

Quantitative Determination of Residual Glufosinate, Glyphosate and AMPA in Rice Matrix by Direct LC-MS/MS Method

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User Benefits

- ◆ A direct LC-MS/MS method with QuPpe method for sample preparation is established for quantitative determination of herbicides glufosinate, glyphosate and AMPA (metabolite) in rice matrix on LCMS-8060NX.
- ◆ The method achieves the required LOQ of 0.1 mg/kg in reference to the MRL of glyphosate in rice by EU regulation. The performance evaluation results indicate the acceptable linearity, accuracy, precision, matrix effect and recovery.

Introduction

Glyphosate and glufosinate are herbicides widely-used in farming of rice, tea and other agricultural products in South Asia and many other countries. The maximum residue limits (MRLs) of glyphosate in various products have been set by the authorities of EU, USA, Japan, etc., and their values are varied greatly with different products. For example, the MRLs of glyphosate in wheat, oats and soybean are 30, 30 and 20 mg/kg, respectively, whereas it is as low as 0.1, 0.5 and 2 mg/kg in rice, grape and tea respectively by the EU regulation. Aminomethyl-phosphonic acid (AMPA) is a toxic degradation product of glyphosate in soils, which is always monitored along with glyphosate by LC-MS/MS [1-5]. In this application notes, a sensitive MRM method is described for the quantitative determination of glyphosate, glufosinate and AMPA in rice matrix on LCMS-8060NX, a tandem LC-MS/MS system with an ion-focus ESI interface.

Experimental

Reagents and standards

Glyphosate, glufosinate and AMPA of high purity grade were purchased from Sigma-Aldrich. Ammonium formate (>99.0%) and formic acid of LC/MS grade were used as additives in the mobile phase prepared from LC/MS grade acetonitrile and Milli-Q water.

LC-MS/MS conditions

The analytical conditions on LCMS-8060NX are compiled in Table 1. The three compounds studied are very polar and may adsorb on metal surface. The stainless-steel tubing between the LC autosampler and the column was replaced with PEEK resin tube. In addition, polypropylene plastic sample vials and tubes were used in standard and sample preparation instead of glass vials, tubes and bottles.

The MRM transitions (quantifier ion and reference ion) and their optimized collision energy (CE) of the three compounds are compiled into Table 2. The ion intensity ratios between quantifier ion and reference ion are used as confirmation criteria.

Sample preparation

Basmati rice was purchased from local supermarket as matrix in method development and performance

Table 1 Analytical conditions on LCMS-8060NX

LC Conditions (Nexera™)	
Column	Acclaim Trinity Q1 column (3 x 100 mm, 3 μm)
Flow Rate	0.5 mL/min
Mobile Phase	A: Water with 50mM ammonium formate and 1% formic acid (pH 2.8) B: Acetonitrile
LC program	Gradient elution, 10 minutes
Oven Temp.	40°C
Injection Vol.	30 μL
MS Conditions (LCMS-8060NX)	
Interface	HESI IonFocus 1.0 kV
Interface Temp.	400°C
DL Temp.	300°C
Heat Block Temp.	400°C
Nebulizing Gas	3 L/min
Heating Gas Flow	20 L/min
Drying Gas Flow	15 L/min
Mode	MRM, negative mode

Table 2 MRM transitions and optimized CE

Name	Quantifier	CE (V)	Ref. Ion	CE (V)	Ratio
AMPA	110.0>78.9	27	110.0>63.0	22	100/100
Glufosinate	180.1>63.0	37	180.1>95.0	17	100/87
Glyphosate	168.1>63.0	23	168.1>149.9	37	100/45

evaluation. The rice was first powdered at cold temperature. The QuPpe method recommended by EURL was adopted [2]. A schematic procedure of sample preparation is shown in Figure 1, which was used in preparation of rice matrix blank, pre-spiked and post-spiked samples for method development and performance evaluation.

To avoid absorption of the polar compounds to glass vial, polypropylene plastic tubes, sample vials and Eppendorf tubes were used in preparation and storage of samples and standards. A disposable polypropylene plastic sample vial (P/N: 228-31600-91) was used in the LC-MS/MS analysis.

- 1) Weigh 1 g of rice sample (ground powders) into a 50 mL centrifuge tube
- 2) Add 9 mL of water and keep for 15 min at room temperature
- 3) Add 100 μ L of mixed standard solution of desired concentration
- 4) Add 9.9 mL MeOH containing 1% formic acid & 100 μ L formic acid, shake the mixture for 1 min
- 5) Add 1 mL of 10% aqueous EDTA solution; shake the tube thoroughly for 15 min with a mechanical shaker
- 6) Centrifuge under refrigerated condition (-9 $^{\circ}$ C) at 11,000 RPM for 10 min
- 7) Filter the supernatant with 0.22 μ m nylon filter
- 8) Transfer 2 mL of the extract to a tube containing 2 mL of acetonitrile, vortex for 1 min
- 9) Ultrafiltrate supernatant through a 3 kDa cut-off filter and transfer to polypropylene plastic vial
- 10) Inject to LC-MS/MS for analysis

Figure 1 Sample preparation procedure of pre-spiked sample in rice matrix in reference to QuPPE method [2].

Results and Discussion

Optimization of LC-MS/MS

To retain and separate effectively the three highly polar compounds, various LC columns with appropriate mobile phases conditions have been used such as HILIC [3], hybrid mode column [1, 4] and Phenyl column (metal free) with in-vial ion pairing reagent [5]. In this study, a hybrid mode column (Acclaim Trinity Q1) was used and the LC conditions were well-optimized to obtain proper retention, peak shape and stability in the presence of rice matrix. Figure 2 shows the MRM chromatograms of mixed standards in pure solvent and spiked in rice matrix.

Calibration curve and sensitivity

The calibration curves were established with mixed standards in pure solvent from 0.5 ng/mL to 100 ng/mL (Figure 3). Good linearity was obtained with r^2 of 0.9995, 0.9997 and 0.9977 for AMPA, glufosinate and glyphosate, respectively. The accuracy results of the whole range are shown in Table 3.

The sensitivity of the method for the standards in pure

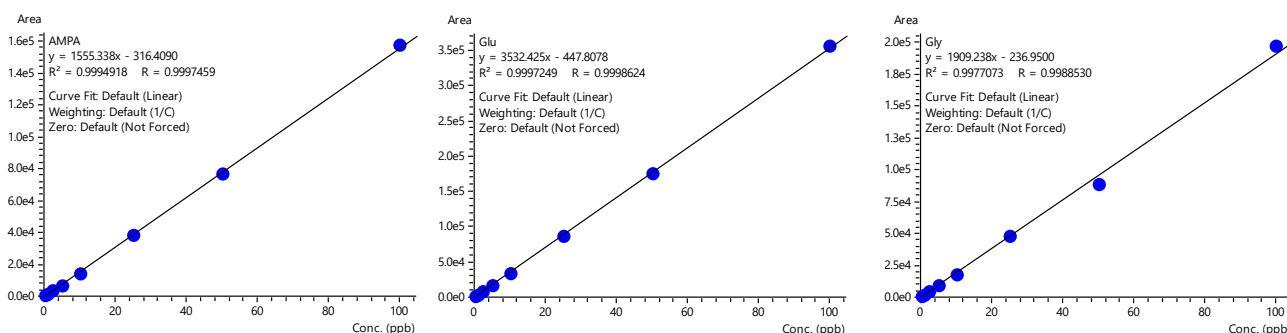


Figure 3 MRM calibration curves of AMPA, glufosinate and glyphosate for the range of 0.5~100 ng/mL (ppb).

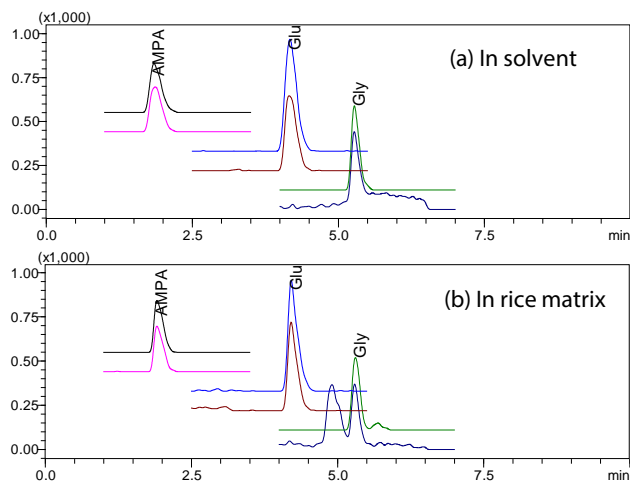


Figure 2 MRM peaks of mixed standards (each 2.5 ng/mL) in pure solvent (a) and in rice matrix (b).

Table 3 Accuracy results of three herbicides on LCMS-8060NX

Level	Calibrant (ng/mL)	AMPA (meas.)		Glufosinate (meas.)		Glyphosate (meas.)	
		(ng/mL)	A [%]	(ng/mL)	A [%]	(ng/mL)	A [%]
1	0.5	0.51	101.5	0.55	109.2	0.55	109.7
	0.5	0.58	115.0	0.58	115.4	0.51	101.9
2	1	1.13	113.1	0.96	96.1	0.97	97.1
	1	1.01	101.0	0.99	98.7	0.98	98.0
3	2.5	2.40	95.8	2.52	100.9	2.50	100.0
	2.5	2.42	96.8	2.45	97.8	2.39	95.6
4	5	4.73	94.7	4.74	94.8	5.30	106.1
5	10	9.38	93.8	9.59	95.9	9.51	95.1
6	25	24.83	99.3	24.93	99.7	25.29	101.2
7	50	49.59	99.2	49.77	99.5	46.53	93.1
8	100	101.45	101.5	100.95	100.9	103.42	103.4

solvent is estimated to be LOD at 0.5 ng/mL and LOQ at 1 ng/mL based on the MRM peaks. The MRM peaks of the standards of 0.5, 1.0 and 2.5 ng/mL are shown in Figure 4a. The sensitivity in the presence of rice matrix is estimated to be 1 ng/mL as LOD and 2.5 ng/mL as LOQ. These values correspond to 0.04 mg/kg and 0.1 mg/kg in rice sample, respectively, which meet the MRL for glyphosate recommended by EU regulation. The MRM peaks of 1 ng/mL in pure solvent, pre- and post-spiked samples are shown in Figure 4b.

Matrix effect, recovery and precision

Without use of internal standards, it is critical to evaluate the matrix effect and recovery. The rice used in this study is free of the target compounds, as such used as the blank matrix. The method performance evaluation was

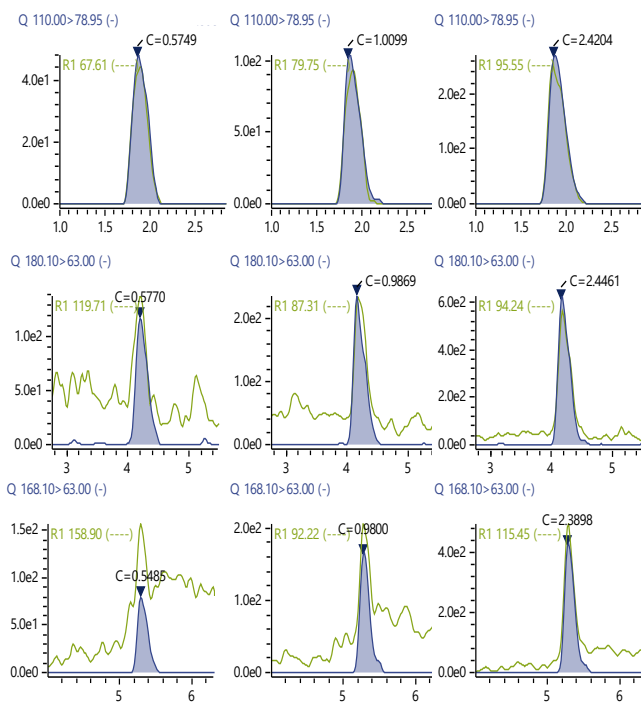


Figure 4a MRM peaks of low concentrations from left to right: 0.5, 1.0 and 2.5 ng/mL of AMPA (top), glufosinate (middle) and glyphosate (bottom)

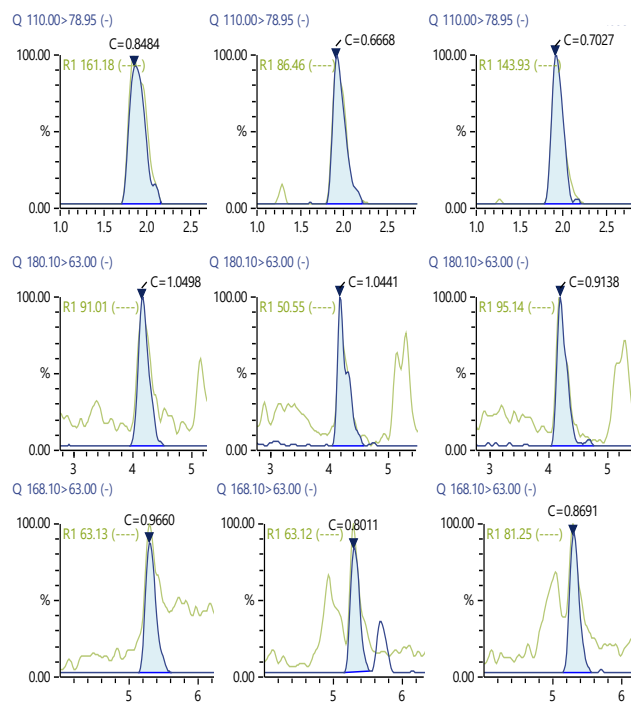


Figure 4b MRM peaks of 1 ng/mL samples, from left to right: in pure solvent, pre-spiked and post-spiked of AMPA (top), glufosinate (middle) and glyphosate (bottom)

Table 4 Results of method performance evaluation: precision (RSD%, n=6), matrix effect (ME), recovery (Rec) and process efficiency (PE)

Level	Compound	Mix std in solvent (n=6)		Mix std post-spk (n=6)		Mix std pre-spk (n=6)		Performance (n=6)		
		Conc. (ng/ml)	RSD (%) Conc.	Conc. (ng/ml)	RSD (%) Conc.	Conc. (ng/ml)	RSD (%) Conc.	ME (%)	Rec (%)	PE (%)
1 ng/mL (0.04 mg/kg)	AMPA	0.90	12.1	0.84	9.6	0.83	10.8	93.3	98.9	92.3
	Glufosinate	1.03	5.0	0.98	6.1	1.01	4.9	95.2	103.2	98.2
	Glyphosate	0.89	11.4	0.93	11.5	0.93	11.0	104.7	100.2	105.0
2.5 ng/mL (0.1 mg/kg)	AMPA	2.43	4.0	2.08	7.2	1.92	9.2	85.9	92.3	79.2
	Glufosinate	2.45	2.8	2.06	5.9	2.04	6.6	83.8	99.2	83.1
	Glyphosate	2.45	2.8	2.38	7.1	2.02	6.3	97.0	85.2	82.6
5 ng/mL (0.2 mg/kg)	AMPA	4.75	2.1	4.43	4.9	4.21	8.1	93.3	94.9	88.6
	Glufosinate	5.07	1.1	4.45	2.7	4.52	4.2	87.8	101.5	89.2
	Glyphosate	5.00	3.2	4.90	6.8	3.79	3.8	98.1	77.4	75.9

performed with three concentration levels, 1, 2.5 and 5 ng/mL. The results of Day 1 are summarized in Table 4. The evaluation was carried out for three days from sample preparation to LC-MS/MS analysis. The inter-day performance results are essentially close to the results as shown in Table 4, which indicate the acceptable stability and reliability.

Conclusion

A direct LC-MS/MS method was established for the quantitative determination of polar herbicides glufosinate, glyphosate and AMPA (metabolite) in rice matrix. The QuPPE sample preparation procedure recommended by EURL was adopted. The LOQ of the method for the targets in rice matrix was 2.5 ng/mL or better, which achieves the MRL (0.1 mg/kg) for glyphosate set by EU regulation. The method

performance in terms of precision, matrix effect and recovery was evaluated. The results of intra-day and inter-day evaluation indicate that the method is acceptable in precision, matrix effect and recovery.

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