A Sensitive, Specific and High-Throughput LC/MS/MS Assay to Quantify Glyphosate in Protein, Non-protein, and Botanical Dietary Supplements

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INTRODUCTION

Glyphosate, a broad-spectrum systemic herbicide, is one of the most widely used herbicides with the signal word WARNING on the label due to its toxicological concern. The glyphosate is amphoteric, low mass, high water soluble, and does not have a chromophore. It is not easily retained on a reversed-phase HPLC and detection by UV or fluorescence is difficult. Instead, various LC-MS assays were published for different matrices. In this presentation, we show development of a sensitive, specific and high-throughput LC/MS/MS assay for different matrices, including protein, non-protein, and botanical finished goods.

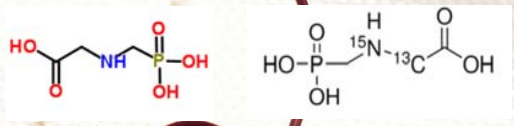

Figure 1: Chemical structure of glyphosate and internal standard (IS, glyphosate $^{13}C^{15}N$)

METHODOLOGY

Sample Preparation and Extraction:

About 2 g of sample was extracted with water and methanol, followed by an ultrafiltration membrane to remove interfering materials.

UPLC-MS Conditions

UPLC system: Nexera UPLC system including SIL-30AC auto-sampler, controller, column heater and binary pump (SHIMADZU)
Column: 3.0x100 mm, Acclaim™ Trinity™ Q1 3µm (Thermo Scientific)
Mobile Phase A: 50 mM Ammonium Formate, pH 2.9
Mobile Phase B: Acetonitrile
Flow rate: 0.40 mL/min
Pump Gradient Cycle time: 10.0 minutes

MS detector: Triple Quadrupole 5500 MS (AB Sciex)
MS Parameters: see Table 1

MS CONDITIONS				
Scan Mode	Ion Mode	Source Temperature (°C)		Dwell Time (ms)
MRM	Negative	600		125
Compounds Parameters				
Analyte	Q1	Q3	RT (min)	Typical DP	Typical CE
Glyphosate	168.0	63.0	2.45	-45	-30
15N13C-Glyphosate (IS)	169.9	63.0	2.45	-45	-34

Table 1: MS Conditions for Glyphosate and IS

RESULTS and DISCUSSIONS

Specificity

The specificity results indicated that there is no interference between analyte and IS and the method is specific. The representative chromatograms are shown in ***Figure 1***.

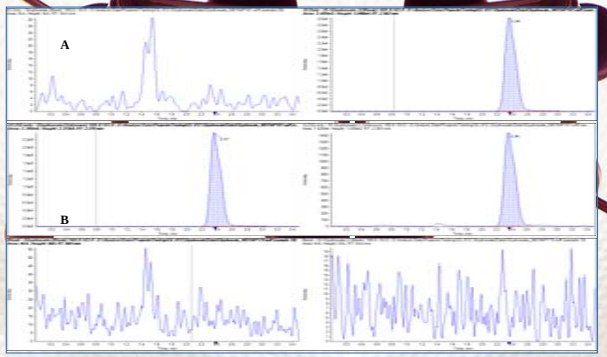

Figure 1: The representative chromatograms of A: IS only sample, B: Glyphosate only and C: blank sample. Analyte is in the left column, IS in the right for each of A, B, and C.

Linearity

The curve range of 10.0-2,000 ng/mL was successfully validated. The regression is linear with 1/x as the weighing factor (***Figure 2***). The Correlation Coefficient R2 = 0.99978. The % variance of LLOQ was 5.61% and all other points on the curve do not exceed 6.54% (***Table 2***). The representative chromatograms for LLOQ and ULOQ were in ***Figure 3***.

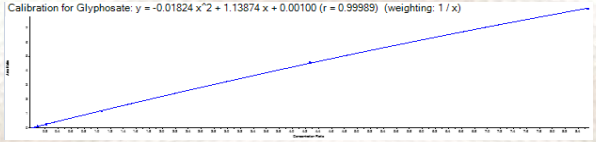

Figure 2: The typical linear standard curve of glyphosate

STD ID	Nominal Conc. (ng/mL)	Measured Conc. (ng/mL)	Accuracy (%)	Variance (%)
STD-1	10.7	11.3	106	5.61
STD-2	21.4	21.6	101	0.93
STD-3	53.5	50	93	-6.54
STD-4	268	261	97	-2.61
STD-5	1070	1085	101	1.40
STD-6	2140	2130	100	-0.47

Table 2: The linearity data for glyphosate

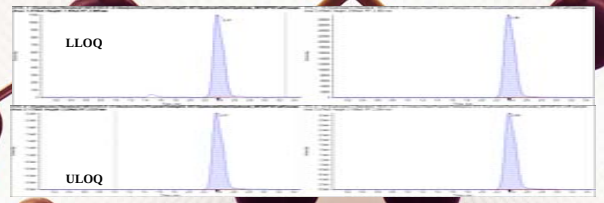

Figure 3: The chromatograms of LLOQ and ULOQ samples

Accuracy and Precision

The accuracy and precision were investigated with post-spiking glyphosate in protein placebo, non-protein placebo, and botanical placebo samples at lower, medium and high regions of the established range of the calibration curve.

Botanical Matrix SID 477773			Non-Protein Matrix SID 461179			Protein Matrix SID 463410		
Concentration	Accuracy	%RSD	Concentration	Accuracy	%RSD	Concentration	Accuracy	%RSD
50.0 ng/mL	86.2%		50.0 ng/mL	83.8%		50.0 ng/mL	98.7%	
50.0 ng/mL	81.5%	3.48	50.0 ng/mL	89.3%	3.90	50.0 ng/mL	104%	2.96
50.0 ng/mL	87.0%		50.0 ng/mL	90.2%		50.0 ng/mL	103%	
500 ng/mL	93.8%		500 ng/mL	89.5%		500 ng/mL	95.6%	
500 ng/mL	89.7%		500 ng/mL	87.8%		500 ng/mL	96.5%	
500 ng/mL	91.4%	2.49	500 ng/mL	92.6%	3.20	500 ng/mL	96.4%	2.23
500 ng/mL	91.0%		500 ng/mL	94.6%		500 ng/mL	98.9%	
500 ng/mL	95.6%		500 ng/mL	94.6%		500 ng/mL	92.4%	
500 ng/mL	90.2%		500 ng/mL	94.5%		500 ng/mL	97.1%	
1,500 ng/mL	95.2%		1,500 ng/mL	91.2%		1,500 ng/mL	98.3%	
1,500 ng/mL	94.0%	0.80	1,500 ng/mL	94.9%	3.00	1,500 ng/mL	97.7%	0.800
1,500 ng/mL	93.9%		1,500 ng/mL	96.8%		1,500 ng/mL	96.7%	

Table 4: *The accuracy and precision results in botanical, non-protein and protein matrix.*

Stability

Nine days stability has been established for standard solution and extracted samples under room temperature. Glyphosate stability data is shown in ***Table 5***.

Standard Solution Stability	
STD ID	Accuracy
STD-1	96.8%
STD-2	94.0%
STD-3	99.4%
STD-4	94.9%
STD_5	97.5%
STD-6	98.4%

Botanical Extracted Sample Stability			
Injection	Initial Accuracy	Stability Accuracy	% Diff.
1	94.6%	93.0%	-1.61%
2	88.0%	93.8%	5.79%
3	89.7%	96.8%	7.10%
4	98.2%	87.5%	-10.8%
5	100%	95.6%	-4.57%
6	91.6%	97.6%	5.97%
Average	93.7%	94.0%	0.320%

Protein Extracted Sample Stability			
Injection	Initial Accuracy	Stability Accuracy	% Diff.
1	93.7%	97.0%	3.32%
2	94.1%	96.0%	1.85%
3	95.9%	97.5%	1.56%
4	105%	94.6%	-10.7%
5	92.2%	95.6%	3.43%
6	101%	93.8%	-6.71%
Average	97.0%	95.7%	-1.22%

Non-Protein Extracted Sample Stability			
Injection	Initial Accuracy	Stability Accuracy	% Diff.
1	100%	98.8%	-1.43%
2	100%	92.4%	-8.18%
3	97.7%	96.3%	-1.41%
4	91.5%	95.9%	4.42%
5	97.0%	102.1%	5.16%
6	103%	99.1%	-4.30%
Average	98.4%	97.4%	-0.960%

Table 5: *The stability result of standard solutions and extracted samples.*

CONCLUSIONS

This is the first-known validated, quantitative assay for glyphosate in protein, non-protein, and botanical dietary supplements.