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Application of high-performance gel-permeation chromatography clean-up in the determination of hexaflumuron residues in soil

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ABSTRACT

A high-performance gel-permeation chromatography (HPGPC) column (7.5 mm I.D.), packed with a divinylbenzene cross-linked polystyrene gel (10 μm particles and 5 nm pore size) was used to produce a simple, one-stage clean-up procedure for determination of the insecticide hexaflumuron in three typical agricultural soils.

Conventionally, hexaflumuron extracts are purified by a series of time-consuming liquid–liquid partitions and solid-phase purification prior to high-performance liquid chromatography. HPGPC allows isolation of hexaflumuron from soil matrices with improved sensitivity in a shorter analysis time.

The use of HPGPC methodology has been validated over the range 0.01–1.0 mg/kg with recoveries in the range 73–119% (mean 98%). HPGPC gave excellent separation of hexaflumuron from other extracted materials with significant reduction in clean-up time and solvent consumption. This methodology has been successfully applied to samples derived from field trials.

INTRODUCTION

Hexaflumuron [1-(3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy)phenyl) - 3 - (2,6-difluorobenzoyl) urea] is the active ingredient of the insect growth regulator Consult (DowElanco), and is typically used on orchard fruits and vegetables.

Gel permeation chromatography (GPC) has been used to clean-up environmental samples for over 18 years. During this period the procedure has remained essentially unchanged, using preparative (25 mm I.D.) GPC columns based on 200–400 mesh Bio-Beads S-X resins (Bio-Beads S-X3 is the most commonly used), divinylbenzene cross-linked polystyrene gels. GPC is suitable for nearly all types of pesticides [1–5]. Tindle and Stalling [1] developed an automated clean-up apparatus which was evaluated by Griffitt and Craun [6] for use on a wide variety of fats and oils. Van Rhijn and Tuinstra [7], and Tuinstra *et al.* [8] described the use of a miniature (10 mm and 2 mm I.D.) GPC column, which effectively decreased solvent consumption and the collected fraction, but with limited sample loading.

Existing methods for the analysis of hexaflumuron use a number of liquid–liquid partitions which are time-consuming with high solvent consumption. In addition a silica solid-phase clean-up stage is sometimes required. This method still only achieves a lowest determination limit of 0.05 mg/kg.

This study investigates HPLC–UV quantitation of hexaflumuron residues in soil following the use of HPGPC as a clean-up procedure.

EXPERIMENTAL

Reagents and materials

Hexaflumuron analytical standard is from DowElanco Europe. Acetonitrile and water

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were of HPLC grade; hexane and dichloromethane (DCM) were of Distol grade. 0.2 μm Minisart SRP 15 filters (PTFE membrane) were used.

Hexaflumuron standard stock solutions: 100 mg of analytical standard was dissolved in 60 ml of acetonitrile and diluted to 100 ml with water to give 1000 μg/ml stock solution. The solution was diluted to provide appropriate standard solutions.

GPC test solution: for testing the column performance, a mixture of corn oil (6.25 mg/ml), bis(2-ethylhexyl)phthalate (0.25 mg/ml), perylene (0.005 mg/ml) and sulphur (0.02 mg/ml) was prepared in DCM. A typical calibration chromatogram is shown in Fig. 1. GPC optimization solution: 1 μg/ml hexaflumuron standard in DCM.

HPGPC system and conditions

HPGPC was carried out using Spectra-physics SP8810 pump, Waters 486 UV detector, Waters Fraction collector, Waters autosampler, chart recorder, a PLgel 600 mm × 7.5 mm I.D. GPC column packed with polystyrene–divinylbenzene (10 μm particles and 5 nm pore size) and a PLgel 50 mm × 7.5 mm I.D. GPC guard column, packed with polystyrene–divinylbenzene (10 μm particle size) with DCM as the eluent at a flow-rate of 1.0 ml/min. The injection volume was 500 μl.

To establish the collect time (start and end time of the fraction peak) 1 μg/ml hexaflumuron optimization solution was injected onto the GPC column and monitored using UV detector at 254 nm (Fig. 2). A typical elution program for the fraction collector is: wait (dump) 14.3 min, collect 2.4 min.

HPLC system and conditions

Analyses were carried out using a Varian Star system consisting of 9050 UV detector, 9010 solvent delivery system, 9095 autosampler and a 250 mm × 2.1 mm I.D. Kromasil C18 (5 μm) column with following conditions: mobile phase: acetonitrile–water (gradient); solvent programme:

Time (min)	Acetonitrile (%)	Flow-rate (ml/min)
0.0	58	0.2
12.5	58	0.2
26.0	85	0.2
27.0	100	0.4
37.0	100	0.4
38.0	58	0.2

Equilibration time: 6 min; Injection volume: 50 μl; Wavelength: 254 nm; Range: 0.005 AUFS.

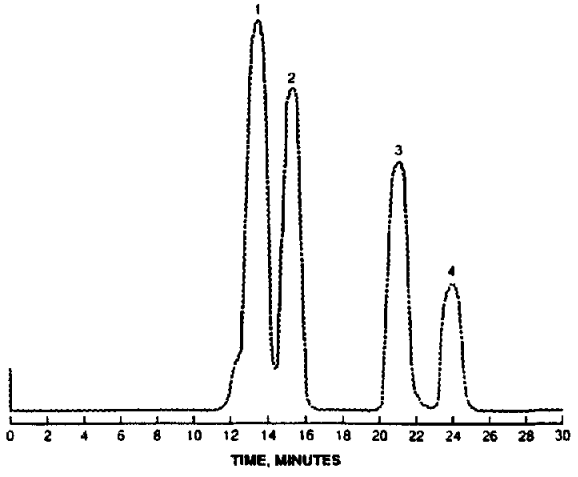

Fig. 1. Typical chromatogram of test solution. 1 = Corn oil (6.25 mg/ml), 2 = bis(2-ethylhexyl)phthalate (0.25 mg/ml), 3 = perylene (0.005 mg/ml), 4 = sulphur (0.02 mg/ml).

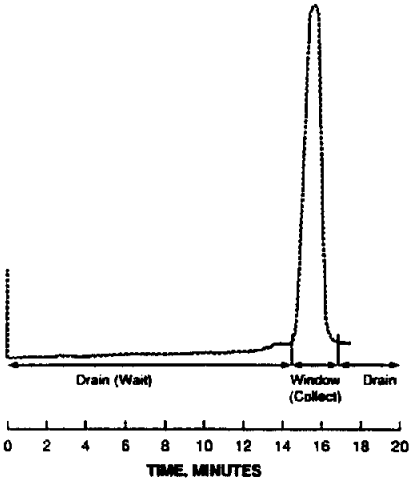

Fig. 2. Chromatogram of 1.0 μg/ml hexaflumuron optimization solution to establish the start and end time of the hexaflumuron fraction peak.

TABLE I

MEAN RECOVERY (%) DATA FOR HEXAFLUMURON

Soil type	Fortification rate (mg/kg)	No. of analysis	Mean recovery (%)
Sandy silt loam	0.01	2	100
Clay loam	0.01	4	91
Sandy loam	0.01	2	109
Sandy silt loam	0.10	2	102
Clay loam	0.10	5	105
Clay loam	1.00	2	88

Extraction and clean-up

Soil samples (10 g) were weighed into 50 ml vials and after addition of 20 ml acetonitrile–water (1:1) were shaken for 30 min. The extracts were centrifuged for 10 min at 1500 rpm (*ca.* 500 *g*) and the supernatants transferred to 240 ml jars. The extraction was repeated twice and the supernatants combined in 240 ml jars. After addition of 120 ml water, hexaflumuron was partitioned into 20 ml of hexane by shaking for 5 min. The extracts were centrifuged at 1500 rpm (*ca.* 500 *g*) and the hexane transferred into 50 ml vials. The partitioning was repeated once and the hexane extracts combined and evaporated to dryness in a gentle stream of nitrogen at 40°C. The residuum was reconstituted in 2.0 ml DCM.

The DCM solution was filtered using a 0.2-μm filter and 500 μl of this solution injected onto the GPC column. The hexaflumuron fraction was collected and evaporated to dryness in a gentle stream of nitrogen at 40°C. The residuum was reconstituted in 250 μl acetonitrile–water (60:40) using ultrasonication for 30 s. A 50 μl aliquot of this solution was chromatographed.

TABLE II

MEAN BACKGROUND VALUES FOR UNTREATED SOIL SAMPLES

Soil type	Mean concentration (mg/kg)
Sandy silt loam	0.0030
Clay loam	0.0000
Sandy loam	0.0018

RESULTS AND DISCUSSION

The analytical procedure was validated using three different untreated soil types fortified at various levels.

A summary of mean recovery data for three different soil types is given in Table I. All recoveries are corrected for the appropriate background values of the untreated samples.

A summary of mean background contribution from untreated soil is given in Table II. These values were used to calculate the lowest fortification and hence the determination limit of the method.

Typical chromatograms of hexaflumuron standard, untreated clay loam soil, untreated sandy silt loam and clay loam soil fortified at 0.1 mg/kg are shown in Figs. 3–6.

The use of HPGPC proved to be a very effective technique for the clean-up of agricultur-

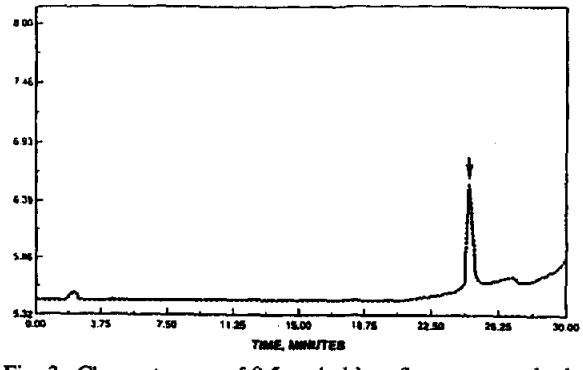

Fig. 3. Chromatogram of 0.5 μg/ml hexaflumuron standard. The *y*-axis represents UV absorbance (AU).

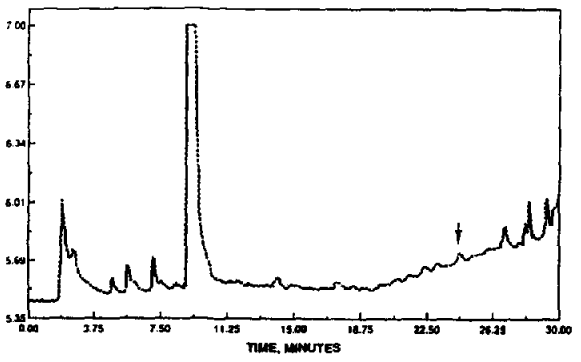

Fig. 4. Chromatogram of untreated soil sample (sandy silt loam). The y-axis represents UV absorbance (AU).

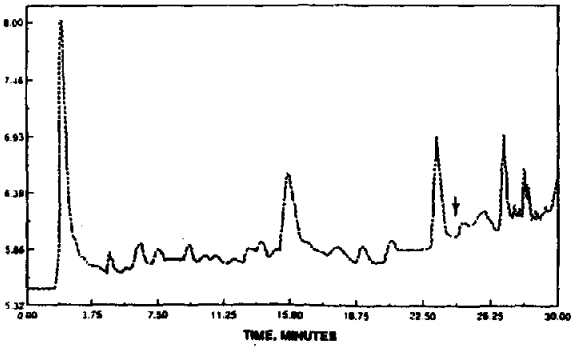

Fig. 5. Chromatogram of untreated soil sample (clay loam). The y-axis represents UV absorbance (AU)

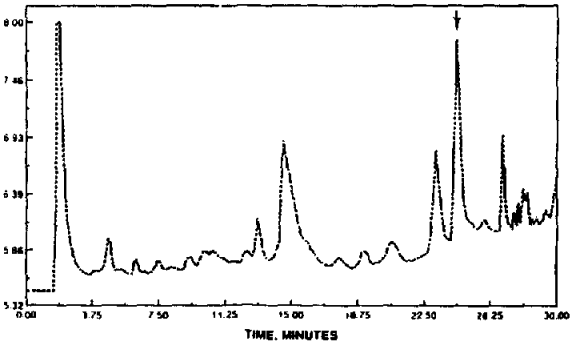

Fig. 6. Chromatogram of fortified soil sample (clay loam) at 0.1 mg/kg, 98% recovery. The y-axis represents UV absorbance (AU).

al soils. Using HPGPC, a determination limit of 0.01 mg/kg was achieved with excellent recovery data. This is an improvement by a factor of 5 on the standard method using conventional clean-up procedures. A different chromatographic profile was obtained for each soil type, clay loam being the "worst case"; however, the target determination limit was maintained with all soil types. The technique was successfully applied to the analysis of soil samples with improved sample throughput.

The use of analytical high-performance GPC column packed with 10 μm particle size and 5 nm pore size divinylbenzene cross-linked polystyrene provides a clean-up which results in increased speed of clean-up, sensitivity, and reduced solvent consumption.

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