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Simultaneous determination of 15 phenylurea herbicides in rice and corn using HPLC with fluorescence detection combined with UV decomposition and post-column derivatization

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ABSTRACT

A method was developed for the simultaneous determination of 15 phenylurea herbicides (fenuron, tebuthiuron, metoxuron, monuron, chlortoluron, fluometuron, isoproturon, diuron, monolinuron, metobromuron, buturon, siduron, linuron, chlorbromuron, and neburon) in rice and corn samples by HPLC with fluorescence detection combined with UV decomposition and post-column derivatization. After extraction with acetonitrile and evaporation, the herbicides were redissolved in *n*-hexane and purified on a Florisil solid-phase extraction column. HPLC separation was carried out on a C18 column with water–acetonitrile gradient elution. UV decomposition was carried out under a 254-nm UV lamp. The method was evaluated in terms of the limits of detection and quantification. The linearity was satisfactory, with a correlation coefficient of >0.9980. Precision and recovery studies were evaluated at three concentration levels for each matrix. Good precision was obtained, with relative standard deviation in the range 1.5–9.6% for spiked rice samples and 0.9–9.9% for spiked corn samples. Recovery ($n = 6$) ranged between 75.3% and 104.3% for rice and between 75.0% and 105.1% for corn. The intra-day precision ($n = 5$) for the 15 herbicides in rice and corn samples spiked at an intermediate level was between 1.5% and 7.1%, and the inter-day precision over 10 days ($n = 10$) was between 6.4% and 15.6%.

1. Introduction

Many herbicides are used for agricultural purposes all over the world. Despite their benefits in increasing agricultural production, herbicides, including phenylurea compounds, can have a negative impact on the environment [1,2]. The wide use of herbicides leads to bioaccumulation through the food chain, so that these compounds can eventually pose a risk to animals and humans. The harmful effects of pesticides on humans include carcinogenesis, nerve disorders and immunological and respiratory diseases. For these reasons, there is a growing demand for fast and reliable pesticide monitoring in agriculture and food production.

Phenylurea herbicides are widely used because of their inhibition of photosynthesis that provides pre- and post-emergence control of many annual and perennial weeds in rice, corn, soybean, cotton and potato crops [1,3]. Owing to their high toxicity [4] and possible carcinogenicity, the determination of phenylurea herbicides at low concentrations in agricultural samples is of prime importance.

Methanol has been used as an extraction solvent for the analysis of phenylurea herbicides in plant samples [5]. Sample preparation was accomplished by liquid–liquid extraction. This technique is common, but is time-consuming and complicated and requires large amounts of organic solvents [6]. In the present study, the extraction method for phenylurea herbicides was significantly simplified to facilitate sample pretreatment and extraction, with consequent good recovery and selectivity [7].

Florisil is very effective in cleaning up sample extracts by adsorbing polar contaminants before determination by HPLC [8]. This technique offers a simple and selective means of purifying extracts before analysis. In addition, it offered the potential of reducing the use of organic solvents normally used in purifying extracts of complex matrices such as rice and corn samples.

Phenylurea herbicide concentrations have been determined using gas chromatography (GC) [1,9–11], HPLC for plant material [12,13], soil [14,15], and water samples [16,17] and electrochemical detection for groundwater samples [4]. However, determination of phenylurea herbicide concentrations by GC is difficult owing to thermal decomposition [3], so various derivatization techniques have been used to solve this problem [11]. HPLC allows direct analysis of phenylurea herbicides [16]. HPLC with UV detection

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has been used to assay water [16,17] and soil samples [14,15]. Because of a lack of selectivity [6,18], no official method for simultaneous direct determination of trace phenylurea herbicides in plant matrices is available, although some methods have been tested in comparative studies [12,13]. This led to the development of a post-column derivatization procedure that greatly improved the analytical selectivity and allowed HPLC assays of trace amounts of phenylurea herbicides in complicated matrices.

HPLC analysis of phenylurea herbicides using UV decomposition and post-column derivatization has been described in the literature, but the three methods could only detect 14 [5], 4 [6] and 10 [19] phenylurea herbicides. In addition, owing to the poor resolution reported in previous studies [5], complete separation for determination of 14 phenylurea herbicides was difficult to achieve. In the present study, we used a similar approach for the analysis of 15 phenylurea herbicides in rice and corn samples. The analytes were well separated and subsequent UV treatment produced primary amines that could be labeled with *o*-phthalaldehyde (OPA) to form fluorescent isoindole derivatives (Fig. 1).

Sensitive and efficient Florisil solid-phase extraction (SPE) was followed by HPLC with fluorescence detection combined with UV decomposition and post-column derivatization for the simultaneous determination of 15 phenylurea herbicides in rice and corn samples.

2. Experimental

2.1. Chemicals and solvents

HPLC-grade acetonitrile and methanol were obtained from Merck (Darmstadt, Germany). Acetone, *n*-hexane and toluene were obtained from Tedia (Fairfield, USA). Sodium chloride was obtained from Shanghai No. 4 Reagent & H.V. Chemical Company (Shanghai, China). OPA and Thiofluor (reagent grade) were purchased from Pickering Labs (USA). Sodium tetraborate and sodium hydroxide were purchased from Acros Organics (New Jersey, USA). HPLC-grade water was obtained from a Milli-Q water purification system (Millipore Corp., USA) and used to prepare all aqueous solutions.

Thiofluor stock solution was prepared by dissolving 2 g of Thiofluor in 10 mL of methanol. OPA stock solution was prepared by dissolving 0.1 g of OPA in 10 mL of methanol. Sodium tetraborate stock solution was prepared by dissolving 7.5 g of ACS-grade sodium tetraborate in 1000 mL of HPLC-grade water and adjusting the pH to 10.5.

The post-column derivatization solution was prepared by adding 5 mL each of the OPA and Thiofluor stock solutions to 500 mL of the sodium tetraborate stock solution. The resulting solution was used within 24 h.

Analytical standards of fenuron, tebuthiuron, metoxuron, monuron, chlortoluron, fluometuron, isoproturon, diuron, monolinuron, metobromuron, buturon, siduron, linuron, chlorbromuron, neburon were obtained from Dr. Ehrenstorfer GmbH, Germany.

Table 1
The time procedure of gradient elution

Time (min)	Acetonitrile (%)	Water (%)
0:0	30	70
15:0	50	50
31:0	90	10
31:5	30	70
40:0	30	70

Table 2
Retention times and resolution for 15 phenylurea herbicides

Herbicides	Retention time (min)	Resolution
Fenuron	9.857	13.40
Tebuthiuron	11.913	5.59
Metoxuron	13.497	3.54
Monuron	15.273	4.42
Chlortoluron	19.073	9.67
Fluometuron	19.822	1.90
Isoproturon	20.489	1.83
Diuron	21.159	1.45
Monolinuron	21.745	1.27
Metobromuron	23.022	3.57
Buturon	24.734	5.02
Siduron I	25.193	1.89
Siduron II	25.736	2.07
Linuron	26.560	2.13
Chlorbromuron	27.278	2.10
Neburon	30.642	9.87

The mobile-phase and post-column derivatization solution were filtered through 0.45-μm cellulose acetate (water) or PTFE (ACN) filters and degassed by sonication prior to use.

2.2. Sample preparation

Samples were finely ground using a model HR-2870 Philips mill (Philips, Zhuhai, China). An accurately weighed 10-g portion was homogenized at high speed for 2 min with 50 mL of acetonitrile in a 150-mL Erlenmeyer flask. The mixture was filtered through filter paper into a mixing cylinder and sealed with a stopper. NaCl (10 g) was added and the cylinder was manually shaken vigorously for 1 min. Then the phases were allowed to stand for 20 min. A 25-mL portion of the supernatant was removed by pipette and evaporated just to dryness using a vacuum evaporator (water bath at 50 °C). The residue was dissolved in 2 mL of *n*-hexane and then subjected to SPE clean-up.

Florisil SPE columns (6 mL, 1000 mg; Agilent Technologies, USA) were conditioned by sequential washing with 5 mL of acetone/*n*-hexane (40:60, v/v) and 5 mL of *n*-hexane. The extract was transferred to a washed column and eluted with 10 mL of acetone/*n*-hexane (40:60, v/v). The eluate was collected in a conical evaporating flask and evaporated to dryness at 50 °C in a vacuum rotary evaporator. The residue was dissolved in 5.0 mL of acetonitrile/water (50:50, v/v), vortex mixed and then filtered through a 0.45-μm Teflon filter. All samples were analyzed soon after preparation.

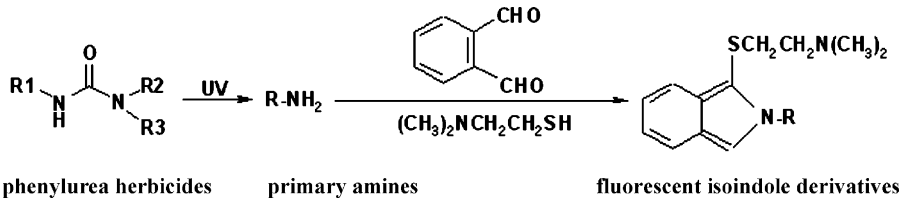

Fig. 1. Analytical scheme for the determination of 15 phenylurea herbicides.

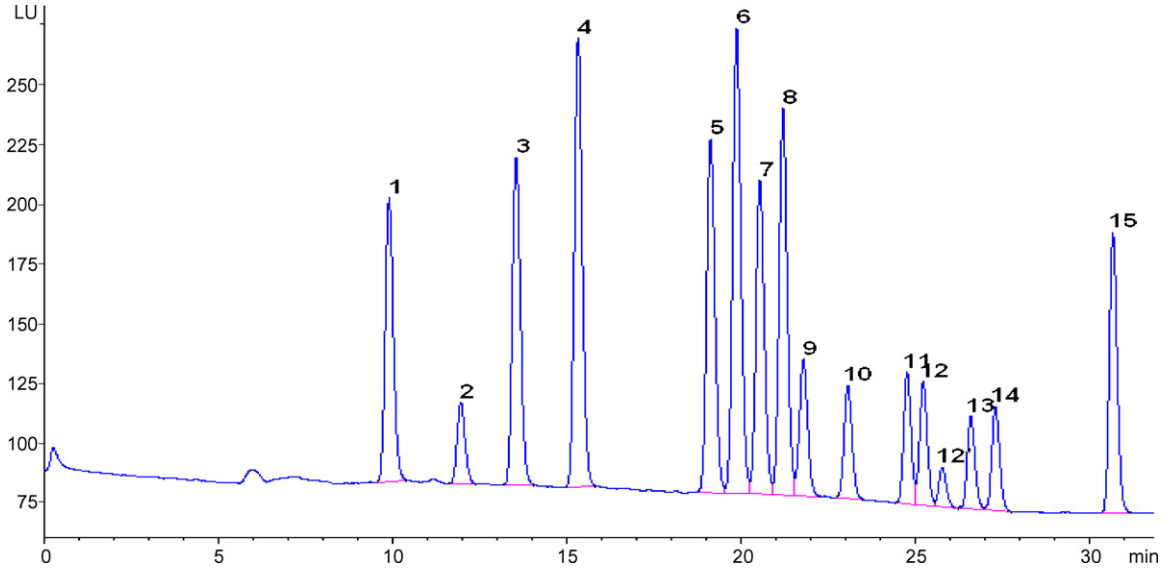

Fig. 2. Representative chromatogram of 15 phenylurea herbicides. Peak identification and concentrations: 1, fenuron (0.5 mg L^{-1}); 2, tebuthiuron (5.0 mg L^{-1}); 3, metoxuron (0.5 mg L^{-1}); 4, monuron (0.25 mg L^{-1}); 5, chlortoluron (0.5 mg L^{-1}); 6, fluometuron (0.5 mg L^{-1}); 7, isoproturon (0.25 mg L^{-1}); 8, diuron (0.5 mg L^{-1}); 9, monolinuron (2.5 mg L^{-1}); 10, metobromuron (2.5 mg L^{-1}); 11, buturon (0.5 mg L^{-1}); 12 + 12′, siduron (2.5 mg L^{-1}); 13, linuron (2.5 mg L^{-1}); 14, chlorbromuron (2.5 mg L^{-1}); and 15, neburon (0.5 mg L^{-1}).

Table 3
Limits of detection and limits of quantification

Herbicides	LOD[a] (mg kg^{-1})	LOQ[a] (mg kg^{-1})	LOD[b] (mg kg^{-1})	LOQ[b] (mg kg^{-1})
Fenuron	0.003	0.010	0.003	0.009
Tebuthiuron	0.030	0.090	0.032	0.096
Metoxuron	0.003	0.010	0.003	0.008
Monuron	0.001	0.004	0.002	0.005
Chlortoluron	0.003	0.010	0.003	0.009
Fluometuron	0.002	0.007	0.003	0.008
Isoproturon	0.002	0.005	0.001	0.004
Diuron	0.003	0.008	0.002	0.007
Monolinuron	0.016	0.050	0.015	0.049
Metobromuron	0.014	0.045	0.016	0.050
Buturon	0.003	0.010	0.002	0.008
Siduron	0.016	0.050	0.015	0.048
Linuron	0.012	0.043	0.014	0.046
Chlorbromuron	0.016	0.050	0.013	0.044
Neburon	0.003	0.010	0.003	0.009

[a] In rice matrix-match solutions.
[b] In corn matrix-match solutions.

Table 4
Analytical parameters of the regression equations in rice matrix-match solutions

Herbicides	Concentration range (mg L^{-1})	Regression equations	r^a	$S_\alpha{}^b$	$S_r{}^c$	$\alpha/S_\alpha{}^d$
Fenuron	0.01–1.0	$y = 205.0x + 3.8$	0.9986	1.45	4.50	2.62
Tebuthiuron	0.10–10.0	$y = 21.7x + 2.0$	0.9997	0.71	2.21	2.82
Metoxuron	0.01–1.0	$y = 259.0x + 2.7$	0.9992	1.38	4.30	1.96
Monuron	0.005–0.5	$y = 700.0x + 3.7$	0.9996	1.35	4.22	2.74
Chlortoluron	0.01–1.0	$y = 271.9x + 3.9$	0.9989	1.73	5.38	2.25
Fluometuron	0.01–1.0	$y = 349.3x + 4.1$	0.9995	1.44	4.48	2.85
Isoproturon	0.005–0.5	$y = 497.5x + 3.1$	0.9987	1.70	5.30	1.82
Diuron	0.01–1.0	$y = 347.7x + 4.6$	0.9994	1.55	4.82	2.97
Monolinuron	0.05–5.0	$y = 115.5x + 2.6$	0.9999	1.22	3.81	2.13
Metobromuron	0.05–5.0	$y = 105.0x + 2.8$	0.9999	0.94	2.94	2.98
Buturon	0.01–1.0	$y = 138.2x + 3.5$	0.9980	1.17	3.65	2.99
Siduron	0.05–5.0	$y = 69.0x + 2.7$	0.9997	1.16	3.63	2.33
Linuron	0.05–5.0	$y = 88.5x + 2.2$	0.9999	0.73	2.17	3.01
Chlorbromuron	0.05–5.0	$y = 45.1x + 1.7$	0.9998	1.33	2.15	1.28
Neburon	0.01–1.0	$y = 289.3x + 1.4$	0.9998	0.84	2.63	1.67

[a] Correlation coefficient.
[b] Standard deviation of intercept.
[c] Standard error of the estimate.
[d] Standard error of intercept.

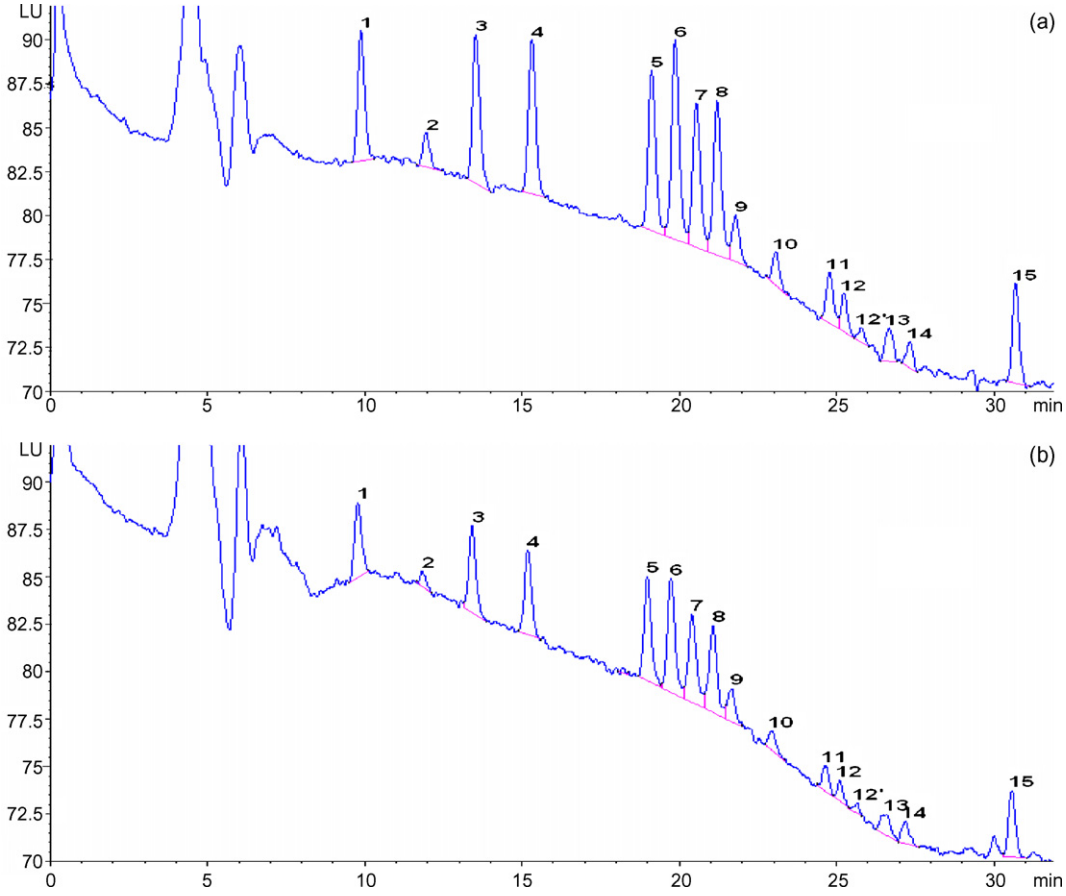

Fig. 3. Chromatogram obtained for (a) rice and (b) corn samples spiked near the detection limits.

2.3. Preparation of standards

Individual stock solutions of fenuron, tebuthiuron, metoxuron, monuron, chlortoluron, fluometuron, isoproturon, diuron, monolinuron, metobromuron, buturon, siduron, linuron, chlorbromuron, and neburon were prepared by accurately weighing appropriate amounts of the standard compounds and dissolving them in toluene. The solutions were protected against light and stored at −20 °C in a refrigerator.

Working solutions were prepared by evaporating aliquots of the stock solutions to dryness under a gentle N_2 stream and redissolving the residue in acetonitrile/water (50:50, v/v) to the required concentration before use.

Calibration standards of fenuron, metoxuron, chlortoluron, fluometuron, diuron, buturon and neburon (0.01, 0.1, 0.5 and 1.0 mg L^{-1}), monuron and isoproturon (0.005, 0.05, 0.25 and 0.5 mg L^{-1}), monolinuron, metobromuron, linuron, siduron and chlorbromuron (0.05, 0.5, 2.5 and 5.0 mg L^{-1}), and tebuthiuron (0.1,

Table 5
Analytical parameters of the regression equations in corn matrix-match solutions

Herbicides	Concentration range (mg L^{-1})	Regression equations	r^a	$S_\alpha{}^b$	$S_r{}^c$	$\alpha/S_\alpha{}^d$
Fenuron	0.01–1.0	$y = 203.7x + 2.2$	0.9986	1.07	3.59	2.06
Tebuthiuron	0.10–10.0	$y = 21.6x + 1.7$	0.9997	0.75	2.35	2.27
Metoxuron	0.01–1.0	$y = 260.2x + 2.4$	0.9990	1.57	4.90	1.53
Monuron	0.005–0.5	$y = 696.6x + 3.0$	0.9997	1.15	3.58	2.61
Chlortoluron	0.01–1.0	$y = 271.1x + 3.5$	0.9991	1.48	4.61	2.36
Fluometuron	0.01–1.0	$y = 348.1x + 3.6$	0.9995	1.52	4.73	2.37
Isoproturon	0.005–0.5	$y = 495.6x + 3.2$	0.9990	1.50	4.68	2.13
Diuron	0.01–1.0	$y = 351.0x + 3.9$	0.9995	1.49	4.64	2.62
Monolinuron	0.05–5.0	$y = 116.1x + 2.0$	0.9999	0.90	2.80	2.22
Metobromuron	0.05–5.0	$y = 105.6x + 2.1$	0.9998	1.22	3.84	1.72
Buturon	0.01–1.0	$y = 140.7x + 2.9$	0.9981	1.26	3.93	2.30
Siduron	0.05–5.0	$y = 68.4x + 2.8$	0.9996	1.28	3.99	2.19
Linuron	0.05–5.0	$y = 88.1x + 2.2$	0.9998	1.20	3.73	1.83
Chlorbromuron	0.05–5.0	$y = 45.9x + 3.3$	0.9998	1.30	4.05	2.54
Neburon	0.01–1.0	$y = 287.3x + 3.0$	0.9996	1.12	3.49	2.68

[a] Correlation coefficient.
[b] Standard deviation of intercept.
[c] Standard error of the estimate.
[d] Standard error of intercept.

Table 6
Mean recovery and precision of the 15 analytes assay ($n = 6$)

Herbicides	Spiked level (mg kg^{-1})	Rice		Corn	
		Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)
Fenuron	0.01	85.4	7.3	84.5	6.5
	0.10	87.9	3.2	95.0	4.0
	1.00	91.2	3.1	99.5	3.0
Tebuthiuron	0.1	85.0	7.2	85.7	8.8
	1.0	91.9	5.3	97.7	6.0
	10.0	94.1	3.9	99.0	3.1
Metoxuron	0.01	83.9	7.1	83.8	6.9
	0.10	88.4	5.0	95.6	4.7
	1.00	91.0	4.9	95.9	2.5
Monuron	0.005	81.9	6.8	75.0	7.4
	0.05	91.0	5.2	97.5	4.8
	0.50	96.4	2.6	100.7	4.0
Chlortoluron	0.01	87.2	8.9	79.9	6.9
	0.10	88.4	5.4	85.3	5.3
	1.00	92.4	3.7	99.7	2.3
Fluometuron	0.01	82.2	7.8	88.0	6.7
	0.10	90.4	6.7	98.2	6.1
	1.00	93.8	4.6	99.1	2.2
Isoproturon	0.005	80.8	8.3	79.8	6.7
	0.05	94.3	6.0	98.9	5.2
	0.50	99.9	4.4	100.2	3.0
Diuron	0.01	78.0	5.5	85.1	6.0
	0.10	80.3	4.8	98.8	3.4
	1.00	95.4	2.9	101.8	2.4
Monolinuron	0.05	79.5	9.6	82.7	7.8
	0.50	85.5	2.9	97.1	4.2
	5.00	100.1	1.7	103.4	3.2
Metobromuron	0.05	87.8	8.0	77.5	7.5
	0.50	99.3	5.9	85.6	6.3
	5.00	104.3	4.6	101.3	4.7
Buturon	0.01	75.3	7.5	81.5	9.9
	0.10	85.9	5.5	90.4	4.1
	1.00	101.9	1.5	96.6	2.8
Siduron	0.05	85.8	6.8	93.6	5.2
	0.50	99.7	4.6	97.8	4.4
	5.00	102.8	3.9	105.1	2.7
Linuron	0.05	80.4	8.4	86.0	9.1
	0.50	89.6	3.2	94.9	4.4
	5.00	103.6	4.4	104.7	0.9
Chlorbromuron	0.05	80.2	6.4	75.3	5.9
	0.50	90.2	3.5	82.1	3.8
	5.00	101.9	2.6	98.7	2.2
Neburon	0.01	79.3	3.9	81.4	2.9
	0.10	90.6	3.6	90.3	1.5
	1.00	95.8	1.9	103.9	1.4

1.0, 5.0 and 10.0 mg L^{-1}) were prepared by dilution with acetonitrile/water (50:50, v/v).

2.4. LC system and conditions

An Agilent 1100 HPLC system equipped with a quarternary pump (model G1311A), a degasser (model G1379A), an autosampler (model G1313A), a column oven (model C1316A) and a fluorescence detector (model G1321A) (Agilent Technologies, USA) was used for chromatography. System control and data collection were performed using Chemstation 9.03 (Agilent Technologies).

Chromatography was performed on a reverse-phase Zorbax Eclipse XDB-C18 column (5 μm particle size, 250 mm × 4.6 mm i.d., Agilent Technologies) equipped with a Zorbax Eclipse XDB-C18 (5 μm particle size, 12.5 mm × 4.6 mm i.d.; Agilent Technologies) guard column. Gradient elution with acetonitrile and water as the mobile-phase was carried out according to the procedure shown in Table 1 at a flow rate of 0.75 mL min^{-1} with the column temperature set at 25 °C.

Photochemical reactions were carried out in a post-column derivatization unit (PCX 520, Pickering Labs, USA) fitted with a knitted open PTFE tube reactor coil (3.0 m × 0.5 mm i.d.; Supelco, USA) and a 4-W 254-nm UV lamp (BHK Inc., USA). The analytes eluting from the HPLC column were photolyzed on-line to primary amines by exposure to UV light. The photodegradation products were reacted on-line with the post-column derivatization solution to form highly fluorescent compounds that were monitored using a fluorescence detector at excitation and emission wavelengths of 350 and 450 nm, respectively.

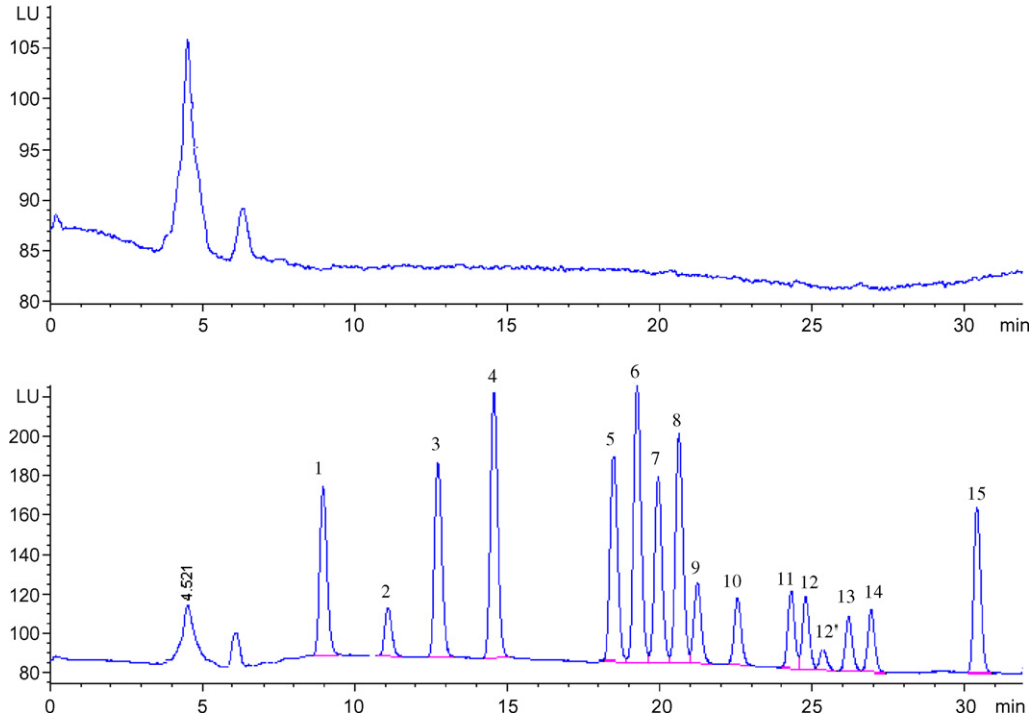

Fig. 4. Chromatograms of *Indica* rice spiked with standard solutions. Peak identification and concentrations: 1, fenuron (0.4 mg L^{-1}); 2, tebuthiuron (4.0 mg L^{-1}); 3, metoxuron (0.4 mg L^{-1}); 4, monuron (0.2 mg L^{-1}); 5, chlortoluron (0.4 mg L^{-1}); 6, fluometuron (0.4 mg L^{-1}); 7, isoproturon (0.2 mg L^{-1}); 8, diuron (0.4 mg L^{-1}); 9, monolinuron (2.0 mg L^{-1}); 10, metobromuron (2.0 mg L^{-1}); 11, buturon (0.4 mg L^{-1}); 12 + 12′, siduron (2.0 mg L^{-1}); 13, linuron (2.0 mg L^{-1}); 14, chlorbromuron (2.0 mg L^{-1}); and 15, neburon (0.4 mg L^{-1}).

2.5. Validation procedure

Validation was performed according to the National Standard of the People's Republic of China [20] and relevant literature reports [21,22]. The analytical figures of merit determined were the limit of detection (LOD), limit of quantification (LOQ), linearity, accuracy and precision. The LOD and LOQ were defined as three and ten times the signal/noise ratio, respectively. The calibration standards described in Section 2.3 were prepared in matrix-matched solutions and analyzed five times. Calibration curves were constructed by plotting the peak area against the concentration. Accuracy was assessed by measuring herbicide concentrations in rice and corn samples spiked at three different levels. The spiked samples were subjected to the sample preparation procedure described in Section 2.2 and analyzed using the experimental and instrumental conditions described in Section 2.4. Recovery was determined by comparing the amount of phenylurea herbicides added to the amount detected.

The intra- and inter-day precision was defined in terms of the relative standard deviation (R.S.D.). The intra-day precision was determined within 1 day by analyzing 5 replicate rice and corn samples spiked with the phenylurea herbicides at 1.0 mg kg^{-1}. The inter-day precision was determined on 10 separate days using 10 rice and corn samples spiked with the herbicides at 1.0 mg kg^{-1}.

3. Results and discussion

3.1. LC separation

Gradient elution was used to completely separate the 15 phenylurea herbicides. The chromatographic conditions were optimized to obtain appropriate separation. Fenuron, tebuthiuron, metoxuron and monuron were easily eluted at low acetonitrile concentrations, and chlortoluron, fluometuron, isoproturon, diuron, monolinuron, metobromuron, buturon, siduron, linuron, chlorbromuron and neburon were easily eluted at high acetonitrile concentrations. Thus, the percentage acetonitrile in the mobile-phase was linearly increased from 30% to 50% in the first 15 min to elute fenuron, tebuthiuron, metoxuron and monuron, and then linearly increased from 50% to 90% in the next 16 min to elute the remaining analytes. The optimum elution procedure for the 15 herbicides is presented in Table 1. The retention time and resolution for each analyte in this system are presented in Table 2 and a representative standard chromatogram is shown in Fig. 2. All 15 herbicides were well separated in a total run time of 31 min, with good peak resolution, sharpness and symmetry. The influence of the flow rate of the mobile-phase was investigated at 0.5, 0.75 and 1.0 mL min^{-1}. At 0.5 mL min^{-1} the sensitivity was highest, but this flow rate led to a longer analytical time and a lower pre-column vs. post-column pressure for 90% acetonitrile in the mobile-phase. A flow rate of 1.0 mL min^{-1} showed the lowest sensitivity. Thus, a flow rate of 0.75 mL min^{-1} was selected as the optimum.

3.2. Optimization of post-column derivatization

The flow rate of the derivatization reagent was varied at a constant mobile-phase flow rate of 0.75 mL min^{-1}. Experimental results revealed that the baseline and intensity of the fluorescence signal increased with the flow rate of the derivatization solution. Higher fluorescence intensity and post-column pressure were observed at 0.3 mL min^{-1} compared to 0.1 mL min^{-1}. However, the signal noise at 0.3 mL min^{-1} was greater and therefore 0.2 mL min^{-1} was chosen as the optimum flow rate for the derivatization solution for herbicide analysis in rice and corn samples.

3.3. Validation

3.3.1. LOD and LOQ

Under optimal conditions, the LOD was determined as the sample concentration producing a peak height three times that of the baseline noise. The LOQ was calculated as the sample concentration producing a peak height 10 times the signal/noise ratio [21,22]. The LOD and LOQ values calculated are presented in Table 3. There were no significant differences in LOD and LOQ values between rice and corn matrix solutions.

Fig. 3 shows a chromatogram of rice and corn samples spiked with the 15 herbicides at concentrations close to their LODs, for which the recovery was 62.8–75.5% and 65.4–78.3%, respectively.

3.3.2. Linearity

Calibration standards of the 15 analytes in matrix solutions were analyzed five times by the standard addition method. Linear relationships between the peak area and concentration were observed for the 15 herbicides. The lower limits of the linear range approximately corresponded to the LOQs calculated and the upper limits were 100 times the LOQs. The linear regression equations, standard deviation for the intercepts and the correlation coefficients (r) are reported in Tables 4 and 5. The linear regression equations were of the form $y = a + bx$, where y is the analyte peak area based on five parallel measurements and x is the concentration ($mg\,L^{-1}$). The correlation coefficient (r) was >0.9980 for all curves in both matrix solutions. Student's t-test was used to determine whether the experimental intercept (α) of each regression equation was significantly different from the theoretical zero value. The test was based on calculation of α and its standard deviation (S_α) and comparison with the t-distribution values. As shown in Tables 4 and 5, the t-values calculated did not exceed the criterion of $t_{0.05,n-2=3} = 3.182$, indicating that the intercept was not significantly different from zero for all regression lines.

3.3.3. Accuracy and precision

To establish the method accuracy, six replicate rice and corn samples were spiked and analyzed. Values for the mean recovery and R.S.D. are presented in Table 6. The mean recovery obtained for all analytes in spiked rice and corn samples was 75.3–104.3% and 75.0–105.1%, respectively, which is within the range expected for residue analysis of rice and corn samples.

The intra-day precision ($n = 5$) for 15 herbicides spiked at an intermediate level in rice and corn sample was between 1.5% and 7.1%, and the inter-day precision over 10 days ($n = 10$) was between 6.4% and 15.6%.

4. Analysis of phenylurea herbicides in real samples

To evaluate the effectiveness of the method, it was applied to the analysis of two rice samples (*Indica* and *Japonica*) and one corn sample. None of the pesticides were detected in these tests. To confirm the efficiency of the method, it was used to analyze a spiked *Indica* sample. Representative chromatograms are shown in Fig. 4.

5. Conclusion

The proposed extraction method is a rapid and simple technique that was suitable for rice and corn samples. The SPE clean-up step was a suitable purification process for determination of the 15 analytes. The method validation results demonstrate that the proposed methodology has good accuracy and precision for multi-residue analysis, with effective baseline separation and limits of detection and quantification that allow determination below the maximum residue limits for the pesticides in rice and corn samples.

Application of the method to the analysis of pesticide residues in rice and corn samples confirmed its efficiency.

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