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Derivatization reactions of carbamate pesticides in supercritical carbon dioxide

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Abstract Supercritical fluid carbon dioxide (SC-CO₂) has been used to dissolve derivatizing agents (e.g. heptafluorobutyric anhydride, HFBA, and pyridine), which also act as a modifier in the fluid phase, for simultaneous extraction and derivatization of carbamates from the sample matrix. The derivatized carbamate pesticides (carbaryl, 3-hydroxycarbofuran, carbofuran, aldicarb, methiocarb) were then analyzed by GC-ECD or GC-MS with excellent sensitivity. Extraction and conversion of the carbamates was complete, as indicated by HPLC with post-column hydrolysis and *o*-phthalaldehyde derivatization then fluorescence detection. GC-MS (ion trap) was also used to confirm the formation of the carbamate derivatives. Compared with the same HFBA reaction in an organic solvent the derivatization reaction time was considerably shorter in SC-CO₂. The described approach, combining both extraction and derivatization, simplifies the analysis of carbamate pesticides and eliminates the use of organic solvents associated with the derivatization step.

Keywords Carbamate · Derivatization · Extraction · Pesticide · Supercritical fluid

Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that might also be suitable.

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Introduction

The analysis of trace levels of pesticides in agricultural, food, and environmental samples is routinely performed by use of gas chromatography (GC) with hetero-element detectors such as the electron capture detector (ECD), high performance liquid chromatography (HPLC), or GC-MS (mass spectrometry). In GC methods the target pesticides are frequently derivatized to increase their volatility and/or their detectability, depending on the mode of detection used. Likewise, derivatization of the pesticide for HPLC analysis facilitates both separation and detection of the target analytes, particularly when using ultraviolet or fluorescence detectors [1, 2].

Derivatization of the analyte is, of course, only one step in the total analytical method and usually follows extraction, cleanup, and concentration of the analyte. In routine pesticide residue analysis extraction has traditionally been performed by using organic solvents for the extraction step. Over the last decade, because of concern about the effect of organic solvents on the environment and on laboratory personnel, other techniques have been developed to supplant the use of copious amounts of organic solvents in trace residue analysis [3]. Among these alternative techniques, supercritical-fluid extraction (SFE), with supercritical carbon dioxide (SC-CO₂), has been extensively investigated for pesticide analysis [4].

As noted by Hawthorne and King [5], additional benefits accrue when the analyte derivatization step is integrated into SFE. Indeed, several researchers have devised SFE/SFR (supercritical fluid reaction) methods, which have been recently reviewed by Fields [6]. Among some of the popular schemes for derivatizing analytes in the presence of supercritical-fluid media are the use of ion-pairing reagents [7, 8], silylation [9], formation of pentafluorobenzyl esters [10], transesterification to form methyl esters [11, 12] and the novel use of chelating agents for metal analysis [13]. In the last of these special fluorinated ligands have frequently been synthesized for the specific purpose of enhancing the solubility of the derivative in non-polar SC-CO₂ [14].

Exploiting SFR for analytical derivatization has frequently been done on an empirical basis with little optimization. This includes the choice of the best derivatizing agent and the optimum conditions for affecting derivatization for a particular class of analyte. In this study, five reagents were initially tested for their efficacy in the derivatization of carbamate pesticides. Heptafluorobutyric anhydride (HFBA) was found to be the best reagent when derivatization was performed in tandem with SC-CO₂ extraction of the target analytes, with identification of the resulting derivative by GC-MS. A standard HPLC post-column derivatization method was used to ascertain the completeness of the reaction and to facilitate comparison of the SFR method with derivatization performed in a typical organic solvent, benzene. Finally, the lower limit of detection afforded by the HFBA derivatization method was estimated by use of ion-trap GC-MS with specific-ion monitoring.

Experimental

Reagents

Derivatization reagents

Acetyl chloride, chloroacetic anhydride, heptafluorobutyric anhydride, hexamethyldisilazane, and trimethylchlorosilane were purchased from Sigma (St Louis, MO, USA). Trimethylphenylammonium hydroxide was obtained from Fluka (Milwaukee, WI, USA). Reagents for the described post column HPLC derivatization scheme were acquired from Pickering Laboratories (Mountain View, CA, USA). The carbon dioxide used for the SFE and derivatization studies was Air Products (Allentown, PA, USA) SFC/SFE-grade CO₂.

Carbamate pesticides

Carbaryl, 3-hydroxycarbofuran, carbofuran, aldicarb, and methiocarb (>98% purity) were obtained from either Chem Service (West Chester, PA, USA) or the EPA Repository (Research Triangle Park, NC, USA).

SFE and derivatization

All SFE and derivatization experiments were performed with a model SFX-2-10 extraction unit from Isco (Lincoln, NE, USA) using 10-mL, 316 SS extraction cartridges. An unheated restrictor calibrated to deliver 1.5 mL min⁻¹ fluid at 5,000 psig (35 MPa) was used in these experiments. The procedure used for HFBA derivatization of the carbamate test mixture was also used to test the derivatization efficacy of the other reagents. In general, the derivatization reagents were placed on a glass wool plug in the first part of the extraction cell and were separated by glass beads from another glass wool plug on to which was injected 50 µL of the carbamate pesticide mixture. The SC-CO₂+cosolvent then flowed over the initial glass wool plug containing the derivatization reagents, through the glass bead bed, and on to the glass wool plug containing the carbamate pesticides.

For the heptafluorobutyric anhydride-pyridine catalyst reaction methanol (30 µL)+HFBA (2 µL)+pyridine (2 µL) and the carbamate mixture (1-5 µg mL⁻¹, 50 µL) on glass wool were packed inside the extraction cartridge. The cartridge was held statically in the extraction cell for 30 min at 80°C and 5000 psig (35 MPa), with 1% (v/v) methanol as cosolvent. Dynamic SFE was then performed for 30 min at a CO₂ flow rate of 1.5 mL min⁻¹ through the

extraction cartridge and with extract and product collection in methanol. After SFE/SFR the methanol was evaporated to dryness, the residue was reconstituted in methanol (1 mL), and the solution was analyzed by GC-ECD and/or ion-trap GC-MS, or alternatively by use of the HPLC method described below. Additional experiments with HFBA were performed at 5000 psig (35 MPa), and 50 and 60°C, using a 4 h reaction time, and yielded similar results to those obtained at 5000 psig (35 MPa) and 80°C. Further optimization studies are discussed in the Results and Discussion section.

By use of a similar procedure acetyl chloride, chloroacetic anhydride, trimethylphenylammonium hydroxide, and a hexamethyldisilazane(HMDS)-trimethylchlorosilane(TMCS) mixture were also tested as derivatization agents under SC-CO₂ conditions. Derivatization with acetyl chloride was performed at 5000 psig (35 MPa) and 60 and 80°C, but for 20 min only, because it was apparent that the corrosive nature of the derivatization agent was damaging both the extraction cartridge and the extractor. Chloroacetic acid was also evaluated under similar conditions, and in benzene and *n*-hexane. Derivatization of the carbamates was also performed recorded by use of trimethylphenylammonium hydroxide in SC-CO₂ at 60°C and 5000 psig (35 MPa), for times ranging from 2 to 6 h. As has been noted in the literature [6], however, there is some debate about whether the reaction occurs before GC, or in the injection port of the GC itself. Derivatization with the mixed silane reagent was also performed at 5000 psig (35 MPa) and 60°C for 4 h.

Derivatization in organic solvents

For comparison with the behavior of the derivatization reactions in SC-CO₂ media, reactions were also performed in benzene and *n*-hexane as solvents. For derivatization in benzene the stock carbamate mixture solution in methanol (approx. 50 µL) was placed in a screw-cap test tube and the residual methanol evaporated in a flow of N₂. Benzene (3 mL) was then added with HFBA (50 µL) and two drops of pyridine. The tube was shaken well and then capped overnight. This reaction mixture was then analyzed by the ion trap GC-MS procedure described below. It was found that the aldicarb peak could not be detected by GC-MS unless a water wash was applied to the reaction mixture, because of interference from the HFBA peak. The procedure was performed identically except with *n*-hexane as reaction solvent, and yielded results identical with those obtained in benzene.

Analysis of carbamate pesticides by GC-MS

Carbamate pesticide derivatives were analyzed by GC-MS by means of a Saturn 4D ion-trap mass spectrometer (Varian, Palo Alto, CA, USA) coupled with a Varian model 3600 gas chromatograph and a model 8200 CX autoinjector (splitless mode). A DB-5MS column, 30 m length, 0.25 mm i.d., 0.25 µm film thickness (J&W Scientific, Folsom, CA, USA) was used with helium as carrier gas. The oven temperature used for the analysis started at 40°C for 1 min and was then ramped at 15° min⁻¹ to 250°C which was held for 1 min. The injector and transfer-line temperature was 150°C.

Analysis of carbamate pesticides by HPLC

An HPLC system consisting of a post-column derivatization module (Pickering Laboratories, Mountain View, CA, USA) and fluorescence detector was used to quantify carbamate pesticide derivatives and extractions performed using SC-CO₂, and conversions performed in benzene. The HPLC pumps (model P2000) and autosampler (model AS3000) were from Thermo Separations Products (San Jose, CA, USA). Acetonitrile and water were used as solvents for the gradient elution separation of the carbamates in conjunction with a reverse phase C₁₈ column (4.6 mm×150 mm, 5 µm particle - Pickering Laboratories). The HPLC mobile phase gradi-

ent was 18% (v/v) acetonitrile in water for 0.5 min when the acetonitrile content was programmed to 70% (v/v) for 31 min and then increased at 32 min to 80% (v/v) which was held for another 8 min. Sodium hydroxide and *o*-phthalaldehyde/thiofluor reagents were delivered from the pumps comprising the Pickering model PCX 5000XX module. Fluorescent detection was accomplished by use of an excitation wavelength of 330 nm with emission detection at 466 nm by use of a Thermo Separations model FL2000 detector. The HPLC injection volume was 20 μ L of a mixture of five carbamate pesticides in the concentration range 30–150 ppb.

Results and discussion

Assessment of the various derivatizing agents for carbamates under SC-CO₂ conditions led to the conclusion that derivatization with acetyl chloride was not possible, because of its highly corrosive action on the extractor parts and extraction cartridges. Although derivatives formed with chloroacetic anhydride in SC-CO₂ were identified for three of the five carbamate pesticides, the GC-MS total ion profiles were very complex, rendering the method questionable for the analysis. Although trimethylphenylammonium hydroxide formed the expected derivatives with the carbamates, this might be because of a reaction occurring in the GC injection port rather than in the SC-CO₂ media. Silylation yielded complex irreproducible chromatograms, possibly because of the sensitivity of the derivatizing agents to the presence of moisture. Only reaction with HFBA resulted in high conversion rates for the determination of carbamate pesticides, particularly when ion-trap GC-MS was used as the method of detection.

Table 1 shows the percentage conversion of the five carbamate pesticides to their HFBA derivatives in SC-CO₂ and benzene. Derivatization in SC-CO₂ was accomplished at 8000 psig (55 MPa) and 80 °C for 3 h. The pesticide derivatives formed in both SC-CO₂ and the organic solvent were quantified by use of the post-column HPLC method described above. Note that for all five pesticides conversion to the respective derivatives was >98% for triplicate runs (RSD=2.0%), for both benzene and SC-CO₂.

Using the HFBA reaction a series of derivatizations was performed at 60 and 80 °C at three different pressures – 3000 (20.7 MPa), 5000 psig (35 MPa), and 8000 psig (55 MPa) for 3 h, in triplicate (RSD=2.0%), to ascertain the effect of pressure on the derivatization reaction. The extent of conversion to the derivatives, relative to complete conversion in benzene, as a function of pressure at 60 and 70 °C, is shown in Figs. 1 and 2, respectively. At

Table 1 Percentage conversion of carbamates to HFBA derivative in SC-CO₂ and benzene

Pesticide	SC-CO ₂ ^a	Benzene
Aldicarb	98.7	98.5
Carbofuran	98.6	98.8
3-Hydroxycarbofuran	98.3	98.3
Carbaryl	98.3	98.6
Methiocarb	98.5	98.9

^a8000 psig (55 MPa), 80 °C, 3 h

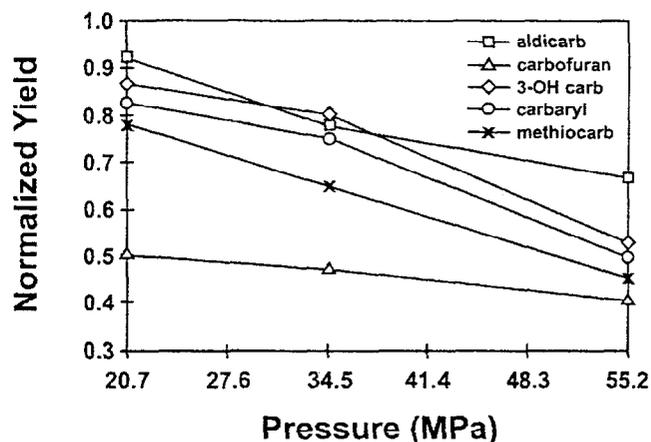


Fig. 1 Yield of the HFBA carbamate derivatization reaction at 60 °C, in 3 h, as a function of pressure (normalized to the yield obtained in benzene)

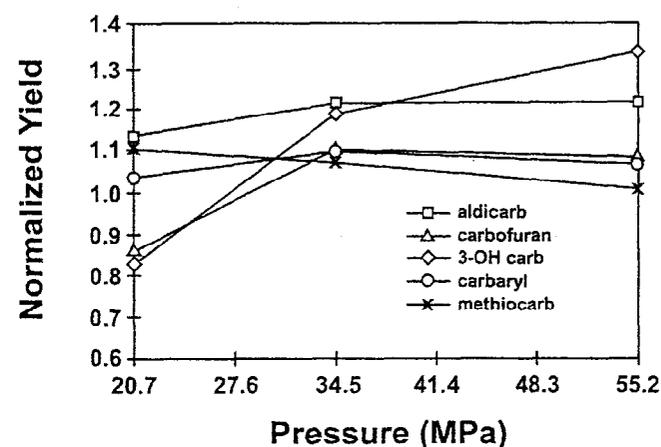


Fig. 2 Yield of the HFBA carbamate derivatization reaction at 80 °C, in 3 h, as a function of pressure (normalized to the yield obtained in benzene)

60 °C increasing the CO₂ pressure resulted in lower conversion to the HFBA derivative (Fig. 1); the same was also true over the same range of pressures for HFBA derivatization at 40 °C. These results, although somewhat surprising considering the often-held belief that application of pressure should always increase the rate of reaction and hence the extent of conversion, are, however, consistent with recent findings by Oakes et al. [15], who found that the rate of reaction is optimum at a specific pressure, rather than just increasing as the applied pressure of SC-CO₂ was increased.

Figure 2 shows that at 80 °C increasing the pressure of the SC-CO₂ increases the conversion of the carbamate derivatives, and at pressures >4500 psig (31 MPa) conversion even exceeds that in benzene. On the basis of these data the optimum conditions chosen for HFBA derivatization were 80 °C and 8000 psig (55 MPa). Under these conditions it was found that halving the reaction time to 1.5 h did not reduce the conversion for four out five of the carbamate pesticides.

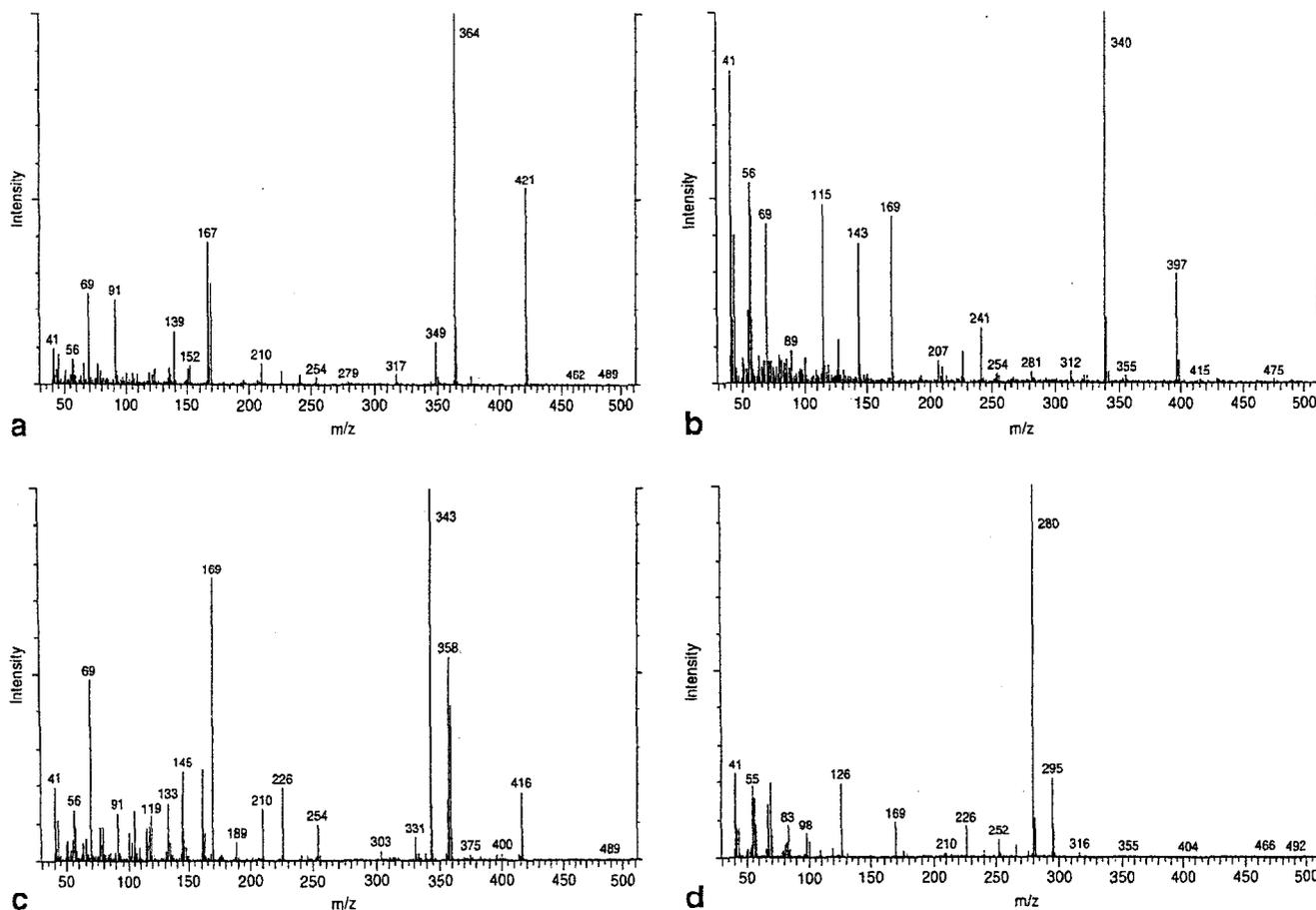


Fig. 3 Mass spectra of: (a) methiocarb HFBA derivative, (b) carbaryl HFBA derivative, (c) 3-hydroxycarbofuran HFBA derivative, and (d) aldicarb HFBA derivative

To verify the identities of the HFBA derivatives formed in SC-CO₂, mass spectra were acquired; they are presented in Figs. 3a, 3b, 3c, and 3d. Figure 3a shows the mass spectrum of the HFBA derivative of methiocarb. The molecular ion peak is apparent at $m/z=421$; the most intense ion fragment in the spectrum is, however, at $m/z=364$, loss of 57 amu representing cleavage of the $-\text{CH}_3\text{NC}=\text{O}$ group. Similar spectra were also recorded for the HFBA derivatives of carbaryl (Fig. 3b) and 3-hydroxycarbofuran (Fig. 3c). Molecular ions are apparent at $m/z=397$ and 416 for the HFBA derivatives of carbaryl and 3-hydroxycarbofuran, respectively. For these two compounds the most intense peaks in the mass spectra occur 57 amu less than the molecular ion and corresponding to the same loss of the $-\text{CH}_3\text{NC}=\text{O}$ group as recorded for methiocarb. In addition, cleavage of an additional $-\text{CH}_3$ group from the HFBA derivative of 3-hydroxycarbofuran is apparent at $m/z=343$. Finally, the HFBA derivative of aldicarb (Fig. 3d) yields peaks at $m/z=295$ and 280, the latter being the most intense in the spectrum.

These molecular or most abundant ions obtained for the carbamate HFBA derivatives provide the basis for quantification of carbamates by ion-trap GC-MS. As shown in Fig. 4, derivatization with the HFBA reagent can

produce spurious peaks in the total ion chromatogram obtained from ion trap GC-MS. By single-ion monitoring for the specific ions, however, the individual HFBA derivatives of the carbamate pesticides can be detected and quantified, as is shown in Fig. 4. GC-MS detection limits obtained by use of this approach are comparable with those of the HPLC fluorescence derivatization method described above. The lower limits of quantification for the five HFBA carbamate pesticide derivatives using the GC-MS-HFBA derivatization method in SC-CO₂ are

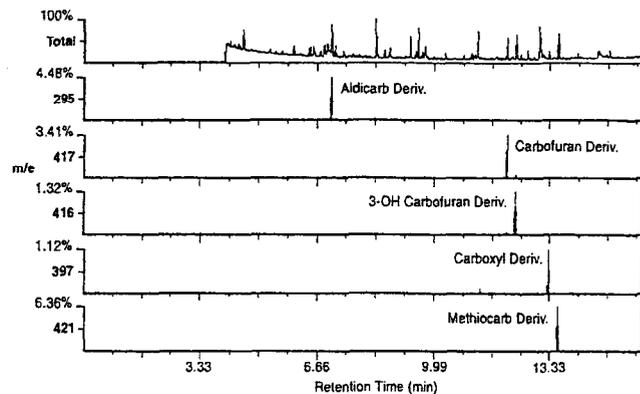


Fig. 4 GC-MS of the five carbamate HFBA derivatives obtained by SFE/SFR

Table 2 Limit of quantification (LOQ) by GC-MS, by use of the molecular ion (M+) and the most abundant ion, after HFBA derivatization in supercritical carbon dioxide

Pesticide	LOQ (most) ^a	LOQ (M+) ^a
Aldicarb	0.48	1.2
Carbofuran	4.2	8.4
3-Hydroxycarbofuran	4.3	15.0
Carbaryl	3.7	22.0
Methiocarb	5.4	14.0

^ang mL⁻¹

given, in ppb, in Table 2, where limits of quantification (LOQ) [16] are presented both for the most abundant ion peak in the spectra and the molecular ion. LOQ obtained by use of the most abundant ion peak in the mass spectra enable detection from 0.48 to 5.4 ppb whereas LOQ obtained by use of the molecular ion range from 1.2 to 22 ppb. The carbamate HFBA derivatives synthesized in SC-CO₂ have been found to be stable in *n*-hexane (the SFE/SFR collection and injection solvent) for at least 6 h. Reproducibility for this GC-MS method ranged from 3–10% RSD (relative standard deviation).

Conclusions

Derivatization reactions in SC-CO₂ should be chosen carefully to ensure their compatibility with the conditions used for SFE of the target analytes. The optimum reagent, reaction conditions, and final analytical method must all be considered when integrating a derivatization reaction into a coupled SFE/SFR scheme. Certain combinations of extraction pressure and temperature might be deleterious to formation of the derivative desired, and this should be

determined initially on neat analytes before attempting derivatization in-situ. Formation of the HFBA derivatives of carbamate pesticides in SC-CO₂ is a feasible method for ppb-level determination, particularly when using ion trap GC-MS as the final analytical technique. Use of the most abundant ion in the mass spectrum of the derivatives enables a lower limit of quantitation ranging from 0.5–5.0 ppb.

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