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Journal of Chromatography A, 818 (1998) 138–143

JOURNAL OF CHROMATOGRAPHY A

Short communication

Supercritical fluid extraction and off-line clean-up for the analysis of organochlorine pesticide residues in garlic

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Received 18 March 1998; received in revised form 29 May 1998; accepted 5 June 1998

Abstract

An approach using supercritical fluid extraction (SFE) followed by clean-up with a $AgNO_3$-loaded Florisil column was utilized for the analysis of four organochlorine pesticides (OCPs) in garlic. The organic sulfur extracted by SFE from garlic was removed by $AgNO_3$ allowing OCPs to be determined by GC–electron-capture detection without interferences. All OCPs recoveries ranged from 85.0% to 110.0% and relative standard deviations were in the range of 3.9–7.2% for spiked samples. The described method may be used to analyze OCPs in garlic on a routine basis. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Sample handling; Food analysis; Pesticides; Organochlorine compounds

1. Introduction

Organochlorine pesticides (OCPs) as a class are some of the most persistent organic contaminants in the environment. Currently, supercritical fluid extraction (SFE) has shown to be an efficient and rapid method for the isolation of OCPs from vegetable-based matrices [1–3]. Garlic as a vegetable is somewhat unusual, since when it is cut and homogenized, the active enzyme converts the organic sulfur-containing compound alliin into allicin. Allicin is not a stable compound and readily degrades to form the secondary products consisting of various sulfides, which contribute to the characteristic flavor and odor of garlic [4]. Unfortunately, the aggregates of elemental sulfur exhibit both partition behaviour and chromatographic characteristics similar to organochlorine compounds. In addition, sulfur-containing compounds possess high affinity for thermal electrons and give strong electron-capture detection (ECD) responses [5]. Therefore, a major problem in monitoring garlic OCPs using GC–ECD is the interferences caused by elemental sulfur. Directly using a selective adsorbent of Cu [5] or $AgNO_3$-loaded silica [6] in the SFE extraction cell for simultaneous extraction of OCPs and on-line clean-up in sulfur-containing soils have been developed. Okihashi et al. [8] reported a method whereby garlic was treated in a microwave to deactivate enzyme, and then OCPs were extracted by organic solvents and the extracts were cleaned-up by traditional analytical methods. Unfortunately, the method used hazardous reagents and the OCPs recoveries were low. To date, the determination of

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PII: S0021-9673(98)00498-1

OCPs in garlic by SFE has not been reported. In the present work, we describe a method for extraction OCPs in garlic by SFE, followed by off-line clean-up with $AgNO_3$-loaded Florisil. The method is simple, practical and produces less pollution.

2. Experimental

2.1. Chemicals

The $AgNO_3$ (purity 99.8%), Cu (purity, 99%) powder as well as anhydrous $MgSO_4$ (99%) were obtained from Shanghai Chemical Reagent Factory No.1 (Shanghai, China). The Cu was treated prior to use by rinsing it with 20% (v/v) HNO_3 for 1 min, followed by a thorough rinsing with deionized water, acetone and *n*-hexane [5]. Florisil (Alltech Associates, Deerfield, IL, USA; P.R. grade, 60–100 mesh; 0.25–0.149 mm) was ignited at 650°C for 2 h before use. The $AgNO_3$-loaded Florisil was prepared by dissolving 2.5 g of $AgNO_3$ in 30 ml of deionized water and then homogeneously mixing the solution with 22.5 g of Florisil. The mixture was dried in an oven at 50°C and then activated at 150°C [6] and kept in darkness until used. Pesticide standards [hexachlorobenzene (HCB), aldrin, dieldrin, endrin, heptachlor epoxide, purity >99%] were supplied by National Center of Certified Reference Materials (Beijing, China). For each pesticide, a stock solution was prepared in hexane. Heptachlor epoxide was used as internal standard. Working standard mixture solutions in hexane, containing 1.0 μg/ml for three pesticides except 0.72 μg/ml for HCB, were used for spiking samples and preparing calibration standards. Carbon dioxide with a purity of 99.995% was purchased from Longkou Qiaofeng Chemical Factory (LongKou, China)

2.2. Supercritical fluid extraction

All extractions were performed using a ISCO 2200 SFE (Lincoln, NE, USA) equipped with two Model 260D syringe pumps and controller, a SFX 2-10 dual chamber extractor (temperature control, ambient to 150°C), and a restrictor temperature controller (ambient to 240°C). An uncoated, deactivated fused capillary column (30 cm×50 μm I.D.) was attached to the outlet of the extractor as a restrictor. The restrictor temperature was fixed at 70°C.

2.3. Gas chromatography

GC–ECD analyses were carried out using a Hewlett-Packard Model HP 5890 series II gas chromatograph equipped with a ^{63}Ni electron-capture detector, using a DB-5 capillary column (25 m×0.53 mm I.D., 1.5 μm film thickness) (J&W Scientific, Folsom, CA, USA). Samples were introduced into the GC column via electronic pressure control, cold on-column injector mode. The chromatographic conditions were as follows: injector temperature, 230°C; detector temperature, 300°C; nitrogen flow-rates, 9.00 ml/min (carrier gas) and 26 ml/min (make-up gas); column temperature program, 12 min at 190°C 10°C/min to 225°C. The data were acquired and processed with a Model HP 3396 II integrator.

2.4. Sample preparation and analysis

Ten grams of chopped and blended fresh garlic sample was thoroughly mixed with 40 g anhydrous magnesium sulfate in a glass beaker immersed in an ice–water bath (the ice–water bath was used because anhydrous $MgSO_4$ mixed with water generates heat). After 5 min, the mixture was thoroughly ground on a porcelain mortar until a drying and homogeneous powder mixture was obtained. The mixture was designated as an SFE sample [7]. SFE was done using a 10-ml extraction cartridge packed with 5.00 g of SFE sample with 2×1 g $MgSO_4$ on the top as well as at the bottom of the cartridge. A 10-ml test tube, containing 5 ml of hexane, was used as the collection system. In the spiking experiment, adequate working standard mixture solutions were added to blank SFE sample in the cartridge and then the solvent was allowed to evaporate over 4 h. SFE was performed using conditions described in Section 3. This extractant solution was transferred onto a glass mini-column (20 cm×1 cm I.D.) packed with 2 g of $AgNO_3$-loaded Florisil, which was prewashed with 2 ml of hexane. The column was eluted dropwise with 10 ml of 20% ethyl acetate in hexane. The eluate was concentrated to drying under nitrogen at 60°C. A 1-ml volume of internal standard solution (0.3 μg/ml) was added and a 2 μl of portion of the

Fig. 1. GC–ECD chromatograms of SFE extractions of blank garlic. (A) No trapping material in the extraction cell; (B) using Cu powder as trapping material in the extraction cell; (C) using $AgNO_3$-loaded Florisil as trapping in the extraction cell; (D) off-line $AgNO_3$-loaded Florisil clean-up.

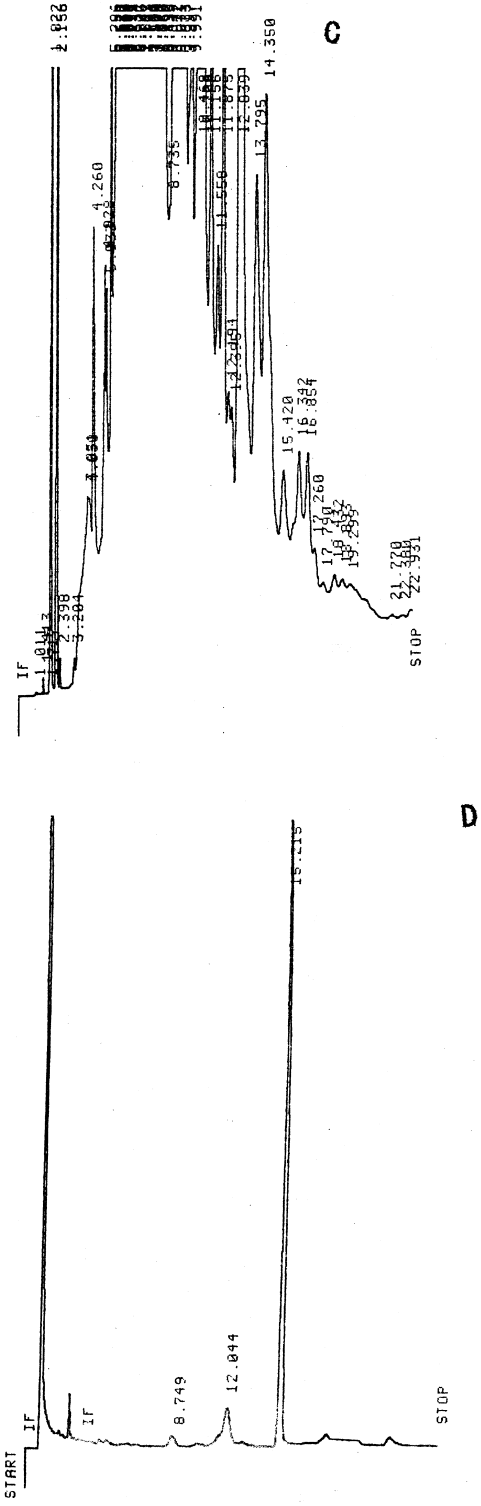

Fig. 1. (*continued*)

solution was analyzed by GC–ECD. Quantification of analytes was carried out using internal standard method.

3. Results and discussion

An SFE optimization study was carried out using 5.0 g $MgSO_4$ as inert support onto which working standard mixture solutions were fortified with the aim of determining the conditions that would provide maximum recovery. Instrumental parameters were varied to determine the effect of extraction conditions on extraction efficiencies: extraction fluid, CO_2; extraction pressure (20.2, 30.3 and 40.4 MPa), extraction temperature (40, 50 and 60°C), static time (1 or 2 min), CO_2 volume utilized (20, 25 and 30 ml). The experimental conditions were optimized using an orthogonal assay [3]. Optimized extraction conditions for organochlorine pesticides were as follows: extraction pressure, 30.3 MPa; extraction temperature, 40°C; CO_2 volume, 25 ml; and 1 min static time. Garlic contains ca. 80% water (as determined by oven drying at 105°C for 2 h), which can easily cause the cartridge outlet frit and capillary restrictor to plug, due to ice formation [9]. Anhydrous $MgSO_4$ was used as both sample dispersant and sorbent trap for water in vegetables [7] and experiments were conducted to determine the amount of anhydrous $MgSO_4$ required to remove water. The ratio of drying agent-to-sample was 4:1 (w/w). The saturation of the ECD chromatogram (Fig. 1A) of the SFE extracts from blank garlic indicated that serious interferences were present. Direct addition of solid-phase adsorbent (Cu or $AgNO_3$-loaded Florisil) to the SFE extraction cartridge to remove the organic sulfur interferences in garlic appeared not to be very effective. Strong sulfur interferences as shown by the huge background peaks were still apparent (Fig. 1B, Fig. 1C), indicating that certain residual sulfur-containing compounds existed in the extracts. In fact, the garlic odor could be smelled in the collection solution when adsorbents were not used or on-line adsorbents were used. The sulfur interfering peaks disappeared while the SFE extracts were reanalyzed after five days, which was consistent with that reported by Ling and Liao [6]. Hence, off-line clean-up methods were investigated. When only Florisil or

Table 1
Mean recovery and relative standard deviation of pesticides from spiked garlic at two fortification levels (n=5)

Pesticide	Concentration (mg/kg)	Mean recovery (%)	Relative standard deviation (%)
HCB	0.072	96.1	4.1
	0.036	105.0	5.2
Aldrin	0.100	89.0	4.4
	0.050	110.0	6.1
Diedrin	0.10	100.0	7.2
	0.050	89.7	3.9
Endrin	0.100	85.0	6.1
	0.050	93.1	5.1

Cu was employed, the interfering peaks were still observed in the chromatogram. When off-line $AgNO_3$-loaded Florisil was used, sulfur interfering peaks could be easily eliminated (Fig. 1D). When malathion and parathion, two sulfur-containing pesticides, passed through the $AgNO_3$-loaded Florisil column and were eluted with 20% ethyl acetate in hexane, they were not detected in the eluent. How-

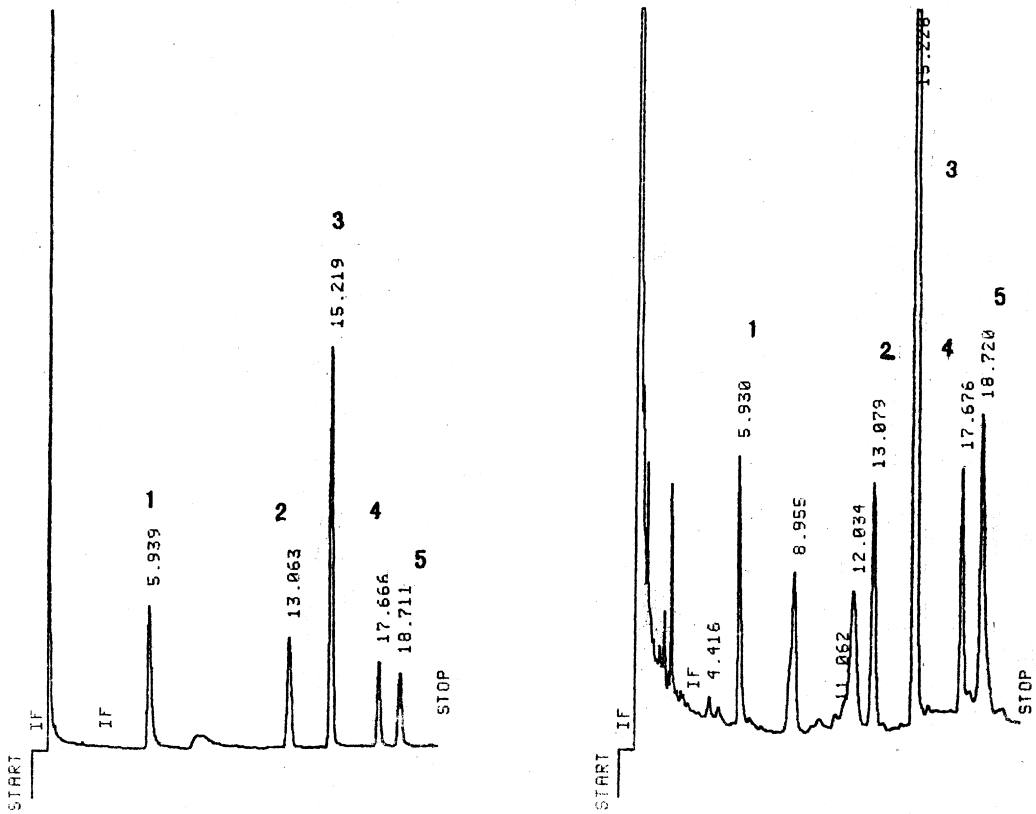

Fig. 2. Chromatograms obtained for standard pesticides (A) and 1-g aliquot of garlic fortified with 0.05 μg pesticides except for HCB (0.036 μg) (B). Peaks: 1=HCB; 2=aldrin; 3=heptachlor epoxide (I.S.); 4=diedrin; 5=endrin. Values at peak indicate retention times in min.

ever, malathion and parathion could be detected in the eluent when only Florisil was utilized. This proved that $AgNO_3$ was used off-line in removing elemental sulfur. $AgNO_3$-loaded Florisil column activity was checked in advance of each series of run by injecting four organochlorine standard mixture solutions.

Table 1 shows mean recoveries and repeatabilities obtained for the pesticides added to blank garlics. A spiked sample chromatogram is shown in Fig. 2B. As can be seen, all the pesticides investigated are recovered satisfactorily with values ranging between 85 and 110%.

In conclusion, the proposed approach resolves the problem of serious interferences of organic sulfur in monitoring garlic OCPs. False positive results can be avoided. The method may be used to analyze OCPs in garlic on a routine basis.

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