RESIDUES AND TRACE ELEMENTS

Determination of Six Pesticides in the Medicinal Herb *Cordia salicifolia* by Matrix Solid-Phase Dispersion and Gas Chromatography/Mass Spectrometry

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A simple and effective extraction method based on matrix solid-phase dispersion was developed for acephate, chlorpropham, pyrimicarb, bifenthrin, tetradifon, and phosalone in leaves of the medicinal plant *Cordia salicifolia*, whose extracts are commercialized in Brazil as diuretic, appetite suppressant, and weight loss products. The determination method was GC/MS with selected-ion monitoring. Different parameters of the method were evaluated, such as type of solid phase (C18, alumina, silica gel, and Florisil) and the amount of solid phase and eluent (dichloromethane, ethyl acetate, chloroform, and cyclohexane). The best results were obtained using 0.5 g herb sample, 0.5 g neutral alumina as the dispersant sorbent, 0.5 g C18 as the cleanup sorbent, and cyclohexane–dichloromethane (3 + 1, v/v) as the eluting solvent. The method was validated using herb samples fortified with pesticides at different concentration levels (0.3, 0.5, and 1.0 mg/kg). Average recoveries (seven replicates) ranged from 67.7 to 129.9%, with relative standard deviations between 6.3 and 26%. Detection and quantitation limits for the herb ranged from 0.10 to 0.15 and 0.15 to 0.25 mg/kg, respectively.

Medicinal plants are widely consumed as home remedies and used as raw materials in the pharmaceutical industry for the production of phytopharmaceuticals (1). The herbs are usually prepared using natural and cultivated plants that are collected, dried, and packaged without any effective hygienic, sanitary, or chemical contamination control. Therefore, it is important to know the risk that their consumption may pose to health (2). *Cordia salicifolia* Cham (Boraginaceae family syn. *Cordia ecalyculata* Vell.), also known by several common names such as "porangaba," "chá do bugre," or "café do mato," is a small tree producing as its peculiar feature red fruits resembling coffee beans, which are roasted and brewed into tea as a coffee substitute. It is indigenous to Brazil but can be found also in tropical forested areas of Argentina and Paraguay. *C. salicifolia* is widely used in the Brazilian ethnomedicinal traditions, in particular in the regions of Minas Gerais, Bahia, Acre, and Goiás. Extracts of the plant are commercialized in Brazil as diuretic, appetite suppressant, and weight loss products. The partially purified extract from the whole plant has shown an inhibitory effect on Herpes Simplex type I virus, and the methanolic extract of branches and leaves appears to exert cytotoxic activity against cancer cells. Studies using rabbits and guinea pigs have indicated cardiotonic properties, and hypolipidemic effects have been observed in normal and alloxan-induced diabetic rats. It has been shown that the administration of commercially available extracts of *C. salicifolia* for a prolonged period does not cause toxic effects in animals (3). Despite the popularity of *C. salicifolia* in Brazilian folk medicine, to our knowledge, no work has been undertaken to determine possible chemical contamination of this medicinal herb.

Few analytical methods for the determination of pesticide residues in medicinal plants have been described in the recent literature (4–7). The determination of pesticides in medicinal herb matrixes is usually accomplished using chromatographic techniques, mainly GC with different detector systems (electron capture, nitrogen-phosphorus, or flame photometric detection). Preliminary steps include sampling, then extraction and cleanup based on different liquid–liquid extraction (LLE), matrix solid-phase dispersion (MSPD), solid-phase microextraction, microwave-assisted extraction, or supercritical fluid extraction procedures. These are the most common extraction techniques that have been used for multiclass pesticide analyses of medicinal herbs (8–12). MSPD, in particular, is a valuable extraction method that provides a useful alternative to traditional extraction techniques before chromatographic analysis (13–15). Moreover, MSPD can be carried out simultaneously with sample homogenization, extraction, and cleanup and requires only a small sample size and small amounts of solvent (16, 17). It avoids the drawbacks generally associated with LLE, such as the use of large volumes of solvent, the occurrence of troublesome emulsions, and slow speed (18–20).

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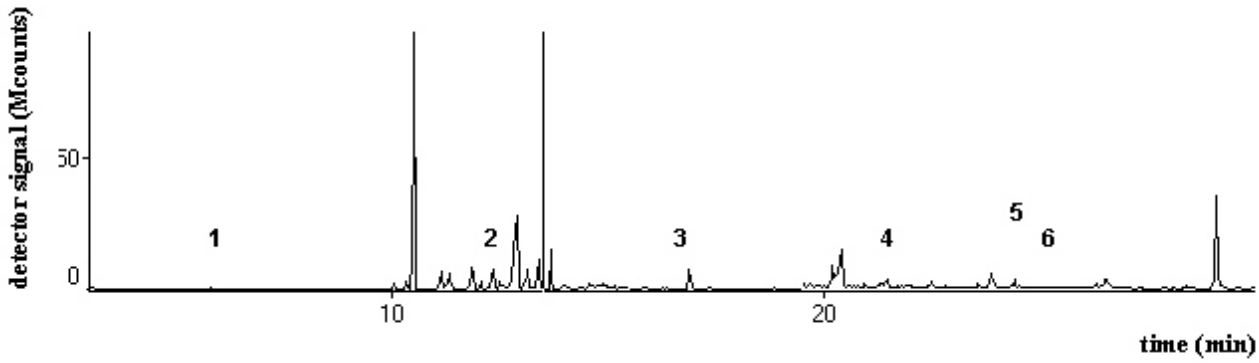

Figure 1. GC/SIM-MS chromatogram of a pesticide standard solution (0.3 mg/kg) in dichloromethane. The numbered peaks are: 1, acephate; 2, chlorpropham; 3, pyrimicarb; 4, bifenthrin; 5, tetradifon; and 6, phosalone.

Acephate, chlorpropham, pyrimicarb, bifenthrin, tetradifon, and phosalone are among the pesticides most commonly used for pest control in different cultivations near medicinal herb plantations in the state of Sergipe (Brazil). Hence, this paper reports a simple methodology for simultaneous determination of these six pesticides in *C. salicifolia* using MSPD and GC/MS.

Experimental

Chemicals and Solvents

The HPLC grade solvents dichloromethane, ethyl acetate, cyclohexane, and chloroform were purchased from Mallinckrodt Baker (Paris, KY). Certified standards of acephate, chlorpropham, pyrimicarb, bifenthrin, tetradifon, and phosalone were purchased from Dr. Ehrenstorfer (Augsburg, Germany); all standards were at least 97.0% pure. Analytical grade anhydrous sodium sulfate was from Mallinckrodt Baker, and research grade Florisil (80–100 mesh) was from Sigma (Büchs, Switzerland). C18-bonded silica (50 m) was obtained from Phenomenex (Torrance, CA), silica gel 60 (70–230 mesh) from Merck (Darmstadt, Germany), and neutral alumina (70–290 mesh, activity I) from Macherey-Nagel (Düren, Germany).

Pesticide Standard Solutions

Stock standard solutions of pesticides were prepared by exactly weighing and dissolving the corresponding compounds in dichloromethane at 500 g/mL and storing at –18 C. These standard solutions were stable for a period of 2 months. Periodically, the stability of the pesticide standards was evaluated by measuring the variation of the

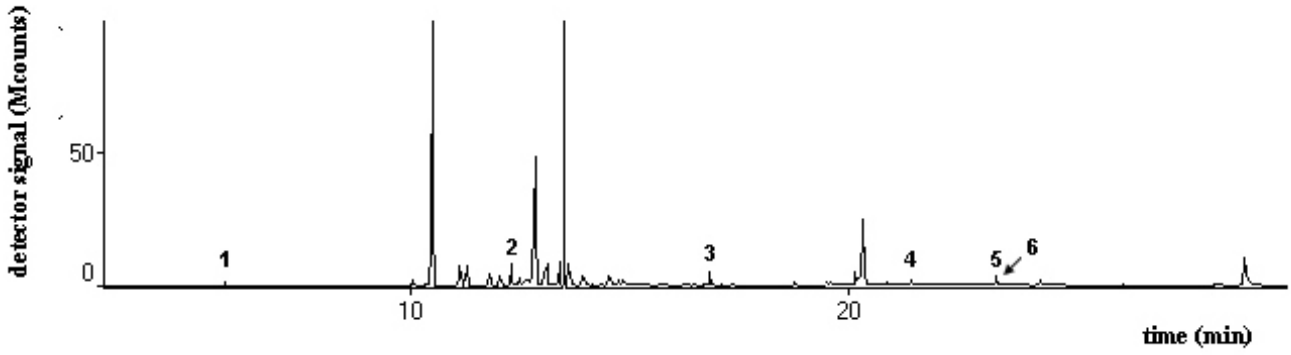

Figure 2. GC/SIM-MS chromatogram of a typical porangaba (*C. salicifolia*) extract, fortified at a concentration level of 0.3 mg/kg, using 0.5 g porangaba + 0.5 g sorbent + 1.0 g cosorbent and cyclohexane–dichloromethane (3 + 1, v/v; 30 mL). The numbered peaks are: 1, acephate; 2, chlorpropham; 3, pyrimicarb; 4, bifenthrin; 5, tetradifon; and 6, phosalone.

Table 1. Influence of different mixtures of solvent on pesticide recovery in the MSPD procedure[a]

Pesticide	Concentration, mg/kg	Recovery, % (RSD, %)		
		Cyclohexane–chloroform	Cyclohexane–dichloromethane	Cyclohexane–ethtyl acetate
Acephate	1.0	75.9 (2.6)	69.4 (8.5)	105.3 (11.6)
Chlorpropham	1.0	56.1 (3.6)	113.1 (12.2)	57.3 (2.1)
Pyrimicarb	1.0	49.1 (5.9)	53.0 (6.6)	190.4 (10.2)
Bifenthrin	1.0	221.7 (3.8)	103.6 (7.0)	134.8 (10.3)
Tetradifon	1.0	262.3 (9.6)	125.8 (14.1)	142.4 (2.2)
Phosalone	1.0	56.1 (6.4)	102.3 (6.8)	161.6 (12.8)

[a] 0.5 g porangaba + 0.5 g neutral alumina sorbent; $n = 2$.

chromatographic peak area, the RSD of which ranged from 2.5 to 3.9%. The working standard solutions were prepared by diluting the stock solutions in dichloromethane as required. Matrix-matched standards were prepared at the same concentrations as those of calibration solutions by adding appropriate amounts of standards to the control matrix extract.

GC/MS System and Operating Conditions

A Shimadzu system (Kyoto, Japan) consisting of a QP-5050*A* mass spectrometer, GC-17A gas chromatograph, AOC 20i autoinjector, and split/splitless injector was used for the identification and quantification of the pesticides studied. A fused-silica DB-5MS column (5% phenyl–95% polydimethylsiloxane; 30 m 0.25 mm id, 0.25 m), supplied by J&W Scientific (Folsom, CA), was used with helium (99.999% purity) as the carrier gas at a flow rate of 1.8 mL/min. The column temperature was programmed as follows: 60 C for 1 min, then ramped to 300 C at 10 C/min, and held for 3 min. The solvent delay was 5 min. The injector port was maintained at 250 C, and 1 L sample volumes were injected in splitless mode (0.7 min). The data were acquired and processed with a personal computer with Shimadzu class 5000 software. The total analysis time was 28 min and equilibration time 2 min.

The eluate from the GC column was fed via a transfer line heated at 280 C, and fed into a 70 eV electron impact ionization source also maintained at 280 C. The analysis was performed in the selected-ion monitoring (SIM) mode. For the first acquisition window (5.0 to 10.0 min), the ions monitored were *m/z* 136, *142*, and 168 (acephate). For the second acquisition window (11.0 to 20.0 min), the ions monitored were *m/z 154*, 171, and 213 (chlorpropham) and *m/z* 152, *166*, and 238 (pyrimicarb). For the third acquisition window (20.0 to 28.0 min), the ions monitored were *m/z* 165, *181*, and 322 (bifenthrin); *m/z* 227, *356*, and 362 (tetradifon); and *m/z* 121, *257*, and 367 (phosalone). Values of *m/z* in italic type correspond to the quantification ion for each analyte.

Sample Preparation and Fortification

Dried porangaba leaf (*C. salicifolia* Cham) samples used for method development were purchased in bulk package format from a local market in the municipality of Aracaju, state of Sergipe, Brazil. No indication of the geographical origin of the plant samples was given on the labels. A representative portion of medicinal plant (100 g) was homogenized using a household blender, sieved (1–2 mm), and stored in jars away from light and moisture at –18 C until used for analysis. Fortified samples were prepared by adding 500 L of a mixture of the standard solutions to 0.5 g sample, resulting in final concentrations ranging from 0.3 to 1.0 mg/kg of pesticides in the sample. The fortified plant samples were left to stand for 30 min at room temperature to allow the solvent to evaporate before extraction. Seven replicates were analyzed at each fortification level.

Optimized Extraction Procedure

An aliquot of dried and powdered medicinal plant (0.5 g) was placed into a glass mortar (ca 50 mL), and 0.5 g neutral alumina was added. The medicinal plant was then gently blended into the alumina with a glass pestle until a homogeneous mixture was obtained (ca 1 min). The homogenized mixture was introduced into a 100 20 mm id polypropylene column filled with 0.1 g silanized glass wool at the base, followed, in order, by 1.0 g anhydrous Na_2SO_4 and 0.5 g C18 sorbent. A 30 mL portion cyclohexane–dichloromethane (3 + 1, v/v) was added to the column, and the sample was allowed to elute dropwise. Columns were placed on an 18-port vacuum manifold with eluent flow rate set at 0.5 mL/min. The eluate was collected in a graduated conical tube and concentrated to 1 mL using first a rotary vacuum evaporator (40 C), followed by a gentle flow of nitrogen. A 1 L portion of the extract was then analyzed by GC/MS.

Results and Discussion

GC/MS Conditions

Preliminary experiments to optimize the GC/MS conditions were performed in full scan mode by direct injection of 1 L of the standard mixture at 5 g/mL and varying the oven temperature. In these evaluations, the characteristic ions were chosen, and the MS system was then programmed in the SIM mode for quantification of each

Table 2. Influence of different sorbents on pesticide recovery in the MSPD procedure[a]

Sorbent/cosorbent	Recovery, % (RSD, %)					
	Acephate	Chlorpropham	Pyrimicarb	Bifenthrin	Tetradifon	Phosalone
C18/Florisil	62.8 (1.5)	116.8 (6.6)	182.3 (14.5)	94.3 (12.8)	114.6 (6.9)	56.1 (2.3)
Silica/Florisil	484.1 (16.0)	36.4 (1.9)	152.6 (5.7)	102.6 (2.0)	316.6 (11.6)	71.6 (3.2)
Alumina/Florisil	68.5 (4.8)	97.2 (3.2)	65.1 (4.0)	61.1 (2.2)	7.3 (3.4)	3.3 (5.1)
C18/alumina	183.4 (3.0)	51.2 (2.3)	33.1 (7.2)	50.9 (4.4)	125.1 (1.8)	331.6 (22.3)
Florisil/alumina	339.1 (19.6)	103.6 (2.6)	57.3 (2.6)	74.4 (2.6)	63.8 (2.6)	51.6 (2.6)
Silica/alumina	121.1 (8.9)	69.5 (5.5)	105.1 (5.4)	105.1 (3.7)	186.1 (15.6)	183.1 (9.9)
Florisil/C18	63.1 (3.4)	209.9 (18.8)	80.2 (2.1)	31.3 (4.0)	21.1 (9.4)	1.8 (3.5)
Silica/C18	116.4 (7.2)	56.6 (6.6)	25.4 (11.2)	30.9 (8.1)	76.1 (2.4)	31.1 (2.0)
Alumina/C18	47.1 (6.9)	42.9 (5.1)	57.4 (4.3)	58.7 (15.0)	71.8 (12.3)	51.6 (1.6)
C18/silica	61.0 (9.8)	52.7 (12.6)	96.9 (6.0)	12.3 (4.5)	1.3 (3.3)	1.1 (5.6)
Florisil/silica	27.6 (12.2)	18.6 (10.3)	107.5 (12.6)	9.3 (9.3)	1.6 (8.5)	1.3 (6.9)
Alumina/silica	25.1 (10.6)	9.5 (6.9)	62.9 (5.4)	7.3 (5.9)	130.7 (7.1)	13.1 (5.4)

[a] 0.5 g porangaba + 0.5 g sorbent + 1.0 g cosorbent; $n = 2$; eluting solvent: cyclohexane–dichloromethane (3 + 1, v/v; 30 mL).

pesticide. The choice of the ions for SIM acquisition was based on the best S/N values.

A difficulty is that matrix components can cause variation in the detector response to pesticides (20–22). Therefore, the matrix effect was evaluated by comparing the detector response for pesticide standards prepared in dichloromethane with that for standards prepared in herb extract. When standards were prepared by spiking blank herb samples with known amounts of pesticides, higher peak areas were obtained for the same pesticide concentrations. Consequently, the quantification of pesticide residues was carried out using matrix-matched standards.

Figures 1 and 2 show chromatograms of a standard solution and sample extract, respectively.

Optimization of the MSPD Procedure

The proposed extraction method was based on the MSPD procedure. The most suitable extraction parameters were evaluated to achieve the highest recoveries for acephate, chlorpropham, pyrimicarb, bifenthrin, tetradifon, and phosalone from the dried herb *C. salicifolia*. The type of the sorbent and polarity of the elution solvent are known to be key factors in MSPD, since they determine both the efficacy of the extraction and the purity of the final extracts (14–16). Preliminary investigations for optimization of the MSPD procedure were performed using dried herb samples spiked with pesticide standard solution at 1.0 mg/kg, and C18, neutral alumina, silica gel, or Florisil as solid-phase sorbent. Cyclohexane was the eluting solvent. The results from these experiments indicated that extraction recovery for all pesticides varied between 5.3 and 440%. Silica, neutral alumina, and Florisil provided cleaner extracts than C18. Comparison of C18 and silica material showed that silica produced better results than the C18-bonded silica sorbent, which gave recoveries between 13 and 180% for most of the target compounds. Used with cyclohexane, the Florisil sorbent produced high recoveries (over 100%) for all of the target analytes. The larger peak areas can be explained by a matrix effect that enhances the chromatographic response to pesticides, as previously reported for the matrix effect in the determination of pesticides in different foodstuffs (20–22).

In order to evaluate the influence of the polarity of the extractant, mixtures of chloroform, dichloromethane, or ethyl acetate with cyclohexane in the proportion 3:1 (v/v) were tested using neutral alumina, and a constant sorbent to *C. salicifolia* matrix ratio of 1:1 (m/m). Results showed that cyclohexane–dichloromethane resulted in the cleanest extracts for pesticide extraction from the *C. salicifolia* matrix, while elution of the MSPD column with cyclohexane–chloroform and cyclohexane–ethyl acetate mixtures produced not only an enhancement of recoveries, but also a higher background and more interfering peaks from the medicinal plant compared to the cyclohexane–dichloromethane. These tests also resulted in inadequate recovery values for the pesticides, ranging from 49.1 to 262.3%, with lower recoveries for the pesticides found with cyclohexane–dichloromethane (3 + 1, v/v) compared to the other eluting solvents (Table 1). The high recovery ($R >$ 100%) can be attributed to the presence of interfering endogenous compounds. Additional tests were performed adding a cosorbent (1.0 g of C18, silica gel, alumina, or Florisil) to the sorbent–herb matrix blend to obtain additional fractionation and assist in sample cleanup in order to obtain satisfactory recoveries. This approach gave improved results: all pesticides were recovered almost quantitatively and with good reproducibility using cyclohexane–dichloromethane (3

Table 3. Average recoveries and RSD of fortified pesticides in medicinal plant from the MSPD method with GC/MS analysis[a]

Pesticide	Fortification level, mg/kg	Mean recovery, %	RSD, %
Acephate	0.3	67.7	12.1
	0.5	85.7	19.7
	1.0	62.9	15.8
Chlorpropham	0.3	113.3	8.6
	0.5	115.8	10.6
	1.0	129.9	11.3
Pyrimicarb	0.3	98.0	12.2
	0.5	117.6	12.4
	1.0	81.3	7.7
Bifenthrin	0.3	99.3	16.4
	0.5	94.6	26.0
	1.0	84.1	6.3
Tetradifon	0.3	103.8	5.2
	0.5	108.5	24.1
	1.0	82.5	11.0
Phosalone	0.3	67.7	14.5
	0.5	105.1	19.7
	1.0	78.5	15.9

[a] $n = 7$.

+ 1, v/v). Elution of the pesticides using cosorbents showed different responses ranging from 1.1 to 484.1% (Table 2). The extraction column prepared with the neutral alumina–herb blend and C18 as cosorbent produced an extract that showed minimal interferences for most of the pesticides studied, while the use of alumina as cosorbent in the MSPD method produced some recoveries higher than 300%, similar to those obtained with Florisil, considering the same proportion between solid phase and plant matrix. All analyses were performed in duplicate. Overall, the results indicated that the combination of neutral alumina as the solid phase, C18 as the cleanup layer, and cyclohexane–dichloromethane (3 + 1, v/v) as the eluent was a suitable extraction procedure for determination of acephate, chlorpropham, pyrimicarb, bifenthrin, tetradifon, and phosalone in *C. salicifolia* leaves.

Recovery Study

Considering that there are no Brazilian regulations concerning maximum permissible pesticide residue concentrations in medicinal herbs, recovery experiments were performed (seven replicates) at three arbitrary fortification levels (0.3, 0.5, and 1.0 mg/kg) by adding known volumes of pesticide standards in dichloromethane to the medicinal plant matrix. Concentrations were calculated by comparing peak areas from extracted ion current profiles with those obtained from matrix-matched standards. Blank analyses were performed in order to check interferences from the matrix. Table 3 presents recoveries of the six pesticides at three concentration levels. Average recoveries ranged from 67.7 to 129.9%, with RSD values of 6.3 to 26%.

Table 4. Linearity, LOQ, and LOD obtained by GC/MS analysis

Pesticide	Linearity		LOQ, mg/kg	LOD, mg/kg
	Linear range, mg/kg	r		
Acephate	0.2–20.0	0.9989	0.25	0.15
Chlorpropham	0.2–20.0	0.9993	0.15	0.10
Pyrimicarb	0.2–20.0	0.9985	0.15	0.10
Bifenthrin	0.2–20.0	0.9994	0.15	0.10
Tetradifon	0.2–20.0	0.9995	0.15	0.10
Phosalone	0.2–20.0	0.9996	0.25	0.15

Linearity, LOD, and LOQ

The linearity of a method is a measure of the range within which detector response is directly proportional to the concentration of analyte in standard solutions or samples. Linearities for all compounds were determined using blank medicinal plant samples fortified at 8 concentration levels (0.2, 0.6, 0.8, 1.2, 1.6, 4.0, 16.0, and 20.0 mg/kg). The slope and intercept values, together with their standard deviations, were determined using regression analyses. Values of r for all pesticides ranged from 0.9985 to 0.9996, indicating that good linearity was obtained at these concentration levels.

The LODs for the pesticides studied were calculated considering the SD of the analytical noise (a value of 7 times the SD of the blank) and the slope of the regression line, and ranged from 0.10 to 0.15 mg/kg. The LOQs were determined as the lowest concentration giving a response of 10 times the average of the baseline noise, calculated using seven unfortified samples. The LOQ values for the different compounds ranged from 0.15 to 0.25 mg/kg (23). These data are summarized in Table 4.

Application of the Method to Real Samples

The MSPD procedure developed was applied to the determination of pesticides in the medicinal plant *C. salicifolia*. Four different samples of this medicinal plant, obtained from local markets in the city of Aracaju (Brazil) and originating from conventional agriculture, were analyzed using this procedure. No pesticide residues at concentrations above the detection limit, were found in these samples.

Conclusions

The proposed MSPD procedure followed by GC/SIM-MS can be applied to determine acephate, chlorpropham, pyrimicarb, bifenthrin, tetradifon, and phosalone residues in

dried leaves of the medicinal plant *C. salicifolia*. The method uses neutral alumina in the MSPD column with C18 cosorbent, and cyclohexane–dichloromethane (3 + 1, v/v) as the eluent. The results demonstrated that the accuracy, precision, and selectivity of the proposed method are acceptable for quantitative analyses of pesticide residues of different chemical classes in the medicinal herb.

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