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Quantitative analysis of imazamox herbicide in environmental water samples by capillary electrophoresis electrospray ionization mass spectrometry

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Abstract

Capillary electrophoresis–mass spectrometry (CE–MS) with an electrospray ionization interface was applied for the quantitative analysis of imazamox pesticide in well water, potable water, and pond water. The detector response for imazamox was determined to be linear over the concentration range of 50–1 ng/ml. The limits of quantitation and detection of the method were determined to be 200 and 20 ng/l for imazamox compound in each type of water sample, respectively. The total sample preparation and CE–MS analysis time was under 2 h.

Keywords: Water analysis; Environmental analysis; Validation; Imazamox; Pesticides

1. Introduction

Imazamox, $C_{15}H_{19}O_4N_3$, (Fig. 1) is an experimental imidazolinone herbicide developed by BASF (formerly American Cyanamid Company, Princeton, NJ, USA; the information provided about imazamox in this section is obtained from American Cyanamid's bulletin for RAPTOR Herbicide). Imazamox is a relatively polar compound with a low *n*-octanol–water partition coefficient (K_{ow}) of 5.36. The relatively high polarity of the compound may be the reason for imazamox's weak adsorption to soil. Imazamox is hydrophilic in the pH range encountered in the environment. The solubility of imazamox in hexane, dichloromethane, methanol and acetone is 0.0006, 14.3, 6.68 and 2.93 g/100 ml, respectively. Imazamox is not classified as readily biodegradable and is stable to hydrolysis but susceptible to photolysis. Imazamox is selective for many leguminous crops such as soybeans, field peas, alfalfa, dry beans, and peanuts.

Currently, there are several general techniques used for the selective extraction of agrochemicals from environmental samples [1,2]. All of the approaches require significant sample cleanup and preparation procedures to avoid signal suppression and enhancement, which may result in irreproducible analytical data [3–5]. Capillary electrophoresis has been used for the rapid and quantitative analysis at ppb levels of imidazolinone herbicides and their metabolites from soil [6]. A reversed micellar electrokinetic chromatography (MECC) method was developed to simultaneously assay imazamox and its two polar metabolites (hydroxy and glucose conjugate) in adzuki beans [7]. Penmetsa et al. [8] used cyclodextrin as an additive in the running buffer to simultaneously separate the enantiomeric and isomeric forms of imidazolinones (imazaquin and imazamethabenz) and diclofop.

The application of CE–MS for trace analysis of environmentally sound herbicides has been the topic of several studies [9,10]. The applicability of CE–MS for polar herbicides such as the sulfonylureas was demonstrated by Garcia and Henion [11], using on-line CE–MS equipped with a pneumatically assisted electrospray (ion-spray) interface. They separated eight sulfonylurea herbicides in complex crop matrices at 1 pmol level. Recently, the mass spectrometer has

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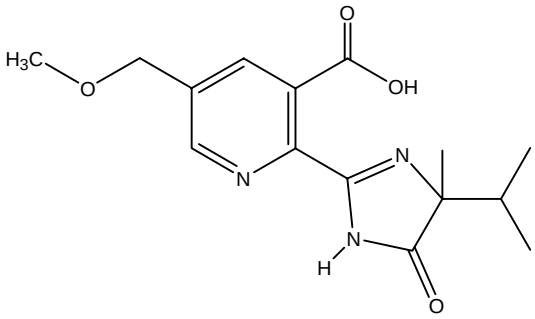

Fig. 1. Molecular structure of imazamox (MW = 305.1).

been gaining acceptance as a quantitative tool by regulatory agencies [US Environmental Protection Agency (EPA), European Union (EU), etc.]. MS offers less potential for matrix interference along with high sensitivity and selectivity.

This investigation explores the application of CE–MS for the qualitative and quantitative determination of imazamox herbicide in water. Residues of imazamox are extracted from the water samples using a reversed-phase solid-phase extraction (SPE) cartridge, RP-102. Measurement of the residues is accomplished by CE–MS using an electrospray ionization (ESI) source with selected-ion monitoring (SIM) in the positive-ion mode. Results are calculated by direct comparison of peak response of imazamox to those of external standards. The validated sensitivity (limit of quantitation (LOQ)) of the method is 200 ng/l for imazamox compound in each type of water sample. The limit of detection (LOD) of the method is 20 ng/l for imazamox in each type of water sample.

2. Experimental

2.1. Chemicals and reagents

Herbicide standards [imazamox: nicotinic acid, 2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)- 5 - (methoxymethyl)] were provided by BASF. Ammonium formate was purchased from Sigma (St. Louis, MO, USA), methanol was obtained from EM Science (Gibbstown, NJ, USA) and/or Burdick & Jackson (B&J) (Muskegon, MI, USA), and formic acid was acquired from Fluka (Ronkonkoma, NY, USA) and/or Sigma. The purity of the standard was $\geq$97.5%. Stock standard solutions were prepared by dissolving a known amount (15 mg) of imazamox in methanol followed by repeated dilutions in methanol–water. The calibration standard solutions were prepared by further dilutions of the stock standard solutions in 0.01% formic acid in purified water.

2.2. Apparatus

Capillary electrophoretic–mass spectrometric separations were performed using a capillary electrophoresis system HP^{3D}CE, Agilent Technologies, Waldbronn, Germany; detection was accomplished by coupling the HP^{3D}CE to HP 1100 series mass-selective detector, Agilent Technologies, Palo Alto, CA, USA. Bare-fused silica capillary columns with 50 μm internal diameter (i.d.) and variable lengths (55–85 cm) were used. The instrument was controlled via HP ChemStation software. The specific connection between CE and MS was made possible by using a CE–MS capillary cassette. The orthogonal flow sprayer was used in all experiments. An Agilent isocratic 1100 series liquid pump was used to deliver the sheath liquid.

2.3. Water samples

Twenty-six samples of natural water were collected from the Farm Pond of Cook College (Passion Puddle Pond), Rutgers University, New Brunswick, NJ, USA, and drinking well water from 86 Rocktown Road, Ringoes, NJ, USA, from March to June 2000. The samples were collected in PTFE bottles of ~1.2 l capacity, stored at 4 °C for approximately 6 h and placed in a freezer at ≤ -8 °C. In addition, 13 samples of tap water from BASF were collected and analyzed on the same day.

2.4. Residue sample preparation

Water samples: 1 l of each sample was fortified at 200 ng/l (LOQ) and 20 ng/l (LOD) with imazamox. The control and fortified samples were subjected to the same sample preparation methods prior to analysis by CE–MS. The pH of the water samples was measured and ranged between 6.4 and 6.7. The pH of the samples was then adjusted to 2.5 with concentrated formic acid. The samples were passed through a 0.5 g RP-102 SPE cartridge (RP-102: Spe-ed 500 mg/6 ml cartridge, catalog no. 4210, Applied Separations, Allentown, PA, USA; adapters: Isolute, PTFE adapters, catalog no. 120–1100, IST International, Lakewood, CO, USA; reservoirs: Isolute, empty, 25 and 70 ml capacity, catalog nos. 120–1007-E and 120–1008-F, respectively, IST International), which was previously conditioned with methanol followed by 1% formic acid in water. The imazamox residues remained on the cartridge while the eluent was discarded. The RP-102 cartridge was washed with two column volumes of water and then dried with air to remove any remaining moisture in the sorbent bed. The residues were then eluted with one column volume (5 ml) of methanol. The eluate was evaporated to dryness using a rotary evaporator/vacuum pump and re-suspended in appropriate volumes of 0.01% formic acid prior to CE–MS analysis.

Seven 1 l samples of the unsupplemented well, potable and pond water were fortified with 200 ng of imazamox (200 ng/l fortification level, LOQ). Two 1 l samples of unsupplemented potable, pond, and well water were fortified with 20 ng imazamox (20 ng/l fortification level, LOD). Another duplicate 1 l sample of unsupplemented potable, pond, and well water were assayed as controls. The fortified and unsup-

plemented potable, pond, and well water samples were acidified with formic acid to approximately pH 2.5 (subsequent discussion on unsupplemented waters will be referred to as control water). Each sample was then passed through a conditioned 0.5 g RP-102 SPE cartridge, onto which the analyte was retained. The cartridge sorbent was washed with purified water to remove any remaining salt. The analytes were then eluted with methanol. The eluate was evaporated to dryness and the residues were re-dissolved in 0.01% formic acid in water for CE–MS analysis.

2.5. Standard solution preparation

2.5.1. Stock imazamox solutions

A 100 μg/ml imazamox was prepared with methanol–water (10:90, v/v). This solution was used to prepare a 1000 ng/ml imazamox stock solution in purified water. The exact concentrations were corrected for the standard purity.

2.5.2. Fortification solutions

Two hundred and 20 ng/ml LOQ concentrations were prepared with 0.01% formic acid in water using the 1000 ng/ml imazamox stock solution. The 20 ng/ml standard was also used as the working (bracketing) standard for injection between samples.

2.5.3. CE–MS calibration standards

Fifty, 40, 10, 4, 2, and 1 ng/ml imazamox standards were prepared with 0.01% formic acid in water. The 1000 ng/ml stock solution was used to prepare the 50, 40, and 10 ng/ml imazamox standards. The remaining standards were prepared using the 10 ng/ml imazamox solution.

2.6. Experimental conditions

Reproducible separations were achieved for water samples with the electrolyte buffers of 10, 25 or 50 mM ammonium formate in 0.01% MeOH–water, pH 7.0. The sheath liquid buffer was methanol ammonium formate (5 mM) (50:50, v/v), pH adjusted to approximately 3.7 with formic acid. The sheath liquid was pumped at 4 μl/min to the ESI source.

In general, the system was operated under varied applied voltages of 10 kV for 55–58 cm, 15 kV for 75 cm and 20 kV for greater than 75 cm capillary lengths. For quantitative analysis, longer capillaries provided superior resolution. Frequent cropping of the column was necessary at the end of each sequence due to equipment design. The MS system would load a default method at the end of each sequence-run (overnight sequence runs) which would boost source temperature from 100 to 300 °C causing polyimide melt down and silica column to be brittle. Sample injections were at a fixed 5 kPa pressure, injection times varied from 10 or 50 s and column temperature was set at 25 °C. All acquisitions were performed under positive polarity. In general, in the full scan acquisition mode, the amount of sample injection was 50 s at fixed 5 kPa, while in the SIM acquisition mode, the sample injection was 10 s at fixed 5 kPa. The abundance in sensitivity or high signal-to-noise ratio in the SIM scanning mode requires shorter injection time than the full scan acquisition mode, which has limited sensitivity or low signal-to-noise ratio. All injections were made with capillary cassette temperature set at 25 °C. Quantitation of imazamox in water samples was performed by direct peak area comparison with bracketing external standard solutions.

Linearity of response was determined during the analysis of imazamox samples. The mass centroid of the ion at 306.1 *m/z* imazamox was determined by making 5 kPa and 10 s injections of the 20 ng/ml CE–MS standard. Equal volumes (5 kPa and 10 s) of the 50, 40, 20, and 10 ng/ml CE–MS calibration standards (imazamox) were injected for LOQ determinations. Also, equal volumes (5 kPa and 10 s) of the 10, 4, 2, and 1 ng/ml CE–MS calibration standards (imazamox) were injected for LOD determinations. The response ratios for all injections were determined by dividing the peak response by the amount (ng) injected.

The validity of the procedure was demonstrated by recovery tests of one fortified control, which was analyzed concurrently with each set of samples. These fortifications should cover the range of expected residue values. For single fortified control samples, the recovery sample was at the sensitivity (LOQ) of this method.

3. Results and discussion

3.1. Limits of quantitation for imazamox in water samples

Laboratory fortifications of the test systems (well, drinking potable and farm pond water) using imazamox at the LOQ level of 200 ng/l were prepared. The test systems and the fortified samples of the test systems were analyzed using CE–MS, under the conditions discussed before, to determine the percent recoveries of imazamox. The fortification and analysis experiments of the water samples from different sources were done on different days. The overall mean and standard deviations for imazamox were as follows: $81.2 \pm 2.1\%$ ($n = 7$) in pond water, $82.6 \pm 3.0\%$ ($n = 7$) in well water, and $87.0 \pm 2.7\%$ ($n = 7$) in potable water. The overall mean recovery for imazamox in all test systems at 200 ng/l was $83.6 \pm 3.6\%$ ($n = 21$). A typical spiked recovery farm pond water sample followed by a control and a 20 ng/ml working standard are shown in Fig. 2. It is apparent that there is no interference in the imazamox window, which indicates that imazamox can be specifically determined in the given pond water at a LOQ of 200 ng/l. The same was observed with the well and potable water samples.

In order to demonstrate reproducibility and accuracy, seven recovery samples of each representative water samples (i.e., potable, well, and farm pond), were assayed using

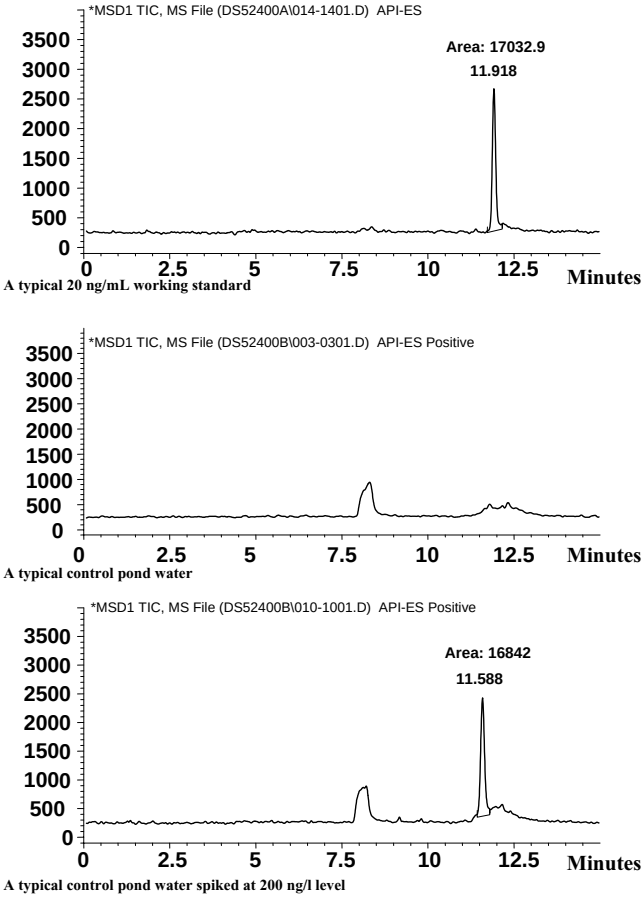

Fig. 2. A typical electropherogram of 20 ng/ml imazamox working standard followed by a control and spiked pond water samples at 200 ng/l LOQ. Fused silica column, 73 cm × 50 μm i.d.; inlet buffer, 10 mM ammonium formate, pH 6.7–7.0; sheath liquid, methanol ammonium formate (5 mM) (50:50, v/v), pH 3.7; applied voltage, 20 kV; detection, ESI-MS; hydrodynamic injection, 10 s at 5 kPa.

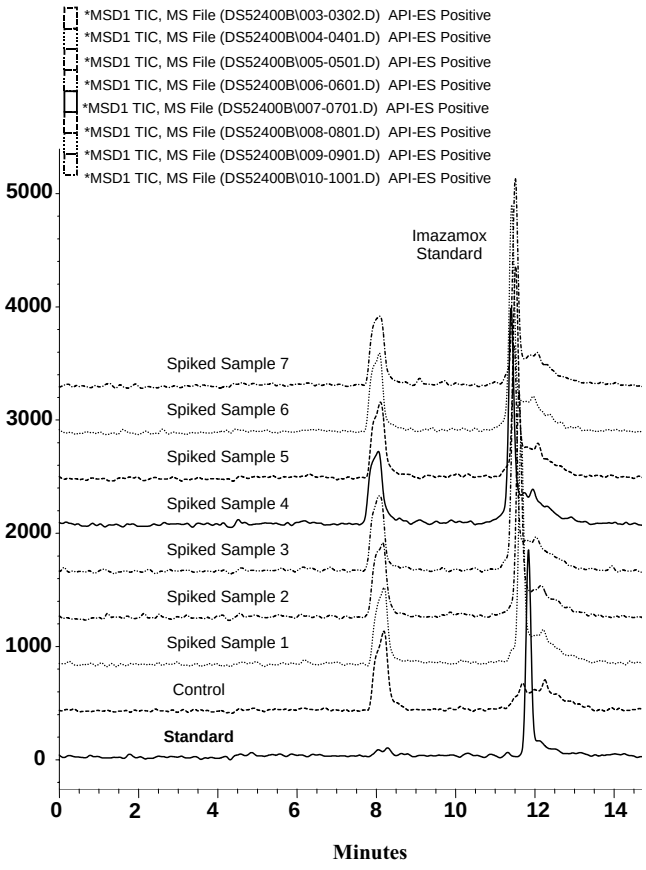

Fig. 3. A signal overlay of typical 20 ng/ml imazamox working standard followed by a control and seven spiked pond water samples at 200 ng/l LOQ; signals are offset by 20% based on intensity to demonstrate migration time alignment of peaks (reproducibility) after multiple simultaneous injections of different pond spiked water samples. Fused silica column, 73 cm × 50 μm i.d.; inlet buffer, 10 mM ammonium formate, pH 6.7–7.0; sheath liquid, methanol ammonium formate (5 mM) (50:50, v/v), pH 3.7; applied voltage, 20 kV; detection, ESI-MS; hydrodynamic injection, 10 s at 5 kPa.

the CE–MS method. A signal overlay of a typical 20 ng/ml working standard, followed by a control and seven spiked recovery farm pond water samples at 200 ng/l level, are shown in Fig. 3. The signals are offset by 20% based on their intensity. A sorbent discoloration, from white to dark brown, was observed during the analysis of the farm pond water. The color change was caused by the multiple sample injections and was attributed to the matrix components, in particular organic compounds, retaining in the RP-102 cartridge. Nevertheless, as it is evident from Fig. 3, the method was reproducible at the LOQ level. This is supported by the insignificant shift in migration time, no gross affect on area counts, and no significant change in resolution and peak efficiencies, even after nine injections. The migration time of imazamox in well and potable water samples (relatively simpler matrices) remained stable throughout the run and the system maintained its reproducibility. The total analysis time for the single RP-102 sample pre-concentration step, combined with CE–MS method, was less than 2 h.

3.2. Limits of detection for imazamox in water samples

Laboratory fortifications of the well water using imazamox at levels of 0 ng/l (control) and 20 ng/l were carried out. The methodology was similar to that of LOQ. The criterion for establishment of LOD was based on the appearance of an interfering peak in the imazamox migration window. In this work, at 20 ng/l a constant 50% interference was observed, which was set as the LOD in the method. The residues were measured by CE–MS and the product ion *m*/*z* 306.1 was monitored with multiplier gain set to 10 V. The results were calculated for imazamox by direct comparison of the peak area in the sample to those of the bracketing external standards. Due to existence of interference in the imazamox window, the peak area of the interference was subtracted from the spiked well water sample. This interference could not be separated from the parent compound despite the use of wide ranges of ammonium

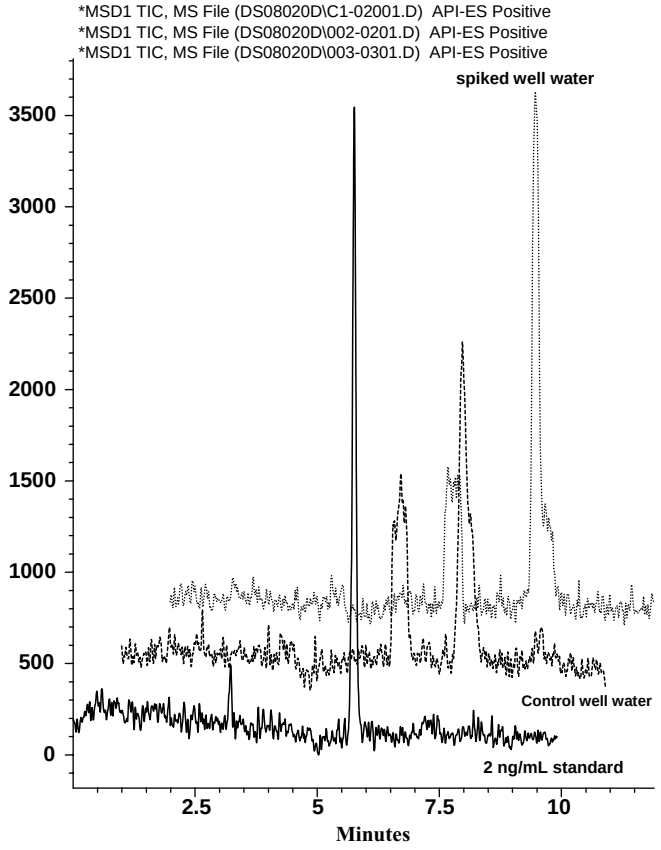

Fig. 4. A typical control and spiked well water mass electropherograms signal overlay at 20 ng/l LOD level (gain = 10 V); signals are offset by 10% based on time lapse. Fused silica column, 58 cm × 50 μm i.d.; inlet buffer, 10 mM ammonium formate, pH 6.7–7.0; sheath liquid, methanol-ammonium formate (5 mM) (50:50, v/v), pH 3.7; applied voltage, 15 kV; detection, ESI-MS; hydrodynamic injection, 10 s at 5 kPa.

formate concentrations (10–50 mM) and pH (3.7–7.0). This led to the inference that at such ultra trace levels (20 ng/l), cross contamination from glassware and the purity level of chemicals and solvents can be a major issue, thereby making any attempt of separation ineffectual. However, since the interference comprised ≤50% of the imazamox peak and did not increase in size between different water samples, the recoveries were considered acceptable and the method LOD of 20 ng/l was established. Depicted in Fig. 4 is a signal overlay of a 2 ng/ml working standard followed by typical control well water and control well water spiked at 20 ng/l with imazamox. The signals are offset by 10% based on time lapse. The percent recovery of imazamox was approximately 50%. The 50% recovery is acceptable for qualitative assessment provided that the level of detection is extremely low (i.e., 20 ng/l). The low sample recoveries could have originated from matrix co-extractives imparting suppression of imazamox ionization. Also, a shift in the migration time was observed which could be due in large part to a change in sample viscosity. If quantitation is necessary at this level, perhaps more selective cleanup procedures should be employed to alleviate matrix interference.

3.3. Precision

The precision of the instrument was measured based on three consecutive injections of 1000 ng/ml and two sets of 20 ng/ml imazamox external standard solutions under two different multiplier voltage conditions, 1 and 10 V [the electron multiplier voltage (gain) is a parameter used to manipulate signal intensity]. The precision is expressed in percent relative standard deviation (R.S.D.). The normalized response [average peak area/(imazamox concentration × gain)] ratios of nine injections are presented in Table 1. The R.S.D. for the nine standards was less than 2%. In general, increase in gain increases the signal intensity as well as the noise.

3.4. Migration time reproducibility

The migration time reproducibility was studied among different concentrations of imazamox standards and recovery samples. Three 1000 ng/ml and three 20 ng/ml standards were injected consecutively at gain voltage of 1 V, while three 20 ng/ml external standard were injected consecutively at gain voltage of 10 V. In all cases, migration time shift were less than 2%. Three consecutive injections of 1000 ng/ml standards demonstrated a migration time shift of less than 1%. The gross migration time shift was less than 2% for all of the standards and seven recovery samples at 200 ng/l in well water.

3.5. System linearity

In this method, imazamox is quantitated using a single-point external bracketing standard. Accordingly, the detection responses must be linear and independent of sample concentration over the approximate range of 50% to 150% of the working external standard concentration. This corresponds to a three-point calibration check that was injected along with the samples. The system linearity was calculated based on percent agreement between calibration check points. The percent agreement is defined in the method as the percent difference between the highest (or the lowest) specific response (SR) of each individual standard value and the average specific response divided by the average SR for all of the solutions over a specific range of concentrations. The detector response for imazamox was determined to be linear over the concentration range of 50–1 ng/ml. This covered the broad range of standards for both LOQ and LOD determinations. The percent agreement of SR for imazamox standards used in LOQ determination (imazamox standard concentrations: 50–10 ng/ml) was calculated to be 3.25%. The linear regression equation of $y = 126{,}192x - 52{,}573$, $r = 0.9924$ was obtained from the plot of area response versus imazamox concentration. These results indicated that the method is linear and has no obvious systematic deviation from linearity. The percent agreement of SR for imazamox standards used in LOD determination (imazamox standard

Table 1
Precision test results from three consecutive injections of imazamox calibration standard at two different concentrations and gain values

Imazamox concentration (ng/ml)	Gain (V)	Average peak area ($N = 3$)	Normalized specific response[a]	R.S.D. (%)
1000	1	9.67×10^5	9.67×10^5	2.00
20	1	2.47×10^4	1.23×10^6	0.87
20	10	2.12×10^5	1.06×10^6	0.54

Fused silica column, 58 cm × 50 μm i.d.; inlet buffer, 10 mM ammonium formate, pH 6.7–7.0; sheath liquid, methanol ammonium formate (5 mM) (50:50, v/v), pH 3.7; applied voltage, 15 kV; detection, ESI-MS; hydrodynamic injection, 10 s at 5 kPa.
[a] Normalized specific response = average peak area/(imazamox concentration × gain).

concentrations: 10–1 ng/ml) was calculated to be 4.04%. The linear regression equation of $y = 24{,}507x - 22{,}734$, $r = 0.9496$ was obtained from the plot of area response versus imazamox concentration. The lack of complete linearity indicates the "pushing" of the limit of detection. Although, this is ±10%, which at ultra trace residue levels is more than acceptable, however, this is the indication of lower limit. An *r*-value of 0.9496 for imazamox at such ultra trace levels indicated a reasonable fit to a linear function. The linear regression equation, $y = 24{,}507x - 22{,}734$ obtained from imazamox standards was used in LOD determination.

4. Conclusions

CE–MS with electrospray ionization interface showed remarkable sensitivity to imazamox and was proved to be an ideal technique for such a polar compound. One of the most important advantages of CE–MS was a significant enhancement in detection signal and migration time reproducibility. The increased sensitivity due to the application of MS allowed the use of larger final volumes. In retrospect, this helped to stabilize the viscosity of sample matrix and resulted in better control over the migration time. Although, migration time shift was not a major issue at the LOQ level, even with various lengths of capillary column, more precise migration times were noticed with the use of shorter columns.

A rapid sample pre-concentration, using an RP-102 cartridge combined with CE–MS, permitted the assay of water samples at ng/l levels within 3 h. The CE–MS quantitative analysis of imazamox at 200 ng/l level (LOD in 20 ng/l) in water samples, was both robust and reproducible. Application of this method for imazamox degradation products in all environmental compartments, such as water and soil, would be ideal. Since imazamox is polar and AHAS enzyme selective pesticide and has a very low field application rate (ounces/acre), it can be assumed that its residues and possible degradation products will appear at low levels in different environmental compartments. Thus, detection and quantitation of low levels of such polar residues will require extremely selective and sensitive technique such as the CE–MS method described in this work.

The trends in development of more selective agrochemical products have introduced relatively new classes of chemicals that lie more in the range of polar to charged species. These classes of compounds pose many analytical challenges, especially at trace levels. The combination of rapid SPE, combined with CE–MS, will provide the tools needed to meet the analytical expectations required in the new age of compound discovery and development.

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