Development of an Analytical Method for the Determination of Glufosinate, Glyphosate and AMPA in Soybean Milk by CE-MS/MS

Application Note

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Abstract

This application note describes an analytical method for the determination of gluphosinate, glyphosate, and AMPA residues in soybean milk by capillary electrophoresis-tandem mass spectrometry (CE-MS/MS).

Introduction

Glyphosate and glufosinate are nonselective herbicides widely used for weed control in agricultural applications and urban landscape management. Aminomethylphosphonic acid (AMPA) is the principal degradation product of glyphosate. Their high efficacy and low cost, compared with other herbicides, have led to their wide useage in several crops. The Codex Alimentarius Commission [1] established the MRL as 20 mg/kg for glyphosate and 2 mg/kg for glufosinate in soybean. The National Health Surveillance Agency (ANVISA) in Brazil set the MRL as 20 mg/kg for glyphosate and 0.05 mg/kg for glufosinate in soybean [2].

Some methodologies for these compounds in food matrices have been proposed, among them by HPLC and LC/MS/MS [3]. However, the high polarity of these compounds and the absence of chromophore groups has made using these methods a complex challenge. This application note presents the development of an analytical method for the determination of glufosinate, glyphosate, and AMPA in soybean milk by CE-MS/MS.

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Experimental

CE conditions	
Instrument	Agilent 7100 CE System
Background electrolyte	25 mM ammonium bicarbonate, pH 7.5
Applied voltage	25 kV
Capillary	PVA coated 50 µm × 85 cm MS
Injection	12 seconds at 100 mBar
Temperature	25 °C
MS conditions	
Instrument	Agilent 6430 Triple Quadrupole LC/MS System
Ion mode	ESI, negative ionization
Sheath liquid	5 mM methanol-ammonium bicarbonate (50:50 v/v), pH 9.0
Flow rate	0.6 mL/min
Capillary voltage	4,000 V
Drying gas (N_2)	12 L/min
Drying gas temperature	150 °C
Nebulizer	10 psi

Table 1 shows a CE-MS/MS method developed for glufosinate, glyphosate, and AMPA analysis using two MRM transitions for each compound.

Table 1. MRM Conditions for the Analysis of Glufosinate, Glyphosate, and AMPA

Analyte	Transition	CE (V)	Dwell time (ms)	Fragmentor
Glufosinate	180>85	26	100	20
	180>63	52	100	20
Glyphosate	168>150	10	100	20
	168>124	15	100	20
AMPA	110>79	20	100	20
	110>63	20	100	20

Sample Extraction:

The sample was cleaned using Captiva ND Lipids filter cartridges (p/n A5300635). Extracts obtained with Captiva ND Lipids from whole soybean milk were transparent and could be introduced into the CE-MS/MS system without risk of contamination.

Results and Discussion

Figure 1 shows the MRM electropherograms obtained for mixture of standards of glufosinate, glyphosate, and AMPA at 10.0 ppm.

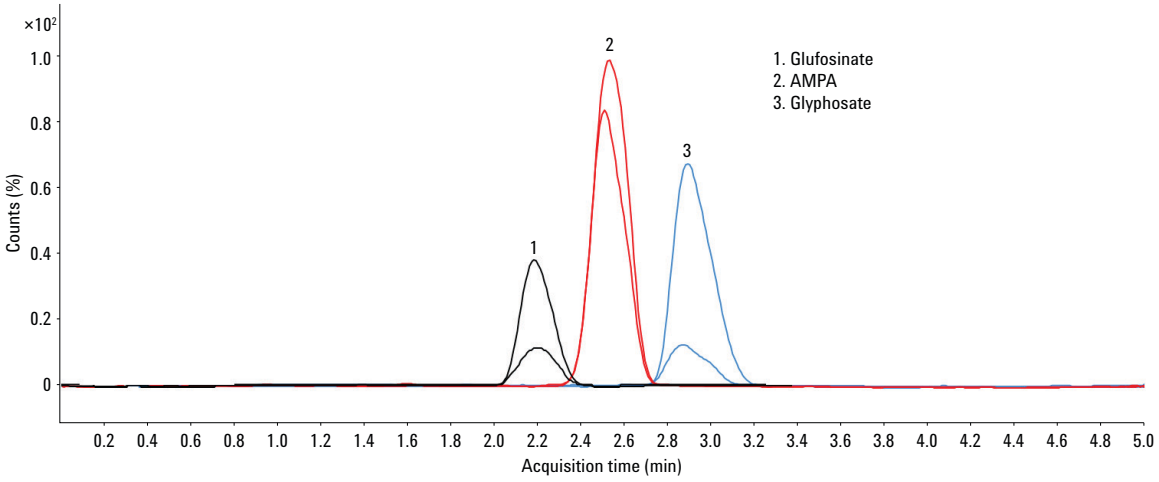

Figure 1. CE-MS/MS electropherogram of glufosinate, glyphosate, and AMPA standards at 10.0 ppm each.

The linearity of the analytical curve was studied using matrix-matched pesticide standard solutions in seven concentrations ranging from 1.0 to 100.0 ppm. For glufosinate (*m/z* 180 > 85), glyphosate (*m/z* 168 > 150), and AMPA (*m/z* 110 > 63), the coefficients of determination (R^2) calculated by linear regression were 0.997, 0.996, and 0,998, respectively. Figure 2 shows an example of the response for glufosinate, glyphosate, and AMPA in soybean milk.

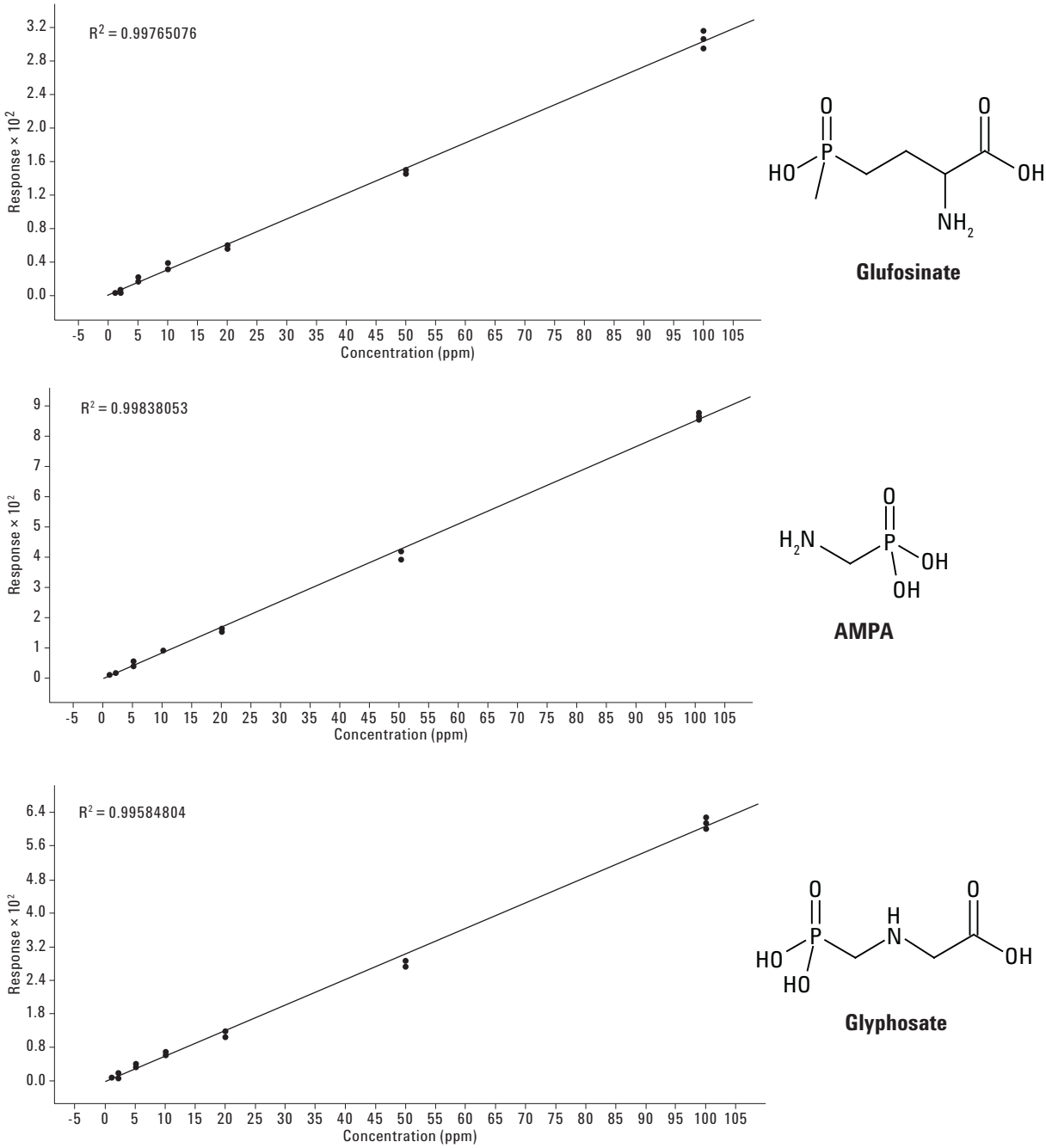

Figure 2. Calibration curves of glufosinate, AMPA, and glyphosate from 1.0 to 100.0 ppm.

Method accuracy and precision data were obtained for pesticides spiked at concentrations of 1.0, 10.0, and 20.0 ppm in soybean milk. Table 2 summarizes the linear range in solvent, the matrix extract, the method Limits of Detection (LOD), and the averages of the results.

Table 2. Linear Range in Solvent and in Matrix Extract, LOD, recovery (%), and RSD (%) for n = 5, Obtained by CE-MS/MS Analysis of Soybean Milk

Compound	Linear range (solvent) ppm	Linear range (matrix) ppm	Method LOD ppm	Recovery (%)	RSD (%)
Glufosinate	0.5–100	1–100	0.3	97.2	1.3
Glyphosate	0.5–100	1–100	0.3	89.9	2.7
AMPA	0.5–100	1–100	0.3	79.7	4.3

The Limits of Quantification (LOQ) were considered as being the lowest level of concentration spiked, with acceptable recovery and precision. Values of LOD were calculated as the LOQ value divided by 3.33, resulting in a concentration of 0.30 ppm for all compounds. The method was successfully applied for determination of glufosinate, glyphosate, and AMPA in soybean milk.

Conclusion

A CE-MS/MS method was developed and validated for the simultaneous quantitation of glufosinate, glyphosate, and AMPA in soybean milk matrix. The sensitivity and specificity of the method are suitable to meet the residue limits established in most countries for soybean derivative samples. The proposed methodology is simple, fast, and gives linear calibration curves and excellent precision data for replicate injections. This shows that the method is a good alternative for classical methods of analysis.

References

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2. National Health Surveillance Agency (ANVISA) at http://www.anvisa.gov.br
3. L. Grey; B. Nguyen and P. Yang, J. AOAC, 86 (6), pages 1770–1780, 2001.

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