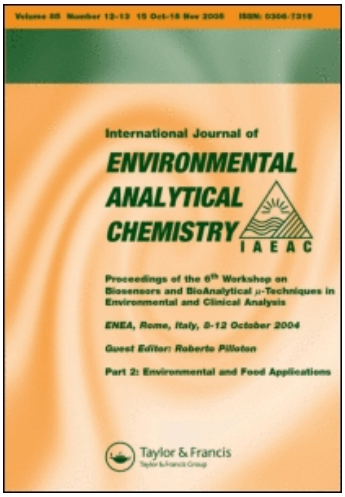

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Pesticide enrichment factors and matrix effects on the determination of multiclass pesticides in tomato samples by single-drop microextraction (SDME) coupled with gas chromatography and comparison study between SDME and acetone-partition extraction procedure

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In this study a single-drop microextraction (SDME) method was extended for the determination of multiclass pesticides (metribuzin, vinclozolin, fosthiazate, procymidone, fludioxonil, kresoxim-methyl, fenhexamid, iprodione, bifenthrin, λ-cyhalothrin, indoxacarb and azoxystrobin) in tomatoes and validated in comparison with a robust solvent extraction method in order to estimate the feasibility of SDME in more complicated determinations in terms of extraction efficiency, pesticides chromatographic stability and chromatographic induced matrix effects in pesticide residue analysis in food samples. Both sample preparation methods: (i) a single-drop microextraction method (SDME) developed recently in our laboratory, and (ii) a modified acetone-partition extraction procedure (APE) method that is being applied today for routine analysis of fruits and vegetables in many laboratories of pesticide residues analysis, were validated under ISO 17025 norms and SANCO Guide recommendations. For all pesticides studied, with the exception of pyrethroids, SDME exhibited good analytical characteristics by reporting from similar to 138 times lower LODs as compared with APE. The enrichment factors of the SDME procedure applied in tomato extracts ranged from 0.7 for bifenthrin to 812 for fenhexamid whereas, the concentration factors for the whole SDME studied ranged from <0.1 for bifenthrin and λ-cyhalothrin to 52 for fenhexamid. Relative recoveries ranged from 67 to 90% for SDME and from 90 to 120% for APE. Matrix effects assessment performed for both methods studied indicated that matrix matched standards should be used for quantitation purposes. However, the estimation of the gas chromatographic matrix effects by SDME indicated that SDME is a more selective sample preparation method than APE.

Keywords: single-drop microextraction; liquid-liquid extraction; vegetables; residue analysis; food analysis; sample preparation; matrix effects

1. Introduction

Over the last decade a significant number of miniaturised approaches have emerged, as viable sample preparation methods resulting in more efficient sample enrichment, faster sample preparation and easier automation with resultant solvent and sample savings as

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compared with classical methods [1,2]. The simplest (in terms of instrumental demands and time of preparation) of these miniaturised sample preparation methods is single-drop microextraction (SDME). The principal advantages of this technique over other widely applied miniaturised sample preparation methods (e.g. solid-phase microextraction (SPME), and use of active sorbents) are the range of solvents that can be used, the scope for preparing highly selective extraction phases by combining solvents and the ease of quantification afforded by introducing an internal standard into the drop.

Recently, several studies have been published on SDME technique for the determination of pesticide residues in food samples [3–8]. Research concern on current studies of SDME application in pesticide residues analyses in food samples has been focused on the optimisation of the main parameters that may influence the SDME and on the optimisation of extra steps employment for the liquefaction of the solid food samples prior the SDME. However, although the SDME analytical protocols exhibit good analytical characteristics, analytical performance data are difficult to be compared since matrix, analytes and calibration methods differ among the different reports (Table 1). In addition, authors' settlements on the optimisation parameters of the non-exhaustive microextraction techniques may lead in significant different analytical data among the different reported methods whereas pesticide enrichment factors of the different SDME are not always reported. Thus, the comparison of the different SDME analytical protocols is difficult for the proper choice of a SDME for a certain determination.

The aim of this work was to study the analytical performance of a SDME determination of pesticide residues in vegetables by validating the method for the determination of 12 pesticides from different chemical families (among others, pyrethroids, strobilurines and dicarboximides) and compare the analytical data with those obtained by a robust method that has been applied for routine analysis of fruits and vegetables in many laboratories of pesticide residues analysis. The SDME procedure used in this validation study was recently developed in our laboratory for the determination of nine organophosphate insecticides and five pesticides from other chemical families in vegetable samples and involves the initial sample extraction by means of a mixture of acetone/water and the following SDME with a drop of toluene [8]. The classic robust method used for the comparison of the results obtained by SDME was originally presented by Luke [9] and uses acetone for sample extraction followed by a liquid–liquid partition with dichloromethane and petroleum ether (1:1, v/v) [10]. Target pesticides in this study were selected to range widely in their physicochemical properties (e.g. λ-cyhalothrin and metribuzin) (Table 2) to examine the feasibility of SDME in more complicated determinations in terms of extraction efficiency, pesticides chromatographic stability and chromatographic induced matrix effects of pesticides determination.

2. Experimental

2.1 *Chemicals and standards*

Pesticide standards, of high purity (97.0–99.9%) were purchased from Riedel-de Haën (Seelze, Germany). All solvents used were of *Pestiscan* grade (pesticide residue analysis grade) and were obtained from Labscan (Dublin, Ireland). Water used for sample preparation by SDME was of HPLC grade and was purchased from Sigma–Aldrich (Steinheim, Germany).

Table 1. SDME applications for pesticide residues analysis in food samples.

Matrix	Analytes/Determination	Drop (Type/volume)	SDME (Time/stirring rate)	LOD (Limit of detection)	Ef (Enrichment factor)	Ref.
Juice	Ethoprophos; diazinon; parathion methyl; fenitrothion; malathion; isocarbophos; quinalphos/ GC-FPD	TOL/1.6 μL	15 min /400 rpm	0.98–2.20 μg L^{-1}	–	[3]
Vegetables	α-BHC; β-BHC; γ-BHC; δ-BHC; dicofol; dieldrin; DDE; DDD; p,p′-DDT/ GC-MS	AC:*p*-xylene/1 μL	30 min /400 rpm	0.05–0.2 μg L^{-1}	–	[6]
Water/fruit juice	Dichlorvos; phorate; fenitrothion; malathion; parathion; quinalphos/ GC-FPD	TOL/1.5 μL	20 min /600 rpm	0.21–0.56 μg L^{-1}	–	[4]
Fish	4,4′DDE; 2,4′-DDD; 4,4′-DDD/GC-MS	TOL/0.6 μL	7 min /400 rpm	0.5 μg kg^{-1}	–	[7]
Water/wine	Chlorothalonil; tradimenol; hexaconazole; diniconazole/ GC-ECD	Xylene/1.6 μL	15 min /800 rpm	Water: 0.0006–0.0010 μg L^{-1} Wine: 6.4–9.4 μg L^{-1}	56.5–105	[5]
Vegetables	Dimethoate; chlorpyrifos methyl; fenitrothion; malathion; chlorpyrifos; quinalphos; methidathion; phosalone; pyrimethanil; procymidone; buprofezin; trifloxystrobin; pyriproxyfen/GC-NPD, GC-ECD	TOL/1.6 μL	25 min /350 rpm	GC-NPD: 0.6–10.3 μg kg^{-1} GC-ECD: 0.03–5.38 μg kg^{-1}	–	[8]

TOL = toluene; AC = acetone.

Table 2. Chemical groups and physicochemical properties of the selected pesticides and their individual concentrations ($\mu g\,L^{-1}$) in F working standard solution.

	Pesticide	Chemical group/use	Vapour pressure (mPa)	Sol. in water (mg L^{-1})	log K_{ow}	F ($\mu g\,L^{-1}$)
1	Metribuzin	1,2,4-triazinone/H	0.058 (25°C)	1050 (21°C)	1.6	10
2	Vinclozolin	Dicarboximide/F	0.13	2.6 (20°C)	3.00	10
3	Fosthiazate	Organophosphorus/N	0.56	9850	1.68	500
4	Procymidone	Dicarboximide/F	10.5 (20°C)	4.5 (25°C)	3.14	50
5	Fludioxonil	Phenylpyrrole/F	0.00039	1.8	4.12	500
6	Kresoxim methyl	Strobilurin type; oximinoacetate/F	0.0023	2	3.40	50
7	Fenhexamid	Hydroxyanilide/F	0.0004	20	3.51	100
8	Iprodione	Dicarboximide/F	0.0005	13	3.00	100
9	Bifenthrin	Pyrethroid/I,A	0.024	<0.001	>6.00	50
10	λ-Cyhalothrin	Pyrethroid/I	0.0002	0.005	7	10
11	Indoxacarb	Oxadiazine/I	0.000025	0.20	4.65	50
12	Azoxystrobin	Strobilurin type; methoxyacrylate/F	0.00000011	6	2.5	50

H = herbicide; F = fungicide; N = nematicide; I = insecticide; A = acaricide.

Stock standard solutions of each pesticide were prepared in acetone at $1000\,\mu g\,mL^{-1}$ and stored in glass tapered bottles at −20°C. A working standard solution of different individual concentrations for each pesticide studied was prepared in acetone at $100\,F\,\mu g\,L^{-1}$ and used in method validation studies. Individual concentrations of each pesticide studied in an F working solution are also shown in Table 2. Other working standard solutions were obtained by appropriate solvent dilutions of 100 F. Ethion was prepared in toluene at $250\,\mu g\,L^{-1}$ and used as internal standard (I.S.) in SDME analyses.

2.2 *Samples*

Method validation studies were performed using tomato samples collected from local organic cultivations (free from target pesticide residues) as blank tomato samples. SDME and APE application study was performed using 20 tomato samples purchased from local food stores. Samples were handled according to the guidelines on good laboratory practice in pesticide residue analysis (Codex Alimentarius Commission 1993) [12] and analysed the same day by both methods studied.

2.3 *Fortified samples preparation*

Fifteen units of raw tomatoes were weighted, chopped, and homogenised in a blender. Appropriate amounts of 100 F or 50 F ($\mu g\,L^{-1}$) working solutions were spiked in suitable portions of homogenised samples for recovery experiments and linearity studies. After agitation, the samples were allowed to equilibrate for 60 min prior to different extraction assays.

2.4 *Analytical procedures*

2.4.1 *Single-drop microextraction (SDME)*

Two grams (2.00 ± 0.01 g) of the homogenised tomato samples were weighted in a glass centrifugation tube and 25 mL of a mixture of acetone/water, 10/90, v/v was added. The solution was homogenised by an Ultra-Turrax (IKA, Werke, Staufen, Germany) for 1 min at 13,500 rpm for 1 minute and then centrifuged at 4000 rpm min^{-1} for 10 min. Seven millilitres of the supernatant donor solution were placed into a 10 mL glass vial (6.5 cm high $\times$ 1.8 cm wide) equipped with a PTFE-coated magnetic stir bar (7 mm $\times$ 2 mm) and screw capped with a PTFE-faced silicone septum. A 10 µL microsyringe with a bevel needle tip (10F, SGE Australia) was used for introducing microdrop to the sample. Before each extraction, the microsyringe was washed at least 10 times with the drop extraction solvent (containing I.S.) in order to eliminate the bubbles in the barrel and the needle. The sample solution was agitated with a magnetic stirrer (at 250 rpm). Then 1.6 µL of organic solvent was drawn into the microsyringe before the extraction. The microsyringe fixed with a stand and clamps was inserted through the septum of the sample vial and immersed into the sample. The plunger was pushed down to expose the microdrop in the stirred solution (the distance of the microdrop from the surface of the stirred donor solution was set at 1.5 cm) for 30 min. When the extraction was finished, the drop was retracted into the micro syringe adjusted to 1 µL and injected directly into the GC inlet for further analysis [8].

2.4.2 *Acetone-partition extraction (APE)*

Seven and a half grams (7.5 ± 0.01 g) of homogenised sample were placed in a glass centrifugation tube and mixed thoroughly with 15 mL of acetone by means of an Ultra-Turrax (IKA, Werke, Staufen, Germany) for 30 sec at 6000 rpm min^{-1}. In this mixture, 15 mL dichloromethane and 15 mL petroleum ether were added and extracted by homogenising another 30 seconds. The homogenate was centrifuged for 2 min at 4000 rpm min^{-1}. 0.2 mL of the supernatant was placed into a 2 mL glass vial. The extract was evaporated to dryness under a gentle stream of nitrogen. The residue was reconstituted in 1.0 mL of toluene/2, 2, 4-trimethylpentane, 10/90, v/v [10].

2.5 *Gas chromatography*

Analyses were performed on a Hewlett Packard model 6890 Gas Chromatography system equipped with a micro electron capture detector (µECD). Pesticides were separated on a fused silica capillary column HP-5 (5% phenyl Methyl Siloxane), 30 m $\times$ 320 µm I.D. $\times$ 0.25 µm df film thickness. The chromatographic temperature program was: 100°C for 1 min, raised to 210°C (5°C min^{-1}) and held for 16 min; then raised to 285°C (3°C min^{-1}) and held for 10 min. The carrier gas (helium) flow rate was 1.7 mL min^{-1} for BPX-5 columns; injector temperature was at 230°C and the splitless injection (1 µL injection volume) was carried out with purge valve on at 1 min. Nitrogen 60 mL min^{-1} and helium 6 mL min^{-1} were used as auxiliary gases for the µECD. Detectors temperature was set at 310°C.

Quantification carried out on GC-µECD was performed using matrix matched calibration standards with both methods studied. Matrix matched standards for SDME were used as donor solutions and were prepared by spiking tomato extracts free from pesticide residues (obtained by the procedure described in Section 2.4.1) with appropriate

amounts of a working standard solution. Matrix matched standards for APE were prepared by diluting the residue of the tomato extract obtained by the APE procedure (Section 2.4.2) in 1 mL of an appropriate working standard solution prepared in toluene/2, 2, 4-trimethylpentane, 10/90, v/v.

A GC-MS system from Thermo Electron Corporation consisting of a Trace GC Ultra gas chromatograph and a Polaris Q mass spectrometer system was used for the confirmation of the positive detections in samples analysed for pesticide residues. Chromatographic separation was performed on a 30 m × 250 μm I.D. HP-5MS capillary column coated with 5% diphenyl and 95% dimethylpolysiloxane (G&W Scientific Products) with a film thickness of 0.25 μm. The chromatographic temperature program was: 100°C for 1 min, raised to 210°C (5°C min^{-1}) and held for 16 min; then raised to 285°C (3°C min^{-1}) and held for 20 min. The carrier gas (helium 99.999%) flow rate was in constant flow mode at 1.0 mL min^{-1}. Split/splitless injection of a 1 μL volume was carried out at 250°C. The interface line and ion source temperatures were maintained at 300 and 200°C, respectively. Analyses were performed in the full-scan mode whereas electron ionisation mass spectra in the range of 45–450 Da were recorded at an electron energy of 70 eV.

3. Results and discussion

3.1 *Analytical performance of SDME and APE analytical procedures*

The SDME analytical protocol developed in a previous work performed in our laboratory was applied and validated for the determination of 12 extra pesticides (Table 2) in tomato samples. Optimised extraction conditions in this determination were set at a lower stirring rate (250 rpm instead of 350 rpm) and longer extraction time (30 min instead of 25 min) mainly due to the increased incidence of drop damage observed using different laboratory equipment. By that change the response areas of selected pesticides were not significantly changed (Figure 1). The validation of the selected determination was also performed by APE and results were further discussed. Both methods were validated under ISO 17 025 norms and SANCO Guide recommendations [12].

The enrichment factor, Ef, of SDME can be defined as the ratio of the concentration of analytes in organic phase to the original concentration of analytes in the aqueous phase (donor solution). The enrichment factors were obtained by three replicate extractions of tomato extracts (donor solutions) spiked with metribuzin, viclozolin and λ-cyhalothrin at 10 μg L^{-1}, procymidone, kresoxim methyl, bifenthrin, indoxacarb and azoxystrobin at 50 μg L^{-1}, fenhexamid and iprodione at 100 μg L^{-1} and fosthiazate and fludioxonil at 500 μg L^{-1}. The concentration of analytes in the organic phase was obtained from calibration curve which was plotted with direct injection of toluene standard solutions. As shown in Table 3, the Efs were high for all pesticides studied (40–812) except for pyrethroid analytes that unexpectedly were found to have poor recoveries when extracted by the proposed SDME analytical protocol (0.7 for bifenthrin and 1.0 for λ-cyhalothrin).

Similarly, the concentration factor, Cf, of the whole SDME procedure was defined as the ratio of the concentration of analytes in organic phase to the original concentration of analytes in the fortified analytical sample. The concentration factors were obtained by three replicate extractions of fortified tomato samples with metribuzin, viclozolin and λ-cyhalothrin at 10 μg kg^{-1}, procymidone, kresoxim methyl, bifenthrin, indoxacarb and azoxystrobin at 50 μg kg^{-1}, fenhexamid and iprodione at 100 μg kg^{-1} and fosthiazate and fludioxonil at 500 μg kg^{-1}. The concentration of analytes in the organic phase after

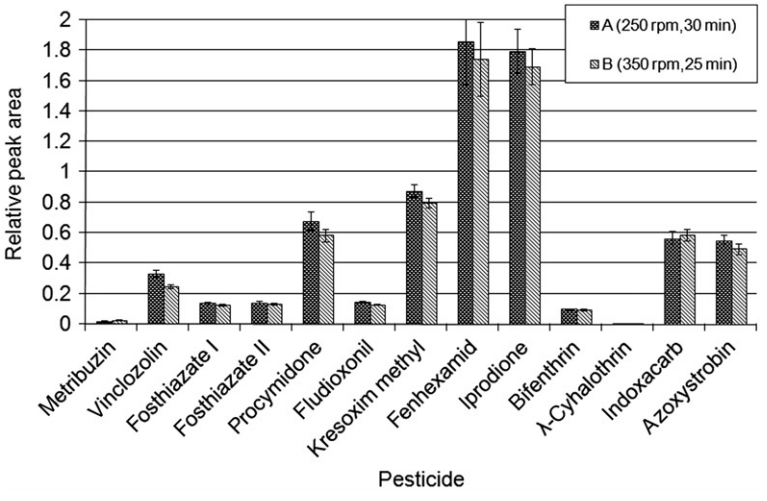

Figure 1. Extraction efficiency ($n = 3$) of the selected pesticides by SDME at different conditions. (A) 30 min extraction with 16 μL toluene, stirring rate 250 rpm and (B) 25 min extraction with 16 μL toluene, stirring rate 350 rpm. Extraction was performed with 7 mL donor standard solution (acetone/water, 10/90, v/v) spiked with metribuzin, vinclozolin and λ-cyhalothrin at 1.6 μg L^{-1}, procymidone, kresoxim methyl, bifenthrin, indoxacarb and azoxystrobin at 8 μg L^{-1}, fenhexamid and iprodione at 16 μg L^{-1}, and fosthiazate and fludioxonil at 80 μg L^{-1}.

SDME was obtained from calibration curve which was plotted with direct injection of toluene standard solutions. The respective Cfs as determined from the whole SDME determination of target pesticides were ranged from <0.1 for bifenthrin and λ-cyhalothrin to 52 for fenhexamid. The highest concentration factor was detected for fenhexamid.

Other analytical data of both methods validated are also shown in Table 3. The limits of detection (LOD) were calculated experimentally from a signal-to-noise ratio of 3.3 by spiking at low concentrations suitable portions of homogenised tomato samples and subjecting them to SDME and APE sample preparations reported. Blank tomato extracts were used for the estimation of the background noise of the chromatographic analysis. LOD of the selected pesticides were found significant lower when extracted by SDME than those obtained with the APE procedure for all pesticides studied except of those of bifenthrin and λ-cyhalothrin.

Fosthiazate, has two chiral centres at the phosphorus and carbon atoms (consist of four stereoisomers) and, therefore, the GC-μECD chromatographic profile for fosthiazate consists of two almost equal peaks (named as fosthiazate I and II). Analytical data shown in Table 2 for the quantification of fosthiazate by both methods studied were calculated using peak areas from both the two chromatographic peaks observed. Fosthiazate at low fortification levels (<2450 μg kg^{-1}) was detected only by SDME.

The selectivity of the method was judged from the absence of interfering peaks at the elution times of the analytes in blank chromatograms of different tomato samples without any spiking for both methods studied. Both methods were free from interferences at the retention times of the selected pesticides. Typical chromatograms of the methods tested are shown in Figure 2.

Precision expressed as repeatability (%RSD) was determined by performing seven consecutive extractions of selected pesticides from fortified homogenate tomato samples at

Table 3. Analytical data for SDME and APE analytical methods used for the determination of selected pesticides in tomato samples.

		SDME		SDME					APE				
Pesticide	MRL[13] ($\mu g\,kg^{-1}$)	Cf	Ef	LOD ($\mu g\,kg^{-1}$)	LOQ ($\mu g\,kg^{-1}$)	Linear range ($\mu g\,kg^{-1}$)	R^2	RSD (%)	LOD ($\mu g\,kg^{-1}$)	LOQ ($\mu g\,kg^{-1}$)	Linear range ($\mu g\,kg^{-1}$)	R^2	RSD (%)
Metribuzin	300	2	40	0.6	2.0	5–100	0.9877	9.0	8.0	25.0	50–1000	0.9950	6.0
Vinclozolin	50	5	93	0.1	0.5	5–200	0.9904	10.3	7.0	23.0	20–1000	0.9998	5.7
Fosthiazate (sum of isomers)	ns	4	77	57.4	190	250–5000	0.9869	15.8	2450	8085	–	–	–
Procymidone	2000	4	84	0.3	1.0	2–500	0.9947	12.3	6.0	19.8	50–5000	0.9977	11.3
Fludioxonil	1000	3	64	116	382	500–5000	0.9885	15.7	181	597	1000–5000	0.9914	13.7
Kresoxim methyl	500	10	110	1.0	3.0	5–1000	0.9989	12.6	11.0	36.3	50–5000	0.9998	12.7
Fenhexamid	1000	52	812	0.5	1.7	5–1000	0.9889	11.9	69.0	228	200–10,000	0.9986	11.3
Iprodione	5000	9	158	0.9	3.0	5–1000	0.9878	9.6	27.0	89	100–10,000	0.9957	17.0
Bifenthrin	2000	0.06	0.7	20.3	67.0	150–5000	0.9857	17.8	10.0	33	50–5000	0.9991	19.4
λ-Cyhalothrin	100	0.05	1.0	4.0	13.0	25–1000	0.9894	15.3	4.0	13.2	10–1000	0.9994	15.6
Indoxacarb	500	3	51	6.3	20.0	50–5000	0.9904	8.7	10.0	34.0	50–5000	0.9987	18.1
Azoxystrobin	2000	6	100	17.2	51.0	100–5000	0.9883	6.9	228	752	2500–5000	1.0000	2.27

Cf = ratio of the concentration of the target analyte in the toluene microdrop to the concentration of the analyte in the tomato sample (using fortified tomato samples with metribuzin, viclozolin and λ-cyhalothrin at $10\,\mu g\,kg^{-1}$, procymidone, kresoxim methyl, bifenthrin, indoxacarb and azoxystrobin at $50\,\mu g\,kg^{-1}$, fenhexamid and iprodione at $100\,\mu g\,kg^{-1}$ and fosthiazate and fludioxonil at $500\,\mu g\,kg^{-1}$, $n = 3$), Ef = SDME enrichment factor defined as the ratio of the concentration of the target analytes in the microdrop to the concentration of the target analyte in the aqueous donor solution (using fortified tomato extracts with metribuzin, viclozolin and λ-cyhalothrin at $10\,\mu g\,L^{-1}$, procymidone, kresoxim methyl, bifenthrin, indoxacarb and azoxystrobin at $50\,\mu g\,L^{-1}$, fenhexamid and iprodione at $100\,\mu g\,L^{-1}$ and fosthiazate and fludioxonil at $500\,\mu g\,L^{-1}$, $n = 3$), LOD = method limit of detection, RSD = relative standard deviation of mean value calculated for fortified tomato samples at the limit of quantification of each pesticide, $n = 7$, ns = not specified, – = not determined.

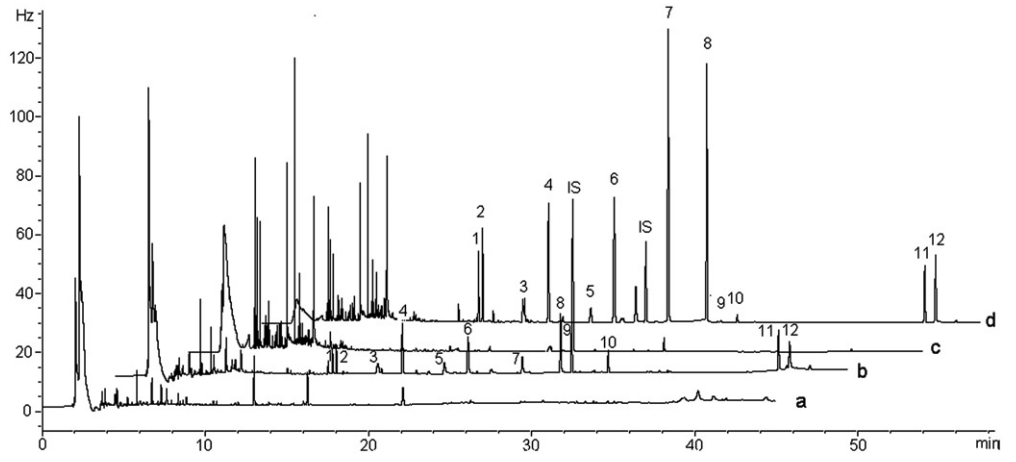

Figure 2. Representative GC chromatograms of: (a) a blank tomato sample extracted by APE, (b) a spiked tomato sample with metribuzin, vinclozolin and λ-cyhalothrin at 50 μg kg^{-1}, procymidone, kresoxim methyl, bifenthrin, indoxacarb and azoxystrobin at 250 μg kg^{-1}, fenhexamid and iprodione at 500 μg kg^{-1} and fosthiazate and fludioxonil at 2500 μg kg^{-1} and extracted by APE, (c) a blank tomato sample extracted by SDME and (d) a spiked tomato sample with target pesticides at metribuzin, vinclozolin and λ-cyhalothrin at 50 μg kg^{-1}, procymidone, kresoxim methyl, bifenthrin, indoxacarb and azoxystrobin at 250 μg kg^{-1}, fenhexamid and iprodione at 500 μg kg^{-1} and fosthiazate and fludioxonil at 2500 μg kg^{-1} and extracted by SDME. Peak numbers correspond to the compounds in Table 2.

the limit of quantification (LOQ) of each individual pesticide. The RSD for all target pesticides were below 20% in all cases (by both methods studied).

Recovery data obtained by the two methods studied are summarised in Table 4. The average relative recoveries of three determinations at four spiked concentrations of pesticides ranged from 66.6 (indoxacarb) to 90% (metribuzin) for SDME, whereas the respective absolute recoveries for APE were ranged from 90% (metribuzin) to 120% (fenhexamid).

3.2 *Effect of matrix in SDME*

The matrix effect for the studied pesticides was evaluated by comparing the slopes of standards prepared in pure solvent (in acetone/water, 10/90, v/v for SDME and in toluene/2,2,4 trimethylpentane, 10/90, v/v for APE) and matrix matched standards calibration graphs for different tomato samples as obtained by linear regression analysis. A statistical study was carried out to compare the slope values of the standards in tomato extracts and the standards prepared in pure solvent. ANOVA test showed that there were statistically significant differences among the slope values for both methods studied and in consequence matrix matched standards should be used to avoid quantitative errors (Table 5). By that statistical analysis both positive (metribuzin, vinclozolin, fosthiazate, procymidone, kresoxim-methyl, fenhexamid, iprodione, indoxacarb and azoxystrobin, $p<0.050$) and negative matrix effects (bifenthrin and λ-cyhalothrin, $p<0.050$) were detected in the determination of the selected pesticides in tomato samples by the SDME analytical protocol, whereas detected matrix effects on pesticides determination by APE

Table 4. Per cent recoveries ($n = 3$), of pesticides spiked to tomato samples at different concentration levels ($\mu g\,kg^{-1}$) as determined by the two methods studied (in parentheses is shown the %RSD of the three determinations performed).

Pesticide	F_1 $\mu g\,kg^{-1}$	SDME	APE	F_2 $\mu g\,kg^{-1}$	SDME	APE	F_3 $\mu g\,kg^{-1}$	SDME	APE	F_4 $\mu g\,kg^{-1}$	SDME	APE
Metribuzin	10	97.5 (8.0)	BQL	20	85.6 (6.8)	BQL	50	95.2 (5.6)	97.0 (6.0)	100	81.8 (8.4)	91.0 (3.1)
Vinclozolin	10	62.9 (4.3)	BQL	20	60.3 (7.3)	96.0 (5.7)	50	71.7 (4.9)	97.0 (6.9)	100	78.9 (9.4)	98.0 (2.3)
Fosthiazate	500	70.4 (5.8)	BQL	1000	64.2 (7.4)	BQL	2500	75.9 (7.2)	BQL	5000	84.1 (4.4)	BQL
Procymidone	50	65.9 (7.3)	115.0 (11.3)	100	66.7 (9.1)	109 (3.8)	250	67.7 (9.0)	108 (3.6)	500	74.7 (7.3)	109 (1.8)
Fludioxonil	500	65.7 (11.7)	BQL	1000	75.9 (4.7)	110 (13.7)	500	68.5 (4.8)	117 (3.4)	5000	76.2 (7.1)	96 (3.7)
Kresoxim-methyl	50	71.8 (9.4)	106.0 (12.7)	100	71.5 (4.9)	111 (5.1)	250	79.7 (6.9)	110 (3.1)	500	91.2 (5.9)	113 (2.5)
Fenhexamid	100	76.5 (11.9)	BQL	200	66.7 (14.7)	111 (11.3)	500	86.3 (13.6)	92.0 (13.1)	1000	88.3 (11.8)	148 (4.8)
Iprodione	100	76.9 (9.6)	BQL	200	73.2 (7.8)	113 (6.4)	500	91.2 (3.3)	104 (5.4)	1000	90.6 (8.8)	105 (4.4)
Bifenthrin	50	BQL	123.0 (19.4)	100	80.1 (4.9)	111 (3.8)	250	85.7 (4.7)	110 (3.4)	500	86.3 (3.7)	109 (3.3)
λ-Cyhalothrin	10	BQL	BQL	20	69.0 (7.0)	110 (3.9)	50	71.0 (5.1)	109 (3.9)	100	76.3 (11.9)	113 (1.7)
Indoxacarb	50	71.8 (5.5)	114.0 (18.1)	100	59.1 (8.7)	119 (6.9)	250	70.4 (5.2)	99.0 (8.2)	500	65.3 (9.9)	102 (5.3)
Azoxystrobin	50	81.5 (1.7)	BQL	100	69.5 (6.9)	BQL	250	74.6 (8.7)	BQL	500	84.3 (0.7)	BQL

F = fortification level, BQL = below method quantification limit.

Table 5. Relative responses (R%) ± RSD ($n = 3$) of selected pesticides indicating different matrix effects in their determination by SDME and APE analytical methods studied.

	SDME			APE	
Pesticide	GC matrix effect[a] (R%)	Effect of matrix in SDME-GC[b] (R%)	ANOVA[c] p	GC matrix effect[d] (R%)	ANOVA[c] p
Metribuzin	49.1 ± 9.6	155.7 ± 7.5	0.044	124.7 ± 9.6	0.018
Vinclozolin	88.1 ± 4.7	270.9 ± 10.3	0.022	111.3 ± 10.2	0.014
Fosthiazate I	61.6 ± 7.2	164.7 ± 8.3	0.038	–	–
Fosthiazate II	61.9 ± 7.0	164.1 ± 8.0	0.047	–	–
Procymidone	87.6 ± 6.0	214.0 ± 11.0	0.045	101.7 ± 10.8	0.718
Fludioxonil	87.0 ± 7.8	89.4 ± 9.0	0.156	108.1 ± 21.2	0.488
Kresoxim methyl	89.5 ± 5.6	212.7 ± 10.4	0.044	101.0 ± 14.3	0.512
Fenhexamid	427.9 ± 13.6	1017 ± 15.9	0.021	219.5 ± 25.2	0.036
Iprodione	134.9 ± 9.8	410.5 ± 14.4	0.023	124.1 ± 14.5	0.046
Bifenthrin	90.4 ± 8.8	42.4 ± 6.2	0.037	106.3 ± 3.4	0.220
λ-Cyhalothrin	90.6 ± 7.2	43.8 ± 5.6	0.027	121.1 ± 17.2	0.023
Indoxacarb	105.8 ± 3.4	155.6 ± 6.5	0.034	150.0 ± 2.0	0.033
Azoxystrobin	103.3 ± 6.5	212.4 ± 9.5	0.040	112.0 ± 22.0	0.048

[a]R%, average relative responses ($n = 3$) of pesticide standards (containing metribuzin, viclozolin and λ-cyhalothrin at 25, 50 and 100 μg L^{-1}, procymidone, kresoxim methyl, bifenthrin, indoxacarb and azoxystrobin at 125, 250 and 500 μg L^{-1}, fenhexamid and iprodione at 250, 500 and 1000 μg L^{-1} and fosthiazate and fludioxonil at 1250, 2500 and 5000 μg L^{-1}) in toluene used as acceptor phase and subjected to the SDME from donor solution of blank tomato extract. 100% = response of pesticide after SDME from solvent acetone/water, 10/90 v/v, as donor solution.

[b]R%, average relative responses ($n = 3$) of pesticides after SDME of fortified tomato extracts with metribuzin, viclozolin and λ-cyhalothrin at 2, 4 and 8 μg L^{-1}, procymidone, kresoxim methyl, bifenthrin, indoxacarb and azoxystrobin at 10, 20 and 40 μg L^{-1}, fenhexamid and iprodione at 20, 40 and 80 μg L^{-1} and fosthiazate and fludioxonil at 100, 200 and 400 μg L^{-1}. 100% = response of pesticide after SDME from spiked solvent (acetone/water, 10/90, v/v) donor solution at the same fortification level.

[c]ANOVA = analysis of variance performed among the slope values obtained by linear regression of calibration graphs constructed from calibration standards prepared in solvent and in matrix matched standards.

[d]R%, average relative responses ($n = 3$) of pesticide standards in tomato extracts (containing metribuzin, viclozolin and λ-cyhalothrin at 25, 50 and 100 μg L^{-1}, procymidone, kresoxim methyl, bifenthrin, indoxacarb and azoxystrobin at 125, 250 and 500 μg L^{-1}, fenhexamid and iprodione at 250, 500 and 1000 μg L^{-1} and fosthiazate and fludioxonil at 1250, 2500 and 5000 μg L^{-1}). 100% = response of pesticide in toluene/2,2,4-trimethylpentane, 10/90, v/v.

where all positive. For APE the positive matrix effect was observed for almost all of the selected analytes ($p < 0.050$) except of procymidone, fludioxonil, kresoxim-methyl and bifenthrin that were not found to be affected by matrix constituents in the extract. The highest positive response by both methods studied was observed for fenhexamid (Table 5).

It is well known that the extent of positive matrix-induced effects is related to the high polarity of the analytes, the type of co-extracts in sample (most distinct detector response enhancement for matrices with high content of essential oils and waxes), the analyte/matrix concentration and the state (history) of the GC system [14–17]. Matrix effects

Table 6. Pesticide residues detected in the 20 tomato samples analysed by SDME and APE analytical methods studied.

		SDME				APE			
Pesticide	MRLs [13] ($\mu g\,kg^{-1}$)	LOQ ($\mu g\,kg^{-1}$)	C[a] ($\mu g\,kg^{-1}$)	Concentration range ($\mu g\,kg^{-1}$)	Positive samples[b] (No.)	LOQ ($\mu g\,kg^{-1}$)	C ($\mu g\,kg^{-1}$)	Concentration range ($\mu g\,kg^{-1}$)	Positive samples[a] (No.)
Procymidone	2000	1.0	9.3	5.7–11.6	3 (0)	19.8	BQL	BQL	1 (0)
Iprodione	5000	3.0	70.6	37.9–145.9	6 (0)	89.0	120.4	BQL–145.9	3 (1)
Bifenthrin	2000	67.0	–	–	0 (0)	33.0	BQL	BQL	2 (2)
λ-Cyhalothrin	100	13.0	BQL	BQL	1 (1)	13.0	BQL	BQL	1 (1)

[a]Mean concentration ($\mu g\,kg^{-1}$) of the positive detections above the quantification limit (LOQ) of the method.
[b]Number (No.) of samples where the residue was detected. In brackets is shown the number of samples that were positive and below method quantification limit (BQL).

observed for APE are exclusively involved with the above mentioned factors (GC matrix effects) and are well known [18]. For SDME the negative effect is usually attributed to the presence of dissolved organic matter (such as humic acids for water environmental samples) and/or suspended solids in aqueous samples subjected to the SDME procedure [19] whereas the most possible explanation for the positive effect is that certain matrix components (e.g. minerals, fructose, organic acids) in donor solution may also infect the extraction efficiency of pesticides by SDME [8]. However, it is not easy to distinguish matrix effects from the chromatographic system and effect of matrix in SDME.

In order to estimate the matrix effects from the gas chromatographic system (GC matrix effect) the SDME was applied in donor solutions of pure acetone/water (10/90, v/v) and blank tomato extracts using a microdrop of a standard solution at 2.5, 5 and 10 F $\mu g\,L^{-1}$ (metribuzin, viclozolin and λ-cyhalothrin at 25, 50 and 100 $\mu g\,L^{-1}$, procymidone, kresoxim methyl, bifenthrin, indoxacarb and azoxystrobin at 125, 250 and 500 $\mu g\,L^{-1}$, fenhexamid and iprodione at 250, 500 and 1000 $\mu g\,L^{-1}$, and fosthiazate and fludioxonil at 1250, 2500 and 5000 $\mu g\,L^{-1}$) in toluene ($n = 3$) and results were expressed as relative response (%R, 100% = response of pesticide in pure solvent). Furthermore, a comparison between calibration standards prepared in pure acetone/water (10/90, v/v) and spiked matrix extracts (used as donor solutions) at 0.2, 0.4 and 0.8 F (2, 4 and 8 $\mu g\,L^{-1}$ with metribuzin, viclozolin and λ-cyhalothrin, at 10, 20 and 40 $\mu g\,L^{-1}$ with procymidone, kresoxim methyl, bifenthrin, indoxacarb and azoxystrobin, at 20, 40 and 80 $\mu g\,L^{-1}$ with fenhexamid and iprodione and at 100, 200, 400 $\mu g\,L^{-1}$ with fosthiazate and fludioxonil) was performed in order to estimate the effect of matrix from the whole SDME analytical protocol (SDME-GC matrix effect). Results from this comparative study as well as the relative responses of pesticides studied in tomato extracts obtained by APE are shown in Table 5.

As can be seen from the results, the number of matrix effects induced by the chromatographic system by SDME in the selected determination were reduced to a negative matrix effect observed for metribuzin and two positive effects observed for fenhexamid and iprodione. The negative matrix effect observed for metribuzin could be attributed to the primary amino group of its chemical structure that may interact with non-volatile sample components traces which are being co-extracted by SDME or to the same interaction of the primary amino group with tomato sample that took place during the SDME in the certain experimental assays. A similar negative effect has been reported for the determination of triazines (negative matrix effect) in food [14,20,21] by gas chromatography. The positive GC matrix effects observed for fenhexamid and iprodione are also in accordance with relative literature since it has been reported that polar analytes that contain hydroxy (–OH) (fenhexamid) and amino (R–NH–) (iprodione) groups are the most susceptible type of analytes to a positive GC effect [14–17]. In conclusion, SDME is a more selective sample preparation method as compared with APE in the certain determination. However, in any case matrix matched standards should be used for the selected pesticides quantitative analysis in tomato samples.

3.3 *Application to real samples*

SDME analytical protocol selected to be studied was further applied in analysis of 20 tomato samples purchased from local market in a three months period time. The same tomato samples were analysed using the APE and results were further compared.

A reagent blank, a standard prepared in acetone and a blank sample were analysed at the beginning of each set of samples, in order to control the cleanness of the instruments and to check the response of the detector. A matrix standard was analysed twice with every set of samples in order to check the performance of the preparation of samples and achieve accurate quantification. The positive identifications of pesticides found are shown in Table 6. Confirmation of the results was conducted using GC-MS in the full scan mode. The results obtained by GC-MS were consistent with those recorded by GC-ECD.

Four from the 12 pesticides tested were detected in 12 of surveyed samples. Procymidone and iprodione were found to be the most frequent detected pesticide residues in tomatoes and the SDME method is a good alternative approach for their determination at low concentrations. However, bifenthrin and λ-cyhalothrin that were also detected in surveyed samples were better analysed by APE method and this is in accordance with validation data already reported.

4. Conclusions

In this study the SDME analytical protocol developed in a previous work was applied and validated for the determination of 12 extra pesticides from different chemical families in tomato samples. The validation of the selected determination was also performed by APE and results were further compared. Comparative results indicated that SDME proved to be efficient for the determination of all dicarboximides selected to be studied, metribuzin, fosthiazate, kresoxim-methyl, fenhexamid and azoxystrobin in tomato samples (showed acceptable recoveries, high precision, and sensitivity and high Ef) at low concentration levels. However, extraction efficiency of pyrethroids was poor and a further optimisation of the SDME should be performed for their determination in tomato samples. Matrix effect assessment performed pointed that SDME is a more selective sample preparation technique as compared with APE (lower GC matrix effects were observed by SDME). However, quantification should be performed using a standard curve of spiked vegetable samples since matrix influence significantly pesticide recoveries by SDME and matrix matched standards prepared in blank extracts by APE.

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