Highly sensitive direct analysis of glyphosate, glufosinate and AMPA in the beverages by LC-MS/MS

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Overview

Development of a direct analysis method by LC/MS/MS for glyphosate, glufosinate and AMPA in beverages.

1. Introduction

Glyphosate and glufosinate are active ingredients in widely used herbicides. It is well-known that glyphosate is degraded into aminomethylphosphonic acid (AMPA) as a metabolite in soil and water. The required LLOQ for glyphosate and AMPA in water is 0.1 ug/L (ng/mL) in the EU. Since glyphosate, glufosinate and AMPA are highly polar compounds, their retention on the reversed phase column are weak. Therefore, a derivatization method with such as FMOC is performed for these compounds. To reduce the complex and time-consuming derivatization, we introduce a high-sensitivity direct analysis of glyphosate, glufosinate and AMPA without derivatization. With limited pretreatment procedures such as filtering and dilution, highly sensitive results could be obtained with good recovery factors for vary of beverages.

2. Methods and Materials

2-1. Analytical conditions

UHPLC-MS/MS analysis was performed on an LCMS-8050 with a heated ESI ion source, equipped with a Nexera™ X2 system (Shimadzu Corporation). Glyphosate, glufosinate and AMPA are difficult to separate in reversed phase column, respectively, due to their hydrophilicity and weak retentions. Thus, hydrophilic interaction liquid chromatography (HILIC) column was used to evaluate the analytical condition for separation and sensitivity. As the result, the chromatographic separation had been optimized using a HILIC column (Shodex® HILICpak® VT-50 2D) and LCMS compatible mobile phases as ammonium bicarbonate aqueous solution with acetonitrile system in 30 minutes gradient elution. Shodex® HILICpak® VT-50 2D has quaternary ammonium group binding on all porous sphere with polyvinyl alcohol base and PEEK body. In order to prevent the adsorption of glyphosate and glufosinate on the surface of the metal tubing, PEEK tubing (0.13 mmi.d.) was used instead of the autosampler standard metal outlet tube.

■UHPLC conditions (Nexera™ X2 system)

Column	: HILICpak® VT-50 2D (150 mm L. × 2.0 mm I.D., 5.0 µm, Shodex®)
Mobile phase A	: 50 mmol/L Ammonium bicarbonate - Water
Mobile phase B	: Acetonitrile
Flow rate	: 0.25 mL/min
Time program	: B Conc. 50 % (0.0 - 3.0 min) →5 % (7.0 - 20.0 min) → 50 % (20.01 - 30.0 min)
Column temp.	: 40 ℃
Injection vol.	: 50 µL

■MS conditions (LCMS-8050)

Ionization	: ESI, Negative MRM mode		
IF voltage	: -3 KV		
DL temp.	: 250 ℃		
Interface temp.	: 300 ℃		
Heat block temp.	: 400 ℃		
Nebulizer gas	: 2 L /min		
Heating gas	: 10 L/min		
Drying gas	: 10 L/min		
CID gas press.	: 325 Kpa		
MRM transition	: AMPA	110.00>78.80	CE: 28 V
	Glufosinate	180.10>62.90	CE: 43 V
	Glyphosate	168.10>62.90	CE: 24 V

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

3. Result

3-1. Analysis of Standard Solution

The chromatogram of each compound at a concentration of 5 µg/L is shown in Figure 1 and the calibration curves are shown in Figure 2. The accuracy and area repeatability (%RSD) values of each calibration point are listed in Table 1. The accuracy of the calibration points are within 95.3 to 106.9 % for each compound, respectively.

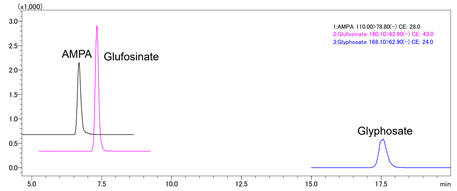

Figure 1 MRM Chromatograms of AMPA, Glufosinate and Glyphosate (each 5 µg/L)

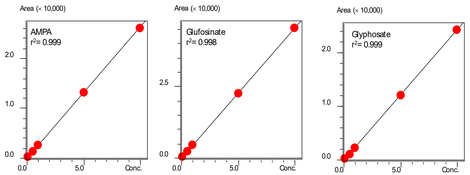

Figure 2 Calibration Curves of AMPA, Glufosinate and Glyphosate (0.1～100 µg/L)

Table 1 The accuracy and area repeatability (%, n=3)

Compound Name	0.1 µg/L		0.5 µg/L		1 µg/L		5 µg/L		10 µg/L	
	Accuracy	Repeatability	Accuracy	Repeatability	Accuracy	Repeatability	Accuracy	Repeatability	Accuracy	Repeatability
AMPA	100.8	9.61	95.3	3.69	101.4	4.60	102.1	4.29	100.4	2.23
Glufosinate	98.8	8.43	106.9	5.82	99.3	7.59	96.8	2.65	98.2	1.90
Glyphosate	100.2	7.32	100.1	5.76	97.8	1.67	100.7	1.94	101.2	0.96

3-2. Analysis of the Beverages

Seven kinds of beverages (coffee, tea, red wine, white wine, apple juice, mineral water and beer) were filtered by membrane filter (0.22 µm). After that they were spiked with AMPA, glufosinate and glyphosate to a concentration of 100 µg/L. Each sample was 100-fold diluted with ultra pure water followed by measuring to determine their recoveries. The obtained values are listed in Table 2.

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

Compound Name	Coffee		Tea		Red Wine		White Wine		Apple Juice		Mineral Water		Beer	
	Recovery	Repeatability	Recovery	Repeatability	Recovery	Repeatability	Recovery	Repeatability	Recovery	Repeatability	Recovery	Repeatability	Recovery	Repeatability
AMPA	89.5	6.83	92.6	5.93	76.2	9.67	76.6	10.38	78.5	1.60	95.5	8.69	84.1	3.76
Glufosinate	83.5	6.80	91.5	4.13	72.9	3.42	77.5	4.76	86.5	5.88	98.1	7.66	77.5	5.08
Glyphosate	97.0	4.57	96.9	6.91	94.8	7.67	104.9	10.34	86.9	1.99	74.4	10.01	92.8	11.90

As an example, the typical chromatograms of the coffee samples are shown in Figure 3. General pretreatment of samples includes a clean-up method such as solid-phase extraction; however, the procedures is often complex and takes time and effort.
This method had very simple pretreatment procedures comprising only filtering and dilution while achieving favorable recoveries ranging from 72.9 to 104.9 % with the samples.

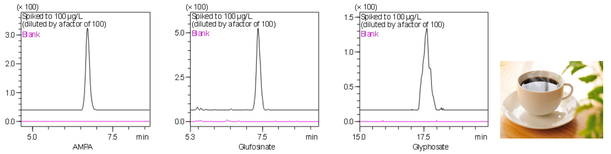

Figure 3 MRM Chromatograms of Spike and Recovery Test Samples (Coffee)

3-3. Trial test for direct analysis of tap water

Analysis of glyphosate group compounds in tap water is important. Tap water contains a variety of anions (Cl^-, HSO_4^-, NO_3^- etc.) as matrix compounds. A typical anion in tap water was monitored by SIM mode. The time program of LC gradient was optimized in order to separate the glyphosate group compounds and the typical anions to decrease the matrix effects (Figure 4). Usually in the pesticide test method, ascorbic acid is added to tap water to eliminate the effect of residual chlorine. Without adding of ascorbic acid, glyphosate group compounds in tap water is not detected. It was confirmed that ascorbic acid was added to the standard solution to be 10 mg/L, it did not affect the target compounds.

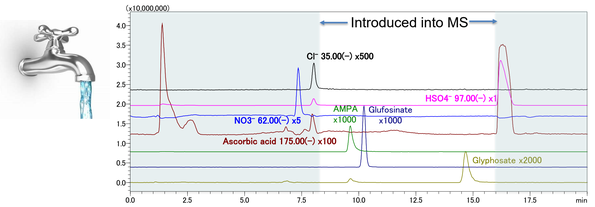

Figure 4 SIM chromatograms of typical anions in tap water

■UHPLC conditions for tap water

Column	: HILICpak® VT-50 2D (150 mm L. × 2.0 mm I.D., 5.0 µm)
Mobile phase A	: 50 mmol/L Ammonium bicarbonate – Water (pH 9)
Mobile phase B	: Acetonitrile
Flow rate	: 0.25 mL/min
Time program	: B Conc. 75 % (0.0 - 2.0 min) → 5 % (18.0 - 23.0 min) → 75 % (23.10 - 30.0 min)
Column temp.	: 40 ℃
Injection vol.	: 50 µL

■MS conditions (LCMS-8060)

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

The recovery rate of tap water from two cities (M and H) was compared (spiked at 20 µg/L). The recovery rate and area repeatability of tap water was shown in table 3. The recovery rate of AMPA and glufosinate of the city-H was less than the city-M. It was considered that the factor affecting the recovery rate of AMPA and glufosinate was NO_3^-. The peaks of the NO_3^- and HSO_4^- of city-M and city-H were shown in Figure 5. NO_3^- of city-H was more than city-M. And it was considered that HSO_4^- was the factor for glyphosate. HSO_4^- were affecting the same degree to city-M and H. We have continued to evaluate the affect by quantitatively adding the anions concentration.

Tap water of city-M was used for the matrix matched calibration solution, the recovery rate of AMPA, glufosinate and glyphosate increased to 108, 104, 112%.
So the standard addition method and the internal standard method were applicable for some tap water containing a variety of anions.

The quantitative lower limit of this method is 0.2 µg/L. The accuracy and area repeatability (%RSD) values of 0.2 and 20 µg/L standard mixture solutions listed in Table 4.

Table 3 The recovery rate and area repeatability of tap water (%, n=3)

Compound Name	Tap water in city-M		Tap water in city-H	
	Recovery	Repeatability	Recovery	Repeatability
AMPA	75.1	2.78	56.8	0.94
Glufosinate	64.6	6.46	57.0	1.18
Glyphosate	68.6	1.81	64.6	1.57

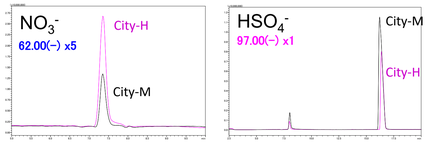

Figure 5 NO_3^- and HSO_4^- peak in tap water

Table 4 The accuracy and area repeatability of standard mixture solution (%, n=3)

Compound Name	0.2 µg/L		20 µg/L	
	Accuracy	Repeatability	Accuracy	Repeatability
AMPA	101.3	5.73	99.3	1.17
Glufosinate	104.9	3.40	101.6	0.92
Glyphosate	119.1	8.72	100.9	0.55

4. Conclusions

- ✓ The direct analysis conditions of AMPA, glufosinate and glyphosate was established by Hilic column and optimized MS parameter.
- ✓ The AMPA, glufosinate and glyphosate in seven kinds of beverages were detected at a high recovery rate (72.9 ～104.9 %).
- ✓ Directly analyze of AMPA, glufosinate and glyphosate in tap water has been investigated. The recovery rate obtained were 56.8 ～75.1% at this moment.

Reference

27th Symposium on Environmental Chemistry (Okinawa, 2018) P-110
Japan Society for Environmental Chemistry