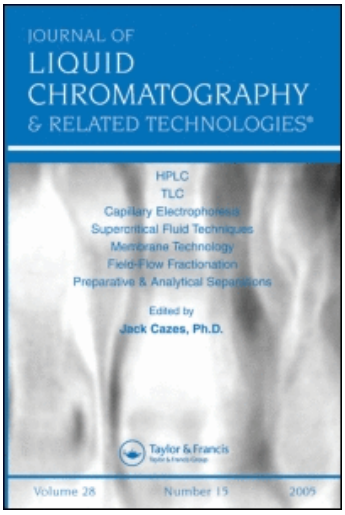

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SIMULTANEOUS DETERMINATION OF ALDRIN, DIELDRIN, ENDRIN, HEPTACHLOR, AND p,p'-DDT IN MEDICINAL PLANT EXTRACTS USING A NOVEL HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD

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ABSTRACT

A high performance liquid chromatographic method for the simultaneous determination of five organochlorine pesticides (aldrin, p,p'-DDT, dieldrin, endrin, and heptachlor) was developed. The method was used to determine the levels of these pesticides in medicinal plant samples. Analysis was carried out using a Merck LiChrospher 100 RP C_{18} (5 μm) column with a gradient solvent system of acetonitrile-water and PDA UV detection (224 nm). Quantification was carried out by the external standard method. The limit of detection for the utilized method was below the local legal limits (ANZFA) for similar plant materials for all 5 pesticides excepting endrin. Medicinal plant extracts were further analyzed by conventional GC-ECD and GC-NPD means using SPE and GPC cleanup as required.

INTRODUCTION

Chlorinated organic derivatives are one of the longest utilized pesticide groups. Many possess high stability and therefore have been found in food products,[1] and medicinal plants[2-4] despite prohibition of use. Organochlorine pesticides and, in particular, aldrin, p.p'-DDT, dieldrin, endrin, and heptachlor, are fat-soluble but water insoluble synthetic chemicals. These properties allow organochlorine pesticides to accumulate in animal fats and milk.

A study reported in 1995 in Victoria Australia[5] found widespread contamination of human breast milk with p,p'-DDT. Dieldrin was found in 43% of samples tested with a number of infants having daily intakes above the accepted daily intakes (ADI) for many of the organochlorine pesticides tested. In the same year a UK study[6] reported almost identical findings. Some countries still do not have legislation in place (for example Australia) concerning organochlorine pesticide levels in phytotherapeutic materials. Acceptable levels can only be estimated by comparison to MRLs (maximum residue levels) set down by the NHMRC for similar phytological products.

Many phytotherapeutic plants are difficult to analyze by GC. This can arise from co-extractives which coelute with pesticide peaks to produce excessive interference. In addition, the presence of low volatility or polymerizing compounds can lead to column and detector contamination. A diverse number of HPLC methods have previously been used to determine pesticide levels in food samples, and these are well documented by Bushway.[7]

Eighteen samples from 9 different commonly used medicinal plants, that were commercially available to the Australian public in 1996, were extracted and analyzed. In the first instance samples were analyzed by a novel reverse-phase with UV detection HPLC method to determine levels of aldrin, dieldrin, endrin, heptachlor, and p,p'-DDT, and then secondly by GC analysis for a further 11 organochlorine pesticides, 18 common organophosphate pesticides, and 5 synthetic pyrethroid pesticides. HPLC methods using reverse-phase chromatography with C18 columns and aqueous mobile phases, followed by UV detection have previously been reported in the analysis of pesticides.[8,9] The described HPLC method may be used to screen samples for the possible presence of the five listed pesticides.

EXPERIMENTAL

Chemicals and Solvents

Methanol was fractionally distilled to be of HPLC quality, diethyl ether (analytical grade) and acetonitrile (HPLC grade) were obtained from BDH.

Hexane was obtained as analytical grade from Ajax chemicals, then fractionally distilled before use. Aldrin, Dieldrin, Endrin, and Heptachlor were obtained as 100 μg/mL solutions in methanol, while p,p'-DDT was obtained as a pure solid, then recrystallised from acetone, GC confirmed purity to be >99%. A 130 μg/mL solution of p,p'-DDT in acetonitrile was made and used immediately. A single stock standard solution was prepared with each of the 5 standards present at 10 μg/mL, excepting p,p'-DDT which was present at 130 μg/mL. The standards were diluted in acetonitrile. Working standards were prepared over the range 1 - 10 μg/mL by serial dilution of the stock with acetonitrile.

HPLC-UV System

The Waters (Millipore, Milford, MA, USA) HPLC system comprised a 717 autosampler, 600 pump and controller, 996 PDA UV detector, and Millenium 2010 software. The system was fitted with a Merck LiChrospher 100 RP-18 (5 μm) 250 x 4 mm column coupled to a Merck LiChroCART guard column. The system ran at ambient temperature; chromatograms at 224 nm.

GC System

Organochlorine screen: GC system comprised a Shimadzu GC14a instrument with ECD detector at 300°C, coupled to DB1701 and SE 54 columns. Carrier gas-hydrogen. Synthetic pyrethroid screen: same instrument and columns as for organochlorine screen. Organophosphate screen: GC system comprised a HP5890 Series II instrument with NPD detector coupled to DB17 and SE 30 columns. Carrier gas-helium. GC analysis was performed at the NSW Agriculture Chemical Residue Laboratories.

Medicinal Plants

Three major commercial wholesale suppliers of phytotherapeutics and one private herb farm supplied samples for this study. Prior to analysis, all samples were stored at 22°C in laboratory desiccators. Alfalfa (*Medicago sativa*-aerial parts, 2 samples, origin Australia), Chamomile (*Matricaria chamomilla*-flowers, 1 sample, origin Australia), *Echinacea spp.* (*purpurea*-whole plant, 3 samples, origin Australia, *angustifolia*-whole plant, 1 sample, origin USA), Feverfew (*Tanacetum parthenium*, 1 raw herb sample- origin Australia, 1 tea preparation-origin USA), *Ginkgo biloba* (1 raw herb sample-origin Australia, 1 tea preparation-origin USA), Green Oats (*Avena sativa*, aerial parts, 1 sample-origin Australia), Liquorice (*Glycyrrhiza glabra*, 1 tea preparation, origin-Overseas) and St John's Wort (*Hypericum perforatum*, 2 samples of raw herb-origin not supplied, 1 tea preparation, origin-overseas) samples were used for analysis.

Sample Extraction

Samples (100 g-dry weight) were subjected to mechanical maceration in hexane (500 mL), followed by filtration through a Whatman (#1) filter paper. The eluate volume was then reduced to 5 mL under vacuo. A literature Florisil Sep-Pak procedure[10] for analysis of pesticide residues was employed with some modification. Thus a Florisil Sep-Pak was pre-conditioned by passing 5 mL of hexane through it, followed by the addition of the sample. The Sep-Pak cartridge was then washed with 85 % hexane/diethyl ether (20 mL). Solvent was removed under vacuo to dryness, then the residue was resuspended in 2 mL of acetonitrile and sonicated for 2 minutes. The sample was then passed through a Maxi-clean C18 cartridge and 0.22 μm filter then injected into the HPLC.

For GC organophosphate analysis, the acetonitrile solution was injected into a dual column GC-NPD. For OC analysis, acetonitrile was evaporated and the sample diluted into petroleum ether, then injected onto a dual column GC-ECD. For SP analysis and samples producing GC interference, the acetonitrile was evaporated and samples diluted into dichloromethane. This solution was cleaned up on a gel permeation column, evaporated to dryness and taken up in petroleum ether. The sample was then analyzed by dual column GC-ECD.

HPLC-UV Analysis

The mobile phase flow rate was 1.3 mL/min, with UV detection at 224 nm. The initial solvent mix was 70:30 CH_3CN:H_2O, with a linear ramp to 85:15 over 11 minutes, a 7 minute hold 85:15, a linear ramp to 100 % CH_3CN over 3 minutes, a 5 minute hold at 100 % CH_3CN, a return to the initial mix over 5 minutes, then a 10 minute equilibration before the next analysis. Quantitative results were obtained by comparing peak areas of samples with those of external standards.

GC Analysis

Organochlorine screen: ECD detector at 300°C. Injection was at 250°C split to 2 columns, with a heating program of: 140°C held for 1 minute then ramped to 250°C at 10°/minute, then held for 10 minutes. Organo chlorine screen (chlordane, dieldrin, p,p'-DDT, o,p-DDT, p,p'-DDD, o,p-DDD, p,p-DDE, o,p-DDE, α-endosulfan, β-endosulfan, endosulfan sulphate, endrin, heptachlor, heptachlor epoxide, lindane).

Synthetic pyrethroid screen: temperature program- 140°C held for 1 min then ramped to 250°C at 10°C/minute, then held for 10 minutes. Synthetic Pyrethroid screen (cyfluthrin, cyhalothrin, cypermethrin, deltamethrin,

fenvalerate). Organophosphate screen NPD detector at 300°C. Carrier gas-helium, injection was at 250°C split to 2 columns, with a heating program of: 143°C held for 1 minute then ramped to 180°C at 10°C/minute, held for 3 minutes, then further ramped to 280°C at 10°C/minute and held for 5 minutes. Organo phosphate screen (azinphos ethyl, azinphos methyl, bromophos ethyl, chlorpyrifos, demeton-s-methyl, chlorfenvinphos, diazinon, dichlorvos, dimethoate, ethion, fenamiphos, fenthion, malathion, methacrifos, methidathion, monocrotophos, parathion, profenofos).

RESULTS AND DISCUSSION

An HPLC-PDA chromatogram of a standard solution of aldrin, p,p'-DDT, dieldrin, endrin, and heptachlor along with elution times is shown in Figure 1. The linearity of the method was verified by injecting the 5 standards in varying amounts as follows: 0.4 μg, 2 μg, 0.1 μg, 0.0 5μg, and 0.02 μg for endrin, dieldrin, heptachlor, and aldrin, and 0.26 μg, 0.13 μg, 0.065 μg, 0.0325, and 0.013 μg for p,p'-DDT. The equations for the lines of best fit (in order of elution from the HPLC column) for the 5 point calibration were as follows (Y variable stands for response, and X for amount of standard in μg):

Endrin,	$Y = 4.55 \times 10^{5}X + 6.22 \times 10^{3}$ (r = 0.9967)
Dieldrin,	$Y = 5.35 \times 10^{5}X - 6.08 \times 10^{3}$ (r = 0.9975)
Heptachlor,	$Y = 3.71 \times 10^{5}X - 8.60 \times 10^{1}$ (r = 0.9999)
p,p'-DDT,	$Y = 1.65 \times 10^{6}X + 1.21 \times 10^{2}$ (r = 0.9999)
Aldrin,	$Y = 4.31 \times 10^{5}X - 7.94 \times 10^{2}$ (r = 0.9999)

The HPLC assay was validated by measuring the recovery of aldrin, dieldrin, endrin, and heptachlor (5.0 μg), and p,p'-DDT (6.5μg) from equivalent blank 100g samples spiked with standards. Overall, the mean recoveries were 94.2% (endrin), 94.9% (dieldrin), 93.0% (heptachlor), 101.5% (p,p'-DDT), and 61.2% (aldrin). The limit of detection for the method was defined as the concentration of analyte that yields a signal to noise ratio of at least 3:1. These values were calculated from recovery experiments (see Table 1).

Aldrin, Endrin, dieldrin, p,p'-DDT, and heptachlor were not detected in the phytotherapeutic samples analyzed, neither in the first instance by HPLC to the levels of detection indicated in Table 1 nor secondly, by the GC methodology described in section 2.7, to ≥ 10 μg/kg. None of the additional organochlorine, organophoshate, or synthetic pyrethroid pesticides analyzed by GC (listed in section 2.7) were detected to a level of 10 μg/kg for OC, and 100 μg/kg for OP, and SP. It should be noted, although, that the described method was not validated for these additional pesticides.

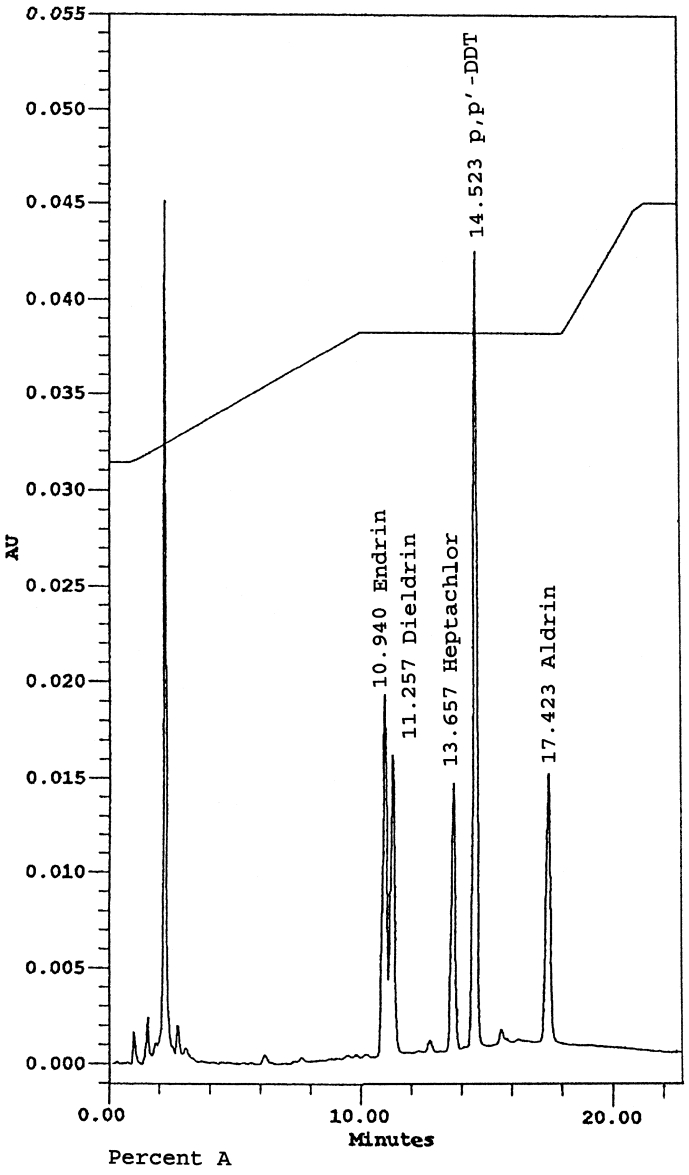

Figure 1. HPLC-PDA chromatogram of a standard solution containing endrin, dieldrin, heptachlor, aldrin (10 μg/g each), and p,p'-DDT (6.5 μg/g). HPLC conditions are described at HPLC-UV analysis section. Chromatogram was generated at 224 nm, with elution times as shown for a 40 μL injection.

Table 1

Recovery of Aldrin, Dieldrin, Endrin, and Heptachlor, and p,p'-DDT, from Blank Spiked Samples

Standard	Amount[a]	% Recovery (Mean)	S.D.	n	Limit of Detection[b]
Endrin	5.0 μg	94.2	2.94	3	0.5 μg/g
Dieldrin	5.0 μg	94.9	4.11	4	0.6 μg/g
Heptachlor	5.0 μg	93.0	6.88	3	0.4 μg/g
p,p'-DDT	6.5 μg	101.5	7.07	4	0.1 μg/g
Aldrin	5.0 μg	61.2	3.75	4	0.8 μg/g

S.D. = Standard deviation. [a] Amount of pesticide standard that blank sample was spiked with for recovery experiments. [b] Limit of detection defined as concentration of analyte that yields a signal to noise ratio of 1:3 at 224 nm.

Australia does not currently have legislation in place specifically concerning pesticide levels in medicinal plants. The ANZFA MRLs[11] (Australia New Zealand Food Authority Maximum Residue Limits) however, provide a general guideline by comparing similar plant products. The limit refers to mg of pesticide residue per kg of phytotherapeutic sample. For example, an MRL of 0.1 mg/kg for aldrin and dieldrin has been set down for carrots, cucumber, lettuce (head and leaf), onion, and potato, with levels of 0.2 mg/kg for heptachlor in carrots, and 1.0 mg/kg of p,p'-DDT in vegetables and fruit. A zero level for endrin in food stuffs has been set down by the ANZFA. In line with these limits for a 100 g phytotherapeutic sample, the present method (whereby 100 g of sample is extracted then purified and concentrated down to 2.0 mL of HPLC analyte) would need to have limits of detection below or equal to 5 μg/g for aldrin, 5 μg/g for dieldrin, 10 μg/g for heptachlor, and 50 μg/g for p,p'-DDT in the injected solution to establish comparative compliance. The limits of detection for aldrin, dieldrin, p,p'-DDT, and heptachlor employing this method, are all below these ANZFA levels as indicated in Table 1.

In conclusion, results obtained from both HPLC and GC analysis did not detect higher than regulatory levels of any of the tested pesticides. All detection levels in tested samples were below those specified by ANZFA. Additionally, this study utilized a novel HPLC analysis. In countries (such as Australia) that do not have legislation in place for organo-chlorine levels in medicinal plants the responsibility rests with the growers or distributors to establish the 'quality' of the material being presented for sale. HPLC instrumentation can be relatively inexpensive to purchase. This makes the HPLC method described above attractive for small-scale laboratories wishing to perform quality control on

medicinal plant samples. The HPLC assay described allows aldrin, dieldrin, p,p'-DDT, and heptachlor to be measured in phytotherapeutic samples to levels that are lower than those set down by recognized bodies for similar food stuffs. For endrin the method can provide information to the level indicated in Table 1. In samples where these pesticides are not detected by this method or the levels detected are near to the limits of detection for the individual components, GC may be used to confirm or determine levels to a greater level of sensitivity.

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