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Short Communication

Mixed adsorbent for cleanup during supercritical fluid extraction of three carbamate pesticides in tissues

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ABSTRACT

A mixed selective adsorbent composed of 7% diol and 93% C_{18} has been used in a cleanup column to remove the supercritical fluid lipid extractables from tissues containing carbamate pesticides. Fatty acid and sterols observed in supercritical fluid chromatographic analysis of supercritical fluid extracts of tissues were essentially eliminated. The supercritical fluid extraction recoveries of three carbamate pesticides (Bendiocarb, Methiocarb and Carbaryl) from chicken muscle ranged from 71 to 96%.

INTRODUCTION

The selective extraction of an analyte from a lipid containing material using supercritical carbon dioxide is extremely difficult. Supercritical carbon dioxide readily solubilizes lipid matter, including free fatty acids, glycerides, and sterols. Since co-extraction of the lipid matter is unavoidable and adversely affects the separation and detection of the analytes, procedures to cleanup the extracts are required before analysis. Murugaverl and Voorhees [1] have employed a solid-phase extraction procedure using a C_{18} column in conjunction with an on-line supercritical fluid extraction (SFE)–supercritical fluid chromatography (SFC) system to retain the co-extracted fat components while passing carbamate pesticides. Similarly, France *et al.* [2] have used a cleanup column packed with deactivated alumina or silica in an off-line application for the analysis of chlorinated pesticides in fat. Other investigators have also reported the use of post-extraction solid-phase cleanup columns [3,4]. Each of these analyses varied in the type of analytes and matrices used, but all employed an analytical scheme involving gas chromatography. The reported stationary phases used in the cleanup step in these studies have performed adequately on sample sizes usually less than 100 mg. For example, 5.7 ng of Bendiocarb in 8.7 mg of fat could be analyzed without significant interferences using the C_{18} cleanup column on the on-line system with flame ionization detection [1]. An increase in the fat sample size above 15 mg on this system resulted in significant interferences in the chromatography from the extracted fat components. The following paper

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discusses the development of a new mixed selective adsorbent that can be used for cleanup with SFE on tissue samples greater than 100 mg.

EXPERIMENTAL

Sample preparation

Tissue samples were chopped into very small pieces and then spiked with a standard solution containing about 1.5 μg/μl levels of Bendiocarb, Methiocarb or Carbaryl (Ultra Scientific) in methanol. The spiked muscle was then ground using a mortar and pestle. Hydromatrix (Varian) (30%, w/w) [5] was then added and grinding continued until a uniform consistency was obtained. All reported ppm and ppb values are w/w.

Cleanup column

Stainless-steel column blanks, 4 cm × 3 mm I.D. with 2 μm frits (Keystone Scientific), were partially packed with about 100 mg of a stationary phase containing 7% diol in C_{18} (Keystone Scientific). The cleanup column was flushed with supercritical CO_2 (Air Products) at 219 atm (1 atm = 101 325 Pa) and 90°C for 30 min. The tissue sample was then placed on top of the stationary phase in the cleanup column and attached to the supercritical fluid carbon dioxide.

Extraction condition

Tissue samples were extracted at 90°C and 219 atm for about 30 min, resulting in the use of about 4 ml of supercritical CO_2. In the on-line mode, the extracts were trapped in a cryogenic trapping device (Fig. 1), while in the off-line experiments, extracts were collected into 2 ml of ice-cooled methanol. A 25 μm I.D. fused-silica capillary restrictor was used in both cases. The methanol solutions from the off-line extractions were evaporated to dryness and then reconstituted to 100 μl with methanol for SFC–mass spectrometry (MS) analysis.

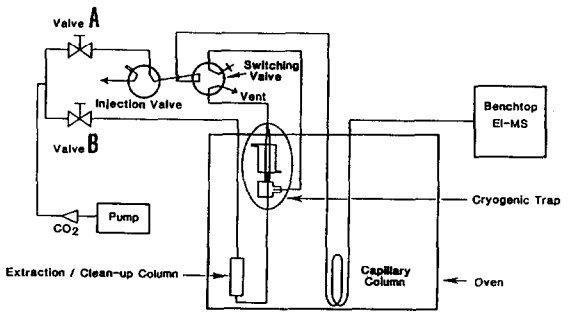

Fig. 1. The on-line SFE–clean-up–capillary SFC–benchtop EI-MS system.

SFC–MS

SFC analysis of off-line extracts was accomplished using a 1.5 m × 50 μm I.D. SB-Biphenyl-30 column (Dionex) with density programming of the CO_2 at 70°C from 0.2 g/ml to 0.7 g/ml at a ramp rate of 0.015 g/ml per min. Extracts were introduced into the SFC system via a Valco injection valve with a 60-nl sample loop. The mass spectrometer (Perkin-Elmer) was operated in the electron ionization mode either in the selected ion monitoring mode or in the full scan mode with a scan range of 75 to 250 u [6].

SFE–cleanup–SFC–MS

The on-line SFE–cleanup–SFC system has been previously discussed [1] and the schematic diagram of this system is shown in Fig. 1. This analysis employed the same Biphenyl column and operating parameters as previously discussed for the SFC–EI-MS analyses.

RESULTS

Several stationary phases including divinylbenzene–styrene, alumina, silica, C_{18}, cyanopropyl, amino and diol, were investigated in this study. Individually, none of these worked satisfactorily. The diol, amino, alumina and silica completely retained the carbamate pesticides investigated, while most of the other phases allowed too much fatty materials through the clean-up column. The optimum stationary phase for the cleanup of fat extracts was found to be a diol–C_{18} (7:93) mixture.

Fig. 2 shows an example of the interferences observed in a total ion chromatogram from 55 mg of beef muscle spiked with 5.5 ppm of Bendiocarb using the on-line system with a C_{18} cleanup column. Analysis of the interference peaks revealed that they consisted mainly of fatty acids and sterols. Selected ion monitoring

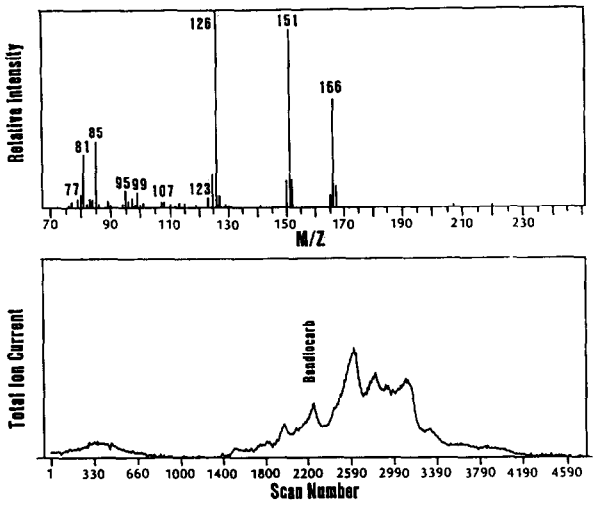

Fig. 2. The on-line SFE–clean-up–SFC–EI-MS total ion chromatogram of 5.5 ppm Bendiocarb in 55 mg beef muscle sample.

of the major Bendiocarb mass spectral peaks, m/z 126, 151 and 166, did not reduce the background signal. This level of interference inhibits a detection level much lower than the observed 5 ppm from Bendiocarb.

Fig. 3 shows the total ion chromatogram of 50 mg of beef muscle spiked with 4.4 ppm Bendiocarb run on the SFE–cleanup–SFC–MS system using a diol–C_{18} (7:93) cleanup column. A

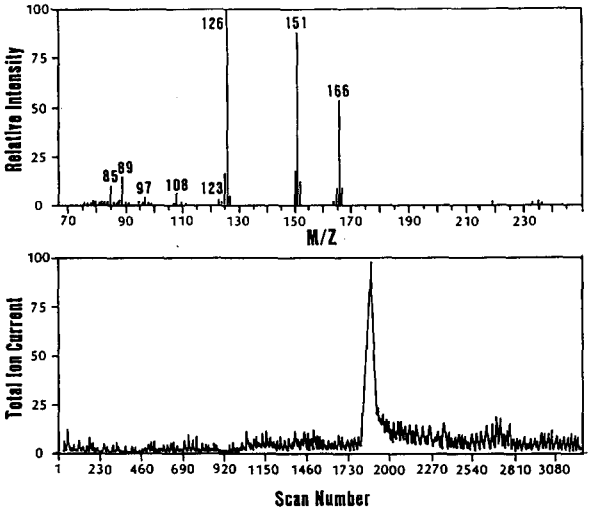

Fig. 3. Total ion chromatogram of the on-line analysis of 50 mg beef muscle sample spiked with 4.4 ppm of Bendiocarb using the diol–C_{18} mixed sorbent in the clean-up column.

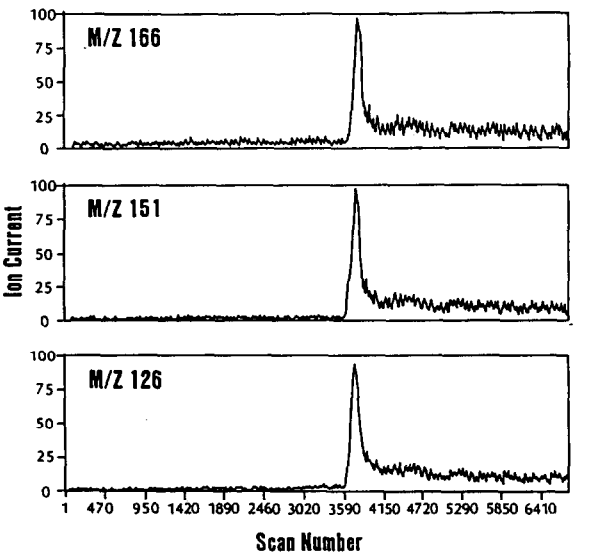

Fig. 4. On-line analysis of 75 mg beef muscle spiked with 1.5 ppm of Bendiocarb using the diol–C_{18} mixed sorbent in the clean-up column. MS operated in the selected ion mode.

comparison between Figs. 2 and 3 clearly shows a dramatic reduction of the background due to the fat components. Fig. 4 illustrates the selected ion trace of m/z 126, 151 and 166 for 1.5 ppm Bendiocarb in 75 mg beef muscle obtained under similar conditions. Using the on-line SFE–cleanup–SFC–MS, muscle samples ranging from 50 to 130 mg spiked with 1 ppm of Bendiocarb have been successfully analyzed. The detection limit for Bendiocarb for these experiments was about 200 ppb.

A series of off-line SFE experiments have been conducted using 95 to 150 mg of chicken muscle. Table I summarizes the means and R.S.D.s from selected ion chromatography results of a 100 mg chicken muscle sample spiked with 260 ppm of Bendiocarb and Carbaryl, and 180 ppm of Methiocarb. Because of the limitations of the injection loop (0.06 μl), higher concentrations of pesticides were used than would normally be analyzed with the on-line system. Using this injection volume, roughly 10 ng of each analyte was injected onto the column. The high R.S.D.s can be attributed to the mass spectrometer integration software. Because of the large peak areas, a slight change in the setting of the baseline by the operator had a

TABLE I

DATA FOR REPETITIVE SFC–MS RUNS OF CARBAMATE PESTICIDES IN 100 mg CHICKEN MUSCLE

	Bendiocarb	Methiocarb	Carbaryl
Sample mass 100 mg			
No. of runs	4	4	4
Mean peak area[a]	$1.91 \cdot 10^6$	$1.05 \cdot 10^6$	$1.41 \cdot 10^6$
R.S.D. (%)	17	22	24
Sample mass 150–300 mg			
No. of runs	5	5	5
Mean peak area[a]	$1.41 \cdot 10^5$	$7.30 \cdot 10^4$	$1.48 \cdot 10^5$
R.S.D. (%)	13	14	15

[a] All peak areas were normalized to a sample mass of 100 mg.

drastic effect on the calculated areas. No effort was made to optimize reproducibility. The signal-to-noise ratio for the peaks was about 20:1. Extraction efficiencies of 85% (Methiocarb), 96% (Bendiocarb) and 71% (Carbaryl) were calculated by comparing the individual means of the extract concentrations against an external standard.

Table I also summarizes similar mean and R.S.D. data for the repetitive off-line analyses using 150 to 300 mg chicken muscle samples spiked with the pesticides at 13 ppm Methiocarb, 19 ppm Bendiocarb and 19 ppm Carbaryl. Following cleanup and the other sample preparations, the quantities of pesticides injected in the 0.06 μl volume onto the SFC was 0.8 to 1.14 ng. A signal-to-noise ratio of 10:1 was observed for the SIM data. The calculated extraction efficiencies for Bendiocarb, Methiocarb and Carbaryl were 77, 80 and 83% respectively.

CONCLUSIONS

The combination of 7% diol and 93% C_{18} is an effective stationary phase for cleanup of supercritical fluid extracts of tissues containing carbamate pesticides. Extraction efficiencies between 71 and 96% were observed. Chicken muscle samples as large as 580 mg containing low ppm levels of pesticides were successfully extracted and then cleaned up using 100 mg of the diol–C_{18} stationary phase. Similar results were also observed for beef muscle.

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REFERENCES

1 B. Murugaverl and K.J. Voorhees, *J. Microcol. Sep.*, 3 (1991) 11.
2 J.E. France, J.W. King and J.M. Snyder, *J. Agric. Food Chem.*, 39 (1991) 1871.
3 K.S. Nam, S. Kapila, A.F. Yanders and R.K. Puri, *Chemosphere*, 23 (1991) 1109.
4 M.L. Hopper and J.W. King, *J. Assoc. Off. Anal. Chem.*, 74 (1991) 661.
5 N. Alexandrou, M.J. Lawrence and J. Pawliszyn, *Anal. Chem.*, 64 (1992) 301.
6 B. Murugaverl, S. Deluca and K.J. Voorhees, *J. Chromatogr.*, 633 (1933) 195.