LC-MS/MS Application Technology on Quantitation of Glyphosate, Aminomethylphosphonic Acid, and Glufosinate in Multiple Food Matrices

Zhong Chenchun; Zhu Huaien; Jin Wenhai;
Sciex JAPAC Solution Innovation Center, China

Glyphosate, the most used herbicide in the world, is a broad-spectrum systemic herbicide and crop desiccant. After absorption in soil, glyphosate is rapidly converted to its main metabolite (aminomethylphosphonic acid, AMPA). Glufosinate is a broad-spectrum herbicide that is used to control important weeds and yellow nut-sedge similar to glyphosate. Due to their strong polarity, these compounds are not amenable to the extraction procedure, chromatographic method or are poor ionizers that require additional single-residue methods which involve time-consuming preparation and separation and often involve derivatization to improve detection. One major drawback is the high amount of manual labor required to produce a clean extract, leading to increased operator-induced error.
This note described a workflow of quantitation of glyphosate, AMPA, and glufosinate in multiple food matrices, like orange, wheat, and tea powder. The target limit of detection of 5μg/kg was achieved in orange and wheat and 10μg/kg in tea powder. According to the validation results, both the accuracy and recovery of the method meet the requirements.

Application benefits

1. **A simplified, developed, high-throughput sample preparation method is presented.**

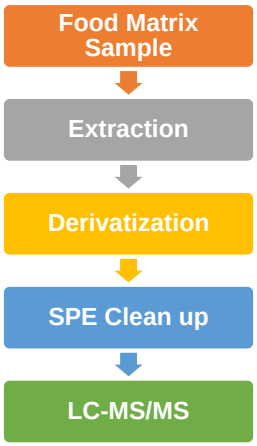

Figure 1. Glyphosate, AMPA, and glufosinate sample preparation of vMethod.

Compared with AOAC 2000.05.10 methods, which require large volume solvent of evaporation, this method provides simplified and developed sample preparation procedures. All the steps are carried out in plastic containers to avoid non-specific adsorption. Instead of evaporation, the step of extraction was succeeded by derivatization, which considerably reduce the time required for sample preparation procedures and therefore increase the analytical throughput. More importantly, the excess substances for derivatization reaction will be washed out during the step of SPE clean-up, which would increase the service lifetime of the analysis column, LC and MS equipment.

2. **A single QTRAP 4500 technique was able to successfully analyze all the analytes in 5 min.**

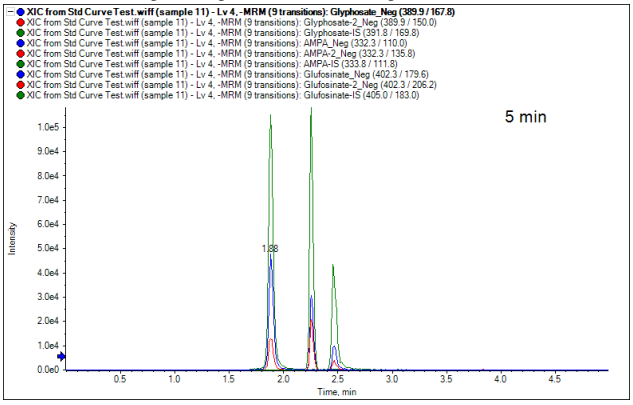

Figure 2. Chromatogram of derivatized three polar pesticides standards and their isotope labeled standards.

Using the SCIEX QTRAP 4500 system, three derivatized polar pesticides were successfully analyzed in 5 min, as seen in Figure 2. Compound and source optimization were performed on the tandem mass spectrometer by continuous infusion of derivatized standards. (Table 1). MS/MS was achieved by using negative electrospray ionization (ESI) conditions under multiple reaction monitoring (MRM) mode. The transition that was unique to a particular analyte was used as its quantifier along with at least one more transition as a qualifier.

Table 1. Optimized MS/MS parameters for the analytes and their internal standards in negative ESI mode.

Q1 (Da)	Q3 (Da)	ID	DP (V)	CE (V)
389.9	167.8	Glyphosate-FMOC 1	-65	-18
389.9	150.0	Glyphosate-FMOC 2	-65	-36
391.8	169.8	Glyphosate-IS-FMOC	-50	-17
332.3	110.0	AMPA-FMOC 1	-56	-14
332.3	135.8	AMPA-FMOC 2	-56	-23
333.8	111.8	AMPA-IS-FMOC	-45	-10
402.3	179.6	Glufosinate-FMOC 1	-60	-16
402.3	206.2	Glufosinate-FMOC 2	-60	-21

405.0	183.0	Glufosinate-IS-FMOC	-50	-16

3. **This method has shown excellent sensitivity, repeatability, and adequate calibration range for regulatory compliance, which enable laboratories to analyze routine samples.**

3.1. Linear dynamic range

In the LC-MS/MS system, the peak area ratios of isotope-labeled standards were linearly proportional to the concentration of glyphosate, AMPA, and glufosinate, respectively, over the nominal concentration range of 0.5-500 ng/mL with a coefficient of determination of r > 0.998 (shown in Figure 3).

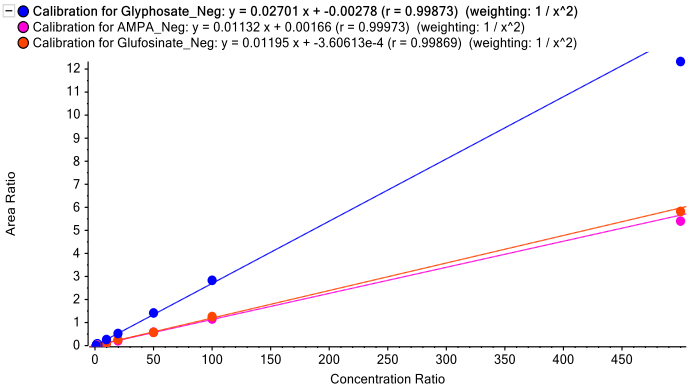

Figure 3. The linear dynamic range was 0.5-500 ng/mL for glyphosate, AMPA, and glufosinate, with relative coefficient (r) 0.99873, 0.99873, and 0.99973, respectively.

3.2. Recovery

For the determination of method precision and accuracy, multiple food matrices samples (orange, wheat, and tea powders) fortified with low, mid and high levels of glyphosate, AMPA, and glufosinate were processed alongside standard samples prepared in aqueous buffer. All samples were processed in the same manner; recovery was calculated based on the ratio of concentrations, calculated versus nominal. By this method, glyphosate, AMPA, and glufosinate average percent recovery from orange, wheat, and tea powder was 82-118, 80-109, and 90-120% at 0.05, 0.1, and 0.5 mg/kg (at 0.1, 0.2, and 1 mg/kg for the sample of tea powder), respectively; the RSD (n=3) was ≤11.8, ≤10.9, and ≤11.6% at 0.05, 0.1, and 0.5 mg/kg (at 0.1, 0.2, and 1 mg/kg for the sample of tea powder), respectively. (shown in Figure 4).

3.3. Robustness

The RSDs of peak areas of all the three compounds in the sample of standard and tea powder at 0.2 mg/kg were 6.20-8.98% and 6.67-7.26% over 100 samples for five days (20 samples per day); the RSDs of retention times were 0.55-1.22% and 0.96-2.00%.

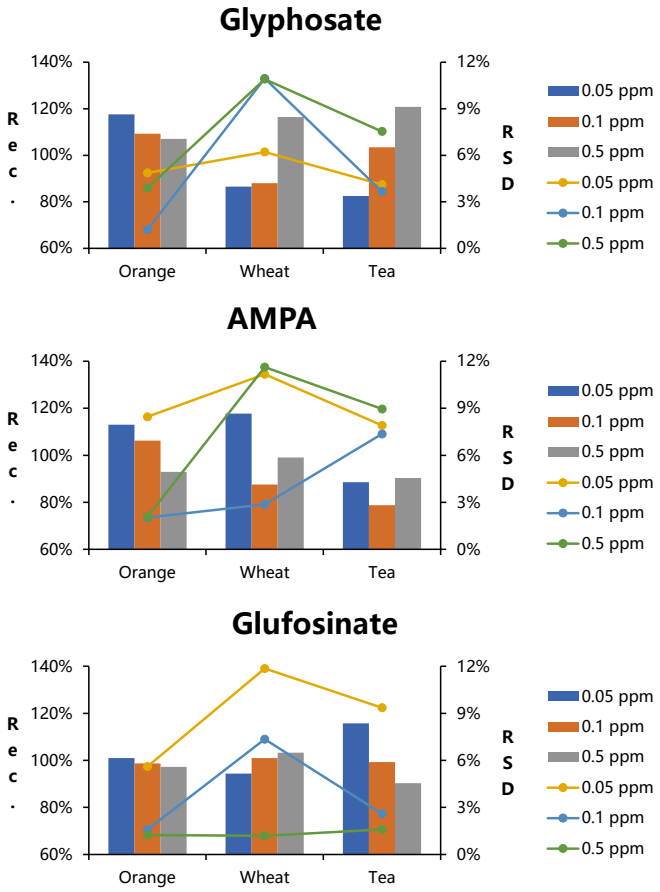

Figure 4. The recovery and repeatability of glyphosate, AMPA, and glufosinate in orange, wheat, and tea powder, respectively, at low, medium, and high fortified concentrations.

Conclusion

A time-saving method for the detection and quantitation of glyphosate, AMPA, and glufosinate was developed and validated with derivatization and SPE clean up. The final method was sensitive, and limits of quantification are sufficiently low to detect limited concentrations of these analytes in multiple food matrices, like orange, wheat, and tea powder.

This method took full advantage of the SCIEX QTRAP 4500 system on quantitation for complex matrices samples and presented it as a useful weapon to analysis polar pesticides with merit of accuracy, reliability, and stability. Above all, this method, as a cost-effective integrated workflow, will facilitate customers' daily work to enhance customer experience.

References

1. For more information on this Method, please contact your local SCIEX sales representative.

Document number: RUO-MKT-02-9381-A

Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
Phone 508-383-7700
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