LC-MS/MS Analysis of Emerging Food Contaminants

Trace Level Detection of Glyphosate in Water and Beer Samples

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Overview

Here we present results of using Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS) to identify and quantify underivatized glyphosate and its metabolite AMPA in water and beer samples.

The enhanced sensitivity of the SCIEX QTRAP® 6500+ LC-MS/MS system and direct injection of 50 µL resulted in limits of quantitation (LOQ) for both compounds of 100 ng/L in water samples and 200 ng/L in beer samples. Beer samples were degassed, diluted 1/1 with water and injected directly. Excellent repeatability and linearity was observed. High confidence in identification was achieved by monitoring 4 MRM transitions per compound.

Introduction

Glyphosate (N-(phosphonomethyl)glycine) is a widely used broad-spectrum systemic herbicide and crop desiccant. Generally, glyphosate is considered as safe and not toxic to humans.[1-3]

However, Glyphosate is a topic with an extraordinary degree of public attention and concerns since the International Agency for Research on Cancer (IARC), a branch of the World Health Organization, classified glyphosate as a probable human carcinogen.[4]

Traces of glyphosate have been found in surface water, many foods (i.e. bread, breakfast cereals, dairy, and beer) and also in human urine and breast milk.[5-9]

Glyphosate can be analyzed using Enzyme-linked immunosorbent assays (ELISA). Although relatively quick and simple to perform, ELISA tests are limited in selectivity and susceptible to cross-reactivity, which can lead to false positive or false negative results.

When analyzed using LC, Glyphosate is derivatized with FMOC to improve its retention, as it is very polar. This derivatization step complicates the analysis and there is a growing need for a method which can detect Glyphosate and AMPA in their underivatized forms. Anion exchange, HILIC, porous graphitized carbon and mixed-mode columns were used with LC-MS/MS to determine underivatized polar pesticides with limited success.[7, 10-12]

Here we used an LC method using a mixed-mode column and a mobile phase at pH 2.9. LOQs as low as 100 ng/L in water and 200 ng/L in beer were achieved by utilizing large volume injection (50 µL) and high sensitivity detection with the SCIEX QTRAP® 6500+ system. The method was successfully applied to the analysis of 40 different beers. Results were compared to previously published data.[9]

Experimental

Samples and Sample Preparation

- Tap water sampled in our laboratory in Concord, Ontario (Canada)
- Store-bought samples from the Liquor Control Board of Ontario (LCBO)
- One home-made ale brewed with Toronto tap water and barley malted in Germany
- Degassed and diluted 2x with LC grade water

LC Separation

- ExionLC™ AD system
- Acclaim Trinity Q1 (100 x 3 mm, 3µm)
- Gradient of water + 50 mM ammonium formate/formic acid (pH=2.9) and acetonitrile
- Injection of 50 µL

MS/MS Detection

- SCIEX QTRAP® 6500+ system and IonDrive Turbo V™ source with Electrospray Ionization (ESI) probe
- Negative polarity
- Multiple Reaction Monitoring (MRM) of 4 transition per analyte (Table 1) and *Scheduled* MRM™ algorithm
- Data acquisition using Analyst® software version 1.6.3
- Data processing in MultiQuant™ software version 3.0.2

Table 1. MRM transitions to detect Glyphosate and AMPA with compound dependent parameters, Declustering Potential (DP) and Collison Energy (CE)

Step	Q1	Q3	DP (V)	CE (V)
Glyphosate	168	63	-30	-26
	168	150	-30	-14
	168	124	-30	-16
	168	81	-30	-20
AMPA	110	63	-15	-26
	110	79	-15	-36
	110	81	-15	-16
	110	80	-15	-24

Results and Discussion

Large volume injection was utilized to achieve the desired LOQ of 100 ng/L. Figure 1 shows MRM chromatograms of AMPA and Glyphosate using 10 and 50 µL injection volume. It can be seen that the larger injection volume increases the glyphosate signal by a factor of 5, but also results in peak broadening of the earlier eluting AMPA.

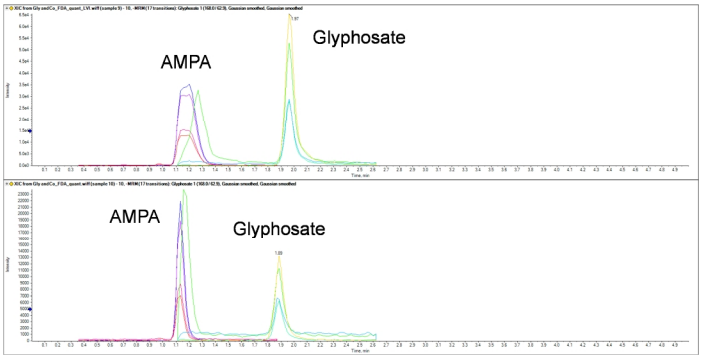

Figure 1. MRM chromatograms of 10 ng/mL of AMPA and Glyphosate using a 10 µL (bottom) and 50 µL injection volume (top)

The LOQ was evaluated by repeat analysis of low level standards spiked into tap water (which was tested previously to not contain glyphosate and AMPA). Figures 2a and 2b show the 4 MRM transitions of both compounds at a concentration of 100 ng/L. After 5 injections the coefficient of variation (%CV) was 3.32% for Glyphosate and 11.4% for AMPA, respectively.

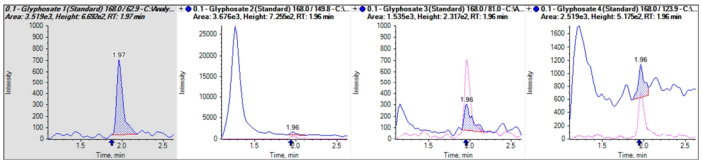

Figure 2a. 100 ng/L Glyphosate spiked into tap water with a %CV of 3.32% (n = 5)

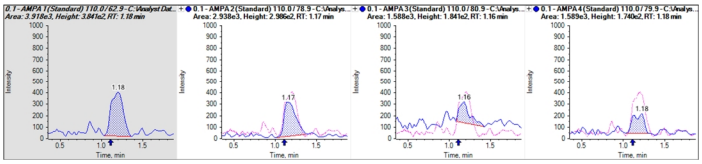

Figure 2b. 100 ng/L AMPA spiked into tap water with a %CV of 11.4% (n = 5)

Linearity for quantitation was evaluated over a range from 100 ng/L to 100 µg/L. Linearity was excellent with coefficients of regression better than 0.999 using linear fit with 1/x weighting (Figure 3). Accuracies were all well between 80 and 120% at all concentration levels.

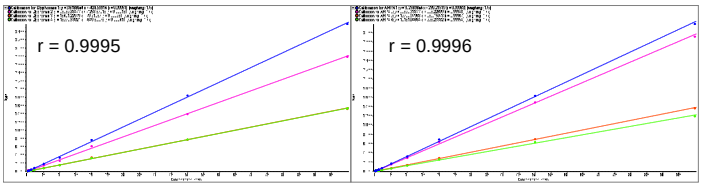

Figure 3. Linearity for glyphosate (left) and AMPA (right) from 100 ng/L to 100 µg/mL using linear fit and 1/x weighting

After initial verification the new LC-MS/MS method was applied to the analysis of glyphosate and AMPA in commercial beers and one home-made beer. Glyphosate was frequently detected. Example chromatograms are shown in Figure 4. AMPA was not detected in any samples.

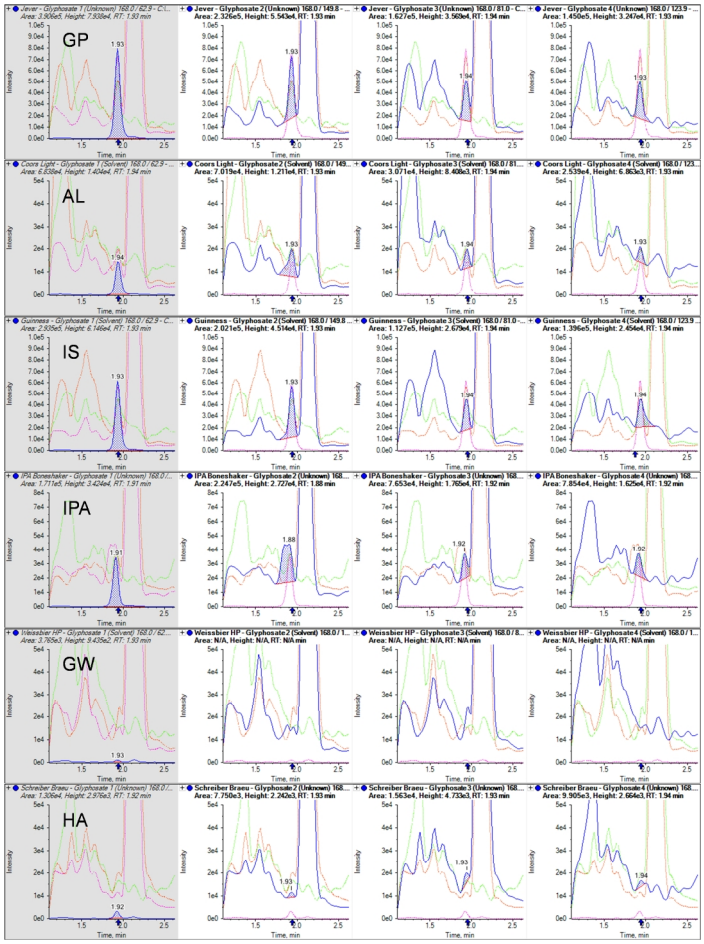

Figure 4. Glyphosate findings in different beers: German Pilsner (GP) 21.6 µg/L, American Light beer (AL) 3.8, Irish stout (IS) 16.2, Canadian craft India Pale Ale (IPA) 9.5, German Weissbier (GW) 0.2; and home-made Ale (HA) 0.7 µg/L

High confidence in identification was achieved by the detection of 4 MRM transitions and calculation of quantifier-qualifier ratios. An MRM ratio tolerance of 30% was applied to identification. Due to interferences, some beer samples had failing MRM transition ratios, but in all cases except the German Weissbeer, at least 2 transitions where present at the correct ratio for all of the beers tested.

Quantitative results are listed in Table 2. Our results correlate well with previously reported data from the Environmental Institute (Umweltinstitut München,Germany), which is surprising, considering that samples were purchased in different stores at different times.

Table 2. Glyphosate concentrations measured in 40 different beers, results are compared to previously reported data[9]

Beer	Glyphosate (µg/L)	Previously reported[9]
American IPA	2.72	
American Light (1)	3.56	
American Light (2)	1.55	
American Light (3)	1.13	
American Light (4)	0.69	
American Light (5)	0.22	
Canadian Ale (1)	9.99	
Canadian Ale (2)	7.54	
Canadian Bock (1)	6.52	
Canadian Bock (2)	4.21	
Canadian IPA (1)	15.71	
Canadian IPA (2)	14.93	
Canadian IPA (3)	13.97	
Canadian IPA (4)	10.63	
Canadian IPA (5)	10.48	
Canadian IPA (6)	9.48	
Canadian IPA (7)	7.10	
Canadian IPA (8)	6.97	
Canadian IPA (9)	6.83	
Canadian IPA (10)	5.61	
Canadian IPA (11)	5.14	
Canadian IPA (12)	5.09	
Canadian IPA (13)	3.06	

Table 2. continued

Beer	Glyphosate (µg/L)	Previously reported[9]
Canadian IPA (14)	2.31	
Canadian Stout	9.84	
Czech Pilsner (1)	13.96	
Czech Pilsner (2)	6.18	
Czech Pilsner (3)	6.15	
Czech Pilsner (4)	3.95	
German Pilsner (1)		29.74
German Pilsner (2)	23.78	23.04
German Pilsner (3)	7.21	20.73
German Pilsner (4)	6.77	12.01
German Pilsner (5)	4.98	
German Pilsner (6)	3.41	0.50
German Pilsner (7)	2.78	
German Pilsner (8)	0.87	2.99
German Pilsner (9)	0.76	0.55
German Pilsner (9)	0.27	
German Pilsner (9)		5.78
German Pilsner (9)		3.86
German Pilsner (9)		3.35
German Weissbier (1)	0.75	
German Weissbier (2)		2.92
German Weissbier (3)		0.66
German Weissbier (4)		0.49
Home-made Ale	18.65	
Irish Stout	13.96	

The glyphosate concentrations in beer analyzed by LC-MS/MS ranged from 0.22 to 23.78 µg/L. There is no obvious correlation between the concentration of glyphosate and the origin or style of the beer. However, beers brewed with adjuncts such as rice (typical for American Light beers) or wheat (German Weissbier) tend to have a lower concentration of glyphosate.

These results support the hypothesis that Glyphosate originated from the malted barley used for brewing and not from other ingredients, such as water, hops, and yeast.

Summary

Here we presented the analytical results for underivatized glyphosate and its metabolite AMPA in water and beer samples using LC-MS/MS.

The method, using a SCIEX QTRAP® 6500+ system and direct injection of 50 µL, provided excellent sensitivity, repeatability, and linearity. Water samples were injected directly resulting in an LOQ of 100 ng/L and beer samples were injected after degassing and 1/1 dilution resulting in an LOQ of 200 ng/L. High confidence in identification was achieved by monitoring 4 MRM transitions per compound.

40 beers samples were analyzed with glyphosate findings between 0.22 to 23.78 µg/L. Results correlate well with previous reported data.

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