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Improved GC-MS/MS Method for Determination of Atrazine and Its Chlorinated Metabolites in Forage Plants—Laboratory and Field Experiments

Chung-Ho Lin

Center for Agroforestry, School of Natural Resources, University of
Missouri, Columbia, Missouri, USA

Robert N. Lerch

U.S. Department of Agriculture, Agricultural Research Service, Cropping
System and Water Quality Research Unit, Columbia, Missouri, USA

Harold E. Garrett and Milon F. George

Center for Agroforestry, School of Natural Resources, University of
Missouri, Columbia, Missouri, USA

Abstract: Analytical procedures using gas chromatography–ion trap tandem mass spectrometry (GC-MS/MS) were developed to analyze atrazine (ATR) and its dealkylated metabolites in four forage species (switchgrass, tall fescue, smooth bromegrass, and orchardgrass). Atrazine, deethylatrazine (DEA), and deisopropylatrazine (DIA) were extracted with methanol (CH_3OH) followed by liquid–liquid extraction and partitioning into chloroform, with additional cleanup by C_{18} solid-phase extraction (SPE). Through the optimization of ionization conditions and ion storage voltages, the background noise of product ion spectra (MS/MS) was reduced dramatically, providing sub-μg/kg detection limits. Mean recoveries of ATR, DEA, and DIA were 94.3, 105.6, and 113.1%, respectively. The estimated limit of detection (LOD) was 0.6 μg/kg for ATR, 1.3 μg/kg for DEA, and 0.3 μg/kg for DIA. These LODs were one to two orders of magnitude lower than those reported for other GC-MS, GC-MS/MS, high pressure liquid chromatography (HPLC)-UV, or HPLC-MS/MS

procedures designed for food-safety monitoring purposes. To validate the developed method, a field experiment was carried out utilizing three replications of four forage treatments (orchardgrass, tall fescue, smooth bromegrass, and switchgrass). Forage plants were sampled for analyses 25 days after atrazine application. DEA concentrations in C3 grasses ranged from 47 to 96 μg/kg, about 10-fold higher than in switchgrass, a C4 species. The ATR and DIA concentrations were similar, ranging from 1.5 to 13.2 μg/kg. The developed method provided sufficient sensitivity to determine the fate of ATR and its chlorinated metabolites via plant uptake from soil or dealkylation within living forage grasses. It also represented significant improvements in sensitivity compared to previous GC methods.

INTRODUCTION

According to the U.S. Department of Agriculture (2001), atrazine (ATR) is the leading preemergence herbicide for broad-leaf weed control in corn production in the United States. About 36.3 million kg of ATR are applied to the soil annually, more than any other herbicide used in U.S. agriculture. In 2000 alone, it was applied on more than 68% of all corn acreage. Recently, a major concern has been raised about contamination of food products and surface and groundwater by ATR and its chlorinated metabolites (Hayes et al. 2002; Swan et al. 2003).

Radiolabeled studies suggest that many plant species have the capacity to accumulate and detoxify ATR (Burken and Schnoor 1997; Burnet et al. 1993). Atrazine can be detoxified or metabolized through nonenzymatic and enzymatic reactions (Ballantine and Simoneaux 1991; Gronwald 1994; Hatton et al. 1999). These include hydrolysis and/or N-dealkylation reactions that yield chlorodealkylated or other hydroxylated ATR metabolites. Enzyme-mediated conjugation with glutathione or cysteine is also possible (Ballantine and Simoneaux 1991). For mature tolerant species (mainly C_4 plants), the nonenzymatic hydrolysis of ATR catalyzed by benzoxazinone is the major detoxification pathway for the formation of nonphytotoxic hydroxylated metabolites, either in free or conjugated form (Ballantine and Simoneaux 1991; Kearney and Kaufman 1975; Palmer and Grogan 1965). In these tolerant plants, glutathione conjugation by glutathione S-transferase is not a major contributor to ATR detoxification (Ballantine and Simoneaux 1991). Similarly, N-dealkylated metabolites account for only a small portion of metabolized ATR in tolerant plants. In ATR-susceptible species (including most C_3 species) the N-dealkylation reactions, possibly mediated by Cytochrome P450, are the major detoxification mechanisms (Burken and Schnoor 1997; Jensen 1982; Kearney and Kaufman 1975), resulting in the production of less phytotoxic metabolites [i.e., deethylatrazine (DEA) and deisopropylatrazine (DIA)].

Most studies on the uptake and detoxification of ATR, including those noted previously, used radiolabeled ATR followed by quantification with thin-layer chromatography (TLC) or HPLC. These methods depend solely on the differences in polarity between the parent and its metabolites for identification. However, coalescing of spots during TLC or peak broadening and/or nonuniform peaks appearing during HPLC make the absolute identification of specific metabolites difficult. Because of the lack of specific structural elucidation by these methods, the absolute identification and quantification of metabolites is often ambiguous. Thus, to evaluate the phytoremediation capability of a species, especially for assessment of herbicide uptake and detoxification under field conditions, more definitive analytical methods are required. This is especially the case when it is necessary to unambiguously identify and quantify ATR and its metabolites in plant tissues at sub-μg/kg levels.

Methods employing GC-MS/MS have been successfully used for quantifying many pesticides and their metabolite residues in grain, fruit, and other agricultural commodities (Cairns and Siegmund 1990; Schachterle and Feigel 1996). Tandem mass spectrometry allows for precise structural confirmation and quantitative analyses that are more accurate because of the elimination of plant matrix interferences. Unlike many pesticide analyses using UV or single MS detection systems, in which intense matrix interferences may preclude identification and accurate quantitation of the analytes, MS/MS instrumentation allows much lower detection limits without painstaking sample cleanup procedures.

Procedures using GC-ion trap MS/MS are well suited for the analyses of volatile, thermally stable compounds in organically rich biological matrices (Lehotay and Eller 1995; Schachterle and Feigel 1996). They involve the following steps: 1) volatilization and separation of molecules by a capillary column during GC, 2) formation of ions by electron impact or chemical ionization, 3) trapping a single precursor ion of desired mass-to-charge ratio (m/z) during the first stage of MS, and 4) dissociation (i.e., fragmentation) of the precursor ion into characteristic product ions during the second stage of MS. Ion trapping is accomplished by scanning the ions with a radio-frequency (RF) signal to trap the precursor ion of interest. The appropriate RF signal traps the desired ion or range of ions between a hyperbolic electrode in the trap while other ions become unstable and are ejected from the trap (Cairns and Siegmund 1990; Schachterle and Feigel 1996). Isolated precursor ions are then exposed to a secondary dissociation excitation energy. Energized ions further collide with an inert gas (often helium) within the ion trap. The resulting conversion of kinetic energy into internal energy is sufficient to cause fragmentation. Following fragmentation, the characteristic product ions are analyzed using an electron multiplier consisting of a series of electrodes or dynodes (Schachterle and Feigel 1996).

Because MS/MS techniques have the capability to select a desired initial mass (i.e., precursor ion) and specific product masses (i.e., product ions), they filter out most of the coeluted interference and reduce background noise

dramatically. Although some ions are lost during the fragmentation process (particularly in quadrupole MS/MS), removal of the interference ions generates an extremely clean baseline (McMaster and McMaster 1998). This provides a much higher resolution and lower limit of detection (LOD) than most UV or single MS systems. It is especially significant when organically rich matrices are to be analyzed. Additionally, in many single MS procedures, the precursor ion is used as the principal ion for quantification (Cairns and Siegmund 1990). This may be satisfactory for a high-molecular-weight precursor ion when it is unlikely that there will be interfering ions of similar mass/charge ratio present in the background; however, when low-molecular-weight analytes are being quantified, it is likely that there will be interfering ions present. During MS/MS, the diagnostic product ions can provide the additional information needed to satisfy the criteria for structural confirmation, elucidation of the fragmentation process, and accurate quantification of the analytes (Cairns and Siegmund 1990).

Many GC and other chromatographic techniques designed for food-safety monitoring programs have been developed to quantify atrazine and its chlorinated metabolites in plant tissue (Battista, Di Corcia, and Marchetti 1989; Lehotay and Eller 1995; Liao, Joe, and Cusick 1991; Lowrance, Vellidis, and Hubbard 1995). However, because of interferences from the coeluted hydrocarbon matrix during separation, the detection limits of these methods often were limited to the range of 10 to 1000 μg/kg. These methods would not provide satisfactory sensitivity to investigate the fate of these compounds taken up by living plants under field conditions. Theoretically, through the optimization of ionization energy, ion storage voltages, and ion selection, the sensitivity and selectivity of these methods could be further improved using ion-trap technology. The objective of this study was to develop GC ion trap–MS/MS analytical procedures to analyze ATR and its chlorinated (dealkylated) metabolites in four different forages at sub-μg/kg levels. In addition, a field lysimeter experiment was carried out to validate the developed method. The developed method will be valuable for predicting and acquiring information regarding the fate and the residue levels of ATR and its metabolites in forage plants under field conditions.

MATERIALS AND METHODS

Tissue Extraction

Three C3 species, orchardgrass (*Dactylis glomerata* L.), smooth bromegrass (*Bromus inermis* Leyss.), and tall fescue (*Festuca arundinacea* Schreb.), and one C4 species, switchgrass (*Panicum virgatum* L.), were grown in 1-m-wide × 0.5-m-deep lysimeters filled with a sandy loam soil. The lysimeters had no history of ATR application. To confirm that the soil solution was free of ATR (<20 ng/L) and its metabolites, the soil leachate was

sampled and analyzed by HPLC using a method described by Lerch, Blanchard, and Thurman (1998). Six replications of fresh grass samples (approximately 200 g) were harvested and homogenized with dry ice using a grinding mill with a 1-mm screen. The homogenized samples were placed in a freezer at $-30°C$ overnight to allow CO_2 sublimation. Grass subsamples containing 10 g of dry weight tissue equivalent were placed into 250-mL high-density polypropylene centrifuge tubes and spiked with ATR, DEA, and DIA at 10 µg/kg using 0.2 mL of a 500-µg/L standard solution of each compound. (Dry weight equivalent was determined by drying a subsample at 60°C until sample weight was constant.) Samples were then held in a refrigerator at 4°C for 2 h. Atrazine, DEA, and DIA were extracted from these test samples by the following three-step procedure: 1) homogenization in 150 mL of 80% aqueous methanol (CH_3OH) (v/v) for 1 min at 10,000 rpm, 2) further extraction for 24 h at ambient temperature (approximately 25°C) using an end-to-end shaker at 200 oscillation/min, and 3) sonication for 20 min. Extracted samples were centrifuged for 20 min at 12,000 g at 0°C, and the supernatants were decanted into a graduated cylinder. The same extraction procedure was repeated on the remaining residue with an additional 100 mL of 80% aqueous CH_3OH. The supernatants were combined and evaporated to remove the CH_3OH using a Savant concentrator (Holbrook, N.Y.). The remaining solution was extracted four times with 50 mL of tri-chloromethane ($CHCl_3$). Trichloromethane portions were combined and evaporated to about 5 mL in the Savant concentrator. Each sample was transferred to a 12-mL test tube and diluted with 5 mL of CH_3OH. Samples were brought to about 0.5 mL under a stream of dry N_2. This residue was extracted twice more with 0.5 mL of CH_3OH using a vortex mixer. After each extraction, the test tube was rinsed with a small volume of distilled deionized (DI) water, and the solution was poured into a 100-mL beaker. The final sample volume was brought to 60 mL with DI water to give a final CH_3OH concentration of 2.5% CH_3OH.

Solid-Phase Extraction

Additional sample cleanup was accomplished using a Varian Sep Pak LRC 1 g C_{18} silica-based SPE cartridge (Varian, Inc., Harbor City, Calif.). The cartridges were preconditioned with 10 mL of CH_3OH followed by 10 mL of DI water at a flow rate of 2 mL/min. A vacuum manifold was used to regulate the flow rate. The 60-mL sample solutions were passed through the cartridges at a 3-mL/min flow rate, and the cartridges were purged with air for 1 h to remove excess water. This was followed by elution with 12 mL of CH_3OH at 2 mL/min. Eluates were concentrated to 2 mL under a stream of N_2 in a temperature bath at 30°C. Samples were then spiked with 200 ng of the internal standard terbuthylazine (TRB) and filtered through an Anotop 0.2-µm syringe filter (Whatman International, Maidstone, UK).

A 250-μL volume of the extract solution was diluted and vortexed with 250 μL of CH₃OH. Of this final solution, 0.4 μL was injected and analyzed by GC-MS/MS.

GC-MS/MS Conditions

The analyses of ATR, DEA, and DIA were performed using a Varian 3400cx GC with a Hewlett Packard cross-linked methylsiloxane capillary column (12.5 m × 0.20 mm I.D.) coupled with a Varian Saturn 2000 ion trap MS/MS system (Varian Inc., Walnut Creek, Calif.). The GC temperature program was 70°C for 1 min, ramped to 120°C at 50°C/min, ramped to 155°C at 3°C/min, and finally ramped to 290°C at 50°C/min and held for 12 min. Injector temperature was held at 250°C for 5 min. Splitless injection was used. Helium was used as the carrier gas at a flow rate of 1 mL/min. The transfer line between the GC and mass spectrometer was held at 280°C, and the ion trap manifold was set to 250°C. A Varian 8200 AutoSampler was used to perform sample injection and solvent flushing of the needle.

Precursor ions (Table 1) were selected by injecting a working standard of each compound into the GC ion trap system operating in the single MS mode. Working standards were 50 μg/L in 100% CH₃OH. Selection of diagnostic product ions and the MS/MS ion trap sensitivity were optimized by utilizing Varian's Automated Methods Development Package (AMD) (Table 1). For MS/MS analyses, electron impact ionization with nonresonant excitation was chosen. Scan mass range was from 10 to 650 m/z with a 0.67-s scan cycle. The maximum ionization time was 65,000 μs, total ion count was 5000, and prescan ionization time was programmed at 1500 μs.

Method Validation using Atrazine-Treated Field Samples

Twelve 1-m wide and 0.5-m deep lysimeters with three replications of four forage treatments (orchardgrass, smooth bromegrass, tall fescue, and switch-grass) were established at the University of Missouri Horticulture and Agro-forestry Research Center, New Franklin, Missouri (longitude 92°46′W; latitude 39°1′N). These lysimeters were arranged as a complete randomized design with three replications of each ground cover. Each lysimeter were filled with a sandy loam soil with an average pH of 7.0, organic matter content of 0.72%, and cation exchange capacity of 3.0 meq/100 g. The interior surfaces of the lysimeters were fluorinated to avoid possible herbicide adsorption. As soon as the vegetation was well established, herbi-cides were applied to the soils by irrigating each lysimeter with 3 L of solutions containing 500 μg/L of atrazine. These concentrations are represen-tative of those expected in the surface runoff under corn-production conditions

Table 1. Optimized ion trap MS/MS conditions for precursor ion selection and quantification using product ions

Compound	Precursor ion (m/z)[a]	Product ions (m/z)[b]	Product ion excitation storage level (m/z)[c]	Emission current[d] (μA)	Nonresonant excitation voltage[e] (V)	Excitation time (ms)
ATR	200	68 + 69 + 94 + 104	48	80	35	50
DEA	171–173[f]	68 + 69 + 94 + 104	48	65	40	20
DIA	171–173[f]	110 + 68	48	80	35	50
TRB	214	214	143	65	0	0

[a]Isolation windows were set at 2 m/z for all compounds. Scan rate was 1 μs/scan.
[b]Analyte quantification based on these product ions.
[c]Radio frequency value.
[d]Ionization energy.
[e]Dissociation excitation energy.
[f]Single-mode MS scanning range was adjusted to account for the natural isotopic variation in chlorine (Cl^{35}, 75.53%; Cl^{37}, 24.47%) (*CRC Handbook of Physics and Chemistry*, 52nd edition, 1971–1972).

in northern Missouri (Alberts et al. 1995). Plant samples were collected after a 25-day period. Samples were stored at $-70°C$ prior to analysis.

RESULTS AND DISCUSSION

Ion Selection and Optimization of GC-MS/MS Conditions

Preliminary experiments indicated that solid phase extraction (SPE) followed by GC using single MS mode was not sufficiently selective to achieve the desired sensitivity (data not shown). When using the ion trap system in the single MS mode, the signals of analytes in the forage samples were overshadowed by the background noise and could not be identified on the chromatogram at the spiked level of 10 $\mu g/kg$. The need for development of a GC-MS/MS method was apparent from these preliminary experiments.

To identify the precursor ions, working standards of 50 $\mu g/L$ ATR, DEA, and DIA in CH_3OH were injected using the single MS mode. The most abundant ions generated during electron ionization were selected as precursor ions. These were m/z 200 for ATR and m/z 173 for both DEA and DIA (Table 1). During MS/MS, many characteristic fragmented product ions for each precursor ion appeared in the mass spectra (Figures 1–3). Diagnostic product ions were identified (Table 1) following the comparison of working standards and blank and spiked forage samples. Chemical structures of the diagnostic product ions m/z 68, 94, 104, and 110 (Figure 4) were derived from previous studies (Lerch et al. 1995; Voyksner et al. 1990). To maximize the sensitivity of detection for the identified diagnostic ions, the instrument was programmed using the AMD software. The optimization methods available in this software are described in detail by Schachterle and Feigel (1996). In the experiments reported here, the nonresonant fragmentation excitation voltage or dissociation excitation energy was incremented in 5- or 10-V steps over the desired range of energies. The isolation windows were set at 2 m/z for all the compounds, and the scan rate was 1 μs/scan. Various combinations of ionization during the first ionization stage of MS, and dissociation excitation energy and excitation time during the second stage of MS, were used for ATR and its dealkylated metabolites (Figures 5–7). The final optimized conditions for the generation of product ions of ATR, DEA, and DIA are shown in Table 1. As the ionization energy or excitation time is increased, a larger number of products ions are produced; however, this occurs at the expense of total ion intensity (Schachterle and Feigel 1996). The AMD procedures allowed the optimization of ionization energy, dissociation excitation energy, and excitation time. For instance, maximum combined product ion intensity for ATR (m/z $68 + 69 + 94 + 104$) was obtained at a dissociation excitation energy of 35 V with an excitation time of 50 ms. This excitation time dramatically improved signal strength (Figure 5) and, therefore, sensitivity for
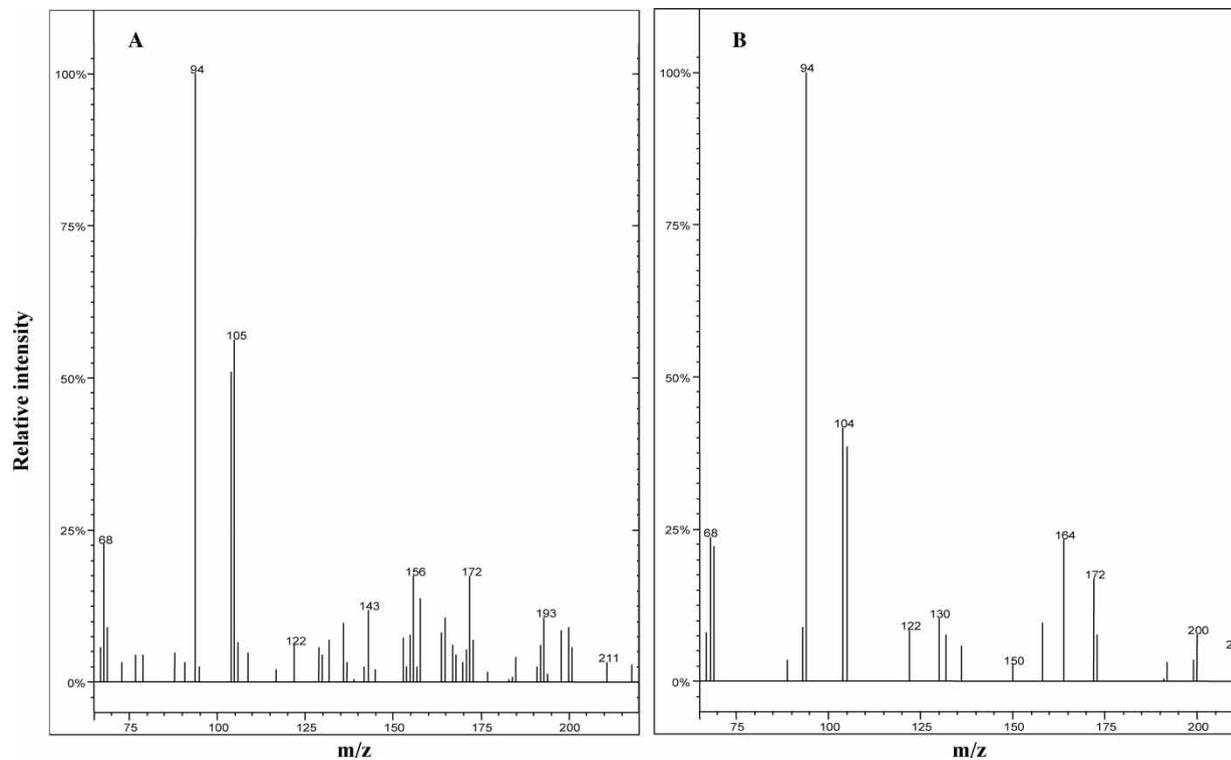
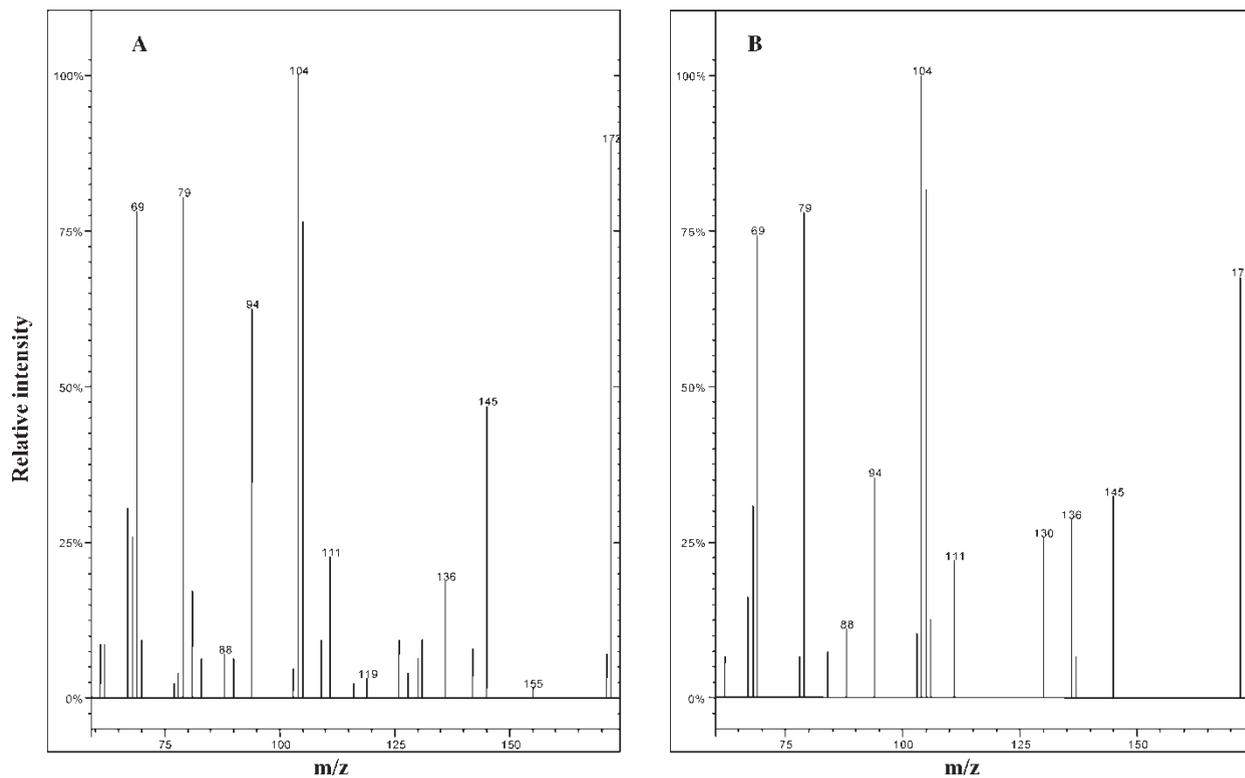***Figure 1.*** Product ion spectra of atrazine (ATR) isolated from smooth bromegrass (A, 10 μg/kg ATR) compared to a standard (B).

Figure 2. Product ion spectra of deethylatrazine (DEA) isolated from smooth bromegrass (A, 10 µg/kg DEA) compared to a standard (B).

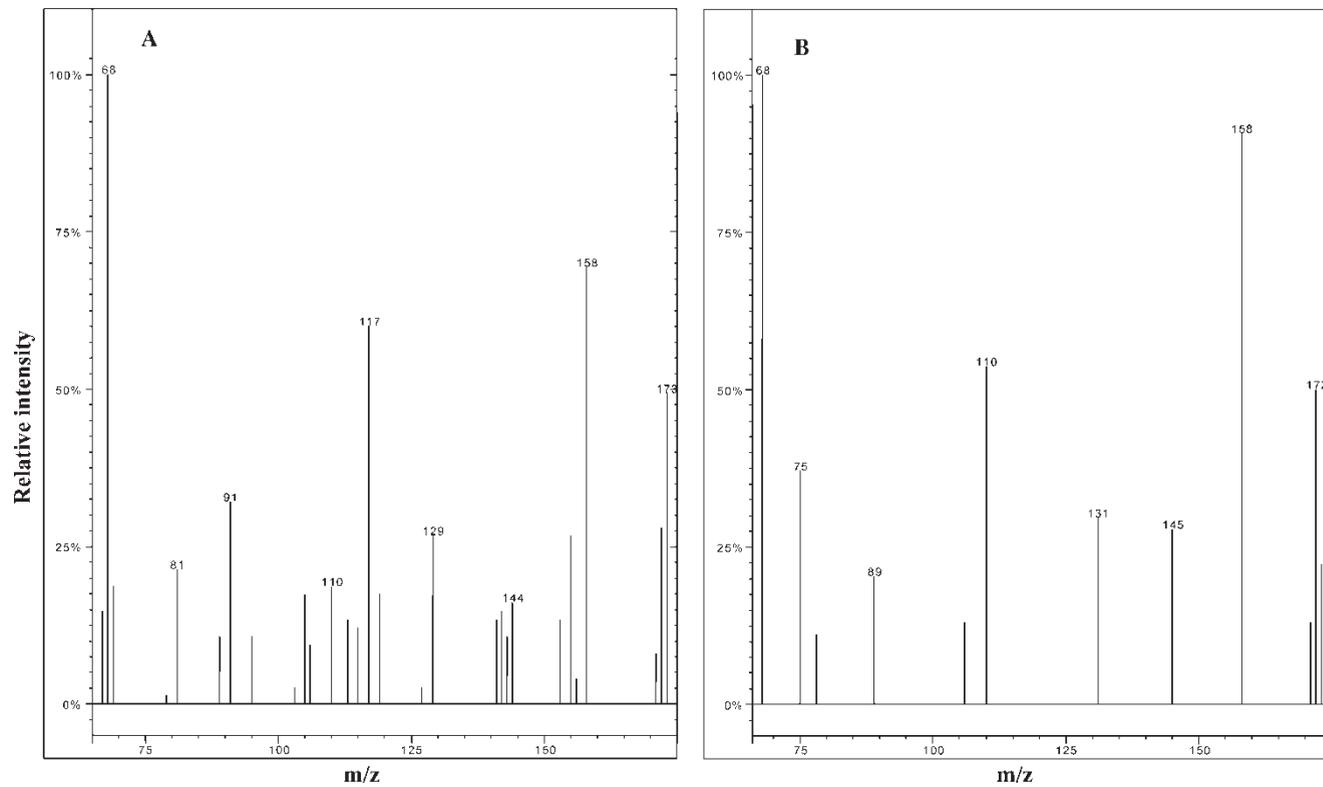**Figure 3.** Product ion spectra of deisopropylatrazine (DIA) isolated from smooth bromegrass (A, 10 μg/kg DIA) compared to a standard (B).
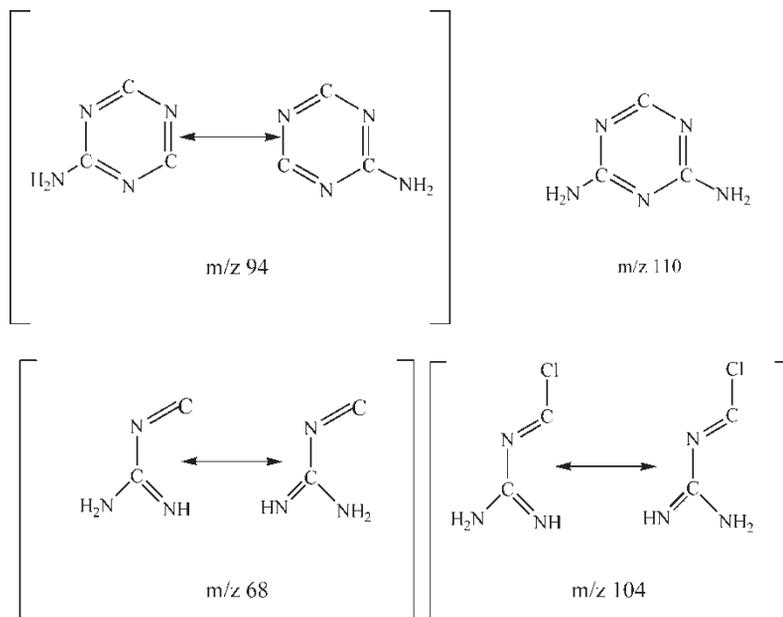
Figure 4. Chemical structures of the diagnostic product ions used for quantification of atrazine, deethylatrazine, and deisopropylatrazine.

Figure 5. Optimization of ionization energy (μA), dissociation excitation energy (V), and excitation time (ms) utilizing Varian's Automated Methods Development Package to maximize the signal strength of atrazine (ATR) diagnostic product ions.
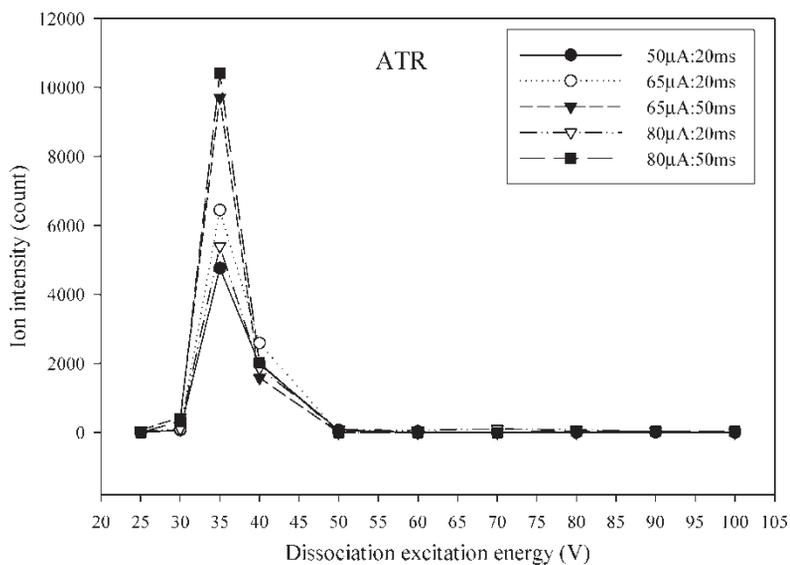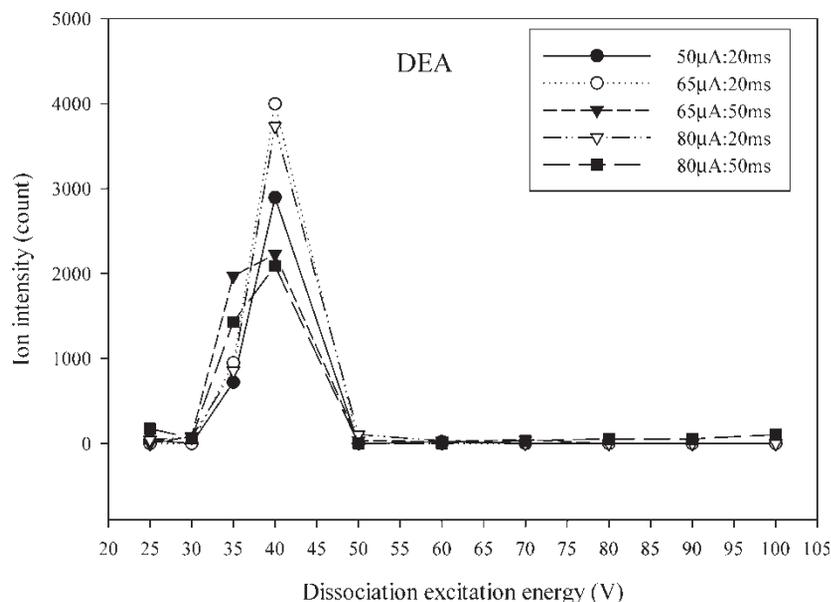
Figure 6. Optimization of ionization energy (μA), dissociation excitation energy (V), and excitation time (ms) utilizing Varian's Automated Methods Development Package to maximize the signal strength of deethylatrazine (DEA) diagnostic product ions.

detecting ATR in plant samples. Similar optimization was performed for the m/z 68 + 69 + 94 + 104 product ions of DEA (Figure 6) and m/z 68 + 110 product ions of DIA (Figure 7). The RF excitation storage level was maintained at m/z 48 to efficiently trap the low m/z diagnostic product ions (m/z 94 for ATR and DEA and m/z 68 for DIA) and to eliminate fragment ions with m/z ratios less than 48 (Table 1). (The AMD procedures recommend an RF value that will eliminate fragment ions that have an m/z value of 1/1.4 of the smallest target product ion.) Although the selected diagnostic product ions were not the most abundant ions present in the product ion spectra, the unique characteristics of these ions essentially eliminated the background noise caused by coeluting interferences. This generated an extremely clean baseline on the chromatograms (Figures 8 and 9). Diagnostic ions commonly used for quantification of ATR, DEA, and DIA in other soil and plant residues could not be used to achieve the sensitivity desired here because of background interferences. For instance, m/z 215, 200, 186, 173, and 172 ions have been used to quantify ATR, whereas m/z 172, 173, 158, and 145 ions have been used to quantify DEA and DIA (Lehotay and Eller 1995; Liao, Joe, and Cusick 1991; Sandra and David 1995). The targeted limits of detection (LOD) for plant extracts in these GC-MS and GC-MS/ MS studies were about 5 to 50 μg/kg based on a signal-to-noise ratio of
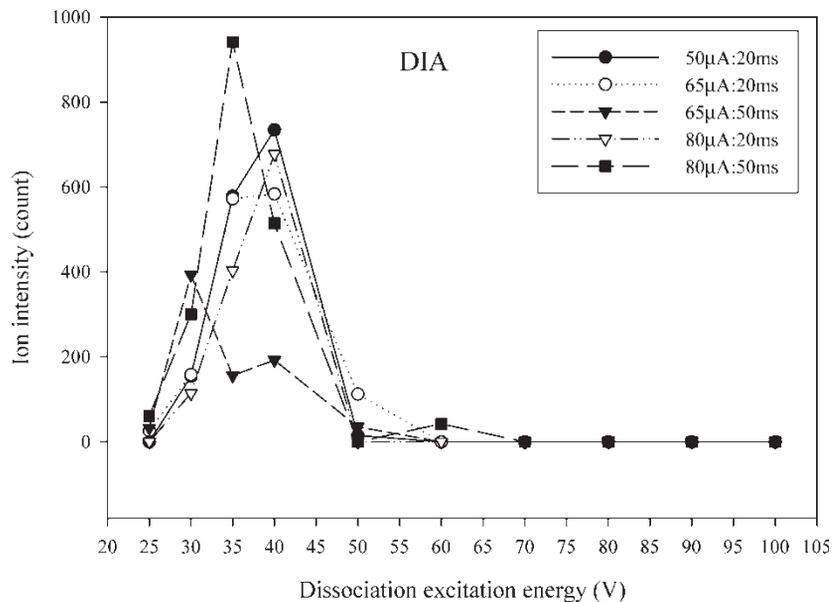
Figure 7. Optimization of ionization energy (μA), dissociation excitation energy (V), and excitation time (ms) utilizing Varian's Automated Methods Development Package to maximize the signal strength of deisopropylatrazine (DIA) diagnostic product ions.

three. In the study here, the ions m/z 172, 173, 158, and 200 were abundant in the background noise and generally obscured the compound signals at the 10-μg/kg spiked level.

Analyses of Spiked Samples

Previous studies have described GC analyses that do not employ intensive sample cleanup procedures (Feigel 1998; Schachterle and Feigel 1996). However, the C_{18} SPE cleanup step was required here to keep the injection sleeve to the GC column free of contamination. In preliminary experiments, this contamination was observed to occur after several sample injections.

To enhance the recovery rate, removal of $CHCl_3$ from the concentrated plant extract is crucial prior to the dilution with DI water and C_{18} cleanup. If $CHCl_3$ remains in the extract, a significant amount of ATR, DEA, and DIA will be partitioned into the $CHCl_3$ fraction. This will interfere with the following dilution, sample loading, and the hydrophobic partitioning process that takes place in the C_{18}-bonded silica resin. The addition of $CHCl_3$-miscible CH_3OH during the final sample evaporation prior to SPE
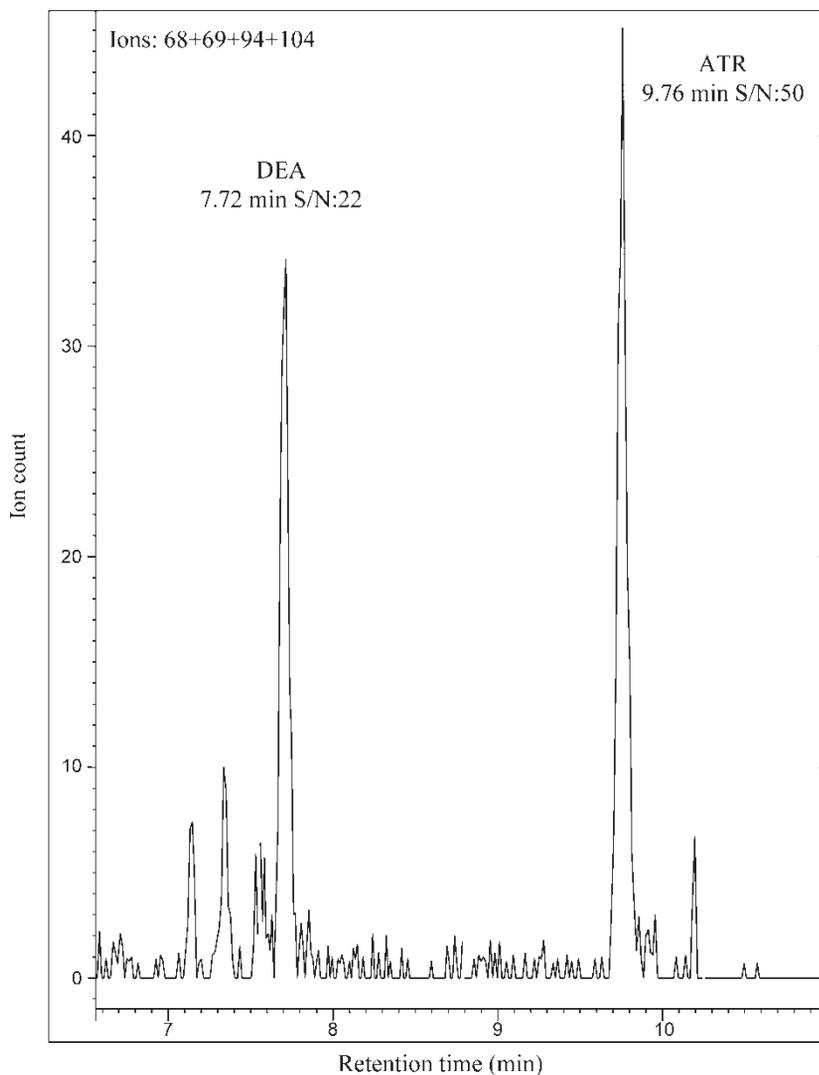
Figure 8. Selected ion storage chromatogram of the diagnostic product ions from GC-MS/MS analysis of atrazine (ATR) and deethylatrazine (DEA) in smooth bromegrass at 10 µg/kg (S/N = signal/noise ratio).

improved the recovery and solubility of the analytes during the subsequent dilution in DI water.

For calculating sample concentrations, a quadratic calibration curve was constructed from GC-MS/MS measurements of 5- to 500-µg/L working standards of each analyte. Correlation coefficients were 0.997 or greater for all standard curves, and the relative standard deviations (RSD) ranged from
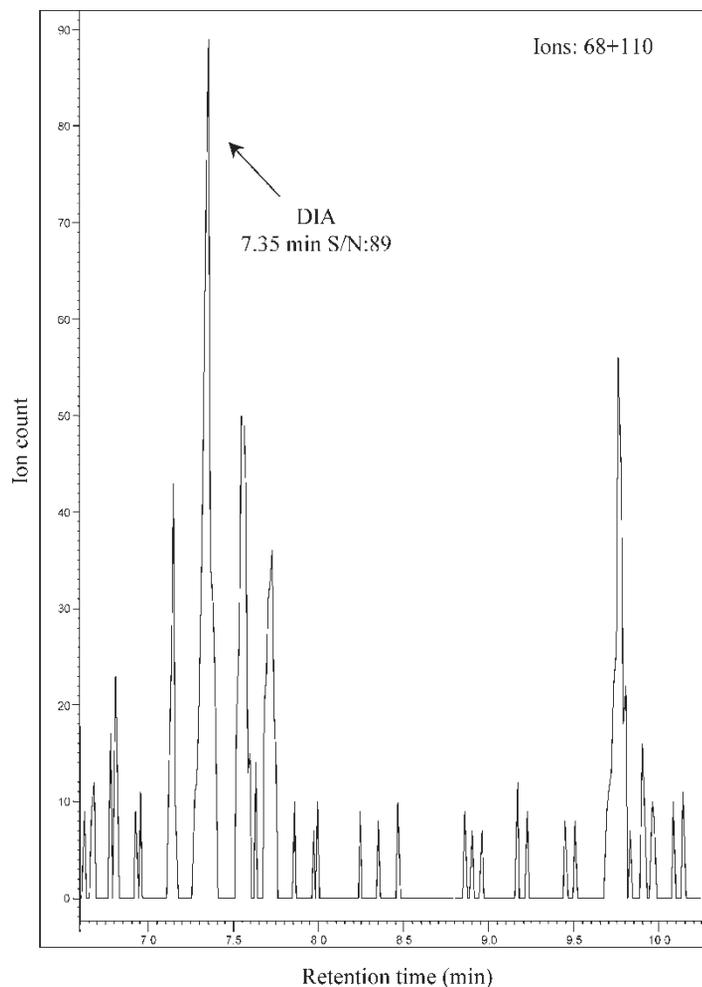
Figure 9. Selected ion storage chromatogram of the diagnostic product ions from GC-MS/MS analysis of deisopropylatrazine (DIA) in smooth bromegrass at 10 μg/kg (S/N = signal/noise ratio).

9.7 to 16.6%. [RSD (%) = 100 · standard deviation/sample average·] Average recoveries from six replicate injections of each forage extract were 94.3% for ATR, 113.1% for DEA, and 105.6% for DIA (Table 2). Sample RSD values were generally low except for DIA (Table 2). Significant signal enhancement was observed for DEA and DIA, particularly the spiked samples of orchardgrass and smooth bromegrass.

Tandem MS product ion chromatograms of spiked bromegrass samples are shown in Figures 8 and 9. Signal-to-noise ratios of each compound were automatically calculated. The SPE GC-MS/MS method successfully

Table 2. Average GC-MS/MS recoveries (%) and limits of detection of atrazine (ATR) and its dealkylated metabolites, deethylatrazine (DEA) and deisopropylatrazine (DIA), in spiked grass samples [spike level was 10 μg/kg (n = 6)]

Parameter	ATR	DEA	DIA
Tall fescue	89.3	109.2	89.0
Orchardgrass	92.0	117.5	123.5
Smooth bromegrass	101.8	122.2	127.8
Switchgrass	79.1	103.3	82.1
Mean recovery (%)	94.3	113.1	105.6
SD	6.6	7.3	23.4
RSD	7.0	6.5	22.1
Limits of detection (μg/kg)	0.6	1.3	0.3

removed and avoided most matrix interferences and provided the desired unambiguous identification of ATR and its chlorinated metabolites. Background noise was less than approximately four counts. Therefore, a signal intensity of 15 counts is sufficient to quantify and confirm the analytes of interest for an LOD based on a signal-to-noise ratio of three. The estimated LODs were 0.6 μg/kg for ATR, 1.3 μg/kg for DEA, and 0.3 μg/kg for DIA. These LODs were one to two orders of magnitude lower than those reported for other GC-MS, GC-MS/MS, HPLC-UV, or HPLC-MS/MS (Battista, Di Corcia, and Marchetti 1989; Lehotay and Eller 1995; Liao, Joe, and Cusick 1991; Lowrance, Vellidis, and Hubbard 1995).

Analyses of Field Atrazine-Treated Samples

The concentrations of ATR, DEA, and DIA in the field forage samples collected from atrazine-treated lysimeters were successfully determined by the developed GC-MS/MS method. The ATR and its two chlorinated metabolites in aboveground forage tissues vary considerably between species (Table 3). Deethylatrazine was the predominant chlorinated species found in all forage plants. The DEA concentration in C3 grasses range from 47 to 96 μg/kg, about 10-fold higher than in C4 switchgrass. Deethylatrazine accounted for 53 to 85% of the total chlorinated residue detected in the forages (Table 3). The ATR and DIA concentrations were of similar magnitude, ranging from 1.5 to 13.2 μg/kg. The significantly lower concentrations of total chlorinated atrazine residues in switchgrass than in other C3 grasses might have resulted from its more advanced biochemical detoxification mechanisms, such as glutathione conjugation and hydroxylation (Hamilton 1964; Kern, Meggitt, and Penner 1975), or it may have resulted from

Table 3. Concentrations (μg/kg) of atrazine (ATR), deethyatrazine (DEA), and deiospropylatrazine (DIA) and total residue (μg/kg) in forage plants collected from atrazine-treated field lysimeters (n = 3)

Grass treatments	DIA	SD[a]	Total residue (%)	DEA	SD	Total residue (%)	ATR	SD	Total residue (%)	Total residue
Orchardgrass	8.90	3.10	7.90	96.4	13.4	84.9	8.30	0.40	7.30	113.6
Tall fescue	3.20	0.80	5.60	47.5	11.6	82.8	6.60	3.30	11.6	57.4
Smooth bromegrass	5.90	1.80	5.50	87.9	11.4	82.2	13.2	2.20	12.3	107
Switchgrass	4.30	0.90	34.4	6.60	2.70	53.0	1.60	0.70	12.5	12.5

[a]SD = standard deviation.

lower uptake rates due to its lower transpiration rates during the experiment (unpublished data). However, in mature switchgrass plants, glutathione conjugation catalyzed by glutathione S-transferase is not a major contributor to ATR detoxification (Ballantine and Simoneaux 1991). N-Dealkylation, especially N-deethylation, has been identified as the major detoxification pathway for triazine herbicides in a wide range of C3 species, for example, annual ryegrass (*Lolium rigidum*) (Burken and Schnoor 1997; Burnet et al. 1993).

In most studies of the fate of atrazine in agronomic applications, expensive radioactively labeled herbicides are applied to the soil or plant under carefully controlled conditions in a greenhouse or growth chamber environment (Burken and Schnoor 1997). In general, the application of the radioactive-labeled atrazine is restricted in most large-scale field studies. On the other hand, most nonradioactive analytical procedures previously reported in the literature were designed for food-safety monitoring purposes. These methods did not provide sufficient sensitivity to study the fate of atrazine and its chlorinated metabolites taken up by living forage plants. In addition, methods developed for food-safety monitoring did not sufficiently eliminate the plant matrix interferences associated with forage grasses. The developed GC-MS/MS methods reported in this work may offer a more sensitive approach to detect and quantify the ATR, DEA, and DIA uptake by living plants and to evaluate their fate and ecological implications in prairie or vegetative buffer systems.

CONCLUSION

The proposed GC-MS/MS method allows for unambiguous identification and quantitative measurement of ATR and its dealkylated metabolites taken up by living forage plants. Average recoveries of ATR, DEA, and DIA during GC-MS/MS ranged from 94 to 113% with estimated LODs of 0.6, 1.3, and 0.3 μg/kg for ATR, DEA, and DIA, respectively. The method developed here is a considerable improvement over previous procedures and it facilitates analysis of forage species that have previously defied efforts to quantify herbicides at sub-μg/kg levels because of the intense matrix interferences associated with these plant species. They will be helpful in determining the fate of ATR within living plants and to evaluate plant uptake and detoxification capacity of various plant species used in vegetative buffers. This developed procedure has been successfully applied to a field lysimeter phytoremediation study with several forage treatments.

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