Rapid, Direct Determination of Imidazolinone Herbicides in Water at the 1 ppb Level by Liquid Chromatography/Electrospray Ionization Mass Spectrometry and Tandem Mass Spectrometry

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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 analytical methodologies for the determinations of individual imidazolinones in water at the 1 ppb level involve processing several hundred milliliters of water through a series of solid-phase extraction cartridges and solvent-partitioning steps. By combining liquid chromatography with electrospray ionization mass spectrometry or electrospray ionization tandem mass spectrometry, all imidazolinones can be monitored simultaneously at the 1 ppb level with only a simple filtration prior to analysis. Recoveries from tap, lake, and well waters were essentially quantitative for each imidazolinone in both modes of analysis.

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

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 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, alfalfa, and other leguminous crops. Imazapyr, on the other hand, is a total vegetation control agent that has found use in forestry management.

Current analytical methodologies for the determinations of imidazolinones in water at the 1 ppb level are targeted at individual members of the group. Typically, several hundred milliliters of water are processed through a series of solid-phase extraction (SPE) cartridges and solvent-partitioning steps with final analysis by liquid chromatography with UV detection (Devine, 1991; Wells and Michael, 1987). Alternatively, the final extract can be methylated and analyzed by gas chromatography with nitrogen/phosphorous detection (Devine, 1991; Mortimer and Weber, 1993). Obviously, these clean-up and concentration steps require both time and organic solvents in loading and eluting SPE cartridges and in subsequent solvent removal. An attempt at the direct determination of imazapyr in water by LC/UV gave a detection limit (*not* limit of quantitation, LOQ) of 10 ppb (Liu et al., 1992).

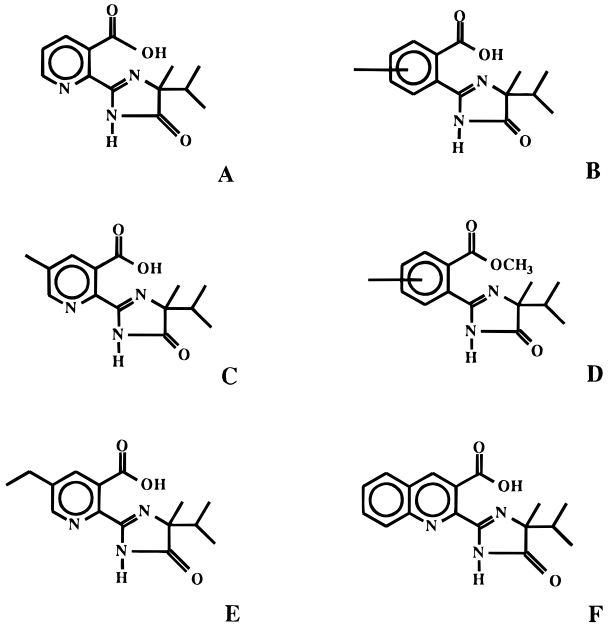

Figure 1. Structures of the imidazolinone herbicides: (A) imazapyr, (B) imazamethabenz, (C) imazmethapyr, (D) imazamethabenz-methyl, (E) imazethapyr, and (F) imazaquin.

Recent reports have indicated that liquid chromatography/electrospray ionization mass spectrometry (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). Our goal in this study was to ascertain if LC/

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S0021-8561(96)00125-2 CCC: $12.00

ESMS could be used to monitor all imidazolinones simultaneously in water at 1 ppb (LOQ) without any concentration or cleanup of the water. With the absence of any sample cleanup, we also wanted to evaluate liquid chromatography/electrospray ionization coupled with tandem mass spectrometry (LC/ESMS/MS) in case additional specificity might be required (Johnson and Yost, 1985; Covey et al., 1986; Voyksner et al., 1987). We also needed to determine the effect on electrospray ionization performance of repeated injections of environmental waters. With no sample cleanup to remove salts and minerals, their potential buildup in the electrospray interface could be deleterious.

EXPERIMENTAL PROCEDURES

Solvents and Standards. CH_3OH (catalog no. 230-4) and H_2O (catalog no. CP80285-4) were high-purity solvents suitable for LC and spectrophotometry from Burdick & Jackson. Glacial acetic acid was "Baker Analyzed" reagent grade (catalog no. 9507-00) from J. T. Baker. Imidazolinone standards were obtained from American Cyanamid Company, Agricultural Products Research Division, Princeton, NJ.

LC/ESMS and LC/ESMS/MS. LC/ESMS and LC/ESMS/MS were 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 (catalog no. P-728, Upchurch) followed by a low-volume static mixer (catalog no. CMA0110113T, Lee Scientific). An ~50-cm length of 0.007-in.-i.d. PEEK LC tubing conducted the mobile phase from the static mixer to the Rheodyne Model 7725 injector provided with the API system on the mass spectrometer. The injector was fitted with a 100-μL loop. A very short length (~10 cm) of 0.005-in.-i.d. PEEK LC tubing connected the injector to a 5-cm × 4.6-mm i.d. TosoHaas TSK-GEL Super-ODS column. A second ~1-cm length of 0.005-in.-i.d. LC tubing connected the column outlet to the inlet of the Finnigan-MAT electrospray interface. Two gradients were employed, each at a flow rate of 0.5 mL/min. A gradient of 10% organic (1% HOAc/CH_3OH) to 60% organic over 10 min with a 2-min hold followed by a 1-min reset to 10% organic was used for the simultaneous analysis of all imidazolinones. A faster gradient over the same range in 5 min with a 2.5-min hold and a 0.5-min reset was used for the analysis of individual imidazolinones.

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 1.0 s/scan total cycle time, the following (M + H) ions were monitored: *m/z* 262 (imazapyr), *m/z* 275 (imazamethabenz), *m/z* 276 (imazmethapyr), *m/z* 289 (imazamethabenz-methyl), *m/z* 290 (imazethapyr), *m/z* 312 (imazaquin).

Additional instrumental parameters for LC/ESMS/MS included the following: collision gas and pressure, Ar at 1.0 mTorr; collision energy, −30 eV; preamplifier gain, 10^{-9} A/V. Using the parent scan mode in LC/ESMS/MS, the first quadrupole successively passed the (M + H) ion of each imidazolinone (as done in LC/ESMS), while the third quadrupole was kept fixed on the common product ion at *m/z* 86. Unit mass resolution had to be maintained on both quadrupoles when simultaneously determining all imidazolinones.

Preparation of Solutions. From an initial stock solution of 1000 ng/mL (equivalent to 1000 ppb) of each analyte in H_2O, successive dilutions were made to give standard solutions of 100, 10, 1, and 0.5 ng/mL. Fortified environmental water samples were prepared by pipetting (Falcon 7551 disposable serological pipet, 10 mL in 1/10 mL) 9.9 mL of the water into a 20-mL disposable scintillation vial and adding 100 μL of the appropriate standard solution. As a result, the concentration of the fortified sample was 100-fold lower than the concentration of the standard solution used for spiking.

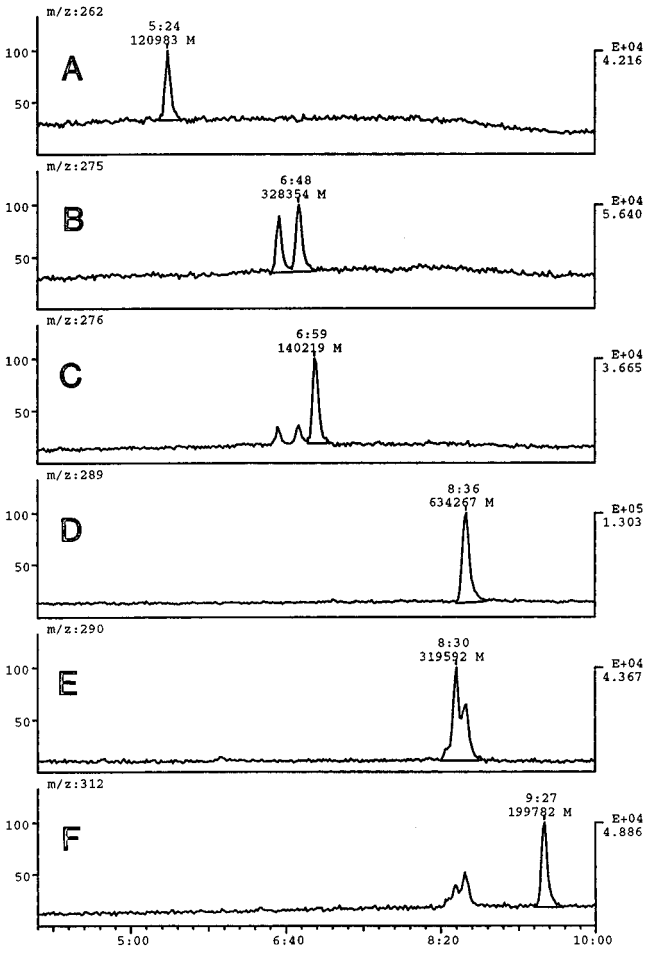

Figure 2. Extracted ion current profiles of the imidazolinones from LC/ESMS of 1 ppb fortified lake water: (A) imazapyr, (B) imazamethabenz, (C) imazmethapyr, (D) imazamethabenz-methyl, (E) imazethapyr, and (F) imazaquin.

Sample Preparation for Analysis. Between 5 and 10 mL of the water for analysis (the exact volume was *not* important) was poured into a disposable 10-cm^3 syringe barrel (catalog No. 309604, Becton-Dickinson) fitted with a 0.22-μm Millex-GS filter (catalog No. SLG S025OS, Millipore Corp.). The syringe plunger was inserted into the barrel and the water forced through the filter and into a 20-mL scintillation vial. The sample was then ready for analysis.

RESULTS AND DISCUSSION

LC/ESMS Analyses. By LC/ESMS, each imidazolinone generated essentially only an (M + H) ion. Monitoring these ions from a lake water fortified at 1 ppb (Figure 2) showed excellent responses, and there was a dearth of any other chromatographic responses. This performance resulted not only from the sensitivity and specificity provided from LC/ESMS but also from the enhanced chromatographic performance of the 2-μm silica-based column. Placing the column between the injector provided with the API system and the ES interface and removing the UV detector reduced chromatographic dead volumes to a minimum. Consequently, chromatographic peak widths at half-height were ~4 s with the slower gradient and ~3 s with the faster gradient. With narrower LC peaks, signal/noise increased as much as 8 times compared to that obtained

Table 1. Recoveries[a] (Percent) of Imidazolinones from Water Using LC/ESMS

	well			tap			lake		
analyte	cont[b]	1 ppb	10 ppb	cont	1 ppb	10 ppb	cont	1 ppb	10 ppb
imazapyr	<0.1	108.5	101.8	0.11	115.9	100.9	<0.1	101.0	104.1
imazamethabenz	<0.1	105.7	96.6	<0.1	107.0	100.8	<0.1	97.0	98.9
imazmethapyr	<0.1	95.8	107.2	<0.1	99.2	96.7	<0.1	101.6	103.2
imazamethabenz-methyl	<0.1	96.2	97.0	<0.1	100.5	94.3	<0.1	93.6	94.8
imazethapyr	<0.1	100.6	100.1	<0.1	88.0	90.1	<0.1	101.0	104.1
imazaquin	<0.1	96.0	95.8	<0.1	103.2	99.7	<0.1	97.0	98.9

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

in our laboratory with a 3-mm × 10-cm C-18 column connected to a UV detector prior to the ES interface and with a 25-min gradient. This latter chromatographic system was used in some earlier preliminary evaluations.

The only interferences that caused a potential problem were those generated by certain imidazolinones with other imidazolinones. These "cross-interferences" arose from certain pairs of imidazolinones eluting close to each other and differing by only 1 u in molecular weight. As evident in Figure 2, this effect was most pronounced in the selected ion chromatogram of *m/z* 290 for imazethapyr. While the *m/z* 290 ion was monitored as the (M + H) ion of ^{12}C-imazethapyr, the *m/z* 290 ion was also the (M + H) ion of the ^{13}C isotope (or ^{15}N isotope) of imazamethabenz-methyl which eluted shortly after imazethapyr. This same effect was also evident in the selected ion chromatogram of *m/z* 275 for imazmethapyr. Here, the ^{13}C isotopes of the two imazamethabenz isomers generated a pair of peaks preceding imazmethapyr. In fact, the reason for developing the slower gradient was to effect a chromatographic separation between this latter pair of analytes. With the faster gradient, the second peak of imazamethabenz was pushed into the front of the imazmethapyr peak. Unfortunately, no amount of adjustment to the gradient gave any better separation between imazethapyr and imazamethabenz-methyl.

However, since the relative amount of ^{13}C (and also ^{15}N) is fixed in a population of a given molecule by the number of C and N atoms in the molecule, a correction can be made to compensate for this situation. The ^{13}C and ^{15}N contribution of imazamethabenz-methyl to the *m/z* 290 response was calculated by multiplying the *m/z* 289 peak area by 0.183, $(P + 1)/P$ for 16 C's and 2 N's. The peak area for imazethapyr was then obtained by subtracting this correction factor from the total area of the merged peaks at *m/z* 290.

This concern may actually be mostly hypothetical in nature because imazamethabenz-methyl, a grain herbicide, has a dramatically different use spectrum from imazethapyr. Thus, in real-world situations, it is highly unlikely that imazamethabenz-methyl and its metabolite, imazamethabenz, would appear in the same water sample as imazethapyr and, most likely, other imidazolinones. Under these circumstances where only certain imidazolinones need to be determined, the faster LC gradient can be employed to cut analysis time approximately in half with a concomitant 30−50% increase in analyte peak height. Additional improvement in *S/N* can also be achieved by devoting more instrument scan time to only those analytes of interest.

To validate the method, control water samples from three different sources (tap, lake, and well) were spiked with the appropriate standard solution to give fortified samples at 1 and 10 ppb. 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 over the range of 0.5−10 ng/mL. Overall, the recoveries expressed as the average ± 1 standard deviation for each analyte across all fortified samples were 105 ± 5.9% for imazapyr, 101 ± 4.5% for imazamethabenz, 101 ± 4.3% for imazmethapyr, 96 ± 2.6% imazamethabenz-methyl, 98 ± 7.5% for imazethapyr, and 97 ± 3.4% for imazaquin. Controls showed apparent levels of <0.1 ppb for all analytes.

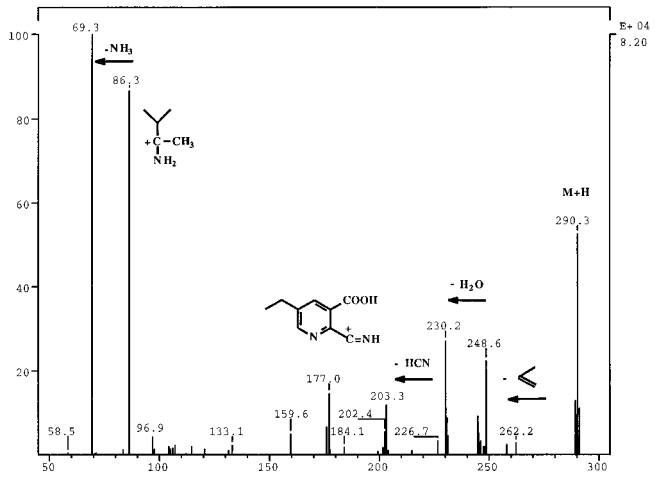

Figure 3. Product ion spectrum from collisionally activated dissociation of the (M + H) ion of imazethapyr.

LC/ESMS/MS Analyses. Collisionally activated dissociation (CAD) of the (M + H) ions of the imidazolinones generated a number of structurally significant "product ions". Over a range of fragmentation energies with 1 mTorr of Ar as the collision gas, the two most intense and consistent product ions were *m/z* 86 and 69. These ions were produced from the imidazolinone ring and were, thus, common to all imidazolinones. The product ion spectrum of the (M + H) ion of imazethapyr is shown in Figure 3. Even though slightly less intense than *m/z* 69, the *m/z* 86 ion was selected for monitoring because retention of the NH_2 group in the ion was deemed to yield a more structurally significant product ion. By fixing the third quadrupole (second mass analyzer) on the product ion at *m/z* 86, the parent ions of the imidazolinones could be scanned as in LC/ESMS with the first quadrupole. Thus, to be detected as a particular imidazolinone by this highly specific detection technique, a compound would have to simultaneously elute at the correct LC retention time, generate the appropriate parent ion, and fragment that parent ion under CAD to the *m/z* 86 product ion.

As encountered with LC/ESMS, the only interferences that caused a potential problem were those generated by certain imidazolinones with other imidazolinones. Since the (M + H) ions of all imidazolinones generated the same product ion, this additional element of specificity, usually useful for dealing with matrix co-extrac-

Table 2. Recoveries[a] (Percent) of Imidazolinones from Water Using LC/ESMS/MS

	well			tap			lake		
analyte	cont[b]	1 ppb	10 ppb	cont	1 ppb	10 ppb	cont	1 ppb	10 ppb
imazapyr	<0.13	102.9	95.7	<0.15	94.5	85.7	<0.12	98.9	97.5
imazamethabenz	<0.1	109.1	111.2	<0.1	109.2	101.5	<0.1	96.9	90.1
imazmethapyr	<0.13	83.2	96.3	<0.15	109.6	104.2	<0.16	89.0	95.8
imazamethabenz-methyl	<0.1	85.2	93.0	<0.1	95.6	92.4	<0.1	98.5	96.3
imazethapyr	<0.1	94.2	99.7	<0.1	111.0	81.6	<0.1	100.8	93.4
imazaquin	<0.1	100.6	109.4	<0.1	86.8	97.8	<0.1	96.9	90.1

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

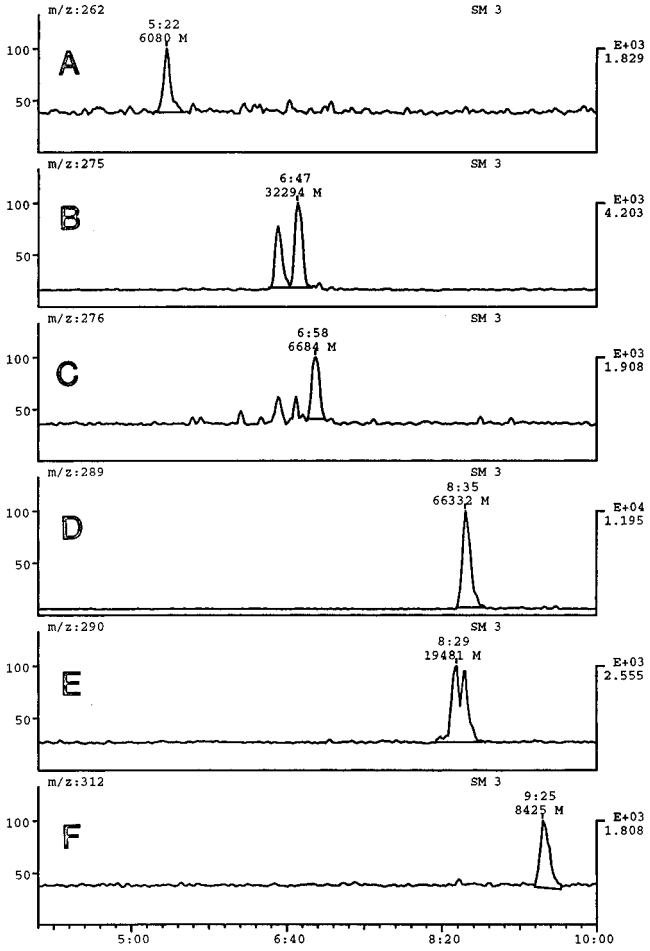

Figure 4. Extracted ion current profiles of the imidazolinones from LC/ESMS/MS of 1 ppb fortified lake water: (A) imazapyr, (B) imazamethabenz, (C) imazmethapyr, (D) imazamethabenz-methyl, (E) imazethapyr, and (F) imazaquin.

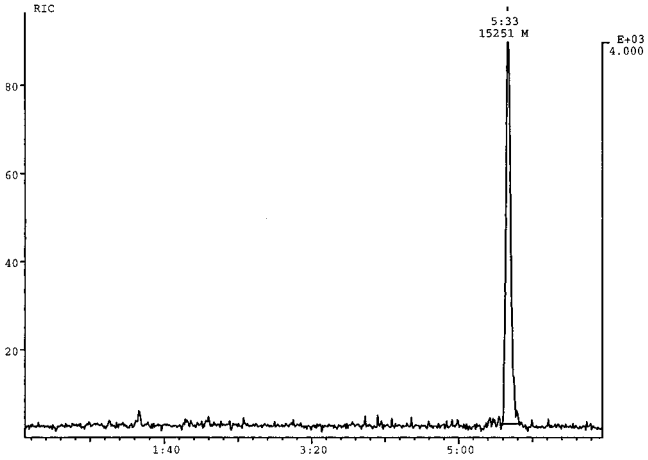

Figure 5. Extracted ion current profile of imazethapyr at 1 ppb in well water using LC/ESMS/MS with quadrupole detuning and a faster LC gradient.

tives, did not eliminate the type of interference seen in LC/ESMS. In fact, this situation led to the additional downside of being unable to detune the quadrupoles to increase signal response, a common procedure in MS/MS (Hunt et al., 1980, 1981). With detuning the resolution of the first quadrupole, the higher mass imidazolinone of the cross-interfering pairs started to show response in the chromatogram of the imidazolinone 1 u lower. This problem could not be solved by a simple consideration of ^{13}C isotope ratios. Consequently, even with eliminating chemical noise and, thus, being able to run the detector electronics at maximum sensitivity, the absence of detuning reduced absolute signal responses in LC/ESMS/MS ~10 times from those obtained in LC/ESMS. One minor compensating feature with LC/ESMS/MS was the leveling of the baseline offsets of the individual analytes compared to the widely disparate ones of LC/ESMS.

The correction factor for the response of imazamethabenz-methyl in the selected reaction chromatogram of *m/z* 290 → 86 was also somewhat more complicated in LC/ESMS/MS. Generation of the *m/z* 86 product ion from *m/z* 290 from ^{13}C-imazamethabenz-methyl depended upon the odds that all ^{12}C was retained in the product ion. If the ^{13}C was retained, the product ion would occur at *m/z* 87 and not be detected. The odds that ^{13}C (or ^{15}N) was *lost* in the CAD process to give *m/z* 86 were the number of C's and N's in the neutral fragment *lost* with CAD (11 C's and 1 N) vs the number of C's and N's in the parent ion (16 C's and 2 N's). Thus, the correction factor was 11/16 × 0.176 (^{13}C contribution of imazamethabenz-methyl to the *m/z* 290 ion) plus 1/2 × 0.0074 (^{15}N contribution) or 0.125. This correction factor was applied in the same manner as discussed for LC/ESMS.

Figure 4 shows data from LC/ESMS/MS of the same fortified lake water analyzed by LC/ESMS and shown in Figure 2. With the lower absolute responses of LC/ESMS/MS, the signal/noise was somewhat less than LC/ESMS even with the reduced noise from tandem MS detection. This effect was most noticeable in the chromatograms of imazapyr. LC/ESMS/MS was validated with the same control and fortified solutions used in the LC/ESMS validation. The results are given in Table 2. Overall, the recoveries expressed as the average ± 1 standard deviation for each analyte across all fortified samples were 96 ± 11% for imazapyr, 103 ± 9.8% for imazamethabenz, 96 ± 9.8% for imazmethapyr, 94 ± 5.4% for imazamethabenz-methyl, 97 ± 11% for imazethapyr, and 100 ± 7.4% for imazaquin. Controls generally showed apparent levels of <0.1 ppb for all analytes.

As discussed for LC/ESMS, the potential for imidazolinone cross-interference in the analysis is largely a hypothetical consideration. If the quadrupoles are

detuned and the faster gradient employed, greatly improved response can be achieved as shown in Figure 5 for imazethapyr at 1 ppb in well water.

Conclusions. Rapid, direct methods for the simultaneous determinations of imidazolinones in water at 1 ppb (LOQ) have been developed using LC/ESMS and LC/ESMS/MS. The only sample preparation was a simple filtration. No organic solvents were used other than those for LC. While both analytical approaches gave essentially quantitative recoveries, the precision from LC/ESMS/MS was approximately half that of LC/ESMS and probably resulted from the lower absolute responses in LC/ESMS/MS. Perhaps the loss of response from not being able to detune may be overcome with newer LC/ESMS/MS instrumentation based on ion trap technology where ion transmission loses are negligible in MS/MS (McLuckey et al., 1991). For the samples analyzed in this study, no benefit was accrued by using the more specific detection of LC/ESMS/MS. However, for "dirtier water" samples or for confirmatory purposes, LC/ESMS/MS may still prove highly useful. Concerns about salt or mineral deposits adversely affecting instrumental performance have so far proved unwarranted. Having analyzed not only water samples but also soil and plant extracts for over 6 months, analyte responses have been remarkably stable and no maintenance of the ES interface has been required over this period of time.

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Received for review February 23, 1996. Accepted May 8, 1996.[⊗]

JF960125G

[⊗] Abstract published in *Advance ACS Abstracts,* July 15, 1996.