Rapid Analysis of Glyphosate and its degradation Products in Surface Water Using UPLC-MS in Selected Ion Recording Mode

Anthony A. Provatas*, Steven L. Kolakowski, Laleen C. Bodhipaksha, Andrew S. Bell, James D. Stuart and Christopher R. Perkins.
University of Connecticut, Center for Environmental Sciences and Engineering, Storrs, Connecticut, USA. *Corresponding author: anthony.provatas@uconn.edu

Glyphosate, is one of the most widely used nonselective, broad-spectrum herbicides for vegetation controls globally due to its low toxicity. Although glyphosate, an organophosphorus compound has a very low toxicity in mammals, it can be harmful to aquatic life in surface water at higher concentrations. A rapid, efficient and rugged analytical methodology using a Waters® UPLC-MS system in selected ion recording mode was developed for quantitative analysis of glyphosate in surface water providing high sensitivity and eliminating complications associated with analyte derivatisation. This method also enabled qualitative monitoring of the glyphosate degradation products including aminomethylphosphonic acid (AMPA). Sample preparation was simplified, in comparison to similar analysis methods, minimising cost and reducing preparation time from days to hours without compromising analytical sensitivity. Surface water (20 mL) fortified with glyphosate was evaporated on a Genevac® EZ-2 evaporator, and reconstituted with 200 µL of HPLC grade water prior to analysis. The method detection limit for glyphosate was 1.49 ng/mL with 91% accuracy and relative standard deviation less than 2.9%. The linearity of the calibration curve was R2=0.9999. The proposed method was applied to various glyphosate fortified surface water samples yielding recoveries of 78% - 135%.

Introduction

Glyphosate, a nonselective, broad-range weed killer, is one of the most widely used herbicides in the United States and globally in applications for vegetation controls due to its low toxicity to mammals and lack of bioaccumulation [1, 2]. Glyphosate's efficacy is derived from its interference with the shikimate pathway in plants and bacteria. Humans and mammals lack this pathway, explaining glyphosate's relatively low toxicity and safety of use on crops and areas frequented by humans and other mammals. Recent studies, however, have shown secondary effects in mammals, such as reproductive dysfunction, and it also can be harmful to aquatic life in surface water at higher concentrations [3, 4, 5]. There is also evidence that glyphosate inhibits gut bacteria found in humans from participating in crucial gastrointestinal processes, such as digestion, synthesising vitamins, and detoxifying xenobiotics. The effect of glyphosate on gut bacteria can ultimately lead to several neurological diseases [4]. Additionally, glyphosate may remain in treated soil for an extensive period of time after the initial application due to glyphosate's high solubility and relatively long half-life [6]. Growing concerns about the potential health effects of glyphosate have brought the analysis of glyphosate and its breakdown products including aminomethylphosphonic acid (AMPA), its main metabolite, to the forefront of method development and analytical research.

Glyphosate and AMPA are polar, relatively small, non-volatile molecules that lack either a strong chromophore or fluorophore group, rendering direct analysis of these compounds difficult. The analysis of glyphosate utilising traditional methods requires a derivatisation step. Methods typically used to analyse glyphosate include: HPLC- fluorescence detection, GC-MS or HPLC-MS/MS [7]. However, there are significant downsides attached to the derivatisation process, which can be long and tedious. Additionally, if HPLC is selected as the analytical technique, it is very challenging to retain underivatised glyphosate utilising reverse phase chromatographic conditions.

There is precedent for direct analysis of glyphosate and its breakdown products in water. An acknowledged method for direct analysis includes a procedure involving capillary electrophoresis with electrochemiluminescence detection [8, 9]. However, this particular method necessitates specialised equipment, and is, as such, not suited to the typical analytical laboratory. Methods involving hydrophilic interaction chromatography and mass spectrometry (HILIC-MS) have been used to determine glyphosate and AMPA without derivatisation [10]. Additionally, methods utilising reversed-phase liquid chromatography coupled with electro-spray ionisation tandem mass spectrometry (LC/ESI-MS/MS) have also been developed to enable direct analysis of glyphosate. These methods are performed either in the presence or the absence of a suppressor molecule [11]. Finally, procedures involving ion chromatography (IC) with suppressed conductivity, integrated pulsed amperometric detection, and condensation nucleation light scattering detection have been developed. Though these methods avoid having to use a derivatisation step, they displayed very low sensitivity [12]. While these methods have proven to be effective, they are unsuitable for the average analytical laboratory due to the time-consuming and difficult nature of column switching setups involved in the successful execution of the majority of these methods. Given the difficult and impractical nature of most of these previously developed methods for the direct analysis of glyphosate and its breakdown products, there exists a need for a rapid, simple and rugged method to determine these compounds.

The purpose of this study was to develop a rapid and simple analytical method for direct (without derivatisation) quantitative analysis of glyphosate in surface water utilising a Genevac EZ-2 evaporator in the sample preparation/analyte concentration step and UPLC-MS for analyte detection. Furthermore, an effort became necessary to also determine the feasibility of the method for qualitative identification of Glyphosate's degradation products including AMPA. The separation and retention of analytes were achieved on a Waters® Acquity™ UPLC BEH-Amide column and selective detection was achieved by selected ion recording (SIR). The presented analytical approach is significantly faster compared to the existing methods and eliminates any complications associated with analyte derivatisation. It is simple to execute and utilises ubiquitous laboratory equipment to analyse a compound that ordinarily requires complex analytical steps.

Experimental

Chemicals and Reagents

HPLC grade water and acetonitrile (>99.9%) were obtained from Sigma Aldrich (St. Louis, Missouri, USA). Glyphosate (98.5%) was acquired from AccuStandard (New Haven, CT, USA).

Calibration and Quality Control Samples

Due to a lack of a suitable compound as an internal standard, external calibration was utilised. Glyphosate stock solution was prepared in HPLC-grade water at a concentration of 1000 µg/mL. A calibration curve was established from 0.5 to 1000 ng/mL with a R^2 value of 0.9999. A glyphosate solution prepared in HPLC-grade water at a concentration of 500 ng/mL was used as the laboratory control sample.

For method detection limit and precision and accuracy studies seven surface water samples spiked with glyphosate to yield 25 ng/mL and four surface water samples with spiked glyphosate concentration of 250 ng/mL were used, respectively. The method was validated by analysing various surface water samples fortified with glyphosate whose concentrations ranged 300 – 240x10^3 ng/mL. Samples with concentrations above the developed calibration curve were diluted accordingly with HPLC water prior to analysis.

Direct evaporation

A solvent evaporation step was used for samples preparation. A 20 mL aliquot of HPLC grade water or surface water was pipetted into a 50 mL glass tube. All samples were spiked with the glyphosate solution to yield the required initial concentration. The samples were then evaporated to dryness using the Genevac™ EZ-2 (Stone Ridge, NY, USA) evaporator utilising the manufacturer's 'aqueous' presetting (8.0 mbar at 30° C for 8 hours). Following evaporation, the residues were reconstituted with 200 µL of UPLC grade water and sonicated for about 10 seconds each. The samples were then transferred to LC vials for analysis by UPLC-MS.

UPLC and Mass Spectrometric Conditions

The samples were analysed using a Waters Acquity™ UPLC® coupled with an Acquity™ TQD tandem mass spectrometer (Waters Co., Milford, MA). An Acquity™ UPLC BEH-Amide (1.7 µm, 2.1 x 100 mm) column was utilised to achieve the separation and retention of analytes. The column was at 25ºC, was injected with a sample volume of 5.0 µL with a flow rate of 0.300 mL/min, a mobile phase isocratic at 30% water/70% acetonitrile with a run time of 5 minutes.

The detection and quantification of glyphosate and AMPA were performed in negative ESI-MS mode. The IntelliStart™ software was utilised to optimise the conditions for the determination of glyphosate and AMPA and improved manually to achieve optimal results. The selected monitoring ions for glyphosate were m/z 337.6 and m/z 168.7, with a cone voltage 50V and dwell time of 0.05 seconds whereas for AMPA were m/z 220 and m/z 110, with a cone voltage 50V and dwell time of 0.05 seconds. Statistical analysis for obtaining calibration and quantification results for glyphosate were performed using Waters QuanLynx™, included in MassLynx software v.4.2. Parameters for the mass spectrometer were set as follows: capillary voltage, – 3.20 kV; desolvation temperature, 400°C; source temperature, 120°C; desolvation gas flow, 650 L/h; collision gas flow, 0.2 mL/min.

Results and Discussion

Simple Sample Preparation Using a Genevac® EZ-2 Evaporator. This developed method for glyphosate analysis is simple and cost-effective partially due to the utilisation of a Genevac ES-2 evaporator. The use of this instrumentation allowed for quick and hands-free evaporation of the surface water samples and glyphosate isolation from the water for increase of analyte detection limit. Previous report on degradation of glyphosate under various conditions in water suggested that glyphosate may degrade to CO_2 in water due to the presence of microorganisms [12]. The later study also claimed that increased temperature enhanced the breakdown of glyphosate. The results of our study showed that, although there was minor sample loss throughout the course of the experimental process, the retained levels of glyphosate were acceptable. Furthermore, glyphosate degradation during sample evaporation under the selected conditions, was not observed. Therefore, the use of the Genevac EZ-2 evaporator allowed the quantitative detection of parts per billion levels of glyphosate by UPLC-MS and enabled the reduction of overall analysis time from days to hours.

Application of the UPLC Amide Column and SIR for Glyphosate Analysis. The simplification of the developed method for glyphosate analysis was partially accredited to the use of a UPLC amide column (BEH-Amide 2.1 x 100 mm, 1.7 µm) based on its capability of retaining extremely polar compounds using a reverse phase solvent system. The method also utilised single ion recording (SIR), which enabled the selective determination of glyphosate *(Figure 1)* and its degradation products including aminomethylphosphonic acid *(Figure 2)*. As a result, there was significant decrease of matrix interferences and an increase in the overall analyte sensitivity.

Method Validation Studies. A statistical analysis was performed to validate whether the proposed methodology was suitable for trace analysis of glyphosate in surface water. Method detection limit (MDL), precision, and accuracy studies were performed according to the EPA guidelines [13]. Seven surface water replicates with a glyphosate concentration of 25 ng/mL were used in the MDL study. The obtained average concentration of the analyte was 23.2 ng/mL that accounts for an average recovery of 92.9% *(Table 1)*. The method detection limit derived from this study was determined to be 1.49 ng/mL, presenting the sensitivity of the method.

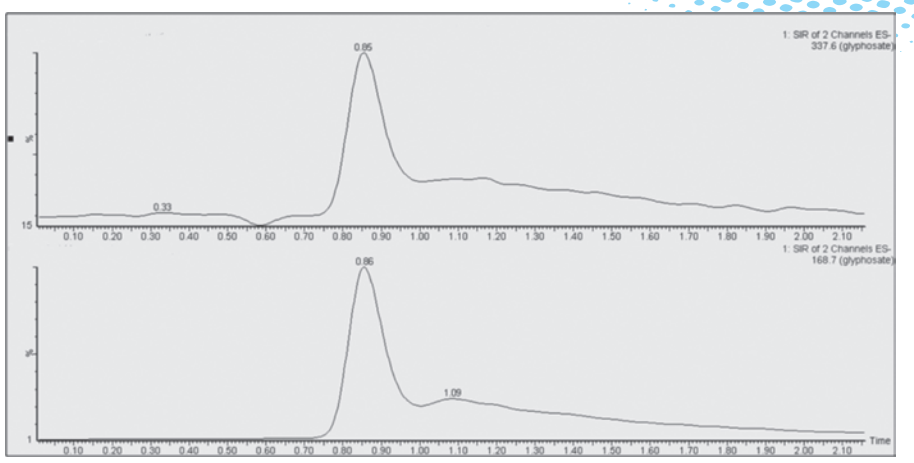

Figure 1. Single ion recording of glyphosate in a surface water sample fortified with glyphosate at 100x103 ng/mL. Retention time at 0.85 min. Top - SIR: m/z 337.6, bottom - SIR: m/z 168.7.

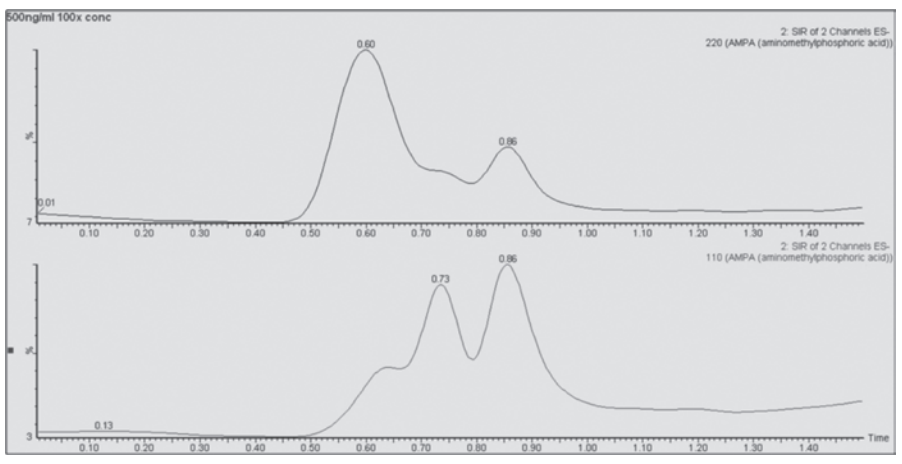

Figure 2. Single ion recording of Aminomethylphosphonic Acid and other glyphosate degradation products. Retention time = 0.5 – 1.0 min. Top - SIR: m/z 220.0, bottom - SIR: m/z 110.0.

Precision and accuracy of the method were determined by analysis of four replicated surface water samples, 20 mL each, spiked with the glyphosate stock solution to yield a concentration of 250 ng/mL. The data derived from these samples provided a standard deviation of ±6.6 ng/mL, relative deviation of 2.9% and uncertainty of 5.8 ng/mL *(Table 1)*. The observed deviations from the expected value seemed to largely associate with systematic errors. The average glyphosate recovery obtained for this test was 91%. This expressed the relative effectiveness of the developed method.

In order to apply the developed method, five surface water samples fortified with glyphosate whose concentrations ranged 300 – 240x10^3 ng/mL and a surface water blank were analysed *(Table 2)*. The average glyphosate recovery measured for these samples were between 78% and 135%. These results also exemplify the high sensitivity and selectivity that can be achieved rapidly and without the need of an analyte derivatisation step.

Table 1. Results for method detection limit, precision and accuracy studies

Method detection limit study									
Measured glyphosate concentration (ng/mL)							MDL (ng/mL)	Average Recovery (%)	Initial [glyphosate] (ng/mL)
S#1	S#2	S#3	S#4	S#5	S#6	S#7	1.49	92.9	25.0
23.8	22.8	22.4	22.9	23.7	23.7	23.2			
Precision and accuracy study									
Measured glyphosate concentration (ng/mL)					Average Recovery (%)	STDEV	Relative STDEV (%)	Uncertainty	Initial [glyphosate] (ng/mL)
S#1	S#2	S#3	S#4	Average	91.0	6.6	2.9	5.8	250.0
226.4	229.7	238.1	219.7	228.8					

(note: S# denotes the sample number and STDEV denotes the standard deviation)

Table 2. Results for screening surface water samples fortified with glyphosate

Surface water samples fortified with glyphosate					
Fortification of Glyphosate (ng/mL):					
Surface Water Blank	Surface Water #1	Surface Water #2	Surface Water #3	Surface Water #4	Surface Water #5
ND	300	3000	300 x10^2	120 x10^3	240 x10^3
Measured Value (ng/mL):					
ND	407	3367	306 x10^2	126 x10^3	189 x10^3
Percent Recovery:					
ND	135%	112%	102%	105%	78%

Conclusions

A validated analytical methodology was developed for the direct quantitative analysis (without derivatisation) of glyphosate in surface water, utilising a Genevac EZ-2 evaporator for sample preparation and a UPLC-MS equipped with a UPLC amide column and single ion recording (SIR) for analyte detection. Furthermore, this method also enabled qualitative monitoring of glyphosate degradation products including aminomethylphosphonic acid (AMPA). Since the focus of this method was the analysis of glyphosate, any degradation products including AMPA were monitored qualitatively. Consequently, further studies are on-going to quantitatively access AMPA and other breakdown products.

The presented simple analytical approach is significantly faster compared to the existing methods and eliminates any complications associated with analyte derivatisation. It can be successfully utilised for routine high throughput screening of surface water samples and other similar matrices.

References

[1] A. Royer, S. Beguin, J. C. Tabet, S. Hulot, M. A. Reding and P. Y. Communal, Anal. Chem., 3826 (2000).

[2] H. C. Steinrucken and H. Amrhein, Biochemical and Biophysical Research Communications, 1207 (1980).

[3] M. N. Marques, E. A. Passos, M. T. S. Da Silva , F. O. Correia, A. M. O. Santos, S. S. Gomes and J. P. H. Alves, J Chromatogr Sci., 822 (2009).

[4] A. Samsel and S. Seneff, Entropy, 1416 (2013).

[5] M. T. K. Tsui and L. M. Chu, Chemosphere, 1187 (2003).

[6] K. Qian, T. Tang, T. Shi, F. Wang, J. Li and Y. Cao, Analytica Chimica Acta , 222 (2009).

[7] M. Abdullah, J. Daud, K. Hong and C. Yew, 363 (1995).

[8] S. Y. Chang and C.-H. Liao, J. Chromatogr. A, 309 (2002).

[9] H.-Y. Chiu, Z.Y. Lin, H.L. Tu and C.W. Whang, 195 (2008).

[10] X. Li, J. Xu, Y. Jiang, L. Chen, Y. Xu and C. Pan, Acta Chromatographica, 559 (2009).

[11] K.-H. Bauer, T. P. Knepper, A. Maes, V. Schatz and M. Voihsel, J. of Chromatogr. A, 117 (1999).

[12] E. Mallat and D. Barcelo, J. Chromatogr. A, 126 (1998).

[13] Federal Advisory Committee on Detection and Quantitation Approaches and Uses in Clean Water Act Programs, Environmental Protection Agency, (2007)

Acknowledgements

We acknowledge valuable assistance provided by Steve Harrington and Kim Lilley of Waters® Corporation (USA) and Jay Oakley of GeneVac® (UK). Special thanks to Gary Ulatowski of University of Connecticut who provided invaluable assistance during the method development and sample preparation. The information presented in this article does not constitute an endorsement of any instrument, consumable or manufacturer by the authors, University, or the State of Connecticut.