Bull Environ Contam Toxicol (2008) 81:604–607
DOI 10.1007/s00128-008-9545-z

Multiple Residue Extraction for Organochlorine Pesticides in Medicinal Plants

P. C. Abhilash · Nandita Singh

Received: 5 June 2008 / Accepted: 26 August 2008 / Published online: 5 September 2008

Abstract A rapid, multiresidue, multimatrix analytical method for the determination of aldrin, endrin, dieldrin and hexachlorocyclohexane isomers (α-, β-, γ- and δ-HCH) residues in medicinal plants has been developed. Samples were extracted by matrix solid-phase dispersion (MSPD) followed by gas chromatographic-electron-capture detection (GC-^{63}Ni-ECD). The validation of the proposed approach was carried out by comparison with the European Pharmacopoeia reference extraction method obtaining similar or even better efficiencies by the proposed approach.

Keywords Organochlorine pesticides · Extraction method · Matrix solid-phase dispersion extraction · European Pharmacopoeia method

Scientific advances in the use of medicinal plants and phytomedicines have led to the need for more accurate, faster and more sensitive analytical methods for their quality analysis and quality assurance (Zuin and Vilegas 2000). Phytomedicines have been used in medical practice for thousands of years and recognized especially as a valuable and readily available resource for health care in developing countries (Tewary et al. 2004). World Health Organization report indicated that about 70–80% of the world population rely on non-conventional medicines mainly of herbal sources in their primary health care (Akerele 1993). Traditionally herbs and herbal products have been considered to be gentle, non-toxic and even harmless mainly because of their natural origin. Like other crops, medicinal plants are susceptible to insect and disease attacks both in field and storage, so pesticides are widely used for their protection. Contamination of crude medicinal plants as well as their products or preparations by pesticides has brought fears regarding the quality and safety of the phytomedicines available in the market. (Tewary et al. 2004).

Millions of Indian rural households use medicinal plants in a self-help mode. Over one and a half million practitioners of the Indian System of Medicine in the oral and codified streams use medicinal plants in preventive, promotive and curative applications. There are estimated to be over 7,800 manufacturing units in India. Phytomedicinal safety analysis is essential for quality assurance (WHO 1992). Phytomedicinal safety assessments necessarily involve the development of adequate analytical procedures to determine endogenous toxic compounds in plants (Chan 2003). In addition, exogenous toxic compounds such as pesticide residue should be analyzed in phytopharmaceuticals (Bisset 1994). However, most conventional methodologies for pesticide residue analysis of medicinal plants and their products, such as the European Pharmacopeia (EP) procedure, are costly, time-consuming and require larger samples and greater volumes of hazardous solvents. Therefore, the present work focuses on the suitability of matrix solid-phase dispersion extraction (MSPD) for the analysis of organochlorine pesticides in various parts of medicinal plants. The analyses were carried out with a Gas chromatograph equipped with electron-capture detector (GC-^{63}Ni ECD). The analytical results confirmed that the proposed method is sensitive and selective for determining organochlorine pesticide residues in medicinal plants giving sensitive limits well below those set by international

P. C. Abhilash (✉) · N. Singh
Eco-Auditing Group, National Botanical Research Institute, Council of Scientific and Industrial Research, Rana Pratap Marg, Lucknow, Uttar Pradesh 226 001, India
e-mail: pcabhilash@gmail.com; pcabhilash@hotmail.com

N. Singh
e-mail: nanditasingh8@yahoo.co.in

regulations like European Union (EU), and World Health Organization.

Materials and Methods

Florisil (60–100 mesh) obtained from Supelco (Bellefonte, PA, USA) and neutral alumina (60–230 mesh, activity I), anhydrous Na_2SO_4, NaCl and $MgSO_4$ from Merck (Darmstadt, Germany). Certified analytical standards of pesticides, purity >99%, were obtained from Supelco. Acetone, ethyl acetate, n-hexane and cyclohexane (gas chromatography grade) were purchased from Merck. Individual stock solutions (1.0 mg mL^{-1}) of each isomer were prepared in cyclohexane and stored in a freezer at −20°C. Mixed working standard solutions in cyclohexane at concentration of 0.001–1 mg mL^{-1} were prepared by diluting the stock solutions with cyclohexane and stored at 4°C in the dark. Matrix matched standard solutions were prepared at the same concentrations as that of the calibration solutions by adding appropriate amounts of standard solutions to blank matrix extracts.

Medicinal plants *Withania somniferea* (L.) Dunal, *Ocimum sanctum* L. and *Achyranthes aspera* L. were collected from Botanic Garden of National Botanical Research Institute (NBRI). Collected plants were washed with deionised water and separated in to various parts like root, stem, leaf, fruits, etc. Extracts from all the matrices were pre-checked to confirm the absence of any pesticide before fortification and sample processing. For the recovery studies, blank samples were fortified before the extraction by addition of mixed standard solution of alpha, beta, gamma, and delta HCHs, aldrin, dieldrin, and endrin to give 0.001 of each compound. They were then subjected to the procedures described in under sections.

A Perkin Elmer (Norwalk, CT, USA) gas chromatograph Model Clarus 500 series equipped with ^{63}Ni electron capture detection (ECD) system and split-splitless injector was used. Nitrogen of purity greater than 99.99% was used as carrier gas at a programmed flow of 1.5 mL/mints. For separation, a 35% diphenyl and 65% dimethyl polysiloxane capillary column (30 m × 0.32 mm I.D., 0.5 μm) Elite 35 (Perkin Elmer, USA) was employed. GC-conditions: splitless injection of 1 μL was carried out at 250°C, Column temperature programme: initial temperature 100°C, held for 2 min, then increased at rate of 15°C minutes to 300°C, held for 5 min. Detection was performed at 300°C using a ^{63}Ni electron capture detection (ECD).

Matrix Solid Phase Dispersion Extraction

Leaf, stem and root samples were dried at 35°C for 24 h, powdered, sieved (1–2 mm) and stored at 4°C for posterior analysis. Plant matrices (5 g) were gently ground with 0.5 g Florisil (deactivated with 3% acetone) in a pestle and mortar for 5 min and 1 g MgSO4 and 0.5 g NaCl was added to this mixture and ground firmly for 5 more minutes. This mixture was transferred into a glass column filled with neutral alumina deactivated with 3% acetone (2 g) and anhydrous sodium sulfate (0.5 g). A mixture of *n*-hexane-ethyl acetate solvent 70:30 (v/v, 10 mL) was utilized for elution in the column and repeated with another 10 mL of same solvent mixture. The resulting extract was concentrated, resuspended with *n*-hexane (1 mL) and kept in 4°C for posterior analysis.

Modified European Pharmacopoeia (EP) Method

About 30 mL of acetone: dichloromethane (3:1 v/v) suspension was added to 10 g of dried and powdered medicinal parts. After 20 min maceration at room temperature, the sample was homogenized in a vortex mixer with 3 g anhydrous Na_2SO_4 for 5 more minutes. The extraction process was followed by a clean up by solid phase extraction with Florisil. Glass column (30 cm × 1.5 cm i.d.) were packed from the bottom with glass wool plug/cotton and 5 g of Sigma-Aldrich branded Florisil (60–100 mesh size) with a top layer of 2 g anhydrous Na_2SO_4. Samples were eluted with 30 mL of same solvent mixture (acetone: dichloromethane 3:1 v/v), concentrated by using a rotary evaporator and then reconstituted in 1 mL toluene and kept in 4°C for final analysis.

Results and Discussion

The variables requiring to be optimized in MSPD were type and quantity of solid-phases and sorbent, sample volume, elutropic strength and the volume of the elution solvent. Therefore, in an earlier study, we focused systematically on the development of optimal analytical methods based on MSPD techniques to determine the combined residues of hexachlorocyclohexane isomers in plant matrices (Abhilash et al. 2007, 2008, in press). In the present study, we extended the optimized method for the extraction of different OCPs (aldrin, endrin, dieldrin and HCH isomers). Precision, LOD, and MDL of different analytes are shown in Table 1. Precision, expressed as relative standard deviation, ranged between 5.19% for aldrin, and 9.85% for δ-HCH. The instrumental limit of detection ranged between 2 ng for α-HCH and 6 for δ-HCH. The method detection limit was varied from 0.465 to 1.136 ng for various analytes studied. Mean recoveries for different pesticides were found in the range of 88–98% (Table 2). Matrix effect expressed as the signal from the pesticides in different plant matrix compared to the signal in solvent was tested in all matrices. In general,

Table 1 Precision, detection limits, method detection limits and linearity ranges of various analytes

Pesticide	Precision (RSD%)	LOD (ng)	MDL (ng)	Linear range (μg ml^{-1})	r^2
Aldrin	5.19	3.0	0.60	1–1000	0.9978
Endrin	7.05	2.0	0.47	1–1000	0.9985
Dieldrin	6.95	3.0	0.57	1–1000	0.9969
α-HCH	5.40	3.0	0.69	1–1000	0.9939
β-HCH	9.85	6.0	1.14	1–1000	0.9980
γ-HCH	6.15	3.0	0.60	1–1000	0.9984
δ-HCH	7.95	4.5	0.90	1–1000	0.9979

the measured matrix effect for different analyte is quite small, with a mean value of 94–102% and a relative standard deviation of 8%.

The EP method, the recent official procedure to determine pesticides in medicinal plants was adapted and employed as the referential methodology throughout this study. Although the amount of medicinal parts employed for the analysis was reduced to about a fifth of the amount suggested by EP, which was accomplished by reducing the consumption of all the reagents with no loss to the method's performance, the modified procedure consumed a large amount of reagents and time. Table 3 shows the pesticides extracted using both methods as well as the factor f defined as the amount of analyte extracted by MSPD/amount of analyte extracted by the EP method. As can be seen, the efficiency provided by MSPD is similar or even better than obtained by the reference method, thus showing the suitability of MSPD for the routine analysis of pesticide residues in medicinal plants.

The apparent current explosion of interest in commercial utilization of herbal products should be followed by accurate quality control. Prompt analytical methods would be quite useful for setting up realistic MRLs and other regulatory guidelines in the management of pesticide residues in herbal products. Considering the above aspects, a rapid and sensitive method has been proposed for the extraction of organochlorine pesticides in medicinal plants prior to GCECD. The use of MSPD provides the following advantages:

Table 2 Statistical pesticide recovery (%) from medicinal plants. Mean percentage recovery of organochlorine pesticides from various medicinal plants at a fortification level of 0.001 mg kg^{-1} of pesticides

Medicinal part	Aldrin	Dieldrin	Endrin	α-HCH	β–HCH	γ-HCH	δ-HCH
Withania-leaf	91.56	98.41	87.75	94.31	89.30	91.54	96.25
Ocimum-leaf	97.55	93.35	91.90	93.09	94.36	90.46	95.56
Achyranthes-leaf	95.43	92.89	91.95	92.78	91.65	91.48	99.30
Withania-root	94.56	89.90	89.94	91.29	93.78	81.95	94.68
Ocimum-root	90.40	93.78	87.86	98.71	96.39	96.19	99.50
Achyarnthes-root	96.78	94.67	95.39	86.19	91.96	84.15	95.75
Withania-stem	97.89	87.46	92.43	94.50	93.40	88.19	99.39
Ocimum-stem	97.90	91.55	96.54	94.45	93.24	90.19	98.49
Achyranthes-stem	95.51	94.37	95.89	98.79	94.89	96.55	98.70

Table 3 Comparative pesticides recovery from medicinal plant *Withania somnifera* (L.) Dunal (fortified at a concentration of 0.001 mg kg^{-1}) by MSPD and EP methods

Pesticide	*Withania somnifera* (L.) Dunal								
	Leaf (μg g^{-1})			Stem (μg g^{-1})			Root (μg g^{-1})		
	MSPD	EP	f^a	MSPD	EP	f^a	MSPD	EP	f^a
Aldrin	0.945	0.911	1.037	0.933	0.910	1.025	0.968	0.945	1.024
Endrin	0.915	0.892	1.025	0.949	0.905	1.048	0.945	0.932	1.013
Dieldrin	0.973	0.945	1.030	0.896	0.886	1.011	0.931	0.918	1.014
α-HCH	0.916	0.890	1.029	0.975	0.963	1.012	0.917	0.901	1.017
β-HCH	0.925	0.899	1.029	0.979	0.954	1.026	0.940	0.928	1.013
γ-HCH	0.933	0.947	0.985	0.962	0.957	1.005	0.988	0.980	1.008
δ-HCH	0.945	0.898	1.052	0.893	0.889	1.004	0.942	0.932	1.010

f^a: MSPD/EP

(i) Shortening of the extraction time.
(ii) Less sample volume.
(iii) Higher recoveries than the reference method.

Acknowledgments The authors are grateful to Director, National Botanical Research Institute, for providing facilities. P.C Abhilash thankfully acknowledges University Grants Commission, Government of India, for Doctoral Fellowship (UGC-JRF-SRF).

References

Abhilash PC, Jamil S, Singh N (2007) Matrix solid-phase dispersion extraction versus solid-phase extraction in the analysis of combined residues of hexachlorocyclohexane isomers in plant matrices. J Chromatogr A 1176:43–47

Abhilash PC, Jamil S, Singh V, Singh A, Singh N, Srivastava SC (2008) Occurrence and distribution of hexachlorocyclohexane isomers in vegetation samples from a contaminated area. Chemosphere 72:79–86

Abhilash PC, Singh V, Singh N (in press) Simplified determination of combined residues of Lindane and other HCH isomers in vegetables, fruits, wheat, pulses and medicinal plants by matrix solid phase dispersion (MSPD) followed by GC-ECD. Food Chem. doi:10.1016/j.foodchem.2008.07.004

Akerele O (1993) Nature's medicinal bounty: don't throw it away. World Health Forum 14:390–395

Bisset NG (1994) Herbal drugs and phytopharmaceutical: a handbook on practice on a scientific basis. CRC Press, London

Chan K (2003) Some aspects of toxic contaminants in herbal medicines. Chemosphere 52:1361–1371

Tewary DK, Kumar V, Shanker A (2004) Leaching of pesticides in herbal decoction. Chem Health Saf 670:1–5

WHO (1992) Quality control methods for medicinal plant material. WHO/PHARMA/92.559, World Health Organization, Geneva

Zuin VG, Vilegas JHY (2000) Pesticide residues in medicinal plants and phyto-medicines. Phyto Res 14:73–88