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Extraction and clean-up methods for the determination of organochlorine pesticide residues in medicinal plants

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Abstract

The application of various extraction solvents with an Ultra-Turrax system and ultrasonication with a probe and solid-phase extraction (SPE) with octadecyl (C_{18})-bonded silica and Florisil cartridges to linden samples for the gas chromatography–electron capture detection (GC–ECD) of α-hexachlorocyclohexane (α-HCH), β-HCH, γ-HCH, hexachlorobenzene (HCB), heptachlor and its epoxide, *p,p'*DDE, *p,p'*DDD, *o,p'*DDT, *p,p'*DDT, α-endosulfan, β-endosulfan, endosulfan-sulfate, aldrin, dieldrin and endrin is described. Better results were obtained using ultrasonication with *n*-hexane and SPE Florisil cartridges. Limits of quantification ranged from 1 μg/kg for α-HCH to 10 μg/kg for endrin. An attempt to apply this methodology to other medicinal plants such as senna, common balm, german camomile, high mallow and orange flowers revealed that the SPE Florisil clean-up was not enough on its own and it was necessary to use 2 g of 3% deactivated Florisil.

Keywords: Pesticides; Hexachlorocyclohexane; Hexachlorobenzene; Heptachlor; DDE; DDD; DDT; Endosulfan; Aldrin; Dieldrin; Endrin

1. Introduction

Organochlorine pesticide residues still exist as pollutants in countries like Portugal, despite their prohibition seven years ago [1]. A lot of herb teas are imported from non-EU countries, escaping its legislation. In these cases we must consider that these plants are submitted to prohibited or undesirable products [2]. Moreover the organochlorine group still remains in the environment, some members depending on the oil content in the plant, while others are spread by vectors such as dust and rain particles. Therefore an investigation of these compounds in medicinal plants needs to be undertaken and this report presents the results of the study of the validation methods which present adequate accuracy and precision for sixteen organochlorine pesticides.

With regard to the establishment of maximum limits for pesticide residues in teas by Codex [3], we found only dicofol (5.0 mg/kg for dry product) and endosulfan-sum (30.0 mg/kg). In contrast with these levels, EEC legislation also addresses other residues for black tea and similar substances. Portuguese legislation [4] does not observe these particular values.

Few investigations have been undertaken and only a small number of analytical methodologies on pesticide residues in infusion plants exist.

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In the past decade we found some papers that describe the classic methodology in medicinal plants, solvent extraction and column adsorption chromatography [1,5], in black tea [6,7] or in its infusion [8]. These methods are often lengthy, labour intensive and costly.

More recently methodology such as supercritical fluid extraction (SFE) is described for the extraction of those compounds in camomile [9].

In spite of some use of solid-phase extraction (SPE) in vegetable products such as animal feeds [10] and raw crops [11] no comprehensive data is available on the clean-up of herb teas or black tea for the determination of organochlorine pesticides.

The purpose of this investigation was to find a method that permits quantitative, adequate, recoveries of sixteen organochlorine pesticides from infusion plants.

Several extraction procedures based on different extraction solvents using an Ultra-Turrax system or ultrasonication with a probe were tried.

The experimental methods were tested for the residues of insecticides such as hexachlorocyclohexane (HCH) isomers (α, β, γ), 1,1,1,-trichloro-2,2-bis(*p*-chlorophenyl) ethane (*p,p'*DDT), 1,1,1,-trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl) ethane (*o,p'*DDT), 1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethylene (*p,p'*DDE) and 1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethane (*p,p'*DDD), aldrin, dieldrin and endrin, heptachlor and its epoxide (HE), endosulfan (α, β and sulphate) and a fungicide, hexachlorobenzene (HCB).

2. Experimental

2.1. Reagents

Anhydrous sodium sulphate [granulated for residue analysis (Merck)]; *n*-hexane, acetonitrile, dichloromethane and ethyl ether (Carlo Erba, Milan, Italy) were of pesticide residue grade; water purified via Milli-Q (Millipore, Bedford, MA, USA); pesticide standards (Dr. Ehrenstorfer, Germany); elution solvents: *n*-hexane, ethyl ether–*n*-hexane (1:9), ethyl ether–*n*-hexane (5:5) and dichloromethane–*n*-hexane (85:15) were prepared daily. Florisil (60–100 mesh, Fluka Chemika, USA) was heated to 300°C in a furnace for 3 h, cooled in a desiccator prior to appropriate deactivation with water (3%) and used within the next 48 h.

A standard pesticide mixture consisting of a stock standard solution (approximately 500 mg/ml) of each pesticide was prepared separately in *n*-hexane, except β-HCH which was prepared in *n*-hexane–acetone (95:5) and γ-HCH and endosulfan-sulphate which was supplied at 10 ng/μl in cyclohexane. Standard work solutions (10 mg/ml) were prepared in *n*-hexane. Two fortification solutions were also prepared in *n*-hexane and their concentrations ranged between 50 ng and 1250 ng.

2.2. Materials and apparatus

A gas liquid chromatograph Carlo Erba Mega HRGC 5300 equipped with an ^{63}Ni electron capture detector was used. Two fused-silica capillary columns, 30 m×0.25 mm I.D., 0.25 μm, with chemically bonded phases DB-5 and DB-17 (J&W Scientific), were used. The first column was used for quantification and the second as a confirmation column. In both columns, a 1-μl sample was injected in the splitless mode and the splitter was opened after 60 s. Chromatographic conditions were 280°C for the detector, 220°C for the injector and 150°C held for 1 min and programmed at 10°C/min to 210°C, held for 1 min and programmed at 3°C/min to 230°C, held for 5 min and finally programmed at 3°C/min to 250°C, held for 3 min for the first column and 10 min for the second column. Gases used were: carrier gas helium N60 carrier at 2 ml/mim, split valve 100 ml/min, purge valve 2 ml/min, make-up gas, nitrogen at 120 kPa.

For quantification, a Spectra-Physics 4270 integrator was used, which compared peak areas in samples and standard solutions.

An Ultra-Turrax system from Ystral (West Germany) (velocity 6; 25–240 V) was also used.

A 100 W ultrasonic processor VC 100 from Vibra Cell-Sonic and Material (Danbury, CT, USA) with a standard probe of titanium alloy: TI, 6 AL, 4 V (6×108 mm), 117 V, 50/60 Hz was used under the following conditions: amplitude 100; output 32 W; timer 2 min and pulser 6 s. Samples were centrifuged using a Centrífuge Model Meditronic (Selecta) at 1000 *g* for 5 min. Other apparatus included a water

bath at ≅35°C and a nitrogen U for extract concentration. The vacuum system for SPE and the vacuum pump B-160 were from Vacobox Büchi (Switzerland); Si-C_{18} glass cartridges (6 ml) and Florisil glass cartridges (6 ml) were from J.T. Baker (Phillipsburg, NJ, USA). Centrifuge pyrex test tubes with PTFE-lined screw caps were from Schott (Germany); pyriform flasks (50 ml) and a rotary vacuum evaporator (Heidolph VV 2001) were also used. Glass columns (8×150 mm) filled with 2 g of 3% deactivated Florisil were used in each experiment.

2.3. Sample preparation

The dry sample was cut into very small pieces with scissors, ground into a fine powder with a mortar and pestle and homogenized.

2.4. Extraction methods

A 2.5-g amount of linden sample, weighed in a 50-ml cup was used for the different extraction methods. In method C, for orange flowers, common balm, camomile, high mallow and senna only 2.0 g was used.

The used extraction solvents were: method A_1, 12.5 ml acetonitrile + 12.5 ml *n*-hexane; method A_2, 1.875 ml water + 10.625 ml acetonitrile + 12.5 ml *n*-hexane; method A_3, 8.75 ml water + 16.25 ml acetonitrile; method A_4, 12.5 ml water + 12.5 ml acetonitrile; method B_1 = method A_4; method B_2 = method A3; and method C, 25 ml *n*-hexane.

In methods A_2, A_3 and A_4, following the addition of water the sample was allowed to stand for 15 min before addition of acetonitrile and/or *n*-hexane, according to the method used. Homogenize for 1 min with the Ultra-Turrax system for methods A. The sample was then placed in a pyrex centrifuge test tube. The cup and the Ultra-Turrax were washed with 5 ml ACN and the sample centrifuged at 1000 *g* for 5 min. In methods A_1 and A_2 the upper phase (*n*-hexane extract) and the lower phase (acetonitrile extract) of the supernatant were decanted into different graduated tubes and concentrated to 6 ml with the aid of a gentle stream of nitrogen at ≅35°C.

In methods B_1 and B_2, 2.5 g of linden was transferred to a centrifuge pyrex test tube and allowed to stand for 15 min after addition of water. Acetonitrile was added and the sample was sonicated with a probe according to the previously described conditions with the tube immersed in a ice/salt bath. The sample was centrifuged at 1000 *g* for 5 min. The acetonitrile extract was decanted to graduated tubes and concentrated to 6 ml.

In method C, different medicinal plants were placed in the centrifuge tube, *n*-hexane added and the sample allowed to stand for 15 min. The sample was sonicated as described in methods B_1 and B_2. The sample was centrifuged at 1000 *g* for 5 min and the *n*-hexane extract decanted into 50-ml pyriform flasks and concentrated to 6 or 2 ml in a rotary vacuum evaporator.

2.5. Recoveries

For recoveries, 1 ml of fortification solution was added to a sample of linden (*Tilia cordata* Mill.) and allowed to stand for 15 min before extraction, over five replications.

Method C was extended to five medicinal plants with a more generalized use in the Portuguese population: common balm (*Melissa officinalis* L.), German camomile (*Matricária chamomilla* L.), high mallow (*Malva sylvestris* L.), orange flowers (*Citrus aurantium* L.) and species of *Cassia* (senna) (*C. angustifolia* Vahl, *C. sennal*, *C. obavata* Colladon).

2.6. SPE clean-up

2.6.1. Methods A and B

For the acetonitrile extract the C_{18} cartridge was conditioned by washing with 10 ml acetonitrile and 10 ml water. The column was not allowed to dry after the final water wash; a layer of water was left on the column top. A 6.0-ml volume of the concentrated acetonitrile extract was transferred to the column. The vacuum was adjusted to elute the extract at <5 ml/min. The tubes were rinsed with 2×1 ml 25% acetonitrile–water and eluted through the column. The column was vacuum dried for 5 min and all the eluates were discarded. The sample was eluted with two different eluents, 2×5 ml *n*-hexane (E_1) and 2×5 ml ethyl ether–*n*-hexane (1:9) (E_2). E_1 was concentrated to 2 ml for quantification by HRGC–ECD and E_2 was concentrated to 6 ml. Ca. 1

cm of sodium sulfate was added to a Florisil column and washed with 2×5 ml *n*-hexane. The column was not allowed to dry. E_2 was placed on the column and allowed to elute through the column before being discarded. The Florisil column was then eluted with 3×5 ml ethyl ether–*n*-hexane (1:9) (E_{1a}) by gravity flow and the eluate concentrated to 1 ml for determination by GC–ECD.

For the *n*-hexane extract, the sample extract was concentrated to 6 ml with the aid of a stream of nitrogen at ≅35°C; 1 cm of sodium sulphate was added to a Florisil SPE cartridge and the column washed with 10 ml *n*-hexane without letting it dry. The concentrated *n*-hexane extract was transferred to the column and let flow by gravity. Three different eluents were used: E_{1b} 2×5 ml of *n*-hexane, E_{2b} 2×5 ml of ethyl ether–*n*-hexane (1:9) and E_{3b} 2×5 ml of ethyl ether–*n*-hexane (5:5). The eluates were collected in three different graduated centrifuge tubes and concentrated to 1 ml for quantification.

2.6.2. Method C

A clean-up procedure similar to that used for the *n*-hexane extract in methods A and B was used for linden; 1 cm of sodium sulphate was added to a Florisil SPE cartridge and the column washed with 10 ml *n*-hexane without letting it dry. The concentrated *n*-hexane extract was transferred to the column and let flow by gravity. Two different eluents were used: E_1 10 ml *n*-hexane and E_2 10 ml *n*-hexane–dichloromethane (85:15). The eluates were collected in two different graduated centrifuge tubes and concentrated to 1 ml for quantification.

For senna, orange flowers, camomile, common balm and high mallow adsorption chromatography columns (8×150 mm) filled with glass wool, 2 g of 3% deactivated Florisil and 1 cm of sodium sulphate were used. Eluents similar to those used to elute pesticide residues were used, but in greater quantity: 20 ml instead of 10 ml. The eluates were concentrated to 1 ml for GC.

3. Results and discussion

Sample extracts containing the organochlorine pesticide residues were analysed on a GC system with DB-5 and DB-17 columns. The first column separated the sixteen compounds, but in the second column *p,p'*DDD and *o,p'*DDT are coeluted.

In order to optimize the extraction conditions, we first tried, in method A_1, two different polarity solvents like acetonitrile and *n*-hexane (Fig. 1) because pesticides with different polarities are involved. The acetonitrile and hexane extracts were cleaned up with octadecyl bonded silica and Florisil SPE cartridges, respectively, because of the abundance in pigments that can raise problems to HRGC–ECD detection. Very low recoveries for compounds like α-HCH, γ-HCH, aldrin, heptachlor epoxide (HE), *p,p'*DDE, β-endosulfan, *p,p'*DDD and *o,p'*DDT were obtained with method A_1 (Table 1). Low recoveries for HCB, β-HCH, dieldrin, endrin, endosulfan-sulphate and *p,p'*DDT, varying from 49% for endrin to 66% for endosulfan-sulphate were obtained (Table 1). This method only offers good recoveries for heptachlor, distributed over the four eluates and for α-endosulfan which is eluted in eluates E_{1b} and E_{3b}. Besides these problems, the acetonitrile extracts of linden contain high proportions of water-soluble pigments observed by yellow colored eluates, originating from eluate 2 which raises problems in ECD detection. This eluate was

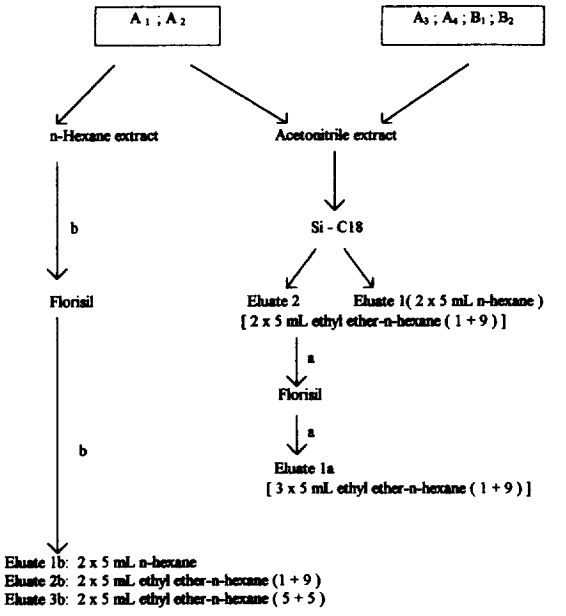

Fig. 1. Linden extracts purification scheme obtained from experimental methods A and B.

Table 1
Recovery [mean±R.S.D.(C.V.) (%) ($n=5$)] of sixteen organochlorine pesticides from fortified linden herb using methods A_1 and A_2

Compound	Concentration in sample (μg/kg)	A_1					A_2		
		C_{18}	Florisil				C_{18}	Florisil	
		E_1	E_{1a}	E_{1b}	E_{2b}	E_{3b}	E_1	E_{1a}	$E_{1b}+E_{2b}$
α-HCH	20	nd	nd	15±2 (16)	nd	nd	nd	nd	44±0 (1)
HCB	40	nd	nd	58±5 (8)	8±0 (5)	nd	1±0 (25)	nd	45±5 (12)
β-HCH	20	nd	nd	61±6 (9)			81±2 (2)	nd	35±1 (2)
γ-HCH	100	19±5 (24)	nd	7±2 (23)	nd	nd	108±16 (15)	nd	16±1 (9)
Heptachlor	100	44±2 (4)	nd	42±3 (7)	6±1 (12)	19±1 (6)	63±4 (6)	nd	22±1 (6)
Aldrin	200	9±1 (6)	nd	15±1 (5)	nd	3±2 (44)	13±1 (11)	nd	15±2 (17)
Heptachlor epoxide	80	nd	nd	12±0 (2)			nd	nd	46±1 (2)
α-Endosulfan	40	nd	nd	85±8 (9)	nd	26±2 (8)	nd	nd	37±2 (7)
*p,p'*DDE	500	8±2 (22)	nd	16±1 (3)			15±1 (7)	nd	10±1 (8)
Dieldrin	214.2	24±3 (11)	nd	39±3 (7)			36±3 (7)	nd	48±5 (10)
Endrin	200	18±2 (11)	nd	31±2 (7)			34±3 (10)	nd	45±2 (3)
β-Endosulfan	80	16±4 (23)	nd	nd	nd	nd	22±2 (11)	nd	23±4 (16)
*p,p'*DDD	500	6±1 (21)	nd	8±1 (7)			102±9 (9)	nd	9±1 (15)
*o,p'*DDT	500	12±3 (28)	nd	21±1 (4)			26±2 (6)	nd	18±3 (19)
Endosulfan-sulphate	80	nd	nd	nd	nd	66±2 (3)	nd	nd	nd
*p,p'*DDT	500	16±3 (18)	nd	37±4 (3)			44±4 (9)	nd	27±3 (10)

nd: Not detected.

cleaned-up with SPE Florisil cartridges. Chromatographic difficulties such as an unstable baseline and interferences in the zone of HCH isomers, HCB, heptachlor and its epoxide, are observed in Fig. 2. This phenomenon does not take place with the *n*-hexane extract.

The addition of water to other solvents to improve extraction efficiency is recognized as a procedure in pesticide residue studies [12–16]. Water proportions employed by different investigators vary between 5% and 50% [17]. Some of them have said that, at least 5% of water was found to be essential for good recoveries [13]. In dry products, removal of pesticides is a special problem because dehydratation results in greater concentration of plant material. That makes extraction of the pesticides more difficult than in high moisture products and raises problems in clean-up due to high concentrations of coextractives [12]. So, addition of water brings about the deactivation of the cellulose active sites, improving the extraction efficiency [16]. Consequently in methods A_2, A_3 and A_4 different water proportions were used, 7.5%, 35% and 50%, respectively, to improve the recoveries of more polar compounds.

Results obtained for method A_2 (Table 1) compared with method A_1 give good recoveries for β-HCH, γ-HCH, dieldrin, *p,p'*DDD and endrin. Better recoveries were obtained for α-HCH, HE and *p,p'*DDT, but for the two first compounds they were lower than 50% (44% and 46%, respectively). For *p,p'*DDT it was 71%. Recoveries remained good for heptachlor, identical for HCB, very low for aldrin, *p,p'*DDE and *o,p'*DDT, and lower for α-endosulfan. Endosulfan sulphate was not recovered in this method because the eluate E_{3b} was not experimented with. In method A_2 the extraction efficiency was better than A_1 essentially with respect to more polar organochlorine pesticides in study.

The use of 35% of water added to acetonitrile in method A_3 (Table 2) was expected to improve the recoveries of the more polar compounds, but this did not happen. A similar result was observed for method A_4 (Table 2) in which a 50% water proportion was used. In both methods, the only studied pesticide that presented admissible recoveries was endrin.

To verify the difference between the two extractive apparatus, Ultra-Turrax and ultrasonic processor with a probe, we tried two water proportions (50% and 35%) using methods B_1 and B_2 in comparison with methods A_4 and A_3 (Table 2). Relating method B_1 with A_4, better recoveries are

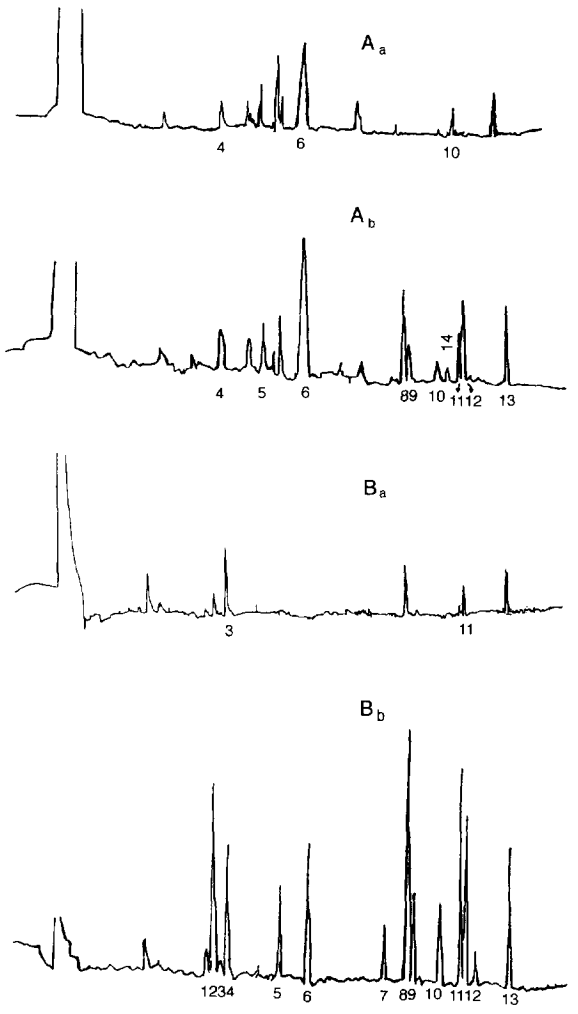

Fig. 2. Representative gas chromatograms (DB 5 column) of method A1: unspiked linden (A_a) and spiked linden (A_b) (acetonitrile extracts) and of method C: unspiked linden (B_a) and spiked linden (B_b) (*n*-hexane extracts). Peak identification: 1 = α-HCH; 2 = HCB; 3 = β-HCH; 4 = γ-HCH; 5 = heptachlor; 6 = aldrin; 7 = α-endosulfan; 8 = *p,p'*DDT; 9 = dieldrin; 10 = endrin; 11 = *p,p'*DDD; 12 = *o,p'*DDT; 13 = *p,p'*DDT; 14 = β-endosulfan.

obtained in method B_1 for HCB, β-HCH, α-endosulfan, dieldrin, endrin and β-endosulfan. However, for other pesticides, recoveries remain lower than in the A_4 method, like γ-HCH, heptachlor, aldrin, *p,p'*DDE, *p,p'*DDD, *o,p'*DDT and *p,p'*DDT and HE. Endosulfan-sulphate was not recovered. Correlating method B_2 with method A_3, the values obtained for recoveries with method B_2 are higher for α-HCH, heptachlor and its epoxide, aldrin, α-endosulfan, *p,p'*DDE, dieldrin, β-endosulfan, *p,p'*DDD, *o,p'*DDT, these values staying lower than 80% for HE, *p,p'*DDE, *p,p'*DDD and *o,p'*DDT.

Comparing methods B_1 and B_2 we can observe that better results are obtained with the second method, in which the water proportion used in acetonitrile was 35%.

Similar methodology was used for seafood products [18] and eggs [19] and the recoveries obtained for both extracts were good for all pesticides except for *p,p'*DDE in eggs (<80%) at 0.05 ppm spiked levels.

All these results for linden show a better extraction efficiency for ultrasonication with a probe than with the Ultra-Turrax system. We tried to improve recoveries by using *n*-hexane like an extraction solvent with ultrasonication and modifying the second eluent by replacing ethyl ether by dichloromethane: 2×5 ml of ethyl ether–*n*-hexane (1:9) [E_{2b}] by 10 ml *n*-hexane–dichloromethane (85:15) [E_2]. The recovery values obtained with method C and observed in Table 3 reveal that this method has adequate accuracy for most studied compounds. Heptachlor epoxide was not recovered in both eluates, and the recovery of β-endosulfan was 15%. Recoveries for β-HCH, endosulfan-sulphate and *o,p'*DDT, ranging from 50 to 70%, were 53%, 66% and 71%, respectively. Method C was extended to other different kind of medicinal plants, but in orange flowers, in spite of extracts being discolored, interferences appear in GC chromatograms. For senna and high mallow, the E_1+E_2 eluates from Florisil cartridges are intense green-yellow, and for common balm and camomile are yellow. The samples were reduced to 2 g, and 2 g of a 3% deactivated Florisil miniaturized column was employed to retain the cited pigments that interfere with ECD detection and quantification. Table 4 shows the recoveries of sixteen organochlorine pesticide residues. Recovery experiments at the same levels used for linden gave up to 75% recoveries for most of the compounds. For dieldrin and endrin those values are lower than 70% except for common balm. The β-endosulfan and endosulfan-sulphate were not recovered in all matrixes and heptachlor epoxide was not recovered in senna and orange flowers.

Limits of quantification for linden with method C

Table 2
Recovery [mean±R.S.D.(C.V.) (%) ($n=5$)] of sixteen organochlorine pesticides from fortified linden herb using methods A_3, A_4, B_1 and B_2

Compound	Concentration in sample (μg/kg)	A_3		A_4		B_1		B_2
		C_{18}	Florisil	C_{18}	Florisil	C_{18}	Florisil	C_{18}
		E_1	E_{1a}	E_1	E_{1a}	E_1	E_{1a}	E_1
α-HCH	20	29±8 (28)	nd	nd	nd	nd	nd	111±9 (8)
HCB	200	21±24 (11)	nd	5±1 (21)	nd	75±7 (10)	nd	65±3 (4)
β-HCH	20	nd	nd	nd	nd	36±3 (9)	nd	32±4 (10)
γ-HCH	60	37±4 (21)	nd	48±8 (16)	nd	26±4 (14)	nd	57±5 (6)
Heptachlor	100	32±3 (9)	nd	52±2 (4)	nd	25±3 (11)	nd	91±17 (18)
Aldrin	200	34±14 (42)	nd	16±3 (18)	nd	4±1 (20)	nd	112±11(10)
Heptachlor epoxide	100	24±3 (12)	nd	26±3 (11)	nd	nd	nd	40±6 (15)
α-Endosulfan	40	nd	nd	149±34 (23)	nd	100±12 (12)	nd	106±20 (19)
*p,p'*DDE	500	27±2 (7)	nd	33±4 (12)	nd	8±1 (18)	nd	51±5(9)
Dieldrin	100	41±3 (7)	nd	38±3 (9)	nd	75±4 (6)	nd	88±10 (12)
Endrin	200	91±10 (21)	nd	75±22 (15)	nd	106±7 (6)	nd	93±10 (11)
β-Endosulfan	80	18±2 (13)	nd	45±5 (12)	nd	92±8 (9)	nd	94±10 (10)
*p,p'*DDD	500	40±2 (4)	nd	27±3 (12)	nd	16±3 (20)	nd	61±4 (6)
*o,p'*DDT	500	34±3 (10)	nd	39±4 (9)	nd	12±2 (13)	nd	78±5 (7)
Endosulfan-sulphate	80	nd	nd	nd	nd	nd	nd	nd
*p,p'*DDT	500	39±7 (18)	nd	50±3 (7)	nd	20.±4 (19)	nd	23±2 (8)

nd: Not detected.

ranged from 1 μg/kg for α-HCH to 10 μg/kg for endrin.

To verify the recoveries of sixteen pesticide residues in study without interferences of the matrix, a study was conducted adding 1 ml of fortification solution in *n*-hexane at the two different columns used in this study, 2 g of 3% deactivated Florisil and 1 g Florisil SPE cartridges. The elution was con-

Table 3
Recovery [mean±R.S.D.(C.V.) (%) ($n=5$)] of sixteen organochlorine pesticides from fortified linden herb using method C

Compound	Concentration in sample (μg/kg)	C	
		Florisil	
		E_1	E_2
α-HCH	20	90±1 (1)	nd
HCB	200	98±3 (3)	nd
β-HCH	20	53±4 (7)	nd
γ-HCH	60	121±5 (5)	nd
Heptachlor	100	112±3 (2)	nd
Aldrin	200	70±10 (15)	31±6 (20)
Heptachlor epoxide	100	nd	nd
α-Endosulfan	40	110±24 (22)	nd
*p,p'*DDE	500	84±10 (12)	nd
Dieldrin	100	82±8 (10)	12±0 (3)
Endrin	200	89±14 (16)	nd
β-Endosulfan	80	nd	15±4 (5)
*p,p'*DDD	500	95±3 (3)	nd
*o,p'*DDT	500	71±10 (14)	nd
Endosulfan-sulphate	80	nd	66±11 (11)
*p,p'*DDT	500	88±8 (13)	nd

nd: Not detected.

Table 4
Recovery [mean±R.S.D.(C.V.) (%) ($n=5$)] of sixteen organochlorine pesticides added to different kinds of medicinal plants

Compound	Type of medicinal plants				
	Senna	Common balm	German camomile	High mallow	Orange flowers
α-HCH	75±10 (14)	87±9 (16)	95±16 (17)	93±17 (19)	96±15 (16)
HCB	92±16 (17)	108±15 (14)	97±12 (13)	79±4 (5)	82±0 (0)
β-HCH	94±9 (18)	98±3 (3)	82±14 (17)	82±1 (1)	90±7 (7)
γ-HCH	82±6 (14)	103±7 (7)	84±4 (4)	84±4 (6)	83±3 (8)
Heptachlor	84±18(19)	96±4 (4)	85±13 (15)	97±7 (8)	77±9 (12)
Aldrin	88±3 (12)	118±5 (4)	88±6 (7)	87±0 (0)	101±21 (21)
Heptachlor epoxide	nd	100±6 (6)	75±4 (5)	89±19 (21)	nd
α-Endosulfan	90±5 (8)	103±10 (9)	97±9 (9)	90±5 (5)	96±12 (12)
*p,p'*DDE	76±10 (21)	113±8 (7)	89±9 (10)	89±2 (2)	87±4 (5)
Dieldrin	58±5 (18)	87±9 (17)	35±2 (4)	49±5 (10)	53±2 (4)
Endrin	56±8 (26)	75±5 (6)	20±1 (5)	42±5 (11)	41±13 (23)
β-Endosulfan	nd	nd	nd	nd	nd
*p,p'*DDD	80±9 (11)	110±11 (10)	79±2 (2)	89±7 (8)	84±4 (10)
*o,p'*DDT	82±7 (15)	91±4 (4)	79±2 (3)	83±7 (9)	86±7 (16)
Endosulfan-sulphate	nd	nd	12±2 (13)	nd	nd
*p,p'*DDT	82±10 (22)	86±2 (2)	80±7 (8)	82±4 (5)	82±2 (4)

nd: Not detected.

ducted, in the first case, according to method C for camomile, common balm, orange flowers and high mallow, and according to E_1 and E_2 of method C for linden, adding E_3: 10 ml *n*-hexane–dichloromethane (50:50) when the second column was used. Results, observed in Table 5, are identical to those observed

Table 5
Recovery [mean±R.S.D.(C.V.) (%) ($n=5$)] of sixteen organochlorine pesticides from different Florisil columns

Compound	Concentration in sample (μg kg^{-1})	2 g of 3% deactivated Florisil	1 g Florisil SPE cartridges	
		$E_1^a+E_2^b$	$E_1^c+E_2^d$	E_3^e
α-HCH	20	90±8 (5)	89±10 (10)	nd
HCB	200	87±10 (11)	77±9 (7)	nd
β-HCH	20	45±5 (6)	57±13 (13)	nd
γ-HCH	60	80±5 (6)	90±6 (6)	nd
Heptachlor	100	87±4 (5)	90±10 (10)	nd
Aldrin	200	92±10 (10)	73±4 (6)	nd
Heptachlor epoxide	100	70±9 (10)	nd	nd
α-Endosulfan	40	94±13 (13)	101±7 (9)	nd
*p,p'*DDE	500	85±9 (10)	93±6 (8)	nd
Dieldrin	100	77±7 (7)	88±9 (11)	nd
Endrin	200	90±12 (13)	88±12 (13)	nd
β-Endosulfan	80	3±0 (0)	11±3 (4)	4±1 (2)
*p,p'*DDD	500	74±12 (12)	89±7 (8)	nd
*o,p'*DDT	500	77±7 (9)	81±6 (7)	nd
Endosulfan-sulphate	80	27±3 (4)	61±6 (6)	54±7 (8)
*p,p'*DDT	500	90±8 (9)	100±10 (12)	nd

[a] 20 ml *n*-hexane.
[b] 20 ml *n*-hexane–dichloromethane (85:15).
[c] 2×5 ml *n*-hexane.
[d] 2×5 ml *n*-hexane–dichloromethane (85:15).
[e] 2×5 ml *n*-hexane–dichloromethane (50:50).
nd: Not detected.

in Tables 4 and 3 when the first and the second column were used, respectively.

4. Conclusions

The proposed method C and GC procedures described are suitable for multi-residue screening of linden for fourteen organochlorine residues.

Method C is more rapid than methods A and B, and shows us that ultrasonication gives better results than the Ultra-Turrax system.

As with most SPE methods, this method requires small quantities of solvents per sample, 53.5 ml of *n*-hexane (10 ml for cartridge activation, 25 ml for extraction and 18.5 ml for elution) and 1.5 ml of dichloromethane.

For other medicinal plants, the sample size was reduced from 2.5 g to 2 g, the employed adsorbent was 2 g of 3% deactivated Florisil and the solvent elution volume was doubled from 10 ml to 20 ml.

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