183	179	177	0.9970	10	1.1	1.9	3.8	6.4
Aldrin	OC		365	263	227	193	0.9988	10	1.6	2.1	5.2	7.0
α-Endosulfan	OC		407	195	191	170	0.9956	50	2.5	2.4	8.4	8.0
p, *p*′-Dichlorodiphenyldichloroethylene (DDE)	OC		318	318	299	281	0.9969	50	1.0	1.2	3.4	4.0
Dieldrin	OC		381	243	211	176	0.9990	10	1.1	1.3	3.5	4.3
Endrin	OC		245	245	209	173	0.9976	10	10.8	9.5	36.1	31.7
β-Endosulfan	OC		407	195	191	170	0.9998	50	0.8	0.7	2.7	2.3
p, *p*′-Dichlorodiphenyldichloroethane (DDD)	OC		320	235	165	199	0.9962	50	1.5	1.7	4.9	5.7
o, *p*′-Dichlorodiphenyltrichloroethane (DDT)	OC		355	235	165	199	0.9971	50	1.2	2.9	4.1	9.7
Methoxychlor	OC		346	227	169	197	0.9971	10	12.0	11.5	40.0	38.5
Bifenthrin	PY	INS	423	181	165	164	0.9974	500	1.3	1.5	4.3	5.01
Bupirimate	PYR	FUN	316	208	178	129	0.9992	1,000	1.7	2.1	5.8	5.7
Chlorpyrifos	OP	INS; NEM	351	197	114	148	0.9956	200	0.9	1.1	3.1	3.7
Cyprodinil	PYR	FUN	225	224	208	197	0.9980	5,000	5.2	4.9	17.3	16.5
Dazomet	UN	FUN; NEM; FUM	162	162	89	44	0.9988	20	4.3	4.0	14.4	13.4
Deltamethrin	PY	INS	505	172	141	88	0.9961	200	4.4	3.9	14.8	13.1
Diazinon	OP	INS	304	179	167	110	0.9951	10	0.3	0.7	1.0	2.3
Fenhexamid	AN	FUN	302	177	166	153	0.9998	5,000	2.5	2.1	8.3	7.0
Fluazifop-*p*-butyl	APA	HER	383	254	249	163	0.9993	200	0.2	0.4	0.8	1.3
Fludioxonil	UN	HER	248	248	235	191	0.9918	3,000	0.8	3.8	2.5	12.7
Iprodione	DICA	FUN	330	314	271	153	0.9989	15,000	1.2	1.7	4.2	5.7
Malathion	OP	INS	330	173	134	106	0.9993	20	1.4	2.0	4.8	6.7
Mepanipyrim	PYR	FUN	223	222	207	221	0.9976	2,000	0.2	0.7	0.5	2.3
Methiocarb	MC	INS; MOL	225	168	153	109	0.9976	1,000	0.7	2.8	2.4	9.4
Myclobutanyl	AZ	FUN	289	152	150	151	0.9975	1,000	0.8	0.6	2.5	2.0
Pendimethalin	DN	HER	281	252	191	162	0.9967	50	2.8	1.3	9.4	4.4
Procymidone	DICA	FUN	284	283	281	282	0.9994	20	1.2	1.2	4.0	4.0
Pyrimethanil	PYR	FUN	199	198	183	158	0.9959	5,000	1.1	1.0	3.5	3.4
Quizalofop-*p*-ethyl	APA	HER	299	223	151	255	0.9987	50	3.2	2.4	10.6	8.0
Tetraconazole	AZ	FUN	372	336	249	105	0.9934	200	0.2	1.0	0.6	3.4
Tolylfluanid	PS	FUN; INS	347	138	137	136	0.9942	5,000	0.1	0.3	0.3	1.0
Vinclozolin	DICA	FUN	286	212	172	145	0.9968	50	0.9	1.1	2.9	3.6

OC, organochlorine; PY, pyretroids; PYR, pyrimidine; OP, organophosphorus; UN, unclassified; AN, anilide; APA aryloxyphenoxy propionic acid; DICA, dicarboximide; MC, *N*-methyl carbamate; AZ, azole; DN, dinitroaniline; PS, phenylsulfamide; INS, insecticide; FUN, fungicide; HER, herbicide; NEM, nematocide; FUM, fumigant; MOL, molluscicide.

[a] LOD is based on an S/N ratio of 3 (where S is the signal of the target ion and N is the noise intensity).

[b] LOQ is based on an S/N ratio of 10 (where S is the signal of the target ion and N is the noise intensity).

Method validation

Six matrix standards were used for matrix-matched calibration standards that included all 36 pesticide analytes at 10, 50, 100, 200, 400 and 500 μg/kg. The recovery experiments were carried out for strawberry sample matrix in three replicates each at three spiking levels (10, 50 and 200 μg/kg) for each sample preparation protocols (d-SPE and DPX cleanup) for strawberries from OF and IPM. Samples were then prepared according to the aforementioned procedure. The recoveries were calculated using the matrix-matched standard at the given spiking level for each concentration. The solvent calibration standards were used to assess MEs. The absolute MEs were calculated on the basis of the ratio between the response of the matrix standards and response in pure solvent standard.

Statistical analysis

All tests were conducted at least in triplicate. Data were analyzed using the GraphPad software 5 based on ANOVA tests.

Results

Optimization of the MS/MS method was performed for 36 pesticides using standard solutions injected in the EI ionization mode. The GC–MS/MS conditions, including precursor ion and qualifier ions (Q) of all target compounds, are shown in Table I.

This study was designed to compare different clean–up methods and also to validate QuEChERS GC–MS/MS method. Recovery validation experiments were conducted in each matrix at three spiking levels (10, 50 and 200 μg/kg). Table II lists the overall recoveries of each pesticide in the study. The results include the combination of data from strawberries spiked at three levels using two cleanup types and two crop types. Majority of the pesticides gave satisfactory recoveries (ranging from 70 to 120%). HCHs, aldrin, β-endosulfan, fludioxonil and myclobutanyl showed recoveries >120% with DPX tips. No low recoveries (<70%) were reported.

Table I summarizes the DPX matrix-matched coefficient of determination, along with LOD and LOQ values for the pesticides studied obtained with d-SPE and DPX cleanup. The DPX calibration plots exhibit good linearity for all the pesticides, and on average, the coefficients of determination were >0.997. The LOD and LOQ values obtained from DPX and d-SPE methods are similar. For all the 36 studied pesticides, the LODs were <12 μg/kg. In this study, the DPX method achieved LODs between 0.1 and 12 μg/kg for strawberries. However, for endrin and methoxychlor (MRL = 10 μg/kg), the DPX method revealed LODs higher than the listed tolerance levels.

Matrix effects for strawberry samples were also evaluated. The study was performed by comparing the response of reference

Table II

Percent Recoveries and Relative Standard Deviation (RSD) (in Parentheses) Based on Three Spiking Levels Obtained with DPX and d-SPE Extraction Versions in Strawberries *Camarosa* Variety from OF and IPM

Pesticides	Recoveries (%) OF (*n* = 3)						Recoveries (%) IPM (*n* = 3)					
	10 (μg/kg)		50 (μg/kg)		200 (μg/kg)		10 (μg/kg)		50 (μg/kg)		200 (μg/kg)	
	DPX	d-SPE	DPX	d-SPE	DPX	d-SPE	DPX	d-SPE	DPX	d-SPE	DPX	d-SPE
α-HCH	127 (10)	99 (11)	130 (12)	103 (10)	132 (11)	100 (10)	129 (4)	93 (3)	130 (3)	97 (5)	128 (5)	98 (5)
β-HCH	120 (8)	97 (9)	124 (10)	100 (11)	127 (8)	107 (11)	123 (7)	99 (3)	126 (6)	101 (5)	125 (6)	110 (5)
δ-HCH	119 (11)	109 (8)	121 (11)	114 (9)	124 (12)	117 (9)	121 (4)	110 (6)	121 (2)	112 (4)	119 (3)	114 (4)
HCB	115 (7)	94 (11)	117 (9)	98 (11)	116 (7)	95 (11)	114 (3)	100 (7)	112 (6)	99 (7)	110 (5)	102 (7)
γ-HCH (lindane)	123 (12)	115 (7)	127 (13)	119 (10)	124 (12)	111 (7)	119 (5)	106 (5)	119 (7)	109 (6)	120 (4)	108 (6)
Aldrin	115 (6)	102 (9)	119 (7)	107 (11)	117 (6)	110 (9)	120 (5)	107 (5)	122 (7)	109 (6)	123 (4)	111 (6)
α-Endosulfan	112 (6)	99 (6)	119 (10)	105 (8)	120 (6)	109 (6)	112 (4)	98 (7)	119 (3)	99 (4)	115 (3)	102 (4)
p, *p*′-DDE	101 (9)	95 (8)	109 (12)	99 (10)	110 (9)	97 (8)	106 (7)	102 (6)	109 (5)	100 (4)	103 (4)	102 (4)
Dieldrin	79 (9)	70 (9)	85 (7)	74 (8)	86 (9)	78 (9)	85 (5)	75 (3)	88 (8)	73 (3)	83 (6)	76 (3)
Endrin	115 (11)	100 (5)	117 (8)	106 (8)	119 (11)	110 (5)	116 (5)	99 (2)	117 (8)	100 (3)	120 (5)	104 (3)
β-Endosulfan	123 (12)	113 (7)	129 (7)	116 (11)	125 (12)	112 (7)	120 (4)	119 (6)	125 (6)	120 (2)	122 (3)	122 (2)
p, *p*′-DDD	112 (5)	98 (10)	110 (9)	102 (11)	111 (5)	100 (10)	112 (6)	95 (5)	119 (6)	97 (3)	115 (7)	99 (3)
o, *p*′-DDT	112 (7)	100 (8)	115 (10)	103 (13)	117 (7)	109 (8)	110 (6)	107 (7)	118 (4)	110 (5)	115 (6)	112 (5)
Methoxychlor	105 (7)	88 (11)	109 (10)	94 (10)	105 (7)	98 (11)	112 (6)	86 (4)	116 (4)	89 (6)	111 (5)	92 (6)
Bifenthrin	93 (10)	75 (8)	99 (12)	80 (10)	95 (10)	76 (8)	90 (2)	78 (3)	99 (2)	80 (4)	95 (3)	83 (4)
Bupirimate	100 (11)	80 (5)	104 (13)	88 (5)	102 (11)	90 (5)	101 (4)	82 (3)	107 (4)	85 (2)	100 (5)	86 (2)
Chlorpyrifos	95 (11)	70 (6)	98 (9)	77 (5)	101 (11)	79 (6)	104 (4)	73 (6)	104 (5)	75 (4)	100 (4)	75 (4)
Cyprodinil	95 (3)	82 (7)	99 (7)	91 (8)	103 (3)	94 (7)	99 (5)	80 (5)	107 (3)	82 (4)	100 (2)	83 (4)
Dazomet	90 (7)	70 (10)	98 (5)	77 (8)	99 (7)	80 (10)	92 (6)	75 (5)	98 (4)	73 (3)	95 (3)	77 (3)
Deltamethrin	90 (8)	80 (9)	98 (5)	89 (11)	95 (8)	85 (9)	89 (6)	79 (5)	94 (4)	80 (5)	90 (6)	84 (5)
Diazinon	78 (9)	70 (5)	83 (10)	77 (12)	85 (9)	79 (5)	82 (7)	72 (7)	89 (8)	74 (5)	87 (5)	76 (5)
Fenhexamid	102 (10)	90 (7)	106 (8)	97 (7)	110 (10)	95 (7)	103 (7)	90 (7)	112 (8)	92 (6)	110 (4)	95 (6)
Fluazifop-*p*-butyl	99 (7)	73 (10)	103 (9)	78 (5)	100 (7)	80 (10)	104 (8)	75 (3)	110 (5)	73 (2)	108 (5)	77 (2)
Fludioxonil	119 (6)	100 (12)	123 (5)	104 (10)	121 (6)	108 (12)	118 (6)	101 (5)	123 (7)	103 (7)	120 (3)	107 (7)
Iprodione	111 (11)	89 (11)	117 (13)	96 (9)	115 (11)	93 (11)	110 (5)	92 (6)	118 (5)	95 (5)	112 (6)	96 (5)
Malathion	112 (6)	99 (9)	118 (10)	104 (11)	120 (6)	105 (9)	119 (4)	101 (7)	119 (4)	105 (3)	120 (5)	109 (3)
Mepanipyrim	112 (7)	106 (8)	116 (5)	109 (10)	119 (7)	110 (8)	114 (4)	107 (5)	119 (4)	108 (3)	117 (3)	110 (3)
Methiocarb	90 (5)	72 (9)	96 (5)	77 (9)	99 (5)	79 (9)	93 (2)	77 (3)	99 (1)	74 (2)	99 (3)	76 (2)
Myclobutanyl	122 (10)	110 (11)	125 (10)	112 (11)	122 (10)	112 (11)	119 (3)	103 (5)	120 (2)	109 (7)	122 (5)	114 (6)
Pendimethalin	106 (7)	77 (10)	108 (7)	82 (10)	110 (7)	81 (10)	100 (3)	80 (6)	109 (2)	82 (6)	100 (6)	85 (6)
Procymidone	92 (6)	71 (9)	96 (6)	75 (9)	99 (6)	74 (9)	98 (4)	77 (5)	103 (3)	79 (5)	103 (8)	78 (7)
Pyrimethanil	90 (6)	71 (12)	96 (6)	75 (12)	98 (6)	80 (12)	96 (4)	70 (5)	103 (6)	71 (5)	101 (6)	74 (5)
Quizalofop-*p*-ethyl	103 (10)	80 (10)	109 (10)	86 (10)	106 (10)	83 (10)	106 (6)	82 (3)	101 (8)	89 (2)	110 (8)	93 (4)
Tetraconazole	115 (6)	100 (8)	117 (6)	106 (8)	120 (6)	111 (8)	118 (6)	98 (4)	122 (6)	100 (3)	121 (7)	103 (3)
Tolylfluanid	79 (7)	72 (11)	83 (7)	76 (11)	80 (7)	74 (11)	84 (8)	72 (4)	88 (6)	70 (3)	84 (5)	72 (2)
Vinclozolin	90 (11)	72 (8)	95 (11)	74 (8)	97 (11)	75 (8)	97 (8)	71 (4)	100 (4)	75 (4)	101 (4)	75 (3)

standards prepared in pure solvent with the response of matrix-matched standards (prepared as described in "Reagents"). Concentration levels tested for MEs were 50 μg/L for the OCP and 200 μg/L for the others. The ratio between response in matrix and response in pure solvent was taken as absolute ME. In both cases, a ratio of 0.8–1.2 was established as acceptable (22); this means that severe MEs (>2) affected, in this case, the response of six analytes (α, δ-HCH, endrin, iprodione, methiocarb and myclobutanyl) after application of the overall analytical procedure. The chromatographic signal for these six pesticides increased in the presence of the matrix. However, in strawberries, nearly 61% of pesticides suffered significant ME, with response ratio out of the range of 0.8–1.2.

We found that strawberry samples from OF and IPM can be cleaned up using DPX, and 18 strawberry samples were analyzed. Table III shows the detected pesticides in OF and IPM strawberry samples in three consecutive years. In OF strawberry samples, the pesticide residues detected were lindane and also β-endosulfan in 2009. The iprodione was found in higher levels in different varieties of strawberries from IPM.

Discussion

The pesticides were chosen based on the list of pesticides used in the Portuguese strawberries production, and OCPs owing to their persistence in the environment. Although OCPs were banned years ago, they are still detected in several food, environmental and even in human samples (16, 23, 24). The GC–MS/MS analysis indicated that the DPX and d-SPE were effective in the removal of interferences in strawberry samples. It is also noteworthy that 1 mL of extracts of strawberries can be extracted with the DPX tips. The DPX extractions take only a couple of minutes to perform, and the extract is cleaned up onto the sorbent, yielding a pesticide residue extract. GC–MS/MS was used for obtaining lower LODs in order to provide greater selectivity and thus allowing confidence in pesticide identification and quantitation (16). The LODs and LOQs are matrix-dependent. It is recommended to perform matrix-matched calibration for quantitative analysis. The LOD and LOQ are sufficiently low and adequate for the development of food safety studies. According to the European Union pesticide database, the lower MRLs for OCP range from 10 to 50 μg/kg (*o*, *p*′-DDT, *p*, *p*′-DDE, *p*, *p*′-DDD and α,β-endosulfan) in strawberries. The MRLs range from 20 to 15,000 μg/kg for malathion and iprodione.

Most of the pesticides showed an evident signal enhancement in the presence of matrix. In spite of this, a considerable number of pesticides still gave a response out of the 0.8–1.2 range, as Figure 1 illustrates. Statistic studies based on two-way ANOVA (Bonferroni post-tests) showed that no significant differences were observed in the absolute ME between the two cleanup

Table III

Pesticide Concentration Found in OF and IPM Strawberry Samples in Three Consecutive Years

Strawberry samples 2009		Strawberry samples 2010		Strawberry samples 2011	
OF (μg/kg)	IPM (μg/kg)	OF (μg/kg)	IPM (μg/kg)	OF (μg/kg)	IPM (μg/kg)
Lindane (27) *β*-Endosulfan (7)	Fludioxonil (117) Bifenthrin (55) Mepanipyrim (630) Tolylfluanid (49) Cyprodinil (36–43) Tetraconazole (35–37) Malathion (27) Lindane (5–7)	Lindane (13)	Iprodione (539–1,057) Cyprodinil (19–26)	Lindane (5–7)	Iprodione (498–520) Cyprodinil (23) Tetraconazole (22–25)

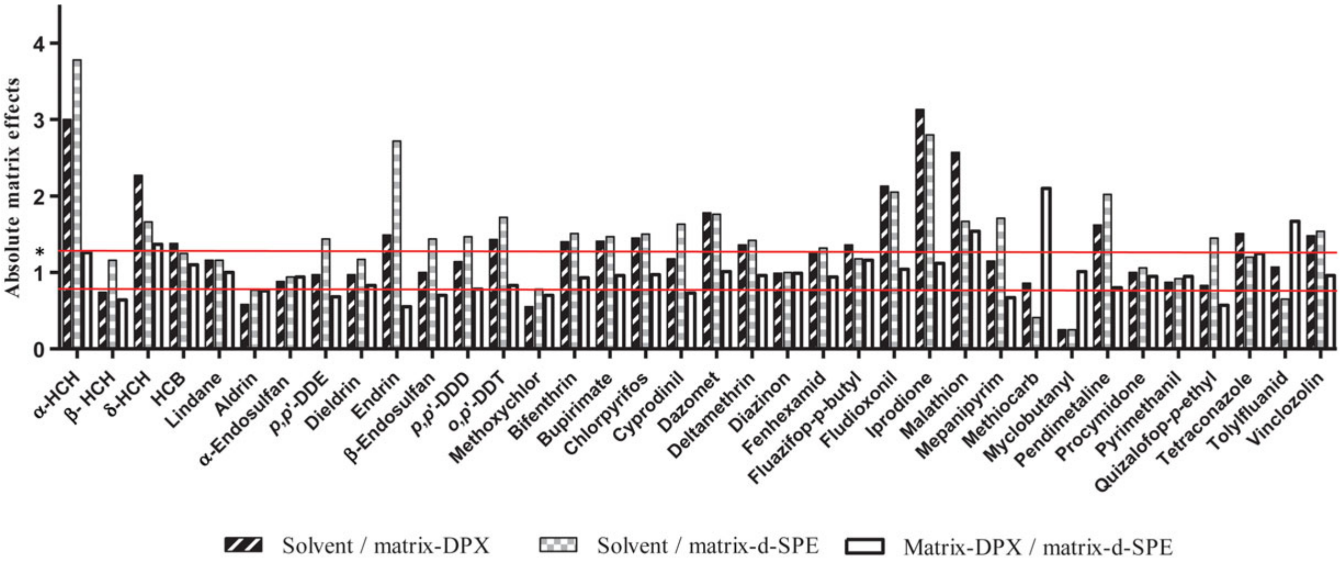

Figure 1. Absolute ME for strawberry samples in the GC–MS/MS determination of selected pesticides. *0.8–1.2: acceptable absolute ME.

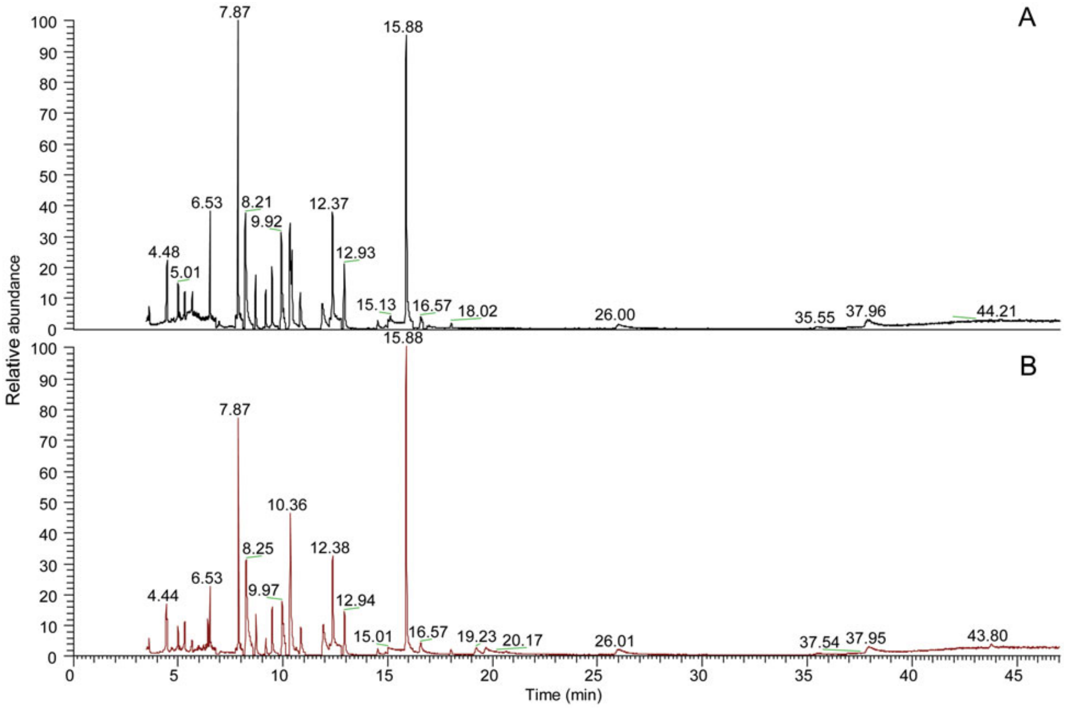

Figure 2. GC–MS chromatograms of a real strawberry sample spiked with a pesticide mixture at 200 μg/kg: (A) cleanup: dispersive solid-phase extraction; (B) cleanup: disposable pipette extraction.

versions (DPX and d-SPE) from OF. The same result was obtained in samples from IPM. These results showed that two extraction versions are similar and can be applied in this kind of sample. The chromatograms from DPX and d-SPE are very similar (Fig. 2). At the same time, the absolute MEs between the two agricultural management practices (OF and IPM) of strawberry DPX extracts were compared and no differences were observed. These results were obtained using a citrate version of QuEChERS and cleanups (d-SPE and DPX) without GCB. In other studies, the DPX was reported as having good performance in different samples and similar to d-SPE. However, those authors preferred to use acetate buffering with d-SPE as the final version (19).

Furthermore, it can be concluded that for the correct quantification of pesticides in strawberry samples, matrix-matched standards calibration using absolute matrix responses would have to be used.

According to our previous study, the strawberries from OF and IPM showed differences in their compositions (20). A statistic study based on one-way ANOVA (Newman–Keuls) has shown that the recoveries obtained from DPX and d-SPE methodology are significantly different in strawberries from both OF and IPM agricultural management practices. The results also showed that d-SPE recoveries in both the agricultural management practices were lower when compared with DPX recoveries. All the steps during d-SPE (vortex, centrifugation) and also the matrix (OF and IPM) can affect the efficiency of the extraction. Although the DPX cleanup generally showed chromatograms similar to those from the d-SPE version, the DPX tips extractions showed main advantages: the methodology is easy to perform, no conditioning steps are necessary, minimal elution solvent volumes are used, rapid extraction times and minimal training are required.

Pesticides were detected in concentrations lower than the MRL established by the European Union for strawberries. Except for lindane and β-endosulfan that are persistent pesticide in the environment, most of the others are commonly used in conventional and IPM practices of strawberry cultivation. Most of them are fungicides, which is the class most widely used in strawberry crops.

Conclusion

A simple, reliable and sensitive method for the analysis of 36 pesticides in strawberries from OF and IPM has been demonstrated using DPX. The results from this work demonstrate the potential for routine use of QuEChERS and DPX combined with GC–MS/MS to achieve faster individual sample turnaround time and higher throughput. Furthermore, the use of the citrate version of QuEChERS for extraction with either d-SPE or DPX cleanup using a standardized combination of $MgSO_4$, PSA and C18 powders was applied to strawberry matrices from different crop types for the first time. The recovery results showed significant differences between each other, but the percentage recovery values were in a satisfactory range. The DPX cleanup showed that some pesticides achieved recoveries >120%. In terms of MEs, no differences were observed. The different strawberry matrices (OF and IPM) also showed a performance relatively analogous. We conclude that the main advantages in applying extraction methodology with DPX are the rapid extraction time and the easy way to perform the extraction.

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