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Direct aqueous determination of glyphosate and related compounds by liquid chromatography/tandem mass spectrometry using reversed-phase and weak anion-exchange mixed-mode column

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ABSTRACT

Analysis of the broad-spectrum herbicide glyphosate and its related compounds is quite challenging. Tedious and time-consuming derivatization is often required for these substances due to their high polarity, high water solubility, low volatility and molecular structure which lacks either a chromophore or fluorophore. A novel liquid chromatography/tandem mass spectrometry (LC/MS–MS) method has been developed for the determination of glyphosate, aminomethylphosphonic acid (AMPA) and glufosinate using a reversed-phase and weak anion-exchange mixed-mode Acclaim® WAX-1 column. Aqueous environmental samples are directly injected and analyzed in 12 min with no sample concentration or derivatization steps. Two multiple reaction monitoring (MRM) channels are monitored in the method for each target compound to achieve true positive identification, and $^{13}C,^{15}N$-glyphosate is used as an internal standard to carry out isotope dilution mass spectrometric (IDMS) measurement for glyphosate. The instrument detection limits (IDLs) for glyphosate, AMPA and glufosinate are 1, 2 and 0.9 μg/L, respectively. Linearity of the detector response with a minimum coefficient of determination (R^2) value ($R^2 > 0.995$) was demonstrated in the range of ~10 to 10^3 μg/L for each analytes. Spiked drinking water, surface water and groundwater samples were analyzed using this method and the average recoveries of analytes in three matrices ranged from 77.0 to 102%, 62.1 to 101%, 66.1 to 93.7% while relative standard deviation ranged from 6.3 to 10.2%, 2.7 to 14.8%, 2.9 to 10.7%, respectively. Factors that may affect method performance, such as metal ions, sample preservation, and storage time, are also discussed.

1. Introduction

Glyphosate is a non-selective, broad-spectrum herbicide used for control of annual and perennial plants including grasses, sedges, broad-leaved weeds, and woody plants [1,2]. It is used primarily in corn, soybean production and landscaping in urban areas. Several different forms of glyphosate are in widespread use including isopropylamine (e.g., Roundup Original®), potassium salt (e.g., Roundup WeatherMAX®) and the trimethylsulfonium salt (e.g., Touchdown®). Aminomethylphosphonic acid (AMPA) is a major metabolite of glyphosate and is often monitored due to its occurrence in environmental samples. Glufosinate or its ammonium salt (e.g., Basta, Rely, Finale, Challenge and Liberty) is also a broad-spectrum, non-selective herbicide [3] used in nurseries, vineyards, and orchards. As shown in Fig. 1, glyphosate and glufosinate are similar in structure. However, they exhibit completely different modes-of-action. Glyphosate works by inhibiting an enzyme involved in the synthesis of the aromatic amino acids (tyrosine, tryptophan and phenylalanine) while glufosinate works by blocking the enzyme glutamine synthase, a central enzyme in plant metabolism. Glyphosate resistance encountered in problematic weeds, such as rye grass, can be overcome by applying glufosinate. The recent development of crop plants that have been genetically modified to tolerate glufosinate is expected to increase its usage in the future [4].

Glyphosate and related herbicide glufosinate are considered moderately toxic to animals and humans, and have therefore been used extensively worldwide [5,6]. The frequent applications of these compounds generate concerns about their long-term influence and necessitate their monitoring. Since the 1980s, a wide range of analytical techniques have been employed for the determination of glyphosate and related compounds [7], including gas chromatography (GC) [8–10], high-performance liquid chromatography (HPLC) [11–13], capillary electrophoresis (CE) [14–16] coupled with, most commonly, fluorescence or mass spectrometry (MS) detection. The high polarity, high water solubility, low volatility, and the lack of chromophore or fluorophore in the molecular structure of these compounds made derivatization

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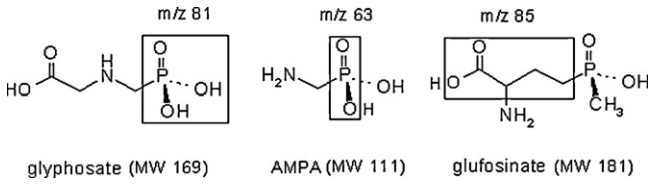

Fig. 1. Structures of glyphosate, AMPA and glufosinate.

a standard procedure employed for their determination to improve volatility, enable chromatographic separation, and/or to increase detection sensitivity. Commonly used derivatization agents include trifluoroacetic anhydride (TFAA)/trifluoroethanol (TFE) [17,18], chloroformates/diazomethane [19], 9-fluorenyl methoxycarbonyl chloride (FMOC-Cl) [20,21], and orthophthaldehyde (OPA)/mercaptoethanol [22,13].

A few attempts to eliminate the tedious and time-consuming derivatization step have been reported by using ion chromatography (IC) technology [23–25]. Though IC is a very powerful tool for ionic substance separation, co-elution of the target compounds has been reported previously, which indicates that IC also had difficulties in separating these high polarity hydrophilic organic acids. In addition, the non-volatile buffers or the 100% aqueous mobile phases used in standard IC procedures are not favourable for mass spectrometric detection. Post-column organic solvent addition was used to help the electrospray ionization process by Dahlmann et al.

Taking advantage of the unique multimode (reversed-phase and weak anion-exchange combined) separation mechanism offered by the Acclaim® Mixed-Mode WAX-1 column, a novel (LC/MS–MS) method to determine glyphosate, AMPA and glufosinate in aqueous environmental matrices by direct injection with no sample concentration or derivatization is presented here. The analysis is carried out by using an electrospray ionization source (ESI) in negative ionization and multiple reaction monitoring (MRM) scan mode. The MRM channel of the molecular ion and the most abundant product ion is chosen for target compound identification and quantification, while a second MRM channel is employed for further confirmation.

2. Experimental

2.1. Chemicals and reagents

Custom-made standard stock solutions of glyphosate, AMPA and glufosinate ammonium were purchased from Accustandard Inc. and Absolute Standards Inc. The standards from independent sources were used to monitor calibration accuracy to meet an accreditation requirement of the Canadian Association for Laboratory Accreditation Inc. (CALA). Custom-made solutions with certificates of analysis were chosen so no further verification for concentrations needed. A weight factor of 0.914 was used to convert the concentration of glufosinate ammonium to the concentration of glufosinate. A stock solution (>96% purity) of ^{13}C,^{15}N-glyphosate was obtained from Cambridge Isotope Chemicals (Andover, MA, USA) and used as an internal standard (all standards and samples ready for analysis contained 100 μg/L of ^{13}C,^{15}N-glyphosate) to carry out isotope dilution mass spectrometric (IDMS) analysis for glyphosate. Intermediate standard solutions from separate sources were prepared by mixing the corresponding stock solutions. Then one intermediate standard solution was used to prepare calibration standard solutions and spiking solution while the other intermediate standard solution from a different source was used to prepare control standard solutions by serial dilution with high purity water. Due to the high capacity of the target compounds to form a coordination complex with metal ions, plastic labwares or silanized glasswares were used to avoid their adsorption onto the glass surfaces. Clean glassware was silanized first by rinsing/filling with Sylon CT silanizing solution (Supelco, Mississauga, Ontario) for 1 min, then rinsed twice with toluene, three times with methanol and finally with NANOpure™ water. The silanized glassware was air-dried in a fumehood overnight.

Methanol, ammonium acetate (>99%), and ethylenediaminetetraacetic acid disodium salt ($EDTANa_2$, ACS reagent grade) were purchased from Sigma Aldrich (Oakville, ON, Canada). 10-mL 25% w/v sodium thiosulphate solution dropper bottles were purchased from ACP Chemicals Inc. (Montreal, QC, Canada).

The high purity water used to prepare standard solutions and mobile phases was produced by passing reverse osmosis water through a Barnstead NANOpure™ water purification system (Mississauga, ON).

2.2. Sample analysis

All standards and samples were stored in polypropylene or polyethylene bottles at 5 ± 3 °C. Samples were allowed to condition to room temperature before processing. Each sample was homogenized by manual shaking before transferring 1 mL of sample to a 1.8 mL plastic HPLC vial. Then 10 μL of a 10 μg/mL internal standard solution was added into each vial. The LC/MS–MS determination was achieved using a Shimadzu Prominence/20 series (Columbia, MD) HPLC system coupled to an Applied Biosystems (Foster City, CA) 4000 Q-trap mass spectrometer. Aqueous samples were injected into an Acclaim® Mix-mode WAX-1 (reversed-phase/weak anion-exchange) 3 μm 50 mm × 3 mm LC column (Dionex, Sunnyvale, CA, USA) for separation. Column temperature used was 30 °C and the injection volume was 70 μL. Mobile phases were 50:50 methanol:water (A) and 300 mM ammonium acetate in 50:50 methanol:water (B). The total flow rate was 0.4 mL/min. The initial gradient was 40% B, increased to 100% B at 4 min, maintained at 100% B for another 2 min, and then returned to 40% B at 6.5 min. The LC column was then conditioned for another 5.5 min resulting in a total run time of 12 min. Multiple reaction monitoring (MRM) data were acquired and processed in negative ESI mode. Table 1 listed the two MRM transitions and collision energies used for each target compound during the analysis. Isotope dilution was used for the quantitation of glyphosate, while external standard quantitation [26] was used for glufosinate and AMPA. Curtain, collision, nebulizer, and auxiliary gases of the MS–MS were set at 15, 6, 45 and 55 psi, respectively. Source temperature and entrance potential were kept at 600 °C and 10 V, respectively. Ion spray voltage, declustering potential, and collision cell exit potential used were −4500, −50 and −5 V, respectively.

3. Results and discussion

3.1. Column selectivity and mass spectrometric identification points

As demonstrated by the total MRM chromatogram (top plot) and reconstructed individual MRM chromatograms in Fig. 2, the novel column chemistry of the Acclaim® WAX-1 column, which combined reversed-phase and weak anion-exchange properties on one column, provided excellent retention and selectivity for glyphosate and related compounds without derivatization. However, it was observed that after running several batches of samples, the peaks for target compounds, especially for glyphosate, became broader, with excessive tailing, which indicated stronger retention of target compounds. The broader MRM chromatography peaks for glyphosate and ^{13}C,^{15}N-glyphosate were shown in the smaller inserted plots in the figure. Possible reasons that might cause peak broadening had been investigated, and it was believed that the

Table 1
MRM schemes for the target compounds are listed in the table below.

Compound name	Formula	CAS #	Q1 Mass	Q3 Mass	Quantification/confirmation	Collision energy (eV)
Glyphosate	$C_3H_8NO_5P$	1071-83-6	168	63	Quantification	−30
			168	81	Confirmatiom	−20
AMPA	CH_6NO_3P	1066-51-9	110	63	Quantification	−35
			110	81	Confirmatiom	−20
Glufosinate	$C_5H_{12}NO_4P$	51276-47-2	180	63	Quantification	−50
			180	85	Confirmatiom	−30
$^{13}C,^{15}N$-glyphosate	–	–	170	63	Internal standard	−30
			170	81	Confirmatiom	−20

accumulation of ubiquitous metal ions on the column during sample analysis was the source of peak degradation. To remove metal activity, the column was conditioned with a 50 mM disodium EDTA solution in NANOpure water followed by a 50:50 methanol:water solution offline for about 3 h at a flow rate of 0.4 mL/min (~200 column volume of solvent). This flush was done when broader peaks were observed or after 80–100 samples were analyzed as a column maintenance procedure. Retention time (RT) shift might be observed for target compounds after EDTA reconditioning. The RT shift maintained consistent for each analyte between column flushes so it would not affect the analysis. Chromatography resolution could be fully returned within 6-month of column usage even though baseline separation was not necessary for the mass spectrometry detector.

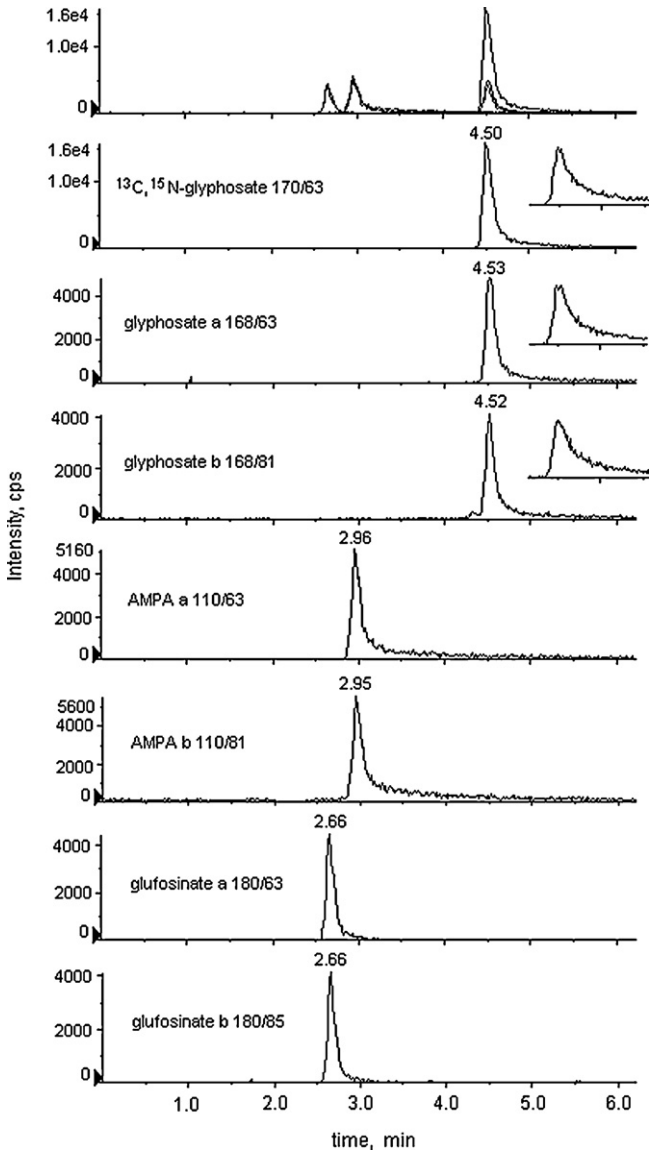

Fig. 2. The total MRM chromatogram (top) and reconstructed individual MRM chromatogram of a standard solution with preservative added NANOpure water at a concentration of 20 μg/L for glyphosate, 40 μg/L for AMPA, 18.3 μg/L for glufosinate and 100 μg/L for $^{13}C,^{15}N$-glyphosate.

The concept of identification points was introduced for LC/MS based residue identification to eliminate false positive results [27]. The two MRM channels used for each target in this method provide four identification points (1 point from the molecular ion plus 1.5 point from each product ion) that were enough to ensure the unambiguous identification of a compound. The usage of more MRM channels than just the most abundant one usually sacrifices the sensitivity of the method. Fortunately, the impact on the sensitivity was not great in this method since the intensities of the two MRM chromatograms for each target were not too far apart, as shown in Fig. 2.

3.2. Sensitivity, linearity, accuracy and precision

Using diluted standard solutions it was determined from chromatograms that the instrument detection limits for glyphosate, AMPA and glufosinate were 1.0, 2.0 and 0.9 μg/L, respectively, with signal-to-noise ratios ≥5. Quantitation limits were then set at 10 times instrument detection limits, therefore 10, 20 and 9 μg/L for glyphosate, AMPA and glufosinate, respectively. Calibration standard solutions were prepared by serial dilution with preservative added Nanopure water (details and reasons for preservative would be explained later) at seven concentration levels (L1 to L7): 10, 20, 50, 100, 200, 500 and 1000 μg/L for glyphosate, 20, 40, 100, 200, 400, 1000 and 2000 μg/L for AMPA and 9.14, 18.28, 45.7, 91.4, 182.8, 457 and 914 μg/L for glufosinate. The set of calibration standard solutions were analyzed in replicate ($N = 6$). Average accuracy (Avg. Acc.% = calculated concentration/theoretical concentration × 100%, an accuracy of 100% means that the calculated value is exactly the same as the theoretical value), relative standard deviation of accuracy (Acc. RSD), average peak area (Avg. Area), and relative standard deviation of peak area (Area RSD) were determined and are listed in Table 2. The correlation coefficient (R) and the coefficient of determination (R^2) values derived from the six replicate results with linear fit and $1/x$ weighting were also summarized for each MRM channel in the table.

As listed in Table 2, the calculated results for all target compounds had an average accuracy of 100 ± 10% with less than 10% relative standard deviation, and a good linear regression as demonstrated by R^2 value > 0.995. It was noticed that the relative standard deviation of chromatographic peak areas for glyphosate was much higher than those for AMPA and glufosinate. As glyphosate has a stronger retention on the column, it was eluted much later with a higher ammonium acetate concentration. This high salt concentration impacted the ionization process of glyphosate and caused inconsistency of its signal, hence the high deviation of peak areas. The use of $^{13}C,^{15}N$-glyphosate as an internal standard

Table 2
Instrument performance data for accuracy, precision and linearity.

N = 6	Glyphosate a	Glyphosate b	AMPA a	AMPA b	Glufosinate a	Glufosinate b	Int. std
	168/63	168/81	110/81	110/83	180/63	180/85	170/63
L 1							
Avg. Acc.%	96.3	100	102	101	94.0	95.8	–
Acc RSD	3.86%	3.66%	5.02%	4.53%	5.06%	5.36%	–
Avg. Area	3.32E + 04	1.67E + 04	3.25E + 04	3.80E + 04	1.70E + 04	1.55E + 04	2.32E + 05
Area RSD	12.2%	9.91%	6.61%	5.11%	4.55%	5.01%	10.6%
L 2							
Avg. Acc.%	96.5	98.5	99.2	98.6	96.3	96.9	–
Acc RSD	4.91%	3.12%	5.23%	5.68%	4.76%	4.23%	–
Avg. Area	4.64E + 04	2.54E + 04	5.30E + 04	6.04E + 04	2.55E + 04	2.30E + 04	2.39E + 05
Area RSD	15.5%	12.8%	7.96%	7.90%	6.18%	5.60%	10.1%
L 3							
Avg. Acc.%	101	98.0	97.8	98.6	102	101	–
Acc RSD	4.76%	5.48%	5.12%	6.20%	5.49%	6.05%	–
Avg. Area	1.45E + 05	9.00E + 04	1.91E + 05	2.07E + 05	8.68E + 04	7.65E + 04	2.57E + 05
Area RSD	11.2%	11.9%	5.35%	6.36%	5.38%	5.96%	11.5%
L 4							
Avg. Acc.%	103	103	101	102	104	102	–
Acc RSD	4.31%	4.44%	6.46%	6.05%	4.94%	6.16%	–
Avg. Area	2.99E + 05	1.99E + 05	4.07E + 05	4.32E + 05	1.76E + 05	1.53E + 05	2.71E + 05
Area RSD	9.03%	7.47%	6.51%	6.18%	5.02%	5.96%	10.0%
L 5							
Avg. Acc.%	104	101	100	101	104	103	–
Acc RSD	3.62%	3.65%	5.32%	5.48%	4.88%	4.98%	–
Avg. Area	6.31E + 05	4.19E + 05	8.13E + 05	8.63E + 05	3.50E + 05	3.10E + 05	2.89E + 05
Area RSD	7.41%	8.83%	5.29%	5.49%	4.86%	4.93%	8.49%
L 6							
Avg. Acc.%	100	98.7	98.9	99.8	102	105	–
Acc RSD	2.27%	3.54%	5.84%	4.98%	4.16%	4.19%	–
Avg. Area	1.74E + 06	1.18E + 06	2.03E + 06	2.15E + 06	8.58E + 05	7.90E + 05	3.33E + 05
Area RSD	11.7%	9.67%	5.96%	5.08%	4.14%	4.30%	12.4%
L 7							
Avg. Acc.%	98.9	100	100	99.8	97.8	96.5	–
Acc RSD	2.11%	2.93%	5.51%	6.59%	5.17%	5.50%	–
Avg. Area	3.49E + 06	2.45E + 06	4.12E + 06	4.30E + 06	1.65E + 06	1.45E + 06	3.41E + 05
Area RSD	9.34%	8.27%	5.57%	6.61%	5.28%	5.50%	8.10%
R	0.999	0.999	0.998	0.998	0.999	0.998	–
R^2	0.999	0.999	0.997	0.996	0.997	0.996	–

for glyphosate measurement compensated for the variation hence improved accuracy of calculated results for glyphosate. It was also noticed that the peak area of $^{13}C,^{15}N$-glyphosate increased with glyphosate concentration in the solution due to the contribution of the [M+2] isotope in the glyphosate molecule. As demonstrated by the results, this increase did not affect measurement accuracy within the selected concentration range.

3.3. Sample analysis for environmental water and inter-laboratory study

During our study, it was found that AMPA and glufosinate were more sensitive to chlorine residues in tap water. Their signals totally disappeared seven days after being spiked into drinking water without adding preservative while glyphosate held its level well. To quench the chlorine residue in treated water, 50 μL of 25% w/v sodium thiosulphate solution was added to 50 mL tap water as a preservative before the target compounds were spiked. It was also found that the sodium thiosulphate solution added would suppress the signal intensity of glufosinate to about 50%. To compensate for this signal suppression, sodium thiosulphate solution was added to Nanopure water which was used to prepare standard solutions. Sodium thiosulphate was also added to composite surface and composite groundwater before the target compounds were spiked.

The target compounds and/or internal standard $^{13}C,^{15}N$-glyphosate were spiked into tap water, composite surface water and composite groundwater from various locations in Ontario to evaluate matrix effects on method performance. Samples were spiked with target compounds (concentration of each target was described in Table 3) and 100 μg/L of $^{13}C,^{15}N$-glyphosate. Blanks were spiked only with 100 μg/L of $^{13}C,^{15}N$-glyphosate. No target compounds were detected in any blank, and analytical results for these freshly spiked environmental water samples were summarized in Table 3. As can be seen from the table, glyphosate had the best results overall in all matrices. Ion suppression was observed for glyphosate in the three matrices in an increasing order: groundwater < drinking water < surface water. Because of the isotope-labelled internal standard used, the matrix effects were compensated. However, the results of AMPA and glufosinate did show matrix effects. Signals for AMPA and glufosinate were suppressed in environmental water comparing with the ones in Nanopure water. Their average recoveries (Avg. *R*%) in environmental water ranged from 63 to 82% and relative standard deviation (RSD%) ranged from 9 to 15%. The presence of matrix effects was an expected trade-off for the elimination of sample preparation work. Data quality would be improved for AMPA and glufosinate by the use of isotope-labelled standards as well. $^{13}C,^{15}N$-AMPA has been purchased from Cambridge Isotope Chemicals and will be used in the method for this purpose. Unfortunately, isotope-labelled glufosinate is still not available yet. "Standard addition is the most suitable method for compensating matrix effects" in this case [28]. Recoveries of $^{13}C,^{15}N$-glyphosate and $^{13}C,^{15}N$-AMPA will be used as an indicator for standard addition in the future. When recovery of any isotope-labelled compound in a sample is outside of the acceptable range of 50–150%, standard addition is mandatory to ensure accurate results.

This method was developed mainly for monitoring drinking water quality in Ontario. The method detection limit (MDL) for each

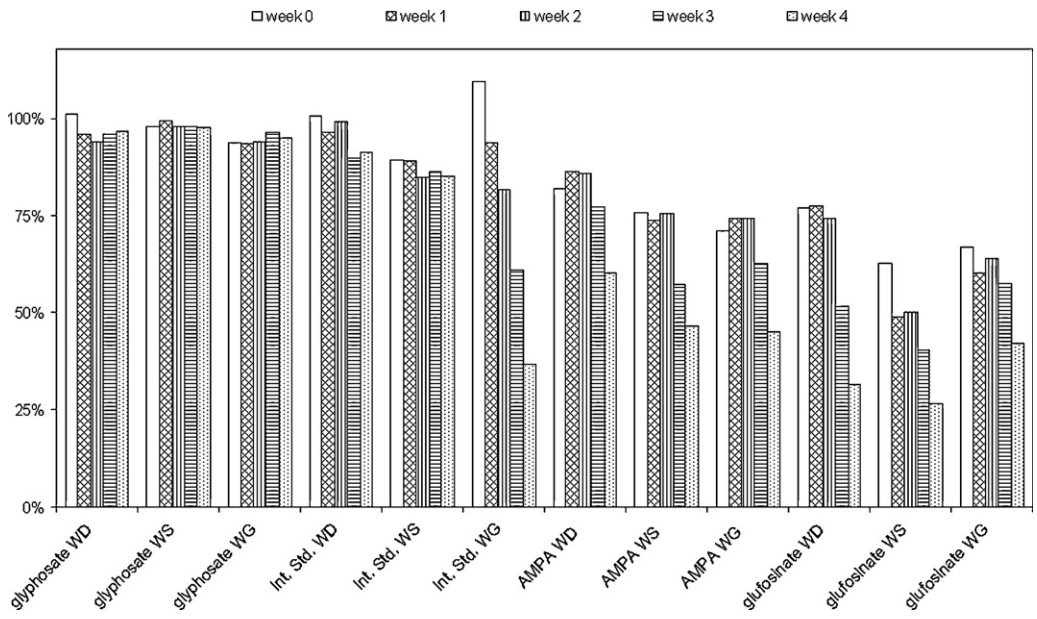

Fig. 3. The recoveries of spiked target compounds and $^{13}C,^{15}N$-glyphosate in drinking water (WD), surface water (WS) and groundwater (WG) from week 0 to 4. Samples were stored in the dark at $5 \pm 3\,^{\circ}C$.

Table 3
Results for freshly spiked tap water, surface and groundwater samples.

Compound name	Spiked level (μg/L)	Tap water $N = 9$			Composite surface water $N = 10$			Composite groundwater $N = 9$		
		Avg. *R*%	Std dev (μg/L)	RSD%	Avg. *R*%	Std dev (μg/L)	RSD%	Avg. *R*%	Std dev (μg/L)	RSD%
Glyphosate (a)	100	101	6.39	6.32	97.9	2.69	2.75	93.7	4.07	4.34
Glyphosate (b)	100	102	6.40	6.30	101	3.52	3.50	94.3	2.73	2.89
AMPA (a)	200	81.9	14.5	8.86	75.7	15.6	10.3	71.1	14.5	10.2
AMPA (b)	200	78.3	13.9	8.86	73.7	16.9	11.5	70.4	14.8	10.5
Glufosinate (a)	91.4	77.0	7.27	10.3	62.5	8.34	14.6	66.8	6.23	10.2
Glufosinate (b)	91.4	79.5	7.44	10.2	62.1	8.39	14.8	66.1	6.46	10.7

target compound in drinking water was calculated according to the U.S. Environmental Protection Agency (EPA) protocol [29] with 10 replicates of freshly spiked tap water samples and listed in Table 4. The method quantification limit (MQL) in the table was defined as MQL = 3 MDL. To evaluate method performance over time, average inter-day accuracy of calculated results for freshly spiked tap water samples and RSD% was monitored for ~6 months and the results were summarized in Table 4. As can be seen from the table, glyphosate had the lowest MDL at 1.5 μg/L and the best average accuracy of 102% with 4% RSD during 6 months period. Lower inter-day accuracy of ~80% and ~70% with higher RSD of ~13% and ~19% were observed for AMPA and glufosinate.

Dr. Hanke et al. reported an ultratrace-level method that offered limits of detection in ng/L range to assess fate and behavior of these compounds in groundwater and surface water. This direct injection method was less sensitive compared to these methods with enrichment and derivatization steps. Also due to the higher sample load on column for detection, the dynamic range is only 10^2 instead of 10^3. The lower sensitivity with narrower linear range was again an expected trade-off for the elimination of sample preparation work. This method provides a quick, easy and reliable approach to satisfy the needs for emergency response, water quality monitoring and regulation enforcement in North America, where Maximum Contaminant Level (MCL) in drinking water for glyphosate is set at 700 μg/L in the United States (280 μg/L in Ontario) and the Water Quality Guideline for the protection of aquatic life is set at 65 μg/L in Canada (no limits are set for glufosinate and AMPA).

Table 4
MDL, MQL, inter-day accuracy ($N = 33$) and RSD of the accuracy for freshly spiked tap water samples.

Compound name	MDL (μg/L)	MQL (μg/L)	Inter-day accuracy%	RSD%
Glyphosate (a)	1.51	4.53	102	4.05
Glyphosate (b)	1.52	4.56	102	4.28
AMPA (a)	3.85	11.5	80.2	13.1
AMPA (b)	3.91	11.7	77.7	14.1
Glufosinate (a)	1.85	5.55	70.4	19.4
Glufosinate (b)	1.68	5.04	70.6	20.7

A storage study was also carried out for the target compounds in environmental water matrices. Spiked samples and blanks were refrigerated ($5 \pm 3\,^{\circ}C$) and stored in the dark for a month. 1 mL of the spiked samples and blanks was taken out every week for analysis. The average recoveries of target compounds in spiked drinking water (WD), surface water (WS) and groundwater (WG) samples and the recovery of $^{13}C,^{15}N$-glyphosate in WD, WS and WG blanks from week 0 to 4 were plotted in Fig. 3. The results showed that recoveries for $^{13}C,^{15}N$-glyphosate in WD and WS were fairly steady, but decreased significantly with time in WG. By week four, only less than 50% of spiked $^{13}C,^{15}N$-glyphosate were detected in WG. Similar phenomena were also observed for glyphosate by Ibáñez et al. [30] and Freuze et al. [31] due to slow complexation with cations in WG. Our storage study indicated that the measured results for glyphosate should be acceptable if analysis was completed within 3 weeks of the environmental occurrence. It was also noticed from Fig. 3 that recoveries for AMPA and glufosinate were

quite stable for the first two weeks, and started to drop from week three in all matrices. This also required that samples should be collected in a timely manner and analyses should be completed within 14–21 days after the collection date to ensure the best data quality.

Since native and isotope-labelled glyphosate went through the same change in the storage study, the internal standard corrected recoveries for native glyphosate in spiked samples were very consistent over the four week period in all three matrices. Although impossible to add ^{13}C,^{15}N-glyphosate in real samples at the same time as glyphosate occurs, it is still better to do the addition as early as possible so the isotope-labelled standard can behave as closely as possible to the native compound. Therefore, another good practice for sample analysis is to add ^{13}C,^{15}N-glyphosate as soon as samples are received, especially for WG samples. To reduce possible changes during storage, it is also better to store all samples frozen from the time they are collected until they are analyzed.

CALA completed on-site assessment on the method in October 2010. As one CALA accreditation requirement, this method participated in two inter-laboratory studies for glyphosate administered by Environmental Resource Associates (ERA) in August 2010 (WS-169) and April 2011 (WS-177). The reported result for sample WS-169 using this method was 715 μg/L, which was 103% of the "grand mean Target" at 692 μg/L (RSD 5.18%) from seven participated laboratories. The reported result for sample WS-177 using this method was 380 μg/L, which was 99.2% of the "grand mean Target" at 383 μg/L (RSD 6.23%) from ten participated laboratories. Method performance will be continuously monitored in the future by regularly participating in such inter-laboratory studies.

4. Conclusion

This paper describes a quick, easy and reliable 12-min LC/MS–MS method for the measurement of glyphosate, AMPA and glufosinate in environmental water with IDLs of 1.0, 2.0 and 0.9 μg/L, respectively. Aqueous environmental samples were directly injected and analyzed without going through tedious and time-consuming derivatization and concentration steps. The method reported here performed well in two inter-laboratory studies and was accredited by CALA for the analysis of drinking water, surface water and groundwater samples.

References

[1] J.P. Giesy, S. Dobson, K.R. Solomon, Rev. Environ. Contam. Toxicol. 167 (2000) 35.
[2] A.D. Baylis, Pest Manage. Sci. 56 (2000) 299.
[3] G. Hoerlein, Rev. Environ. Contam. Toxicol. 138 (1994) 73.
[4] C. Cox, J. Pest Reform. 16 (1996) 15.
[5] L. Goodwin, J.R. Startin, B.J. keely, D.M. Goodall, J. Chromatogr. A 1004 (2003) 107.
[6] K.R. Solomon, D. Thompson, J. Toxicol. Environ. Health B 6 (2003) 289.
[7] C.D. Stalikas, C.N. Konidari, J. Chromatogr. A 907 (2001) 1.
[8] E. Börjesson, L. Torstensson, J. Chromatogr. A 886 (2000) 207.
[9] J. Struger, D. Thompson, B. Staznik, P. Martin, T. McDaniel, C. Marvin, Bull. Environ. Contam. Toxicol. 80 (2008) 378.
[10] Z.H. Kudzin, D.K. Gralak, J. Drabowicz, J. Luczak, J. Chromatogr. A 947 (2002) 129.
[11] H. Roseboom, C.J. Berkhoff, Anal. Chim. Acta 135 (1982) 373.
[12] L. Grey, B. Nguyen, P. Yang, J. AOAC Int. 84 (2001) 1770.
[13] J. Patsias, A. Papadopoulou, E. Papadopoulou-Mourkidou, J. Chromatogr. A 932 (2001) 83.
[14] M.G. Cikalo, D.M. Goodall, W. Matthews, J. Chromatogr. A 745 (1996) 189.
[15] M. Molina, M. Silva, Electrophoresis 22 (2001) 1175.
[16] S.Y. Chang, H.C. Liao, J. Chromatogr. A 959 (2002) 309.
[17] C.L. Deyrup, S.M. Chang, R.A. Weintraub, H.A. Moye, J. Agric. Food Chem. 33 (1985) 944.
[18] P.S. Mogadati, J.B. Louis, J.D. Rosen, J. AOAC Int. 79 (1996) 157.
[19] H. Kataoka, S. Ryu, N. Sakiyama, M. Makita, J. Chromatogr. A 726 (1996) 253.
[20] H.A. Moye, A.J. Boning, Anal. Lett. 12 (1979) 25.
[21] I. Hanke, H. Singer, J. Hollender, Anal. Bioanal. Chem. 391 (2008) 2265.
[22] J.E. Cowell, J.L. Kunstman, P.J. Nord, J.R. Steinmetz, G.R. Wilson, J. Agric. Food Chem. 34 (1986) 955.
[23] Y. Zhu, F. Zhang, C. Tong, W. Liu, J. Chromatogr. A 850 (1999) 297.
[24] K.H. Bauer, T.P. Knepper, A. Maes, V. Schatz, M. Voihsel, J. Chromatogr. A 837 (1999) 117.
[25] J. Dahlmann, K. von Czapiewski, A. Schreiber, Y. Ozeki, F. Werres, Application Biosystems Publication 114AP51-01, 2006.
[26] J. Wang, in: J. Cazes (Ed.), Encyclopedia of Chromatography, CRC Press, 2005, p. 1401.
[27] A.A.M. Stolker, R.W. Stephany, L.A. van Ginkel, Analysis 28 (2000) 947.
[28] M. Stüber, T. Reemtsma, Anal. Bioanal. Chem. 378 (2004) 910.
[29] US EPA, Title 40 of the US Code of Federal Regulations, Part 136, Appendix B, Office of the Federal Register National Archives and Records Administration, Washington, DC, 1993.
[30] M. Ibáñez, O.J. Pozo, J.V. Sancho, F.J. López, F. Hernández, J. Chromatogr. A 1134 (2006) 51.
[31] I. Freuze, A. Jadas-Hecart, A. Royer, P.Y. Communal, J. Chromatogr. A 1175 (2007) 197.