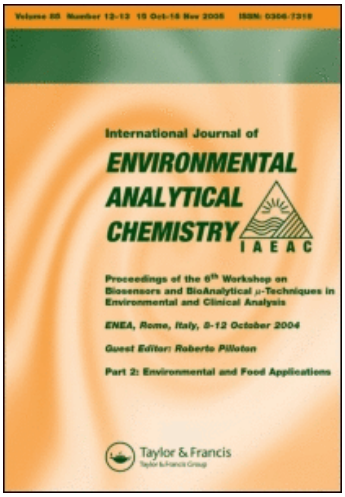

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GC-MS DETERMINATION OF ORGANOCHLORINE PESTICIDES IN FIVE MEDICINAL PLANTS

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Capilllary gas chromatography-mass spectrometry (GC-MS) in the electron impact (EI) mode and using selected ion monitoring (SIM) has been used to determine the main organochlorine pesticides in five commonly used medicinal plants (mint, vervain, camomile, lime tree and tea). Solid-phase extraction (SPE) is proposed for the treatment of these medicinal herbs. Validation of this step using factorial discriminant analysis (FDA) has shown that these plants can not be considered as one homogeneous group with regard to the extraction procedure. Consequently, two procedures have been designed. In each procedure, the plant is infused prior to SPE. Elution is achieved with n-hexane-dichloromethane (85:15, v/v). If necessary, the organic extract is treated with trifluoroacetic acid (TFA) to reduce matrix interferences.

KEY WORDS: GC-MS, organochlorine pesticides, SPE, medicinal plants, factorial discriminant analysis.

INTRODUCTION

The increasing risks to human health, generated by the widespread use of pesticides in our environment is well established[1-4]. In consequence, their determination in water, plants, soils, foodstuffs, etc. is of major importance. Numerous analytical methods have been proposed to reach this goal. These include gas chromatography (GC) coupled with ECD

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(electron capture) or NPD (nitrogen-phosphorus) detection for organochlorine and organophosphorus compounds[5–7], respectively. Pesticides of higher polarity, such as carbamates, are usually determined by high-performance liquid chromotography (HPLC)[8]. More recently, supercritical fluid chromatography (SFC) was proposed[9], which can also be used for organochlorine[10] and organophosphorus[11] compounds. Our interest was focused on the determination of organochlorine pesticides in medicinal herbs because of the large use of plants at home and even in hospital with the new trend (or fashion) of 'soft medicine' in many countries. Only a few studies were published in this area of pesticide research[12,13]. This is partly due to the complexity of the matrix (essential oils, resins, tannins, chlorophylls and mucilages). Liquid/liquid (L/L) extraction of pesticides is therefore tedious and in most cases not too rewarding, with extracts heavily contaminated by numerous endogenous compounds. Actually, the identification and quantification of organochlorine pesticides in this material is difficult, even when using high-resolution capillary gas-chromatography (HRGC) combined with ECD detection[14]. Some papers recommend chemical pretreatment to reduce matrix interferences whatever the chromatographic procedure chosen[15,16].

This paper describes a procedure based on solid-phase extraction (SPE) which economizes solvent use and time. The procedure includes a simple new chemical treatment with trifluoroacetic acid (TFA) in order to diminish matrix interferences. GC-MS (EI-SIM mode) was selected for obvious reasons to obtain sufficient selectivity and sensitivity[17–19].

MATERIAL AND METHODS

Chemicals

Aldrin, purity 98%; dieldrin, purity 99%; endrin, purity 95%; heptachlor, purity 99%; heptachlor epoxide, purity 99%; and hexachlorobenzene (internal standard) (HCB), purity 99%; were purchased from Promochem (Wesel, Germany); p, p'-DDD, purity 99% and p,p'-DDT, purity 99%, were from Aldrich, (Deisenhofen, Germany); p,p'-DDT, purity 99.6%; and endrin ketone, purity 96%; from Chem Service (West Chester, PA, USA); α-endosulfan, purity 96%, β-endosulfan, purity 96%, endrin aldehyde, purity 96%, α-hexachlorocyclohexane (HCH), purity 96%, β-HCH, purity 96%, γ-HCH (Lindane), purity 96%, δ-HCH, purity 96%, and methoxychlor, purity 96%, were from Supelco (Houston, TX, USA).

All solvents were of analytical grade (Merck, Darmstadt, Germany) and carefully checked under the standard GC-MS conditions used. Trifluoroacetic acid (TFA) for spectroscopy, and trichloroacetic acid (crystal extrapure), sodium hydroxide, sodium hydrogen carbonate, sodium bisulphite and anhydrous sodium sulphate were purchased from Merck. Sodium hypochlorite solution was prepared according to the U.S. Pharmacopoeia (USP XXII, p 1261).

Commercial trademark bags of medicinal plants were tested for the evaluation of the proposed method.

Material

Extraction devices C_8, C_{18} Spe-ed™ mini columns (0.5 g of support/6 ml were from Applied Separations (Bethelehem, PA, USA). All the columns were used on an ACCUBOND™ vacuum manifold from J & W Scientific (Folsom, CA, USA).

A Hewlett Packard gas chromatograph (HP 5890) equipped with a HP-5 fused silica capillary column (25 m × 0.22 mm id, coated with 0.33 μm of 5 % phenylmethylsilicone stationary phase) and a split-splitless injection port, was coupled with a HP 5970 mass selective ion detector. Mass spectra were analysed with a HP 59970 MS-Chem Station with a HP 59973 NBS mass spectral library.

The split-splitless injection port temperature was used only in the splitless mode (0.7 min), and set at 270°C; the column was flushed by helium (Ultrapur™; Air Liquide, Paris, France) at a flow rate of 3.5 ml/min. The oven temperature was programmed as follows: 45°C (0.8 min) increased up to 180°C at 30°C/min, the plateau held at 2 min and then increased at 5°C/min, the plateau held 2 min and then increased at 5°C/min to a final temperature of 260°C (7 min plateau); the temperatures of the transfer line and the MS source were 260°C and 200°C, respectively. The electron impact energy was set at 70 eV, the dwell time was 30 ms, and selected ion monitoring (SIM) performed.

Methods

Extraction procedure The extraction procedure starts with the infusion of the raw vegetal material followed by a SPE process.

Statistical analysis (see below) and chemical differences between the medicinal herbs[20] prompted us to propose two different procedures, i.e., Procedure I (mucilage-free plants) for mint, vervain and tea, and Procedure II (plants containing mucilages) for camomile and lime-tree.

Procedure I. Infusions of the mentioned plants were prepared after weighing 5 g of commercial finely powdered herb into an appropriate beaker. Infusion was done with 200 ml of boiling deionized water; after cooling, 10 ml of acetone were added. This solution was passed through a C_8 SPE extraction column previously activated with 5 ml of n-hexane and 5 ml of methanol and then with 5 ml of deionized water.

Prior to extraction, the residue on the SPE column was washed with 10 ml of deionized water. The pesticides were eluted with 3 ml of hexane-CH_2Cl_2 (85:15, v/v). The organic extract was reduced to exactly 0.5 ml under a gentle stream of nitrogen at 45°C (complete evaporation of solvent must be avoided). This solution was finally spiked with 25 μl of a n-hexane solution of HCB (50 μg/ml) used as an internal standard; 3 μl of this solution were injected in the GC-MS.

Procedure II. Procedure II is similar to Procedure I, except for the addition of 1 g of $NaHCO_3$ and 0.5 g of cysteine instead of an addition of 10 ml of acetone.

With both procedures, an additional specific purification step can be added. Three purification procedures were evaluated, the TFA treatment (water-soluble endogenous

compounds eliminated by coprecipitation, and selective acidic destruction of some organic material) being finally selected.

TFA treatment The organic extract from SPE (3 ml)is mixed with 3 × 300 µl of TFA and vortexed each time for 1 min, and the aqueous layer discarded. If the aqueous layer does not appear (depending on the nature and origin of the plant), 50 µl of deionized water are added and the same process as described above is followed. In both cases, washing with 0.5 ml of 0.1 M NaOH is performed to neutralize the excess of TFA. The organic phase is concentrated to 500 µl before adding the internal standard as mentioned in Procedure I.

TCA treatment (specific destruction of water-soluble endogenous compounds). The TCA reagent is prepared with 1 g of trichloroacetic acid in 10 ml of 1 M NaOH. The procedure is similar to the TFA treatment except that 3 × 300 µl of TCA instead of TFA, reagent, is used.

Chlorine oxidation (selective oxidation of endogenous compounds). 1 ml of sodium hypochlorite solution is added to the 3 ml of organic extract and vortexed for 2 min. The aqueous layer is discarded and the remaining organic phase washed with 0.5 ml of 30 % (w/v) $NaHSO_3$ to eliminate the excess of chlorine. Then, concentration of the organic phase to 500 µl is performed as described above.

Statistics

Recovery studies[21]. The recovery (R_i %) of each pesticide (i) is calculated according to the following equation:

$$\% R_i = \frac{A^i \pm \sigma^i}{A_s^i \pm \sigma_s^i} \cdot 100 \quad (1)$$

where A^i is the area of (i) in the sample, A_s^i is the area of (i) in the standard solution, and σ_s^i and σ_s^i are the standard deviations of (i) in the population sample and standard, respectively.

Because of the propogation of errors, the relative standard deviation of the recovery of (i) (SR_i/R_i) is defined by

$$RSD = \frac{S_{R_i}}{R_i} = \sqrt{\frac{1}{n}\left(\frac{S_i}{A_i}\right) + \frac{1}{n'}\left(\frac{S_s^i}{A_s^i}\right)} \quad (2)$$

with n = 3 (triplicate analysis), n' = 8 (number of injections of standard solution), whole Si and S_s^i are the estimated standard deviations in the sample and the standard respectively. Finally, % RSD = SR_i/R_i . 100.

Factorial discriminant analysis (FDA) and One-way analysis of variance (ANOVA) were

performed by using a PC IBM compatible STAT ICTF™ software. For FDA, only the first plane (defined by the axes 1 and 2) was used in order to interpret the data.

RESULTS AND DISCUSSION

Analytical characteristics

Figure 1A shows a typical TIC (total ion chromatogram) of the pesticides (1 µl/ml each; spiked vervain infusion). The repeatability (expressed as RSD, n = 6) of the retention time of each compound is better than 0.5%. The detector response was linear from the limits of detection up to 0.20 µg of injected compound: all 17 calibration curves exhibited a linear correlation coefficient (r) better than 0.999. The concentration range nicely agrees with the level of organochlorine pesticides found in medicinal herbs[22–24].

Complying with the official threshold levels of organochlorine pesticides found in medicinal plants[25] (< 5 ppb) is obviously one of the main aim of our study. The instrumental limit of detection (LOD) (3 SD of the background signal[26]; in pg injected) for each compound is included in the legend of Figure 1.

The use of a Ross injector (Chrompack®) was studied, but abandoned because of the less good repeatability (RSD > 7 % for all pesticides (n = 6)) compared with the splitless device (RSD < 7 % for 11 pesticides (n = 6)).

Extraction procedure

Infusion is not the most efficient process for extracting pesticides from plants[27]. Despite this, infusion was chosen as the preliminary step of the method because it corresponds to the usual way of intake of the plants studied.

Choice of the SPE support. In order to evaluate the importance of the nature of the stationary phase, two types of reversed phase (RP) supports (C_8 and C_{18}) were tested with hexane-dichloromethane (85:15, v/v) as eluent.

No statistically significant difference was found between the two RP supports in the recoveries of the pesticides. The C_8 support was chosen because it provides of a cleaner chromatographic profile. As an example, Table 1 reports the calculated recoveries for the tested pesticides extracted from mint infusion with the two supports.

SPE eluting system. Recovery studies were carried out by spiking infusions of the five medicinal plants (vervain, camomile, tea, mint and lime-tree) with 0.5 ml of the pesticide standard mixture (1 µg/ml of each pesticide in ethyl acetate).

Three elution systems selected on the basis of literature data (hexane, light petroleum and n-hexane-dichloromethane (85:15, v/v)[5–7,28] were tested. n-hexane-dichloromethane was preferred because it gave better recoveries for β-endosulfan, endrin aldehyde and endrin

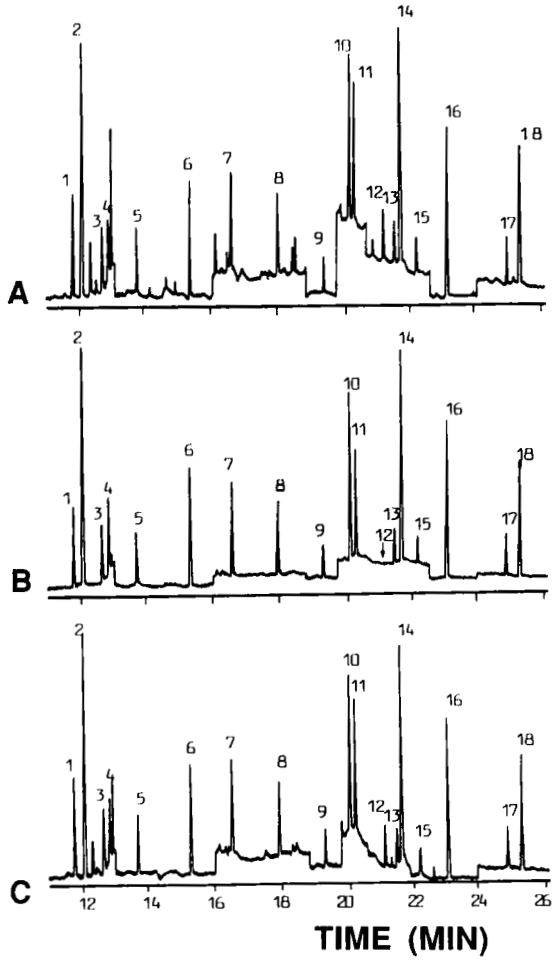

Figure 1 Typical total ion chromatogram with selected ion monitoring (SIM) in m/z and LOD values in pg injected of a spiked infusion of vervain; without ultimate purifications step of the organic extract (A), with TFA procedure (B), and with TCA procedure (C). Chromatographic conditions: see text. 1:α-HCH (m/z = 181, 183; LOD = 30 pg), 2: HCB (*internal standard*: m/z = 284, 3: β-HCH (m/z = 181, 183; LOD = 30 pg), 4: γ-HCH (m/z = 181, 183; LOD = 30 pg), 5: δ-HCH (m/z = 181, 183; LOD = 30 pg), 6: heptachlor (m/z = 100, 272, 274; LOD = 25 pg), 7: aldrin (m/z = 66, 79, 265; LOD = 30 pg), 8: heptachlor epoxide (m/z = 81, 353, 355; LOD = 25 pg), 9: α-endosulfan (m/z = 195, 207, 241; LOD = 100 pg), 10: p,p'-DDE (m/z = 246, 248, 318; LOD = 30 pg), 11: dieldrin (m/z = 79, 81, 263; LOD = 30 pg), 12: endrin (m/z = 81, 263; LOD = 100 pg), 13: β-endosulfan (m/z = 159, 195, 235, 237; LOD = 100 pg), 14: p,p'-DDD (m/z = 235, 237; LOD = 30 pg), 15: endrin aldehyde (m/z = 67, 250; LOD = 70 pg), 16: p,p'-DDT (m/z = 235, 237; LOD = 20 pg), 17: endrin ketone (m/z = 67, 227, 317; LOD = 80 pg), 18: methoxychlor (m/z = 227; LOD = 60 pg).

Table 1 Calculated recovery (n=3) of each pesticide on a spiked mint infusion extracted using C_{18} and C_8 cartridges (each pesticide: 1 $\mu g.ml^{-1}$ added) without purification step.

Pesticide	*Spe extraction on c_8 column*	*SPE extraction on C_{18} column*
	R_i (%) ± RSD (%)	*R_i (%) ± RSD (%)*
α-HCH	85 ± 11	88 ± 11
β-HCH	92 ± 6	90 ± 13
γ-HCH	86 ± 9	86 ± 11
δ-HCH	100 ± 3	97 ± 13
Heptachlor	74 ± 6	74 ± 10
Aldrin	68 ± 3	66 ± 9
Heptachlor epoxide	87 ± 4	81 ± 10
α-Endosulfan	91 ± 5	83 ± 10
p,p'-DDE	79 ± 6	73 ± 11
Dieldrin	87 ± 7	79 ± 10
Endrin	88 ± 20	79 ± 16
β-Endosulfan	92 ± 3	86 ± 7
p,p'-DDD	98 ± 7	84 ± 8
Endrin aldehyde	72 ± 11	62 ± 8
p,p'-DDT	85 ± 7	74 ± 13
Endrin ketone	115 ± 16	96 ± 16
Methoxychlor	114 ± 10	95 ± 11

Table 2 Calculated recovery (Ri (%) + RSD (%); n = 3) of each pesticide with three elution systems (tea infusion with C_8 SPE column).

Pesticide	*C_8 Hexane*	*C_8 Light petoleum*	*C_8 Hex/CH_2Cl_2 (85:15)*
α-HCH	90 ± 3	92 ± 4	96 ± 5
β-HCH	86 ± 4	86 ± 5	98 ± 5
γ-HCH	91 ± 3	92 ± 4	99 ± 6
δ-HCH	43 ± 8	39 ± 7	105 ± 8
Heptachlor	72 ± 9	66 ± 7	77 ± 15
Aldrin	53 ± 6	52 ± 7	51 ± 4
Heptachlor epoxide	94 ± 6	98 ± 7	100 ± 8
α-Endosulfan	81 ± 4	91 ± 4	92 ± 7
p,p'-DDE	38 ± 5	45 ± 6	44 ± 8
Dieldrin	83 ± 6	91 ± 8	92 ± 9
Endrin	97 ± 6	101 ± 5	98 ± 14
β-Endosulfan	31 ± 4	30 ± 9	96 ± 10
p,p'-DDD	57 ± 27	74 ± 6	78 ± 9
Endrin aldehyde	6 ± 32	8 ± 18	83 ± 11
p, p'-DDT	47 ± 12	48 ± 15	60 ± 19
Endrin ketone	22 ± 8	23 ± 21	115 ± 14
Methoxychlor	96 ± 7	88 ± 10	131 ± 24

Table 3 Effect of added acetone (in % v/v) on pesticide recovery (Ri (%) + RSD (%); n = 3) from a water spiked sample using C_8 cartridges.

Pesticide	*25 % added acetone*	*5 % added acetone*	*100 % water*
α-HCH	75 ± 11	90 ± 5	84 ± 6
β-HCH	78 ± 12	89 ± 6	87 ± 6
γ-HCH	75 ± 11	71 ± 9	85 ± 9
δ-HCH	78 ± 10	87 ± 6	90 ± 7
Heptachlor	83 ± 7	76 ± 6	75 ± 10
Aldrin	86 ± 9	70 ± 7	67 ± 10
Heptachlor epoxide	82 ± 9	89 ± 5	87 ± 9
α Endosulfan	84 ± 7	91 ± 3	88 ± 5
p,p'-DDE	95 ± 9	76 ± 5	76 ± 8
Dieldrin	82 ± 6	91 ± 4	86 ± 5
Endrin	85 ± 8	94 ± 3	90 ± 7
β-Endosulfan	86 ± 10	96 ± 6	85 ± 8
p,p'-DDD	91 ± 6	102 ± 7	74 ± 7
Endrin aldehyde	82 ± 8	88 ± 6	90 ± 5
p,p'-DDT	88 ± 7	71 ± 6	76 ± 8
Endrin ketone	88 ± 11	101 ± 6	94 ± 4
Methoxychlor	81 ± 10	94 ± 4	90 ± 6

ketone, as is shown in Table 2 for tea infusion as an example. Similar results were found for camomile, mint, vervain and lime-tree infusions. These SPE results are as good as the data reported for liquid/liquid extraction procedures of HCH isomers and DDT performed on medicinal herbs[15].

The influence of the acetone added to the infusion on the SPE process was evaluated because of a literature report[21] which demonstrated that a polar organic solvent can significantly improve the recovery of pesticides from water. In our case acetone was used instead of methanol (data not reported) because of the lower viscosity of the acetone-water mixture[29].

From the data reported in Table 3 two advantages can be drawn from the addition of 5 % (v/v) acetone: a better precision and an improved recovery for heptachlor, aldrin and β-endosulfan and for p,p'-DDD. The use of 25 % (v/v) acetone has a negative effect on analyte recoveries and precision, except for p,p'-DDE of which the extraction increased. All pesticides are lost when pure acetone is used. Finally, 5 % (v/v) of acetone was added to the infusions to obtain a more rapid, exact and reproducible SPE process.

Matrix effects. As displayed in the factorial discriminant analysis (Figure 2), the matrix distinctly influences the recoveries as shown by the large dispersion of the data along the two main axes, 1 and 2. Moreover, it is shown that the gravity centers (G) of the C_8 and C_{18} extraction procedures are, for the same infusion, relatively close to each other. The latter point strongly suggests the similarity of the two SPE processes performed with C8 and C18 columns.

However, when applying the above procedure to camomile and lime-tree, a large increase in duration of the SPE extraction process was observed. This was attributed to the high

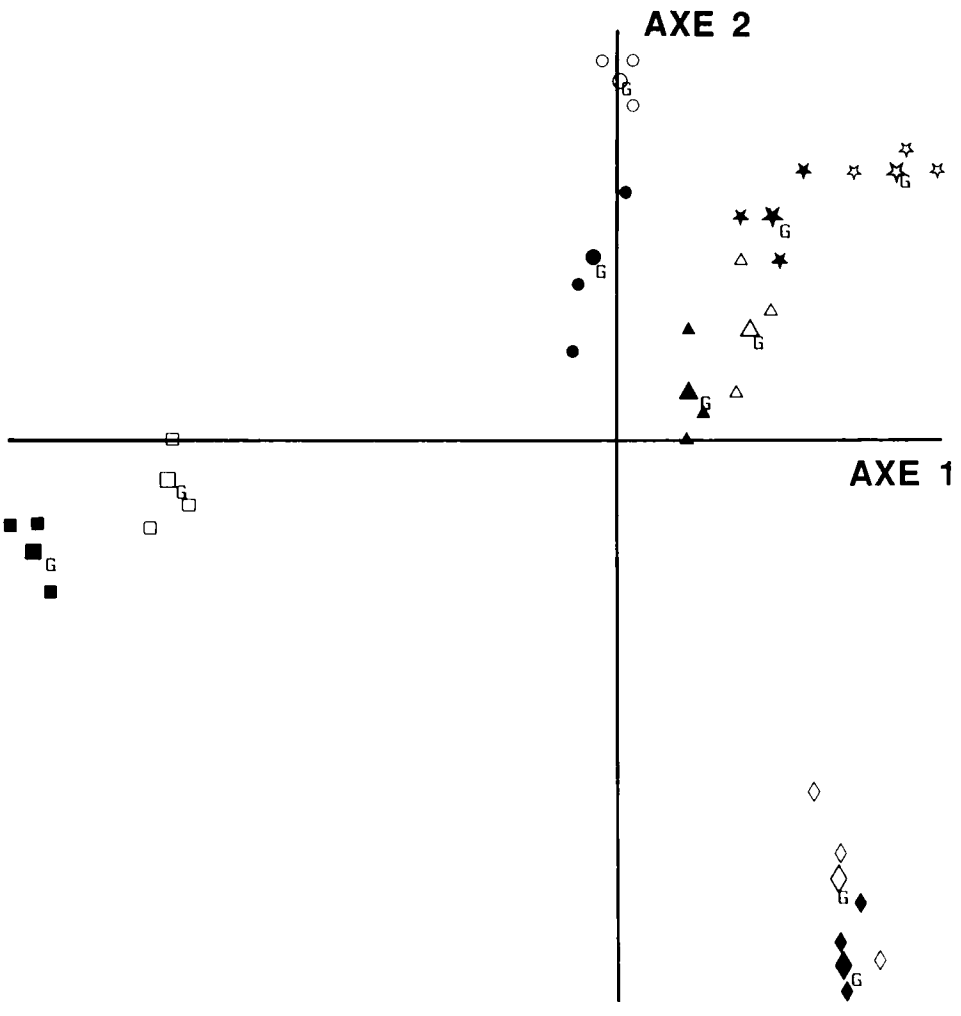

Figure 2 Factorial discriminant analysis of the calculated recoveries as a function of the matrix and the SPE system. Balck symbols refer to the C_8 cartridge, open symbols to the C_{18} cartridge. △, ▲: mint, □, ■: vervain, ○, ●: tea, ◊, ♦: camomile, ☆, ★: lime. The subscripts G refer to the gravity center of each group of extracts (C_8 or C_{18}).

mucilage content of these two plants. To overcome this drawback, strong reductors such as sulphide and thiol-type reagents were added to the infusion. Cysteine was finally selected because its more efficient reduction power on the mucus-like macromolecules[30]. This led to the separate Procedures I and II outlined above. An indirect consequence of the addition is a decrease of the pH of aqueous infusion (down to pH 3). Some papers underline the influence of the pH on the SPE recovery of organophosphorus pesticides, triazine herbicides and polychlorinated biphenyls[7,31-32]. The equilibrium between components of the sample matrix,

analytes and sorbents can be altered by extreme pH values[33]. To avoid matrix-dependent variations in Procedure II, the pH was adjusted by adding sodium hydrogen carbonate.

Ultimate SPE organic extract purification. As seen in Figure 1A which shows a typical SIM chromatogram of a spiked infusion of vervain, numerous small peaks due to endogenous compounds are eluted with the pesticides of interest. This is common although, at a lower level—for all plant extracts obtained using this method. In order to eliminate these disturbing peaks and reduce the noisy background, liquid/liquid purification of the SPE extract was performed.

The liquid/liquid procedure was chosen because of its simplicity and reliability without significant loss of pesticides. As an example, the average yield of HCH isomers without TFA treatment is 91 % (see Table I) in mint extract and 89 % upon TFA treatment, which can be considered a non-significant difference. The same kind of result was observed with all the plants studied for all the pesticides except for endrin and endrin aldehyde whose yield are 0% and 45%, respectively after TFA treatment. The TFA procedure (see Material and Methods) was finally selected because of its high efficiency (Figure 1B) in comparison with the absence of a purification step (Figure 1A) and with TCA treatment (Figure 1C). The major drawback of the purification step was the complete loss of endrin and the partial destruction (55%) of endrin aldehyde. The ability of strong acids as sulphuric acid to destroy endrin is well established[34–35]. Finally, as already mentioned, in all cases, the purified organic extract must be reduced to 0.5 ml for sufficient sensitivity. However, reduction to dryness must be carefully avoided to prevent loss of pesticides such as HCH derivatives[33] by volatilization.

CONCLUSION

The proposed method includes some advantages owing to the use of SPE such as saving of time and solvents and the use of highly selective and sensitive EI-SIM MS detection. It allows LODs as low as 30 pg injected for most of the 16 pesticides studied, which complies with the threshold levels for medicinal herbs. The response is linear over three decades for all compounds, the repeatability is sufficient (RSD < 7 %) for precise determination at the trace level.

The SPE extraction of organochlorine pesticides from tea, vervain and mint infusions using 5 % acetone improves recovery, repeatability and speed (Procedure I). The high viscosity of infusions of camomile and lime-tree, caused by their mucilage content, is a severe drawback when performing SPE. This can be avoided by the addition of cysteine to the infusions (Procedure II).

Finally, the complete analytical procedure including (1) the use of 5 % acetone to mint, vervain and tea infusion, or cysteine in case of chamomile and lime tree, (2) SPE with hexane-dichloromethane (85:15, v/v) elution, and (3) TFA treatment, provides convenient results for all five medicinal herbs.

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