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Multiresidue determination of pesticides in plants by high-performance liquid chromatography following gel permeation chromatographic clean-up

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Abstract

Gel permeation chromatography was applied as a clean-up step in a HPLC multiresidue method for the determination of several pesticides in plants, not amenable to analysis by GC. The pesticides investigated were diflubenzuron, triflumuron, clofentezine, hexythiazox and flufenoxuron. The clean-up technique resulted in a good separation of analytes from co-extractive matrix compounds. Complete HPLC separation of all pesticides was achieved under the conditions selected. The analytical procedure was characterized with high accuracy and precision and acceptable sensitivity to meet requirements for monitoring these pesticides in crops.

Keywords: Sample handling; Fruits; Food analysis; Pesticides

1. Introduction

The changing patterns in pesticide usage requires new approaches in residue analysis. GC is the basic technique in modern multiresidue (MR) methods for analysis of pesticides [1–13]. Liquid–liquid partitioning and adsorption chromatography have been traditionally applied for clean-up of the extracts [13–15] but recently solid-phase extraction (SPE) [11,12,16–20] and gel-permeation chromatography (GPC) [3,4,6,7,10] gained popularity. GPC has proven to be an universal clean-up procedure in MR multimatrix methods and has been adopted in general MR methods of the Netherlands [14], Official Analytical Chemists [21], Germany [22] and Sweden [23]. It has been applied as a clean-up step in HPLC methods for determination of some classes of pesticides not readily determined by GC: carbamates [24,25], benzoyl ureas [26] in fruits and vegetables.

A number of new generation pesticides such as the insecticides which inhibit chitin synthesis, diflubenzuron, triflumuron, flufenoxuron and acaricides clofentezine, hexythiazox, are widely used for pest control in fruit growing and in horticulture. Only a few analytical procedures for the determination of their residues in crops are reported in the literature. They have strong UV absorption and analytical methods for residues based on HPLC with UV detection have been developed for diflubenzuron [27,28] and hexytiazox [29]. A method for the simultaneous determination by HPLC–UV of hexytiazox, clofentezine and fenoxycarb has been reported [30]. These compounds are not amenable directly to GC. Diflubenzuron residues were determined by GC with electron capture detection after derivatization to chloroacetylaniline [31] and by GC–MS after derivatization with heptafluorobutyric acid anhydride [32]. They are not included in the recent MR methods based on GC or GC–MS [1,3–6,8–12,14,21–23,33,34].

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HPLC is the most important alternative to GC for pesticide residues analysis. The aim of this work was to develop a MR method based on HPLC, complementary to the GC MR methods, for determination of the widely used new generation pesticides diflubenzuron, triflumuron, flufenoxuron, clofentezine, hexythiazox in plants.

2. Experimental

2.1. Chemicals and reagents

All reagents and solvents were of reagent grade. Methanol distilled in glass and bidistilled water were used for HPLC. Ethyl acetate and cyclohexane were obtained from Merck (Darmstadt, Germany). Bio-Beads S-X3, 200–400 mesh from Bio-Rad Labs (Munich, Germany) were used for GPC. Pesticide reference standards were supplied by the main manifacturers and were >99% pure. Stock standard solutions (1 mg/ml) were prepared in methanol. Composite working standard solutions were prepared in the mobile phase by diluting the stock solutions as required.

Matrices: Samples of untreated apples and tomatoes were analyzed alone and fortified with analytical standards of pesticides.

2.2. Apparatus

A Pye Unicam (Cambridge, UK) liquid chromatograph was equipped with a PU 4010 pump, a PU 4020 variable-wavelength UV detector and a Rheodyne Model 7125 injector with a 20-μl loop. A LiChrosorb RP-18, 5-μm column (250×4.6-mm I.D.) (Merck) was used for HPLC determination. GPC clean-up was carried out on a stainless steel column (500×8-mm I.D.) Tessek, Separon (Prague, Czech Republic) filled with Bio-Beads S-X3 and connected to a PU 4010 pump and a Rheodyne injector with a 1-ml loop.

2.3. Procedures

(a) Extraction: 100 ml of ethyl acetate and 30 g of anhydrous sodium sulfate were added to 50 g of a homogenized plant material and the extraction was carried out in a high-speed blender for 3 min. The extract was filtered through a sodium sulfate layer.

(b) GPC clean-up: An aliquot, equivalent to a 5-g sample, was taken and the solvent was evaporated under a gentle stream of nitrogen. The residue was dissolved in 5 ml of ethyl acetate–cyclohexane (1:1). One ml of the solution was injected on to the GPC column. The elution was carried out with a mixture of ethyl acetate–cyclohexane (1:1) at a flow-rate 1 ml/min. The first fraction of 12.5 ml was discarded. The second fraction of 15 ml was collected, the solvent evaporated under a gentle stream of nitrogen and the residue was dissolved in the HPLC mobile phase to a final volume of 1 to 5 ml.

(c) HPLC conditions: The analysis were carried out isocratically with a methanol–water (8:2) mobile phase at a flow-rate 1 ml/min. With respect to the UV detection, a wavelength of 254 nm was selected as a compromise between the sensitivity of all five compounds.

(d) Method validation: The recovery tests were carried out by adding 100 μl of standard solutions in methanol to 50 g of chopped and homogenized samples of apples and tomatoes in blender jar. The concentrations of pesticides in the standard solutions were 50 μg/ml, 100 μg/ml, 500 μg/ml and 1000 μg/ml for fortification levels 0.1 mg/kg, 0.2 mg/kg, 1 mg/kg and 2 mg/kg, respectively. The solvent was allowed to evaporate, the samples were mixed and allowed to stand 1 h before extraction. Each recovery test was repeated five times. Untreated control samples were analysed with each matrix and fortification level.

3. Results and discussion

The GPC clean-up technique was evaluated for efficiency in separation of the analytes from the matrix co-extractives and quality of the chromatograms. Under the HPLC conditions selected, the pesticides were completely separated in the analytical column as is shown in Fig. 1. GPC clean-up resulted in effective separation of matrix extractives. No interference was observed with the analytes in the chromatograms of control samples of untreated matrices at final volumes of the samples of 4 ml (Fig. 2). When the final volume of the samples was 1

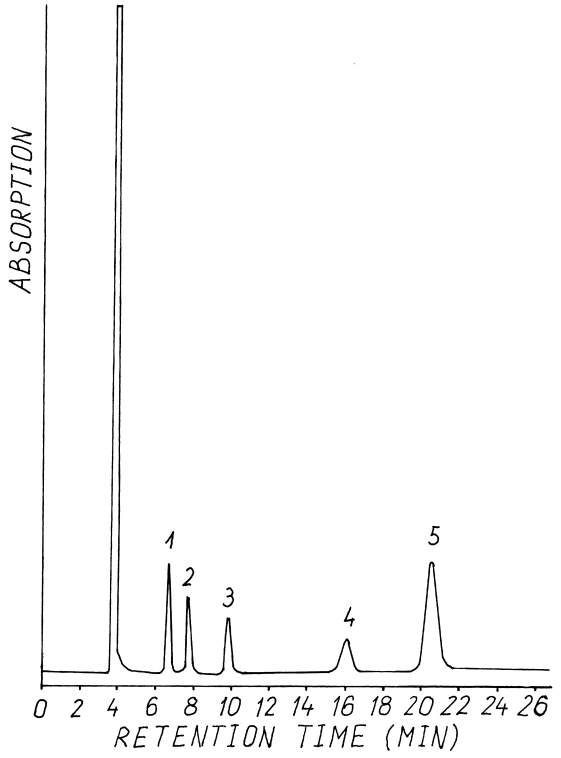

Fig. 1. Chromatogram of mixed standard solution. Peaks: 1=diflubenzuron 4 ng; 2=triflumuron 4 ng; 3=clofentezine 4 ng; 4=hexythiazox 8 ng; 5=flufenoxuron 8 ng.

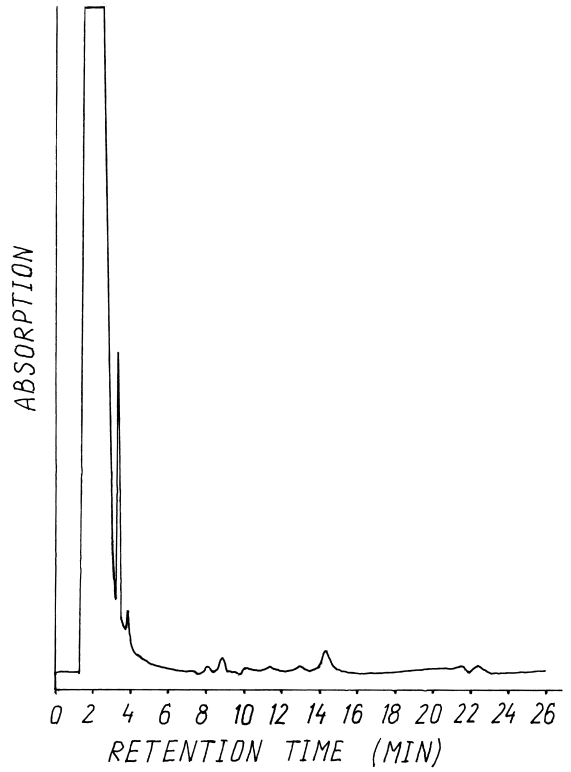

Fig. 2. Chromatogram of untreated tomato sample; final volume 4 ml.

ml, an interfering peak was present only with clofentezine but not with the other pesticides. This interference was due to impurities from the system. When a blank sample of solvents and reagents only was analyzed by the GPC clean-up, a peak interfering with clofentazine was observed at 1-ml sample volume. The impurity peak was reduced to negligible at final volumes of 4 ml or more. The impurities from the system influenced the recovery of clofentezine only at low concentration rates, when the sample final volume was 1 ml, but not at higher concentration rates, when the final volume of the sample was 4 ml or more. For example, at the level of fortification of clofentezine 0.1 mg/kg, measured at a final volume of the 1-ml sample, the mean recovery exceeded 200%. At fortification levels 0.2 mg/kg, measured at the final volume of the 4-ml samples, or at higher concentrations – 0.5–1 mg/kg and final volumes of the 5-ml samples, the mean recoveries for clofentezine were between 84.8–96.1 (Table 1). The GPC clean-up was well applicable to the determination of all compounds including clofentezine but the sensitivity of the latter was limited down to concentration of 0.2 mg/kg.

Chromatograms of apple and tomato samples fortified with a mixed standard and cleaned according to the GPC clean-up procedure are shown as examples (Figs. 3 and 4).

For method validation, recoveries and repeatability were determined via analysis of two types of crops – apples and tomatoes, selected as they were the main crops to which the pesticides, included in the study, were applied. The untreated samples were fortified at two concentration levels – one near the limit of determination of the method and the other – 10 times higher. The low levels of concentrations for recovery studies were 2.5 to 10 times below the maximum residue limits (MRLs) of the pesticides in different

Table 1
Accuracy and precision of the method

Pesticide	Recovery±S.D. (%)		Level of fortification (mg/kg)
	Apples	Tomatoes	
Diflubenzuron	102.6±11.8	85.8±6.0	0.1
	98.2±2.9	97.4±5.4	1.0
Triflumuron	96.1±13.6	84.6±4.8	0.1
	97.1±8.2	95.3±4.9	1.0
Clofentezine	89.2±10.7	84.8±7.9	0.2
	94.8±4.9	96.1±5.2	0.5
	92.7±8.3	87.5±6.8	1.0
Hexythiazox	95.7±10.8	90.7±9.2	0.2
	94.5±94.5	88.3±9.5	2.0
Flufenoxuron	97.7±3.9	76.7±4.8	0.2
	99.5±1.7	79.5±9.1	2.0

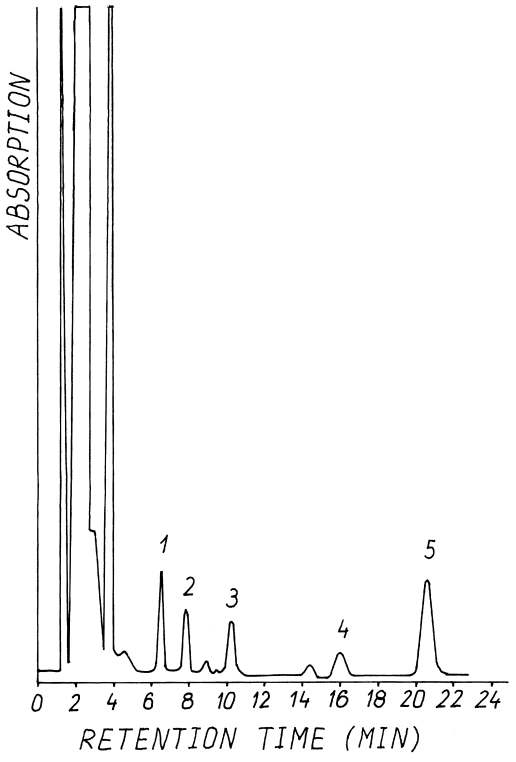

Fig. 3. Chromatogram of tomato sample fortified with mixed standard solution; final volume 5 ml. Fortification levels: 1=diflubenzuron 1 mg/kg, 2=triflumuron 1 mg/kg; 3=clofentezine 1 mg/kg; 4=hexythiazox 2 mg/kg; 5=flufenoxuron 2 mg/kg.

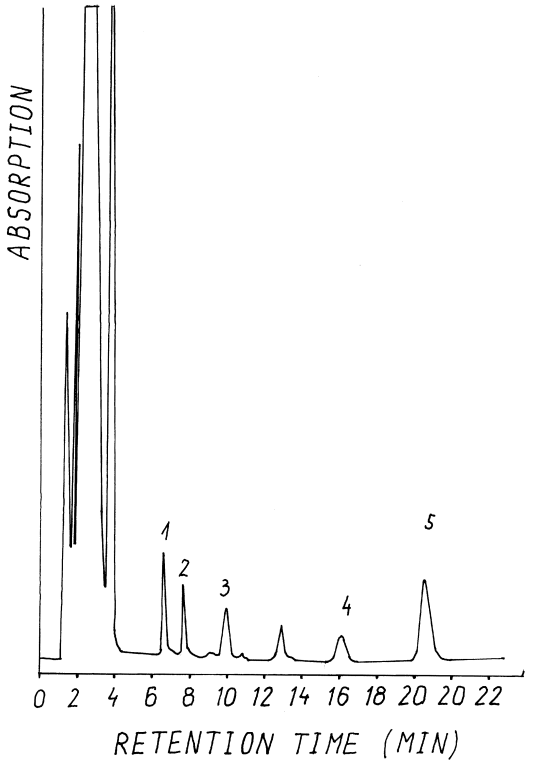

Fig. 4. Chromatogram of apple sample fortified with mixed standard solution; final volume 5 ml. Fortification levels: 1=diflubenzuron 1 mg/kg, 2=triflumuron 1 mg/kg; 3=clofentezine 1 mg/kg; 4=hexythiazox 2 mg/kg; 5=flufenoxuron 2 mg/kg.

crops [35]. The results of recovery studies are given in Table 1. The accuracy and precision were quite acceptable.

Detection at a constant wavelength of 254 nm gave satisfactory results for the sensitivity of all compounds. The limit of quantitation is defined as the quantity able to give a peak, equal to six times the baseline noise of the chromatograms of untreated control samples. The sensitivity of the method is presented in Table 2. The limits of quantitation of the method are below the MRLs of pesticides defined by Codex Alimentarius Commission, FAO [35] as 1 mg/kg for diflubenzuron in apples and tomatoes, for clofentezine in vegetables, and as 0.5 mg/kg for hexythiazox and clofentezine in apples. There are no international MRLs established for triflumuron and flufenoxuron. Since their toxicity is lower than the toxicity of diflubenzuron and clofentezine, it is not

Table 2
Sensitivity of the method

Compound	Minimum detectable amount (ng)	Limit of quantitation (mg/kg)
Diflubenzuron	1	0.05
Triflumuron	1	0.05
Clofentezine	1	0.2
Hexythiazox	4	0.2
Flufenoxuron	2	0.1

likely to expect significant differences in acceptable concentrations of residues in crops. The sensitivity of the method could be considered relevant to regulatory requirements for monitoring these pesticides in crops.

For linearity study, the response of the detector was plotted versus the analyte's amounts over a range of 2 ng to 40 ng. The correlation coefficients for all compounds exceeded 0.999.

In comparison with the classical separating funnel, partitioning GPC is faster, formation of emulsions is avoided and the quantity of hazardous solvents is reduced. The GPC clean-up resulted in a good separation of the matrix co-extractives. The GPC column can be used for the clean-up of many samples, so that less handling is required and the procedure is more cost effective. This study shows that the GPC clean-up procedure has a sufficient efficiency for HPLC determinations of the pesticides studied and is preferable for its generality.

4. Conclusions

A multiresidue method for analysis of five pesticides, not amenable to GC was developed, based on HPLC determination with sufficient sensitivity, accuracy and precision.

GPC clean-up procedure resulted in a good separation of the matrix co-extractives from the analytes. The essential features of this procedure include high efficiency of the process, generality and a reusable clean-up column.

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