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Application News

No. C210A

Liquid Chromatograph Mass Spectrometry

Direct Analysis of Glyphosate, Glufosinate and AMPA in Foods Using a Triple Quadrupole LC/MS/MS

Glufosinate and glyphosate are non-selective herbicides used for various purposes in both the agricultural and domestic sectors. For grains, in particular, they are used as pre-harvest herbicides to reduce the work needed for harvesting, and the maximum residue limits has been established by country or regions.

When degraded in soil and water, glyphosate produces aminomethylphosphonic acid (AMPA) as a metabolite. Glyphosate, glufosinate and AMPA are all highly polar compounds, making retention in the reversed-phase mode with HPLC or LC-MS difficult. Therefore, analysis usually employs derivatization using FMOC.

The C181 issue of Application News introduced a method which allows direct analysis of glyphosate, glufosinate and AMPA in beverages without derivatization . This article reports a direct analysis for food samples, such as grains and fruits, pretreated using the QuPPe (Quick Polar Pesticides) method developed by EURL (Stuttgart, Germany).

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■ Limit Values Prescribed in Individual Countries

The prescribed limit for residual pesticides vary from country to country, and these values differ with agricultural products. The maximum residue limits (MRLs) of glyphosate adopted by the Codex Alimentarius, EU, US and Japanese authorities are shown in Table 1 (as of October 2019).

Table 1 MRLs established by the Codex Alimentarius, EU, US and Japanese Authorities (mg/kg)

Country	Wheat	Oats	Soy bean	Grape
Codex	30	30	20	-
EU	10	20	20	0.5
US	30	30	20	0.2
Japan	30	30	20	0.5

■ Sample Pretreatment

Samples (flour, whole grain flour, oats grain, soybeans and grapes) were pretreated using the QuPPe method. There are two ways in QuPPe method, the first for most commodities and the other for samples containing high level of proteins or lipids. In this article, all samples were pretreated using the latter procedure. For grapes, however, the deproteinization process was skipped. The pretreatment workflow is shown in Fig. 1. Stable isotopes for individual compounds were used as internal standard. In addition, we made a minor change that the amount of sample to be weighed had been adjusted according to the food.

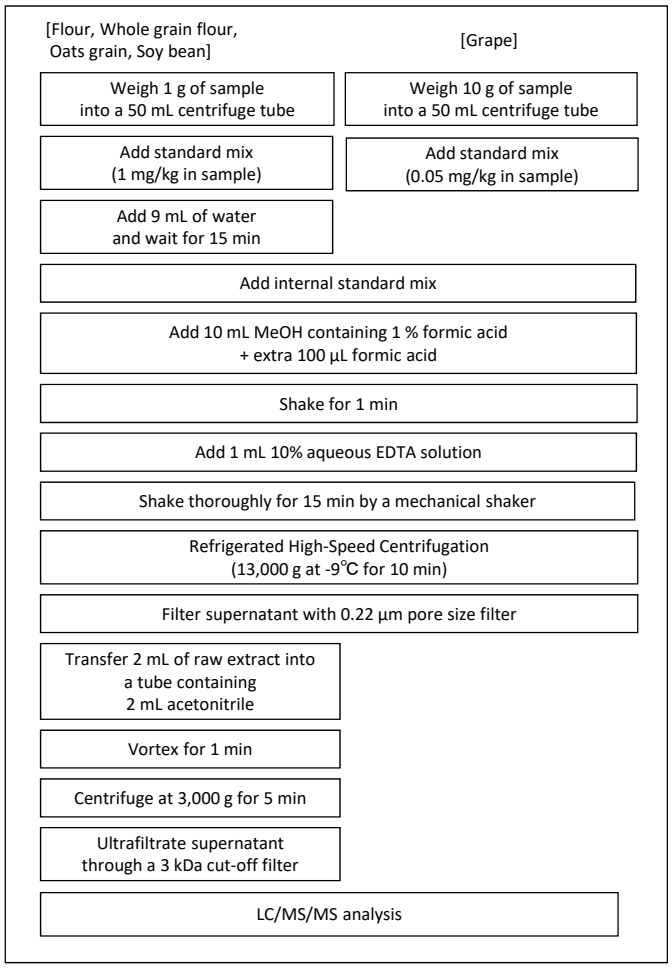

Fig. 1 Pretreatment Workflow

■ Analytical Conditions

The analytical conditions are shown in Table 2 and Table 3. The metallic outlet tubing of the LC autosampler was replaced with PEEK resin tubing in order to prevent the adsorption.

Table 2 Analytical Conditions for LCMS

[HPLC conditions] (Nexera™ X2)		
Column	:	RESTEK® Polar X (2.1 x 30, 2.7 µm)
Mobile phases	:	A) 0.5% formic acid in H_2O
		B) 0.1% formic acid in Acetonitrile
Gradient Program	:	B 60% (0-1 min) – B 5% (2-7 min) – B 60% (7.01-10 min)
Flow rate	:	0.6 mL/min
Column Temp.	:	35℃
Injection volume	:	5 µL
[MS conditions] (LCMS-8060)		
Ionization	:	ESI (Negative mode)
Probe Voltage	:	-3.0 kV
Mode	:	MRM
Nebulizing gas flow	:	3.0 L/min
Drying gas flow *	:	20.0 L/min
Heating gas flow *	:	20.0 L/min
DL/ Heat Block Temp.	:	300℃/500℃
Interface Temp.	:	400℃
CID gas	:	325 kPa

*The limits of the preset values were released after sufficient output from the nitrogen gas supply source to be used was confirmed.

Table 3 MS/MS Parameters

	Compound	Quantitative MRM transition (*m/z*)	Qualitative MRM transition (*m/z*)
Target	AMPA	110.00>78.90	110.00>62.90
	Glufosinate	180.10>62.90	180.10>85.00
	Glyphosate	168.10>63.00	168.10>78.80
IS	[^{13}C, $^{2}H_2$, ^{15}N]-AMPA	114.00>78.90	114.00>78.90
	[$^{2}H_3$]-Glufosinate	183.10>62.90	183.10>85.00
	[1, 2, 3-$^{13}C_3$, $^{2}H_2$]-Glyphosate	173.10>63.00	173.10>78.80

Analytical Results of Calibration Standards

Calibration standards were prepared at concentrations of 0.5, 1, 5, 10, 50 and 100 ng/mL and analysis was repeated six times. The chromatograms of 0.5 ng/mL standard and calibration curves made by the internal standard method are shown in Fig. 2. The accuracy and area repeatability of all calibration points has been confirmed within 80 to 120% and 20% or lower, respectively. For all compounds, good linearity was obtained with a correlation coefficient R of 0.999 or greater. In addition, the analysis results of calibration standards at the minimum concentration of 0.5 ng/mL as the lower limit of quantification for each compound are shown in Table 4.

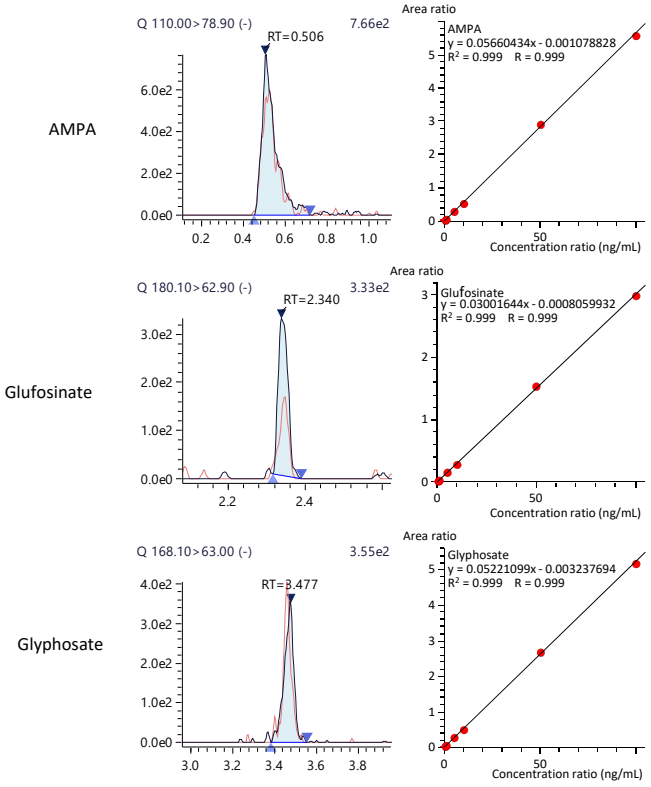

Fig. 2 Chromatograms of 0.5 ng/mL Standard and Calibration Curves

Table 4 Analytical Results of 0.5 ng/mL Standard Samples

Sample	LLOD (pg)	LLOD (ng/mL)	Area %RSD	Area ratio %RSD	Accuracy (%)
AMPA	2.5	0.5	10.9%	9.1%	96.3%
Glufosinate	2.5	0.5	9.4%	9.3%	102.6%
Glyphosate	2.5	0.5	7.2%	8.1%	103.9%

Quantitative Results of Each Food Product

We performed quantification of the compounds by the internal standard method and the recovery was determined. The results are shown in Table 5 and Table 6. For all samples, good recoveries ranging from 90 to 100% were obtained.

Furthermore, the chromatograms of whole grain flour and grape to which 1 mg/kg standard samples were added are shown as representative chromatograms in Fig. 3.

Table 5 Concentrations Quantified in Foods (mg/kg)

Sample	AMPA	Glufosinate	Glyphosate
Flour	< 0.025	-	-
Whole grain flour	0.080	-	0.98
Oats grain	< 0.025	-	-
Soy bean	-	-	-
Grape	-	-	-

Table 6 Recovery in Foods

Sample	AMPA	Glufosinate	Glyphosate
Flour	95.1%	94.4%	97.4%
Whole grain flour	94.2%	92.4%	96.6%
Oats grain	94.3%	93.9%	92.7%
Soy bean	91.8%	94.0%	95.8%
Grape	96.0%	94.8%	97.3%

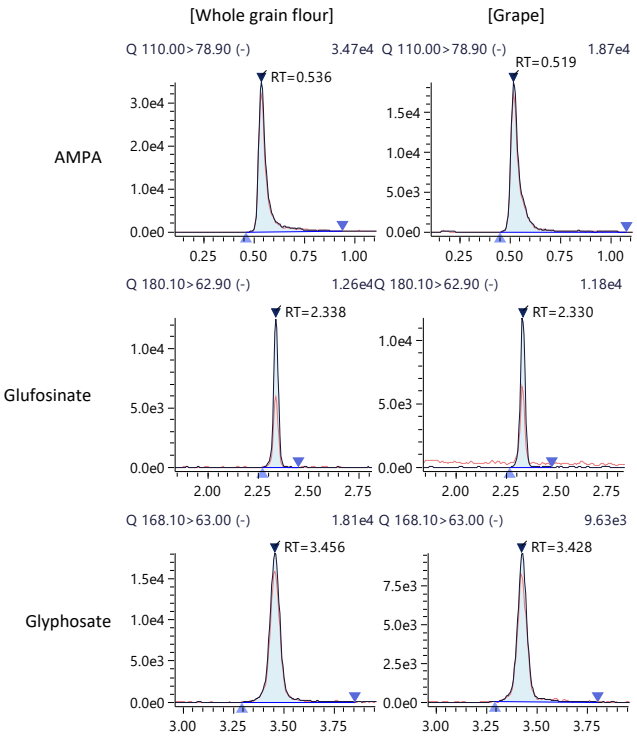

Fig. 3 Chromatograms of Whole Grain Flour and Grapes spiked at 1 mg/kg

Conclusions

- We examined a method that allows direct analysis of glyphosate, glufosinate and AMPA in food products pretreated by the QuPPe method.
- Good linearity ranging from 0.5 to 100 ng/mL was obtained by injecting 5 µL of standard samples.
- Good recovery and repeatabilities for all food products analyzed were obtained.

<References>

1) European Commission, QuPPe Method. http://quppe.eu (accessed on July 10, 2019)
2) Commission Regulation (EU) No 441/2012 of 24 May 2012 https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32012R0441&from=EN (accessed on October 31, 2019)
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4) "Partial Amendment of Specifications and Standards for Foods, Food Additives, etc." (Public Notice of the Ministry of Health, Labour and Welfare No. 361 of 2017)

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