Determination of Halosulfuron-Methyl in Bell Peppers Using QuEChERS and CE/MS/MS

Application Note

Food Testing and Agriculture

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Abstract

This application note describes a fast analytical method for determination of halosulfuron-methyl using QuEChERS and capillary electrophoresis tandem mass spectrometry (CE/MS/MS). The method was developed and validated for halosulfuron-methyl in green and yellow bell peppers. It showed good linearity ($R^2 > 0.999$) from 5.0 to 500 ppb for the analytical curves prepared in background electrolyte and extracts of bell peppers. The limits of quantification (LOQ) for green and yellow bell peppers were 1.3 ppb and 2.3 ppb, respectively. The limits of detection (LOD) were 0.4 ppb and 0.7 ppb, respectively. Precision and accuracy were verified through recovery of spiked samples at 10, 20, and 50 ppb, in five replicates. Recovery values ranged from 98 to 112% with RSD lower than 5%.

Introduction

Some pesticides retain poorly by reversed phase. This makes them challenging to measure accurately by LC or LC/MS due to their elution with much of the matrix in a QuEChERS extract. Certain reference laboratories around the world are tasked with developing alternatives to reversed phase LC for these compounds, which are often polar or fully ionic in nature [1].

Free zone capillary electrophoresis (CE) offers one such alternative because it uses this ionic nature to retain these compounds. The University of São Paulo has previously shown this advantage with respect to glyphosate, glufosinate, and AMPA analysis and how CE interfaces easily with a mass spectrometer (MS) using a commercially available interface [2]. Another problem compound that can be addressed equally successfully by CE-MS is halosulfuron-methyl (HSU), which is a sulfonylurea herbicide registered by the U.S. Environmental Protection Agency for postemergence broadleaf weed and sedge control in a wide range of crops [3]. HSU interferes with acetolactate synthase enzyme, resulting in a rapid cessation of cell division and plant growth in roots and shoots. HSU, like other sulfonylurea herbicides, is a weak acid and highly phytotoxic, and is subject to pH-dependent hydrolysis of the sulfonylurea linkage. Figure 1 shows the structure of halosulfuron-methyl.

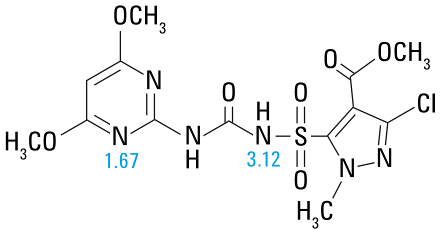

Figure 1. The structure halosulfuron-methyl with pKa values (www.chemicalize.org).

Regulatory agencies monitor residues of HSU in food using established maximum residue limits (MRLs). The tolerable MRL value for halosulfuron-methyl in bell pepper is set at 0.05 ppm in the United States and 0.01 ppm in the European Union [4]. In Brazil, there is a strong need to monitor this compound given its widespread use with tomatoes, bell peppers, and sugarcane, which are important export commodities. Therefore, laboratories are looking for a faster and more reliable method than has previously been available.

LC/MS is an important tool for multiresidue screening. The attraction of using CE, for problem compounds that do not fit the multiresidue suite, is that it can be wheeled up to the same mass spectrometer when such compounds are required for analysis. This mix-and-match strategy ensures the LC is kept clean for what it does best while the CE picks up the rest. CE has other advantages, such as being regarded as an environmentally friendly or greener technique, due to lower solvent expense and waste generation, and also for providing best-in-class peak shape/efficiency.

We used capillary electrophoresis coupled to electron spray ionization tandem mass spectrometry (CE-ESI/MS/MS) as an alternative to LC/MS/MS for the determination of HSU in various types of food matrices [5]. The method is fast, with low reagent consumption, and exhibits high sensitivity to HSU in bell peppers.

Experimental

CE conditions

Instrument:	Agilent 7100 CE
Background electrolyte:	20 mM NH_4HCO_3, pH 8.5, adjusted with NH_4OH
Applied voltage:	25 kV
Capillary:	Fused-silica, 50 µm id × 50 cm (p/n 190-0131)
Injection:	12 seconds at 100 mBar
Temperature:	25 °C

MS conditions

Instrument:	Agilent 6430 Triple Quadrupole LC/MS
Ion mode:	ESI, positive ionization
Sheath liquid:	5 mM NH_4HCO_3:MeOH (50:50 v/v).
Flow rate:	6.0 µL/min
Capillary voltage:	4,500 V
Drying gas flow (N_2):	3 L/min
Drying gas temperature:	280 °C
Nebulizer pressure:	7 psi

The ions monitored for halosulfuron-methyl are listed in Table 1. The most intense transition (435 to 182 *m/z*) was used as a quantifying ion, and the others were used as a qualifying ion for the confirmation of the analysis. The HSU molecule contains multiple pKa values, two of which are in a pH range most commonly used for separations conducted by CE.

The versatility of CE/MS allows molecules to explore this feature in a unique way: enabling CE to separate using one molecule ionized form and detection by MS with the molecule in another form. In this example, HSU was separated by CE in an anionic form and detected by MS in a cationic form.

Table 1. Migration time (m_t) and MRM conditions of halosulfuron-methyl.

Analyte	**Migration time (min)**	**Precursor (*m/z*)**	**Product (*m/z*)**	**Dwell time (ms)**	**Fragmentor energy (V)**	**Collision energy (V)**	**Cell accelerator (V)**
Halosulfuron-methyl	2.1	435	182	100	30	16	4
			139	100	30	56	4
			83	100	30	64	4

Sample preparation

Extractions of halosulfuron-methyl from yellow and green bell peppers were performed using an Agilent Bond Elut QuEChERS AOAC Extraction Kit (p/n 5982-5755). Then, dispersive cleanup was done with a Bond Elut QuEChERS Dispersive SPE Kit for Fruits and Vegetables (p/n 5982-5022CH). A 10 g aliquot of homogenized sample was placed into a 50 mL centrifuge tube followed by extraction using 10 mL acetonitrile. The partition step was performed by adding 6.0 g anhydrous magnesium sulphate ($MgSO_4$) and 1.5 g sodium acetate with consecutive shaking for 1 minute and centrifugation for 5 minutes at 4,000 rpm. Cleanup was accomplished by transferring 2 mL of the supernatant to a 15 mL polypropylene tube containing 50 mg PSA sorbent and 150 mg $MgSO_4$. The tube was vortexed for 1 minute and centrifuged for 5 minutes at 4,000 rpm. The extract was then filtered, and was ready for analysis.

Results and Discussion

Figure 2 shows the MRM electropherogram obtained from a sample of green bell pepper spiked with 50 ppb halosulfuron-methyl.

The linearity of the analytical curve was studied in background electrolyte and matrix-matched halosulfuron-methyl standard solutions in concentrations ranging from 5.0 to 500 ppb, shown in Figure 3. For all calibration curves, the correlation coefficients (R^2) calculated by linear regression presented values greater than 0.999 for background electrolyte, yellow, and green bell peppers.

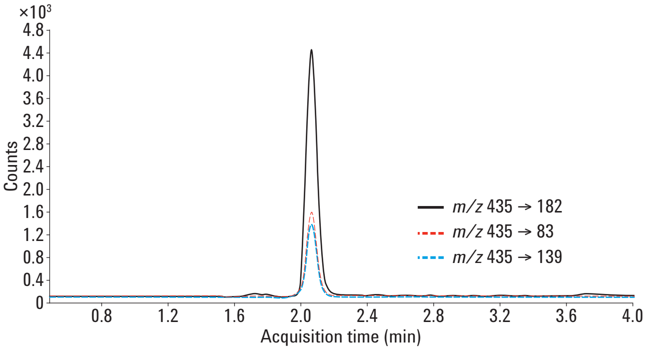

Figure 2. CE/MS/MS electropherogram from a sample of green bell pepper spiked with 50 ppb halosulfuron-methyl.

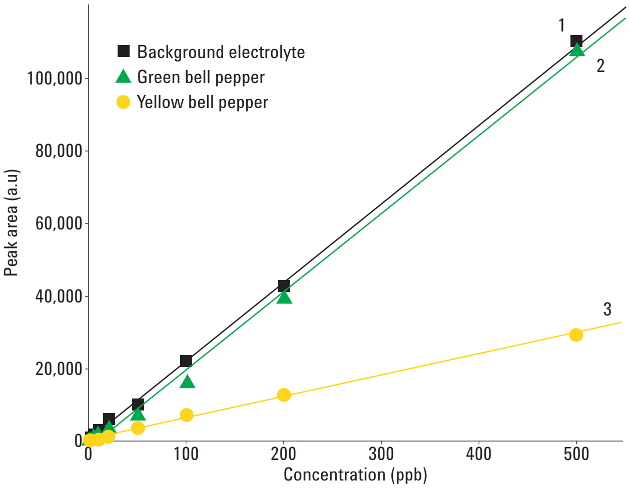

Figure 3. Calibration curves of halosulfuron-methyl in (1) background electrolyte (20 mM NH_4HCO_3), (2) green bell pepper, and (3) yellow bell pepper.

The limit of detection (LOD) and limit of quantification (LOQ) were determined, taking the LOD as three times the baseline noise, and the LOQ as 10 times the baseline noise. This was done in a time close to the migration time of the analyte. Precision and accuracy, expressed in terms of recovery from green and yellow bell pepper samples, were studied by analyzing spiked samples at three different concentrations in five replicates. These results, and the LOD and LOQ obtained, are summarized in Table 2.

Table 2. Percentage of recoveries for three fortification levels (n = 5), LOD, and LOQ of HSU in green and yellow bell peppers.

Matrix	Level (ppb)	Recovery (%)	RSD (%)	LOD (ppb)	LOQ (ppb)
Green bell pepper	10.0	105	1	0.4	1.3
	20.0	109	1		
	50.0	99	3		
Yellow bell pepper	10.0	112	3	0.7	2.3
	20.0	98	5		
	50.0	101	2		

Conclusions

We demonstrate that CE/MS/MS is a powerful tool for the quantitative and qualitative determination of halosulfuron-methyl in bell pepper. The proposed methodology is simple and fast (< 3 minutes), with linear calibration curves and excellent precision data for replicate injections. The method should be a good alternative to LC/MS methods for food analysis. The sensitivity and specificity of the method are suitable to inform international legislation on the maximum residue limits of halosulfuron-methyl in bell peppers, and can be extended to other food matrices [5].

References

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