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Journal of Chromatography A, 791 (1997) 119–126

JOURNAL OF CHROMATOGRAPHY A

Determination of carbendazim in soil and lake water by immunoaffinity extraction and coupled-column liquid chromatography–tandem mass spectrometry

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Received 17 December 1996; received in revised form 28 July 1997; accepted 28 July 1997

Abstract

A method employing a high-performance protein G immunoaffinity column coupled to a reversed-phase analytical column through the use of a trapping column and INTEGRAL Micro-Analytical Workstation for the extraction of carbendazim from lake water samples and soil extracts is described. Characterization of the target analyte is achieved by on-line mass spectrometric analysis. The specificity of immunoaffinity extraction makes it possible to detect low levels of carbendazim in soil samples without interference from matrix components. The use of selected reaction monitoring allows for the achievement of the highest possible sensitivity with a minimum of chemical interference. Carbendazim is enriched from soil extracts at the 100 ppb level. It is possible to detect trace levels (25 pptr) of carbendazim in lake water samples. This is probably due to fewer matrix interferences in water than in soil. Very little sample preparation is required for environmental water samples while a liquid–solid extraction method is required prior to immunoaffinity extraction for soil samples. The ability to pump sample through the protein G column at high flow-rates (10 ml/min) and to automate the column-switching procedure makes the method rapid and efficient. Multiple samples can be analyzed in a relatively short period of time with minimum sample preparation. With the described protocol, three soil or water samples can be analyzed per hour without operator intervention.

Keywords: Soil; Water analysis; Carbendazim

1. Introduction

Benomyl, methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate, has been widely used as a systemic fungicide for disease control in crops for many years. Its mode of action and movement in the environment, however, are not clearly understood [1,2]. The primary reason for this is because benomyl readily decomposes in many common organic solvents [3] and also in water [4,5] to carbendazim, methyl 1H-benzimidazol-2-ylcarbamate (MBC) [4]. Carbendazim itself is fungicidal and it is thought that the fungitoxicity of benomyl is due to the presence of carbendazim [6]. While not used in the United States, it has been registered for use in over fifteen countries [7]. In soil, the conversion of benomyl to carbendazim is believed to be rapid; therefore, analytical determinations for benomyl focus on residues of carbendazim [8]. Carbendazim is a moderately weak acid with a pK_a of 4.48 [9]. It forms complexes with

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0021-9673/97/$17.00
PII S0021-9673(97)00819-4

simple, hydrated and basic metal ions important in soil [10]. The importance of such complexation is not known [8]. However, adsorption of organic cations is generally strong and depends on soil composition, mineralogy and exchangeable ions [8].

It has been demonstrated that benomyl and carbendazim [11,12] as well as a variety of pesticides [13] can be determined using high-performance liquid chromatography (HPLC). However, sample preparation still took place off-line from the HPLC analysis and lacked specific characterization of the fungicides. Immunoaffinity extraction (IAE) has been employed for the determination of trace pesticides in environmental samples using ultraviolet (UV) detection [14,15]. Rule and Henion [16] and Rule et al. [17] have used IAE coupled with mass spectrometric detection to characterize drugs in urine and carbofuran in water and potato extract. The method involves delivering the sample through the antibody column where it interacts with the antibody forming antibody–analyte complexes. These complexes are then disrupted and the analytes of interest desorbed from the pre-column by lowering the pH of the wash solution and are flushed onto a second small column where they are trapped. The trapping column used in this work is packed with particles that have a semipermeable surface (SPS). The outer surface of the particle is hydrophilic while the inner surfaces are hydrophobic. This design allows large, unwanted compounds to pass through the column while small, nonpolar compounds will be trapped on the hydrophobic surfaces inside the particles. HPLC mobile phase is then pumped in the reverse direction through the trapping column and the trapped analytes are flushed onto the analytical HPLC column for separation. The work presented here demonstrates the application of automated on-line immunoaffinity extraction coupled with tandem mass spectrometric detection (IAE–LC–LC–MS–MS) for the ultra-trace determination of carbendazim in soil and lake water.

2. Experimental

2.1. Chemicals

The reference standard of carbendazim was purchased from the US Environmental Protection Agency (EPA) repository (Research Triangle, NC, USA). Methanol, acetonitrile and water of HPLC-grade, sodium phosphate, sodium chloride, sodium hydroxide and ammonium acetate were obtained from Fisher Scientific (Fair Lawn, NY, USA). Purified mouse monoclonal IgG_1, cell line 72S/10.3.9.85.7.5, was generously provided by Dr. Robin Charlton of E.I. DuPont de Nemours. Antiserum was diluted with phosphate buffered saline (PBS) consisting of aqueous 0.15 *M* NaCl and 0.01 *M* NaH_2PO_4 and stored at 4°C. Soil samples and lake water were collected from local resources.

2.2. Extraction of carbendazim from soil

The method used to extract carbendazim from soil was developed by Clemons and Sisler [4]. 20 ml of methanol were added to a 10 g soil sample and shaken. This suspension was then fortified at the 100 ppb level with authentic reference standard of carbendazim and shaken for 2 h at ambient temperature. The resulting solution was concentrated to dryness under vacuum in a Savant Instruments (Farmingdale, NY, USA) SpeedVac SVC100. Thirty ml of a MeOH–0.05 *M* NaOH (75:25) solution were added to the dried soil and this solution was shaken for 3 h. Five ml of a filtered aliquot of the soil solution was neutralized by diluting it 1:25 with 0.005 *M* HCl. Hundred ml of this solution were used for IAE. Adding salt in concentrations of 0.15 *M* NaCl and 0.01 *M* NaH_2PO_4 resulted in the solution having a similar ionic strength to the phosphate buffered saline to be used in the IAE. The addition of salt greatly improved the recovery of the extraction.

2.3. Preparation of lake water for IAE

Lake water was collected in a glass bottle and stored at 4°C. The only sample preparation required prior to IAE was suction filtration through a 0.45 μm Nylon-66 filter (Rainin, Woburn, MA, USA). Aliquots (150 ml) of filtered lake water were fortified at the 0, 10, 25, 50, 100 and 200 parts-per-trillion (pptr) levels. Again, salt was added to these solutions to adjust the ionic strength to that of PBS. 100 ml of the fortified and blank water samples were analyzed by on-line IAE–LC–LC–MS–MS.

2.4. IAE and HPLC

IAE and coupled column chromatography were achieved using a PerSeptive Biosystems INTEGRAL Micro-Analytical Workstation (Framingham, MA, USA) equipped with an autosampler, two reciprocating HPLC pumps, a reagent pump and two automated switching valves. Experiments involving UV detection used an Applied Biosystems (Ramsey, NJ, USA) Model 757 variable-wavelength absorbance detector set at 220 nm. The injection valve was fitted with a 5 μl sample loop. The extraction itself was accomplished on a 33×2.1 mm I.D. ChromatoChem Hi Pac Protein G column packed with 30 μm particles (Missoula, MT, USA) through which phosphate buffered saline was pumped at 2.0 ml/min by pump 1 (Fig. 1). Twenty μg of anti-MBC IgG were loaded onto the protein G column via the autosampler. A 10×3 mm I.D. Regis Technologies (Morton Grove, IL, USA) SPS C_{18} guard column with 5 μm particles was used as the analyte trapping column when the 2% (v/v) acetic acid stripping buffer was pumped through the protein G column. The antibody–analyte complex denatures and is desorbed from the protein G column. MBC is trapped on the retentive SPS C_{18} packing under the aqueous conditions while the unwanted protein is pumped to waste. The trapping column effectively acts as the ''injector'' for the analytical column when the valves are rotated and the flow of mobile phase is pumped across it in the reverse direction. Pump 2 continuously delivered MeCN–water (40:60), 10 m*M* NH_4OAc at a flow-rate of 200 ml/min through a Keystone Scientific (Bellefonte, PA, USA) Betasil C_{18} 150×2 mm I.D. column packed with 5 μm particles. The effluent from the column was directly transferred to the ion spray interface without a post-column split. While the selected reaction monitoring (SRM) LC–LC–MS analysis took place, the reagent pump delivered 20% (v/v) aqueous acetic acid to the

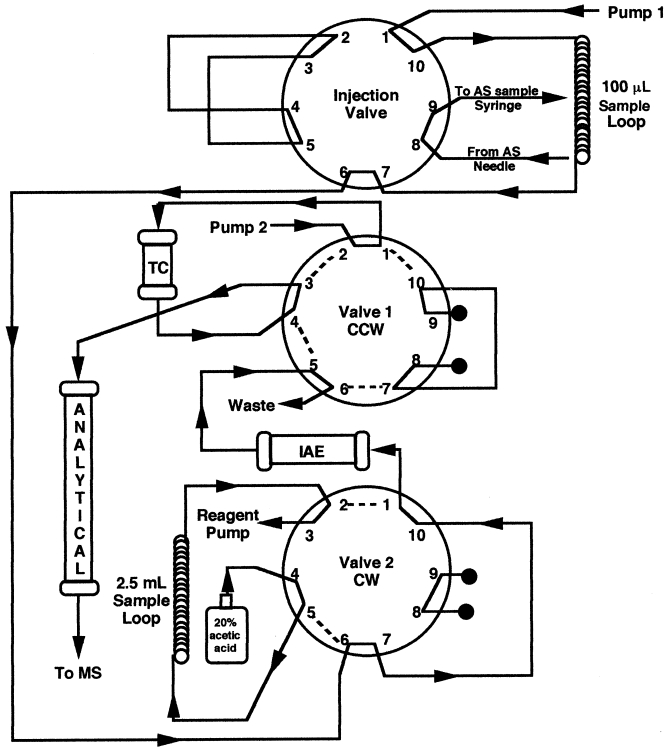

Fig. 1. INTEGRAL micro-analytical workstation. Switching valve positions and eluent flow paths for loading of antibody onto protein G immunoaffinity column.

protein G column to ensure that all protein on the column was stripped away and that it would be ready for the next run at the end of the current analysis.

2.5. Mass spectrometry

The mass spectrometer, a PE-Sciex API-300 (Thornhill, Ontario, Canada), was operated in the positive-ion detection mode. Collision-induced dissociation (CID) reactions were accomplished with nitrogen as the collision gas with a collision energy of 23 eV in the laboratory reference frame. The ion spray interface was maintained at 4.0 kV and received 200 μl/min from the chromatographic column effluent without a post-column split. SRM was based on the observation of the product ion *m/z* 160 derived from *m/z* 192 $[M+H]^+$ for MBC (Fig. 2). The dwell time for ions in the SRM mode was 2000 ms. Carbendazim was also introduced into the atmospheric pressure ionization (API) source by infusion of a 1 ng/μl standard solution in MeCN–water (40:60), 10 m*M* NH_4OAc at 8 μl/min using an infusion pump (Harvard Apparatus, South Natick, MA, USA) to obtain a representative product ion spectrum (Fig. 2).

3. Results and discussion

3.1. IAE

IAE involves molecular recognition for concentrating a trace amount of a substance from a large volume of sample. However, IAE alone is not powerful enough to separate the analyte of interest from the protein of the antibody or from more specific binding components once it has been desorbed from the protein G column. The effluent from the protein G column is chemically complex and requires further clean-up. Coupled-column liquid chromatography (LC) following IAE lends the method the selectivity required to tackle ultra-trace analysis. Fig. 3A shows the result of IAE–UV determination of 260 p*M* of the analytical standard of carbendazim that was directly injected onto the IAE column. It is obvious that due to background interference, carbendazim cannot be distinguished from the other components desorbed from the protein G column using UV detection. When a trapping column and analytical column are added to the sample analysis system, the chromatographic peak for carbendazim is readily apparent (Fig. 3B).

While coupled-column chromatography affords

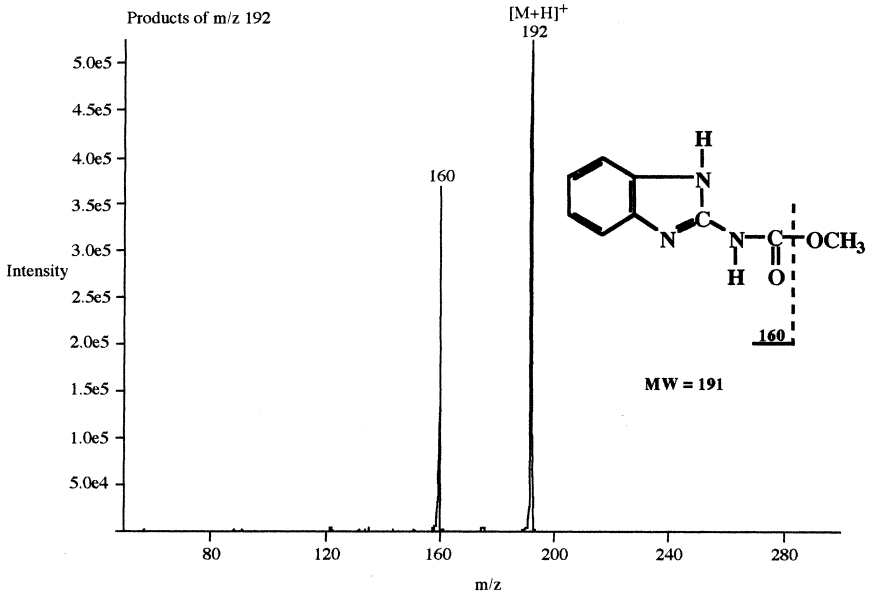

Fig. 2. Full-scan product ion spectrum from 50–300 u of carbendazim. For mass spectrometric conditions, refer to Section 2.5.

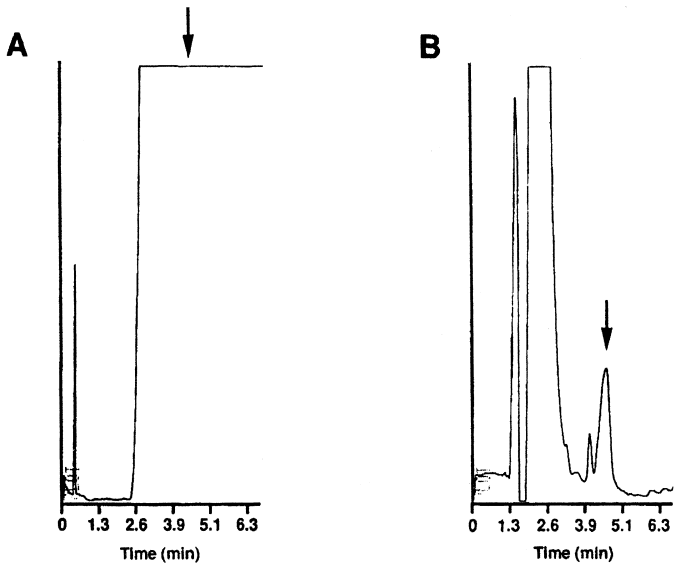

Fig. 3. (A) IAE–UV determination of 260 p*M* carbendazim injected directly onto antibody treated protein G column. (B) IAE–LC–LC–UV of 260 p*M* carbendazim injected directly onto antibody treated protein G column.

greater selectivity than IAE alone, there is still interference from background components in the LC separation. These interferences arise from compounds in the solvents which absorb UV and other small molecules that may be trapped from the sample on the trapping column. Tandem mass spectrometry (MS–MS) lends much greater selectivity to IAE than UV. By monitoring a specific transition in the CID mass spectrum of carbendazim, it is possible to eliminate virtually all background noise from the chromatogram and detect only those analytes which produce that transition. Fig. 4 shows the IAE–LC–LC–MS–MS ion current profile of 2.4 p*M* carbendazim directly injected onto the antibody-treated protein G column. There is a significant improvement in S/N for Fig. 4 which represents 100-times less sample than shown in Fig. 3B. The most convincing experiments, however, are those involving the extraction of carbendazim from a complex matrix such as soil and from environmental water.

3.2. Determination of carbendazim in soil extracts and lake water

The specificity of IAE makes it possible to detect low levels of carbendazim in soil extract samples while reducing interference from matrix components. Table 1 lists the sequence of timed events for the IAE of soil extracts and lake water. The ion current profile resulting from the SRM IAE–LC–LC–MS analysis of soil fortified at 100 ppb with carbendazim is shown in Fig. 5. At sub-ppb levels, however, it is found that recovery of carbendazim from soil becomes more difficult. This is most likely due to adsorptive losses of analyte on the soil. The use of SRM allows for the achievement of the highest possible sensitivity with a minimum of chemical interference.

Fig. 6 shows that it is possible to detect ultra-trace levels of carbendazim in lake water samples. This is probably due to fewer matrix interferences in water than in soil. Very little sample preparation is required for environmental water samples. The lake water samples analyzed for this work required only filtration prior to analysis to remove particulate matter which could cause damage to the HPLC pumps and to the columns used for extraction and chromatography.

A standard curve of carbendazim in lake water was prepared to demonstrate the ability of this method to reliably recover carbendazim over a range of concentrations. The linear behavior of the peak

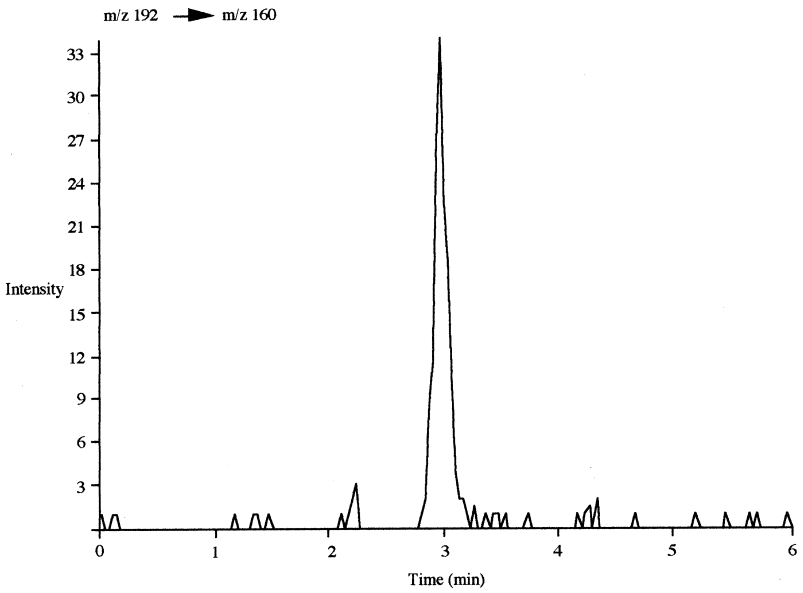

Fig. 4. SRM IAE–LC–LC–MS of 2.4 p*M* carbendazim directly injected onto antibody treated protein G column. For mass spectrometric conditions, refer to Section 2.5.

height of the transition of m/z 192 fragmenting to m/z 160 as a function of carbendazim concentration in lake water over a concentration range of 10–200 pptr gives the equation $y=0.312x-1.82$ with $r^2=$ 0.991. The dynamic range of this curve is only a factor of twenty, but it demonstrates that IAE may be a useful tool for ultra-trace concentration of similar environmental samples.

Table 1
Timed events for the determination of carbendazim from soil and lake water

Time (min)	Event
0	Pump 1 delivers PBS through IAE column and mobile phase through analytical column. Valve 1 in counter clockwise position
0	Antibody l aded nto AE olumn rom utosampler
1	Pump 1 delivers sample through IAE column at 10 ml/min
11	Switch valve 1 to clockwise position. Pump 1 delivers 2% (v/v) acetic acid and antibody and analyte are desorbed from IAE column onto trapping column
14	Switch valve 1 to counter clockwise position. Pump 2 delivers mobile phase to trapping column which backflushes analyte onto analytical column for separation and MS detection. Simultaneously switch valve 2 counter clockwise to flush IAE column with 20% acetic acid
14	Switch valve 2 clockwise to recondition IAE column with PBS in preparation for subsequent analysis

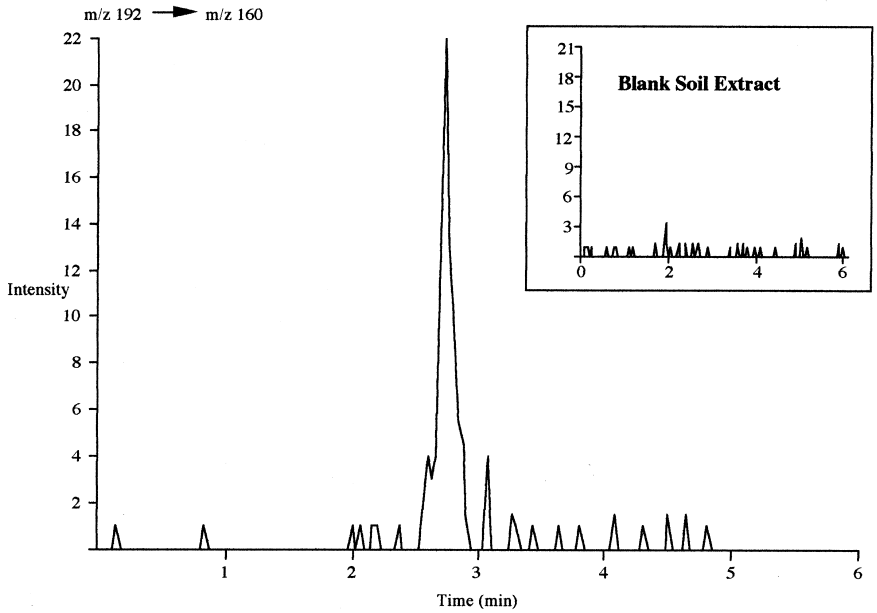

Fig. 5. Result of SRM IAE–LC–LC–MS analysis of soil fortified at 100 ppb with carbendazim. Blank soil extract is shown in the inset. For mass spectrometric and chromatographic conditions, refer to Sections 2.4 and 2.5.

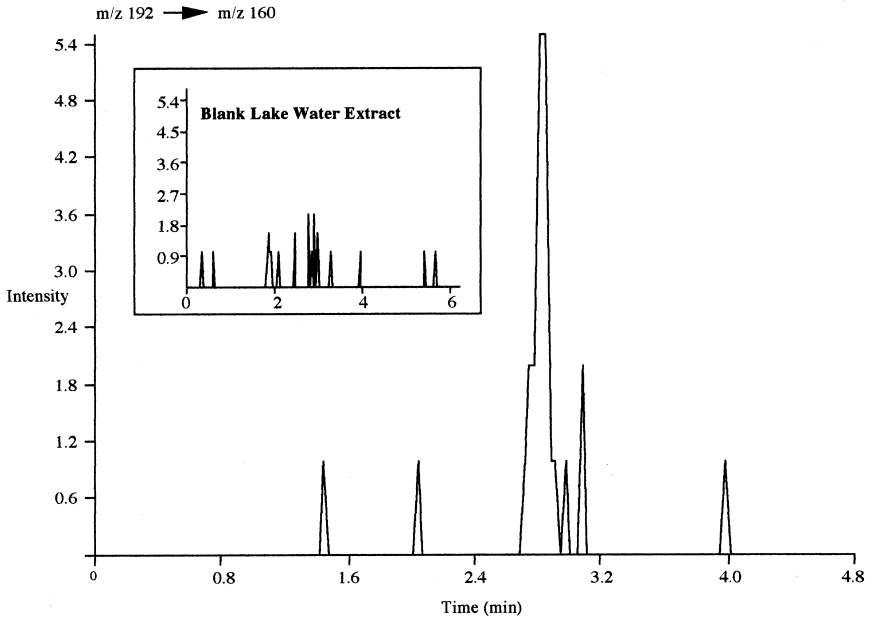

Fig. 6. Result of SRM IAE–LC–LC–MS analysis of lake water fortified at 25 pptr with carbendazim. Blank lake water extract is shown in the inset. For mass spectrometric and chromatographic conditions, refer to Sections 2.4 and 2.5.

4. Conclusions

The specificity of IAE makes it possible to detect low levels of carbendazim in soil samples without interference from matrix components. At sub-ppb levels, however, it is found that recovery of carbendazim from soil becomes more difficult. This is most likely due to adsorptive losses of analyte on the soil. It has been found that adsorption of carbendazim is strong depending upon the composition of the soil [8]. For the levels of carbendazim that are recovered from the soil, the use of IAE–LC–LC coupled with SRM achieves the highest possible sensitivity and specificity with a minimum of chemical interference. In contrast, recovery of carbendazim from the lake water samples was possible at the low pptr level. The complexity of the sample matrix for the water samples is less than that for the soil samples, making recovery of carbendazim from the lake water matrix more feasible.

The ability to pump relatively crude sample through the protein G column at high flow-rates (10 ml/min) and to automate the column-switching procedure makes the method rapid and efficient. Sample preparation for aqueous samples takes place on-line reducing the amount of time normally spent on manual extractions. By using on-line IAE, multiple samples can be analyzed in a relatively short period of time with minimum sample preparation. With the described protocol, three samples can be analyzed per hour without operator intervention.

Acknowledgements

We wish to thank PerSeptive Biosystems for the generous loan of the INTEGRAL Micro-Analytical workstation. Our gratitude also extends to PE-Sciex for the loan of the API-300 mass spectrometer. We also thank Dr. Richard Hammen of Chromatochem for providing the Protein G immunoaffinity column and Dr. Robin Charlton of E.I. DuPont de Nemours for the use of the anti-carbendazim IgG. Funding for this work is made possible by E.I. DuPont de Nemours.

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