Trace level detection of glyphosate in water and beer samples

Using LC-MS/MS analysis on the QTRAP® 6500+ System

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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 concern 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 (such as bread, breakfast cereals, dairy, and beer) and also in human urine and breast milk.[5-9]

Glyphosate can be analyzed using an enzyme-linked immunosorbent assay (ELISA). Although relatively quick and simple to perform, ELISA tests are limited in selectivity and are 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 that 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]

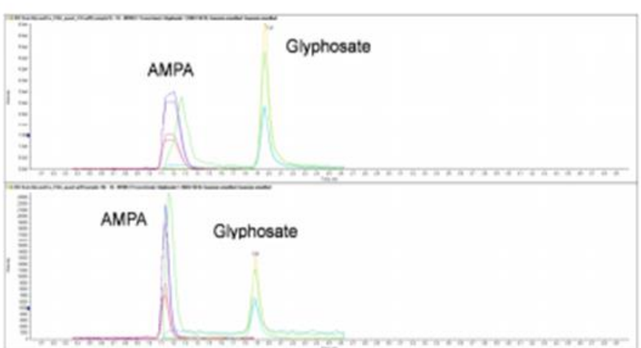

Figure 1. Separation of AMPA and glyphosate. MRM chromatograms of 10 ng/mL of AMPA and glyphosate using a 10 µL (bottom) and 50 µL injection volume (top).

Here, an LC method was developed using a mixed-mode column and a low pH mobile phase (pH 2.9). LOQs as low as 100 ng/L in water and 200 ng/L in beer were achieved by utilizing large volume injections (50 µL) and high sensitivity detection with the SCIEX QTRAP 6500+ System. The method was successfully applied to the analysis of 40 different beers, yielding comparable results to previously reported results where available.[9]

Key features of the method

- Enables the identification and quantification of underivatized glyphosate and its metabolite AMPA in water and beer samples
- High sensitivity achieved using the SCIEX QTRAP 6500+ LC-MS/MS System
- Very simple sample preparation using large volume injections of either water or diluted beer
- High confidence in identification was achieved by monitoring 4 MRM transitions per compound
- Limits of quantification (LOQ) for both compounds of 100 ng/L in water samples and 200 ng/L in beer samples
- Excellent repeatability and linearity was observed

Experimental

Sample preparation: Tap water was obtained from the laboratories in the SCIEX office in Concord, Ontario (Canada). Store-bought samples were obtained from the Liquor Control Board of Ontario stores (LCBO). One home-made ale brewed with Toronto tap water was obtained in addition to a commercial barley malted beer. All samples were degassed and diluted 2x with LC grade water.

Chromatography: The ExionLC™ AD System was used to perform the separation, using an Acclaim Trinity Q1 column (100 x 3 mm, 3 µm). Mobile phase A was water with 50 mM ammonium formate/formic acid (pH = 2.9) and mobile phase B was acetonitrile. Injection volume was 50 µL.

Mass spectrometry: Mass analysis was performed on a SCIEX QTRAP 6500+ System equipped with an IonDrive™ Turbo V Ion Source and an electrospray ionization (ESI) probe. Both analytes were detected using negative polarity using the MRM transitions outlined in Table 1. Data acquisition was done using Analyst® Software 1.6.3.

Data processing: Data processing was performed using MultiQuant™ Software 3.0.2.

Table 1. MRM transitions.

Compound	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

Large volume injection was used to achieve the desired LOQ of 100 ng/L. Figure 1 shows MRM chromatograms of AMPA and glyphosate using 10 and 50 µL injection volumes. 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.

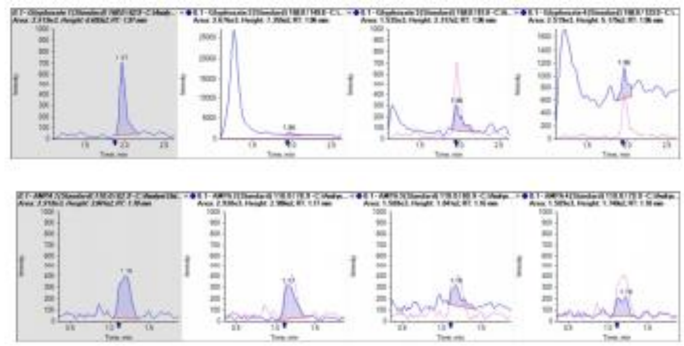

Figure 2. Tap water results. (Top) Glyphosate was spiked into tap water and the signal for the 4 different MRM transitions at the LOQ of 100 ng/mL is shown. Replicate injections had %CV of 3.32% (n=5). (Bottom) Similar data is shown for AMPA. Here, the %CV was 11.4% at the LOQ of 100 ng/mL.

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.

Linearity for quantification 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 between 80 and 120% at all concentration levels.

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

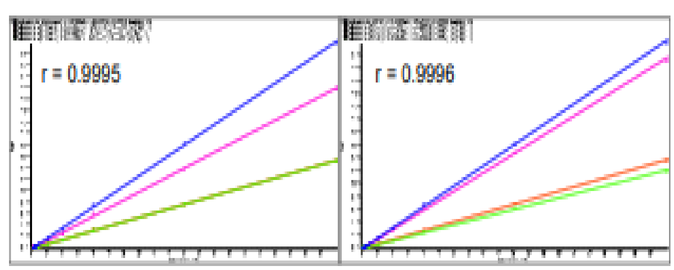

Figure 3. Linear dynamic range explored. The linearity for glyphosate (left) and AMPA (right) across the concentration range of 100 ng/mL to 100 µg/mL is shown, using a linear fit and a 1/x weighting.

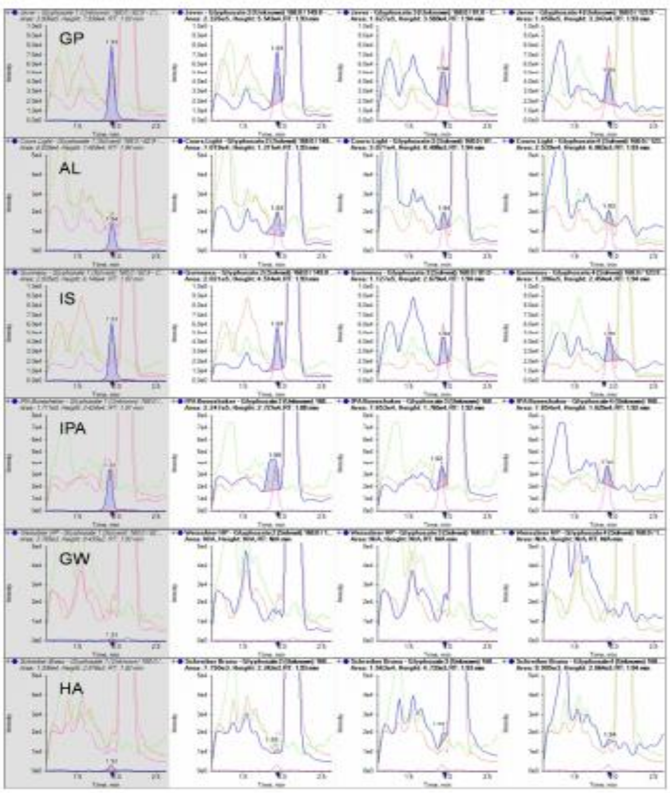

Figure 4. Glyphosate findings in different beers. Glyphosate levels were measured in the various beverage samples: German pilsner (GP), 21.6 µg/L; American light beer (AL), 3.8 µg/L; Irish stout (IS), 16.2 µg/L; Canadian craft India pale ale (IPA), 9.5 µg/L; German weissbier (GW), 0.2 µg/L; and home-made 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. These 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.

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.

Table 1. Glyphosate concentrations measured in 40 different beers. Where comparison was possible, results correlated well with published values.

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*	
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*

Table 1 continued. Glyphosate concentrations measured in 40 different beers. Where comparison was possible, results correlated well with published values.

Beer	Glyphosate (µg/L)	Previously reported[9]
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*	

Summary

Here, the analytical results for underivatized glyphosate and its metabolite, AMPA, in water and beer samples using LC-MS/MS was investigated. The method, using a SCIEX QTRAP 6500+ System and direct injection of 50 µL of liquid, 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 with water 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.[9]

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