Analysis of the residues of herbicides and metabolites in tea and honey

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INTRODUCTION

Glyphosate is the most widely used broad-spectrum herbicide in the world. After being absorbed by soil, it is quickly transformed into its main metabolite, aminomethylphosphonic acid (AMPA). Glufosinate is a polar herbicide with similar structure and properties to glyphosate. Due to the high efficacy and low cost of these herbicides, they are widely used in the cultivation of various crops. Compared with residue detection in fruits and vegetables, honey and tea matrices are more complex and can limit detection. Here, we established an efficient method for the determination of glyphosate, its metabolite AMPA and analogue glufosinate in tea and honey by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The method demonstrated excellent sensitivity, high recovery and good reproducibility.

MATERIALS AND METHODS

Sample preparation: The sample was placed in a plastic centrifuge tube and an aqueous solution containing mixed internal standards added. Ultrasonic extraction was performed, and the sample was then purified by SPE column. Sodium tetraborate solution and FMOC-Cl acetonitrile solution were added to the solution for derivatization at 55ºC for 4 hours. Formic acid was added to quench the reaction. The supernatant was removed and purified by SPE, and the eluate was analyzed by LC-MS/MS.

HPLC conditions: The eluate was separated using an ExionLC AC system with a Gemini NX-C18 column (2.0 x 50 mm, 3.0 µm, Phenomenex). The mobile phases used were 5 mM ammonium bicarbonate in water and 50% acetonitrile in methanol. Figure 1 shows a representative chromatographic separation of a mixed standard.

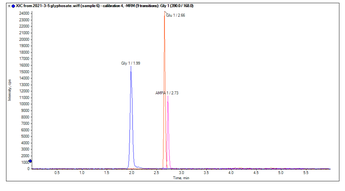

Figure 1. Chromatographic separation of glyphosate, AMPA and glufosinate.

MS/MS conditions: Samples were analyzed using the SCIEX Triple Quad 4500 system. The optimal MS conditions for each target compound were determined by injecting standard solution (50 ng/mL) directly to the mass spectrometer. Analyst software, version 1.7 was used for data acquisition and processing, and SCIEX OS-MQ software, version 2.0 was used for data analysis. The MS source conditions were optimized as follows: curtain gas (CUR), 30 psi; collision gas (CAD), medium; nebulizing gas (GS1), 55 psi; heater gas (GS2), 55 psi; ion spray voltage (IS), -4500V in negative mode; and source temperature, 550ºC. The related parameters for multiple-reaction monitoring (MRM) acquisition, declustering potential (DP) and collision energy (CE) for each compound are presented in Table 1.

Table 1. Optimized MS parameters.

Compound	Precursor ion (m/z)	Fragment ion (m/z)	DP (V)	CE (eV)
Glyphosate-FMOC	390.0	168.0	-65.0	-18.0
	390.0	150.0	-65.0	-36.0
Glyphosate-IS-FMOC	392.0	170.0	-65.0	-18.0
AMPA-FMOC	332.0	110.0	-56.0	-14.0
	332.0	136.0	-56.0	-23.0
AMPA-IS-FMOC	334.0	112.0	-56.0	-14.0
Glufosinate-FMOC	402.0	180.0	-60.0	-16.0
	402.0	206.0	-60.0	-21.0
Glufosinate-IS-FMOC	405.0	183.0	-60.0	-16.0

RESULTS

In this study, a sensitive and reliable LC-MS/MS approach for the simultaneous, rapid, qualitative and quantitative analyses of glyphosate, its metabolite AMPA and analogue glufosinate in tea and honey was developed. The chromatographic and mass spectrometry conditions, and pretreatment methods were systematically optimized. EDTA was added to the extraction solution to complex the metal ions in the matrix and stabilize the state of the analyte. The pH of the purified solution was adjusted by sodium hydroxide solution to enhance the derivatization conditions of FMOC-Cl. After the derivatization process, SPE purification and enrichment steps were added to reduce the excess derivatization reagents and other impurities to minimize potential contamination of the chromatographic column and analytical system and to improve the stability of the whole method.

The MS source conditions were optimized as described to optimize sensitivity of detection.

Recoveries, linearity, precision and sensitivity were assessed for method validation. The application of isotope-labeled standards ensured good method validation results. The recoveries in honey and tea ranged from 74.9% to 104.4% (Table 2) and the relative standard deviations (RSDs) ranged from 1.6% to 5.5%. Lower limits of quantification were obtained, and different ranges of linear calibration curves were established with R^2>0.998 (Figure 2).

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Table 2. Recoveries and RSDs for 3 pesticides. Recoveries (%) and reproducibility (%CV) at 2 different spike levels are shown.

Sample		Glyphosate		AMAP		Glufosinate	
Honey sample 1	Spike level（mg/kg）	0.02	0.04	0.02	0.04	0.02	0.04
	Recoveries %	*74.9*	*90.2*	*97.5*	*101.8*	*100.9*	*104.4*
	RSD %（n=6）	*2.7*	*3.2*	*5.5*	*4.5*	*2.3*	*1.6*
Honey sample 2	Spike level（mg/kg）	0.02	0.04	0.02	0.04	0.02	0.04
	Recoveries %	*102.9*	*101.5*	*92.5*	*97.0*	*100.2*	*101.4*
	RSD %（n=6）	*1.8*	*0.9*	*2.6*	*3.0*	*3.3*	*1.6*
Tea	Spike level（mg/kg）	0.02	0.04	0.02	0.04	0.02	0.04
	Recoveries %	*100.3*	*102.9*	*98.8*	*95.5*	*97.8*	*101.1*
	RSD %（n=6）	*4.9*	*2.5*	*5.0*	*4.7*	*4.3*	*2.7*

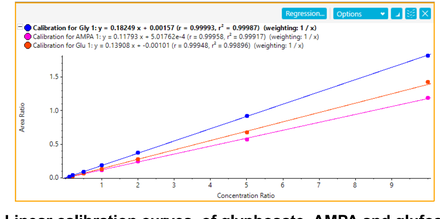

Figure 2. Linear calibration curves of glyphosate, AMPA and glufosinate.

CONCLUSIONS

This method can be successfully applied to the accurate determination of glyphosate, AMPA and glufosinate in honey and tea. The method has good specificity, repeatability and high sensitivity.

TRADEMARKS/LICENSING