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Column Switching and Liquid Chromatographic Technique for the Rapid Determination of Fenoxycarb Insecticide Residues in Apples

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Abstract

Fenoxycarb residues are analyzed by column switching and reversed-phase high performance liquid chromatography (RP-HPLC). The active ingredient is extracted from apples on a silica gel column using a n-hexane - diethyl ether mixture. The eluate is evaporated, dry residue dissolved in acetonitrile-deionized water, and injected into the liquid chromatograph with a column switching system (C8 columns), and a UV-photodiode array detector (UV - PDA). The analyte is quantified by the external standard method. The average recoveries of the active ingredient from the spiked sample are 81.3 ± 3.2% and 80.3 ± 5.8%, the coefficients of variation are 3.9% and 7.2% for fortification levels 0.1 mg/kg and 0.05 mg/kg, respectively, and the limit of quantification at λ — 228 nm is 0.05 mg/kg. Labor and organic solvent uses are greatly reduced in comparison to the existing methods. The overall procedure allows a sample throughput of up to 30 samples per day. The method was applied to the determination of fenoxycarb residue in apples from treated orchards.

Keywords: column switching, HPLC, pesticide residue, food analysis

Introduction

Fenoxycarb (ethyl 2-(4-phenoxyphenoxy) ethylcarbamate) is an insect growth regulator with contact and stomach action. It is used for control of Lepidoptera, scale insects, and suckers on fruit, cotton, olives, vines, and ornamentals [1]. Fenoxycarb has low soil mobility, it does not bioaccumulate, and it breaks down relatively quickly in the environment. Previously published methods for the analysis of residue of fenoxycarb [2-3] were based on acetone extraction with silica gel cleanup, Florisil [2], and acetonitrile-water extraction followed by SepPack C18, and liquid-liquid partition [3] with the UV reversed-phase liquid chromatographic determination.

High performance liquid chromatography (HPLC) is especially used for the trace-level determination of pesticides which cannot be analyzed directly by gas chromatography (GC) due to low volatility, high polarity and/or thermal instability. Typical examples are chlorophenoxy acids, carbamates (with fenoxycarb) and phenylureas. Fenoxycarb analyte can be nicely separated and efficiently detected by means of HPLC-UV, while GC analysis requires an often complicated derivatization procedure prior to separation.

Column switching with the HPLC system is still not

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very much in vogue in pesticide residue analysis, so it is an interesting object of study. The possibility to perform an automated and efficient clean up of extract samples is a highly desirable option in analysis.

The major aim of this study is to set up a sensitive and convenient HPLC method, reduce labor, and reduce the use of organic solvents through the combination of silica gel column extraction of the analyte and column switching liquid chromatography with UV-photodiode array detector (UV-PDA) detection with special attention to the elimination of co-extracted interferences.

The relevant aspects in applying column switching in our study were to increase chromatographic resolution, selectivity and sensitivity, to enrich trace amounts of the sample, to protect sensitive UV-PDA detectors, and to speed up column stabilization.

Experimental

Chemicals

Acetonitrile was for HPLC grade from J.T. Baker (Deventer, The Netherlands). Deionized water was purified by Maxima water purification system (ELGA, High Wycombe, England). Both solvents were filtered through 0.45 µm Nylon 66 Membranes (Supelco, Bellefonte, PA, USA) and degassed using helium sparging. n-Hexane and diethyl ether (not stabilized with ethanol) were residue analysis grade, and distilled-in-glass if necessary. The extraction mixture was n-hexane - diethyl ether (93:7, v/v). Inorganic compounds were all reagent grade. Silica gel was Kieselgel 60 extrapure, particle size 0.063 - 0.200 mm (70 - 230 mesh ASTM) (Merck, Darmstadt, Germany) reactivated prior to use at 773 K for 2 h, cooled in a desiccator, kept tightly closed.

Standard and Samples

Fenoxycarb standard (purity 99.2%, Ciba - Geigy AG, Basel, Switzerland) was used for fortification and quantification. Stock solution of fenoxycarb (200 µg/mL) was prepared in *for HPLC* grade acetonitrile. The calibration and working standard solutions of fenoxycarb were prepared by diluting from stock solution with acetonitrile - deionized water (40:60, v/v). These solutions were stored in the refrigerator at 277 K.

Samples of apples were collected fresh from private apple orchards.

Apparatus

A food cutter (Hobart) and high-speed commercial blender with stainless steel container (Waring Products Division, New Hartford, USA). Rotary-vacuum-evaporator was Rotavapor-R type W (Buchi, Flawill, Switzerland) with 323 K water bath. Shaker was type 358S (Elpan, Lubawa, Poland). Extraction columns were from polypropylene cartridge, 130 x 25 mm id with a glass wool plug (Pharma-Plast A/S, Rodby, Denmark).

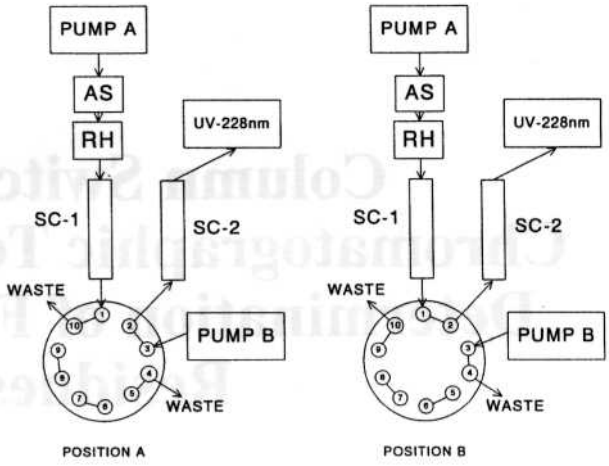

Fig. 1. Diagram of column switching liquid chromatographic system.

The HPLC system consisted of CM 3500 and 3200 pumps, autosampler Milton Roy type 713 (AS), UV - PDA detector type SM 5000 set at λ = 228 nm (TSP, Riviera Beach, FL, USA); programmable, 10 port (or equivalent) column switching valve type WEC10WK (VICI, Valco Instruments, Houston, TX, USA); 100 µL injection loop (Supelco, Bellefonte, PA, USA); Rheodyne Pneumatic Sample Injector Model 7126 (RH) (Rheodyne, Cotati, CA, USA). The data were collected and analyzed with LCtalk computing system (TSP LCtalk™ HPLC software, version 2.03.02).

Chromatographic Conditions

Separating columns were: clean-up column (SC-1) Supelcosil LC-8 DB, 150 x 4.6 mm id, 5 µm (Supelco, Bellefonte, PA, USA) and analytical column (SC-2) Zorbax Rx-C8, 250 x 4.6 mm id, 5 µm (Rockland Technologies, Nuenen, The Netherlands). Mobile phases were: A, acetonitrile-deionized water (60:40, v/v) and B, acetonitrile-deionized water (75:25, v/v). Flow-rates for both pumps were 0.5 mL/min; injection volume was 100 µL. The chromatographic system is presented in Figure 1.

Column Switching Procedure

Sample extract was automatically injected by AS via Rheodyne valve (RH) (Figure 1), fitted with a 100 µL loop, into clean-up column 1 (SC-1), through which pump A was pumping mobile phase A. At the same time pump B was pumping mobile phase B, through analytical column 2 (SC-2) (Position A). At a predetermined time the switching valve changed into Position B, at which a portion of effluent from SC-1 was transferred into SC-2. The valve was then automatically switched back into Position A. Switching and retention times were 15.7-16.8 min and 24.1 min, respectively.

Sample Preparation

Fresh apple samples: 1 kg of fresh fruit was chopped into small pieces, carefully mixed and kept frozen at 255K. A representative portion (200 g) was transferred into a blender, 50 mL distilled water was added, and sample material disintegrated by high speed blending in order to obtain the homogenous pulp. Pulp subsample of 6.25 g (representing 5 g sample) was weighed into a mortar of ca. 10 cm diameter, 10 g silica gel was added and ground to obtain the consistency of the free-flowing powder.

The weight of subsample pulp (W_p) taken for extraction and the amount of silica gel (W_s) depend on the volume (V) of water added to the sample. They can be calculated in grams by the following formula [4]:

$$W_p = (200 + V) / 40 \quad (1)$$

$$W_s = 1.6 \times W_p \quad (2)$$

Extraction

The extraction column was plugged with glass wool, the powdery sample was transferred through a widemouth polypropylene funnel (10 cm top id), and the column tapped to form a packed bed ca. 3 cm high. The column was topped with 7 g anhydrous sodium sulfate. Mortar and pestle were rinsed with 20 mL n-hexane - diethyl ether (93:7, v/v), and rinsates were carefully poured into the column. The fenoxycarb residues were extracted with total volume of 130 mL eluent and collected in round-bottomed flasks. The solvent was evaporated to dryness using a rotary evaporator, and the dry residue was dissolved in 2 mL acetonitrile - deionized water (40:60, v/v) for 10 minutes in a shaker.

Results and Discussion

Determination

The compositions of both mobile phases were chosen in such a manner that analysis time was kept to a reasonable minimum, and peak broadening was avoided through analyte preconcentration on column heads. The retention time of fenoxycarb on SC-1 was determined by connecting SC-1 directly to the UV-PDA detector. The chromatogram obtained in this way allowed the determination of the width at the base of fenoxycarb peak, and thus the required switching valve opening window. This window had to be experimentally checked each time the new mobile phases were prepared.

Instrument Calibration

The instrument was then calibrated by measuring the peak height of a series of standard solutions of varying concentrations of fenoxycarb in order to find the linearity range of the UV-PDA detector response, and to ensure that the valve opening window was sufficiently wide for complete transfer of the analyte at maximum concentrations from the calibration curve. We found that in the range of 0.06-1.00 µg/mL, the response of the detector was linear with a correlation coefficient (R^2) $R^2 > 0.999$. We also determined that up to the fenoxycarb concentration of 1.00 µg/mL the switching valve cut off neither the front nor the back of the peek. Calibration of the detector is presented on Figure 2.

Recovery and Validation Studies

Method recovery of the active ingredient was studied by analyzing seven replicates of samples spiked at 0.1 mg/kg and 0.05 mg/kg levels. Recoveries were in the range 77.2-85.4% for 0.1 mg/kg level and 71.9-85.4% for

Table 1. Method validation data. Recovery and precision.

Active ingredient	Fortification level (mg/kg)	n	Recovery (%)		SD (%)	CV (%)	Limit of quantification (mg/kg)
			Range	Average			
Fenoxycarb	0.05	7	85.4 83.8 85.1 71.9 72.4 80.9 82.4	80.3	5.8	3.9	0.05
Fenoxycarb	0.1	7	82.5 77.2 78.5 80.9 85.4 85.2 79.1	81.3	3.2	7.2	0.05

n – number of replicates at each spiked level; SD – standard deviation; CV – coefficient of variation.

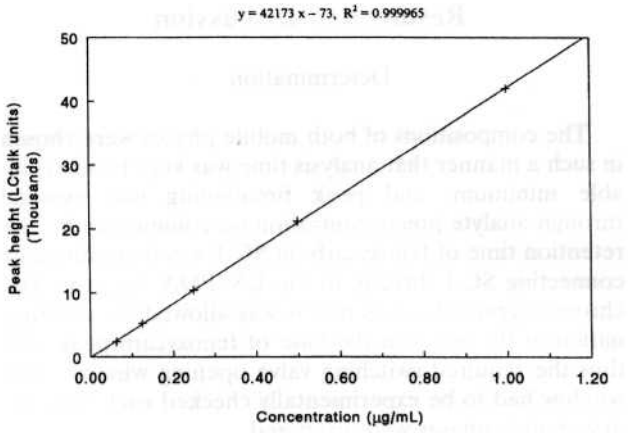

Fig. 2. Peak height calibration over concentration range 0.06 - 1.00 µg/mL for fenoxycarb.

0.05 mg/kg level. The average recoveries of the active ingredient from the spiked samples and standard deviations were 81.3 ± 3.2% and 80.3 ± 5.8%, the coefficients of variation were 3.9% and 7.2% for fortification levels 0.1 mg/kg and 0.05 mg/kg respectively. Neither the reagent blank nor the control sample had interfering peaks at the fenoxycarb retention time (Figure 3). The minimum detectable amounts under UV-PDA detection at λ = 228 nm was defined as three times the baseline noise, corresponding approximately to 6 ng of fenoxycarb. The limit of quantification was 0.05 mg/kg. Validation data i.e. recovery, precision, and limit of quantification are summarized in Table 1.

Residues of fenoxycarb in apple picked from the trees were determined. We didn't find residues of fenoxycarb in treated apple.

Performance of the Method

The application of silica gel column extraction technique allows the analyst to avoid troublesome extract filtration, and liquid-liquid separatory funnel partitioning. This technique has proven very successful in our laboratory in cases of gas-liquid chromatographic analyses of pesticide residue in fruits and vegetables [5]. However, the combination of silica gel column extraction with single column liquid chromatography, no matter whether isocratic or gradient, did not allow quantification due to the heavy load of coextractives (Figure 4, A). The column switching approach has alleviated this problem and allowed adequate quantitication of the analyte. The chromatograms of the sample extract on SC-1 and portion of SC-1 effluent on SC-2 are presented in Fig. 4.

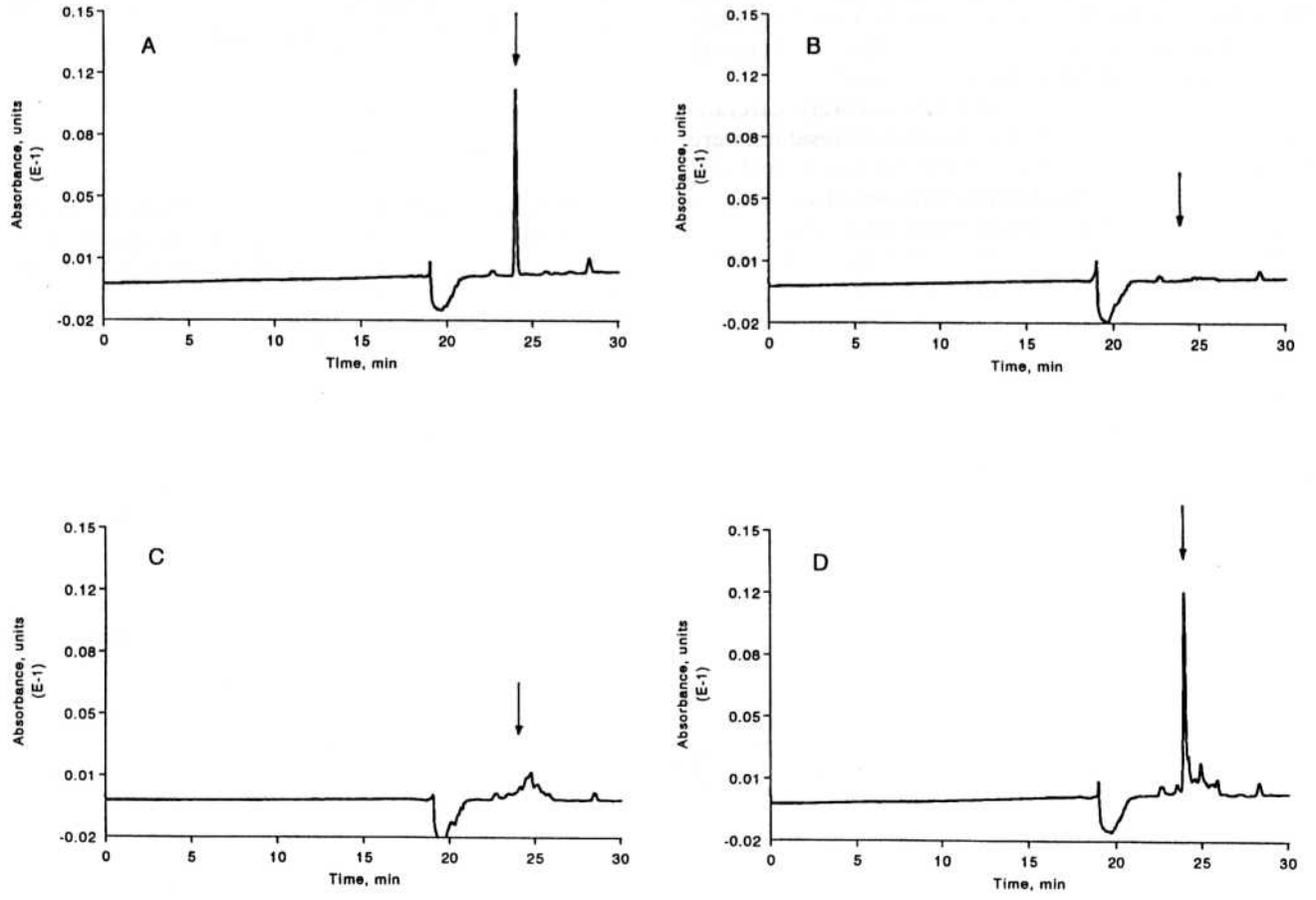

Fig. 3. Typical chromatograms (arrow indicates fenoxycarb): (A) - fenoxycarb standard 0.2 µg/mL; (B) - reagent blank; (C) - 5 g untreated control apples; (D) - 5 g control apples fortified at 0.1 mg/kg (80.9% recovery).

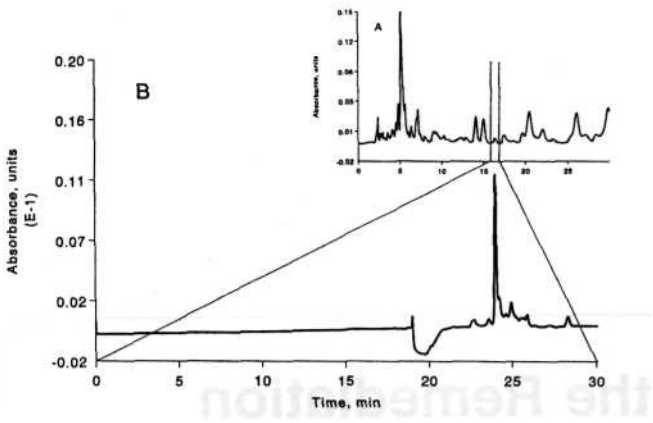

Fig. 4. The chromatogram of the sample extract on SC-1 clean-up column 1 coupled directly to UV-PDA detector (A) and the chromatogram of SC-1 effluent portion on SC-2 analytical column 2 (B). The fenoxycarb peak is identified by its retention time (24.1 min).

We found that labor and use of organic solvents in the three methods compared were 3.5 hours per analysis (hpa) and 880 mL, 2.0 hpa and 160 mL, and 1.5 hpa and 165 mL, for [2], [3], and our study, respectively. Our method avoided extract filtration and separatory funnel liquid-liquid partitioning, thus greatly improving analyst efficiency and increasing sample throughput.

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