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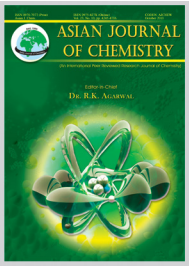

Determination of Herbicide Residues in Grain and Soil by Gel Permeation Chromatography

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Multi-residue analysis method of herbicides in wheat, rice and soil was studied. Semi-automatic gel permeation chromatography (GPC) equipment was used to clean-up the extracts and the residues were analyzed by TLC-Hill reaction and GC-NPD methods. The R_f values, the relative R_f values to atrazine (RRf) and the minimum detectable quantities (MDQs) in silica gel-ethyl acetate system were determined. The average recoveries were 84.4-98.7 % with CV of 3.0-14 % by TLC at 0.1 mg/kg fortification levels and, with the exception of isoproturon, 80-110 % with a CV of 0.67-13 % by GC at three different levels. The performance of these two methods was compared.

Key Words: Herbicide, TLC, Gas chromatography, Gel permeation chromatography.

INTRODUCTION

Urea and triazine herbicides are used extensively to protect a large number of crops, such as wheat, maize and vegetables against weeds. Monitoring of their residues in grain and soil is important to protect public health and control agricultural production. Most published methods for separation, detection and quantitation of urea and triazine herbicides in grain, soil and water are based on gas chromatography and liquid chromatography[1]. However, gas chromatography determination of urea herbicides without prior derivatization is difficult because of their low response and thermal instability.

We studied the determination of herbicide residues in rice, wheat and soil by TLC[2,3] and GC-NPD. The samples were extracted with ethyl acetate and cleaned up with GPC according to DFG method S19[4]. The TLC chromatographic separation and detection was performed on silica gel plates with a photosynthesis inhibition bio-assay (TLC-Hill reaction) detection method[5].

The minimum detectable quantities (MDQs), R_f and RR_f values and recoveries of residues of five selected urea and triazine herbicides (isoproturon, atrazine, metobromuron, metribuzin and prometryn) were determined in rice and wheat grains and soil samples.

EXPERIMENTAL

Isoproturon (purity, 97.0 %), atrazine (purity, 99.9 %), metobromuron (purity, 99.4 %), metribuzin (purity, 99.5 %), prometryn (purity, 98.1 %) and diuron (purity, 99.0 %) analytical standards were obtained from Institute for Control of Agrochemicals of Ministry of Agriculture. All other chemicals and reagents were analytical grade or better and obtained from the Beijing Chemical Company (Beijing, PRC).

Wheat leaves were used to obtain chloroplast for the Hill reaction according to the basic procedure[5].

Sample preparation: Weigh 20 g of ground wheat (or rice) or soil sample into the Erlenmeyer flask. Add 20 mL distilled water and stir vigorously. Add 15 g NaCl, 80 mL acetone and 50 mL cyclohexane and ethyl acetate (1:1). Homogenize the mixture in Warring Blender. Add 30 g of anhydrous sodium sulphate to the sample, shake for 0.5 h. Decant and filter 65 mL aliquot (equal to 10 g sample). Evaporate the extract to dryness. Dissolve the residue in 1 mL mixture of cyclohexane and ethyl acetate (1:1) for GPC cleanup. Fill the 200 × 10 mm glass column with 8 g swollen Bio-Rad SX-3 gel. Calibrate new GPC columns before use. Inject 1 mL sample extract into the column and elute the sample with cyclohexane and ethyl acetate (1:1). Collect the pesticide fraction, evaporate it to nearly dryness and take it up in 1-2 mL ethyl acetate.

Development of TLC plate: Use freshly activated 20 × 20 cm silica gel TLC plates. Prepare a spotting plan in advance and spot 10-20 µL pesticide solution in order of spots numbered from 1-11 starting from the left side of the plate. Apply the similar volume from the plant extracts as well as from the standard solutions on the plate. Fill the tank with acetyl acetate to obtain 1 cm immersion depth for the plate. Place filter paper into developing tanks for 0.5 h before eluting the plates to

obtain saturated vapour phase in the tank. Place the developing tank into water basin and keep the temperature within ± 2 °C between 20 and 30 °C for improving reproducibility of retention values. Elute plates up to 11-12 cm from the origin. Adjust the volume of the mobile phase after each elution. In order to obtain good separation of the spots for the determination of residues in rice, wheat and soil samples, develop the plate in the same direction with two different developing solvents: first with petroleum ether/ethyl acetate (8:2), then with petroleum ether/ethyl acetate/methanol (8:1:1).

TLC detection: Air dry the developed plate and spray it uniformly with the detecting reagent prepared according to the basic procedure[5]. Place the plate about 20 cm below a 60 W wolfram lamp (ordinary bulb) for a few minutes. The inhibition should occur resulting in bluish spots in greenish background within 10 min. The spots are usually visible after some minutes and reach optimum after *ca.* 5 min. The quantitation should be performed immediately after appearance of the spots as they disappear within a few minutes.

GC detection: An VARIAN-3800 GC-NPD and HP-5/MS fused silica capillary column (30 m × 0.25 mm × 0.25 μm) were used. Injection volume: 1 μL; injection temperature: 250 ºC; detector temperature: 300 ºC.The oven temperature was initially held at 80 ºC and increased at 10 ºC/min to 160 ºC and increased at 3 ºC/min to 180 ºC and then increased at 10 ºC/min to 220 ºC, which was held constant for 5 min. The nitrogen carrier gas flow was kept constant at 1.0 mL/min. The hydrogen and air gas flow were 4.0 and 175 mL/min, respectively.

RESULTS AND DISCUSSION

GC separation and detection: Retention time (Rt), linear range of standard curve and limit of detection (LOD) of 5 herbicides obtained with GC-NPD method are listed in Table-1. The results showed that they could be separated under the experimental conditions described. The regression coefficients of the linear calibration range were higher than 0.9946. The sensitivity of atrazine, metribuzin, prometryn and metobromuron was high and their LODs were in the range of 3×10^{-11} - 5×10^{-10} g but the sensitivity of isoproturon was low and its LOD was 6×10^{-9} g.

Elution pattern of herbicides by GPC: The amount of herbicides was determined by GC method described above. Results of the elution of herbicides by GPC (Table-2) showed that pesticides were not eluted at the first 9 mL, but nearly all of the co-extracted materials were eluted in this fraction. Consequently, when the sample extracts were eluted the first 9 mL of the eluent was discarded and the 10-25 mL was collected for further determination.

Recovery of herbicides from fortified samples by GC method: The test portions of rice, wheat and soil samples were fortified with the herbicides at three different levels, from 0.1 to 5 mg/kg in three replicates at each level. The samples were extracted and cleaned up by the method described. The results of three replicate analyses (Table-3) showed that the average recoveries of atrazine, prometryn, metribuzin and metobromuron in rice, wheat and soil were in the range of 80.2-110 % with CV 0.67-13 %. The recoveries of isoproturon were high because of the interference from the impurities. The limit of quantification (LOQ) of atrazine, prometryn and metribuzin in rice, wheat and soil samples were lower than 0.08 mg/kg. Limit of quantification of metobromuron in rice and wheat samples was 0.3 mg/kg. The determination of limit of quantification of isoproturon was not possible in these samples because of the interferences from the matrices.

TLC detectability and recovery of compounds: The RR_f values for atrazine, linearity, MDQs and LOQs of five herbicides obtained with Hill reaction after elution in silica gel ethyl

TABLE-1
RETENTION TIME, LINEARITY, LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION OF FIVE HERBICIDES BY GC-NPD METHOD

Pesticide	Rt (min)	Linear range (ng)	Linear equation	r	Limit of detection (g)	Limit of quantification (mg/kg)	
						Grain	Soil
Isoproturon	5.022	6-60	Y = 0.0083x + 0.0174	0.9946	6×10^{-9}	–*	–*
Atrazine	10.955	0.4-10	Y = 0.0567x + 0.0056	0.9966	4×10^{-11}	0.03	0.01
Metobromuron	12.778	4-40	Y = 0.0123x – 0.0004	0.9953	5×10^{-10}	0.30	0.06
Metribuzin	13.344	3-30	Y = 0.02x + 0.0101	0.9971	4×10^{-11}	0.05	0.08
Prometryn	13.991	1-13	Y = 0.053x + 0.0061	0.9997	3×10^{-11}	0.03	0.08

*Note: The recoveries of isoproturon were very high because of the interference from the matrices. Its LOQ could not be determined.

TABLE-2
ELUTION PATTERN OF FIVE HERBICIDES FROM GEL PERMEATION CHROMATOGRAPHIC TECHNIQUE

Fraction No.	Elution volume (mL)	Isoproturon (%)	Atrazine (%)	Meto-bromuron (%)	Metribuzin (%)	Prometryn (%)
1	1-7	–	–	–	–	–
2	8	–	–	–	–	–
3	9	–	–	–	–	–
4	10	19.35	12.54	–	–	–
5	11	30.47	31.10	17.57	18.36	21.40
6	12-13	32.69	35.05	31.58	31.84	30.39
7	14-15	17.49	21.30	29.73	25.34	22.40
8	16-17	–	–	21.12	24.46	10.99
9	18-19	–	–	–	–	8.75
10	20-21	–	–	–	–	6.25
11	22-23	–	–	–	–	–

TABLE-3
RECOVERY OF HERBICIDES IN FORTIFIED SAMPLES BY GC-NPD METHOD

Pesticide	Spike level (mg/kg)	Rice		Wheat		Soil	
		Average recovery (%)	CV (%)	Average recovery (%)	CV (%)	Average recovery (%)	CV (%)
Isoproturon	5.0	93.7	15.0	101.0	18.0	88.0	5.70
	1.0	126.0	4.0	130.0	7.7	140.0	4.30
	0.1	151.0	2.7	150.0	5.7	141.0	1.10
Atrazine	4.0	104.0	2.2	92.7	6.9	84.3	2.70
	1.5	87.9	2.2	103.0	2.5	99.6	0.67
	0.1	80.2	7.8	101.0	13.0	85.6	2.80
Metobromuron	5.0	87.0	3.4	91.8	6.9	84.0	3.80
	1.0	88.9	11.0	83.6	10.0	87.8	3.00
	0.1	ND	–	ND	–	89.2	4.60
Metribuzin	5.0	110.0	5.2	101.0	4.3	85.1	3.70
	1.0	93.3	7.8	107.0	1.4	98.2	5.50
	0.1	89.7	8.0	108.0	3.2	98.0	5.10
Prometryn	5.0	103.0	2.7	81.0	8.0	81.2	0.93
	1.5	86.0	2.8	88.1	5.6	101.0	1.40
	0.1	81.8	4.3	102.0	1.7	102.0	4.40

TABLE-4
RR_f, LINEAR RANGE, MDQ AND LOQ OF HERBICIDES BY TLC METHODS

Pesticide	RR_f	Linear range (ng)	Linear Equation	r	MDQ (ng)	LOQ (mg/kg)
Isoproturon	0.61	1-100	Y = 0.4123x + 0.2357	0.9890	1	0.03
Atrazine	1.00	1-40	Y = 0.2760x + 0.6422	0.9834	1	0.03
Metobromuron	0.94	20-150	Y = 0.8158x – 0.4124	0.9779	20	0.60
Metribuzin	1.013	2-100	Y = 0.6581x + 0.1759	0.9750	2	0.06
Prometryn	0.97	3-100	Y = 0.3368x + 0.3903	0.9918	3	0.10

TABLE-5
RECOVERIES OF TESTED PESTIDES FROM FORTIFIED SAMPLES BY TLC METHOD

Sample	Pesticide	Spike (mg/kg)	Recovery (%)			Average recovery (%)	CV (%)
			1	**2**	**3**		
Rice	Isoproturon	0.1	87	81	93	86.8	7.2
	Atrazine	0.1	105	103	80	95.9	14
	Metobromuron	0.1	ND	ND	ND	–	–
	Prometryn	0.1	96	91	88	91.6	4.9
	Metribuzin	0.1	102	97	96	98.5	3.0
Wheat	Isoproturon	0.1	95	91	98	94.7	4.0
	Atrazine	0.1	96	96	84	91.8	7.8
	Metobromuron	0.1	ND	ND	ND	–	–
	Prometryn	0.1	88	82	86	85.2	3.6
	Metribuzin	0.1	93	110	93	98.7	9.8
Soil	Isoproturon	0.1	94	92	83	89.8	6.4
	Atrazine	0.1	103	92	84	92.9	10.0
	Metobromuron	0.1	ND	ND	ND	–	–
	Prometryn	0.1	89	77	86	84.4	7.6
	Metribuzin	0.1	101	97	84	93.9	9.2

acetate system are listed in Table-4. The results showed that the RR_f values of metobromuron, metribuzin, prometryn and atrazine were very close and they could not be separated on one plate after developing in ethyl acetate. The RR_f values of isoproturon, atrazine, metribuzin and prometryn in silica-gel-ethyl acetate system were 0.61, 1.0, 1.013, 0.97. Therefore, first with petroleum ether/ethyl acetate (8:2), then with petroleum ether/ethyl acetate/methanol (8:1:1) elution was performed in the same direction. The R_f values of isoproturon, metribuzin, atrazine and prometryn after the second development were 0.088, 0.353, 0.449, 0.581, respectively, which made their simultaneous detection possible.

The detection sensitivity of isoproturon, atrazine, metribuzin and prometryn was high and their MDQs were in the range of 1-3 ng. The sensitivity of metobromuron by TLC-Hill reaction method was low (MDQ 20 ng).

The replicate recoveries of four herbicides in rice, wheat and soil samples at 0.1 mg/kg spiking level are listed in Table-5. Metobromuron was not detectable at 0.1 mg/kg fortification level.

The average recoveries of four herbicides in rice, wheat and soil samples were in the range of 84.4-98.7 % with CV 3.0-14 %. The limit of quantitation of isoproturon, atrazine, metribuzin and prometryn in rice, wheat and soil samples were

in the range of 0.03-0.1 mg/kg, the limit of quantitation in rice, wheat and soil samples of metobromuron was 0.6 mg/kg (Table-4).

Comparison of TLC and GC methods: Urea and triazine herbicides in wheat, rice and soil were detected with GC-NPD and TLC methods. The results (Tables 3 and 5) showed that the recoveries of herbicides from the samples spiked at 0.1 mg/kg level were nearly the same with GC and TLC except urea herbicides, isoproturon and metobromuron. For the latter compounds, the largely different sensitivity of detection did not allow their determination at 0.1 mg/kg spike level with GLC and TLC, respectively.

The results showed that the TLC-Hill method was sensitive and reproducible for most of the compounds tested. It could be used by monitoring laboratories to carry out the preliminary screening of samples in order to complement instrumental analyses.

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