

Simultaneous Determination of Organophosphate and Phenoxy-based Herbicides in Black Tea using LC-MS/MS and QuPpe Pretreatment

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User Benefits

- ◆ An LC-MS/MS method is established for simultaneous analysis of highly-polar organophosphate-based herbicides (glyphosate, glufosinate, AMPA) and less-polar phenoxy-based herbicides (MCPA, 2,4-D, 4-CPA) in black tea on LCMS-8060NX.
- ◆ The results of intra-day and inter-day performance evaluation indicate the acceptable sensitivity, precision and stability against the MRLs set by the EU and JP authorities.

Introduction

Organophosphate and phenoxy-based herbicides are commonly used in agriculture and tea plantation [1]. Due to potential health hazards, maximum residue limits (MRLs) of these herbicides in various food products have been established by authorities around the world. Concurrent screening of multiple herbicides is desired, but different LC-MS/MS methods and columns are often used for analysis of different groups of herbicides due to the substantial differences in properties and retention chemistry. For instance, glyphosate and glufosinate (organophosphate) cannot be well retained and separated with C18 or phenyl LC columns due to their high polarity. The EURL-SRM has recommended specific LC-MS/MS methods and QuPpe pretreatment to handle such highly-polar herbicides and pesticides in food commodities [2]. In this application news, a sensitive LC-MS/MS method is described for simultaneous analysis of five commonly-used herbicides (and a toxic metabolite) in tea plantations in South Asia countries. A mixed-mode LC column was used for analysis of glyphosate, glufosinate, AMPA (metabolite), MCPA, 2,4-D and 4-CPA in black tea on the LCMS-8060NX, a tandem LC-MS/MS system with an ion-focus ESI interface.

Experimental

Reagents and standards

Glyphosate, glufosinate, AMPA, MCPA, 2,4-D and 4-CPA of high purity grade were purchased from Sigma-Aldrich. Mobile phases were prepared with LC/MS grade acetonitrile and Milli-Q water. LC/MS grade formic acid and ammonium formate (>99.0%) were added in Milli-Q water as additive.

LC-MS/MS conditions

The analytical conditions on LCMS-8060NX are compiled in Table 1. Due to the chelating properties of organophosphorus herbicides, stainless-steel tubing between the LC autosampler and the column was replaced with PEEK resin tube.

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

Table 1 Analytical conditions on the 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, 20 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 (negative mode) and optimized CE

Name	Quantifier	CE (V)	Ref. Ion	CE (V)	Ratio
AMPA	110.0>63.0	22	110.0>79.0	27	100:80
Glufosinate	180.1>95.0	17	180.1>85.1	19	100:56
Glyphosate	168.1>63.0	23	168.1>79.0	37	100:56
MCPA	199.1>141.0	15	201.0>143.0	16	100:36
2,4-D	219.0>161.0	15	221.0>163.0	11	100:55
4-CPA	185.1>127.0	15	187.0>129.0	15	100:47

Sample preparation

Black tea samples were purchased from a local supermarket. Extraction of herbicides from tea was done using the QuPpe method with minor modifications [2]. Figure 1 shows a schematic procedure of the sample preparation, which was used in the preparation of black tea matrix blank, pre-spiked and post-spiked samples for method development and performance evaluation. Extraction duration (3 min) was based on recommended steep time for black tea.

- 1) Weigh 500 mg of tea leaves into a 15 mL centrifuge tube
- 2) Add 2.5 mL of water mix and keep for 3 min at room temperature to hydrate the tea leaves.
- 3) Add 100 μ L of mixed standard solution of desired concentration
- 4) Add 2.5 mL of MeOH containing 1% formic acid and vortex mix for 3 min
- 5) Centrifuge under refrigerated conditions (-10 $^{\circ}$ C) at 11,000 RPM for 10 min
- 6) Filter 3.0 mL of the supernatant with 0.22 μ m PTFE filter
- 7) Add 3.0 mL of acetonitrile and store at -20 $^{\circ}$ C for 2 hr.
- 8) Transfer 1.0 mL of extract to a tube containing 1.0 mL of mobile phase A
- 9) Ultrafilter 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 tea matrix in reference to the QuPpe method [2].

Results and Discussion

Optimization of LC-MS/MS method

In a previous study, quantification of organophosphate-based herbicides in rice was established using a tri-mode column [3]. In this study, the same column was adopted and the LC conditions were re-optimized to analyze simultaneously both highly-polar organophosphate-based herbicides and less-polar phenoxy-based herbicides within 20 min as shown in Figure 2. Traditionally, these two classes of herbicides are always analyzed with different methods [4].

Calibration curves

The calibration curves were established with mixed standards in pure solvent from 0.5 ng/mL to 100 ng/mL for organophosphate-based herbicides and 0.05 ng/mL

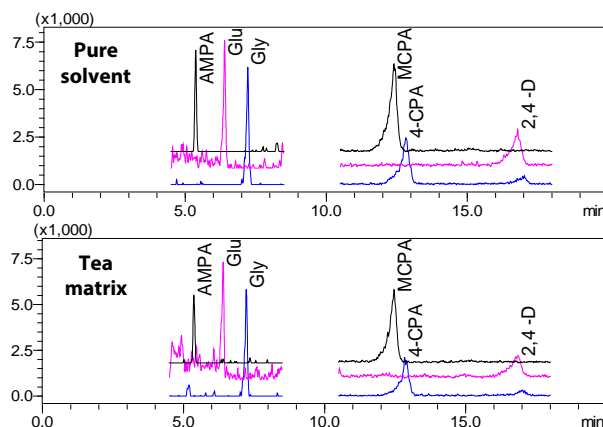


Figure 2 MRM peaks of mixed standards. Organophosphate-based herbicides (2.5 ng/mL) and phenoxy-based herbicide (0.25 ng/mL) in neat standard (top) and in tea matrix (bottom).

Table 3 Summary of quantitative parameters of six herbicides in solvent on the LCMS-8060NX

Name	Range (ng/mL)	Accuracy (%)	R ²	LOQ (ng/mL)
AMPA	0.5 – 100	92.2 – 114.9	0.9991	0.42
Glu*	0.5 – 100	81.6 – 113.5	0.9987	1.61
Gly*	0.5 – 100	90.0 – 102.3	0.9991	0.30
MCPA	0.05 – 10	79.7 – 114.2	0.9989	0.04
2,4-D	0.05 – 10	79.7 – 103.9	0.9991	0.12
4-CPA	0.05 – 10	84.9 – 107.6	0.9994	0.10

*Abbreviations used for Glufosinate (Glu) and Glyphosate (Gly)

to 10 ng/mL for phenoxy-based herbicides. Good linearity was obtained with r² between 0.998 and 0.999 for all six target compounds as shown in Figure 3. The calibration range, accuracy, linearity and LOQ are summarized in Table 3. The sensitivity of this method for AMPA, glufosinate, glyphosate, MCPA, 2,4-D, and 4-CPA in pure solvent has an estimated LOQ of 0.42 ng/mL, 1.61 ng/mL, 0.30 ng/mL, 0.04 ng/mL, 0.12 ng/mL and 0.10 ng/mL respectively. The LOD

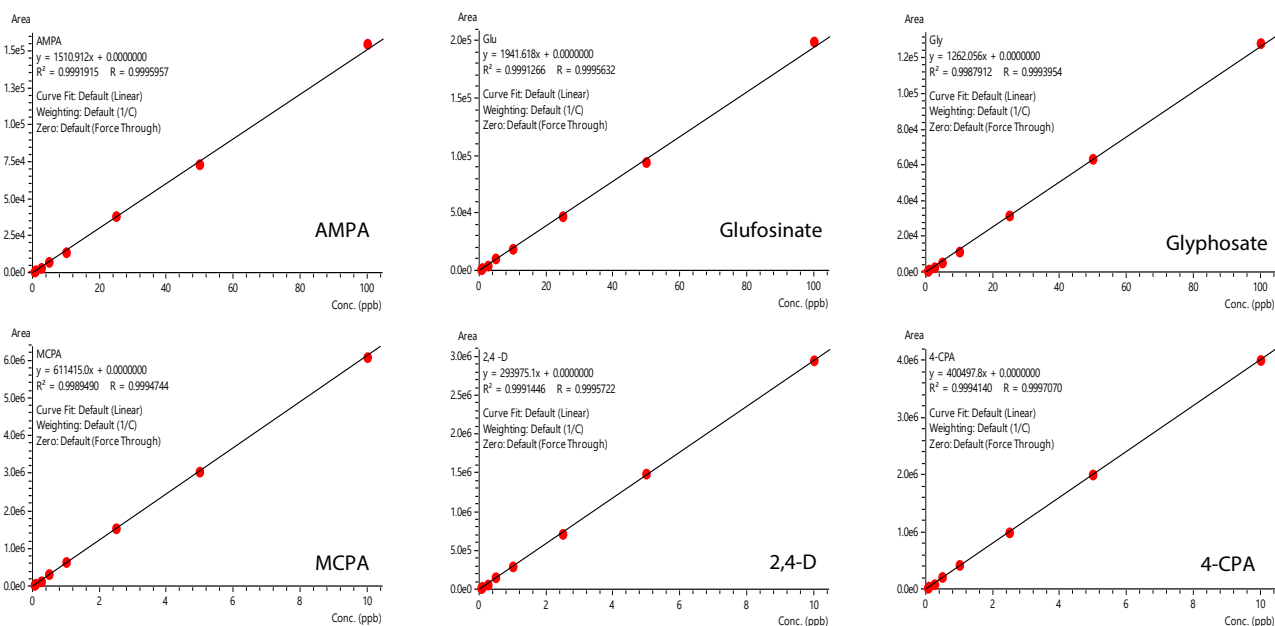


Figure 3 MRM calibration curves of AMPA, glufosinate and glyphosate, ranging from 0.5 ng/mL to 100 ng/mL and calibration curves of MCPA, 2,4-D and 4-CPA, ranging from 0.05 ng/mL to 10 ng/mL.

and LOQ of these herbicides in tea matrix are presented in the later section.

Precision, matrix effect and recovery

Without the use of internal standards, it is critical to evaluate the matrix effect and recovery. Hence, it is crucial to screen for tea samples that could be used as the blank matrix. Four different brands of Ceylon (Sri Lanka) black tea samples were screened for herbicide content (Table 4). Although trace amounts of herbicides were detected in all tea samples, they are far below the MRLs set by the EU and JP regulations. Tea brand E, which contains the lowest concentration of herbicides, was chosen and used as the tea blank for method evaluation. It has been reported that black tea matrix exhibits significant ion suppression to certain herbicides in LC-MS/MS analysis [5]. In this study, we also observed ion suppression following the QuPpe procedure. Hence, dilution (50%) of the tea extract with mobile phase was done before analysis to reduce the ion suppression effect [6]. The subsequent intra-day and inter-day

Table 4 Herbicide content in 4 different commercial black teas

Compound	Brand D (ng/mL)	Brand E (ng/mL)	Brand M (ng/mL)	Brand T (ng/mL)
AMPA	N.D.	N.D.	N.D.	N.D.
Gly*	5.2	N.D.	3.93	1.76
Glu*	N.D.	N.D.	N.D.	N.D.
MCPA	0.061	0.02	0.03	0.01
2,4-D	N.D.	N.D.	N.D.	N.D.
4-CPA	N.D.	N.D.	N.D.	N.D.

*Abbreviations used for Glufosinate (Glu) and Glyphosate (Gly)

method evaluation was performed at three spiked concentration levels over three consecutive days. The results of day 1 (n=6) are shown in Table 5. Except ME at 52.7~58.0% for AMPA, the results of intra-day and inter-day evaluation (not shown) indicate that the precision and stability of the method are acceptable.

Table 5 Results of method performance evaluation: precision (RSD%), matrix effect (ME), recovery (Rec) and process efficiency (PE)

Level	Compound	Mix std in solvent (n=6)		Mix std post-spik (n=6)		Mix std pre-spik (n=6)		Performance (n=6)		
		Conc. (ng/ml)	RSD (%)	Conc. (ng/ml)	RSD (%)	Conc. (ng/ml)	RSD (%)	ME (%)	Rec (%)	PE (%)
2.5 ng/mL (0.1 mg/kg)	AMPA	2.31	16.7	1.22	8.5	1.23	9.8	52.7	100.7	53.1
	Glufosinate	2.50	7.7	2.71	15.2	3.27	16.8	108.6	120.4	130.9
	Glyphosate	2.75	9.5	2.67	8.9	2.82	11.5	97.1	105.8	102.7
0.25 ng/mL (0.01 mg/kg)	MCPA*	0.27	4.5	0.219	3.1	0.215	1.2	74.0	98.2	72.6
	2,4-D	0.27	4.3	0.215	8.0	2.07	4.1	80.5	96.3	77.5
	4-CPA	0.27	3.3	0.217	1.9	0.213	3.5	81.9	98.2	80.4
5.0 ng/mL (0.2 mg/kg)	AMPA	4.56	14.7	2.64	7.45	2.20	10.9	58.0	83.1	48.2
	Glufosinate	4.99	4.1	4.87	9.34	5.28	8.5	97.4	108.4	105.6
	Glyphosate	4.93	4.5	4.59	4.76	4.60	5.7	93.1	100.2	93.3
0.5 ng/mL (0.02 mg/kg)	MCPA*	0.55	2.3	0.453	1.78	0.469	6.0	77.0	103.5	79.7
	2,4-D	0.47	2.6	0.375	2.96	0.353	5.8	79.8	94.1	75.1
	4-CPA	0.56	4.4	0.447	3.15	0.467	4.1	80.5	104.5	84.1
10.0 ng/mL (0.4 mg/kg)	AMPA	9.25	7.6	5.07	8.4	3.72	9.0	54.8	73.4	40.2
	Glufosinate	10.21	3.1	9.11	5.7	10.42	3.4	89.2	114.4	102.1
	Glyphosate	9.23	5.6	9.04	5.2	10.81	6.7	97.9	119.7	117.2
1.0 ng/mL (0.04 mg/kg)	MCPA*	0.95	3.8	0.719	1.8	0.827	1.6	75.0	115.0	86.3
	2,4-D	1.03	4.5	0.881	2.3	1.02	2.47	85.9	115.3	99.0
	4-CPA	0.93	2.3	0.726	2.9	0.830	3.0	78.4	114.3	89.6

*MCPA was originally detected in the tea blank at approximately 0.02 ng/mL.

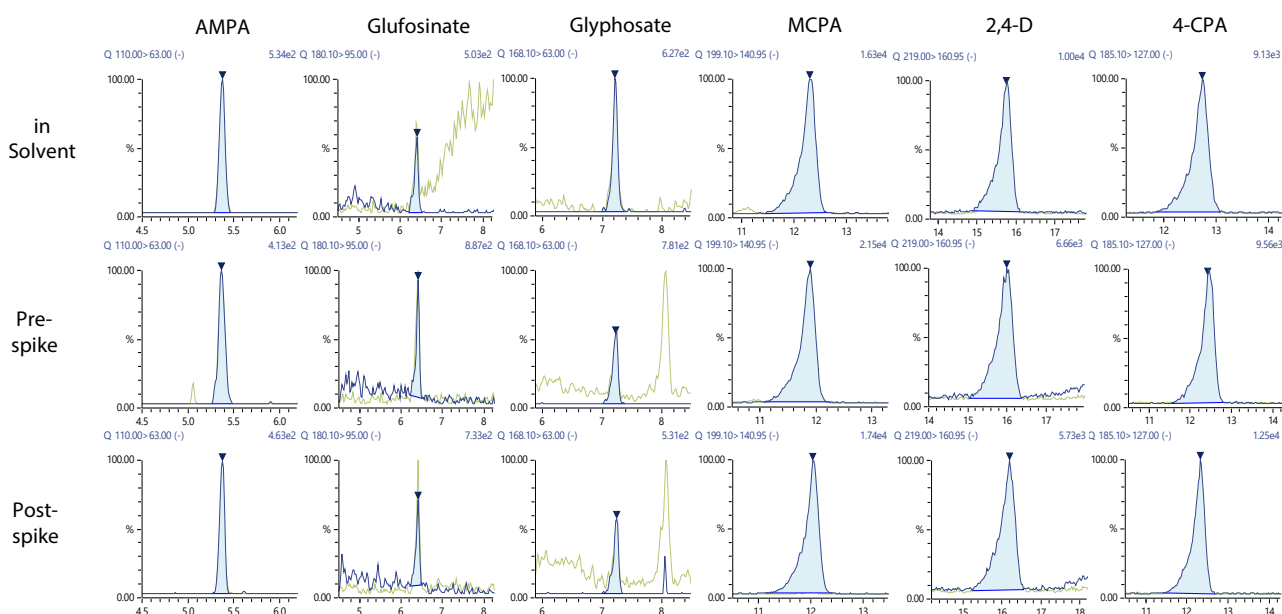


Figure 4 Representative MRM peaks of AMPA, glufosinate and glyphosate (2.5 ng/mL or 0.1 mg/kg) and MCPA, 2,4-D and 4-CPA (1.0 ng/mL or 0.04 mg/kg).

Sensitivity (LOD and LOQ in black tea matrix)

The evaluation of sensitivity of the method was based on spiked samples as displayed in Figure 4. The LOD and LOQ values of the herbicides in black tea matrix obtained are tabulated in Table 6, along with the MRLs stipulated by JP and EU. The estimated LOQs for AMPA (metabolite) and the five herbicides are at 0.06~2.62 ng/mL, which are converted to 0.0024~0.1048 mg/kg. The LOQs meet or are far below the MRLs set by both the EU and JP authorities.

Table 6 Sensitivity (LOD and LOQ) of five herbicides and AMPA in black tea matrix by LC-MS/MS method and the MRLs values

Compd.	LOD in tea		LOQ in tea		MRLs for tea	
	ng/mL	mg/kg	ng/mL	mg/kg	JP mg/kg	EU mg/kg
AMPA*	0.17	0.0068	0.48	0.019	N.A	N.A.
Glu	0.97	0.0388	2.62	0.105	0.3	0.1
Gly	0.34	0.0136	1.06	0.042	1.0	2.0
MCPA	0.02	0.0008	0.06	0.002	N.A	0.1
2,4-D	0.09	0.0036	0.28	0.011	N.A	0.1
4-CPA	0.04	0.0016	0.11	0.004	0.02	N.A.

Conclusion

An LC-MS/MS method was established for simultaneous quantitation of both highly-polar organophosphate-based herbicides (glyphosate, glufosinate, AMPA) and less-polar phenoxy-based herbicides (MCPA, 2,4-D, 4-CPA) in black tea on the LCMS-8060NX. The method performance in terms of precision, matrix effect and recovery was evaluated both intra-day and inter-day. The estimated LOQs for AMPA (metabolite) and the five herbicides are at 0.06~2.62 ng/mL, which are converted to 0.0024~0.1048 mg/kg. The LOQs meet or are far below the MRLs set by both the EU and JP authorities.

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