Determination of Thiazopyr and Its Metabolite in Florida Groundwater Using Graphatized Carbon Solid Phase Extraction and Liquid Chromatographic Determination

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A study comparing two liquid−solid extraction methods for the analysis of thiazopyr and its monoacid metabolite in groundwater is reported. Method A is a modification of a method developed by the Monsanto Co. which is based on liquid−solid extraction with silica-based octadecyl (C_{18}) extraction disks followed by gas chromatographic (GC) determination. Method B was developed in our laboratory and is based on liquid−solid extraction with graphatized carbon black followed by high-performance liquid chromatographic determination. Using method B, the parent and acid metabolite of thiazopyr are analyzed simultaneously without the need for metabolite derivatization. Subsequent GC confirmation and quantification are achieved through solvent exchange and derivatization of acid metabolite using the same sample extract. Recovery studies at 1.0, 5.0, and 10.0 ppb indicated excellent percent recovery for thiazopyr, 88 ± 13, 95 ± 3, and 92 ± 5; and for thiazopyr monoacid, 104 ± 10, 100 ± 6, and 101 ± 3, both respectively. Method detection for each compound is 1.0 ppb.

Keywords: *Thiazopyr; thiazopyr monoacid; groundwater; leaching potential; graphitized carbon black*

INTRODUCTION

Thiazopyr [2-(difluoromethyl)-5-(4,5-dihydro-2-thiazolyl)-4-(2-methylpropyl)-6-(trifluoromethyl)-3-pyridinecarboxylic acid methyl ester, THZ] will be sold as Visor by Rohm and Haas. It is a relatively new herbicide originally developed by Monsanto Agricultural Co. that has use against annual grasses and certain broadleaf weeds. The herbicide is efficacious if applied preplant incorporated, preemergent, or early postemergent. The rate of application is a maximum of 1.0 lb/acre on citrus. This compound's low application rate, in conjunction with its excellent water solubility, presents a twofold problem for Florida's environmental regulation professionals: the ability to detect residues at low parts per billion levels and the imminent threat to Florida's groundwater resource in areas of the state that have uniquely permeable soil types (Caldwell and Johnson, 1982).

A 2-year field study of THZ was conducted under the guidelines outlined in Florida's Draft State Management Plan (SMP). As part of the SMP, registration of new or reformulated pesticides and herbicides must undergo a field study if the target compound meets certain criteria outlined in a Hazard Assessment Study (Britt et al., 1992; *Pesticide Registration Guidelines and Procedures*, 1991). Additionally, methodology for analysis in various environmental matrices must be provided by the manufacturer. The groundwater analysis method provided by Monsanto utilized solid phase extraction (SPE) of THZ and its major monoacid (MA, demethyoxylated) metabolite (Fuhrman, 1993). Our laboratory was not equipped with the sophisticated instrumentation called for in the Monsanto method. Consequently, we were forced to develop alternate procedures for determination of these compounds. Attempts at classical liquid−liquid extractions (LLE) for THZ and MA were complicated by bifurcating procedures for each compound. Hence, the LLE approach was dismissed as too labor intensive. Two other approaches were subsequently explored. A modification of the Monsanto method, which successfully demonstrated the use of bonded silica C_{18} SPE for compound extraction, was developed. In addition, we explored the use of graphitized carbon SPE as another alternative for the compounds extraction.

Use of graphatized carbon black (GCB) SPE cartridges has been described for the analysis of environmental water samples for a variety of pesticides and herbicides (Di Corcia and Marchetti, 1991). In a subsequent work, Di Corcia and Marchetti (1992) demonstrated the utility of these columns by extending the capability to 89 pesticides including base-neutral and acidic compounds. The GCB material has two major advantages over the most common silica-based bonded phase materials. First, there are no silinol interactions because compound adsorption is based solely on carbon. Accordingly, organic compounds containing carbon backbones will adsorb readily to the materials surface. However, this can be an unfortunate disadvantage in that adsorption is nonselective, which, consequently, may limit the utility of the material for analysis of certain environmental matrices such as soil and/or vegetation. Fortuitously, groundwater in Florida does not contain appreciable concentrations of dissolved organic interferences. The second major advantage of the GCB material deals with its ability to retain organic acids. Ironically, the ability to extract ionizable compounds is apparently afforded by oxygen contamination within the GCB material (Di Corcia et al., 1980; Campanella et al., 1982; Andreolini et al., 1987; Crescenzi et al., 1995). These investigators have suggested that the mechanism of retention is related to oxygen con-

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S0021-8561(96)00841-2 CCC: $14.00

tamination that, due to material processing, results in the formation of complexes similar to benzpyrylium salts, capable of retaining acidic compounds. The combination of these two characteristics afforded us the opportunity to extract both the THZ parent and the MA metabolite simultaneously.

EXPERIMENTAL PROCEDURES

Materials. The pesticides chosen for this study were provided by the manufacturer. Individual standard solutions were prepared by dissolving 100 mg of each pesticide in 100 mL of ethanol and subsequently dilution to working concentrations of 10 and 1 ng/μL in methanol containing individual and mixtures of like pesticide compounds.

Octadecyl (C_{18}) SPE disks, 47 mm, available from Varian Analytical, were fitted in a glass SPE manifold containing a stainless steel support available from Kontes (Vineland, NJ). GCB (120/400 mesh), ENVI-carb SPE cartridge with 250 mg bed and 3 mL tube size was commercially available from Supelco Inc. (Bellefonte, PA). Cartridges were fitted into a standard 24 port SPE vacuum manifold also available from Supelco Inc. Vacuum for both apparatus was supplied by the laboratory's house vacuum system.

Analytical water (polished water, 18.3 MΩ) was obtained by purifying distilled water through an E-pure (Barnstead/Thermolyne Corp., Newton, MA) system. Solvents used (acetonitrile, methanol, methylene chloride, iso-octane, ethyl acetate, and acetone) were all of Optima grade, from Fisher Scientific (Atlanta, GA). Reagents included SPE eluting solution (0.016 M KOH in methylene chloride/methanol), made by mixing 0.9 g of KOH into 1.0 L of methylene chloride/methanol (60:40), and GCB conditioning solution, 2% glacial acetic acid solution, made by adding 2.0 mL of acid into a 100 mL volumetric flask and diluting with polished water. Trimethylsilyldiazomethane (2.0 M in hexane) was used as the derivatization agent for GC confirmation and is available from Aldrich Chemical Co. (Milwaukee, WI).

Well water was collected in clean empty 1 L amber collection bottles stored at 4 °C until analysis. Lysimeter water (soil water) was collected in a similar fashion and analyzed depending on volume available.

Procedure. Two methods were developed for extraction of thiazopyr and its monoacid metabolite.

Method A is a modification of the Monsanto method (Fuhrman, 1993). Prior to extraction, well water samples were allowed to come to room temperature and were shaken to ensure complete mixing and suspension of the sample. A 1.0 L sample was acidified to pH 2 with 1.0 N HCl. The C_{18} disk was conditioned through the successive additions of 10 mL of methylene chloride/ethyl acetate (50:50), 10 mL of methanol, and finally 10 mL of analytical water. The disk was prevented from drying during the latter two washings. The sample was allowed to pass through the disk by inverting the collection bottle over the apparatus reservoir and allowing gravity to create a seal during extraction. Sufficient vacuum was applied to allow the sample to pass at the rate of 100 mL/min. Following sample addition, the sample bottle was removed and the disk allowed to aspirate for an additional 5 min. The reservoir was removed and the disk rolled into a cylinder and placed into a clean 15 mL screw-cap culture tube containing ca. 1.0 g of anhydrous powdered sodium sulfate. Five milliliters of methylene chloride/ethyl acetate (50:50) was added to the culture tube and sonicated for 10 min to allow dissolution of the target analytes into the extraction solution. The extract solution was transferred to a graduated conical centrifuge tube. An additional 2.0 mL of eluting solution was used to rinse the culture tube and then added to the centrifuge tube. The sample was evaporated to dryness under a stream of nitrogen at 37 °C and reconstituted to 1.0 mL with absolute ethanol. A 0.5 mL portion was withdrawn and placed in a 2.0 mL GC vial along with 0.75 mL of *N*,*N*-dimethylformamide diethyl acetyl (DMF-DEA). One microliter of sample was injected into a GC fitted with split alternate columns and electron capture detectors.

For method B the ENVI-Carb GCB cartridges were conditioned by washing sequentially with ca. 6 mL of 0.016 M KOH in methylene chloride/methanol (60:40), ca. 5 mL of methanol, ca. 3 mL of 2% acetic acid solution, and finally 3 mL of analytical water. The cartridges were prevented from drying during the addition of the latter three solutions. Prior to extraction, samples were allowed to come to room temperature and were shaken to ensure complete mixing and suspension of the sample. A 75 mL reservoir was attached to the cartridge before sample addition, and a 200 mL aliquot was allowed to pass through the bed at the rate of 100 mL/min. Following sample extraction, the cartridge was allowed to aspirate for an additional 5 min to remove excess water. Thiazopyr was eluted into a 13 mL graduated centrifuge tube by adding 1.0 mL of methanol to the column bed and carefully applying enough vacuum to result in a dropwise flow. The MA was eluted by dropwise flow into the same 13 mL tube by two successive 3.0 mL additions of 0.016 M KOH in methylene chloride/ethyl acetate (60:40). Three hundred microliters of 2% acetic acid was added to the collection tube and mixed well. The organic layer was evaporated under a stream of nitrogen at 37 °C. (Note: the organic layer must be removed to avoid liquid chromatographic interferences. Approximately 300 μL of volume should remain.) The sample was reconstituted to 1.0 mL with methanol, and 20 μL of the sample extract was subsequently injected into an HPLC apparatus.

GC Confirmation Method. The remaining sample extract from method B was transferred to a 13 mL graduated centrifuge tube and evaporated to dryness under a stream of nitrogen at 37 °C. The sample was reconstituted in 0.9 mL with iso-octane/ethyl acetate (50:50) and 50 μL of methanol and 10 μL of trimethylsilyldiazomethane in hexane (Supelco) were added. The contents of the tube were mixed well, and 30 min was allowed for derivatization. One microliter was injected into a GC apparatus for confirmation.

GC Apparatus. An AutoSystem 9000 equipped with split/splitless injector and dual electron capture detection (ECD) was used to determine residues from the method A extract. A 5 m, phenyl-bonded guard column was connected in series from the injector to a quartz Y split (Restek Corp., Bellefonte, PA). DB-5 and DB-17 analytical columns (J&W Scientific, Folsom, CA), both 30 m × 0.025 mm id, 0.25 μm film, were then connected to respective EC detectors. Sample extracts were autoinjected with retention times and peak areas obtained using a fully automated PE Nelson TurboChrom integrated software (Perkin-Elmer, Norwalk, CT) program.

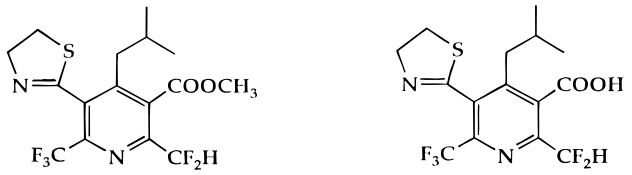
Temperature programming consisted of initial temperature of 80 °C held for 1 min then increased to 180 °C at a rate of 9 °C/min, held for 2 min; increased to 190 °C at a rate of 2 °C/min, held for 9 min; and, finally, increased to 270 °C at a rate of 10 °C/min, held for 15 min, for a total run time of 56 min. A five-point linear standard curve was constructed from peak areas to calculate herbicide residues detected in the well water samples and control spikes.

LC Apparatus. A Model 9012 (Varian, Walnut Creek, CA) LC pump equipped with Model 9100 autosampler and Model 9065 photodiode array detector was employed for the GCB/LC method. A 25 cm × 4.5 mm id, 5 μm, LC-C18-DB analytical column (Supelco) fitted with a μ-Bondapak C_{18} guard column insert (Waters, Milford, MA) was used to resolve incurred sample residues. A five-point linear standard curve was constructed from target analyte peak areas to calculate residues detected in well water samples.

The mobile phase consisted of a programmed gradient, initially with 50% acetonitrile and 50% 0.025 M phosphoric acid in water, programmed linearly to 75% acetonitrile in 18 min, held for 2 min, and then returned to 50% acetonitrile. A 20 μL injection, monitored at 220 nm, and a flow rate of 1.2 mL/min allowed separation of both parent and acid metabolite test compounds within 25 min.

RESULTS AND DISCUSSION

Our laboratory's contribution to this study was to ascertain the level and distribution of THZ and MA residues that may potentially leach into groundwater



Thiazopyr (methyl ester) **Thiazopyr Monoacid**

Figure 1. Structures of THZ and the MA metabolite.

from product application. Figure 1 illustrates the chemical structures of THZ and MA. Hydrolysis of the methyl carboxylate ester to the carboxylic acid was previously demonstrated to occur during environmental fate studies conducted by Monsanto. Therefore, analytical strategies utilizing GC for the detection and quantification the MA compound required derivatization, other than methylation, of the MA metabolite. For this reason the methodology developed by Monsanto (Fuhrman, 1993) employed ethylation of MA metabolite with DMF-DEA prior to GC analysis with a Fisons Trio-1 GC/MS/DS in SIM and negative CI mode, alternately. Use of this instrumentation allowed the analytes to be detected at 0.02 ppb. Fortunately, there was no urgent requirement for our laboratory to achieve a detection limit this low, since the health advisory limit for the study was initially set at 175 ppb. Our laboratory group decided that a 1.0 ppb method detection limit (MDL) would be sufficient to detect residues from the product's low rate of application (1.0 lb/acre). Method A was developed, as a modification of the Monsanto method, to meet the 1.0 ppb MDL. These procedures were coupled with the GC determination procedures mentioned above.

A number of problems were encountered with method A. Figure 2 illustrates the result of 1.0 μL injections of standard, blank, and fortified control (spiked) samples on a DB-17 analytical column. Standard injections indicated no interferences. However, laboratory blank and spike chromatograms were difficult to interpret at the trace level ($\leq$1.0 ppb) and resulted in higher MDLs for sample analysis. Similar chromatograms were obtained from 1.0 μL injections on the DB-5 analytical column. Excessive background noise throughout the blank and spiked chromatograms was determined to result from the DMF-DEA derivatizing agent. Some of the reagent peaks coeluted with the ethylated MA, resulting in difficult confirmation on the DB-17 analytical column (see Figure 2B,C). Furthermore, use of DMF-DEA required labor intensive maintenance of GC injector and detectors, resulting in excessive instrument down time. In addition, recoveries indicated large variances in observed laboratory spiked control samples. Table 1 indicates method performance at three fortification levels for THZ and MA. Although the standard deviations (SD) at the 1.0 ppb level did not exceed reported guidelines used by the laboratory (Horwitz, 1983), the SDs of the 5.0 and 10.0 ppb levels were unacceptable for an analytical method.

The difficulties experienced with method A prompted us to investigate another approach for analysis of the target compounds. Our goals were to improve extraction recovery performance and obtain improved sample cleanup. Di Corcia and Marchetti (1991, 1992) previously reported successful extraction of a number of pesticides and herbicides in water and environmental

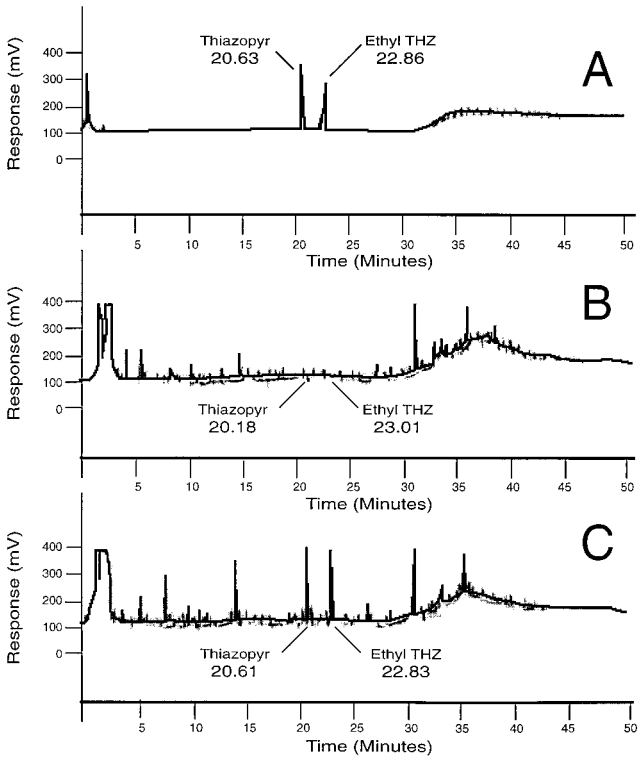


Figure 2. Chromatograms obtained from 1.0 μL injection onto DB-17 analytical column of (A) 0.5 ng/μL mixed THZ and MA, (B) reagent blank, and (C) 5.0 ppb laboratory control spike.

Table 1. Method Performance Comparison for THZ and MA Metabolite[a]

pesticide	spike level (ppb)	% recovery	SD	CV%
method A				
THZ	1.0	83	±22	27
	5.0	68	±30	44
	10.0	72	±32	44
MA	1.0	76	±24	32
	5.0	62	±28	45
	10.0	73	±43	59
method B				
THZ	1.0	88	±13	15
	5.0	95	±3	3
	10.0	92	±5	5
MA	1.0	104	±10	10
	5.0	100	±6	6
	10.0	101	±3	3

[a] Mean values and standard deviations calculated from $n = 16$ determinations.

water using GCB SPE cartridges with LC determination. The ability to extract both ionic and nonionic species simultaneously proved to be an attractive advantage of the GCB material. Of particular interest were the results achieved with acidic pesticides. For example, recoveries for the chlorophenoxy acid herbicides ranged from 95 to 100% with SDs ranging from 2.7 to 5.1 (Di Corcia and Marchetti, 1992). This was important since the MA metabolite presented the greatest challenge to extraction and could be achieved without pH adjustment of the sample. Consequently, procedures for the simultaneous extraction of the THZ parent and MA metabolite were developed. Concurrent with the development of the extraction procedures, our laboratory group also tested the possibility of employing an LC method, with UV detection, as a viable alternative for the compounds' determination. The goal was to eliminate the need for derivatization of MA and improve the quality of the chromatographic determination.

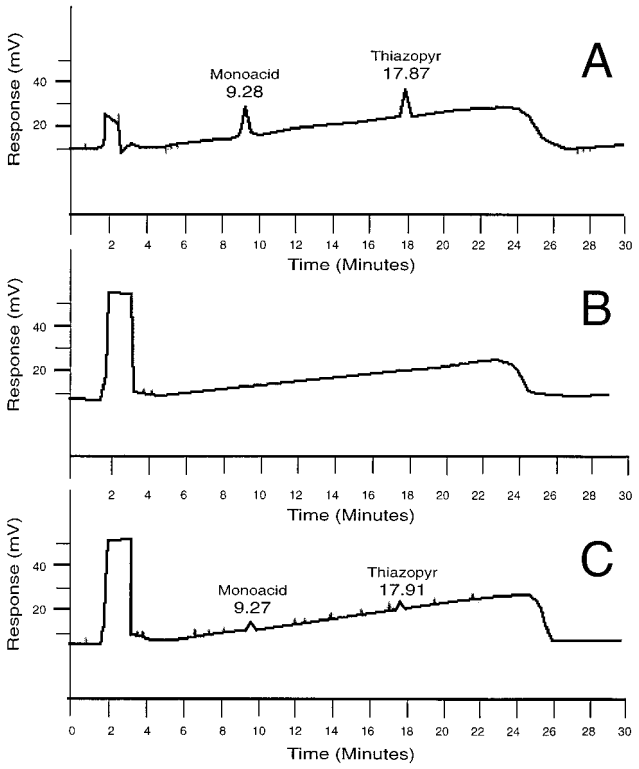


Figure 3. Chromatograms obtained from 20 μL injection onto C18-LC-DB analytical column for (A) 1.0 ng/μL mixed THZ and MA, (B) reagent blank, and (C) 1.0 ppb laboratory control spike.

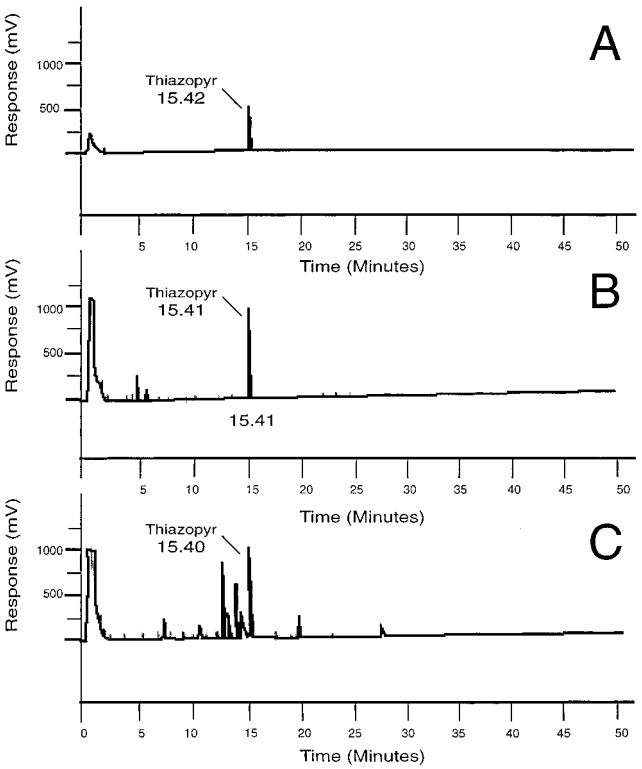


Figure 4. Chromatographic profiles from the GC confirmation method determination procedures and derivatization with trimethylsilyldiazomethane reagent. Chromatograms were obtained from 1.0 μL injections of (A) 0.5 ng/μL THZ standard, (B) 5.0 ppb methylated MA laboratory control spike, and (C) well water sample 5 confirmed to contain 5.0 ppb of MA residue.

Table 2. Real Sample Comparison between Methods A and B for Positive Detections of THZ MA[a]

	sample no.						
method	1	2	3	4	5	6	7
A	7.0	11.0	5.1	4.9	0.8	4.8	2.8
B	8.3	13.6	7.3	8.1	4.7	9.4	5.6

[a] Samples analyzed by both methods simultaneously.

Success was achieved through the combination of method B extraction procedures and the LC apparatus determination procedures. Figure 3 shows the result of standard, blank, and spike analysis using this method. These chromatograms clearly indicate the improvements achieved through this method combination. Method performance results for method B are also found in Table 1. This method shows excellent recovery and coefficient of variation (CV%) for the test compounds.

To compare the efficacy of the two methods, simultaneous extractions and analyses using the two methods were performed independently on the same day. Table 2 contains the results of seven positive detections of MA found in the sample set. We were surprised to find that, in all cases, the results obtained from method B were greater than those from method A. This trend was also observed in two subsequent method comparison studies. These findings indicate an apparent weakness with method A that could present a problem when results at or near the MDL are reported. For example, the result obtained by method A for sample 5 would be reported as less than the MDL (<1.0 ppb). This would be incorrect, since the true value for this particular sample was confirmed to be approximately 5.0 ppb. In fact, samples 3−7 are additional indicators of the apparent weakness in method A.

Use of the PDA detector was hoped to provide spectral information to augment confirmation of incurred THZ and/or MA residues. Unfortunately, there were no distinctive wavelength maxima that could be exploited for monitoring purposes. This was somewhat of a disappointment since many compounds of structural similarity are known to have distinctive UV profiles. We therefore chose a wavelength of 220 nm for monitoring to achieve our stated MDL of 1.0 ppb. Consequently, the inability to use a UV spectra for confirmation prompted our laboratory group to investigate alternate procedures for confirmation of THZ and MA. We thought it prudent to attempt derivatization of MA and subsequent GC analysis using the extract obtained from method B. Methylation was chosen since it was known, and confirmed throughout the duration of the study, that the THZ parent rapidly degraded to the MA in the environment. These facts, and the initial analytical determination of samples, justified our choice of methylation for the MA compound. Of those derivatization agents tested, a trimethylsilyldiazomethane reagent proved to be the most successful. It was both easier and safer than the method employed for diazomethane generation described by conventional EPA methods (EPA Method 515.2, 1992). In addition, this reagent decreased chromatographic interferences and was not as aggressive to the GC apparatus as the DMF-DEA reagent used in method A. Figure 4 illustrates the results of the confirmation analysis for sample 5 of the method comparison study. The value determined for MA-methyl (thiazopyr) from these procedures was 5.0 ppb, which indicates excellent agreement with the method B determination of 4.7 ppb for this sample.

In summary, we have described procedures for the successful extraction and LC analysis of THZ and MA.

The inclusion of GC confirmation procedures using the same sample extract also enhances the utility of this method.

ACKNOWLEDGMENT

We thank the following for their mentorship: Dr. Lambros Tterlikkis, Dr. Magdi Soliman, and Dr. Thomas Fitzgerald. Also helping with the manuscript were Dr. Ashok Shahane and Mr. Wyndham Riotte. We give special thanks to Ms. Connie Holland, whose love, friendship, and concern made this work possible.

LITERATURE CITED

Andreolini, F.; Borra, C.; Caccamo, F.; Di Corcia, A; Samperi, R. *Anal. Chem*. **1987**, *59*, 1720−1725.

Britt, J. K.; Dwinell, S. E.; McDowell, T. C. *Environ. Toxicol. Chem*. **1992**, *11*, 721−728.

Caldwell R. E.; Johnson, R. W. General Soil Map of Florida. Soil Conservation Service of the U.S. Department of Agriculture, 1982.

Campanella, L.; Di Corcia, A.; Samperi, R.; Gambacorta, R. *Anal. Chem*. **1982**, *7*, 429−438.

Crescenzi, C.; Di Corcia, A.; Madbouly, M. D.; Samperi, R. *Environ. Sci. Technol*. **1995**, *29*, 2185−2190.

Di Corcia, A.; Marchetti, M. *Anal. Chem*. **1991**, *63*, 580−585.

Di Corcia, A.; Marchetti, M. *Environ. Sci. Technol*. **1992**, *26*, 66−74.

Di Corcia, A.; Samperi, R.; Sebastini, E.; Severini, G. *Anal. Chem*. **1980**, *52*, 1345−1350.

EPA Method 515.2. U.S. EPA Environmental Monitoring Systems Laboratroy, Cincinnati, OH, Aug 1992, revision 1.0.

Fuhrman, J. D. Monsanto Method RES-DRAFT, version 0, Monsanto Co., 1993.

Horwitz, W. *J. Assoc. Off. Anal. Chem*. **1983**, *66*, 1295−1301.

Pesticide Registration Guidelines and Procedures. Florida Department of Agriculture & Consumer Services: Tallahassee, FL, 1991.

Received for review November 5, 1996. Accepted May 8, 1997.[⊗]

JF960841I

[⊗] Abstract published in *Advance ACS Abstracts,* July 1, 1997.