Microwave-Assisted Extraction Coupled with Liquid Chromatography/Electrospray Ionization Mass Spectrometry for the Simplified Determination of Imidazolinone Herbicides and Their Metabolites in Plant Tissue

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The imidazolinones are a significant new class of low-use-rate, reduced-environmental-risk herbicides for protection of a wide variety of agricultural crops. Current residue methodologies for determining the imidazolinones and their metabolites in crops involve laborious, time-consuming cleanup procedures after an aqueous/organic extraction. Using imazethapyr, the most widely utilized member of the class, and its metabolites as representatives, liquid chromatography/electrospray ionization mass spectrometry was found to permit processing as little as 1% of the extract normally processed through the existing methods. Microwave-assisted extraction aided in the shortened cleanup procedure by providing a 100% aqueous initial extract. Over a range of 11 different commodities, average recoveries for imazethapyr, its hydroxy metabolite, and the glucose conjugate were 100%, 102%, and 97%, respectively. The simplicity and general applicability of this analytical approach reduced not only sample analysis time but also, perhaps even more importantly, method development time.

Keywords: *Imidazolinone; herbicides; determination; MAE; LC/ESMS*

INTRODUCTION

The imidazolinones are a significant new class of low-use-rate, reduced-environmental-risk herbicides for the protection of a wide variety of agricultural commodities (Shaner and O'Connor, 1991). As shown in Figure 1, the members of this class of herbicides have similar structural features centered around the imidazolinone ring and an attached aromatic system bearing a carboxylic acid moiety. In general, the imidazolinones have excellent activity against annual and perennial grasses and broad-leaved weeds when applied either pre- or postemergence. They function by inhibiting acetohydroxy acid synthase, the feedback enzyme in the biosynthesis of the branched-chain essential amino acids (Shaner et al., 1984; Anderson and Hibbert, 1985). This enzyme is not present in animals. Generally, the imidazolinone herbicidal selectivity between weed species and crops is attributable mainly to the differential metabolic rates or in some cases to the absorption rate at different growth stages rather than differential sensitivity of the target site (Shaner and Mallipudi, 1991). Thus, tolerant plant species are capable of metabolizing imidazolinone herbicides at a substantially faster rate than susceptible weeds and crops. The use range of a particular imidazolinone depends upon the susceptibilities of both the selected crop and its associated spectrum of weeds. For instance, imazethapyr controls annual and perennial grasses and broad-leaved weeds in such crops as soybeans, peas, beans, and alfalfa and other leguminous crops. Imazapyr, on the other hand, is a total vegetation control agent that has found use in forestry management and has potential for weed control in imidazolinone-tolerant crops.

Current analytical methodologies for the determination of imidazolinones in crop commodities follow a generally similar, laborious, time-consuming route (Devine, 1991; Mortimer and Weber, 1993). Following extraction, 2 gram-equivalents (g-equiv) of the crop commodity is processed through a series of precipitation, filtration, partition, and evaporation steps. Final cleanup is achieved with a strong cation exchange (SCX) or strong anion exchange (SAX) solid phase extraction (SPE) cartridge followed by a final CH_2Cl_2 partitioning step. Instrumental determination of residues at the 50 or 100 ppb level is accomplished with either liquid chromatography with UV detection or gas chromatog-

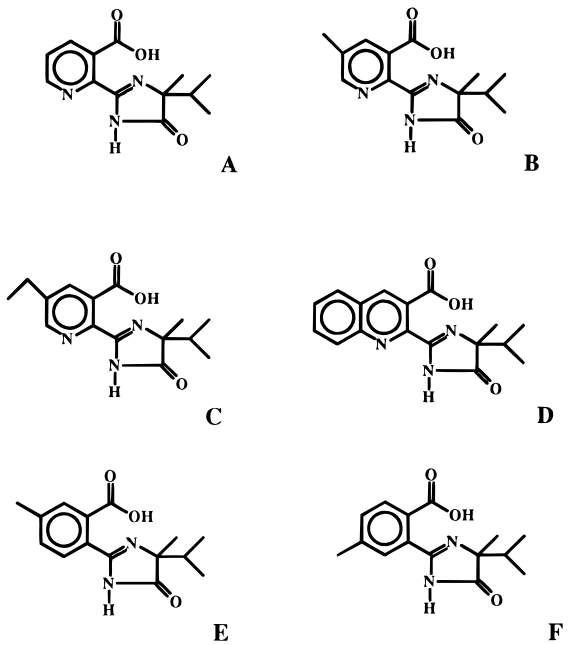

Figure 1. Structures of the imidazolinone herbicides: (A) imazapyr, (B) imazmethapyr, (C) imazethapyr, (D) imazaquin, (E) *m*-imazamethabenz, and (F) *p*-imazamethabenz.

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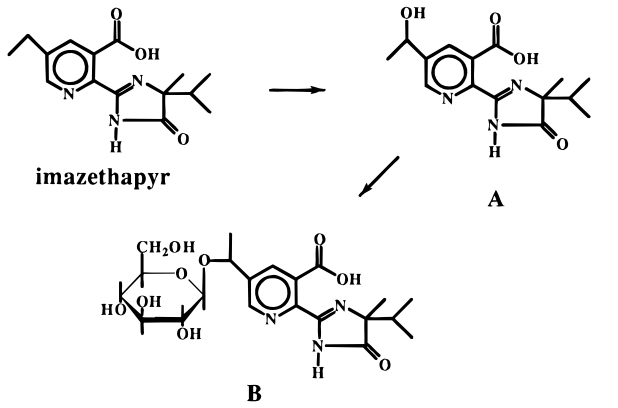

Figure 2. Plant metabolites of imazethapyr: (A) hydroxy metabolite and (B) glucoside of the hydroxy metabolite.

raphy with nitrogen/phosphorus detection following methylation.

Recently, we reported the development of greatly simplified analytical methods for the determination of imazethapyr in soil (Stout et al., 1996a) and imazapyr in imidazolinone-tolerant corn commodities (Stout et al., 1996b). These achievements largely resulted from coupling a more selective elution of a single, small C18 SPE cartridge with the sensitivity and specificity of gas chromatography/electron capture negative ion chemical ionization mass spectrometry (GC/ECNICI). With similar chemical structures and thus similar conventional workup procedures and negative ion responses, this analytical approach should be applicable to the determinations of parent imidazolinones in crop and soil matrices. However, for imidazolinones containing an alkyl side chain, hydroxylation and subsequent glucosidation in certain crops yield metabolites (Figure 2) which must also be determined (Lee et al., 1991). While the hydroxy metabolites could be processed and analyzed by the same analytical procedure as the parent, the polar glucoside could not be eluted from the C18 cartridge with CH_2Cl_2, gave multiple methylated products including the methylated aglycone with the methylating agent, and gave only ~1% of the negative ion response of the parent. Thus to achieve analysis of an imidazolinone and its metabolites by GC/ECNICI would have required eluting the parent and hydroxy metabolite from C18 with CH_2Cl_2 for GC/ECNICI, eluting the glucoside with CH_3OH, stripping the CH_3OH, hydrolyzing the glucoside in boiling 2 N HCl, and processing the released aglycone through a second C18 cartridge for a second GC/ECNICI analysis.

In an effort to develop a simplified procedure for analyzing an imidazolinone and its metabolites simultaneously in crop commodities, we directed our efforts at evaluating liquid chromatography/electrospray ionization mass spectrometry (LC/ESMS) in conjunction with microwave-assisted extraction (MAE). Recent reports have indicated that LC/ESMS can yield as much as a 100-fold improvement in response over that generated by previous LC/MS ionization techniques (Voyksner, 1994; Molina et al., 1994). We recently succeeded in applying this performance enhancement to the rapid determination of imidazolinones in water at the 1 ppb level without any sample cleanup or concentration (Stout et al., 1996c). With an initially cleaner extract from our soil work with MAE, we also wanted to examine the utility of this technique for providing the initial extract for cleanup.

To evaluate the potential general applicability of an analytical procedure based on LC/ESMS and MAE, we targeted imazethapyr and its metabolites as representative analytes and selected a wide range of crop commodities. Although imazethapyr is not used on some of the commodities and its metabolites need *not* be determined on others, the intention was to assess the effect of matrix variability on a fixed set of analytes. The primary mission of this study was to develop a broadly applicable analytical methodology for determining imidazolinones and their metabolites in crop commodities that could be fine-tuned to a specific application as needed.

EXPERIMENTAL PROCEDURES

Solvents and Standards. All solvents were distilled in glass and suitable for pesticide analysis (Burdick & Jackson Laboratories, Inc.). Water was purified by a Milli-Q water purification system (Millipore Corp.). Concentrated hydrochloric acid and glacial acetic acid were analytical reagent grade (J. T. Baker). Saturated KCl/CH_3OH was prepared by weighing ~50 g of KCl (reagent grade; J. T. Baker) into a 1-L bottle and adding ~1 L of CH_3OH. Additional KCl and CH_3OH were added as the solution was depleted.

Stock solutions of 1000 μg/mL imazethapyr and its metabolites (American Cyanamid, Agricultural Products Research Division, Princeton, NJ) were prepared in acetone. Aliquots from each stock standard solution were pooled and diluted with H_2O to give a 1.0 μg/mL fortification standard solution. Further dilutions with H_2O were performed to give analytical standard solutions containing 5, 10, and 50 ng/mL of each analyte. When stored in a refrigerator, these solutions were stable for at least 1 month.

Apparatus. The microwave extractor was an MES 1000 (CEM Corp.). A small laboratory centrifuge (Adams Compact II, Becton-Dickinson) was used with 10-mL disposable centrifuge tubes (Kimble). The vacuum processing station was an IST VacMaster equipped with a PTFE stopcock/needle system (International Sorbent Technology). The SPE cartridges were a 500-mg (3-mL column vol) Isolute SCX (International Sorbent Technology) and a 200-mg (3-mL column vol) Bond Elut C18 (Varian). The sample concentrator was a Techne Dri-Block Model DB-3D.

LC/ESMS was performed on a Finnigan-MAT TSQ70 (functionally upgraded over time to the equivalent of a TSQ700) triple-stage quadrupole mass spectrometer equipped with a Finnigan-MAT atmospheric pressure ionization (API) system. Two Shimadzu Model LC-10AD pumps controlled by a Shimadzu SCL-10A system controller delivered 1% acetic acid/H_2O and 1% acetic acid/CH_3OH to a tee (Cat. NO. P-728, Upchurch) followed by a low-volume static mixer (Cat. No. CMA0110113T, Lee Scientific). A gradient of 20% organic (1% HOAc/CH_3OH) to 50% organic over 20 min with a 5-min hold was delivered at a flow rate of 0.5 mL/min to a Rheodyne Model 7125 injector fitted with a 50−μL loop and onto a 3-mm × 10-cm Upchurch α-chrom C18 column. After exiting the column, the effluent flowed through a Shimadzu Model SPD-10AV UV detector at 254 nm before entering the inlet of the Finnigan-MAT electrospray interface.

Operational parameters specific to the electrospray interface included the following: electrospray voltage, 5 kV; capillary temperature, 300 °C; capillary voltage, 30 V; tube lens, 70 V; octapole offset, −2.0 V; N_2 sheath gas, 80 psi; N_2 auxillary gas rotometer setting, 30. Mass spectrometric operating parameters for LC/ESMS included the following: mode of operation, Q1MS; conversion dynode voltage, −15 kV; electron multiplier voltage, 1350 V; preamplifier gain, 10^{-8} A/V. Using a dwell time of 1.0 s/ion (3 s total cycle time), the (M + H) ions were monitored as follows: *m*/*z* 290 (imazethapyr), *m*/*z* 306 (hydroxy metabolite), *m*/*z* 468 (glucose conjugate).

General Procedure for Sample Preparation. Four grams of the crop commodity was weighed into a microwave extractor vessel. For fortified samples, an appropriate volume

of the fortification standard solution was added; 40 mL of H_2O was added by pipet to the extraction vessel and stirred with the plant material. The vessel was placed in the microwave oven and extracted at 125 °C for 3 min (TAP = time at parameter). After cooling, a few milliliters of the extractant was filtered through a disposable 25-mL fritted reservoir fitted on the vacuum processing station and into a 10-mL disposable centrifuge tube. The extractant was centrifuged at 3200 rpm for 5 min; 1 mL of liquid (100 mg-equiv of the crop commodity) was transferred by pipet from the centrifuge tube to a 20-mL disposable scintillation vial and acidified with 1 mL of 0.01 N HCl.

A 500-mg SCX cartridge was prepared by washing with 1 column vol (cv = 3 mL) of CH_3OH followed by 1 cv of 0.01 N HCl. A disposable Pasteur pipet was used to transfer the acidified extract from the scintillation vial to the cartridge. The vial was rinsed with ~1 mL of 0.01 N HCl which was also transferred to the cartridge. After loading the SCX cartridge at a rate of ~1 drop/s, the cartridge was rinsed with 1 cv of 0.01 N HCl followed by 1 cv of CH_3OH. The analytes were eluted into a second scintillation vial with 2 cv's of saturated KCl/CH_3OH. The CH_3OH was evaporated under a stream of nitrogen on a sample concentrator held at 60 °C. The residue was dissolved in 1 mL of H_2O and acidified with 1 mL of 0.01 N HCl.

For desalting the extract prior to LC/ESMS, a 200-mg C18 cartridge was prepared by washing with 1 cv of CH_3OH followed by 1 cv of H_2O followed by 1 cv of 0.01 N HCl. Using a disposable Pasteur pipet, the acidified extract from the SCX cartridge was transferred to the C18 cartridge. The scintillation vial was rinsed with ~1 mL of 0.01 N HCl which was also transferred to the cartridge. After loading the cartridge at a rate of ~1 drop/s, the cartridge was rinsed with 1 cv of H_2O. The analytes were eluted into a third disposable 20-mL scintillation vial with 1 cv of CH_3OH, and the CH_3OH was evaporated under a stream of nitrogen on the sample concentrator (60 °C). Residual H_2O was removed by adding ~3 mL of CH_3CN and reevaporating. The residue was dissolved in 1 mL of H_2O for LC/ESMS. Assuming injection of equal volumes of standard and sample, a 100-pg injection (10 μL of a 10 ng/mL standard solution) was equivalent to 100 ppb of each analyte in the crop commodity.

Specific Modifications for Selected Commodities. For peanut hull and meat and soybean seed, acidification of the initial extract yielded a precipitate. The precipitate was removed by transferring the acidified extract and precipitate from the scintillation vial to a second 10-mL centrifuge tube. After centrifugation for 5 min, the supernatant was transferred by Pasteur pipet to the SCX cartridge and carried through the remainder of the cleanup procedure.

For alfalfa hay, the 100 mg-equiv load on the SCX cartridge gave inadequate retention of the analytes. This problem was overcome by directly placing only 20 mg-equiv (200 μL) of the alfalfa hay extract into the SCX cartridge barrel. Before transferring the extract to the cartridge barrel, the barrel was filled halfway with 0.01 N HCl after the conditioning procedure. After filling the remainder of the cartridge barrel with additional 0.01 N HCl, the extract was loaded onto the SCX cartridge and processed through the rest of the cleanup procedure. To compensate for the 5× reduction in sample workup, 5× more of the final extract (50 μL) was injected for LC/ESMS.

RESULTS AND DISCUSSION

Overview of Extraction and SPE Cleanup. Developing a general analytical approach for determining imazethapyr and its metabolites across a broad spectrum of crop commodities was a challenge and educational experience because of the range of polarities of the analytes coupled with the biological diversity of the crop matrices. Handling all three analytes simultaneously in the existing cleanup procedures entailed significant compromises accompanied by additional work. The glucose conjugate in particular has always been a major problem. As mentioned in the Introduction, the parent and hydroxy metabolite could be reasonably cleaned up for GC/ECNICI with CH_2Cl_2 elution from a C18 cartridge. However, using CH_3OH to elute the conjugate also eluted a significant amount of colored matrix coextractives. In existing methods, the presence of any CH_3OH or salts in the loading solution of the SCX cartridge led to poor retention of the conjugate. Consequently, following evaporation of the organic solvent from the initial extract and a subsequent precipitation step, a C18 cartridge for desalting and another evaporation step had to be used before loading the extract onto the SCX cartridge. Because the conjugate was not retained well on the SCX cartridge with loading from an aqueous solution, the extract had to be reconstituted in dilute aqueous acid (0.01 N HCl) to achieve a successful load.

Our approach at sample cleanup was targeted at using the "workhorse" SCX cartridge for the imidazolinones and eliminating as many sample-processing steps as possible. If successful, this approach would not only save time but also dramatically reduce organic solvent consumption. To eliminate the initial extractant evaporation and precipitation steps, we envisaged directly loading the initial extract onto the SCX cartridge. Such a procedure would only be possible from a 100% aqueous initial extract.

Extraction. Having succeeded with MAE in the analysis of imidazolinones from soil (Stout et al., 1996a), we examined a number of extractants on aged crop commodities from radiolabeled field studies. Using H_2O, CH_3OH, 10% H_2O/CH_3OH, and 0.1 M NH_4OAc/H_2O on corn plant from the imazapyr/corn studies (Stout et al., 1996b), extractabilities of ~90% were obtained from all extractants and were comparable to those of the conventional aqueous/organic extractions. In the extraction of barley straw from a field study of imazmethapyr where the hydroxy metabolite and glucose conjugate were present, MAE with H_2O or 0.1 M NH_4OAc/H_2O gave better extractabilities (~65%) than the conventional extraction (~55%). Unlike the soil extractions, the initial plant extracts from MAE were not cleaner than those from the conventional extractions. Interestingly, the color of the extract from MAE depended upon the extractant and the age (and color) of the crop commodity and was generated *before* applying any microwave energy. Young green plant (forage) gave a dark green color when extracted with CH_3OH but only a faint straw color with H_2O. Dried plant (fodder or hay) gave a medium brown color with H_2O but a light green color with CH_3OH. Thus, one extractant could not be selected to give less initial color over the entire spectrum of commodities. Consequently, given our desires to directly load the SCX cartridge from the initial extract and to minimize organic solvent usage and given that the extractabilities were essentially the same, we selected H_2O as the extractant for MAE.

SPE Cleanup. Even with an aqueous initial extract, the real key to successful implementation of this shortened analytical approach resided with the SCX cartridge. Not only did this cartridge have to provide sufficient separation of the matrix from the analytes, the SCX cartridge also had to retain a collection of analytes spanning a wide range of polarities while being loaded with the matrix. As previously mentioned, any problems with losses on the SCX cartridge would be reflected first in the glucose conjugate. Our hopes for getting an SCX cartridge to tolerate the coextractives

in a direct load of the initial extract were premised on the reduced amount of matrix requiring cleanup for final determination by LC/ESMS. With adequate instrumental response at 100 pg/analyte, a 10−μL injection from a 1-mL final volume indicated that only 100 mg-equiv of extract was needed. This value was 1/20 of the amount of extract normally processed through the existing conventional analytical procedures.

Our initial attempts focused on a direct load of the aqueous extract onto a 200-mg SCX cartridge. This smaller capacity cartridge allowed elution of the analytes in only 1 cv (~3 mL) of saturated KCl/CH_3OH, thereby minimizing organic solvent usage and subsequent evaporation time. However, a low recovery (~35%) from the glucose conjugate even from an aqueous load of a standard solution indicated that acidification of the extract was still required before loading the SCX cartridge. While all analytes in the standard solution gave excellent recoveries through the 200-mg SCX cartridge after acidification, processing a corn fodder extract spiked at 100 ppb gave essentially 100% recoveries for the parent and hydroxy metabolite but only a 65% recovery for the glucose conjugate. Increasing the SCX cartridge capacity to 500 mg solved this recovery problem but necessitated eluting the cartridge with 2 cv's of saturated KCl/CH_3OH.

Since the electrospray interface could not be exposed to large amounts of nonvolatile KCl from KCl/CH_3OH elution of the SCX cartridge, a 200-mg C18 cartridge served as a desalting step. An attempt was made to eliminate the C18 cartridge by eluting the SCX cartridge with a volatile salt (NH_4OAc) which would either evaporate on stripping before dissolution in H_2O for analysis or not matter in LC/ESMS. NH_4OAc (0.1 M) in CH_3OH was found to elute the analytes from the SCX cartridge as effectively as saturated KCl/CH_3OH. Processing a corn fodder extract through the SCX cartridge with NH_4OAc/CH_3OH elution gave a couple of additional early eluting peaks in the LC/UV chromatogram. These peaks presumably arose from polar components left from elimination of the C18 cartridge and its H_2O wash. While these peaks eluted too early to affect the LC/ESMS analysis, an intense broad hump was detected in the chromatograms of the monitored ions suggestive of something overloading the LC column. With this peak spanning the retention time region from the glucose conjugate through the hydroxy metabolite, adjusting the chromatographic separation to remove the offending component from the analytes appeared highly problematic. Consequently, further attempts at streamlining the cleanup to a single cartridge were abandoned for the duration of this study.

LC/ESMS. With the metabolites of imazethapyr also generating essentially only (M + H) ions as previously reported for the parent imidazolinones (Stout et al., 1996c), the entire analytical approach was centered around exploiting the sensitivity and specificity of LC/ESMS. The sensitivity of the technique reduced the amount of extract that needed to be processed, thus reducing the matrix load on the cartridges. Its specificity permitted detection of the analytes without interference from matrix coextractives. Figures 3−5 vividly illustrate these points. In Figure 3, the analytes at the 100-pg level were not detectable by LC/UV, and the control wheat hay extract showed a plethora of coextractives throughout the chromatogram. In sharp contrast, reasonable responses were obtained by LC/ESMS (Figures 4 and 5), and while the extracted ion current

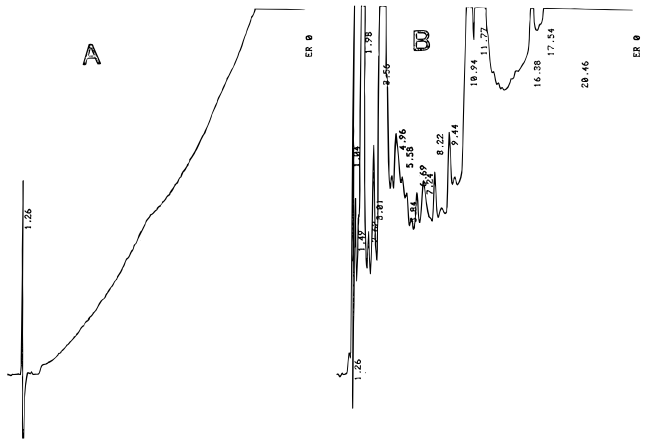

Figure 3. LC/UV chromatograms of (A) a 100-pg standard injection and (B) control wheat hay.

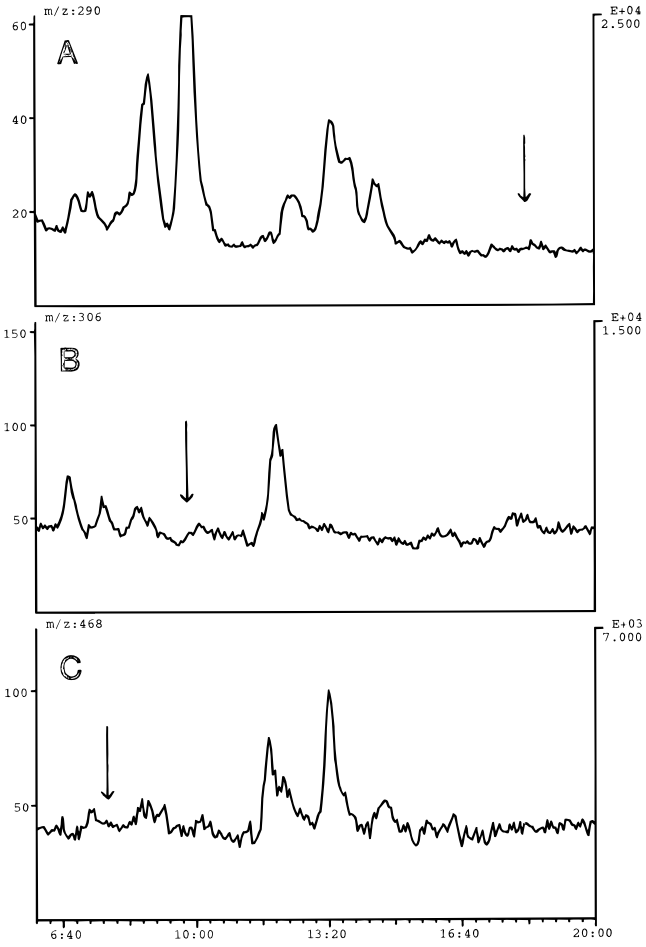

Figure 4. Extracted ion current profiles of imazethapyr and metabolites from LC/ESMS of control wheat hay: (A) imazethapyr, (B) hydroxy metabolite, and (C) glucoside of the hydroxy metabolite.

profiles were hardly devoid of any coextractives, the analytes were well separated from interferences.

Specific Method Modifications. As noted in the Experimental Section, several specific commodities posed special challenges and educational opportunities. In executing the method as originally developed, the peanut commodities (hull and meat) and soybean seed gave precipitates with acidification of their initial extracts. These precipitates ostensibly arose from the insolubility of extracted proteins and/or fatty acids upon addition of acid. Loading the acidified extract with precipitate directly onto the SCX cartridge clogged the

Table 1. Recoveries[a] (%) of Imazethapyr and Metabolites from Crop Commodities

	glucose conjugate			OH metabolite			parent		
commodity	cont[b]	50 ppb	250 ppb	cont	50 ppb	250 ppb	cont	50 ppb	250 ppb
corn fodder	<5	94.8	99.2	<7	115.6	101.4	<10	104.3	106.9
corn forage	<5	92.6	94.2	<7	90.9	89.3	<5	99.7	99.3
corn grain	<5	90.3	90.7	<6	95.5	97.9	<5	100.3	89.1
wheat forage	<6	90.0	93.7	<4	95.4	104.9	<5	103.8	108.2
wheat hay	<6	103.2	102.8	<4	113.7	112.9	<3	102.5	104.2
wheat grain	<6	101.6	93.8	<4	101.8	85.0	<3	102.7	88.5
alfalfa hay	<9	91.5	92.4	<7	110.6	107.1	<10	102.4	113.4
peanut hull	<7	91.4	101.6	<7	88.4	92.2	<3	98.5	92.5
peanut meat	<9	112.4	117.4	<6	90.5	91.1	<6	97.8	94.3
soybean forage	<5	98.4	88.1	<4	96.3	91.4	<3	98.2	96.7
soybean seed	<4	105.4	95.2	<4	130.0	118.6	<3	92.6	89.7
average		97.4	97.2		103.6	99.2		100.6	98.4
std deviation		7.8	9.8		12.9	10.8		5.1	9.0

[a] The average of duplicate samples processed through the procedure. [b] Cont = control value in ppb.

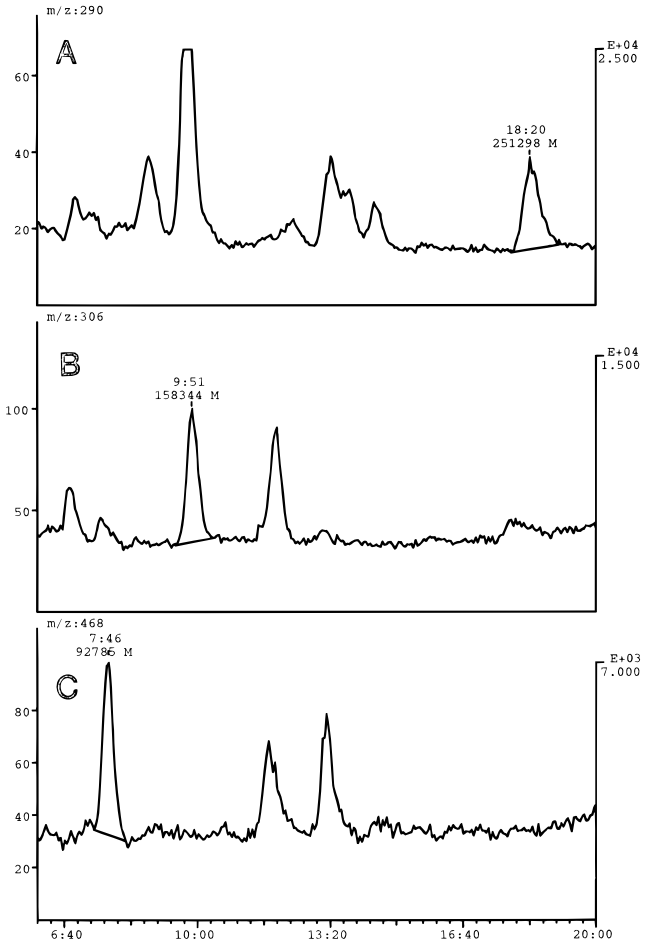

Figure 5. Extracted ion current profiles of imazethapyr and metabolites from LC/ESMS of wheat hay fortified at 50 ppb: (A) imazethapyr, (B) hydroxy metabolite, and (C) glucoside of the hydroxy metabolite.

upper frit of the cartridge resulting in a pathetically slow elution. However, removal of the precipitate was easily effected with a second centrifugation prior to loading the SCX cartridge.

While alfalfa hay offered the greatest challenge, it also provided the most valuable learning experience. With processing 100 mg-equiv of extract, alfalfa hay was the only commodity with which the analytes would not load properly onto the 500 mg SCX cartridge. Only the parent compound gave a recovery of >90%. The recoveries of the hydroxy metabolite and glucose conjugate were ~50% and essentially 0%, respectively. However, since imazethapyr and each metabolite have a 500-ppb tolerance in alfalfa, the easiest way to address the problem was to try loading the SCX cartridge with only 1/10 the usual amount of extract from an alfalfa hay fortified at 500 ppb. With essentially quantitative recoveries for all analytes at this level, we then backtracked to processing only 20 mg-equiv of extract at the 50 and 250 ppb fortification levels. With injecting 50 μL of the final extract, the absolute amount of matrix injected onto the LC/ESMS was no different than before, but the recoveries were now satisfactory as a result of reducing the matrix load on the SCX cartridge.

Reducing the amount of initial extract processed to 20 mg-equiv (1/100 of the amount processed through conventional procedures) and injecting a larger aliquot (50 μL) from the same final volume (1 mL) would appear to be a preferable route for the analyses of the other commodities as well. Preliminary results from the peanut commodities and soybean seed have shown elimination of the precipitate with acidification of this reduced level of matrix and, consequently, the need for a second centrifugation. Additionally, since the extract was directly loaded into the SCX cartridge barrel, the separate acidification and transfer steps were also eliminated. While not attempted in this study, further reducing the amount of initial extract processed to 10 mg-equiv and injecting 100 μL for analysis might permit the use of the originally evaluated 200-mg SCX cartridge with further savings in organic solvent usage and subsequent evaporation time.

Validation Results. To validate the method, control commodities were spiked with the appropriate amount of the fortification standard solution to give fortified samples at 50 and 250 ppb. Unspiked commodities served as controls. Control and fortified samples were processed as described in the Experimental Section and analyzed by LC/ESMS. The results are given in Table 1. Each analyte was linear from 50 to 500 pg (50−500 ppb). Overall the recoveries expressed as the average ± one standard deviation for each analyte across all fortified samples were 100 ± 7.2% for imazethapyr, 102 ± 12% for the hydroxy metabolite, and 97 ± 8.7% for the glucose conjugate. Controls showed apparent levels of <10 ppb for all analytes and were generally <5 ppb.

Conclusions and the Future. A general approach for the simplified determination of imidazolinones and their metabolites in crop commodities has been developed using imazethapyr and its metabolites as representatives. MAE eliminated organic solvents in the extraction and allowed a direct load of the initial extract onto the SCX cartridge for cleanup as long as matrix concentrations were kept at a minimum. The sensitiv-

ity and specificity of LC/ESMS permitted processing as little as 1% of the extract normally carried through conventional analytical procedures. The simplicity and general applicability of the analytical procedure reduced not only sample analysis time and organic solvent consumption but also, perhaps even more importantly, method development time. Sample throughput was increased as much as 6-fold when compared to existing methodologies, and in most cases, the analytical procedure was successfully demonstrated on each commodity in 1−2 days.

Future efforts will be directed at reducing the LC/ESMS analysis time by using methodology recently developed for analyzing the imidazolinones in water (Stout et al., 1996c). As found in that study, commensurate with reduced analysis time will be increased signal/noise. The utility of LC/ESMS/MS will also be explored for confirmatory purposes and in case additional specificity is required with the more rapid chromatographic analysis (Covey et al.,1986; Johnson and Yost, 1985). Perhaps LC/ESMS/MS will enable the removal of the C18 cartridge and permit analysis of the extract after elution from the SCX cartridge with NH_4OAc/CH_3OH.

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