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ORIGINAL PAPER

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Application of supercritical fluid extraction in multi-residue pesticide analysis of plant matrices

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Abstract Sample preparation based on selective supercritical fluid extraction (SFE) prior to gas chromatographic (GC) analysis of several groups of pesticides (organophosphates, organochlorines, pyrethroides and others) in cereals, cereal products, vegetables and fruits has been used. Electron capture detector (ECD) and nitrogen phosphorus detector (NPD) for parallel detection of investigated compounds were used. The obtained results showed satisfactory recoveries for most of compounds (more than 70%) and sufficient selectivity of extraction, namely for GC-NPD amenable compounds. The optimised procedure was successfully compared with conventional liquid extraction followed by GPC clean up in the frame of inter-laboratory study.

Keywords Supercritical fluid extraction · Pesticides · Multi-residue analysis · Food analysis

Introduction

Supercritical fluid extraction has become one of the isolation techniques used in the field of pesticide analysis in food. The process is documented in numerous publications and also summarised in a review [1]. Final tuning of SFE in routine analysis of pesticides with a wide range of physico-chemical properties depends on demands for accuracy of results in relation to the cost of analysis. Some SFE procedures are focused on the most efficient extraction of analytes. This is unavoidably coupled with a post-SFE clean up of extracts [2, 3]. Alternatively, extraction of some sample matrix components may be avoided by addition of suitable sorbent or by in-line clean up [4, 5, 6, 7, 8]. Another possibility is a minimising of matrix co-extractives through the use of as low as possible fluid extraction power (often sacrificing maximum attainable recovery of some analytes) [9, 10]. However, in most cases, the following gas chromatographic separation must be coupled with selective detection of analytes. This fact is reflected in the use of highly selective detectors – i.e. flame photometric detector [3, 8, 10], mass spectrometric detector [4, 5, 6], atomic emission detector [9] and nitrogen phosphorous detector [11]. On the other hand, use of the electron capture detector [8] (very common in pesticide residue analysis) can be rather limited because of its relatively low selectivity, which usually leads to the signal overlap of investigated compounds and interfering substances.

In spite of optimistic expectations during last decade (after development of various analytical applications) SFE is nowadays more severely compared with conventional solvent extraction, usually followed by suitable clean up step. Conventional procedures provide mostly sufficient extraction power for a wide spectrum of analytes without special optimisation. Contrary to that, SFE often needs fine-tuning for various analytes, especially in multi-residue analysis and also in the case of analyte/matrix combination changes. Moreover, the usually emphasized advantages of the SFE applications in respect to protection of the environment often appear less important than practical aspects of its use in real laboratory work. On the other hand, conventional methods are difficult to automate and hence are laborious and time consuming.

The purpose of this study was to extend the spectrum of pesticide/matrix combinations amenable to GC analysis employing selective supercritical fluid extraction as a single step of sample handling and, in suitable cases, to replace conventional procedures. The aim was to optimise the method to as simple a form as possible. Optimised procedure was compared with conventional solvent extraction followed by gel permeation chromatography using samples with incurred pesticides residues, in the frame of inter-laboratory study [12].

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Table 1 Recovery for the initial set of 22 pesticides (extraction temperature: 50 °C)

Pesticide	Matrix Recovery (%) – Standard deviation (n=6)							
	Rice	SD	Wheat flour	SD	Bread	SD	Tomatoes	SD
Bromopropylate *	97	4	94	5	91	3	98	4
Bupirimate #	91	4	77	4	89	3	87	3
Captan *	126	6	120	4	130	5	98	4
Chlorfenvinphos (Σ) #	84	2	83	3	92	3	84	3
Chlorothalonil *	95	5	99	4	97	4	99	6
Chlorpyrifos #	93	3	85	2	89	3	78	4
Chlorpyrifos-methyl #	89	3	81	2	90	3	75	4
Cypermethrin (Σ) *	71	3	89	3	70	2	89	5
Deltamethrin *	85	3	87	4	86	3	91	5
Dichlofluanid *	81	3	91	3	92	4	98	5
Dimethoate #	86	3	70	2	85	3	61	3
Endosulfan-α *	83	4	90	3	86	3	96	3
Endosulfan-β *	84	3	91	3	89	3	97	3
Endosulfan-SO_4 *	97	4	96	4	97	4	98	5
Iprodione *	94	4	95	3	96	4	133	6
Lindane *	85	3	89	2	90	4	89	4
Metalaxyl #	85	4	81	4	89	3	77	3
Omethoate #	90	3	9	2	92	3	0	–
Permethrin (Σ) *	85	4	89	3	79	3	91	5
Phosalone #	94	3	83	3	91	3	98	4
Pirimiphos-methyl #	90	4	80	3	91	4	89	4
Tolylfluanid *	90	4	97	5	99	5	97	6

* Evaluated from ECD record (spiking level 0.05 mg/kg)
# Evaluated from NPD record (spiking level 0.1 mg/kg)
Σ=Sum of all isomers

Experimental

Chemicals

SFE-grade carbon dioxide (99.9993% – without helium in headspace) and technical grade carbon dioxide were obtained from Messer Technogas (CR). Helium (99.996%), nitrogen (99.998%), synthetic air (99.9%) and hydrogen (99.9%) were obtained from Linde Technoplyn (CR). Hydromatrix was obtained from Varian (USA). Toluene and acetone (pesticide analysis grade) were obtained from Merck (Germany). Certified standards of investigated compounds were obtained from Dr. Ehrenstorfer (Germany) – for the list see Tables 1 and 2. Individual pesticide stock solutions (concentration 1000 µg/mL) in toluene and acetone were prepared. Standard mixture containing 10 µg/mL of each of pesticides was prepared in toluene and acetone from the individual standard stock solutions. Acetone solutions were after appropriate dilution used for samples spiking.

Preparation of samples and matrix spiking

Wheat, bread, flour and rice (i.e. low moisture samples) were ground by laboratory mill and afterwards 2 g were weighed directly into extraction thimbles. Unpeeled potatoes, cucumbers, tomatoes, apples (with cores) and oranges (i.e. samples with high water content) were washed with tap water and then 50 g of diced material was homogenised using a laboratory blender. Homogeneous material (2 g) was thoroughly mixed with hydromatrix (in the ratio 1.5 g per 1 g of sample) to obtain a powdered sample that was transferred to the thimble.

Recovery data were obtained by extraction of spiked blank commodity (2 g) with 100 µL of acetone solution of investigated pesticides corresponding to a residue level of approximately 0.05 mg/kg for ECD compounds and 0.1 mg/kg for NPD compounds. Spiking was carried out before the sample transfer to the thimble by gradual mixing of acetone solution with matrix (and/or matrix/hydromatrix mixture). Six replicates of each pesticides/matrix combination were extracted in a sequence together with blank sample of corresponding matrix during the procedure optimisation. Samples were extracted in two parallels in the routine analyses.

Supercritical fluid extraction

A Hewlett-Packard HP 7680 T supercritical fluid extractor (USA) was used for SFE. Samples were extracted with neat supercritical carbon dioxide at 12.3 MPa (123 bar) and 50 °C (density 0.6 g/mL) for a 3 min static extraction period followed by a 30 min dynamic extraction period at a flow rate of 3 mL/min. Alternatively, extraction with neat supercritical carbon dioxide at 20.2 MPa (202 bar) and 80 °C (density 0.6 g/mL) for a 3 min static extraction period followed by a 30 min dynamic extraction period at a flow rate of 3 mL/min was tested. Analytes were collected on stainless steel balls (SST) trap at 5 °C with nozzle temperature 45 °C. Toluene (0.8 mL) was used to elute the SST trap at 25 °C (nozzle set to the same temperature) with a flow rate of 0.4 mL/min into a 2 mL glass vial sealed with a PTFE faced septum. The trap was then rinsed with acetone (5 mL at flow rate 2 mL/min) at 25 °C to waste. During the initial phase of experiments, other parameters were also varied to find optimal conditions (trap temperature, trap type, flow rate, elution volume and flow rate of elution volume). Obtained extracts were quantitatively transferred to a 1 mL volumetric flask and made up to volume with toluene.

GC analysis

Pesticide residues were determined using a Hewlett-Packard HP 6890 gas chromatograph (USA) equipped with electron capture detector (ECD) and nitrogen phosphorus detector (NPD). Samples (equivalent to 1 mg of commodity) were injected using Hewlett-Packard autosampler into a split/splitless injector containing double tapered liner (800 µL).

Table 2 Recovery for the extended set of 36 pesticides (extraction temperature: 50 °C)

Pesticide	Matrix Recovery (%) – Standard deviation (n=6)							
	Wheat	SD	Potatoes	SD	Cucumbers	SD	Apples	SD
Acephate #	81	3	30	4	23	3	17	2
Bifenthrin *	79	4	80	4	88	5	74	5
Bromopropylate *	92	4	88	3	96	4	93	3
Bupirimate #	77	3	92	3	96	4	98	4
Captan *	133	4	91	3	92	2	99	3
Chlorfenvinphos (Σ) #	84	2	77	3	91	4	84	5
Chlorothalonil *	94	4	92	3	97	4	98	5
Chlorpyrifos #	90	2	82	3	78	4	83	5
Chlorpyrifos-methyl #	81	3	72	4	73	3	77	6
Cyhalothrin-λ (Σ) *	81	4	82	5	81	4	80	4
Cypermethrin (Σ) *	82	3	87	5	83	4	85	4
Deltamethrin *	82	3	81	4	85	5	88	4
Diazinon #	81	3	69	4	77	4	77	3
Dichlofluanid *	90	4	94	3	89	3	97	4
Dimethoate #	74	2	71	5	63	4	53	3
Endosulfan-α *	71	3	89	3	88	2	98	3
Endosulfan-β *	73	3	95	3	91	3	98	3
Endosulfan-SO_4 *	92	4	97	3	96	2	99	4
Ethion #	81	4	75	3	92	4	78	3
Fenitrothion #	86	3	75	4	84	5	85	5
Formothion #	83	6	50	4	56	3	58	3
Heptenophos #	79	4	36	5	31	3	28	4
Iprodione *	89	3	116	5	120	6	134	6
Lindane *	77	4	76	3	71	3	90	4
Malathion #	80	3	72	4	84	3	82	4
Metalaxyl #	74	3	84	3	79	4	78	5
Methamidophos #	16	2	4	1	0	–	0	–
Methidathion #	81	3	78	5	85	5	73	4
Mevinphos (Σ) #	89	3	25	4	20	4	18	4
Omethoate #	27	4	3	1	0	–	3	1
Permethrin (Σ) *	79	4	78	5	85	3	73	4
Phosalone #	89	4	81	4	94	4	75	4
Phosphamidon #	78	3	55	4	49	5	50	6
Pirimiphos-methyl #	81	3	70	3	79	4	81	6
Tolclofos-methyl *	78	3	70	4	71	3	78	4
Tolylfluanid *	97	5	92	3	98	5	99	5

* Evaluated from ECD record (spiking level 0.05 mg/kg)
# Evaluated from NPD record (spiking level 0.1 mg/kg)
Σ=Sum of all isomers

The separation of compounds was performed on a fused silica capillary column: DB-5 MS (60 m x 0.25 mm x 0.25 μm – J&W Scientific, USA). The effluent from the column was split to both detectors in the ratio 1:1. GC conditions were as follows: splitless injection (period: 2 min) – 1 μL, carrier gas flow rate (helium) – 0.8 mL/min (constant flow), injector temperature: 250 °C, detectors temperature: 300 °C, oven temperature 1 (first alternative for initial analytes spectrum – Table 1): 90 °C (2 min held), 10 °C/min to 220 °C, 2.5 °C/min to 280 °C (20 min held), oven temperature 2 (second alternative for wider analytes spectrum – Table 2): 90 °C (2 min held), 8 °C/min to 220 °C, 2 °C/min to 280 °C (20 min held).

Results and discussion

The objective of this study was to extend the spectrum of pesticide/matrix combinations amenable to GC analysis by employing selective supercritical fluid extraction for isolation of analytes. Our experimental approach insisted in the use of neat carbon dioxide (no modifier was added) as a supercritical fluid since we aimed at a simple selective extraction procedure and/or extraction providing at least the significant reduction of unwanted co extractives from a sample matrix. Experimental conditions were based on initial method optimisation, which resulted in publication of a study concerned with the determination of organophosphorous pesticides [10]. In the study presented, the only parameter varied was extraction temperature – two experimental series at 50 °C and at 80 °C were carried out. Tables 1 and 2 summarise recoveries of analyte extraction at 50 °C. Recoveries at 80 °C were mostly similar, only the bulk of co-extracted compounds was significantly greater, which was the practical reason for the rejection of this variant for routine analysis.

The values of recoveries shown in Tables 1 and 2 mostly match common criteria set for pesticide residue analysis [13]. Considering the influence of elevated extraction temperature, increased fluid extraction power

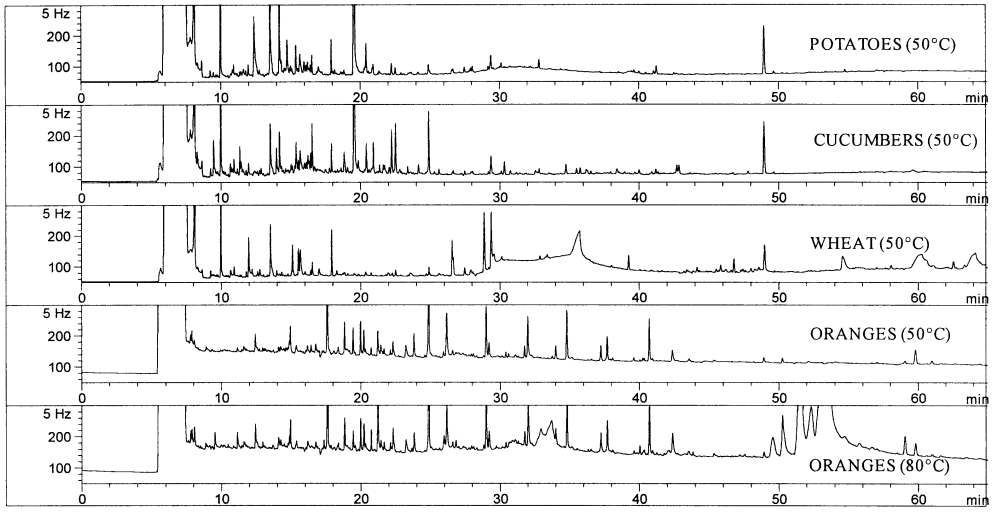

Fig. 1 Typical GC-ECD chromatograms of blank matrices (highly zoomed)

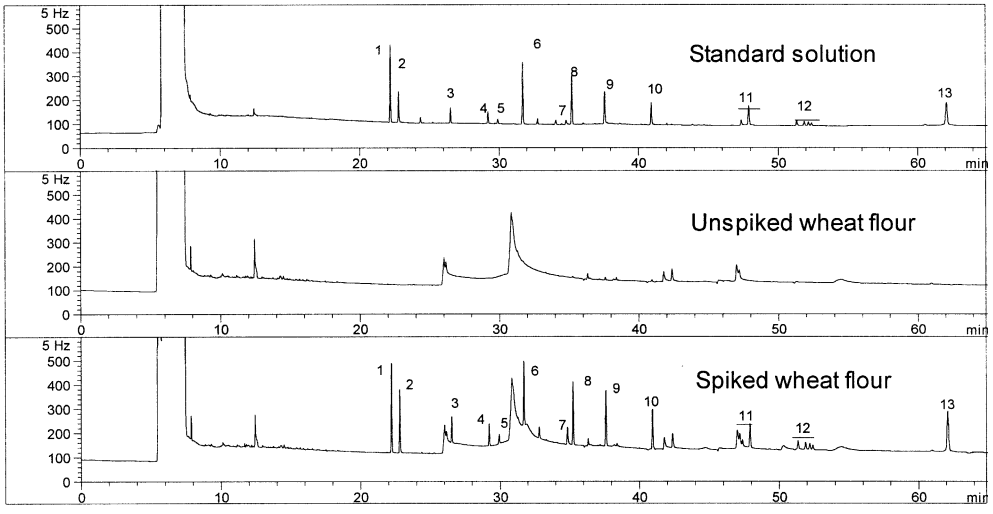

Fig. 2 GC-ECD chromatograms of flour sample extracted at 50 °C. Peak numbering: lindane (*1*), chlorothalonil (*2*), dichlofluanid (*3*), tolylfluanid (*4*), captan (*5*), endosulfan-α (*6*), iprodione (*7*), endosulfan-β (*8*), endosulfan-SO_4 (*9*), bromopropylate (*10*), permethrin (*11*), cypermethrin (*12*), deltamethrin (*13*)

did not usually result in significant improvement of recoveries. Moreover, the greater bulk of co-extractives impaired the amenability of direct GC analysis. Another interesting fact is the dependence of the behaviour of investigated compounds on matrix character: whilst cereals (matrices with low water content) had practically no negative effect on recoveries, other examined matrices caused variability with respect to certain analyte/matrix combinations.

Regarding the results obtained for individual pesticides extracted from cereals, the most troublesome in terms of recoveries were captan, omethoate and methamidophos. Recoveries exceeding 100% determined for the captan may be due to matrix effects [14]. For accurate quantification, the matrix matched standard should be used as a calibrant [3, 13]. Similarly for fruit and vegetables, overestimation of results was observed only for iprodione, which also belongs in the category of "difficult" analytes. For omethoate and methamidophos, low recoveries could be attributed to relatively poor extractability of these polar compounds. In the case of acephate, dimethoate, formothion, heptenophos, mevinphos and phosphamidon, extraction from fruit and vegetables probably plays an important role in their instability under tested conditions.

Figures 1, 2, 3, 4 and 5 illustrate some important points from the study. Comparison of chromatographic backgrounds for chosen GC-ECD records is shown on Fig. 1. At first glance, all chromatograms look very rich in peaks, but there are differences in occurrence of medially and lately eluting compounds. In the case of potatoes and cucumbers, early eluting compounds dominate while for wheat, the co-extractives band at 30–40 min is obvious. In case of oranges, co-extractives are more intensive, especially at 80 °C where it is very significant. Nevertheless, the true importance of the extent of interferences is visible in Figs. 2 and 3, where the relative response of analytes and co-extracted compounds can be directly compared. As regards the chromatographic behaviour of interfering compounds, these mostly do not disturb analytes. This fact tempts the conclusion that it is no problem to directly analyse any extract. However, the amount of co-extracted substances, and subsequently their deposition and pyrolysis in the injector chamber

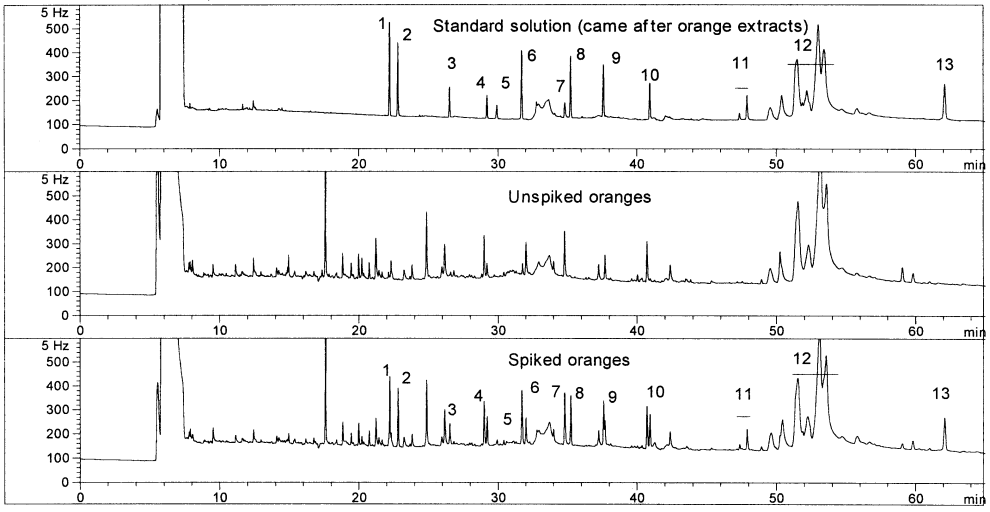

Fig. 3 GC-ECD chromatograms of orange sample extracted at 80 °C. Peak numbering: see Fig. 2

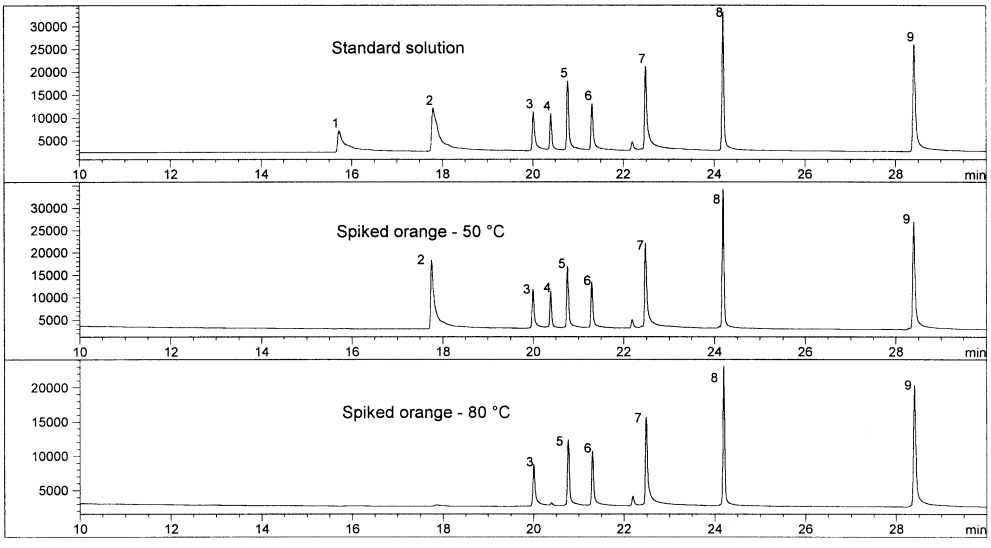

Fig. 4 GC-NPD chromatograms of orange extracts obtained at two different temperatures. Peak numbering: omethoate (*1*), dimethoate (*2*), chlorpyrifos-methyl (*3*), metalaxyl (*4*), pirimiphos-methyl (*5*), chlorpyrifos (*6*), chlorfenvinphos (*7*), bupirimate (*8*), phosalone (*9*)

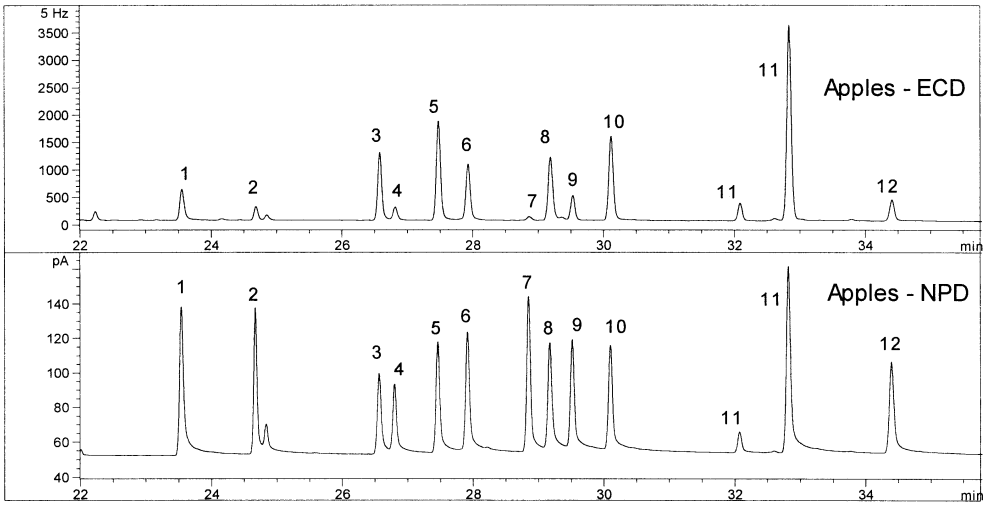

Fig. 5 Comparison of ECD and NPD detectability for some of tested compounds (spiked apple extract, SFE at 50 °C). Peak numbering: dimethoate (*1*), diazinon (*2*), formothion (*3*), phosphamidon (*4*), chlorpyrifos-methyl (*5*), tolclofos-methyl (*6*), pirimiphos-methyl (*7*), fenitrothion (*8*), malathion (*9*), chlorpyrifos (*10*), chlorfenvinphos (*11*), methidathion (*12*)

Table 3 Results of long-term proficiency testing of organophosphates analysis within FAPAS

	Date	Matrix	m	RSZ	SSZ	Critical value	Technique
5	Jul-93	Biscuit	2	1.2	3.7	6.2	LE+GPC
6	Jan-94	Flour	2	–0.2	3.8	6.2	SFE
7	May-94	Flour	2	–1.0	3.0	6.2	SFE
8	Sep-94	Biscuit	1	0.1	0.0	0.0	LE+GPC
9	Feb-95	Biscuit	2	2.3	5.2	6.2	SFE
10	Jun-95	Flour	2	0.2	0.3	6.2	SFE
11	Sep-95	Flour	2	0.4	0.9	6.2	SFE
12	Jan-96	Flour	2	0.0	0.8	6.2	SFE
15	Jan-97	Flour	3	0.4	0.9	8.0	SFE
16	May-97	Flour	2	1.7	3.9	6.2	SFE
17	Oct-97	Flour	2	0.1	1.0	6.2	SFE
18	May-98	Flour	2	–0.4	0.3	6.2	SFE
19	Nov-98	Flour	3	–1.3	6.9	8.0	SFE
20	Apr-99	Flour	2	0.4	0.2	6.2	SFE
21	Nov-99	Flour	3	0.9	0.9	8.0	SFE
22	Apr-00	Rusk	3	–0.2	0.8	8.0	SFE
24	Dec-00	Rusk	3	–0.2	0.2	8.0	SFE

m: Number of analytes, i.e. number of z-scores being combined.
$z=(x–X)/\sigma$; where x is the participant's reported value, X is the assigned value and
σ is the target value for standard deviation. For detailed description see reference [15]
RSZ: The rescaled sum of z-scores ($\Sigma z/\sqrt{m}$) – indicator of bias
SSZ: Sum of squared z-scores – indicator of accuracy
Critical value corresponds to $|z| = 2$ at probability level 4.55%.
$|RSZ| \leq 2$ and/or SSZ ≤ critical value$\Rightarrow$ satisfactory result
LE+GPC – Acetone/methanol extraction + Bio Beads S-X3 clean-up
SFE (50 °C) – see experimental details

Table 4 Results (obtained from two replicates) of comparative study carried out with breadcrumb

Pesticide	Detector	Sample 1*		Sample 2*	
		LE+GPC	SFE	LE+GPC	SFE
		(mg/kg)		(mg/kg)	
Endosulfan-α	ECD	0.46	0.45	0.42	0.44
Endosulfan-β	ECD	0.42	0.40	0.39	0.40
Deltamethrin	ECD	4.35	4.17	3.96	4.13
Diazinon	NPD	0.06	0.06	0.14	0.07
Chlorpyrifos-methyl	NPD	1.84	1.95	1.77	1.88
Pirimiphos-methyl	NPD	2.54	2.52	2.32	2.45
Malathion	NPD	2.63	2.70	2.45	2.72

* Samples 1 and 2 – randomly chosen breadcrumb bags
LE+GPC: Ethyl acetate extraction + PL-gel clean-up
SFE (50 °C): See experimental details

and injector side of GC column (which can cause column phase deterioration), is the usual limitation of any simplified approach. This is documented on Fig. 3, where the chromatogram of standard solution secondary contaminated by the interferences from previous injections of orange extracts can be seen. Figure 4 illustrates the common cleanness of GC-NPD chromatograms, which is practically the same for all tested matrices. We can also observe the changes in the profile of extracted compounds, i.e. disappearance of omethoate at 50 °C and, additionally, loss of dimethoate at elevated temperature (80 °C). It is difficult to estimate whether their higher polarity or instability is the cause of this. Figure 5 illustrates the possibility of simultaneous detection of compounds with both ECD and NPD responses. For these, the use of a dual detection system can facilitate their confirmation and quantification.

As regards the trueness of the data generated by the SFE-based procedure, the verification process was performed by determination of recoveries from spiked samples and confirmed through external quality control. Table 3 documents long-term performance in the proficiency testing scheme FAPAS (Food Analysis Performance Assessment Scheme) organised by CSL MAFF UK (Central Science Laboratory of Ministry of Agriculture, Food and Fisheries of United Kingdom). Summarised results expressed as z-score values prove successful application of the SFE procedure, which appears as a suitable equivalent to LE+GPC (for details see Table 3). The comparison of SSZ and critical values indicates the extent of result trueness in relation to the best estimation of the real analyte concentration in the tested sample. SSZ values were in our case for all rounds lower than the corresponding critical values, which means more or less good results of analyses. Table 4 shows the comparison of results achieved both by SFE procedure and liquid extraction followed by GPC clean up (LE+GPC – for details see Table 4). Results presented in the table were measured for breadcrumb, which was prepared from bread contaminated during dough preparation to obtain incurred pesticide residues [12]. As can be seen, results achieved by both procedures signal the possibility of

Table 5 Comparison of SFE and LE+GPC – advantages and drawbacks

	SFE	LE+GPC
Sample matrix	Porous, dried	Mostly unlimited
Analytes	Less polar, rather volatile	Mostly unlimited
Extraction efficiency	Mostly very high (suitable sample matrix combination)	Mostly very high
Selectivity	Often high	Commonly non-selective
Rapidity	Faster	Slower
Laboriousness	Simple realisation (optimised method), minimal need of solvents and glassware	Simple realisation, high consumption of solvent and glassware
Optimisation	Usually laborious	Usually simple
Cost	Higher	Lower

replacing conventional analytical procedure (LE+GPC) by SFE technique. This is suitable especially in cases where fast and less laborious analysis is needed.

Based on realised experiments and long-term experience with conventional sample preparation procedures, it is possible to evaluate the advantages and drawbacks of SFE, which are summarised in Table 5. If we consider the advantages of SFE as rapidity, selectivity and simplicity, it is also important to mention the drawbacks of laborious optimisation, limited general experience with this technique and limited information exchange, which complicates the spread of SFE. On the other hand, conventional techniques are common in every laboratory, well-described, easily realizable and also well-verified. We can see some drawbacks such as higher consumption of solvents and glassware, but in respect to easy realization with minimal equipment, the conventional techniques are more applicable in laboratory practice.

Conclusions

To summarise all experiences with SFE and conventional LE+GPC sample preparation procedure, SFE provides fast, efficient and sufficiently selective isolation of pesticides. Significant dependence on the physico-chemical properties of analytes and also of the sample matrix is one of its drawbacks, which complicates optimisation for a wide spectrum of pesticide/matrix combinations. Also, the limited number of SFE users gives less opportunity to exchange information and thus the progress in this field is not too significant, especially in routine practice. Those are the main reasons why application of SFE, in spite of its considerable advantages, remains in minority.

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