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# Improved HPLC-MS/MS Method for Determination of Isoxaflutole (Balance) and Its Metabolites in Soils and Forage Plants

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A robust multi-residue procedure is needed for the analysis of the pro-herbicide isoxaflutole and its degradates in soil and plant materials at environmentally relevant (<1 $\mu$g kg$^{-1}$) levels. An analytical method using turbo-spray and heat-nebulizer high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) was developed for the analysis of isoxaflutole (IXF) and its two metabolites, diketonitrile (DKN) and the benzoic acid metabolite (BA), at sub-microgram per kilogram levels in soil and plant samples. The average recoveries of the three compounds in spiked soil and plant samples ranged from 84 to 110% and 94 to 105%, respectively. The limits of quantification were validated at 0.06 $\mu$g kg$^{-1}$ for soil and 0.3 $\mu$g kg$^{-1}$ for plant samples. The limits of detection (LOD) for soil analysis were 0.01, 0.002, and 0.01 $\mu$g kg$^{-1}$ for IXF, DKN, and BA, respectively. Corresponding LOD for the plant analysis method were 0.05, 0.01, and 0.05 $\mu$g kg$^{-1}$. The developed method was validated using forage grass and soil samples collected from a field lysimeter study in which IXF was applied to each of four forage treatments. Forage plants and soils were sampled for analyses 25 days after IXF application to the soil. In soils, IXF was not detected in any treatment, and DKN was the predominant metabolite found. In forage plants, the concentrations of DKN and BA were 10−100-fold higher than that in soil samples, but IXF was not detected in any forage plants. The much higher proportion of BA to DKN in plant tissues (23−53%), as compared to soils (0−5%), suggested that these forages were capable of detoxifying DKN. The developed methods provided LODs at sub-microgram per kilogram levels to determine the fate of IXF and its metabolites in soils and forage plants, and they also represent considerable improvements in extraction recovery rates and detection sensitivity as compared to previous analytical methods for these compounds.

**KEYWORDS: Isoxaflutole; herbicide; tandem mass spectrometry; diketonitrile; benzoic acid metabolite; LC-MS/MS**

## INTRODUCTION

Isoxaflutole (IXF; Balance) is a pre-emergence herbicide belonging to a new family of herbicides called isoxazoles (*1*). This new herbicide was first introduced by Rhône-Poulenc Agro (acquired by Bayer AG in 2001, Research Triangle Park, NC) and is mainly used for corn production. Isoxaflutole appears to perform well at relatively low dosages (about 11−64 g/ha of active ingredient) and offers season-long control against many triazine resistant weeds (*2*, *3*). The U.S. Environmental Protection Agency (EPA) gave conditional regulatory approval to IXF in 1998, and it was commercially introduced for the 1999 growing season in 16 corn-producing states. This herbicide designed as a potential replacement for triazine herbicides has the potential for widespread agronomic use in the next decade. For example, within the first year of introduction, it was estimated that IXF was applied to about 5−10% of the U.S. corn acreage (*4*), with 17% of Iowa corn acreage receiving IXF. In Europe, IXF has also been approved for agricultural application in several countries, including England and The Netherlands (personal communication from TNO Research Institute, Zeist, The Netherlands).

The isoxazole herbicides inhibit 4-hydroxy-phenylpyruvate dioxygenase (HPPD) and thereby indirectly block carotenoid biosynthesis (*1*, *5*). This leads to an absence of mature chloroplast development. The molecular IXF compound applied as a pro-herbicide does not have an effect on HPPD; rather, enzymatic inhibition only occurs when the nitrogen-containing ring of IXF is cleaved and converted to the cyano diketone

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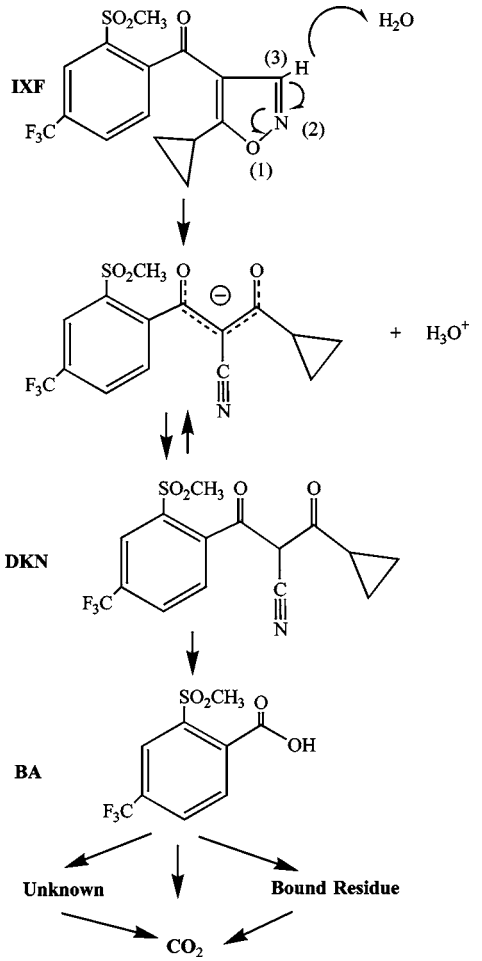


**Figure 1.** Degradation pathway of isoxaflutole and its metabolites (ref *13* and Rhône-Poulenc Agro, personal communication).

**Table 1.** Chemical Properties of Isoxaflutole and Its Metabolites[a]

| | half-life (days) | partition coefficient $K_{oc}$[b] | solubility in water (mg/L) | phytotoxicity | soil half-life (days) |
|---|---|---|---|---|---|
| IXF | <1.4 | 122 | 6.2 | no | 0.5–3 |
| DKN | 8–16 | 92 | 300 | yes | 20–30 |
| BA | 20–60 | 69 | 8000 | no | NA |

[a] Refs *13* and *1* and Rhône-Poulenc Agro, personal communication. [b] $K_{oc}$ is the organic carbon partition coefficient.

structure found in the diketonitrile (DKN) metabolite (**Figure 1**and **Table 1**) (*5*, *6*). Plant resistance to this herbicide is strongly associated with the capacity to convert DKN into the nonbiologically active benzoic acid derivative (BA) (**Figure 1**) (*5*).

Isoxaflutole is nonvolatile and has a half-life of 12−24 h under greenhouse conditions (*1*, *7*). In a field lysimeter study, DKN was the predominant form of the herbicide detected in leachate 36 h after IXF application (*8*). Diketonitrile is much more polar and considerably more stable in the soil environment than IXF (*1*) (**Figure 1** and **Table 1**). Conversion of IXF to DKN occurs photochemically and/or by hydrolysis (*9*). The hydrolysis half-life of IXF to DKN has been measured to be 11.1 h at pH 5, 20.1 h at pH 7, and 3.2 h at pH 9 (*10*). Under anaerobic conditions in an aquatic environment, the half-life of IXF was less than 2 h (*10*). The photolysis half-life of the molecular IXF on or near the soil surface has been observed to be approximately 20−23 h (*9*), while the half-life of IXF was observed to be less than 9 h in an aqueous solution (250 $\mu$g/L) of pH 7.2 exposed to dim visible light at 25 °C (*11*).

The degradation of IXF follows either first- or second-order kinetics. Under dim light (~10 $\mu$einsteins $m^{-2}$ $s^{-1}$), a second-order rate constant of $1.5 \times 10^{-7}$ L $\mu g^{-1}$ $s^{-1}$ has been reported (*11*). Grünanger and Vita-Finzi reported that the hydrolysis of many isoxazoles follows second-order kinetics (*12*). However, in the dark, the degradation pathway of IXF may exhibit first-order kinetics (*13*). The low stability of IXF is associated with the highly unequal electron distribution in the isoxazole ring, resulting in an electron deficiency at the C-3 position (**Figure 1**) (*13*). Because of this electron deficiency, the C-3 hydrogen atom has a high dissociation potential, leading to the opening of the isoxazole ring (*13*). Beltran et al. reported a possible resonance stabilized enolate ion as an intermediate prior to complete conversion to DKN (*13*). A considerably slower degradation of diketonitrile (half-life from 8 to 16 days) produces the polar metabolite, BA. BA further degrades to $CO_2$ and other low molecular weight residues in 20−60 days.

Because of the low application rates and low stability of IXF in soils, a robust analytical procedure is needed to provide subparts per billion detection of IXF and its metabolites in soils and plants. Liquid/liquid extraction (LLE) combined with solid-phase extraction (SPE) has been used for clean up and separation of various aromatic pesticides from fruit, vegetable, and soil residues (*14*). These techniques have been proven to remove interfering compounds associated with plant tissue and soil organic matter. Several analytical procedures using gas chromatography (GC) coupled with mass spectrometry have been developed for the detection of IXF and its two metabolites (*15*, *16*). However, due to the polarity, low volatility, and thermal instability of the IXF metabolites, these methods have been limited to the detection of IXF or total IXF residues in a sample. Determination of total IXF residues requires conversion of IXF and DKN to BA, followed by derivatization of BA to a volatile methyl ester to facilitate the GC analysis. Thus, analysis using GC instrumentation cannot distinguish IXF from its metabolites, providing incomplete information on the environmental fate of IXF.

Reversed-phase HPLC methods are better suited for the separation and quantification of a wide range of nonpolar to polar compounds that are not sufficiently volatile for analysis by GC (*17*). Recently, HPLC has been combined with tandem mass spectrometry for the quantification of pharmaceuticals and agrochemical compounds in complex biological and soil matrices (*18*−*21*). Because triple quadrupole and ion trap MS/MS instruments can screen a specific precursor ion and quantify based on a selected product ion, they filter out most of the matrix interferences present in a sample. This reduces background noise dramatically and improves method sensitivity. Thus, HPLC-MS/MS provides a higher resolution of separation and lower limit of detection (LOD) than single MS instruments. The advantage of high selectivity of MS/MS is especially significant for analysis of organic-rich matrices (*22*). In 1998, Rhône-Poulenc Agro developed an extraction procedure followed by HPLC-MS/MS to determine IXF, DKN, and BA in various agricultural commodities including corn and mustard foliage (*23*). The limit of quantification (LOQ) of this procedure was 10 $\mu$g $kg^{-1}$. The objective of the work reported here was to modify the extraction procedure described by Rhône-Poulenc Agro (*23*) and to develop improved HPLC-MS/MS techniques to determine the residues of IXF, DKN, and BA in both plants and soils at LOQ values less than 1 $\mu$g $kg^{-1}$. The utility of the developed method was then demonstrated by analysis of soil and plant samples collected from a field study.

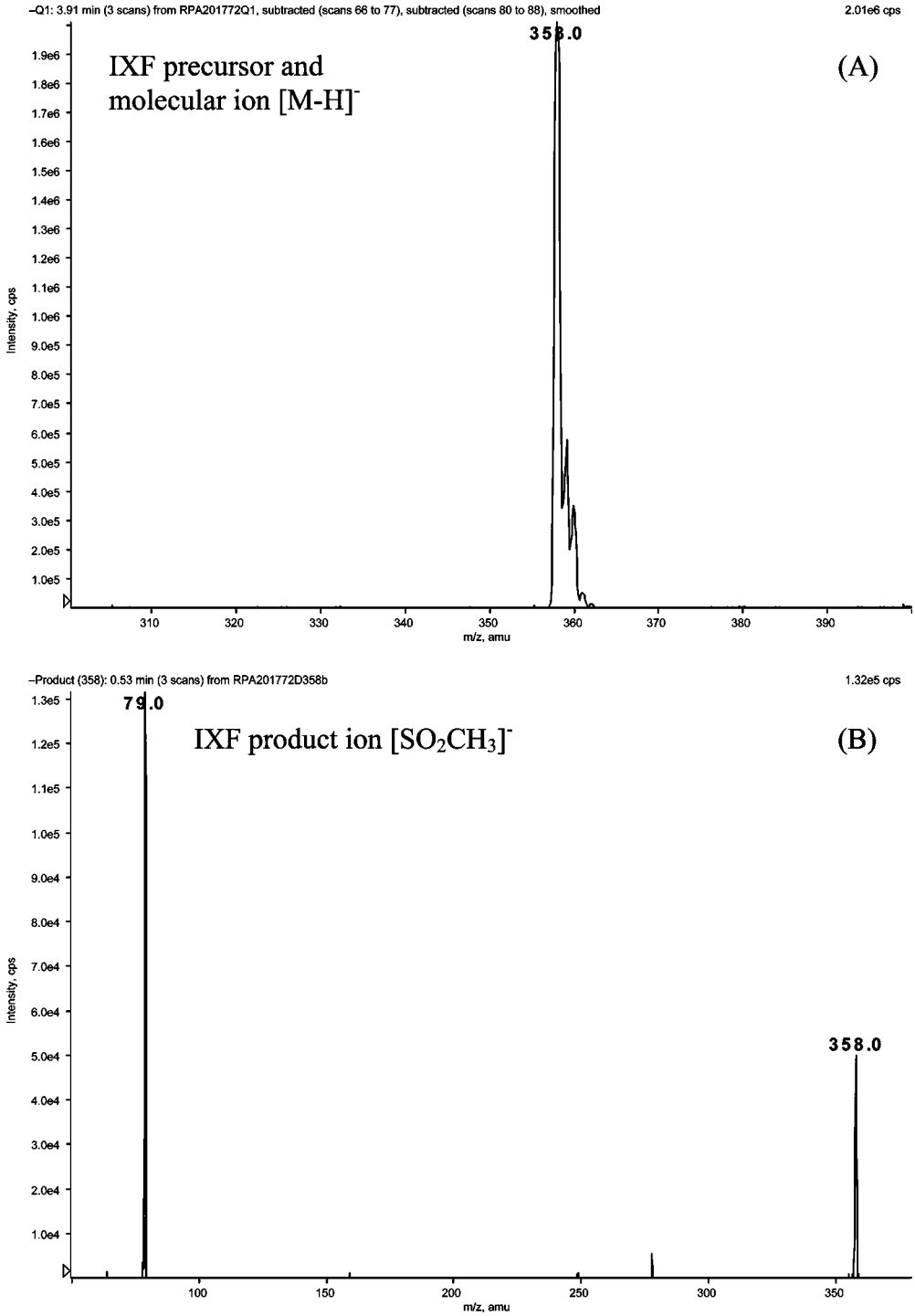


**Figure 2.** Full-scan mass spectra of molecular (A) and product ions (B) for IXF.

## MATERIALS AND METHODS

**Chemicals and Supplies.** Isoxaflutole [5-cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethylbenzoyl)-isoxazole], diketonitrile metabolite [2-cyclopropyl-3-(2-methylsulfonyl-4-trifluoromethylbenzoyl)-3-oxopropanenitrile], and the benzoic acid metabolite [2-methylsulfonyl-4-trifluoromethylbenzoic acid] were obtained in 95−99% purity from Rhône-Poulenc Agro (Research Triangle Park, NC). All solvents used were HPLC grade. Standard stock solutions were prepared using 100% acetonitrile (ACN). The working standards were prepared in 9:1, 1.3% formic acid/ACN (pH 2.1). A Spe-ed RP-102 2.5 g highly cross-linked polystyrene divinylbenzene polymer SPE cartridge (Applied Separations, Inc., Allentown, PA) was used for sample cleanup and concentration in plant methods. This polymer resin has exhibited a higher recovery and better reproducibility for extracting a wide range of moderately to very polar molecules as compared to the $C_{18}$ resin (*24*, *25*). The improved performance of this polymer resin for hydrophobic analytes is related to lower irreversible binding resulting from minimized secondary interactions, such as H-bonding or ion exchange (*26*, *27*).

**Soil Extraction.** Soil extraction procedures were modifications of methods developed for processing plant tissue samples by Rhône-Poulenc Agro (*23*). According to the plant method developed by Rhône-Poulenc Agro, IXF, DKN, and BA were extracted with liquid−liquid extraction using $CH_2Cl_2$ followed by sequential SPE extraction. This method was deemed to be impractical for soil extraction because of the SPE procedure, which called for high volumes of aqueous ACN to elute the analytes and subsequent lengthy evaporation of these eluants to achieve desired sensitivity. Therefore, the SPE procedure was eliminated in our soil method.

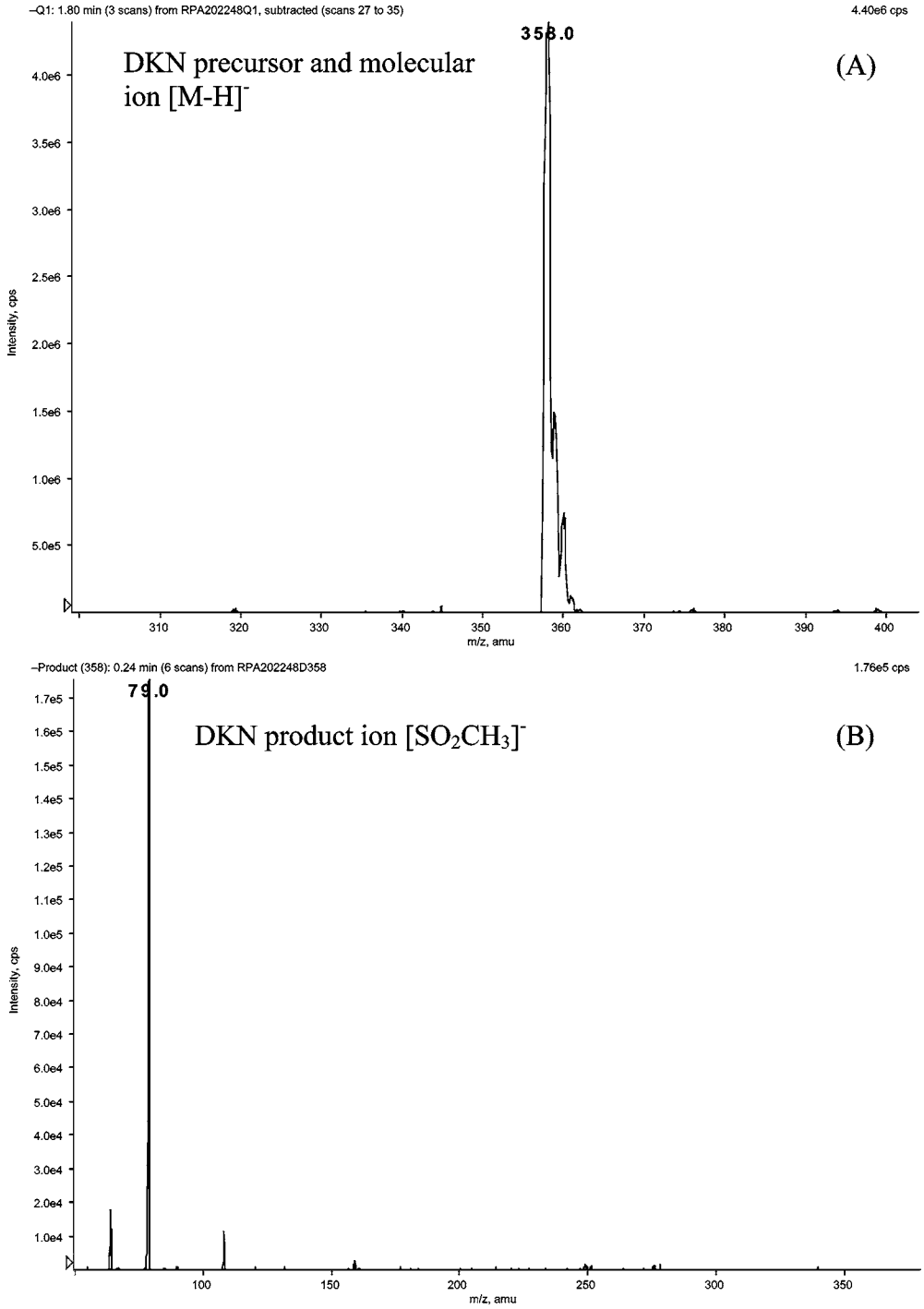


**Figure 3.** Full-scan mass spectra of molecular (A) and product ions (B) for DKN.

Fifty gram soil samples (dry weight equivalent) were placed into 250 mL polypropylene centrifuge tubes and then spiked with IXF, DKN, and BA to create samples containing 0.06, 1, and 1.8 $\mu$g kg$^{-1}$, respectively. There were four replications for each spike concentration and analyte. The samples were sequentially extracted twice with 100 mL of 9:1 methanol/1% formic acid on an end-to-end shaker for 1 h at room temperature. After each extraction, the samples were vacuum filtered through Whatman #42 filter paper using a Buchner funnel. Following the second filtering, the remaining soil residue was rinsed with approximately 20 mL of 100% methanol. The filtrates were transferred to a 500 mL separatory funnel, acidified with 30 mL of 1 N HCl, and then diluted with 150 mL of 10% NaCl. This was followed by liquid−liquid extraction using two separate 100 mL volumes of $CH_2Cl_2$. The $CH_2Cl_2$ fractions were combined in a 500 mL round-bottomed flask, and 4 mL of 1% formic acid was added to this solution. Samples were evaporated using a rotoevaporator until all the $CH_2Cl_2$ was removed. The samples were then adjusted to a final volume of 5 mL with 9:1, 1% formic acid/ACN.

**Plant Extraction.** Plant extraction procedures were also based on modifications of methods developed by Rhône-Poulenc Agro (*23*). In their method, following the liquid−liquid extraction described previously, IXF, DKN, and BA were extracted by sequential SPE elution to further clean up and concentrate the samples. According to the sequential SPE procedure originally described by Rhône-Poulenc Agro (*22*), DKN and BA were eluted with 40 mL of 40% aqueous ACN. After washing the SPE column with 20 mL of 50% ACN, IXF was then eluted with 40 mL of 70% aqueous ACN. In our modified elution scheme, a higher solvent strength and elution volume were utilized in a sequential SPE procedure, and the SPE washing step was eliminated to improve the recovery rates. In addition to a modified SPE elution scheme, the final extracts were also concentrated 100-fold in our method to improve the detection sensitivity.

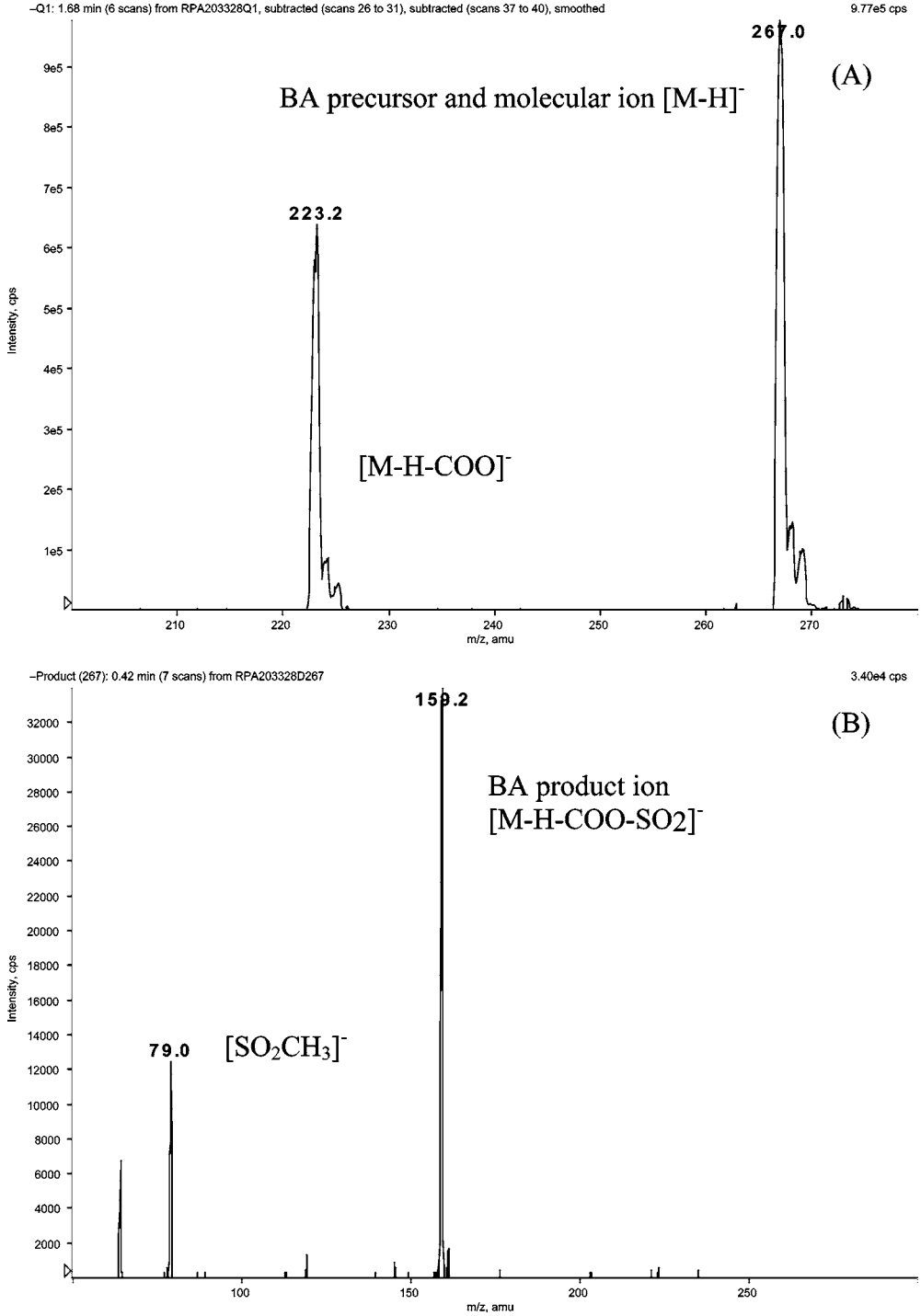


**Figure 4.** Full-scan mass spectra of molecular (A) and product ions (B) for BA.

An approximately equal mixture of tall fescue, smooth bromegrass, and orchardgrass, collected from a field lysimeter study, was ground with small chips of dry ice using a grinding mill with a 1 mm screen. The ground material was then transferred to a refrigerator at 2 °C for approximately 24 h to allow the sublimation of $CO_2$. The total amount of plant material derived from this process was on the order of 100 g. Samples were then stored at −30 °C until further analysis. Duplicate sub-samples of 5 g were fortified with equal amounts of IXF, DKN, and BA by applying standard solutions to the dry ground material. The spike levels were 0.3, 5, and 9 $\mu$g kg$^{-1}$ for each analyte. These samples were homogenized in a laboratory blender for 3 min in 110 mL of a 9:2, methanol/1% formic acid solution. Subsequent sample handling and liquid−liquid extraction were as described previously for soil samples, with the exception of adding 30 mL of 1% formic acid to the $CH_2Cl_2$ solution prior to evaporation. Following evaporation, the final aqueous volume was approximately 25−30 mL. An additional SPE cleanup step was included by passing this volume through a 2.5 g RP-102 cross-linked polystyrene divinylbenzene polymer cartridge. The cartridge was conditioned with 40 mL of ACN, followed by 40 mL of distilled water. Flow rate was 3 mL/min. Samples were then passed through the cartridge at the same flow rate. Diketonitrile and BA were eluted with 65 mL of 40% aqueous ACN into a first fraction at a flow rate of 2 mL/min. Isoxaflutole was eluted with 40 mL of 80% aqueous ACN into the second fraction at the same flow rate. Both fractions were evaporated to about 2 mL and reconstituted to a final volume of 2.5 mL with 1:4 ACN/1% formic acid.

**HPLC-MS/MS Conditions for Analyses of Isoxaflutole.** Isoxaflutole was quantified using a Shimadzu LC-10AT HPLC system (Shimadzu Scientific Instruments, Columbia, MD) coupled with a PerkinElmer SCIEX APCI 365 HPLC-MS/MS system (PerkinElmer Corporation, Foster City, CA). The analytical column for HPLC was a Phenomenex (Torrance, CA) silica-based Luna $C_{8(2)}$ column (30 mm

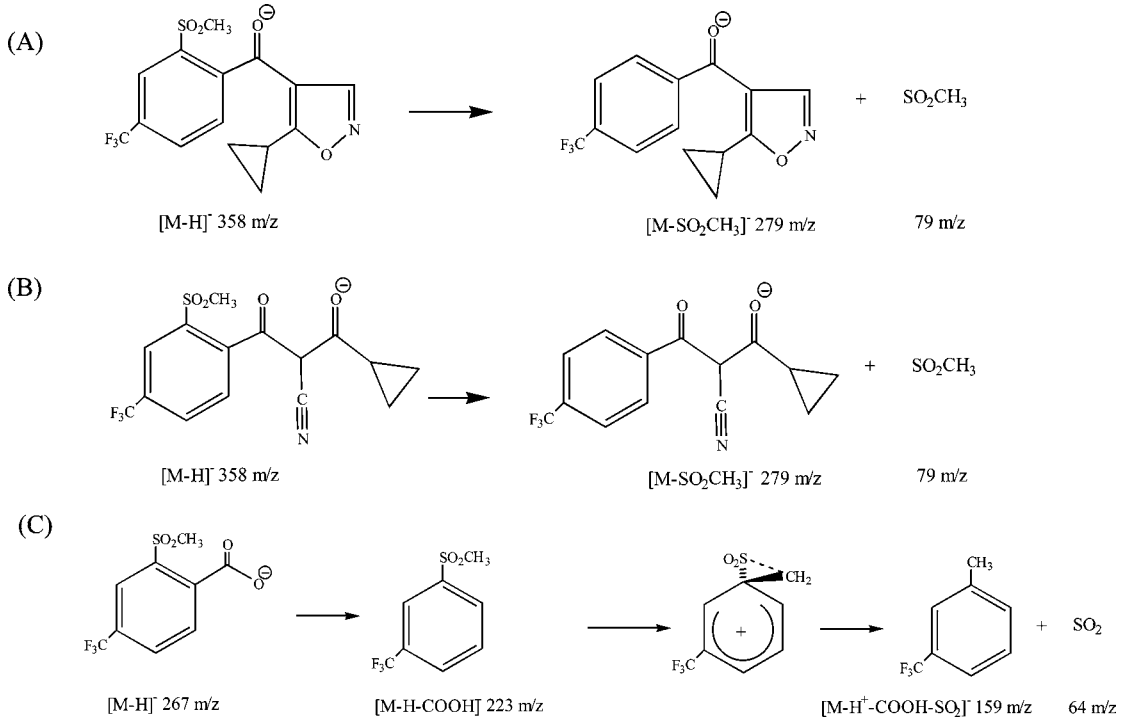


**Figure 5.** Formation of molecular and product ions for IXF (A), DKN (B), and BA (C).

**Table 2.** Average Percent Recoveries and Standard Deviation ($n = 4$) Values for IXF, DKN, and BA from Spiked Soil Samples as Measured by HPLC-MS/MS

| | recovery (%) | | |
|---|---|---|---|
| spike concentration ($\mu$g kg$^{-}$1) | IXF | DKN | BA |
| 0.06 | 106 (6.2)[a] | 97.2 (14.9) | 87.1 (6.6) |
| 1.0 | 109 (9.1) | 99.4 (4.7) | 87.0 (10.4) |
| 1.8 | 112 (10.5) | 99.5 (3.4) | 85.2 (8.1) |
| mean | 109 | 98.7 | 84.4 |
| *p* value for concentration effect[b] | 0.636 | 0.922 | 0.936 |

[a] Mean ± standard deviation. [b] *p* values determined by analysis of variance. No significant concentration effect was observed at the 5% level of probability for recovery of each analyte.

**Table 3.** Average Percent Recoveries and Range ($n = 2$) Values for IXF, DKN, and BA from Spiked Grass Samples as Measured by HPLC-MS/MS

| | recovery (%) | | |
|---|---|---|---|
| spike concentration ($\mu$g kg$^{-}$1) | IXF | DKN | BA |
| 0.3 | 105 (5.0)[a] | 82.2 (11.2) | 102.8 (0.0) |
| 5.0 | 103 (7.1) | 98.8 (13.9) | 87.6 (3.1) |
| 9.0 | 105 (5.0) | 102 (7.7) | 100.7 (12.6) |
| mean | 104 | 94.4 | 97.1 |
| *p* value for concentration effect[b] | 0.954 | 0.299 | 0.236 |

[a] Mean ± range ($n = 2$). [b] *p* values determined by analysis of variance. No significant concentration effect was observed at the 5% level of probability for recovery of each analyte.

× 2 mm) with a 3 $\mu$m diameter particle size. Sample injection volume was 20 $\mu$L. Column temperature was 40 °C. A linear two-part mobile-phase gradient was used. Mobile phase A consisted of water with 1.5% acetic acid and 1 mM ammonium acetate, and mobile phase B consisted of 9:1 ACN/1.5% acetic acid. The mobile-phase gradient program was as follows: 35% B ramp to 60% B in 5 min followed by isocratic conditions of 100% B for 5 min. The flow rate was 0.5 mL/min. Retention time of IXF was 6.4 min.

**Table 4.** Concentrations ($\mu$g kg$^{-1}$) of IXF, DKN, and BA in Soils and Forage Plants Collected from IXF-Treated Field Lysimeters ($n = 3$)

| | soils | | | plants | | |
|---|---|---|---|---|---|---|
| | IXF | DKN | BA | IXF | DKN | BA |
| orchardgrass | 0.0 (0)[a] | 0.18 (0.1) | 0.01 (0) | 0.0 (0) | 2.7 (0.91) | 3.0 (1.78) |
| tall fescue | 0.0 (0) | 0.057 (0.012) | 0.01 (0) | 0.0 (0) | 2.5 (0.19) | 1.9 (0.49) |
| smooth bromegrass | 0.0 (0) | 0.045 (0.021) | 0.0 (0) | 0.0 (0) | 6.8 (1.1) | 3.5 (0.95) |
| switchgrass | 0.0 (0) | 0.026 (0.007) | 0.0 (0) | 0.0 (0) | 1.4 (0.25) | 0.41 (0.12) |

[a] Standard deviation.

The ion source in the MS/MS system was a heated nebulizer atmospheric pressure chemical ionization (APCI) interface. The APCI was operated in the negative ion mode. The MS/MS system was operated in the multi-reaction monitoring (MRM) mode. The nebulizer current and nebulizer temperature were programmed at 3 amps and 400 °C, respectively. The orifice voltage was set at −10 V. The nebulizer gas (nitrogen) was set at 10 L/min with nitrogen auxiliary and curtain gases adjusted to a constant flow rate of 8 L/min. Collision activated dissociation gas value was adjusted to 9 (arbitrary units). Determination of the precursor ion to be screened and the product ion to be used for quantification were determined from spectra obtained by injecting 10−20 $\mu$L of a standard IXF solution containing 1000 $\mu$g/L. Full scans of the first and third quadrupole mass filters are shown in **Figure 2**. The deprotonated molecule ion $[M - H]^-$ (*m*/*z* 358) of IXF was selected as the precursor ion. The predominant fragment ion *m*/*z* 79 was selected as the product ion for quantification. External calibration curves were developed using standard solutions.

**HPLC-MS/MS Conditions for Analyses of Diketonitrile and Benzoic Acid Metabolites.** Chromatography conditions and determination of precursor and product ion spectra for DKN and BA quantification were the same as described for IXF, except that sample injection volume was 10 $\mu$L. Retention times of DKN and BA were 3.50 and 2.15 min, respectively. The ion source for mass spectrometry was a TurboIonspray atmospheric pressure ionization (API) interface (PerkinElmer, Foster City, CA). The API interface was operated in the negative ion mode for DKN and BA. As for IXF, the MRM scanning mode was used for ion detection. The capillary ion source and orifice voltages were programmed at −3.5 kV and −25 V, respectively. The nebulizer temperature was set at 500 °C. The nebulizer

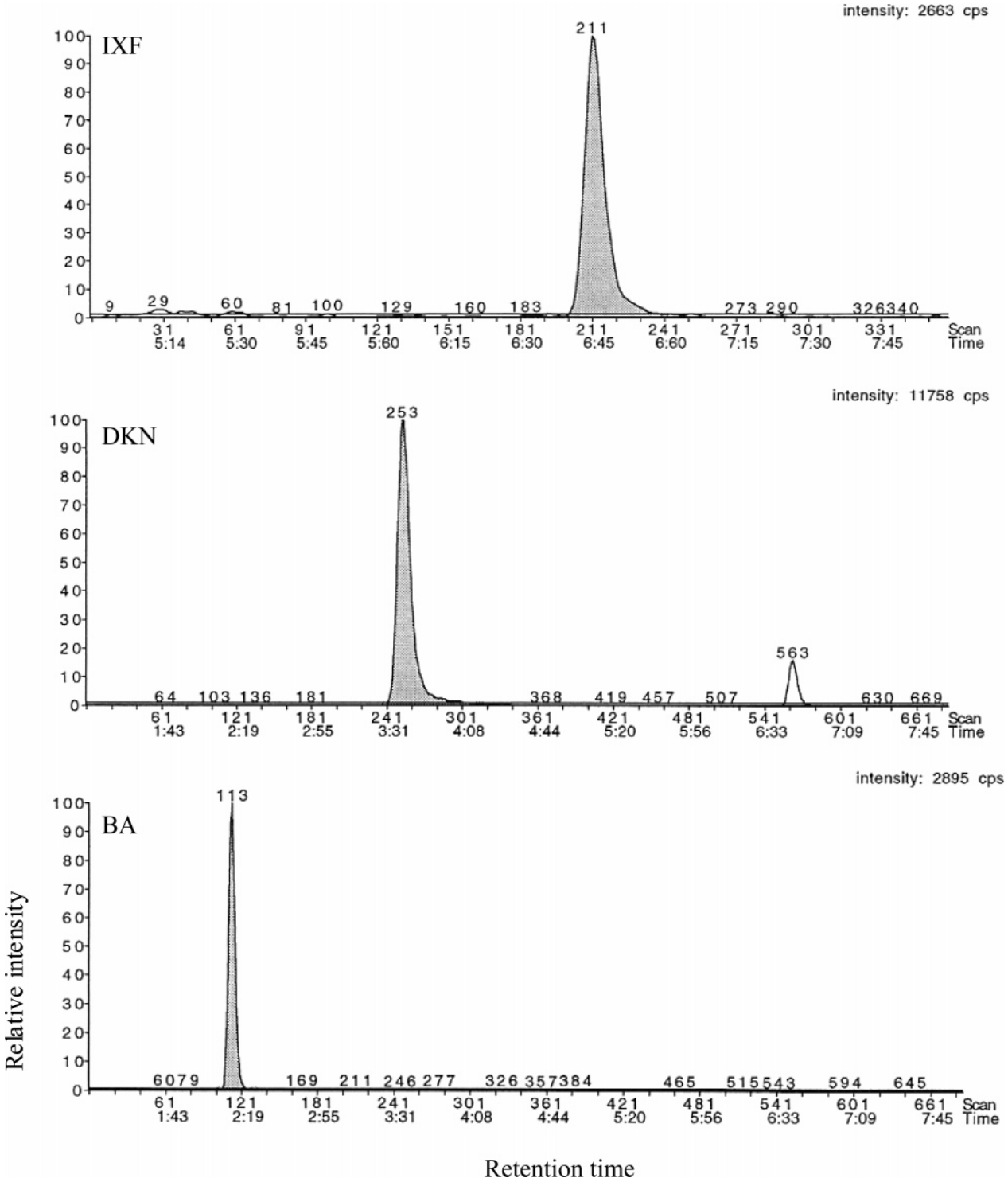


**Figure 6.** High-performance liquid chromatography-MS/MS product ion chromatogram of IXF, DKN, and BA at 1 $\mu$g kg$^{-1}$ in soil extracts.

gas (nitrogen) was set at 13 L/min with nitrogen auxiliary and curtain gases adjusted to a constant flow rate of 8 L/min. Collision activated dissociation gas value was adjusted to 6. The deprotonated molecular ions $[M - H]^-$ (*m*/*z* 358 for DKN and *m*/*z* 267 for BA) were selected as precursor ions (**Figures 3** and **4**). After fragmentation, the predominant product ions *m*/*z* 79 and 159 were selected to quantify DKN and BA, respectively. DKN and BA were quantified using external standard calibrations.

**Source of IXF-Treated Soil and Plant Samples.** Plant and soil samples were obtained from a field lysimeter study established at the University of Missouri Horticulture and Agroforestry Research Center. Details of the field lysimeters and experimental design were previously reported by Lin et al. (*28*). Briefly, three replications of four forages treatments (orchardgrass [*Dactylis glomerata* L.], smooth bromegrass [*Bromus inermis* Leyss.], tall fescue [*Festuca arundinacea* Schreb.], and switchgrass [*Panicum virgatum* L.]) were established in 1 m wide and 0.5 m deep lysimeters. Each lysimeter was 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 mequiv/100 g. As soon as the vegetation was well-established, isoxaflutole was applied to the soils in each lysimeter as a 3 L solution containing 80 $\mu$g/L of isoxaflutole. The site had no previous history of IXF application. Soil and plant samples were collected 25 days after herbicide application. Soil samples were composite samples of five representative 35 cm deep soil cores collected from each lysimeter. Plant material was removed, and the soils were stored at −20 °C until analyzed. For the plant samples, all

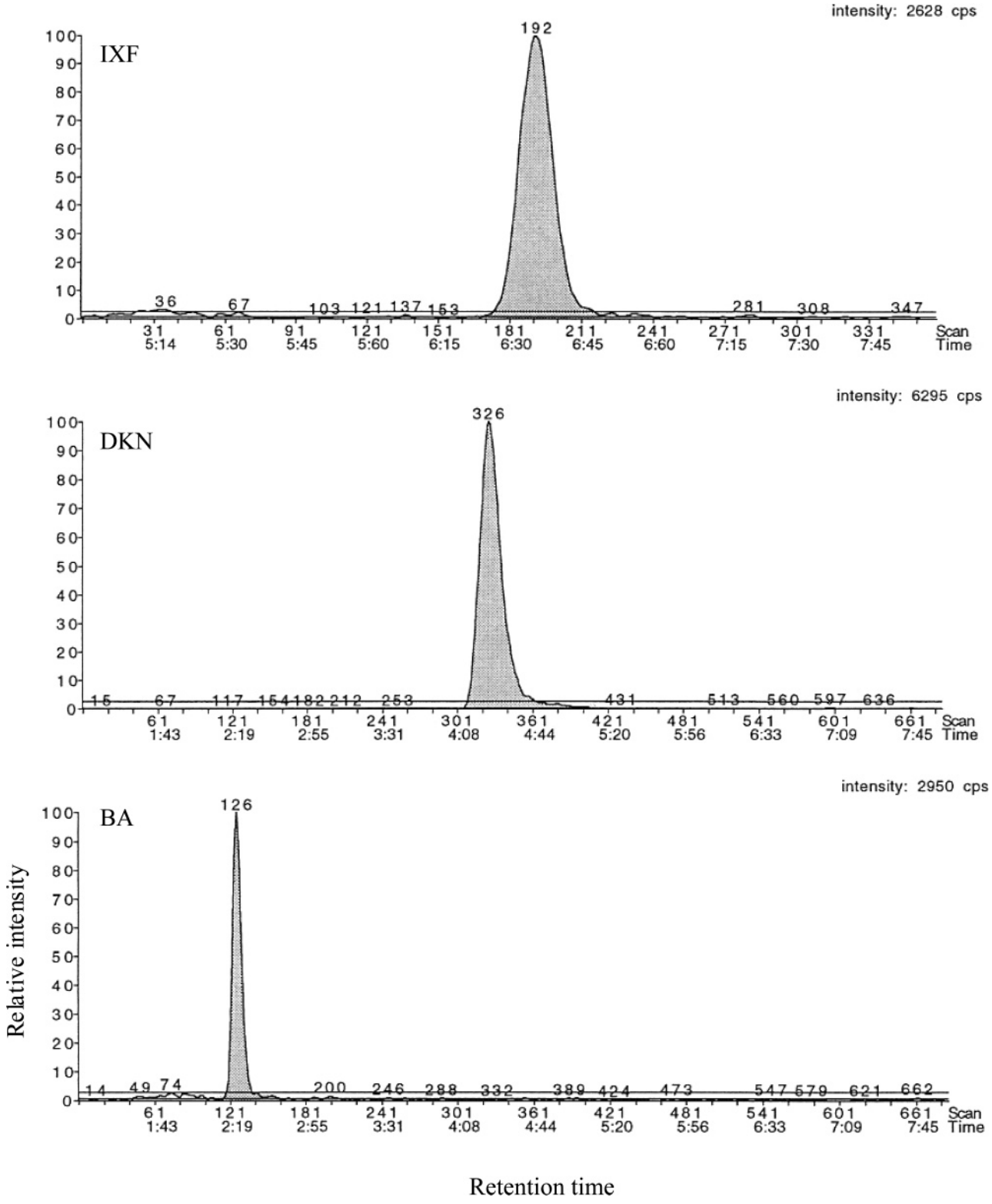


**Figure 7.** High-performance liquid chromatography-MS/MS product ion chromatograms of IXF, DKN, and BA at 5 $\mu$g kg$^{-1}$ in grass extracts.

above-ground plant material was collected, rinsed with water to remove soil, and ground with dry ice to pass through a 1 mm sieve. Plant samples were stored at −20 °C prior to analysis.

## RESULTS AND DISCUSSION

**Recovery from Spiked Soil and Plant Tissue.** For soil analyses, the LLE procedure was sufficient to remove most of the organic interference and to achieve the desired recovery rates. Addition of 150 mL of 10% NaCl was required in this process to disperse an emulsified layer that developed at the interface between the aqueous and the organic fractions. Similar extraction schemes have been developed for other aromatic herbicides, including atrazine (*29*, *30*). Acidification with HCOOH and HCl facilitated the stabilization of the molecular IXF by preventing the cleavage of the N−O bond that leads to the formation of DKN. Additionally, under neutral conditions (pH 7), the metabolites DKN and BA are in the anionic form (p$K_a$ of DKN = 1.1 and p$K_a$ of BA = 2.1, personal com-

munication from Zeneca Agrochemicals, Richmond, CA). Addition of HCl protonates DKN and BA, increasing their hydrophobicity and stability. Under these conditions, both DKN and BA were predominantly partitioned into the $CH_2Cl_2$ fraction. An analogous acidification and extraction scheme was used by Aga et al. (*31*) to quantify the ethanesulfonic acid metabolite of metolachlor.

Average recovery of the analytes from soil ranged from 84.4 to 109% (**Table 2**). The relative standard deviations were acceptable for all three analytes, ranging from 3.4 to 14.9%. Overall, accuracy and precision of recovery were greatest for DKN, followed by IXF and then BA. For the plant analyses, average recovery from plant tissue in our study ranged from 94.4 to 104% (**Table 3**), while those of Rhône-Poulenc's plant method ranged from 65 to 100% with average recover rates of 97.8, 77.6, and 81.1% for IXF, DKN, and BA, respectively. Method accuracy was greatest for BA, but overall precision was greatest for IXF (**Table 3**). There was no significant effect of concentration on recovery ($p > 0.05$). Neither signal suppression nor enhancements due to matrix effects was observed in this work, and therefore, the recoveries were not impacted.

The modified extraction regime, HPLC mobile-phase gradient, and addition of ion modifiers in the method described in this study have resulted in considerable improvements in extraction recovery and detection sensitivity over previous LC-MS/MS methods developed for soil and plant analyses. When compared to Rhône-Poulenc's plant method, the higher solvent strength, elution volume, and elimination of the SPE washing step in our modified elution scheme increased the average extraction recovery of DKN and BA by 21.6 and 19.7%, respectively. However, the average recovery of IXF was comparable between the two methods. In a soil analysis procedure reported by the EPA (RPAC 45750), IXF and its metabolites were simply extracted with 80% ACN, and the filtered raw extract was then directly injected into the LC-MS/MS system (*32*). Because of the lack of sample cleanup, even with the high selectivity of the MS/MS spectrometer, the signal-to-noise ratios of IXF and BA in the EPA method (S/N: 5−10 at 1 $\mu$g kg$^{-1}$) were lower than the method reported here (S/N: >50) due to matrix interferences.

**Tandem Mass Spectrometry: Detection and Structure Elucidation.** Both IXF and DKN have the same molecular weight and deprotonated molecular ion $[M - H]^-$, *m*/*z* 358 (**Figures 2** and **3**). The diagnostic product ion, *m*/*z* 79, for both IXF and DKN was the methylsulfonyl ion $[SO_2CH_3]^-$. This ion is the dissociated functional group produced by fragmentation of the precursor (molecular) ions (**Figure 5A**,**B**). Hence, separation of IXF and DKN by HPLC is required for quantification of these two analytes (**Figures 6** and **7**). The diagnostic product ion of BA, *m*/*z* 159, was derived from the loss of the carboxyl group of BA and the ejection of $SO_2$ following the rearrangement and dissociation of the methyl group from the methylsulfonyl group (**Figure 5C**). The ejection of $SO_2$ was verified by the presence of a product ion at *m*/*z* 64 in the spectrum (**Figure 4B**). The same rearrangement was observed in our previous work when BA in groundwater samples was ionized with a single MS system (*33*). This rearrangement and ejection of $SO_2$ from molecules of the type R-$SO_2$-R was also described by MacLafferty and Zhang et al. (*34*, *35*).

Because of the selectivity of tandem MS filters and unique diagnostic product ions, the background noise was dramatically reduced in the chromatograms for both soil and plant analyses (**Figures 6** and **7**). The limits of quantification (LOQ) were validated at the lowest spiked levels of 0.06 and 0.3 $\mu$g kg$^{-1}$ for fortified soil and plant samples, respectively. A determination of the limits of detection (LOD) was based on a signal-to-noise ratio of 3. The estimated LODs for the soil analysis methods were as follows: 0.01 $\mu$g kg$^{-1}$ for IXF, 0.002 $\mu$g kg$^{-1}$ for DKN, and 0.01 $\mu$g kg$^{-1}$ for BA. For the plant analyses, the LODs were 0.05 $\mu$g kg$^{-1}$ for IXF, 0.01 $\mu$g kg$^{-1}$ for DKN, and 0.05 $\mu$g kg$^{-1}$ for BA. These LODs were 1−2 orders of magnitude lower than those reported for other GC-MS, GC-MS/MS, HPLC-UV, or HPLC-MS/MS (*15*, *16*, *23*, *32*) methods developed for analysis of these three compounds.

The detection sensitivity for IXF quantification was enhanced by approximately 1.5 times by heated nebulizer ionization as compared to electrospray ionization in a preliminary study (C. H. Lin, unpublished data). Our previous work describing the analysis of IXF and its metabolites in groundwater also suggested a lower detection sensitivity of IXF as compared to DKN and BA when they are ionized by an electrospray ionization source in a HPLC-MS system (*33*). The gentle or soft ionizing APCI conditions avoid the fragmentation of IXF produced during electrospray API (*36*). In this type of ion source, the sample aerosol rapidly evaporates. The rapid desolvation and vaporization of the sample droplets prior to entry into the plasma minimize the risk of thermal decomposition and preserve the integrity of the molecule for the following proton transfer. Additionally, the premature fragmentation of the sample ions in the ion source is reduced, given the moderating influence of solvent clusters on the reagent ions and the relatively high gas pressure in the source (*37*). A guide on MS ionization techniques by Hoffmann et al. (*36*) suggests that as a general rule, APCI provides better sensitivity than electrospray ionization for less polar compounds. Watson (*38*) also notes that most applications of electrospray ionization involve protonation of basic sites or deprotonation of acidic sites on analyte molecules. Neither DKN nor BA was detected with the APCI ionization source.

**Analyses of Field IXF-Treated Samples.** The developed HPLC-MS/MS method described in this paper was successfully used to quantify IXF and its metabolites in the IXF-treated lysimeter samples at sub-microgram per kilogram levels. The results from the lysimeter samples suggested that IXF is very unstable, as it was not detected in the soils of any treatment (**Table 4**). The major form of the herbicide in the soil profile was the DKN metabolite, with concentrations ranging from 0.026 to 0.18 $\mu$g kg$^{-1}$. The BA metabolite was detected at very low levels in soils of the orchardgrass and tall fescue treatments (**Table 4**). The concentrations of DKN and BA in plant tissue ranged from 0.41 to 6.84 $\mu$g kg$^{-1}$ and were significantly higher than in the soils. The higher proportion of BA to DKN in plant tissues (23−53%), as compared to soil (0−5%), suggested that living forage tissues were capable of oxidizing DKN to BA. This conversion may represent a detoxification mechanism that relates to the sensitivity of these forage grasses to DKN. Results from preliminary greenhouse studies indicated that the resistance of forages to IXF strongly correlated with their capacity to convert DKN, the biologically active metabolite, to the non-phytotoxic BA metabolite. The same conclusion was reached by Pallett et al. (*6*) in an IXF detoxification study of a broad range of weed species and corn using $^{14}$C labeled IXF (*5*).

**Conclusion.** The development of analytical procedures to detect IXF and its metabolites in soil and plants provides a needed tool for studying the fate of this new herbicide under field conditions. The HPLC-MS/MS method described in this paper allows for unambiguous identification and quantification of IXF and its metabolites in soil and plant samples. Limits of

detection are approximately 1−2 orders of magnitude more sensitive than previously developed soil or plant methods that combined HPLC or GC with mass spectrometry. As compared to the plant method developed previously, our modified extraction scheme increased the average extraction recovery of these compounds in plants by up to approximately 20%. In addition to quantifying the fate of IXF and its metabolites in the environment, these new methods will also be beneficial to determine the resistance and detoxification capacity of plants to IXF as well as for the determination of residue levels in food products.

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