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

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1. Introduction

Glyphosate and glufosinate 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 (MRLs) has been established by country or regions. MRLs of glyphosate adopted by the Codex Alimentarius, EU, US and Japanese authorities are shown in Table 1 (as of October 2019).

When degraded in soil and water, glyphosate produces AMPA as a metabolite. Since glyphosate, glufosinate and AMPA are all highly polar compounds, it is difficult to optimize retention in the reversed phase mode of HPLC. Therefore, analysis usually employs derivatization with active regent such as FMOC. In this study, we examined a method that allows direct analysis in food products, such as grains and fruits, pretreated by the QuPPe method and good recovery and reproducibilities in order to utilize for all food product analysis.

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

2. Experimental

2-1. Reagents

A commercially standard mixture solution was used for standard of Glyphosate, Glufosinate and AMPA . 20 µg/mL mixture standard solution was purchased from FUJIFILM Wako Pure Chemical (Osaka, Japan). Internal standards ([1,2,3-$^{13}C_3$,$^{2}H_2$]-Glufosinate, [$^{13}C_2$,^{15}N]-Glyphosate and [^{13}C, $^{2}H_2$, ^{15}N]-AMPA) were purchased from Alsachim (Illkirch Graffenstaden, Fr)

ALSACHIM
a Shimadzu Group Company

2-2. 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 modification that the amount of sample to be weighed had been adjusted according to the food.

[Flour, Whole grain flour, Oats grain, Soy bean]	[Grape]
Weigh 1 g of sample into a 50 mL centrifuge tube	Weigh 10 g of sample into a 50 mL centrifuge tube
Add standard mix (1 mg/kg in sample)	Add standard mix (0.05 mg/kg in sample)
Add 9 mL of water and wait for 15 min	
Add internal standard mix	
Add 10 mL MeOH containing 1 % formic acid + extra 100 µL formic acid	
Shake for 1 min	
Add 1 mL 10% aqueous EDTA solution	
Shake thoroughly for 15 min by a mechanical shaker	
High-Speed Centrifugation under refrigerated condition (13,000 g at -9℃ for 10 min)	
Filter supernatant with 0.22 µm pore size filter	
Transfer 2 mL of raw extract into a tube containing 2 mL acetonitrile	
Vortex for 1 min	
Centrifuge at 3,000 g for 5 min	
Ultrafiltrate supernatant through a 3 kDa cut-off filter	
LC/MS/MS analysis	

Fig. 1 Pretreatment Workflow

2-3. 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 eliminate the adsorption.

Table 2 Analytical Conditions for LCMS

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

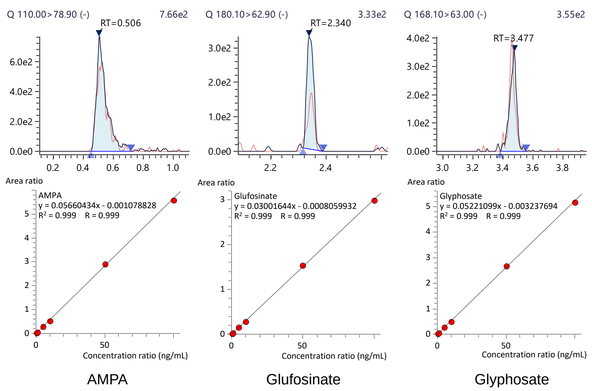
Table 3 MS/MS Parameters

	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
	[1,2,3-$^{13}C_3$,$^{2}H_2$]-Glufosinate	183.10>62.90	183.10>85.00
	[$^{13}C_2$,^{15}N]-Glyphosate	173.10>63.00	173.10>78.80

3. Result

3-1. 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.



AMPA Glufosinate Glyphosate

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

Table 4 Analytical Results of 0.5 ng/mL Standard Samples

	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%

3-2. Quantitation 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%

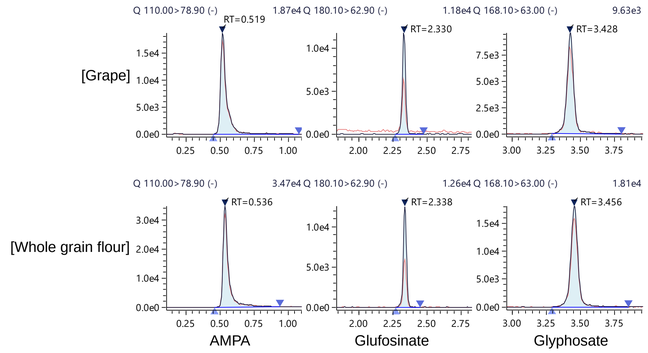


Fig. 3 Chromatograms of Whole Grain Flour and Grapes spiked at 1 mg/kg

4. 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)
3) 158 – Glyphosate, Codex Pesticides Residues in Food Online Database. http://www.fao.org/fao-who-codexalimentarius/codex-texts/dbs/pestres/pesticide-detail/en/?p_id=158 (accessed on October 31, 2019)
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)