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Determination of diflubenzuron in apples by gas chromatography

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Abstract

A method for the determination of residues of the insecticide diflubenzuron, 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea, in apples using gas chromatography with electron-capture detection has been developed and validated. The three solvents ethyl acetate, acetone and dichloromethane were tested for extraction of diflubenzuron residues from apples. Dichloromethane gave the highest recovery and the lowest background and was chosen as the extraction solvent. After extraction the residue of diflubenzuron was derivatized with heptafluorobutyric anhydride. The derivative was purified by silica solid-phase extraction using toluene as the eluent. The external standard calibration was linear over the range 0.05–1.0 μg/ml and the limit of quantification was 0.03 mg/kg apple using 25 g samples. Recovery of diflubenzuron from spiked apples (0.1–0.8 mg/kg) was 80–88% with a relative standard deviation of less than 10% ($n=5$). The method was applied to the determination of diflubenzuron residues in apples from a treated field.

Keywords: Environmental analysis; Food analysis; Apples; Sample preparation; Derivatization, GC; Fruits; Diflubenzuron; Pesticides

1. Introduction

The insecticide diflubenzuron, 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea, is increasingly being used for controlling insect pests in fruits due to its high selectivity and low acute toxicity for mammals [1–3]. Both HPLC and GC methods have been applied for the determination of diflubenzuron in different matrices. Apart from high-performance liquid chromatography–mass spectrometry (HPLC–MS) [4], the lack of a specific detector makes the sample preparation quite time-consuming [3,5]. Due to the thermolability of diflubenzuron, direct determination by gas chromatography (GC) is not feasible. However, GC methods based upon derivatization of hydrolysis products [2] or direct derivatization [6–8] with subsequent electron-capture or mass spectrometric detection have been used for quantification. Determination of diflubenzuron by quantification of heat decomposition products using deuterated diflubenzuron as the internal standard has also been achieved [9].

The objective of this work was to develop a simple and rapid method for the determination of diflubenzuron residues in apples. Due to the higher resolving power of capillary GC as compared to HPLC less laborious sample preparation is necessary. The possibility of using the sensitive electron-capture detector makes GC an attractive alternative for the determination. Solvent extraction was chosen, and the suitability of ethyl acetate, acetone or dichloromethane as the extraction medium was examined. Heptafluorobutyric anhydride (HFBA) and trifluoroacetic anhydride (TFAA) were tested as derivatization reagents. The derivatization products were purified by silica solid-phase extraction before

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analysis by gas chromatography–electron-capture detection (GC–ECD).

2. Experimental

2.1. Materials and reagents

Technical-grade diflubenzuron of 99% purity was obtained from Duphar (Weesp, Netherlands). High-purity anhydrous trifluoroacetic anhydride (TFAA) and heptafluorobutyric anhydride (HFBA) were purchased from Pierce (Rockford, IL, USA).

Acetone, dichloromethane, ethyl acetate and toluene of pesticide grade were purchased from Labscan (Dublin, Ireland) while dried pyridine was purchased from BDH (Poole, UK). Analytical-grade methanol and reagent-grade sodium sulfate were obtained from Merck (Darmstadt, Germany). Technical-grade decane was purchased from Fluka (Buchs, Switzerland).

2.2. Standard solutions

Diflubenzuron stock solutions of 1.0 mg/ml were made by dissolving 100 mg of diflubenzuron in 5.00 ml methanol and diluting with toluene in a 100 ml volumetric flask. Diluted diflubenzuron solutions were prepared from the stock solution by diluting 50 μl and 500 μl to 50.00 ml with toluene, giving solutions of 1.00 μg/ml and 10.0 μg/ml, respectively. Calibration solutions of 0.10, 0.20, 0.50 and 1.00 μg/ml were prepared by appropriate dilution of these solutions. All solutions were kept in the dark at 4°C and the calibration solutions were changed every 3 weeks. For derivatization and subsequent GC determination aliquots of 1.00 ml were used.

2.3. Samples

Apple samples were collected fresh from a 2.5 ha apple orchard at the Agricultural University of Norway at Ås, Norway. The orchard was subdivided into six equivalent plots (numbered 1–6) and the diflubenzuron formulation 'Dimilin SC-48' was applied to each plot with a mistblower. Details of the treatment are given in Table 1. Whole apples from the same plot were homogenized in a blender (Malavasi Professional 15 l, Bologna, Italy) and divided into sub-samples of 25 g each. These sub-samples were kept frozen at −18°C and analyzed within 9 months.

2.4. Extraction

To each sub-sample was added 25 g of anhydrous sodium sulfate and 125 ml of dichloromethane. The mixture was blended at high speed for 5.0 min and filtered under suction through a Buchner funnel using Whatman glass fibre paper. To this filtrate 50 μl of decane was added as a keeper and the filtrate was evaporated to near dryness using a rotary evaporator at 30°C. The residue was redissolved in 10.0 ml

Table 1
Application of 'Dimilin SC-48' and the concentration of residues of diflubenzuron in treated apples

Plot No.	No. of applications	Total amount applied (mg/ha)	Date of application in June 1994	Conc. of diflubenzuron in apples (mg/kg)
1	untreated	untreated	untreated	<0.01
2	1	15.4	10th	0.07
3	1	15.4	20th	0.08
4	1	15.4	30th	0.10
5	2	30.7	10th, 20th	0.15
6	3	46.0	10th, 20th, 30th	0.35

The date of sampling was August 24. At each plot one sample was collected and analyzed.

toluene and 1.00 ml of this solution was derivatized as described below.

2.5. Derivatization

To a sample of 1.00 ml was added 50 μl of pyridine and 100 μl of HFBA. The solution was vortexed for 20–30 s and left at room temperature for 1.5–2 h. Any excess of reagents was removed by solid-phase extraction on a silica column (650 mg Sep-pak from Waters, Millipore, MA, USA). The silica column was conditioned with toluene before application of the reaction solution. In addition, 0.85 ml of toluene was applied, resulting in a total elution volume of 2.0 ml.

2.6. Gas chromatography

A Nordion Micromat HRGC 412 (Nordion Instruments, Helsinki, Finland) equipped with dual columns and dual electron-capture detectors was used. Column A was a NB-1701 column (25 m×0.32 mm I.D. with 0.52 μm film thickness; Nordion Instruments) while column B was a HP-5 column (25 m×0.32 mm I.D. with 0.52 μm film thickness; Hewlett-Packard, USA). The columns were connected to the injector with a two-hole ferrule. Each column was connected to a ^{63}Ni electron-capture detector at 300°C using argon–methane (95:5) as the makeup gas (25 ml/min). Manual splitless injection at 225°C using an injection volume of 2 μl was used throughout. A temperature program from 50°C (2.0 min) to 250°C (5.0 min) at 10°C/min was used with helium as the carrier gas at 1.7 ml/min using constant pressure.

A Hewlett-Packard 5890 series II plus gas chromatograph equipped with a HP 7673 automatic sampler/injector, a HP MS ChemStation and a HP 5971 mass-selective detector was used for GC–MS confirmation of the diflubenzuron derivatives. A HP-1 column (25 m×0.20 mm I.D. with 0.52 μm film thickness) was used for separation with a temperature program from 50°C (2.0 min) to 250°C (5.0 min) at 10°C/min. Helium was used as carrier gas at 0.9 ml/min. Automatic splitless injection of 2 μl was used. The injection port was kept at 240°C while the detection temperature was 260°C. The mass range from 50 m/z to 400 m/z was scanned with a cycle time of 0.5 s.

2.7. Stability

The stability of the diflubenzuron derivative was investigated by adding 0.5 ml of a 1.0 μg/ml lindane (which is a very stable pesticide) to 1.0 ml of a 1.0 μg/ml diflubenzuron–derivative solution resulting in concentrations of 0.33 μg/ml and 0.67 μg/ml, respectively. Lindane was added to compensate for changes in the detector response. The samples were analyzed once every day for 14 days.

2.8. Recovery

Homogeneously blended apples from untreated fields were spiked by adding 0.25, 1.0 and 2.0 ml of a 10.0 μg/ml diflubenzuron solution resulting in concentrations of 0.1, 0.4 and 0.8 mg/kg apple. Extraction and derivatization of these samples were performed as described above.

2.9. Quantitative determinations

The quantification was performed using the external standard method with the diflubenzuron calibration solutions 0.1, 0.2, 0.5 and 1.0 μg/ml. In addition to the samples, a reagent blank and a sample (from plot 1) spiked with diflubenzuron at a concentration of 1.0 mg/kg were analyzed as control samples. Results were plotted in control charts and were not corrected for recovery. Standards were injected after every sixth sample to monitor changes in chromatographic conditions.

3. Results and discussion

The HFBA derivative of diflubenzuron was analyzed using GC–MS. A molecular mass of 323 and additional fragments at m/z 154/156 and 126/128 confirmed that the HFBA derivative was N-(4-chlorophenyl) heptafluorobutylamine [7,8]; see Fig. 1. This derivative was found to be stable when stored in the dark at 4°C for at least 7 days, which is longer than earlier reported [8]. The TFA derivative formed

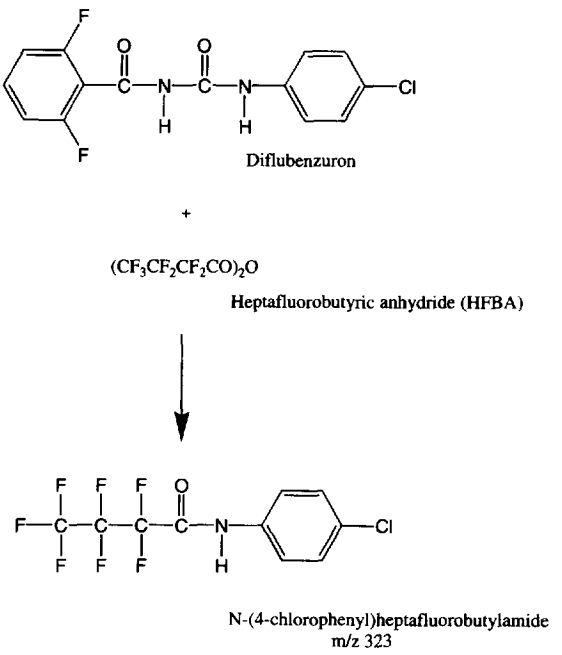

Fig. 1. Derivatization of diflubenzuron (M_r:310.7) with heptafluorobutyric anhydride. Structure of the derivative [7] was confirmed by GC–MS.

when using TFAA as reagent was inferior to the HFBA derivative with respect to detection limit and stability as well as chromatographic efficiency and purity. The chromatography of the HFBA derivative was satisfactory on both column A and B, and since no interfering peaks from the apple samples were observed in the HFBA derivative elution range, both columns are suitable for diflubenzuron determination. These columns are commonly used for multimethod analysis of pesticide residues in our laboratory. In the following, the results obtained using column B (HP-5) are presented. However, quite similar results were obtained on column A, as expected.

3.1. Extraction solvent

A simple solvent extraction method for the isolation of diflubenzuron from the apple matrix was sought. The highest recoveries (80–90%) were found using dichloromethane, while the recoveries using ethyl acetate and acetone were in the ranges 60–70% and 70–80%, respectively (Table 2). Also, a higher background and more interfering peaks in the chromatograms were observed using ethyl acetate and acetone as extraction solvents (Fig. 2). For all solvents the recovery (in %) increased with an increasing amount of spiked diflubenzuron in the investigated concentration range of 0.1–0.5 mg/kg apple. Dichloromethane has also been used by others for extraction of diflubenzuron, both from water [8] and from apples [10]. A recovery of up to 65% was found in the latter case. Recently, a recovery of 92% in apples at the 0.1 mg/kg level was reported using acetone extraction with subsequent partitioning into dichloromethane [3].

3.2. Method validation

3.2.1. Linearity

Replicates ($n=5$) of standard diflubenzuron solutions of different concentrations (0.05, 0.10, 0.20, 0.40, 1.0 and 2.0 μg/ml) were analyzed and the detector response (peak area) was plotted against concentration. Correlations were calculated by least square regression. The correlations were found to be linear in the range of 0.05–1.0 μg/ml with a

Table 2
Recovery, R, (in %) of diflubenzuron using acetone, ethyl acetate and dichloromethane as extraction solvents

μg/g apple	Acetone		Ethyl acetate		Dichloromethane	
	R (%)	R.S.D.	R (%)	R.S.D.	R (%)	R.S.D.
0.1	69	5.3	58	6.1	80	8.2
0.4	78	9.2	64	8.9	80	9.2
0.8	81	7.3	70	7.6	88	7.2

The results are obtained from 5 replicates at each spiked level.

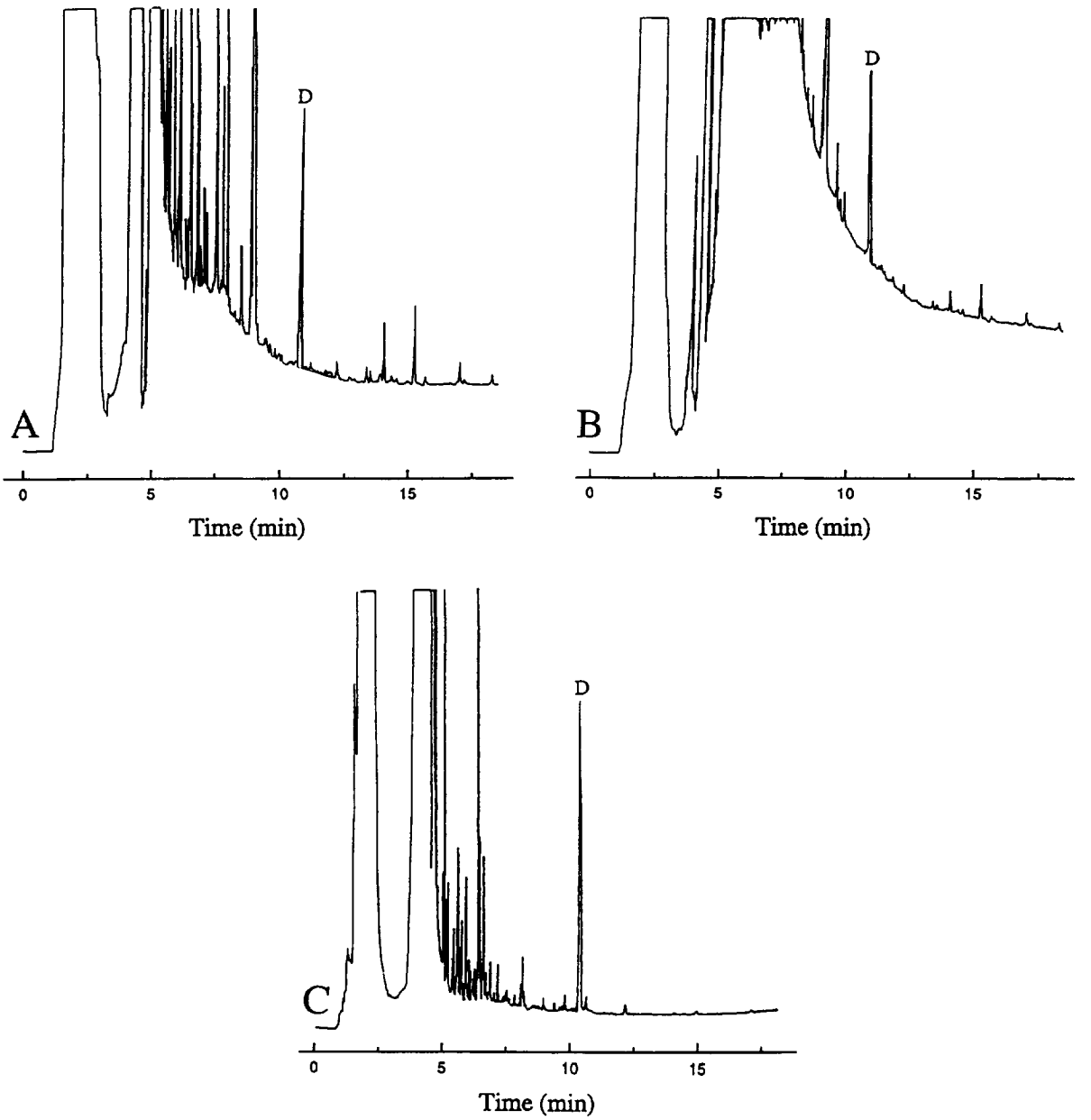

Fig. 2. Chromatograms of 0.5 mg/kg diflubenzuron spiked apple samples extracted with: (A) ethyl acetate, (B) acetone and (C) dichloromethane. The HFBA derivative of diflubenzuron is labelled (D). The HP-5 column and ECD were used, with slightly different temperature programs and attenuation, respectively.

coefficient of correlation larger than 0.99. This corresponds to concentrations from 0.04–0.80 mg/kg apple.

3.2.2. Limit of detection (LOD) and limit of quantification (LOQ)

LODs and LOQs based on signal-to-noise ratios of

3:1 and 10:1, respectively, were found to be 0.008 μg/ml and 0.02 μg/ml for standard diflubenzuron solutions and 0.01 mg/kg and 0.03 mg/kg of apple.

3.2.3. Recovery

The recoveries and R.S.D.s of spiked sub-samples are given in Table 2. The results show that dichloromethane gives better recovery compared to ethyl acetate and acetone. For the three investigated solvents, the recovery increased with increasing concentration of diflubenzuron.

3.2.4. Repeatability and reproducibility

From Table 2 it can be seen that the R.S.D.s are less than 10% in this experiment. No significant difference in results or R.S.D.s were found by repeated analyses of each sub-sample after 3 days of storage at 4°C in the dark.

3.2.5. Robustness

No significant difference in results was found using either column A or column B. Different oven temperature programs did not effect the results significantly.

3.3. Analysis of apples from diflubenzuron treated fields

Residues of diflubenzuron in apples picked from the trees were determined. By comparing the results with the treatment, the residue levels were found to depend on the application dosage and the time after treatment (Table 1).

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