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Application News

SSI-LCMS-074

Liquid Chromatography Mass Spectrometry

Quantitative Analysis of Glyphosate and AMPA using the LCMS - 8060

Liquid Chromatograph Mass Spectrometer

LCMS-8060

Summary

The Lower Limit of Quantitation for Glyphosate and its metabolite Aminomethylphosphonic Acid (AMPA) were tested on the LCMS-8060, reaching an LLOQ of 0.1 ppb without the need for derivatization.

Background

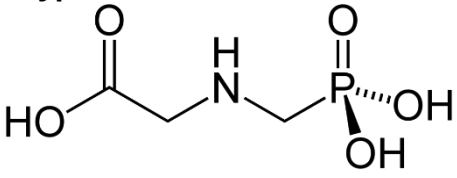
Glyphosate is a widely used weed killer used for crop protection and around homes and gardens. It is an herbicide that acts as an enzyme inhibitor and is only active on growing plants. Once it is absorbed into the soil is converted into its metabolite AMPA. Although Glyphosate is broken down in soil, it can potentially be found in surface water.

Glyphosate is classified as a Level III toxin by the EPA and is regulated to protect public health. Both compounds are low in toxicity to humans but have become a growing concern to the environment and human health.

Many methods used to analyze Glyphosate and AMPA require derivatization due to the analytical challenges posed by these compounds. Glyphosate and AMPA are both highly polar compounds which makes it difficult to analyze them on a standard reverse phase C18 column.

The method presented here shows that in a neat standard Glyphosate and AMPA can be successfully analyzed at the part per billion level without derivatization.

Glyphosate



AMPA

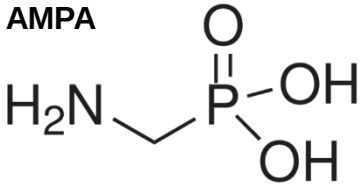


Figure 1: Chemical Structure of Glyphosate and AMPA

Method

Glyphosate and AMPA solids were acquired from Sigma-Aldrich. A stock solution of Glyphosate and AMPA was prepared at 100 ppb. This solution was diluted further with 0.1% formic acid in water. Six calibration levels for LCMS analysis were made ranging from 0.1 ppb to 10 ppb.

Using the MRM optimization tool in LabSolutions, the two analytes were optimized in ESI negative mode. The MRM transitions determined by the software were similar to those found in literature and are shown in the **Table 1** below. The optimized LCMS-8060 interface parameters are shown in **Table 2**.

The two analytes were separated using a Bio-Rad Micro-Guard Cation H+ Cartridge the two analytes were separated. An LLOQ study was preformed to determine the limits of quantitation for Glyphosate and AMPA.

The mobile phases used for the LC conditions were Mobile Phase A – 0.1% Formic Acid in DiH_2O, Mobile Phase B – ACN, and Mobile Phase C - 0.2% Phosphoric Acid in DiH_2O.

All of the tubing post the autosampler injection port was changed from stainless steel to peek tubing to minimize Glyphosate adsorption.

Mobile Phase C was used to passivate the system for 30-60 minutes prior to analysis.

During data processing the peaks were integrated with the standard smoothing method, with both counts and width (sec) set at 3.

Analyte	MRM Transitions	Dwell Time (msec)	Q1 Pre Bias (V)	CE	Q3 Pre Bias (V)
Glyphosate	168.10> 62.90	225	11	23	21
	168.10> 78.95	225	11	39	26
AMPA	110.00 > 79.00	300	12	25	30
	110.00 > 63.00	300	12	21	20

Table 1 MRM parameters for Glyphosate and AMPA

LCMS-8060 System Parameters	
Heating Gas:	20 L/min
Drying Gas:	3 L/min
Nebulizing Gas:	3 L/min
Interface Temperature:	350 °C
Heat Block Temperature:	400 °C
DL Temperature:	250 °C
Injection Volume:	20 µL

Table 2 Interface parameters (ESI-)

Results and Discussion

Following MRM optimization and development of chromatographic conditions a linear six point calibration curve for both compounds was established with n=5. Statistical data for the calibration curves can be found in **Table 3**.

The calibration curves in **Figure 2** have a linearity coefficient of R^2=0.9996 for Glyphosate and AMPA. **Figure 3** shows the chromatograms of the LLOQ which was determined to be 0.1ppb for Glyphosate with a 9.09%RSD and AMPA with a 7.10%RSD. **Figure 4** shows both analytes at the high calibration point of 10 ppb.

Blank samples were injected periodically throughout the batch and showed no detectable levels of carryover for either Glyphosate or AMPA as seen in **Figure 5**.

Glyphosate						
Level	Avg RT	Avg Area	Std Conc.	Avg Conc.	Avg Acc (%)	%RSD
1	1.502	4340.4	0.1	0.0964	96.36	9.09
2	1.494	7698.4	0.2	0.1980	98.98	6.58
3	1.5	19099.0	0.5	0.5430	108.60	3.07
4	1.49	33082.4	1	0.9662	96.62	3.50
5	1.492	164658.6	5	4.9476	98.94	2.34
6	1.498	333226.6	10	10.0486	100.50	5.39

AMPA						
Level	Avg RT	Avg Area	Std Conc.	Avg Conc.	Avg Acc (%)	%RSD
1	4.432	2146.8	0.1	0.0990	99.06	7.10
2	4.428	4088.2	0.2	0.1948	97.34	6.85
3	4.436	11019.6	0.5	0.5362	107.26	2.51
4	4.442	19566.4	1	0.9572	95.72	5.06
5	4.442	102638.0	5	5.0498	101.00	2.58
6	4.442	202360.8	10	9.9628	99.62	6.41

Table 3 Calibration statistics for Glyphosate and AMPA.

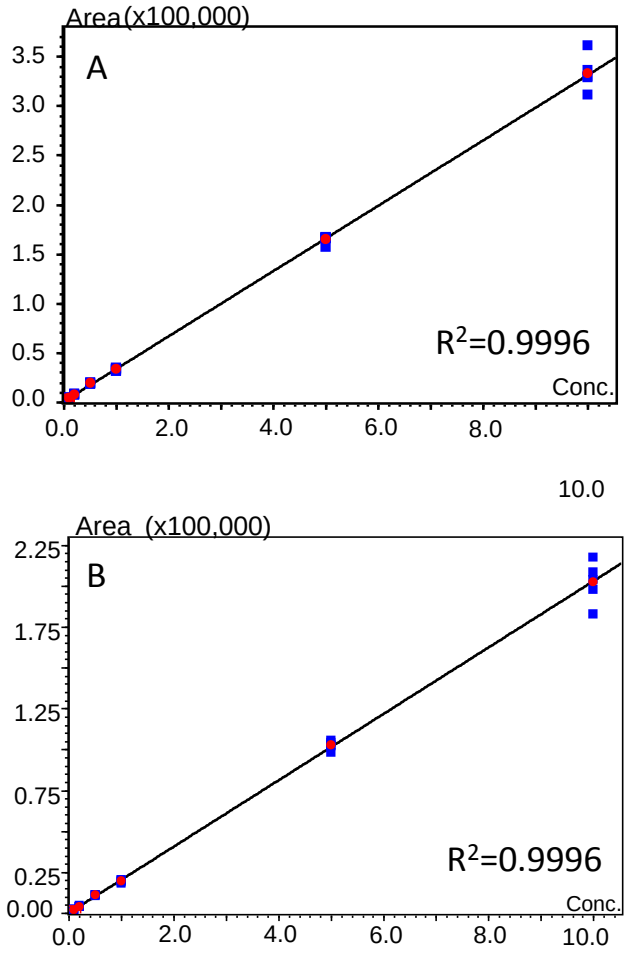


Figure 2 Calibration curve 0.1 ppb to 10 ppb in 0.1% Formic Acid in water. (A) Glyphosate (B) AMPA

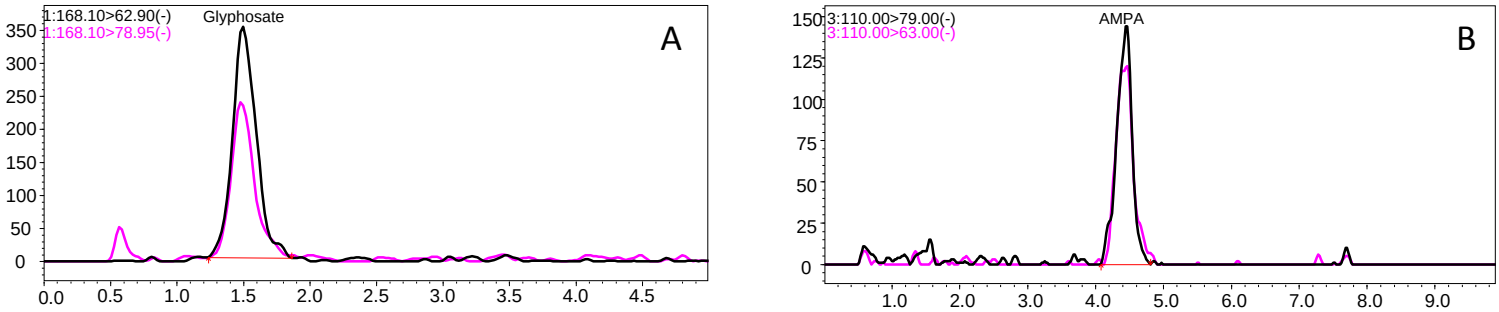


Figure 3 Chromatogram at 0.1 ppb (A) Glyphosate (B) AMPA

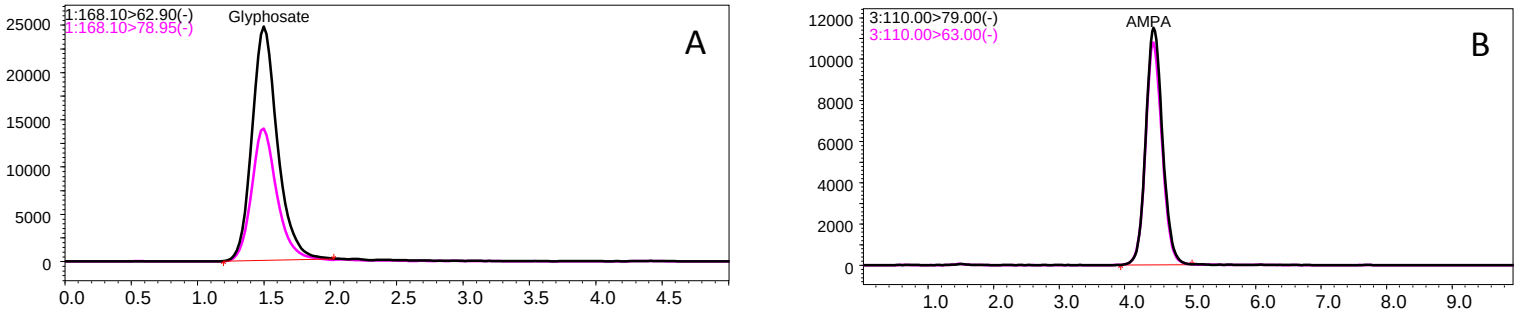


Figure 4 Chromatogram at 10 ppb (A) Glyphosate (B) AMPA

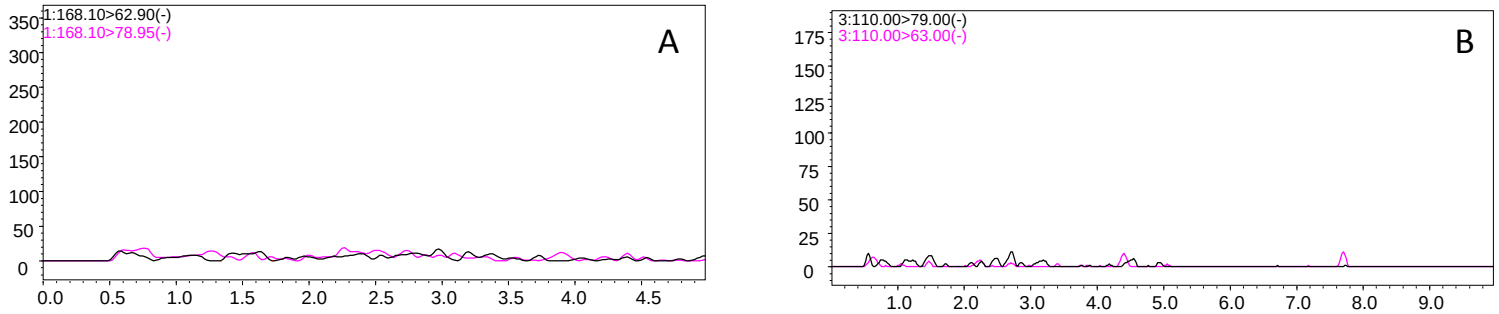


Figure 5 Chromatogram of Blank Sample (A) Glyphosate (B) AMPA

Conclusion

The method and compound optimization parameters determined on the LCMS-8060 for these compounds successfully allowed for accurate quantitation of neat Glyphosate and AMPA at 0.1 ppb without derivatization.

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