SHIMADZU

WP-163

Highly sensitive analysis of glyphosate, glufosinate and AMPA in the tap water and the beverages by LC-MS/MS without derivatization

Kota Ishioka[1], Miho Kawashima[2], Manami Kobayashi[1], Junichi Masuda[1], Yoshihiro Hayakawa[2]

[1]Shimadzu corporation, Hadano, Kanagawa, Japan, [2]Shimadzu corporation, Kyoto, Japan

1. Overview

Direct analysis of glyphosate, glufosinate and AMPA in the drinking water and the beverages was developed with good recovery factors.

2. Introduction

Glyphosate and glufosinate are active ingredients in widely used herbicides for broadleaf weeds and grasses. In the EU, the required LLOQ for glyphosate and its metabolite, aminomethylphosphonic acid (AMPA), in water is 0.1 ug/L. A derivatization process with such as FMOC is performed for these compounds in pretreatment since their retention on the reversed phase column are weak due to their hydrophilicity. Aiming to reduce the complex and time-consuming derivatization procedure, we introduce a high-sensitive direct analysis of glyphosate, glufosinate and AMPA without derivatization. Highly sensitive results could be obtained with good recovery factors for the drinking water and the beverages with limited pretreatment procedures such as filtering and dilution.

3. Methods

UHPLC-MS/MS analysis was performed on an LCMS-8060 with a heated ESI ion source, equipped with a Nexera™ X3 system (Shimadzu Corporation). It is difficult to separate glyphosate, glufosinate and AMPA respectively because of their hydrophilicity and weak retentions. Therefore, using a hydrophilic interaction liquid chromatography (HILIC) column enable us to evaluate the analytical condition for separation and sensitivity. As the result, the chromatographic separation had been optimized using a HILIC column (RESTEK Polar X) and LCMS/MS with appropriate gradient elution.

■ **UHPLC conditions (Nexera™ X3 system)**

Column	: RESTEK Polar X (30 mm x 2.1 mm I.D., 2.7 µm)
Mobile Phase A	: 0.5% formic acid in H_2O
Mobile Phase B	: 0.1% formic acid in Acetonitrile
Flow rate	: 0.6 mL/min
Gradient program	: B conc. 80%(0.0 - 1.0 min) – 25%(2.0 min) – 0%(3.5 – 5.5 min) – 80%(5.6 – 9.5 min)
Column temp.	: 35℃
Injection vol.	: 50 µL

■ **MS conditions (LCMS-8060)**

Ionization	: ESI Negative MRM mode
IF voltage	: -3 kV
DL temp.	: 250℃
Interface temp.	: 350℃
Heat block temp.	: 400℃
Nebulizer gas	: 2 L/min
Heating gas	: 20 L/min
Drying gas	: 20 L/min
CID gas press.	: 325 kPa

■ **MRM transition**

AMPA	*m/z*: 110.00>79.00
Glufosinate	*m/z*: 179.80>63.00
Glyphosate	*m/z*: 167.90>62.80

High Speed Mass Spectrometer
Ultra Fast Polarity Switching - 5msec
Ultra Fast MRM - Max. 555 transition /sec

4. Results

4-1. Analysis of standard solution

The calibration curves for AMPA, glufosinate and glyphosate and the chromatograms of each compound at a concentration of 0.1 µg/L are shown in Fig.1 and Fig.2. The accuracy of the calibration points was within 87.7 to 118.8 % for each compound, respectively. The area repeatability values (%RSD, n=3) of 0.1 µg/L for all compounds were less than 15%.

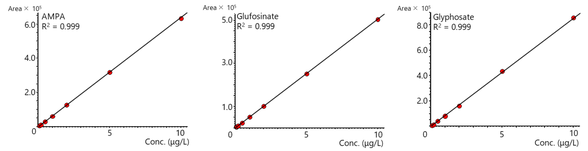

Fig.1 Calibration curves of AMPA, glufosinate and glyphosate (0.1 ~ 10 µg/L)

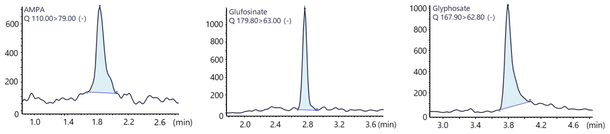

Fig.2 MRM Chromatograms of 0.1 µg/L standard solutions of AMPA, glufosinate and glyphosate

4-2. Analysis of tap water

The gradient program of HPLC was optimized for analysis of glyphosate and related compounds in order to eliminate the matrix effects caused by a variety of anions in trap water (Fig.3). The analysis was evaluated after the pretreatment for dechlorination with 10 mg/L of sodium ascorbate. This simple pretreatment procedure can reduce the time consuming and labor for the analysis.

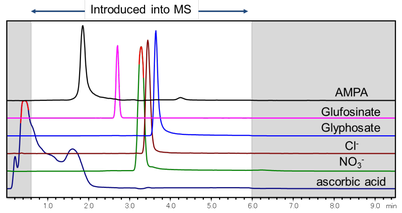

Fig.3 Chromatograms of typical anions in tap water

As the obtained results, the chromatograms of tap water spiked with each compound as 0.1 µg/L are shown in Fig.4. In addition, the recovery rate (%) and area repeatability values (%RSD) were listed in Table 1. These good values indicate that the analytical method for tap water was developed without the complicated procedure for the pretreatment.

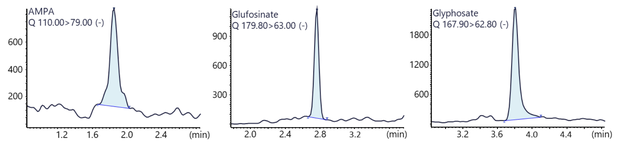

Fig.4 Chromatograms of tap water spiked as 0.1 µg/L

Table 1 The recovery rate and area repeatability of tap water (%, n=5)

	AMPA	Glufosinate	Glyphosate
Recovery	92.4	99.6	70.4
Repeatability	15.7	8.6	4.3

4-3. Analysis of the beverages

The trial test for direct analysis of the beverages (beer, white and red wine) were performed. The beverages contain various impurities as matrix compounds. Therefore the samples were pretreated with dilution followed by filtration using the SPE column (GL Sciences, InertSep Slim-J PLS-3) as shown in Fig.5. After that, the samples were analyzed by the method using internal standard regent purchased from Alsachim (Illkirch Graffenstaden, France) as below.

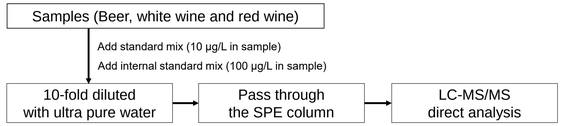

Fig.5 Pretreatment workflow

■ **UHPLC conditions (Nexera™ X3 system)**

Column	: RESTEK Polar X (30 mm x 2.1 mm I.D., 2.7 µm)
Mobile Phase A	: 0.5% formic acid in H_2O
Mobile Phase B	: 0.1% formic acid in Acetonitrile
Flow rate	: 0.6 mL/min
Gradient program	: B conc. 80%(0.0 - 1.0 min) – 0%(9.0 – 11.0 min) – 80%(11.1 – 15.0 min)
Column temp.	: 35℃
Injection vol.	: 5 µL

■ **MS conditions (LCMS-8060)**

Ionization	: ESI Negative MRM mode
IF voltage	: -3 kV
DL temp.	: 250℃
Interface temp.	: 350℃
Heat block temp.	: 400℃
Nebulizer gas	: 2 L/min
Heating gas	: 20 L/min
Drying gas	: 20 L/min
CID gas press.	: 325 kPa

■ **MRM transition**

<Target>

AMPA	*m/z*: 110.00>79.00
Glufosinate	*m/z*: 180.00>63.00
Glyphosate	*m/z*: 167.80>62.80

<Internal standard>

[^{13}C, ^{2}H$_2$, ^{15}N]-AMPA	*m/z*: 114.00>79.00
[1,2,3-^{13}C$_3$,^{2}H$_2$]-Glufosinate	*m/z*: 183.10>63.00
[^{13}C$_2$,^{15}N]-Glyphosate	*m/z*: 173.10>62.80

ALSACHIM
a Shimadzu Group Company

The accuracy of all calibration points in the obtained calibration curves (0.5 ~ 10 µg/L) were within 80 to 120%. The typical chromatograms of the beer spiked at 10 µg/L are shown in Fig.6. The recovery rate (%) and area repeatability values (%RSD) listed Table 2. These values are good for glufosinate and glyphosate in all the beverages. These results indicated that this analytical conditions were more sensitive than our previous study[1].

One the other hand, although the values of AMPA in beer and white wine are good, the peak was not detected in MRM chromatogram in red wine due to ionization suppression by the impurities as matrix compounds. Thus, we need continue to improve the method for analysis of AMPA.

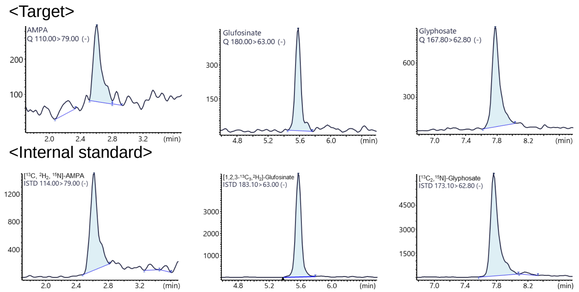

Fig.6 Chromatograms of beer spiked as 10 µg/L (10-fold diluted)

Table 2 The recovery rate and area repeatability of the beverages (%, n=3)

Sample		AMPA	Glufosinate	Glyphosate
Beer	Recovery	90.6	95.2	100.9
	Repeatability	4.2	13.0	8.2
White wine	Recovery	85.4	100.8	82.6
	Repeatability	19.7	3.1	7.6
Red wine	Recovery	N.D.*	109.4	82.1
	Repeatability	N.D.*	13.0	8.4

* not detected

5. Conclusions

- The direct analysis method of AMPA, glufosinate and glyphosate without complicated pretreatment such as a derivatization process was developed with HILIC column.
- As the results of analysis of tap water, we obtained good recovery rate (70.4 ~99.6%) and repeatabily value (%RSD < 20%).
- The recovery rate of glufosinate and glyphosate in three kind of beverage (beer, white wine and red wine) were good values (82.1 ~ 109.4%). The value of AMPA in the beer and white wine were 90.6 and 85.4% respectively.

Reference

[1] 67th ASMS Conference on Mass Spectrometry (Atlanta, 2019) TP-216.