

Determination of Glyphosate, Glufosinate, and AMPA in Green Tea by LC-MS/MS with QuEChERS Cleanup

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Abstract

A QuEChERS-liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was established for the determination of glyphosate, glufosinate, and AMPA in green tea. The samples were extracted by acetonitrile/water solution, and the extracts cleaned up by QuEChERS using a dedicated adsorbent mix for green samples. Analysis and quantification were performed by LC-MS/MS with Multi-Reaction Monitoring Mode (MRM) and external standard calibration. Glyphosate, glufosinate, and AMPA compounds showed a good linear relationship in the range of 1–150 µg/L with correlation coefficients greater than 0.9995, recoveries in the range of 80.0% to 107%, and the Relative Standard Deviations (RSD) were 2.9–5.0%, meeting the requirements of GB 23200.13-2016. The detection limits of the method met the more stringent requirements of SN/T 1923-2007 and were as follows for LOQs (µg/kg): glyphosate: 15.0; glufosinate: 27.0; AMPA: 33.0. An inter-lab comparison fulfilled the requirements of GB/T 27404-2008. The developed method therefore offers the advantages of simple sample pretreatment, short time demand, high sensitivity, and recovery rate, and is suitable for the analysis of the three compounds in food.

Introduction

Glyphosate is a broad-spectrum herbicide with toxicity similar to organophosphorus compounds and is one of the most frequently used herbicides in the world. Furthermore, the chemicals' usage increased after the introduction of genetically modified, glyphosate tolerant crops such as corn, soybeans, and cotton. In the USA, the US Environmental Protection Agency (EPA) regulation document 40 CFR 180 sets the tolerance levels for

the occurrence of glyphosate in food commodities and products. The EPA tolerance for glyphosate residues in dried tea is set at 1 ppm.¹ In rice, the tolerance is 0.1 ppm, whereas in sweet corn it is 3.5 ppm. For glufosinate, a herbicide that is often analyzed alongside glyphosate, the tolerance values are 1.1 ppm for canola meal and 1.0 ppm for rice. These tolerance values include metabolites and degradation products. The EU describes a Maximum Residue Limit (MRL) for tea of 2 mg/kg.² (Aminomethyl) phosphonic acid (AMPA) is the main degradation product of glyphosate and is also included in most analyses targeting glyphosate determination.³⁻⁵

The detection methods reported so far include Ion Chromatography (IC), Gas Chromatography (GC), and Gas Chromatography-Mass Spectrometry (GC-MS), High-Performance Liquid Chromatography (HPLC), and High-Performance Liquid Chromatography-Tandem Mass Spectrometry (HPLC-MS/MS). Because glyphosate and its main metabolite AMPA are highly water-soluble and highly polar amphoteric compounds, the above-mentioned methods display some drawbacks. In particular GC methods typically require derivatization, which is adding complexity and handling hazardous reagents. These also easily react with water and matrix components and form by-products, making quantitative analysis more challenging. Although GC-MS technology is qualitatively accurate and has little interference, excessive use of derivatization reagents may contaminate ion sources.⁶⁻⁷

The tea matrix is complex in composition and contains polar impurities such as polyphenols, amino acids, sugars, and pigments. Conventional sample preparation methods for glyphosate detection are mainly divided into solid phase extraction purification and derivatization.

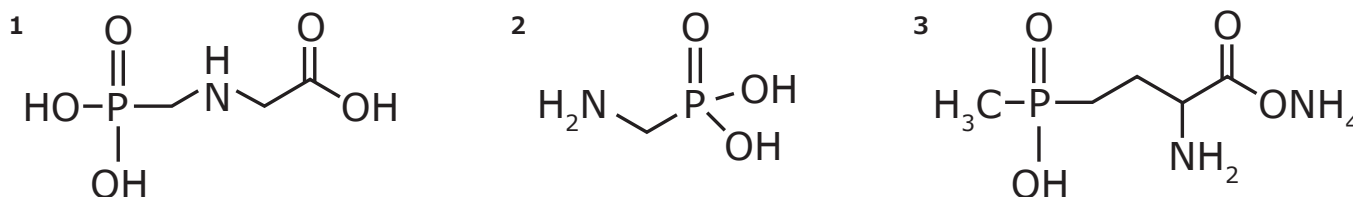
Both method steps are tedious and add costs. In contrast, a cleanup by the QuEChERS methodology is simple and easy to apply. Traditional QuEChERS materials for pigmented samples might tend to over-retain certain pesticides. The Supel™ QuE Verde, a new and improved adsorption and purification mix, allows for a balanced matrix removal and analyte recovery in particular for green samples.

The Supel™ QuE Verde clean-up tube contains Supelclean™ ENVI-Carb™ Y graphitized carbon, PSA (Primary Secondary Amine), and Z-Sep+, a zirconia-coated silica adsorbent packing. This mix allows efficient removal of main matrix components such as organic acids, polyphenolic polar compounds, lipidic impurities, and (green) pigments while maintaining good recovery also for planar pesticides.

Here we describe a method for the determination of glyphosate, AMPA, and glufosinate-ammonium in green tea with QuEChERS sample cleanup, using the Supel™ QuE Verde for efficient matrix removal and analyte

recovery. The HPLC-MS/MS analysis is performed using a Porous Graphitic Carbon (PGC) based HPLC column, the Supel™ Carbon LC, allowing for simpler mobile phase conditions (Reversed Phase, RP) and omitting HILIC conditions.

The results are evaluated against the quality criteria described by two Chinese national standards. For linearity of calibration, reproducibility, and recovery, the criteria stated in GB 23200.13-2016, "National Standard for Food Safety—Determination of 448 pesticides and related chemical residues in tea by liquid chromatography-mass spectrometry"⁸, which is the most authoritative national standard for pesticide detection in tea in China at present, were applied. Regarding sensitivity (LOD & LOQ) the criteria described in SN/T 1923-2007, "Method for the determination of glyphosate residues in food for imported and exported food—the HPLC-MS/MS method"³ were used as a benchmark, as these limit values for glyphosate, glufosinate-ammonium, and AMPA are stricter than those stated in GB23220.13-2006, ensuring the developed method also meets this criteria.



Structural formulas of glyphosate (1), (aminomethyl)phosphonic acid (AMPA, 2) and glufosinate-ammonium (3).

Experimental

Standard, Sample and Reagent Preparation

Sample Pretreatment

About 500 g of a green tea sample was crushed/homogenized with a grinder, screened through a 20 mesh (0.85 mm) sieve, mixed thoroughly, and the resulting coarse powder was stored in a plastic container.

Standard and Sample Preparation

Standard preparation:	<p><i>Glyphosate, glufosinate, and AMPA stock solution I (10 mg/mL):</i> Weigh 0.20 g of each glyphosate, glufosinate, and AMPA standards into a 20 mL amber glass volumetric flask, dissolve with water, and fill up to mark with water. The resulting stock solution with a concentration of 10.0 mg/mL of each glyphosate, glufosinate, and AMPA has to be stored at 0–8 °C and protected from light.</p> <p><i>Glyphosate, glufosinate, and AMPA stock solution II (100 mg/L):</i> Pipette 0.1 mL of glyphosate, glufosinate, and AMPA stock solution I into a 10 mL amber glass volumetric flask and fill up to mark with water to obtain stock solution II containing 100 mg/L of glyphosate, glufosinate, and AMPA. Store at 0–8 °C and protected from light.</p> <p><i>Glyphosate, glufosinate, and AMPA stock solution III (1.00 mg/L):</i> Pipette 0.1 mL of glyphosate, glufosinate and AMPA stock solution II into a 10 mL amber glass volumetric flask and fill up to mark with water to obtain stock solution III containing 1.00 mg/L of glyphosate, glufosinate, and AMPA. Store at 0–8 °C and protected from light.</p> <p><i>Glyphosate, glufosinate, and AMPA standard solutions 1–6:</i> Prepare a total of five standard solutions by pipetting 10 µL, 100 µL, 200 µL, 400 µL, 800 µL and 1500 µL, respectively, of glyphosate, glufosinate, and AMPA stock solution III into five individual 10 mL volumetric bottles. Fill up to mark with acetonitrile/water 40:60 (v:v). Glyphosate, glufosinate, and AMPA concentrations of the resulting six standard solutions is 1.0 µg/L, 10.0 µg/L, 20.0 µg/L, 40.0 µg/L, 80.0 µg/L and 150 µg/L, respectively.</p>
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Reagent Preparation

Extraction solvent: 400 mL of acetonitrile and 600 mL of water were combined to obtain a solution of acetonitrile/water 40:60 (v:v).

Mobile phase A: 1.9217 g ammonium carbonate was weighed into a 1 L volumetric flask and water filled to the mark to prepare a 20 mmol/L ammonium carbonate solution.

Standard and Sample Preparation (cont.)

Sample preparation:	<i>Extraction</i>
	Weigh 2 g \pm 0.005 g of tea sample (accurate to 0.001 g) into a 50 mL centrifuge tube, add 20.0 mL of a mixture of acetonitrile/water (40:60, v:v), vortex at 3500 rpm for 2 min, perform ultrasonic extraction for 30 min and centrifuge at 10000 rpm for 5 min to obtain a tea extract.
	<i>Purification</i>
	Pipette 2.0 mL of the extract into a 15 mL Supel™ QuE Verde (55442-U) centrifuge tube, vortex for 3 min at 2500 rpm, centrifuge for 3 min at 10,000 rpm and filter the supernatant using a 0.22 μ m syringe filter.
	<i>Spiking experiments</i>
	For the determination of recovery and precision, two samples were prepared by combining 2.0 g of a blank tea sample with 100 μ L and 200 μ L of glyphosate, glufosinate, and AMPA stock solution III, respectively. The glyphosate, glufosinate, and AMPA concentration in the two resulting solutions are representing spiked samples of 50 μ g/kg and 100 μ g/kg, respectively.
	The tea sample spiked with a glyphosate, glufosinate, and AMPA concentration of 100 μ g/kg was applied to analyze precision, and the tea sample spiked with a glyphosate, glufosinate, and AMPA concentration of 50 μ g/kg was utilized for the determination of recovery.

LC-MS/MS Analysis

The purified extracts were analyzed by HPLC-MS/MS (Table 1) using a Supel™ Carbon LC column with a volatile bicarbonate buffer in the mobile phase. This column possesses a unique mixed-mode retention mechanism that allows better retention of polar analytes without the need for HILIC conditions.

Note: Before first use, it is recommended to prime the Supel™ Carbon LC columns with sample matrix extracts (20–50 injections) to cover certain active sites on the surface for improved repeatability.

Table 1. HPLC-MS/MS Conditions

HPLC Parameters			
Instrument:	Waters™ Acquity I-class plus and Waters™ Xevo™ TQS UPS with electrospray ion source (ESI source)		
Column:	Supel™ Carbon LC, 2.7 μ m, 10 cm \times 2.1 mm I.D. (59986-U)		
Mobile phase:	[A] 20 mM Ammonium carbonate (pH=9) [B] Acetonitrile:water (95:5, v:v)		
Gradient:	Time (min)	% A	% B
	0	100	0
	1.0	95	5
	3.0	0	100
	13.0	0	100
	13.1	95	5
	15.0	95	5
Flow rate:	0.30 mL/min		
Column Temp.:	30 °C		
Detector:	MSD, ESI, MRM (see Table 2)		
Injection:	5.0 μ L		

Table 2. MS Instrument Parameters

MS-Conditions					
Instrument:	Waters™ Xevo™ TQS UPS				
Capillary voltage:	4.0 kV				
Nebulizing gas flow:	3.0 mL/min				
Drying gas flow:	10.0 mL/min				
Heat block temperature:	400 °C				
DL tube temperature:	250 °C				
Interface temperature:	300 °C				
Ionization mode:	ESI				
Scan:	MRM (see below)				
MRM Transitions					
Compound		Parent (m/z)	Daughter (m/z)	Cone (V)	Collision (V)
Glyphosate	Quant	168	63	-30	-26
	Qual	168	124	-30	-16
AMPA	Quant	110	63	-15	-28
	Qual	110	80	-15	-36
Glufosinate	Quant	180	63	-50	-56
	Qual	180	95	-50	-24

Results and Discussion

After extraction and QuEChERS cleanup, the tea sample was submitted to HPLC-MS/MS analysis (multiple reaction monitoring mode). The quantification was performed by an external calibration approach. A chromatogram for the analysis of glyphosate,

glufosinate, and AMPA standard solution (10 µg/L) is displayed in **Figure 1** showing good resolution. The separation for a spiked tea sample (50 µg/kg) is shown in **Figure 5**.

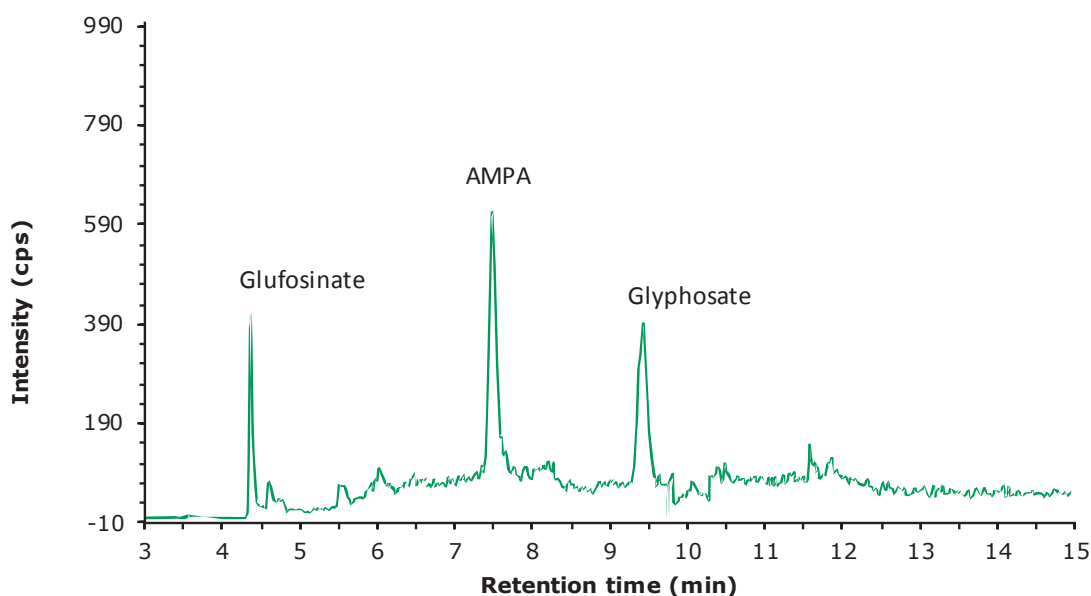


Figure 1. LC-MS/MS chromatogram obtained by the analysis of glyphosate, glufosinate, and AMPA standard solution 1 (10 µg/L).
 1: Glufosinate ($t_r = 4.36$ min), MRM transitions: 180.0 → 63.0 (quantitative);
 2: AMPA ($t_r = 7.48$ min), MRM transitions: 110.0 → 63.0 (quantitative);
 3: Glyphosate ($t_r = 9.41$ min), MRM transitions: 168.0 → 63.0 (quantitative).

Calibration & Sensitivity

The results of the external calibration experiments utilizing glyphosate, glufosinate, and AMPA standard solutions 1-6 (c=1.0, 10.0, 20.0, 40.0, 80.0 & 150 µg/L) showed good linearity for all compounds with R² values

between 0.9987 and 0.9995 (GB 23200.13-2016 criteria R² > 0.9950).⁸ The calibration data for glyphosate, glufosinate, and AMPA are shown in **Figures 2-4** and **Tables 3 & 4**.

Glyphosate

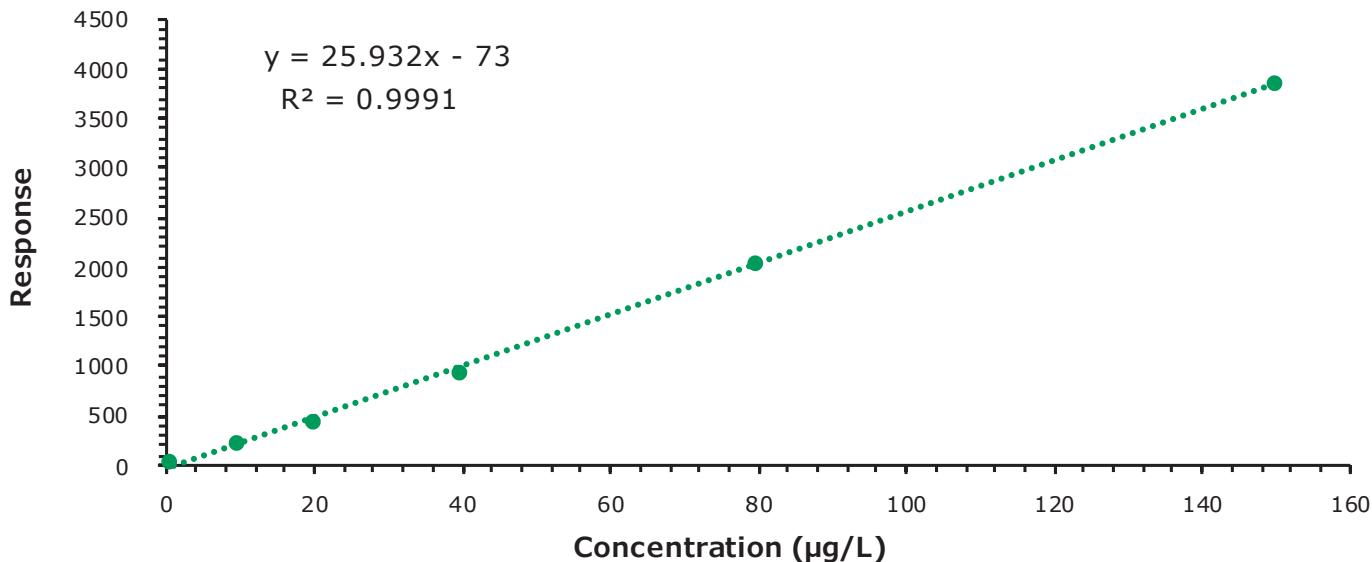


Figure 2. Calibration curve for glyphosate obtained by the analysis of standard solutions 1-6: (c = 1.0, 10.0, 20.0, 40.0, 80.0, and 150 µg/L).

Glufosinate

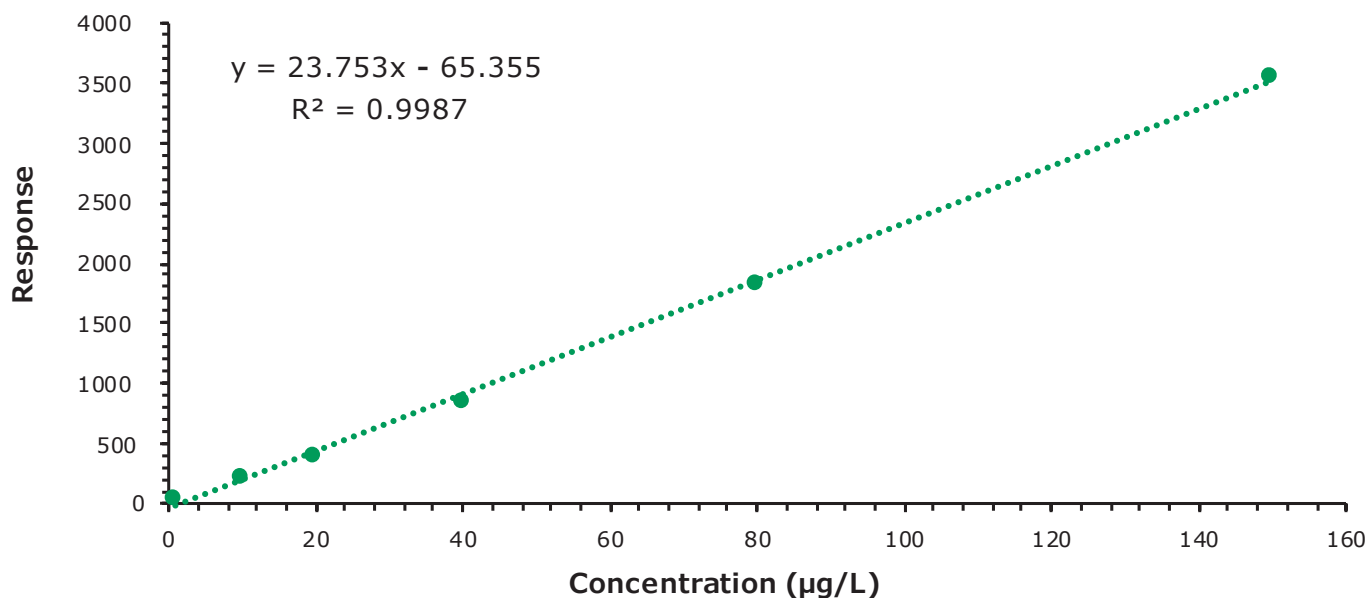


Figure 3. Calibration curve for glufosinate acid obtained by the analysis of standard solutions 1-6: (c = 1.0, 10.0, 20.0, 40.0, 80.0, and 150 µg/L).

AMPA

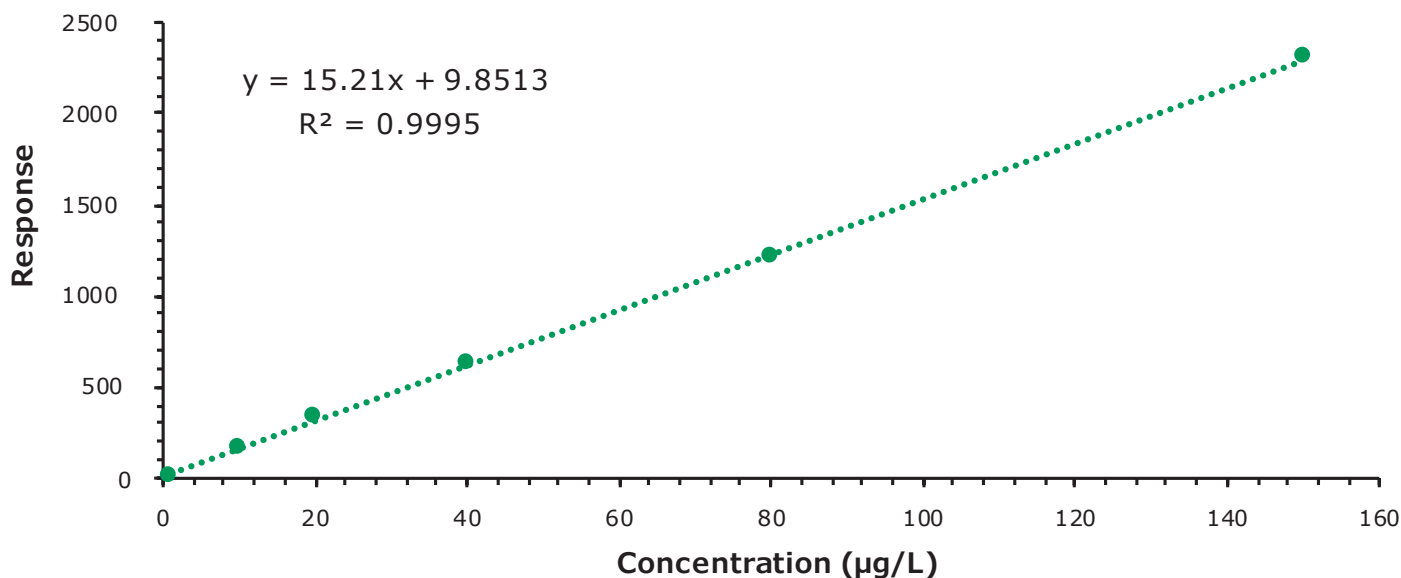


Figure 4. Calibration curve for AMPA obtained by the analysis of standard solutions 1–6: (c = 1.0, 10.0, 20.0, 40.0, 80.0, and 150 µg/L).

Table 3. Responses obtained for glyphosate, glufosinate, and AMPA for the external calibration solutions

Sample	Concentration (µg/L)	Glyphosate Area	Glyphosinate Area	AMPA Area
Standard solution 1	1.0	18.90	19.5	14.5
Standard solution 2	10.0	195.30	200.30	155.31
Standard solution 3	20.0	395.82	380.81	340.82
Standard solution 4	40.0	915.91	815.91	625.91
Standard solution 5	80.0	2011.68	1811.68	1200.58
Standard solution 6	150.0	3829.87	3529.87	2300.07

Table 4. Overview of the calibration data utilizing glyphosate, glufosinate, and AMPA standard solutions 1–6 (c = 1.0, 10.0, 20.0, 40.0, 80.0, & 150 µg/L)

Compound	Calibration	R ²
Glyphosate	y = 25.932x - 73	0.9991
Glufosinate	y = 23.753x - 65.355	0.9987
AMPA	y = 15.21x + 9.8513	0.9995

Precision & Recovery

Seven individual tea samples spiked with glyphosate, glufosinate, and AMPA at a concentration of 100 µg/kg (0.1 ppm) were applied to analyze precision (**Table 5**), and six individual tea samples spiked with 50 µg/kg (0.05 ppm) of each compound were utilized for the

determination of recovery (**Table 6**). The precision/reproducibility was in the range of 2.9 to 5.0% RSD (n=7), meeting the GB 23200.13-2016 criteria of <15%; the average recoveries (n=6) ranged from 85.5 to 97.6%. (GB 23200.13-2016 criteria 60–120%).

Table 5. Precision (RSD) of method at a glyphosate, glufosinate, and AMPA concentration of 100 µg/kg in tea (seven tea samples were prepared as parallel samples for detection and analysis)

Precision (RSD)	Glyphosate (µg/kg)	Glufosinate (µg/kg)	AMPA (µg/kg)
Theoretical value	100.0	100.0	100.0
Measured value 1	106.0	90.1	89.5
Measured value 2	104.0	89.3	89.1
Measured value 3	99.5	88.9	88.2
Measured value 4	104.0	89.7	88.9
Measured value 5	102.0	89.6	89.3
Measured value 6	106.0	89.0	91.3
Measured value 7	98.3	91.1	88.7
Average	103	89.7	89.3
RSD (%)	2.9	4.1	5.0

Table 6. Percent recovery of the method at a glyphosate, glufosinate, and AMPA concentration of 50.0 µg/kg in tea (six tea samples were prepared as parallel samples for detection and analysis)

Recovery	Glyphosate (µg/kg)	Glufosinate (µg/kg)	AMPA (µg/kg)
Theoretical value	50.0	50.0	50.0
Measured value 1	53.4	47.4	42.4
Measured value 2	44.0	44.5	41.7
Measured value 3	49.9	43.9	40.5
Measured value 4	50.6	44.6	42.6
Measured value 5	43.3	42.7	45.1
Measured value 6	51.8	41.8	43.9
Average	48.8	44.2	42.7
% Recovery (average)	97.6%	88.4%	85.5%
RSD (%)	4.2	1.9	1.6

Inter-lab comparison

As per the national standard GB/T 27404-2008 "Laboratory quality control specification-physical and chemical testing of food"⁹, the method was additionally tested in an external lab using a second Supel™ Carbon

LC column. The results showed a deviation of 8.6%, well within the GB requirement for inter-lab comparison set at <15%.

Sample Measurements

LC-MS/MS chromatogram obtained by the analysis of a tea sample utilized for the determination of the

method recovery rate (spiked at 50 µg/kg) is displayed in **Figure 5**.

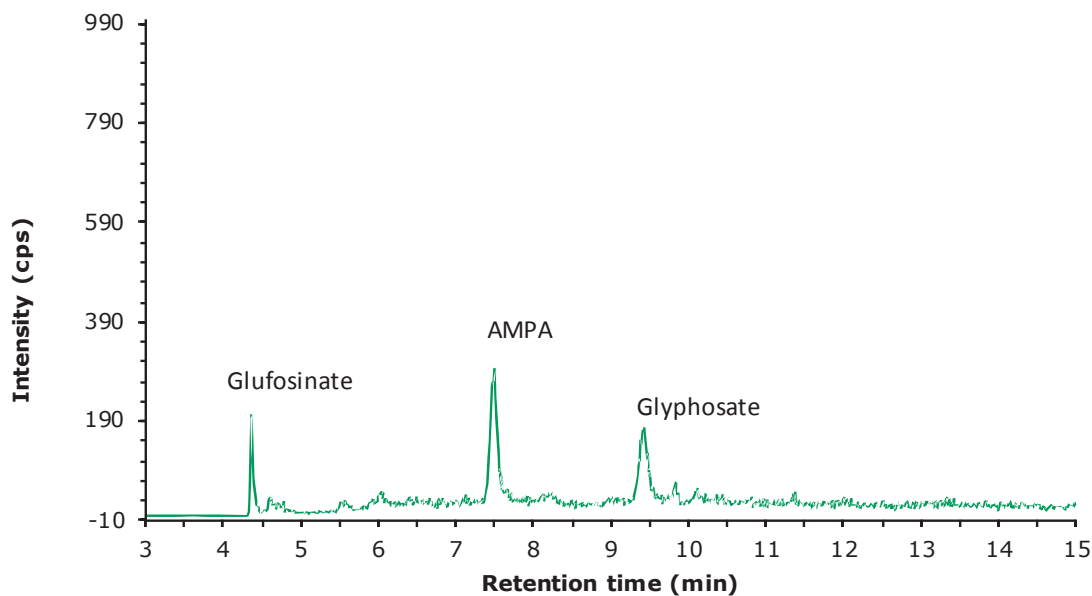


Figure 5. LC-MS/MS chromatogram obtained by the analysis of a tea sample spiked with glyphosate, glufosinate, and AMPA standard solution III at a concentration of 50.0 µg/kg.

Table 7. Chromatographic data for the analysis of a tea sample spiked at 50 µg/kg

Peak no.	Compound	Retention time (min)	Detected mass m/z (g/mol)
1	Glufosinate	4.36	180.0
2	AMPA	7.48	110.0
3	Glyphosate	9.41	168.0

In **Figure 6** the result for the analysis of an unspiked tea sample is shown. No glyphosate, glufosinate, or AMPA peak is visible in this chromatogram.

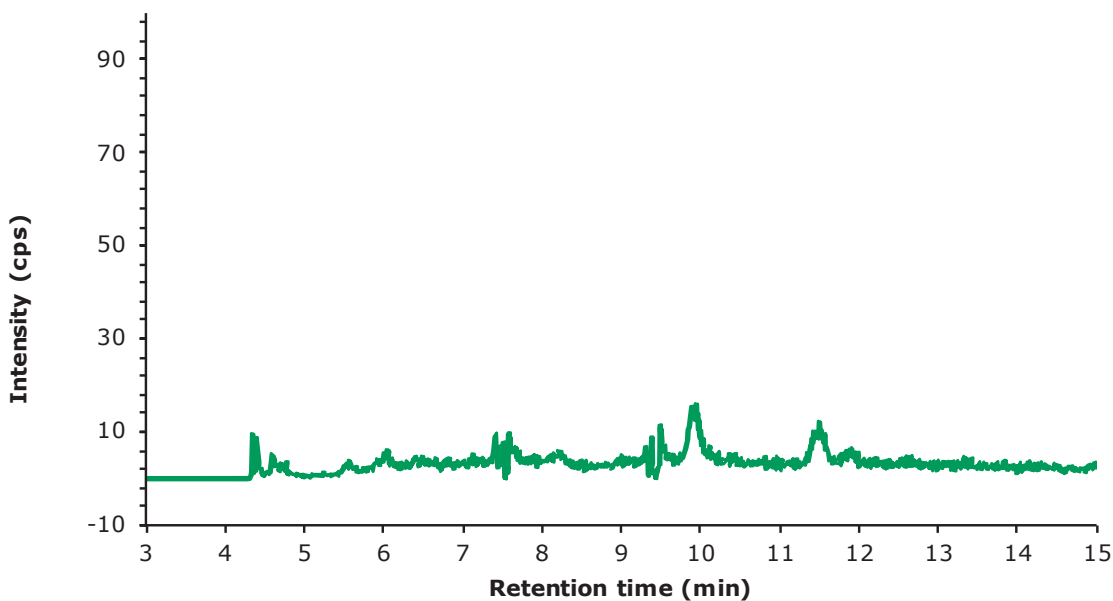


Figure 6. LC-MS/MS chromatogram obtained by the analysis of an unspiked tea sample.

Sensitivity of the Method

The sensitivity was determined using the baseline noise of a blank tea sample, where 3N/X was used to determine LOD and 10N/X was used to determine

LOQ. Determined values and comparison data from the SN/T 1923-2007 method are shown in **Table 8**.

Table 8. LOQ & LOD of glyphosate, glufosinate, and AMPA (determined using baseline noise) and comparison values from the SN/T 1923-2007 method

Peak no.	Compound	Experimental results		SN/T 1923-2007	
		LOD ($\mu\text{g}/\text{kg}$)	LOQ ($\mu\text{g}/\text{kg}$)	LOD ($\mu\text{g}/\text{kg}$)	LOQ ($\mu\text{g}/\text{kg}$)
1	Glyphosate	5.0	15.0	15.0	50.0
2	Glufosinate	9.0	27.0	20.0	60.0
3	AMPA	11.0	33.0	20.0	60.0

Conclusion

A U/HPLC-MS/MS method combined with solvent extraction and QuEChERS cleanup was established to determine glyphosate, glufosinate, and AMPA residues in green tea. The samples were purified using a Supel™ QuE Verde adsorbent mix for green sample matrices and analysed by LC-MS using a Supel™ Carbon LC PGC UHPLC column that enabled RP conditions. Samples were quantified against an external standard calibration. The results showed linear responses in the range of 1.0 to 150 $\mu\text{g}/\text{L}$ for the injected solution with correlation coefficients (R^2) greater than 0.998 for all three analytes. The observed recoveries were ranging from 81% to 107%, and the precision (RSD) was 2.7–5%. Therefore, linearity, RSD, and recovery rate were meeting the GB 23200.13-2016 method criteria. The determined sensitivities were for LODs ($\mu\text{g}/\text{kg}$): glyphosate: 5.0; glufosinate: 9.0; AMPA:11.0; and for LOQ ($\mu\text{g}/\text{kg}$): glyphosate: 15.0; glufosinate: 27.0; AMPA:33.0. These meet or exceed the criteria of the SN/T 1923-2007 method. An inter-lab comparison showed reproducibility meeting the criteria of GB/T 27404-2008.

These results confirm the accuracy and reliability of the method for the detection and analysis of glyphosate, glufosinate, and AMPA in green tea.

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- GB/T 27404-2008 Laboratory quality control specification - Physical and chemical testing of food. GB/T 27404-2008 实验室质量控制规范 食品理化检测在线预览 (foodmate.net)

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