RESIDUES AND TRACE ELEMENTS

Liquid Chromatographic–Diode-Array Detection Multiresidue Determination of Rice Herbicides in Drinking and Paddy-Field Water

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A sensitive, rapid, and simple multiresidue method for the simultaneous determination of six postemergence herbicides currently used in rice cultivation—metsulfuron methyl, bensulfuron methyl, pyrazosulfuron ethyl, bentazone, bispyribac sodium, and cyhalofop butyl—in drinking and paddy-field water is presented. Water samples were extracted with solid-phase extraction cartridges. Final determination was made by LC with diode-array detection. The extraction efficiencies of C18 and HLB cartridges were compared. The average recovery obtained for these compounds for the lowest spiked level (0.1 g/L) varied from 70 to 122% for C18 and 75–119% for HLB, with RSDs of 11 and 8.3%, respectively. The method had good linearity, and the lower detection limit for the pesticides studied varied from 0.03 to 0.04 g/L. The proposed method was also tested in paddy-field water, with recovery studies giving good results with low RSDs at 1.0 g/L.

Herbicides are chemicals designed to kill plants, either selectively or nonselectively, and they comprise more than half of the pesticides employed in agriculture worldwide. Because herbicide molecules are toxic to a greater or lesser extent, they represent not only an environmental risk, but also a health hazard (1). They usually have high mobility in water and soil (2, 3) and can be found far away from the places where they were applied. This is of paramount importance in rice crop production. As a result of the irrigation systems currently used in rice cultivation, it is possible both for cross-contamination between agrochemicals to occur and for these chemicals to spread even to fields where no pesticides have been applied. The postemergence herbicides currently used in the "technological package" for rice crops have different chemical origins: sulfonylureas (metsulfuron methyl, bensulfuron methyl, pyrazosulfuron ethyl), bentazone, bispyribac sodium, and cyhalofop butyl. These herbicides are used at very low doses (<100 g/hectare) and are chemically unstable (in water, time for 50% decline of the initial pesticide concentration = 1–2 months; 4), which makes their determination in environmental water a real analytical challenge. LC–diode-array detection (LC-DAD) has been used for the determination of herbicide residues in surface and groundwaters (5, 6). Sulfonylureas (7, 8), bentazone (9–12), and bispyribac sodium have also been analyzed in water, but no method has been reported for the simultaneous determination of all the herbicides currently used in rice production. Therefore, it is desirable to develop a multiresidue method for the analysis of these herbicides that can either be expanded to include other herbicides and agrochemicals, or be applied to different matrixes of rice production systems, such as water, soil, and grain. In this communication, we present an accurate, reproducible, and sensitive LC-DAD method for the simultaneous determination of these herbicides, which account for 45% of the total of postemergence herbicides used in Uruguay. In addition, the levels of these herbicides need to be determined because of their toxicity. Bentazone was banned by the European Union, and the World Health Organization suggests an oral intake of less than 2 g/L per person/day. Even though the toxicity of these chemicals is not unduly alarming in mammals, it can be dangerous to other species (13).

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Experimental

Instruments and Apparatus

(**a**) *Liquid chromatograph*.—Hewlett Packard (HP; Avondale, PA) system, Series 1050, equipped with quaternary pump, autosampler, and diode-array detector.

(**b**) *LC conditions*.—Mobile phase flow rate: 1.2 mL/min. A gradient of mobile phase A [acetonitrile (ACN)] and mobile phase B (water, pH 3.0, adjusted with phosphoric acid 50%, v/v) was applied, with mobile phase A starting at 48% and increasing from this percentage at $t = 12$ min to 80% A at $t = 13$ min, and then maintained until $t = 20$ min. The injection volume was 20 L, and detection wavelengths were 220, 235, 240, and 247 nm. The analyses were performed at room temperature. The conditions stated above were optimized for each herbicide alone [(, retention time (Rt)] and a mix of 0.5 g/mL was used for adjusting the mobile phase gradient prior to method validation.

(**c**) *Guard column*.—Trident Integral LC Guard Column System with ULTRA C18, 5 m, 4.0 10 mm guard cartridge (Restek Corp., Bellefonte, PA).

(**d**) *Analytical column*.—ULTRA C18, 5.0 m, 100 Å, 4.6 150 mm (Restek Corp.).

Chemicals and Reagents

(**a**) *Deionized water*.—Prepared using a Milli-Q water purification system (Milford, MA).

(**b**) *Phosphoric acid (H_3PO_4)*.—Purity 85% (Fluka-Sigma Aldrich, Steinhein, Germany).

(**c**) *ACN*.—LC grade (Pharmco, Brookfield, CT).

(**d**) *Pesticide standards*.—Bensulfuron methyl, bentazone, bispyribac sodium, cyhalofop butyl, metsulfuron methyl, and pyrazosulfuron ethyl were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany).

(**e**) *Solid-phase extraction (SPE) cartridges*.—Octadecyl-bonded silica sorbent, C18 Bakerbond® SPE (40 m, 500 mg) were obtained from J.T. Baker (Deventer, The Netherlands) and a polymeric phase Oasis-HLB (30 m, 200 mg, 6 cc) from Waters (Milford, MA).

Preparation of Stock and Working Solutions

Stock solutions of the pesticide standards were prepared by accurately weighing 10.0 mg of each pesticide and dissolving it in 10.0 mL ACN, to obtain a 1000 mg/mL stock solution. From this solution, a working standard solution of 100.0 mg/L was prepared in ACN. This solution was then diluted as needed to prepare different standard solutions: 0.1, 0.25, 0.5, 1.0, 2.5, 5.0, and 10.0 mg/L in ACN.

Sample Preparation

Procedure.—Then 250 mL water was filtered through a 0.45 m filter and acidified to pH 3.0 with phosphoric acid. The filtrate was preconcentrated in an Oasis HLB cartridge previously conditioned with 3 mL methanol, pH 3.0, and 3 mL water, pH 3.0. The pH was adjusted with phosphoric acid. Elution was carried out with 2.0 mL of pH 3.0 methanol (2 1 mL) and 2.0 mL ACN (2 1 mL). The eluate was dried under nitrogen flow and redissolved in 0.2 mL ACN in order

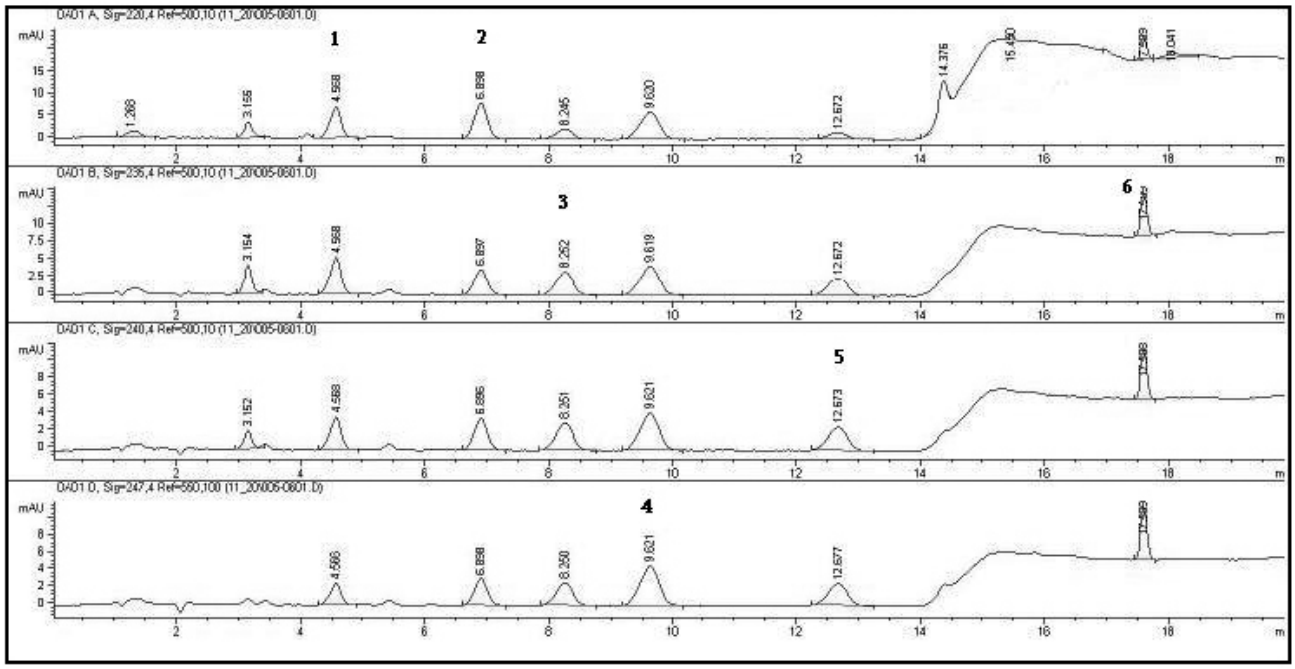


Figure 1. Chromatogram of a 0.5 g/L recovery level in drinking water decomposed in the four quantification wavelengths used; 1, metsulfuron methyl; 2, bentazone; 3, bensulfuron methyl; 4, bispyribac sodium; 5, pyrazosulfuron ethyl; 6, cyhalofop butyl. Each herbicide is signaled at its quantification wavelength.

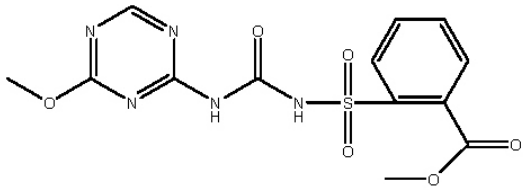
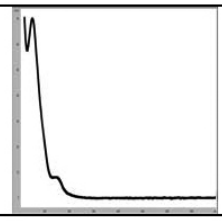
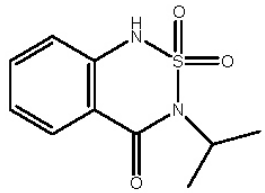
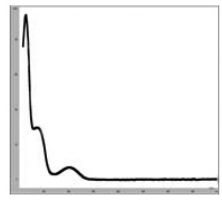
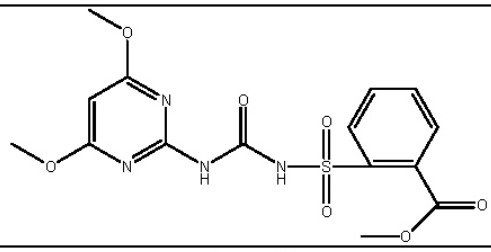
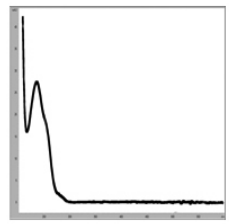
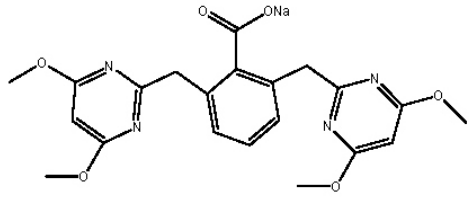
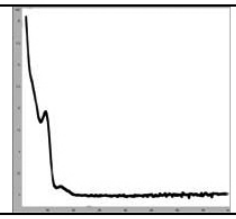
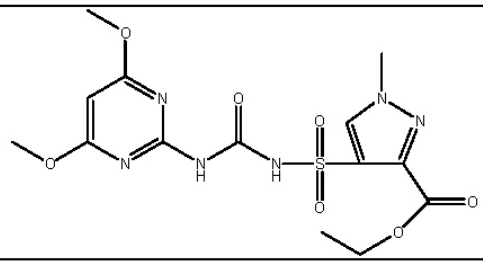
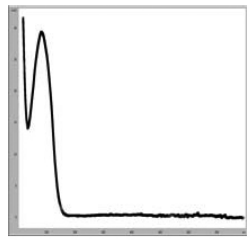
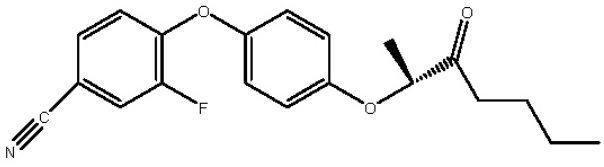
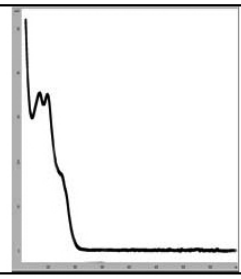
Compound	tR (min)	λmax (nm)	Chemical structure	UV Spectra
Metsulfuron methyl	**4.5**	**220**		
Bentazone	**6.8**	**220**		
Bensulfuron methyl	**8.2**	**235**		
Bispyribac sodium	**9.6**	**247**		
Pyrazosulfuron ethyl	**12.7**	**240**		
Cyhalofop butyl	**17.7**	**235**		

Figure 2. Identification criteria for the compounds analyzed.

Table 1. LOD and LOQ for all investigated compounds in the different matrixes studied

	Drinking water		Paddy-field water			
Pesticides investigated	LOD, g/L	LOQ, g/L	LOD, g/L	LOQ, g/L	Calibration equation	Correlation coefficient (R^2)
Metsulfuron methyl	0.03	0.1	1.7	5.0	88.718*x* – 1.7754	0.9997
Bentazone	0.03	0.1	0.3	1.0	112.46*x* + 0.3271	0.9999
Bensulfuron methyl	0.03	0.1	0.3	1.0	61.447*x* – 1.6489	0.9992
Bispyribac sodium	0.04	0.1	1.7	5.0	94.692*x* – 2.0941	0.9992
Pyrazosulfuron ethyl	0.03	0.1	0.3	1.0	67.328*x* – 8.2178	0.9989
Cyhalofop butyl	0.03	0.1	0.3	1.0	42.623*x* – 0.38	0.9998

to obtain a 1250-fold preconcentration. The extract was then filtered through a 0.45 m pore-size syringe filter and injected in the LC-DAD system.

The same procedure was used with C18 Bakerbond cartridges.

Results and Discussion

Samples showed good stability in the cartridges and could be stored frozen at –20 C for one week. In order to improve the elution of the compounds, it was necessary to treat samples with acid solutions (pH 3.0) and also to use water, pH 3.0, as the one of the mobile phases (2), particularly in order to keep the acidic groups in their protonated form.

Selection of Chromatographic Conditions

Crucial requirements for the development of an LC-DAD pesticide multiresidue measurement method are to eliminate possible interference caused by the matrix and to achieve good resolution between peaks, in order to identify analytes correctly by means of their individual UV spectra. The herbicides analyzed here show a wide range of hydrophobicity, from polar (metsulfuron methyl, bentazone) to relatively nonpolar herbicides like cyhalofop butyl. A combined isocratic–gradient–isocratic solvent system allowed the elution, identification, and quantification of each single herbicide with great accuracy in less than 20 min. A typical chromatogram is shown in Figure 1. During the first isocratic elution, the baseline is clean, without noise. It drifts when the relative amounts of ACN–H_2O change in a minute from 48 + 52 to 80 + 20. The last 5 min also give a straightforward baseline. Blank injections have been run, and no interfering species have been detected at the retention times of the herbicides.

The retention times and UV absorption maxima used for identification are listed in Figure 2.

Table 2. Recovery studies of the six pesticides extracted by SPE from drinking water

		0.1 g/L		0.3 g/L		0.5 g/L	
Pesticide	SPE cartridge	Recovery, %	RSD, %[a]	Recovery, %	RSD, %[a]	Recovery, %	RSD, %[a]
Metsulfuron methyl	C18	89.4	18.4	79.5	15.2	85.4	8.4
	HLB	119.6	8.2	110.3	11.0	75.4	5.6
Bentazone	C18	122.0	7.5	152.4	11.1	144.3	3.3
	HLB	119.1	8.4	119.6	8.4	108.3	8.0
Bensulfuron methyl	C18	93.8	7.7	73.9	12.9	71.4	10.7
	HLB	81.6	7.4	78.7	8.8	62.5	6.7
Bispyribac sodium	C18	70.1	14.4	68.6	7.9	53.0	19.0
	HLB	75.7	4.7	70.3	9.7	49.0	4.8
Pyrazosulfuron ethyl	C18	90.3	12.9	92.1	14.8	79.2	9.9
	HLB	81.7	10.3	92.1	8.4	75.7	6.0
Cyhalofop butyl	C18	121.5	5.7	47.4	17.3	43.8	6.0
	HLB	79.6	10.5	70.0	4.8	78.5	9.2

[a] Number of trials (*n*) = 5.

Method Validation

Linearity.—The DAD response for all herbicides was linear in the concentration range assayed (0.1–10 g/mL) with correlation coefficients >0.998 for all herbicides.

LOD and LOQ.—The LODs of the proposed method were determined at a signal-to-noise (S/N) ratio of 3 for the individual herbicides in water by LC-DAD, and were then experimentally verified; whereas, the LOQs were obtained as the lowest spiked level with acceptable recovery and relative standard deviation (RSD; 14). Table 1 shows the LODs and LOQs obtained for each herbicide, which are similar to values in water previously reported by other authors (15, 16).

Selection of water blanks.—Distilled water was used as the tap water blank and paddy-field water was collected in rice fields where these herbicides had not been used. Both of them were analyzed and the absence of herbicides was verified.

Recovery.—One aim of this study was to compare two SPE cartridges: the C18 Bakerbond and the HLB Oasis (Table 2). Water blanks were fortified at 0.1, 0.3, and 0.5 g/L levels and processed as described above. The recoveries obtained for the majority of the herbicides ranged from 43.8 to 152% for C18, and 70.0–119.6% for HLB. The RSD was 19% in the most unfavorable case for C18 and 11% for HLB.

For paddy-field water (Table 3), at the fortification levels of 1.0 and 5.0 g/L, recoveries for the majority of herbicides ranged from 68.2 to 109.0% for C18 and from 46.7 to 96.3% for HLB, with an average RSD of 5.2 and 8.3%, respectively. There was no significant difference between the results obtained with the two cartridges. Recoveries were better for the lowest levels; this was probably because of the cartridge's limited capacity to retain these herbicides. The calculated RSDs for both cartridges correspond to analyses done the same day (intraday precision).

Repeatability.—The repeatability study was carried out by analyzing a solution of the pesticides in a concentration of 0.1 mg/L, injected seven times, to evaluate the RSDs of the signal intensities, which were below 20% for every pesticide. This complies with the RSD accepted by the DG SANCO/2007/3131 of the European quality control guidelines (14).

Conclusions

The LC-DAD method showed good sensitivity and repeatability. The chromatographic system gave well-shaped and well-resolved peaks in a relatively short analysis time. The method appears to have the flexibility to include other herbicides among the range of substances tested. The lowest LODs and LOQs were obtained for tap water, as expected. For paddy-field water, sensitivity was reduced as a result of matrix effects. Paddy-field water is a complex solution of many biological, organic, and inorganic chemicals that can either interact with the compounds or interfere with the detection system, augmenting the background noise. Consequently, maintaining an S/N ratio of 3:1 requires a higher signal; therefore, only higher agrochemical concentrations can be determined. In light of the fact that LC-tandem mass spectrometry (LC-MS/MS) systems are currently too expensive for most environmental laboratories worldwide, the present LC-DAD method is a reliable tool for the determination of low-dose herbicides in tap and paddy-field water.

Table 3. Recovery studies of the six pesticides extracted by SPE from paddy-field water

		1.0 g/L		5.0 g/L	
Pesticide	SPE cartridge	Recovery	RSD, %[a]	Recovery	RSD, %[a]
Metsulfuron methyl	C18	102.0	5.0	68.2	27.4
	HLB	46.7	1.2	78.0	3.2
Bentazone	C18	100.2	0.7	87.0	0.3
	HLB	89.9	10.5	90.9	1.6
Bensulfuron methyl	C18	109.0	0.1	89.3	4.2
	HLB	81.5	12.2	96.3	1.4
Bispyribac sodium	C18	ND[b]	—	39.3	1.7
	HLB	ND	—	42.9	8.7
Pyrazosulfuron ethyl	C18	104.2	2.6	85.4	3.6
	HLB	80.9	21.2	91.0	2.3
Cyhalofop butyl	C18	89.6	7.0	87.5	4.7
	HLB	72.9	24.0	79.5	4.9

[a] Number of trials (n) = 5.
[b] ND = Not detected.

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