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SBSE-GC-ECD/FPD in the Analysis of Pesticide Residues in *Passiflora alata* Dryander Herbal Teas

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Stir bar sorptive extraction (SBSE) in combination with GC-ECD/FPD analysis is here applied to the determination of the residues of 11 pesticides (hexachlorobenzene, lindane, chlorothalonil, parathion methyl, parathion ethyl, fenitrothion, malathion, dieldrin, α- and β-endosulfan, and tetradifon) in herbal teas prepared with *Passiflora alata* Dryander spiked leaves. The method was optimized using spiked herbal teas in a range from 0.05 to 1 pg/μL for organochlorine pesticides and from 0.15 to 3 pg/μL for organophosphorus pesticides. The method is reproducible and repeatable with recoveries calculated from herbal teas prepared with spiked plant material versus spiked herbal teas, varying from about 30% for tetradifon to about 90% for parathion methyl and malathion. The limits of quantitation (LOQs) ranged from 0.017 pg/μL for lindane to 0.117 pg/μL for malathion.

KEYWORDS: Stir bar sorptive extraction (SBSE); sample preparation; medicinal plants; *Passiflora alata* Dryander; herbal teas; pesticide residues

1. INTRODUCTION

The ever increasing consumption of medicinal plants makes it necessary to control them and the products prepared from them, in terms not only of biologically active components but also of exogenous compounds such as residues of pesticides used for their cultivation. This is particularly true for plants originating from countries where legislation is different or less strict than where they will be consumed. Analysis of pesticide residues in medicinal plants and phytomedicines has recently been reviewed by Zuin and Vilegas (*1*).

The *Passiflora* genus consists of about 400 species belonging to the Passifloraceae family originating from South America. Passiflorae are very popular not only because of their fruits (passion fruits) but also because some of them are used in traditional medicine, mainly against anxiety and insomnia, as well as to treat neuralgia, convulsions, hysterical syndromes, psychogenically derived tachycardia, and asthma. Passiflorae are generally taken as such or mixed with other herbs (e.g. valerian) in the form of infusions of their dried leaves or, less frequently, as tinctures or in tablet form. The most popular species in South America are *Passiflora incarnata* (*P. incarnata*) L., better known as passion flower, and *Passiflora alata* (*P. alata*) Dryander, while *P. incarnata* L. is the most widely used in Europe (*2*).

Pesticides are generally prohibited in medicinal plant production, but, with *Passiflora* species, their residues may be present in dried leaves because they may be used for fruit crop protection; it is therefore necessary to check *Passiflora* leaves used in traditional medicine for residues of the pesticides used in passion fruit cultivation.

Pesticide residues in *Passiflora* leaves are generally determined through the classical multiresidue method reported in the European Pharmacopoeia (*3*) adapted to this plant, although no specific limits are fixed. Recently Zuin applied to *P. alata* and *P. edulis* Sims f. *flavicarpa* Deg. leaves, both maceration/ultrasonic extraction of plant material with ethyl acetate followed by a cleanup on neutral alumine and matrix solid-phase dispersion (MSPD) with Florisil and neutral alumina and *n*-hexane/ethyl acetate as eluent followed by GC-ECD/FPD or GC/MS, to determine the residues of 11 pesticides (hexachlorobenzene (HCB), lindane (γ-HCH), chlorothalonil, parathion methyl, parathion ethyl, fenitrothion, malathion, dieldrin, α- and β-endosulfan, and tetradifon) at the ng/g (ppb) level (*4*). These pesticides were chosen on the basis of those used in Brazil for passion fruit or similar cultivation and included compounds that are not permitted but whose residues have already been found in *Passiflora* leaves. Zuin et al. also developed a method based on SFE extraction using supercritical CO_2 at 100 bar at 40 °C combined with C18 trapping to recover the extracted analytes (*5*). Sample preparation and cleanup by SFE is faster and easier and gave higher recoveries than classical methods (*4*, *5*).

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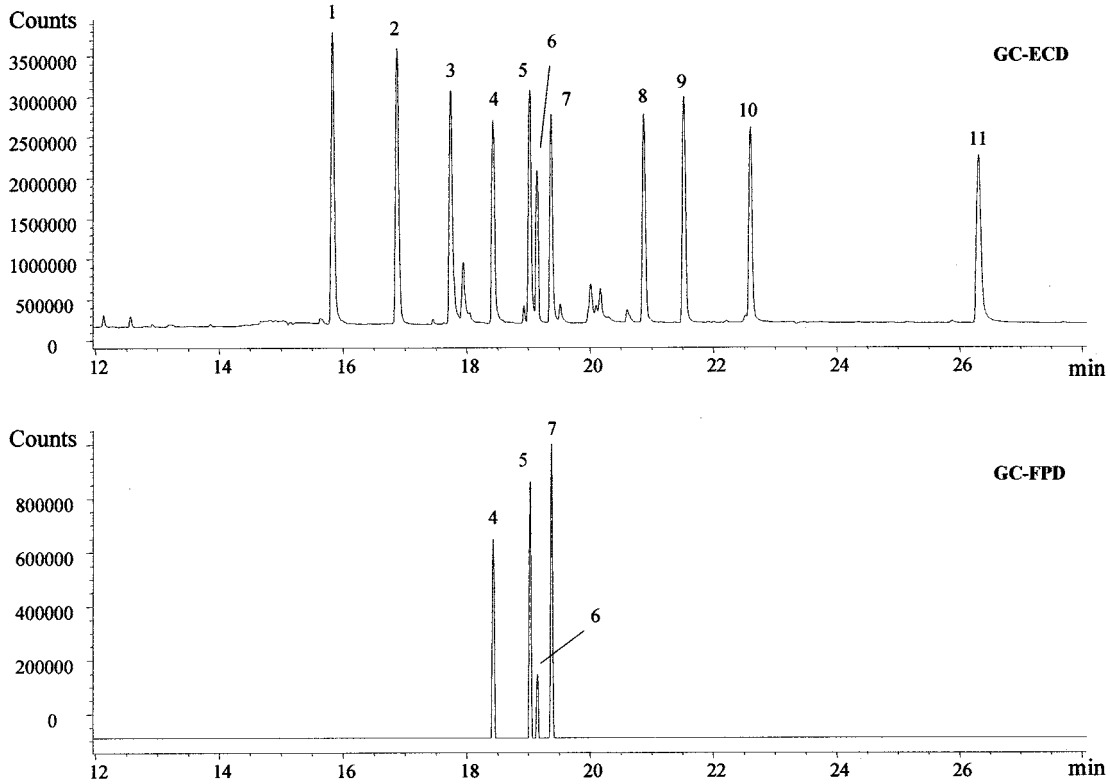

Figure 1. GC-ECD and GC-FPD profiles of the SBSE extract from a water sample spiked with 0.5 pg/μL of OC pesticides and 1.5 pg/μL of OP pesticides: (1) hexachlorobenzene; (2) lindane; (3) chlorothalonil; (4) parathion methyl; (5) fenitrothion; (6) malathion; (7) parathion ethyl; (8) α-endosulfan; (9) dieldrin; (10) β-endosulfan; (11) tetradifon. For chromatographic conditions see text.

Passiflora leaf preparations are commonly taken in the form of infusions; thus, it would be toxicologically interesting to determine the actual amount of pesticide that is transferred from the leaves to the infusion during its preparation, so that the real human intake of pesticides can be measured by making it possible to evaluate their chronic toxicological effect for habitual consumers of herbal teas. The amount of pesticide residues transferred to the infusion is in general quite low and depends on their solubility in hot water; thus, their determination requires a sample preparation technique with high concentration capability.

Recently a new sampling technique has been described (i.e. stir bar sorptive extraction, SBSE) to extract organic analytes from aqueous samples by sorption onto stir bars, consisting of a thick film of poly(dimethylsiloxane) (PDMS) coated on a magnet incorporated in a glass jacket to avoid PDMS decomposition (*6*). The stir bars have been marketed under the name Twister (Gerstel, Mülheim a/d Ruhr, Germany). The analytes are extracted by stirring the bar in the aqueous sample for a fixed time, recovered by desorbing the stir bar thermally either directly into a GC injector liner or into a glass tube inserted in a thermal desorption system and then analyzed by capillary GC or capillary GC/MS. SBSE has been shown to offer very high recoveries and a concentration capability at the ppt (ng/kg) level, because of the high volume of PDMS coating the stir bar (from 25 to 125 μL). SBSE makes it possible to overcome one of the main limitations of IS-SPME (in-the-sample solid-phase microextraction), i.e., its low concentration capability, which is mainly due to the low volume of polymeric coating that in general is around 0.5 μL (e.g. for a 100 μm PDMS fiber is 0.612 μL). SBSE has successfully been applied to the analysis of contaminants in several matrixes, among others: phthalates, nonylphenols, organochloro pesticides (*7*), 2,4,6-trichloroanisole (*8*) and dicarboximide fungicides (*9*) in wine, benzoic acid in soft drinks (*10*), and polychlorobiphenyls in sperm (*11*).

This article reports a method to determine the residues of the 11 pesticides mentioned above in the infusion of *P. alata* through a direct sampling by SBSE combined with GC-ECD/FPD and GC-MS

2. EXPERIMENTAL PROCEDURES

2.1. Materials and Reagents. *2.1.1. Passiflora Samples.* Dried leaves of *Passiflora alata* Dryander collected in March 1999 were supplied by Dr. A. M. Pereira, Grupo do Biologia Vegetal, Universidade de Ribeirão, Preto, Brazil.

2.1.2. Solvents and Chemicals. Solvents were all pesticide-grade from Riedel-de Haen (Seelze, Germany).

2.1.3. Standards. Pure standard sample of hexachlorobenzene (HCH), lindane (γ-HCH), chlorothalonil, parathion methyl, parathion ethyl, fenitrothion, malathion, dieldrin, α- and β-endosulfan, and tetradifon were from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Standard stock solutions in cyclohexane (1 mg/mL) of each pesticide were prepared and stored at −18 °C. Spiking solutions of all pesticides were prepared by diluting suitable volumes of each standard stock solution with hexane.

2.2. Sample Preparation. *2.2.1. General Methods*. The infusion was prepared by suspending plant material (1 g) in 100 mL of boiling water for 5 min and then filtered. For recovery studies and standard addition quantification, 10 g of untreated *Passiflora* leaves were spiked with 10 μL of the spiking solutions corresponding to 5, 10, 50, and 100 ng/g of OC pesticides and to 15, 30, 150, and 300 ng/g of OP pesticides and left to reach the total solvent evaporation; the resulting plant material was used for herbal tea preparation. A 1 μL aliquot of spiking solution was added to 100 mL of boiling water and to 100 mL of infusion prepared as mentioned before corresponding to 0.05, 0.1, 0.5, and 1 pg/μL of OC pesticides and to 0.15, 0.3, 1.5, and 3 pg/μL of OP pesticides.

2.2.2. Stir Bar Sorptive Extraction. The infusion was prepared by suspending plant material (1 g) in 100 mL of boiling water for 5 min

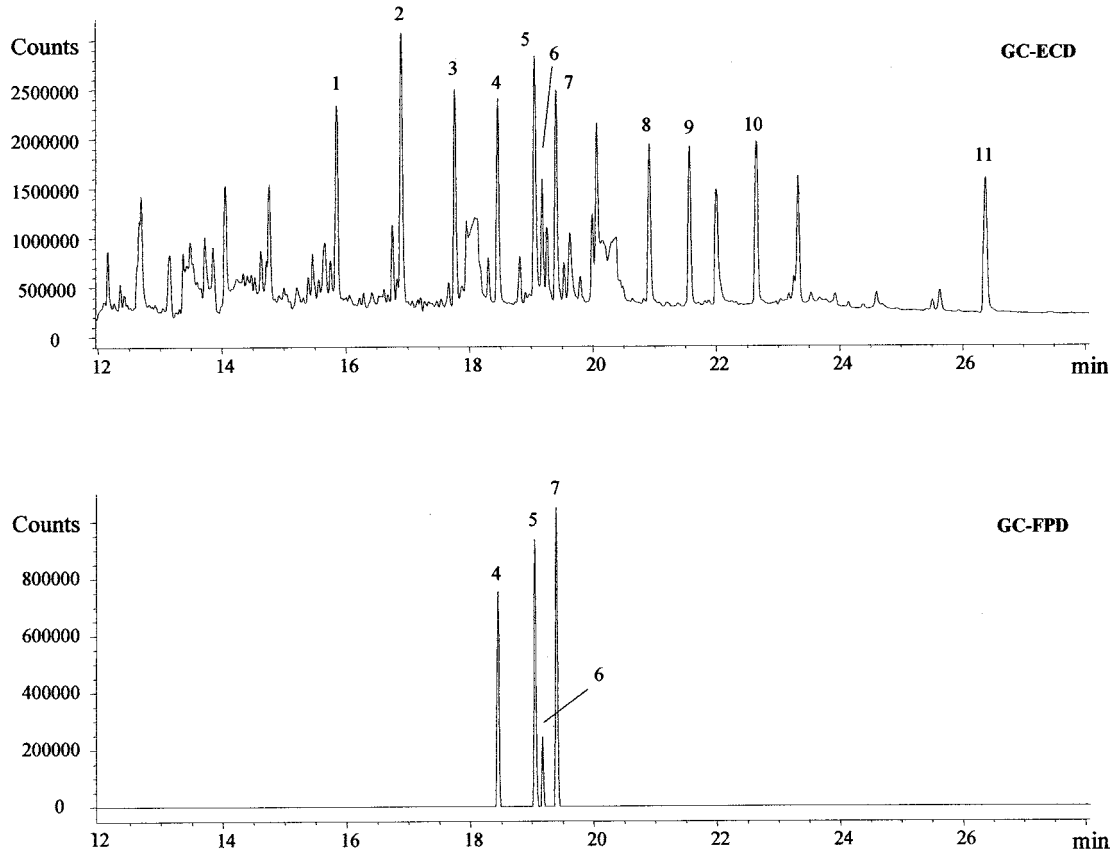

Figure 2. GC-ECD and GC-FPD profiles of the SBSE extract from a *P. alata* herbal tea spiked with 0.5 pg/μL of OC pesticides and 1.5 pg/μL of OP pesticides. For chromatographic conditions see text. For pesticide identification see Figure 1.

Table 1. Detectors (det), Spiking Range in Water, Regression Curve Equations, and the Correlation Coefficients (*r*) for the OC and OP Pesticides Investigated

pesticide	det	spiking range in water (pg/μL)	regression equation	r
HCB	ECD	0.05–1	$y = 1.3 \times 10^7 x + 3.3 \times 10^6$	0.9878
lindane	ECD	0.05–1	$y = 1.1 \times 10^7 x + 4.4 \times 10^6$	0.9833
chlortalonyl	ECD	0.05–1	$y = 1.2 \times 10^7 x + 3.3 \times 10^6$	0.9882
parathion methyl	FPD	0.15–3	$y = 2.9 \times 10^6 x + 2.8 \times 10^6$	0.9859
fenitrothion	FPD	0.15–3	$y = 2.8 \times 10^6 x + 3.8 \times 10^6$	0.9832
malathion	FPD	0.15–3	$y = 1.8 \times 10^6 x + 1.2 \times 10^6$	0.9904
parathion ethyl	FPD	0.15–3	$y = 2.6 \times 10^6 x + 3.2 \times 10^6$	0.9862
α-endosulfan	ECD	0.05–1	$y = 9.9 \times 10^6 x + 2.7 \times 10^6$	0.9848
dieldrin	ECD	0.05–1	$y = 1.1 \times 10^7 x + 3.4 \times 10^6$	0.9827
β-endosulfan	ECD	0.05–1	$y = 9.7 \times 10^6 x + 2.7 \times 10^6$	0.9800
tetradifon	ECD	0.05–1	$y = 1.3 \times 10^7 x + 2.1 \times 10^6$	0.9842

Table 2. Detector (det), Spiking Range in Herbal Tea, Regression Curve Equations, and Correlation Coefficients (*r*) of the OC and OP Pesticides Investigated

pesticide	det	spiking range in herbal tea (pg/μL)	regression equation	r
HCB	ECD	0.05–1	$y = 6.1 \times 10^6 x + 2.1 \times 10^6$	0.9823
lindane	ECD	0.05–1	$y = 9.1 \times 10^6 x + 2.5 \times 10^6$	0.9935
chlorothalonil	ECD	0.05–1	$y = 8.3 \times 10^6 x + 1.4 \times 10^5$	0.9894
parathion methyl	FPD	0.15–3	$y = 2.4 \times 10^6 x + 1.9 \times 10^6$	0.9856
fenitrothion	FPD	0.15–3	$y = 2.5 \times 10^6 x + 3.4 \times 10^6$	0.9895
malathion	FPD	0.15–3	$y = 1.4 \times 10^6 x + 1.0 \times 10^5$	0.9931
parathion ethyl	FPD	0.15–3	$y = 2.4 \times 10^6 x + 2.2 \times 10^6$	0.9840
α-endosulfan	ECD	0.05–1	$y = 6.5 \times 10^6 x + 1.3 \times 10^5$	0.9898
dieldrin	ECD	0.05–1	$y = 6.5 \times 10^6 x + 1.1 \times 10^5$	0.9903
β-endosulfan	ECD	0.05–1	$y = 6.2 \times 10^6 x + 2.3 \times 10^6$	0.9937
tetradifon	ECD	0.05–1	$y = 8.1 \times 10^6 x + 1.1 \times 10^5$	0.9960

and then filtered; the filtrate was submitted to stir bar sorptive extraction (SBSE). For some experiments the residual plant material was dried and submitted to SFE (*4*, *5*). The PDMS stir bar (*L*, 1 cm; vol, 55 μL) was plunged into the water or infusion (100 mL) and equilibrated for 30 min under constant stirring in the thermostatic bath maintained at 50 °C. After extraction, the PDMS stir bar was removed from the sample, dried with filter paper, and then inserted into the GC injector as described elsewhere (*12*), where the analytes were thermally recovered at 280 °C for GC-ECD/FPD analysis. Blank runs of the stir bar were done before and after each analysis, and no memory effects occurred for the target solutions. A series of experiments carried out at different desorption times showed that all analytes were desorbed within 90 s.

2.3. Capillary GC-ECD/FPD Analysis. Capillary GC-ECD/FPD analyses were carried out on a Carlo Erba Mega 5360 GC unit provided with Carlo Erba ECD 40 and FPD 50 detectors. A FSOT 13% phenyl 87% poly(dimethylsiloxane) column (d_f, 0.25 μm; i.d.,0.25 mm; *l*, 50 m) (Mega 13, Mega, Legnano, Milano, Italy) was used. Chromatographic conditions, injection system, splitless; time, 90 s; injector temperature, 280 °C; ECD temperature, 350 °C; FPD temperature, 160 °C; temperature program, from 40 °C (1 min) to 140 °C at 35 °C/min and then to 220 °C (3 min) at 8 °C/min and finally to 280 °C (5 min) at 15 °C/min; carrier gas, helium; flow rate, 1.5 mL/min.

Pesticides were identified in the herbal teas through their retention times by GC-ECD for OC pesticides and by GC/ECD and GC/FPD for OP pesticides, and by standard addition. Their identity was confirmed by comparison of their mass spectra obtained by thermal desorption-GC/MS (TD-GC/MS) analysis with those of standard samples.

2.4. Thermal Desorption−Capillary GC/MS Analysis. Capillary GC/MS analyses in the electron impact mode were performed on an Agilent 6890 GC-5973N MS system (Agilent, Little Falls, DE), applying the same column and conditions as those for the GC-ECD

Table 3. Octanol/Water Partitioning Coefficients ($K_{o/w}$), Water Solubility, Recoveries (R%), and Relative Standard Deviations (RSD %) from Spiked Herbal Teas and from Herbal Teas Made from Spiked Plant Material for the OC and OP Pesticides Investigated

				recoveries from herbal teas vs. spiked water		recoveries from plant material vs spiked water		recoveries from plant material vs herbal teas	
pesticides	$K_{o/w}$	water solubility (mg/L)	spiking amount (pg/μL)	R%	RSD%	R%	RSD%	R%	RSD%
HCB	1.41×10^5	0.005	1.0	50.3	5.3	31.6	6.8	48.5	6.8
			0.5	55.4	5.6	35.4	2.9	43.0	2.9
			0.1	60.3	5.9	35.3	4.1	42.0	4.1
			0.05	63.2	6.2	38.1	6.7	38.6	6.7
lindane	5.25×10^3	7.3	1.0	75.4	4.6	60.7	3.0	67.1	3.0
			0.5	70.2	4.6	56.8	4.3	64.2	4.3
			0.1	69.3	4.3	52.2	4.7	63.2	4.7
			0.05	65.6	2.8	56.6	5.9	64.4	5.9
chlorothalonil	7.76×10^2	0.6	1.0	57.1	7.6	31.8	3.7	39.1	3.7
			0.5	56.7	2.6	28.7	8.0	37.0	8.0
			0.1	50.6	3.6	28.2	8.9	32.9	8.9
			0.05	47.9	2.9	29.6	9.4	34.1	9.4
parathion methyl	1.00×10^3	55	3.0	79.6	8.0	78.3	3.9	95.5	3.9
			1.5	71.7	4.5	65.6	4.0	98.6	4.0
			0.3	74.9	6.9	59.7	5.2	89.3	5.2
			0.15	77.3	5.5	59.3	4.8	82.8	4.8
fenitrothion	3.16×10^3	21	3.0	89.2	7.5	83.2	4.8	88.6	4.8
			1.5	90.6	4.8	82.6	6.0	92.8	6.0
			0.3	89.4	4.9	78.6	6.4	91.1	6.4
			0.15	90.5	6.6	81.1	2.8	89.6	2.8
malathion	5.62×10^2	145	3.0	79.1	9.3	83.1	2.9	93.7	2.9
			1.5	76.6	5.0	71.6	3.2	98.2	3.2
			0.3	77.4	5.8	66.3	4.5	91.3	4.5
			0.15	81.2	6.3	72.4	3.7	83.4	3.7
parathion ethyl	6.76×10^3	11.0	3.0	84.1	7.0	72.6	3.9	73.2	3.9
			1.5	76.2	4.8	54.7	3.7	73.3	3.7
			0.3	77.4	6.1	53.3	4.9	78.2	4.9
			0.15	67.7	4.1	51.8	5.8	68.2	5.8
α-endosulfan	5.49×10^4	0.32	1.0	62.6	7.3	35.2	4.4	49.9	4.4
			0.5	58.9	2.7	37.6	6.0	45.7	6.0
			0.1	56.8	4.7	31.4	5.9	46.9	5.9
			0.05	53.4	6.1	32.6	7.2	51.9	7.2
dieldrin	3.16×10^3	1.52	1.0	55.7	7.5	27.3	6.7	40.6	6.7
			0.5	47.8	3.1	26.6	5.9	42.9	5.9
			0.1	46.5	4.2	25.1	6.9	41.4	6.9
			0.05	43.4	3.9	26.6	6.1	35.2	6.1
β-endosulfan	6.16×10^4	0.33	1.0	70.2	4.1	43.1	4.3	45.8	4.3
			0.5	62.3	4.1	38.9	5.8	49.1	5.8
			0.1	66.4	3.8	44.3	3.9	45.0	3.9
			0.05	70.3	3.8	39.7	7.8	50.9	7.8
tetradifon	4.07×10^4	0.078	1.0	61.4	5.1	29.2	4.0	29.6	4.0
			0.5	54.7	4.7	28.3	4.8	28.6	4.8
			0.1	56.8	6.1	25.6	5.0	29.3	5.0
			0.05	55.9	4.7	27.5	5.5	30.1	5.5

analyses. The inlet was operated in the solvent vent mode, and the carrier gas was helium.

Analyte thermal desorption from the PDMS stir bar was achieved with a TDS-2 unit from Gerstel installed on the 6890 GC unit. For the TDS the following parameters were used: desorption program, from 0 to 280 °C (8 min) at 60 °C/min; carrier gas, He; constant flow, 1.0 mL/min; flow mode, splitless; transfer line, 280 °C. A Gerstel CIS-4 PTV injector was used for cryogenic focusing of the analytes thermally desorbed from the stir bar. The PTV was cooled to −10 °C using liquid CO_2: injection, PTV in *sample remove* mode; injection temperature, −10 °C at 600 °C/min to 280 °C, (5 min).

3. RESULTS AND DISCUSSION

The aim of this study is to evaluate the pesticide residues that are present in an herbal tea, i.e., the pesticides taken with an infusion of a medicinal plant. For these experiments, herbal teas were prepared by suspending 1 g of herb in 100 mL of water to reproduce as closely as possible the conditions in which an herbal tea is consumed, although a larger volume of hot water is generally used in everyday practice. Analyses of the real-world *P. alata* samples showed residues of some of the

Table 4. Detector (det), Spiking Range of Plant Material, Regression Curve Equations, and the Correlation Coefficients (*r*) for the OC and OP Pesticides Investigated

pesticide	det	spiking range in plant material (ng/g)	regression equation	r
HCB	ECD	5–100	$y = 3.5 \times 10^6 x + 1.4 \times 10^6$	0.9984
lindane	ECD	5–100	$y = 7.5 \times 10^6 x + 1.8 \times 10^6$	0.9996
chlorothalonil	ECD	5–100	$y = 3.6 \times 10^6 x + 8.4 \times 10^5$	0.9997
parathion methyl	FPD	15–300	$y = 2.9 \times 10^6 x + 1.1 \times 10^6$	0.9997
fenitrothion	FPD	15–300	$y = 2.5 \times 10^6 x + 2.5 \times 10^6$	0.9956
malathion	FPD	15–300	$y = 1.6 \times 10^6 x + 6.3 \times 10^5$	0.9995
parathion ethyl	FPD	15–300	$y = 2.1 \times 10^6 x + 1.4 \times 10^6$	0.9999
α-endosulfan	ECD	5–100	$y = 3.5 \times 10^6 x + 7.9 \times 10^5$	0.9998
dieldrin	ECD	5–100	$y = 3.1 \times 10^6 x + 6.8 \times 10^5$	0.9994
β-endosulfan	ECD	5–100	$y = 3.7 \times 10^6 x + 1.4 \times 10^6$	0.9992
tetradifon	ECD	5–100	$y = 2.7 \times 10^6 x + 6.3 \times 10^5$	0.9995

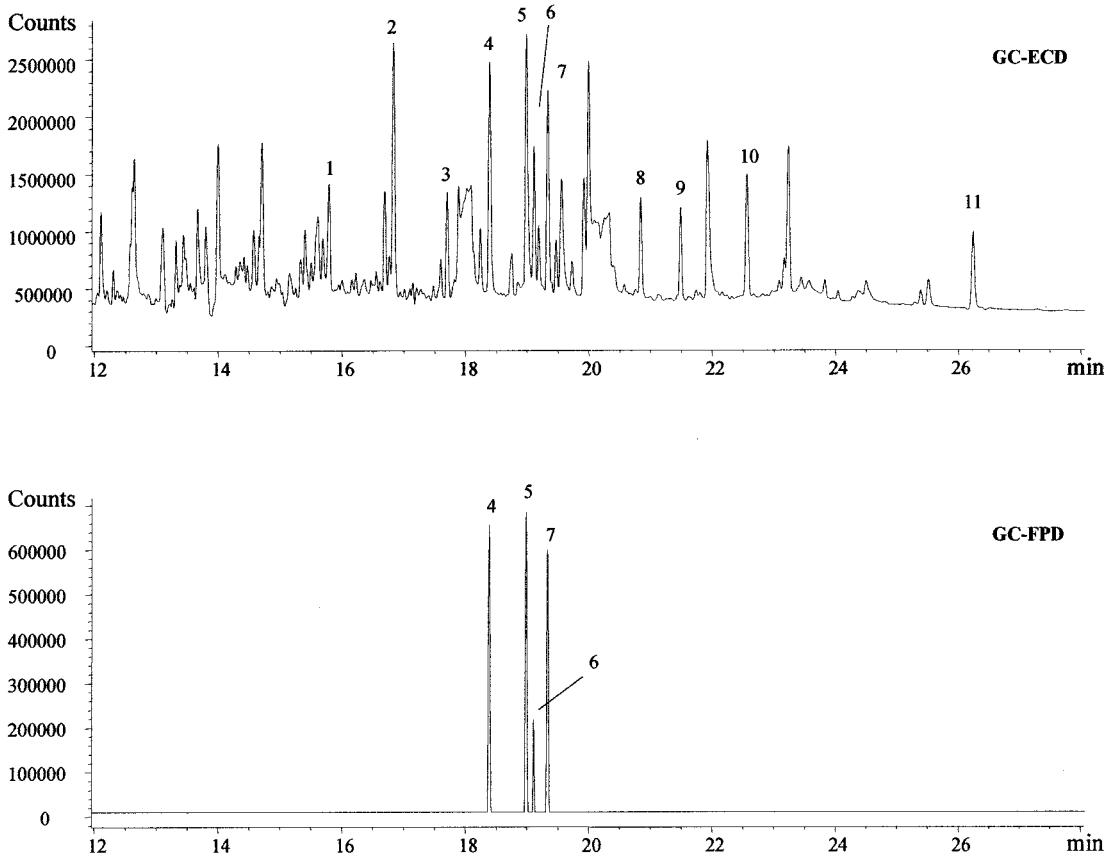

Figure 3. GC-ECD and GC-FPD profiles of the SBSE extract from a *P. alata* herbal tea prepared with plant material spiked with 50 ng/g of OC pesticides and 150 ng/g of OP pesticides. For chromatographic conditions see text. For pesticide identification see Figure 1.

pesticides investigated ranging from 1 to 10 ng/g (*5*): this means that lower concentrations (probably in the ppt order) were expected in the resulting herbal teas.

3.1. SBSE-GC-ECD/FPD Analysis of Spiked Water Samples. In these experiments, water (100 mL) was spiked with amounts of pesticides ranging from 0.05 to 1 pg/μL for OC pesticides and from 0.15 to 3 pg/μL for OP pesticides and submitted to SBSE-GC-ECD/FPD analysis. Spiked water samples were used to evaluate the relative sorptive recovery from spiked *P. alata* herbal teas and *P. alata* herbal teas prepared with spiked plant material. Figure 1 shows the GC-ECD and the GC-FPD profiles of the SBSE extract from a water sample spiked with 0.5 pg/μL of OC pesticides and 1.5 pg/μL of OP pesticides, while Table 1 reports detector (det), spiking range added to water, regression curve equations, and correlation coefficients (r) for each OC and OP pesticide investigated. Each experiment at each pesticide level was repeated six times.

3.2. SBSE-GC-ECD/FPD Analysis of Spiked *P. alata* Herbal Teas. In these experiments *P. alata* herbal teas (100 mL) were spiked with 0.05−1 pg/μL of OC pesticides and with 0.15−3 pg/μL of OP pesticides. Figure 2 shows the GC-ECD and the GC−FPD profiles of the SBSE extract from a *P. alata* herbal tea spiked with 0.5 pg/μL of OC pesticides and 1.5 pg/μL of OP pesticides, while Table 2 reports detector, range of spiked amount in herbal teas, regression curve equations, and correlation coefficients for each pesticide investigated. Each experiment was repeated six times. Table 3 reports octanol/water partitioning coefficients ($K_{o/w}$), solubility, recoveries, and relative standard deviations of the pesticides investigated from spiked herbal teas, calculated by taking the pesticide amounts after SBSE-GC-ECD/FPD analysis of spiked water samples as reference values.

3.3. SBSE-GC-ECD/FPD Analysis of *P. alata* Herbal Teas Prepared with Spiked Plant Material. In these experiments herbal teas (100 mL) were prepared with *P. alata* leaves spiked with 5−100 ng/g of OC pesticides and 15−300 ng/g of OP pesticides. Figure 3 reports the GC-ECD and the GC-FPD profiles of the SBSE extract from a *P. alata* herbal tea prepared with plant material spiked with 50 ng/g of OC pesticides and 150 ng/g of OP pesticides, while Table 4 reports detector, spiking range to plant material, regression curve equations, and correlation coefficients for each pesticide investigated. Each experiment was repeated six times. Table 3 also reports recoveries and relative standard deviations of the pesticides investigated from herbal teas prepared with spiked plant material, calculated by taking the pesticide amounts resulting from SBSE-GC-ECD/FPD analysis of both spiked water samples and of spiked herbal teas as reference values.

As already reported by Sandra et al. (*6*, *8*) for other matrixes, for the same spiked amounts, the calibration curves from water have different slopes than those obtained with spiked herbal teas and then those obtained with herbal teas prepared with spiked plant material. Figure 4 compares the calibration curves of dieldrin and parathion ethyl when analyzed in spiked water, spiked herbal tea, and herbal tea made from spiked plant material. The curves obtained by the background subtraction afford emphasis of the differences in slopes. The slopes for water are much steeper than those for herbal teas, meaning that a significant matrix effect occurs, influencing the partitioning process and recovery. Recoveries should therefore be calculated from herbal teas prepared with spiked plant material versus spiked herbal teas, and calibration (and thus quantitative calculation) should be done using herbal teas prepared from spiked plant material. This is also because the sorptive recovery

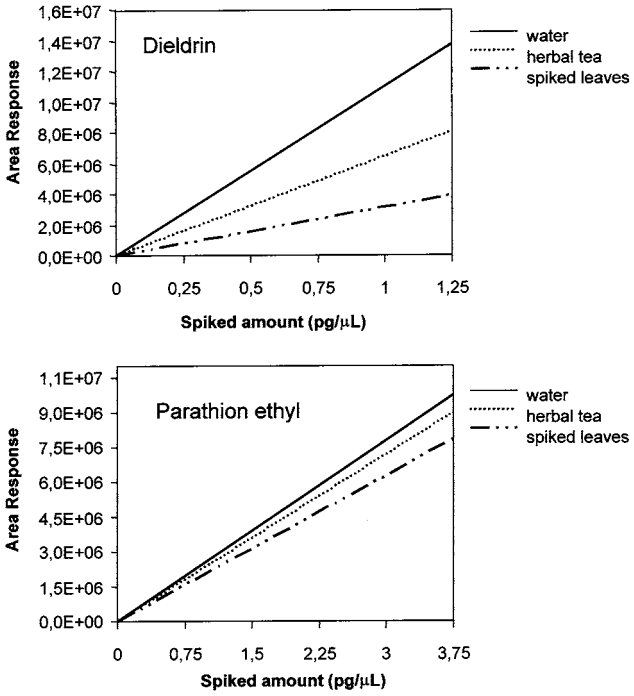

Figure 4. Calibration curves of dieldrin and parathion ethyl when analyzed in spiked water, spiked herbal tea, and herbal tea made from spiked plant material.

of a pesticide from plant material is conditioned by two equilibria (i.e. matrix/water solubilization and water/PDMS partition) involving its interaction with the vegetable matrix, its water solubility, and its water/PDMS partitioning constant (i.e. its $K_{o/w}$ (*6*)). Further reasons for the differences in pesticide recoveries from *Passiflora* plant material may be the pesticide residues remaining with the plant material after herbal tea preparation and the co-solubilization of saponins, which improve water solubility of hydrophobic compounds. Semiquantitative SFE-GC-ECD/FPD analyses showed that the plant material dried after herbal tea preparation still contained significant amounts of pesticides. Recoveries from spiked plant material versus herbal teas varied from about 30% for tetradifon to about 90% for parathion and malathion (Table 3).

This method was validated using herbal teas prepared with spiked plant material. Repeatability of the method was verified by analyzing six samples of herbal teas prepared from spiked *P. alata* leaves at each pesticide concentration and determining relative standard deviation (RSD; Table 3): RSD% varied from 2.8 for fenitrothion at 150 ng/g to 9.4 for chlorothalonil at 50 ng/g.

Intermediate precision was evaluated by controlling recoveries of each OC and OP pesticide at two different fortification levels (OC, 10 and 50 ng/g; OP, 30 and 150 ng/g) from herbal teas prepared from spiked *P. alata* leaves every 2 weeks over a 3 month period, for a total of six determinations. Results are reported in Table 5. By combining all the recovery results at each fortification level, RSDs % were within the chosen range in all cases (up to 10%). This procedure is in agreement with the Guidance Document on Residue Analytical Methods (8064/VI/97-rev 4) issued by the European Commission.

Limits of detection (LODs) and limits of quantitation (LOQs) were determined in agreement with the IUPAC method and are reported in Table 5. LODs were measured by analyzing a standard solution of the pesticides investigated under the GC-ECD/FPD conditions reported above in amounts sufficient to obtain a signal-to-noise ratio of 3:1. LODs ranged from 0.005 pg/μL for lindane to 0.035 pg/μL for malathion.

LOQs were determined through the IUPAC method (*13*) and calculated by analyzing herbal teas prepared from unspiked *P. alata* samples and submitted to six cycles of sorptive extraction. LOQs ranged from 0.017 pg/μL for lindane to 0.117 pg/μL for malathion.

A series of six commercially available samples of *P. alata* and of the corresponding herbal teas were analyzed. Two herbal teas were found to contain pesticide residues, in particular 0.7 pg/μL of dieldrin and 0.9 pg/μL of chlorothalonil, respectively. The analyses of the corresponding dried leaves by the SFE method (*5*) confirmed these results.

These results show that sample preparation by SBSE is able to determine pesticide residues in herbal teas with medium-to-high and reproducible recoveries at the ppt level. The SBSE LODs of HCB and malathion were 0.010 and 0.035 pg/μL, respectively, while LOQs varied from 0.033 and 0.117 pg/μL, respectively, showing how effective is the concentration capability of PDMS stir bars on diluted samples.

In conclusion this application shows how versatile is sorptive extraction in sampling from a high-volume, low-concentration solution, where a highly effective concentration capability is necessary because of the low analyte concentrations, but also

Table 5. Intermediate Precision Expressed as Relative Standard Deviation, LOD, and LOQ for Each OC and OP Pesticide Investigated

			intermediate precision					
pesticide	det	n	R% 10 ng/g	RSD%	R% 50 ng/g	RSD%	LOD (pg/μL)	LOQ (pg/μL)
HCB	ECD	6	40.3	5.4	45.7	4.8	0.010	0.033
lindane	ECD	6	63.8	5.2	65.6	5.1	0.005	0.017
chlorothalonil	ECD	6	33.5	6.7	38.5	5.6	0.010	0.033
α-endosulfan	ECD	6	46.7	6.9	48.8	5.9	0.010	0.033
dieldrin	ECD	6	41.7	7.6	40.9	5.1	0.010	0.033
β-endosulfan	ECD	6	47.6	7.4	46.6	5.7	0.009	0.030
tetradifon	ECD	6	28.4	7.7	28.9	7.1	0.010	0.033

			intermediate precision					
pesticide	det	n	R% 30 ng/g	RSD%	R% 150 ng/g	RSD%	LOD (pg/μL)	LOQ (pg/μL)
parathion methyl	FPD	6	84.6	5.3	96.1	4.6	0.030	0.100
fenitrothion	FPD	6	86.6	5.8	91.8	6.3	0.015	0.050
malathion	FPD	6	84.4	4.8	92.6	5.4	0.035	0.117
parathion ethyl	FPD	6	71.9	6.4	75.7	5.1	0.030	0.100

high sample volumes are necessary to have a sufficient absolute amount of analyte to process. An in-depth study is under way to evaluate the influence on recovery of the water/PDMS phase ratio, the volume of PDMS coated onto the stir bar, and the sampling temperature (*14*).

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