Application Note

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Determination of Multiresidue Pesticides in Soybean by Combining QuEChERS and CE-MS/MS

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Abstract

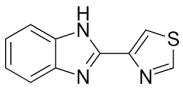
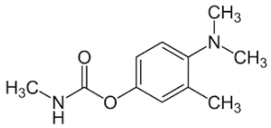
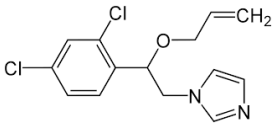
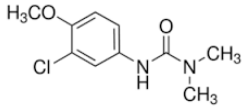
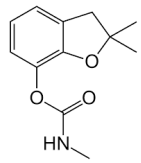
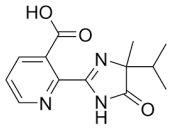
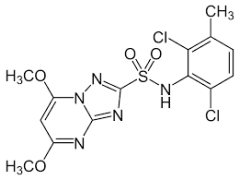
This Application Note describes a sensitive and reliable method for the determination of multiresidue pesticides in soybean. The residues of the seven different pesticides were extracted from soybean samples using the AOAC QuEChERS method without a cleanup step. The best separation of thiabendazole, aminocarb, imazalil, metoxuron, carbofuran, imazapyr, and metosulam was obtained in 100 mM formic acid (pH 2.4) as the background electrolyte (BGE), in less than eight minutes, using a polyvinyl alcohol coated capillary and detection by ESI-MS/MS. The coefficient of determination (R^2) for the matrix-matched calibration curves used for quantification in the range of 5 to 200 µg/L were greater than 0.996. Blank samples were fortified at three levels: 5, 40, and 200 µg/kg; recoveries ranged from 85 % to 120 %. Relative standard deviations were lower than 6.1 % in all cases. The limits of detection (LODs) based on the signal-to-noise ratio (S/N) for all compounds was lower than 0.10 µg/kg, several times lower than the established Maximum Residue Limit (MRL) for soybean.

Introduction

Soybean (*Glycine max*) is one of the most important agricultural crops in the world due to high protein (~38–45 %) and oil (~20 %) content, therefore the demand for soybean and food supplements from soybean has been increasing worldwide[1]. The use of pesticides in soybean crops is a current practice to control insects that could decrease field production. In addition, to minimize risks to human health, it is necessary to control the presence of these residues in soybean and other foods. Residues of pesticides in food are monitored by regulatory agencies, such as the Foreign Agricultural Service (FAS) from the U.S. Department of Agriculture using established MRLs, which is the highest level of a pesticide residue legally tolerated in or on food or feed when pesticides are used in accordance with Good Agricultural Practices[2]. Hence, to ensure that residual levels in soybean are under the safety limits of MRLs, a rapid and reliable control method is essential.

Some pesticides, such as amino group-possessing pesticides, are poorly retained by reversed phase. This makes them difficult to measure accurately by LC/MS due to their elution with much of the matrix in a QuEChERS extract. Free zone capillary electrophoresis (CE) offers an alternative separation mechanism, when these compounds are the focus of the required analysis, because it uses the ionic nature of these compounds to retain them. We have previously shown this advantage with respect to polar pesticides, and how CE is easily coupled to a mass spectrometer (MS) using a commercially available interface[3,4]. Another compound class that can be successfully addressed by CE/MS are amino group-possessing pesticides. The CE approach has many advantages, such as being regarded as an environmentally friendly or greener technique, due to lower solvent expense and waste generation, and for providing best-in-class peak shape/efficiency. We used CE coupled to electrospray ionization tandem mass spectrometry (CE-ESI/MS/MS) as an alternative to LC-MS/MS for the determination of thiabendazole, aminocarb, imazalil, metoxuron, carbofuran, imazapyr, and metosulam in soybean samples using QuEChERS sample preparation. Table 1 shows the molecular structure of pesticides analyzed in this work as well as the MRLs established for soybean[5].

Table 1. Molecular structure, formula, molecular weight and MRLs established for pesticides in soybean.

Compound	Structure	Formula	MW (g/mol)	MRL[a] (mg/kg)
Thiabendazole		$C_{10}H_7N_3S$	201.25	0.05*
Aminocarb		$C_{11}H_{16}N_2O_2$	208.26	ND
Imazalil		$C_{14}H_{14}Cl_2N_2O$	297.18	0.05
Metoxuron		$C_{10}H_{13}ClN_2O_2$	228.68	ND
Carbofuran		$C_{12}H_{15}NO_3$	221.26	0.02
Imazapyr		$C_{13}H_{15}N_3O_3$	261.28	5.0
Metosulam		$C_{14}H_{13}Cl_2N_5O_4S$	418.25	0.01

[a] According to Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels (MRLs) of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. OJ L 70, 16.3.2005, p. 1–16. Part A of Annex I to Reg. 396/2005.

ND, not defined.

All separations were performed at 25 °C using 100 mM formic acid, pH 2.4, as BGE. New polyvinyl alcohol capillaries were preconditioned by flushing with deionized water (10 minutes) and BGE (5 minutes). An additional flushing step with BGE for 90 seconds was included between the runs. Samples were introduced hydrodynamically in 12 seconds at 100 mbar. During the electrophoretic run at 28 kV, a pressure of –20 mbar was applied to the inlet vial to compensate for the ESI suction effect[6]. The mass spectrometer was operated in positive ionization mode, using multiple reaction monitoring (MRM) mode for two specific transitions. Table 2 lists the migration time (t_M), monitored ions, and other MS/MS acquisition parameters used for the identification and quantification of the pesticides in soybean.

Sample preparation

Samples of soybean were bought in local stores in São Paulo State, Brazil and were homogenized using a blender mixer. A 15 g aliquot of homogenized sample was placed into a 50-mL polypropylene tube and fortified with appropriate QC spiking solution (100 µL) when necessary. Afterwards, 15.0 mL of acetonitrile containing 1 % (v/v) acetic acid was added to each tube as well as an extraction salt packet from Agilent Buffered QuEChERS Extraction, AOAC Method 2007.01 (p/n 5982-5755CH), containing 6 g magnesium sulphate ($MgSO_4$) and 1.5 g sodium acetate (NaOAc). The sample tubes were capped tightly, hand-shaken vigorously for one minute, and centrifuged at 5,000 rpm for five minutes. After that, a 1 mL aliquot of the supernatant was diluted with BGE 1:1 (v/v), filtered through a 0.2 µm PVDF and PP membrane (Agilent Captiva filter cartridges p/n A5300002), and analyzed. Recovery tests were carried out by spiking the blank soybean samples after a homogenization step with a known amount of the pesticide standard mixture. Recovery studies were conducted at three different fortification levels: 5, 40, and 200 µg/kg, each in quintuplicate. The recovery samples prepared in this manner were compared against the matrix-matched calibration curves, and the results were reported as percent recovery.

Experimental

CE Conditions

Instrument	Agilent 7100 CE system
Background electrolyte	1,000 mM formic acid, pH 2.3
Applied voltage	28 kV
Capillary	Polyvinyl alcohol (PVA) capillary, 50 µm id with 62 cm total length (Agilent G1600-67219, cut down to 62 cm)
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	20 mM formic acid/methanol (50:50 v/v)
Flow rate	5.0 µL/min
Capillary voltage	4,000 V
Drying gas flow (N_2)	6 L/min
Drying gas temperature	250 °C
Nebulizer pressure	7 psi

Table 2. Migration time (t_M) and MS/MS acquisition parameters used for the identification and quantification of pesticides in soybean.

Compound	t_M (min)	Q1[a] (m/z)	Q3[b] (m/z)	CE[c] (V)	FE[d] (V)
Thiabendazole	4.03	202.0	175.0*/131.0	24/36	130
Aminocarb	4.13	209.1	152.2*/137.2	12/24	105
Imazalil	4.31	297.1	159.0*/41.0	20/36	115
Metoxuron	7.48	229.0	72.1*/42.1	12/16	95
Carbofuran	7.56	222.1	165.1*/123.1	20/30	80
Imazapyr	7.71	262.1	217.1*/69.1	20/40	120
Metosulam	7.80	418.2	175.0*/140.0	32/60	140

[a] Precursor ion (Q1); [b] Fragment ions (Q3); [c] Collision energy; [d] Fragmentor energy;
* Transition used in quantitation

Results and discussion

The BGE and sheath liquid composition, as well as applied potential and hydrodynamic injection, were optimized for separation efficiency and sensitivity. Figure 1 shows a representative electropherogram at optimum conditions of the pesticide standards at 150 µg/L each in BGE.

The linearity of the analytical curve was studied in background electrolyte using matrix-matched calibration solutions prepared by spiking soybean blank extracts to check matrix effects in concentrations ranging from 5.0 to 200 µg/kg. The coefficients of determination (R^2) calculated by linear regression presented values greater than 0.996, and the analytical cycle time was under eight minutes. The LODs and limits of quantification (LOQs) were determined considering the corresponding concentration to produce a signal three and 10 times, respectively, the baseline noise in a close region to the migration time of the analyte. Table 3 shows these results and the coefficients of determination.

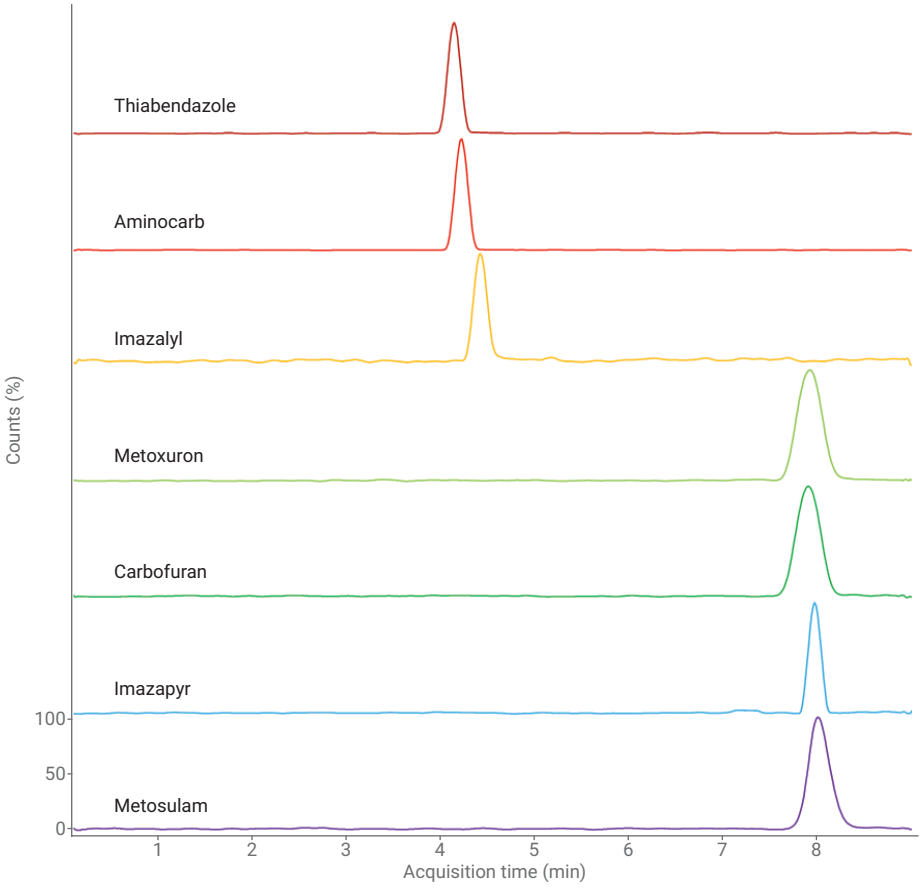


Figure 1. CE-MS/MS electropherogram of a mix of pesticides at 150 µg/L each in BGE.

Table 3. Analytical characteristics of the proposed CE-MS/MS method for pesticides in soybean.

Pesticide	R^2	LOD (µg/kg)	LOQ (µg/kg)
Thiabendazole	0.998	0.15	0.49
Aminocarb	0.999	0.12	0.38
Imazalil	0.997	0.19	0.64
Metoxuron	0.996	0.19	0.63
Carbofuran	0.999	0.10	0.33
Imazapyr	0.996	0.19	0.64
Metosulam	0.997	0.10	0.35

The recoveries and precision of the extraction method were determined as the average of five spiked blank matrix samples analyzed at three concentration levels of 5, 40, and 200 µg/kg. The method exhibited satisfactory performance, with recovery values ranging from 85.2 to 120.3 %, and standard deviation not greater than 6.1 %. Table 4 summarizes these results.

Conclusion

The results indicated that the QuEChERS sample preparation (without a cleanup step) when combined with CE-MS/MS is a powerful tool for the quantitative determination of multiresidue pesticides in food. The sensitivity and specificity of the method were sufficient to meet international MRLs for all pesticides analyzed in this study, with the potential to successfully apply this method to other food matrices. The proposed methodology is simple, fast, and presents linear calibration curves and excellent precision data for replicate injections, which reveals that the method could be a suitable alternative for amino group-possessing pesticides by LC or LC-MS/MS.

Table 4. Concentration (µg/kg) of pesticides in spiked blank soybean samples and recovery tests carried out in these samples (n = 5), as well as the RSD (%) values.

Compound	Added (µg/kg)	Recovery (%)	RSD (%) (n = 5)
Thiabendazole	5.0	99.7	1.6
	40.0	97.1	6.1
	200.0	101.7	0.9
Aminocarb	5.0	90.9	3.1
	40.0	92.7	5.8
	200.0	100.8	5.4
Imazalil	5.0	94.8	4.7
	40.0	85.2	3.0
	200.0	103.5	5.0
Metoxuron	5.0	93.4	4.6
	40.0	120.3	5.2
	200.0	93.5	1.6
Carbofuran	5.0	88.7	4.5
	40.0	108.4	2.5
	200.0	107.9	4.1
Metosulam	5.0	102.2	5.5
	40.0	93.1	3.4
	200.0	95.9	4.1
Imazapyr	5.0	101.5	5.2
	40.0	103.5	3.9
	200.0	105.0	2.6

References

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6. do Lago, C. L.; *et al.* A simple approach to compensate the suction caused by the electrospray ionization source in capillary electrophoresis-mass spectrometry systems. *Electrophoresis* **2014**, *35*, 2412–2416.

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