ising from differences in magnetic susceptibility. Spinning side bands sometimes appear (especially in ^1H spectra), but they can be pushed out of the displayed spectral window by readjusting the rotation speed. Addition of proton-free powder (Al_2O_3, sulfur, etc.) is a helpful experimental hint for better balancing of the spinner and faster rotation.

The use of the high-field instrument is certainly helpful, if the signal to noise ratio must be increased; however, it appears superficial for determination of linoleic acid using ^1H NMR. Since the important peak at 2.78 ppm can be resolved also in lower magnetic fields, it is possible to develop cheaper instruments for large-scale routine measurements.

Registry No. l, 60-33-3; o, 112-80-1; linolenic acid, 463-40-1.

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Received for review November 3, 1987. Revised manuscript received June 28, 1988. Accepted August 3, 1988.

Determination of Fenvalerate in Seawater and Sediment Utilizing Isotopic Dilution and GC/MS

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Fenvalerate, a pyrethroid insecticide commonly applied to many South Carolina agricultural products, is a challenging subject for analytical determination. Exhibiting an exceptional tendency to adsorb onto many surfaces, this analyte resists reproducible trace analysis. This research demonstrates the advantages of using isotopic dilution in a gas chromatography/mass spectrometry (GC/MS) method. Quantitative determinations of fenvalerate in seawater and estuarine sediment represent practical applications of this procedure. Analysis of fenvalerate in seawater at 8 μg/L was performed with an RSD (n = 8) of 3.32%. Concurrently, the syntheses of two deuterium-labeled analogues of fenvalerate are presented.

The controversy encountered in the last 20 years regarding the use of DDT and other chlorinated insecticides has prompted a search for alternative methods of insect control (Carson, 1962; Sanders, 1975; Georghiou and Saito, 1983). There have been many innovative approaches including the use of insect hormones, naturally occurring insecticidal agents, audio disrupters, and electronic insect killers (Quraishi, 1977; Janes, 1985; Mandara, 1985). One class of natural insecticides was determined to be particularly attractive for agricultural development. Pyrethrins I and II (1, 2), first isolated from *Chrysanthemum cinerariaefolium* in 1924, were found to exhibit exceptional potency (Elliott, 1974). These compounds rapidly penetrate insect cuticle and immobilize the pest. This property coupled with efficient toxicity makes these natural products desirable models for a new breed of pesticide. Several synthetic derivatives have been constructed that introduce requisite commercial features such as low volatility, photostability, and even greater toxicity. Fenvalerate (3), a first-generation synthetic analogue, has been proposed for use in South Carolina as a substitute for the banned pesticide toxaphene.

A substantial amount of information regarding the environmental fate of fenvalerate has been reported (Shell, 1984; Reed et al., 1983). Photolytic degradation is the principal vehicle by which the pesticide is depleted after

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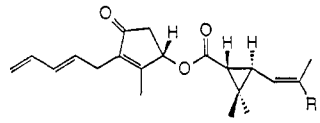
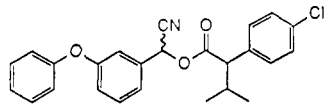


1, R = CH_3, Pyrethrin I

2, R = CO_2CH_3, Pyrethrin II



3, Fenvalerate

application. A significant environmental threat posed by the use of this insecticide is the pronounced ichthyotoxicity. The LC_{50} value for fenvalerate is reported to be 0.008–5.4 μg/L for a range of representative estuarine inhabitants (Schimmel et al., 1983). This observation necessarily dictates a sensitive analytical technique.

The current applied method relies on the use of a gas chromatograph/electron capture detector (GC/ECD) as the principal instrument. Fenvalerate is quite sensitive to electron capture detection, and quantities as low as 5 pg have been detected (Shell, 1978). Metabolites that do not possess the chloride functionality, however, are not detected. This technique also suffers from a lack of precision at the lower levels of detection. Additionally, inconsistent calibration curves have been encountered in the application of GC/ECD to fenvalerate (Wegscheider, 1985).

These disadvantages have prompted this investigation into the application of mass spectrometry to the detection of fenvalerate. The advent of bench top GC/MS units has transformed this instrument into a universal technique. As a result, the development of an effective GC/MS method would be beneficial. This detector is sufficiently sensitive and allows implementation of isotopic dilution. This modification supplies an ideal internal standard to enhance the precision of the method. Confirmation of peak identity is also easily obtained by a computer library match of acquired spectra. This capability could prove to be critical in litigation involving fish kills.

The analytical problems associated with fenvalerate are rooted in its adsorptive behavior. This phenomenon undermines analysis due to adsorption to glass in sample collection bottles and glass-lined GC injection ports. Capillary columns also become a source of sample loss when the stationary phase becomes degraded. This effect, fortunately, may be rendered inconsequential by the application of an internal standard to the analysis. Isotopic dilution has been applied with success to a variety of problematic analyses (Coutts et al., 1979; Mee et al., 1976; Faull et al., 1979; Sjoquist et al., 1979). Integrated results from fragmentograms of selected ions from each species afford the desired quantitative information.

PROCEDURE

Gas Chomatography/Mass Spectrometry. The separations for these analyses were achieved through a Model 9610 Finnigan gas chromatograph equipped with an HP-5 (5% phenyl, 95% methyl) fused silica capillary column (25 m × 0.20 mm, d_f = 1.0 μm). Splitless injection (60-s delay) of a 1-μL sample was followed by 1.8 μL of isooctane. Helium (at 10 psi) was the carrier gas used in these experiments. The carrier gas velocity was calculated at 40 cm/s, on the basis of a hold up time of 63 s. Silanization of the injection port inserts is essential in trace determinations. This was accomplished by submersion of a clean, dry insert into a 10% solution of dichlorodimethylsilane in toluene. The column temperature was increased from 100 to 300 °C at 15 °C/min until 250 °C and then 10 °C/min to 300 °C. The injection port temperature was maintained at 350 °C.

The mass spectra were obtained on a Finnigan a 4521C quadrupole mass spectrometer. The spectrometer was tuned with perfluorotributylamine as the calibration gas. Four ions were selectively monitored over 0.5-s intervals under EI conditions. When the isopropyl-labeled compound was considered, the mass units scanned were 167, 174, 419, and 426 amu. The phenyl-labeled analogue was detected at 225, 228, 419 and 422 amu. Methane (at 0.1 Torr) was the ionization gas selected in the NCI experiments.

Analytical Reagents and Materials. Baker C-8 solid-phase extraction columns (200 mg) and Waters Florisil Sep-Pak cartridges (1000 mg) were used in the extraction process. The experimental procedures detailing the synthesis of each labeled analogue are presented in the supplementary material. Seawater samples were taken from the North Inlet Estuary, Georgetown County, SC, on Nov 22, 1986. Sediment samples from a typical salt marsh were obtained from the marsh surrounding the North Inlet Estuary. The soil was collected at the interface of two geologically different types of soil. The soil is best characterized as a mixture of Leon Sand and Bohicket silty clay loam (Stuckey, 1982).

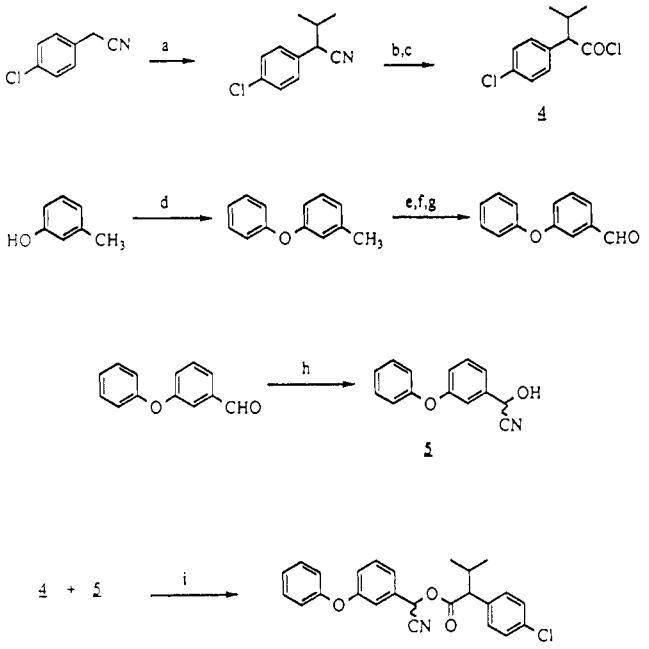
Sample Preparation. *Seawater.* A 100-mL sample of seawater was spiked with 1 mL of a solution containing 800 μg/L nonlabeled fenvalerate. One milliliter of the isotopically labeled specimen (2000 μg/L) was subsequently introduced to the same sample. This solution was well mixed and added to a C-8 extraction column conditioned with 2 mL of methanol followed by 2 mL of distilled water. After the sample had been added, the column was washed with 4 mL of distilled water. The column was then air-dried, and the fenvalerate was eluted with 2 mL of 1:1 hexane–diethyl ether. This eluate was evaporated to dryness under reduced pressure, and the residue was then redissolved in 100 μL of isooctane. A calibration curve was constructed by subjecting varying quantities of fenvalerate to the above procedure.

Salt Marsh Sediment. Fifty grams (50 g) of sediment was spiked with 1.6 μg of nonlabeled fenvalerate and 2.0 μg of labeled fenvalerate. After being mixed well, the sediment was extracted with 100 mL of 1:1 acetone–hexane. The beaker containing the soil and the extraction solvent was submersed in an ultrasonic bath and sonicated for 3 min. The mixture was mixed thoroughly, and the sonication was repeated. The supernatant was decanted and filtered (Whatman No. 1 qualitative filter paper) into a 250-mL round-bottom flask. The residue was extracted with an additional 100 mL of 1:1 acetone–hexane solution, and the two extracts were combined. The extract was concentrated under reduced pressure to approximately 20 mL. Hexane (100 mL) was added to the concentrate, and the resulting solution was again evaporated to dryness. This soil extract was then added to a Florisil Sep-Pak cartridge preconditioned with hexane (20 mL). The column was subsequently washed with 30 mL of hexane, which was discarded. The cartridge was then allowed to dry. The fenvalerate was isolated by elution with 1:1 diethyl ether–hexane (2 mL). The elution solvent was evaporated to dryness under reduced pressure, and the pesticide was dissolved in 100 μL of isooctane.

RESULTS AND DISCUSSION

Synthesis. A versatile route incorporating deuterium

Scheme I[a]



[a] Key: (a) *t*-BuOK, DMSO, NaI, *i*-PrOMs, rt, 36 h; (b) 68% H_2SO_4, reflux, 8 h; (c) $SOCl_2$, reflux 4 h; (d) PhBr, *t*-BuOK, DMSO, 90 °C; (e) NBS, CCl_4, reflux; (f) hexamethylenetetramine, $CHCl_3$, rt; (g) HCl, HOAc, reflux; (h) KCN, EtOH, HOAc; (i) pyridine, hexane, 0 °C.

in either the isopropyl or the phenoxy group was desired. This would readily result in labeled samples of the principal metabolites. The protons at these positions are also nonexchangeable, thereby ensuring the isotopic integrity. The synthesis should ideally introduce the deuterium atoms through relatively inexpensive starting materials. These objectives were attained by pursuing the pathway depicted (Scheme I). Deuteriated bromobenzene-d_5 and 2-propanol-d_8 provided low cost sources of isotopic label.

Method Development. Liquid–liquid partitioning is widely employed in the extraction of fenvalerate from water. More complex samples, such as organic extracts from soil, are clarified by liquid–solid chromatography using Florisil as the adsorbant (Shell, 1978). In this study, these methods were surrendered in favor of solid-phase extraction (SPE) techniques. Sample preparation by SPE decreased both the cost and the analysis time. Reversed-phase SPE columns have been used to concentrate many organic analytes from aqueous samples (Andrews and Good, 1982). Adequate purification of the soil extracts was easily be achieved by using Florisil SPE columns. Comparison of the two extraction techniques to external standards revealed recoveries ranging from 75 to 105%.

The absolute sensitivity of the mass spectrometer was assessed by a variety of operating techniques. Fenvalerate standards were analyzed via electron impact (EI) and negative chemical ionization (NCI) mass spectrometry. Initial investigation of the instrument detection limits indicated that EI analysis was superior to NCI. This conclusion was based on the fact that there was no significant increase in sensitivity with NCI. In each case, multiple ion detection (MID) was also employed. When mass data are acquired at only selected mass windows, the sensitivity and selectivity are enhanced. Inspection of an EI spectrum of fenvalerate (Figure 1A) reveals three high-mass fragments (419, 225, 167) that are relatively intense. The NCI spectrum (Figure 2A) exhibits two potentially useful ion fragments (211,167). It is important to note that the masses derived from NCI correspond to

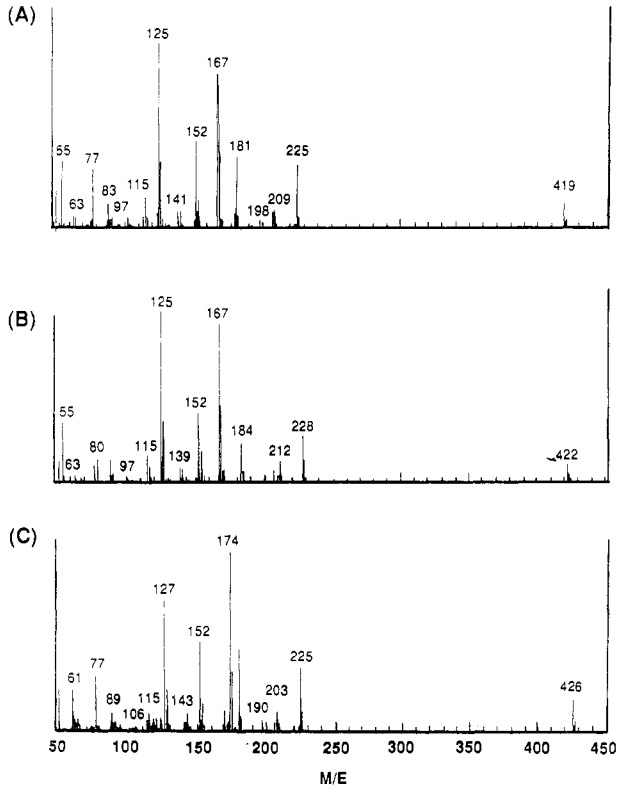


Figure 1. EI spectra: (A) fenvalerate; (B) fenvalerate-d_3; (C) fenvalerate-d_7.

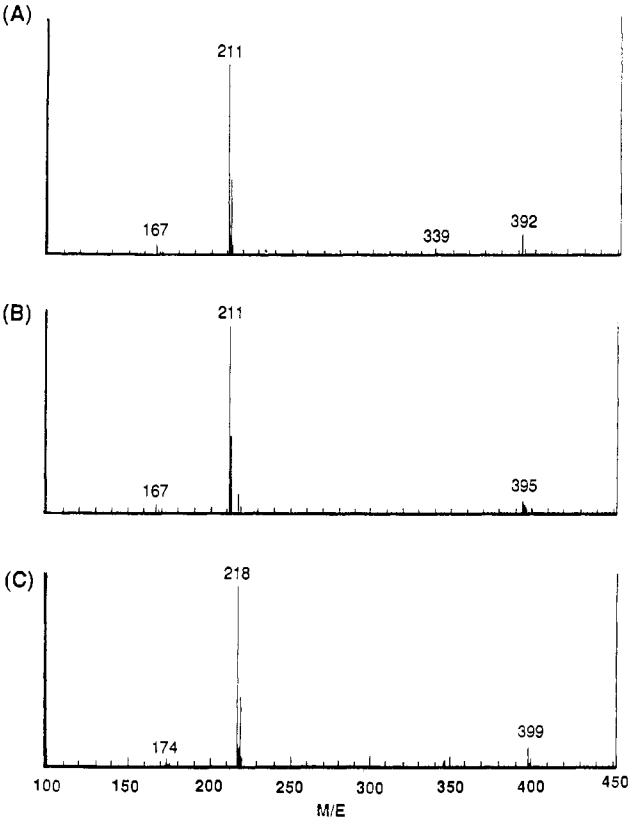


Figure 2. NCI spectra: (A) fenvalerate; (B) fenvalerate-d_3; (C) fenvalerate-d_7.

the portion of the molecule containing the isopropyl group. This would preclude the application of the phenyl-labeled material to NCI determinations. These principal mass fragments (167, 211, 225, 419 amu), as well as the isotopic counterparts, are the ions of interest in the analysis. Matrix interferences may be minimized by utilization of

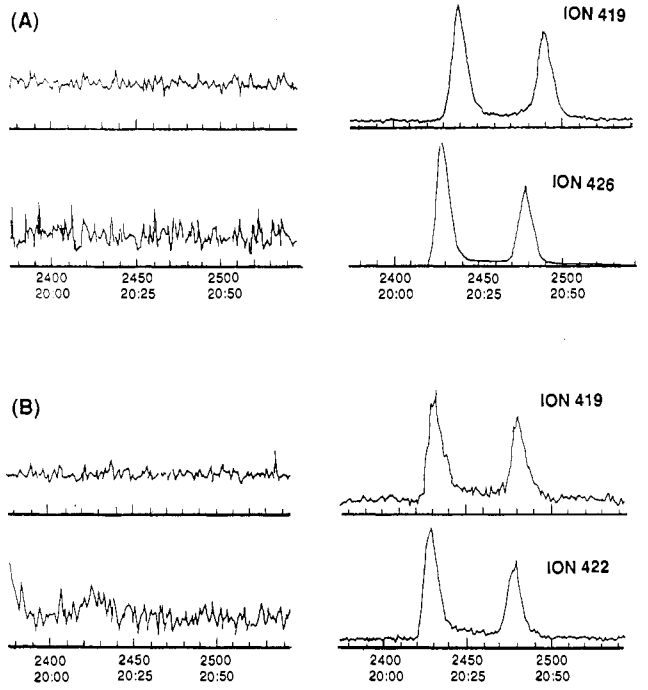


Figure 3. Representative fragmentograms of seawater: (A) spiked to 8 μg/L fenvalerate and 20 μg/L isopropyl-labeled fenvalerate; (B) spiked to 16 μg/L fenvalerate and 20 μg/L phenyl-labeled fenvalerate. The corresponding blanks were amplified by a factor of 10.

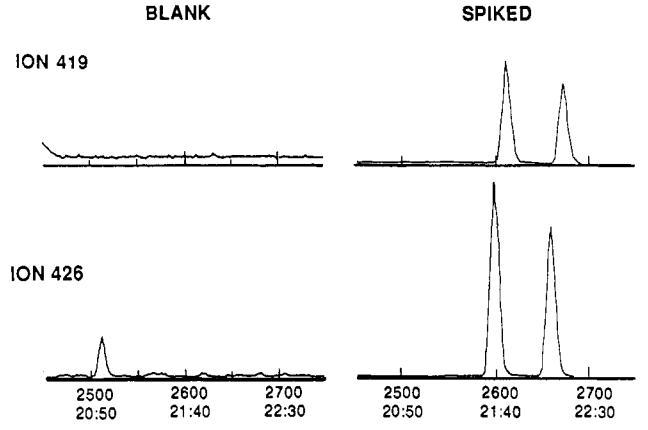


Figure 4. Estuarine sediment spiked to 320 μg/L fenvalerate and 400 μg/L isopropyl-labeled fenvalerate. The blank signal is amplified by a factor of 10.

the parent ion peak in quantification. Another disadvantage of the NCI technique is that the NCI spectrum does not offer a high-mass fragment. In instances where the blank demonstrates no conflicting ion responses, however, the lower masses may be successfully exploited.

A series of calibration standards was prepared by spiking seawater samples (100 mL) with a constant weight of the labeled and a varying amount of the nonlabeled fenvalerate. The pesticide was then isolated in the manner described previously. GC/MS analysis under EI conditions and selected ion monitoring gave fragmentograms of each ion. Figure 3 displays examples of nonlabeled samples spiked with isopropyl- and phenyl-labeled fenvalerate. These fragmentograms were integrated, and the results were compared. The diastereomers were base-line separated by the capillary column, and both peaks were used in quantification. It is interesting to note that even the isotopic analogues are separated by the column. Curiously, the deuteriated derivatives elute earliest. The area of the nonlabeled fenvalerate peak (e.g., 419) was divided by the

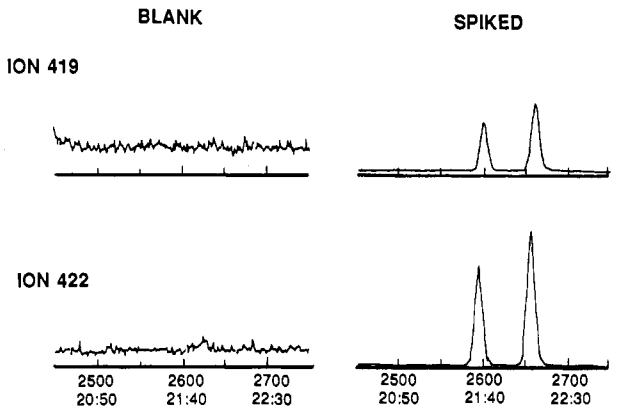


Figure 5. Estuarine sediment spiked to 320 μg/L fenvalerate and 400 μg/L phenyl-labeled fenvalerate. The blank signal is amplified by a factor of 10.

Table I. Replicate Analysis of Seawater Samples Using Isopropyl Label

sample no.	ratio (area 419/area 426)	area 419	concn, μg/L
1	0.231	35546	6.38
2	0.225	25357	6.16
3	0.225	26832	6.16
4	0.234	30809	6.49
5	0.236	40269	6.57
6	0.219	21259	5.95
7	0.227	39470	6.24
8	0.233	52734	6.46

Table II. Replicate Analysis of Seawater Samples Using Phenyl Label

sample no.	ratio (area 419/area 422)	area 419	concn, μg/L
1	0.679	23886	14.7
2	0.734	16383	15.8
3	0.758	14296	16.3
4	0.669	20382	14.5
5	0.566	14165	12.5
6	0.703	14754	15.2
7	0.699	18783	15.1

area of the corresponding labeled peak (e.g., 426, in the isopropyl case). This relationship was plotted against the quantity of the nonlabeled material to give the calibration plots. The method of least squares was used to fit a line to the data points.

Method Application. Replicate analyses of fenvalerate in seawater were performed, investigating each isotopic analogue separately. At a concentration of 8 μg/L, fenvalerate was determined on the isopropyl-labeled spike with a relative standard deviation (RSD, n = 8) of only 3.32% (Table I). Application of the phenyl-labeled standard resulted in an RSD (n = 7) of 8.17% at 16 μg/L (Table II). During the course of this research the analytical problems described previously became evident. In fact, while the isotopic ratio data listed in Table II display impressive precision, the absolute area of the nonlabeled chromatographic peaks fluctuated significantly. If the quantification had been conducted without the benefit of the internal standard, the RSD would have increased from 3.32% to 29.8%. Similarly, the 8.17% observed in the phenyl-labeled series would have inflated to 21.0%.

To extend this method to estuarine sediment, a sample was obtained from a South Carolina salt marsh. Analysis of unspiked extracts indicated that the high-mass regions were unobstructed by coextractants. As expected, however, the lower masses were frequently adulterated. When parent ion peaks were monitored, sediment samples spiked to 320 μg/kg gave useful chromatograms (Figures 4 and

5), employing both the isopropyl- and the phenyl-labeled compound.

The development of this method constitutes a substantial advantage in the quantitative determination of fenvalerate. The mass spectrometer offers adequate sensitivity and selectivity. The utilization of selected ion monitoring negates many undesirable interferences. The application of the isotopic dilution technique yields reproducible results at concentrations near the LC_{50} values of many organisms. The application of the technique to the non-chlorine-containing metabolites is plausible, as the synthetic pathway also yields labeled metabolites. As a result, this GC/MS procedure presents an effective alternative to external standard methods.

ACKNOWLEDGMENT

We thank Dr. Terry Bidleman for many helpful discussions.

Registry No. 3, 51630-58-1; H_2O, 7732-18-5.

Supplementary Material Available: Detailed experimental procedures and the corresponding spectral data of the labeled and nonlabeled products (7 pages). Ordering information is given on any current masthead page.

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