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Note

Chronometric technique for the quantitative analysis of some photosynthesis-inhibiting herbicides

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It is important to develop rapid, sensitive and precise methods suitable for the determination of pesticide residues in agricultural crops. Kováč and Henselová[1] have recently developed an original selective biochemical detection of the residues of herbicides that inhibit the Hill reaction. The method was used for semi-quantitative analysis of prometryne, simazine and atrazine in soil samples and urea herbicides in surface and ground waters[2,3]. It was also employed successfully by Lawrence[4] for the detection of such herbicides in potatoes, carrots and maize. Suzuki and Casida[5] used the technique of Hill reaction inhibition to study the metabolism of phenylurea herbicides. Earlier methods[1–4] enable only semiquantitative evaluation of herbicides. Kováč *et al.*[6] developed a chronometric quantitative multiresidue analysis of Hill reaction inhibitors. The aim of the present work was to verify this chronometric method for the determination of herbicides used in potatoes and carrots.

EXPERIMENTAL

Materials and equipment

All reagents and solvents were reagent grade and the solvents were glass distilled. Stock solutions of herbicides were prepared in acetone in concentrations of 0.05–1.00 $\mu g\ cm^{-3}$ ($\mu g\ cm^{-3}$). A buffer solution of pH 8.6 was prepared by mixing 0.5 *M* borax and 0.1 *M* hydrochloric acid (7:3). The solution of 2,6-dichlorophenolindophenol sodium salt (DCPIP) was prepared at a concentration of 0.4 mg cm^{-3} in buffer solution (pH 8.6) by shaking for 30 min. at laboratory temperature. Three 40-W white neon tubes from Tesla, Holešovice, Czechoslovakia (Type 34 0007 IP 54) were used as a source of light. An HP 9825 A computer was used for the mathematical treatment of the experimental data.

Homogenate of chloroplasts was prepared as follows. A 30-g batch of leaves of 14-day-old bean plants *Phaseolus vulgaris* L ev. Harsgrus, previously washed with distilled water, was quickly dried by filter paper and pulped in a small homogenizer. Glycerine (3 cm^3) and distilled water ice (15 g) were then added and the mixture was homogenized for 30 sec. The homogenate was filtered by hand-press through four layers of dressing gauze and kept in the dark at 2 ± 1°C.

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Thin-layer chromatography

Silufol TLC plates (20 × 20 cm, available from Kavalier Glass Works, Votice, Czechoslovakia) were developed with acetone, dried at room temperature for 20 min and used without activation. Samples and standards were spotted with a capillary micropipette (*ca.* 5 μl), 2.5 cm apart.

Developing solvent systems

S_1, benzene–ethanol (9:1); S_2, chloroform–ethyl acetate (9:1); S_3, chloroform–ethyl acetate–acetone (17:2:1); S_4, light petroleum–acetone (7:3); S_5, chloroform–cyclohexane–ethyl acetate (15:2:3); S_6, chloroform–ethyl acetate–acetone (13:5:3).

Procedure

Blend 25 g of chopped sample (potato, carrot) with 100 cm^3 of acetone for 5 min in a homogenizer (Ultra Turrax). Filter the mixture through cotton wool and evaporate acetone from an aliquot equivalent to a 10-g sample at 40°C under vacuum. Shake the remaining water solution for 1 min with 10 cm^3 of chloroform, remove the organic layer and repeat the extraction with 10 cm^3 of chloroform. Combine organic extracts and dry using filtration through 2 g of anhydrous sodium sulphate. Wash the sodium sulphate with 10 cm^3 of chloroform. Evaporate the combined chloroform solutions (extracts) just to dryness under vacuum at 40°C and dissolve the residue in 0.5 cm^3 of acetone. Spot 5 μl of sample and 5 μl of solutions of the standards on TLC plate and develop in a suitable solvent system. After drying at room temperature spray with the spraying reagent. Mix the DCPIP solution and chloroplast homogenate (2:1) immediately before spraying; 6 cm^3 of the detection reagent (DCPIP plus chloroplast homogenate) are used for spraying one 20 × 20 cm plate. Then expose the plates to white neon light at a distance of 20 cm. The Hill reaction inhibitors show as dark blue-grey spots on a pale yellow-green background within 1–2 min after spraying, then disappear gradually, their lifetime being proportional to the amount of the herbicide in the spot. Use a stopwatch to measure the time from the beginning of the exposure to complete disappearance of the spots. The amount of herbicides in the sample is calculated from the standard curves of the respective herbicides.

RESULTS AND DISCUSSION

The R_F values in different solvent systems of the herbicides under study are listed in Table I, with the detection limits. Recoveries at different levels of herbicides in fortified plant samples are summarized in Table II. Examples of the calibration curves for some of the herbicides are shown in Fig. 1. The analyses of chlorbromuron and linuron in potatoes and carrots, respectively, were evaluated statistically (Table III).

The choice of herbicides was adjusted according to regular agrochemical practice. The method enables simultaneous determination of several herbicides in the same sample following their separation by TLC.

Accurate determination of the herbicides in plant samples depends on the separation of interfering compounds from the plant extracts by choice of a suitable TLC

TABLE I

SEPARATION OF THE HERBICIDE MIXTURE BY TLC ON SILICA GEL IN DIFFERENT SYSTEMS AND DETECTION LIMITS

Herbicide	$R_F \times 100$						*Detection limit* ($\mu g\ 10^{-4}$)
	S_1	S_2	S_3	S_4	S_5	S_6	
Chlorbromuron	38	58	66	64	59	57	2.5
Terbuthylazine	36	17	47	75	36	53	5.0
Simazine	28	17	24	61	13	33	7.5
Metribuzine	42	48	60	61	51	59	2.5
Terbutryne	41	5	48	75	6	47	7.5
Prometryne	42	44	49	74	38	51	7.5
Linuron	38	55	62	64	56	55	2.5
Methoxuron	13	7	16	23	11	21	7.5
Chloroxuron	21	21	25	32	17	30	7.5

developing system so that coloured coextractives do not overlap with the inhibition zones. The evaluation of the herbicides is based on measurement of how long the spot persists. The spotted volumes and the solvents used for samples and standards ought to be the same because the spot persistence depends on the geometry of the spot as well as on the amount of herbicide. A slight change of the zone geometry may occur if there is a high proportion of coextractives (residue levels of 0.01–0.05 mg kg^{-1}). This results in increased recovery values (over 100%). To avoid this, in the case of low residue levels the herbicide standards for the calibration curve were spot-

TABLE II

RECOVERY OF THE ANALYSIS OF THE HILL REACTION INHIBITING HERBICIDES IN POTATOES AND CARROTS

Herbicide	*Fortification level* ($mg\ kg^{-1}$)	*Recovery (%)*	
		Potatoes	*Carrots*
Chlorbromuron	0.05	95	93
	1.00	92	101
Prometryne	0.05	83	74
	1.00		96
Linuron	0.05	101	82
	1.00		96
Methoxuron	0.05		78
	1.00		91
Chloroxuron	0.05		71
	1.00		75
Terbuthylazine	0.05	100	
	1.00	81	
Metribuzine	0.05	98	
	1.00	83	
Terbutryne	0.05	93	
	1.00	80	
Simazine	0.05	94	

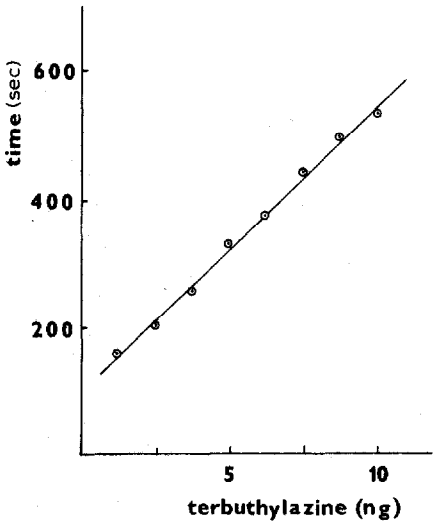

Fig. 1. Calibration graph showing the dependence of the inhibition zone lifetime on the amount of terbuthylazine in the zone.

TABLE III

STATISTICAL EVALUATION OF THE DETERMINATION OF LINURON AND CHLORBROMURON

Number of analyses: 10.

Parameter	*Linuron in carrots (mg kg^{-1})*	*Chlorbromuron in potatoes (mg kg^{-1})*
Sample mean	0.046	0.046
Sample standard deviation	$2.283 \cdot 10^{-3}$	$2.410 \cdot 10^{-3}$
Standard error of sample mean	$7.219 \cdot 10^{-4}$	$7.622 \cdot 10^{-4}$
Confidence interval	0.046 ± 0.013	0.046 ± 0.018

ted together with the cleaned-up extract corresponding to 10 g of control (untreated) material. As for linearity and slope of the calibration curve, the optimum of the zone persistence is the interval 0–600 sec. It is important to evaluate the sample and the calibration curve on the same plate. In this way identical detection conditions for the sample and calibration curve are secured (intensity of illumination, activity of chloroplasts, intensity of spraying). The neon tubes used are of the type regularly used for room illumination, *i.e.* no safety precautions are required.

The precision of the chronometric method was confirmed mathematically and statistically. The coefficients of correlation are 0.976–0.997 (linuron) and 0.969–0.999 (terbuthylazine). The standard deviations are less than 2% of the arithmetic mean. Results were counted from ten analyses.

The coefficients of correlation for methoxuron evaluated by two workers are 0.924–0.997 and 0.993–0.999.

CONCLUSIONS

The method presented has several advantages: it is rapid, simple and selective. The method enables the determination of more herbicides in one sample at the same time and it does not require any time-consuming and complicated clean-up. The procedure was verified by analysing a mixture of herbicides in fortified plant samples at different fortification levels (0.05; 1.00 mg kg^{-1}). The recoveries were 83–101% and 71–93% at the 0.05 ppm level in potatoes and carrots, respectively. The sensitivity and accuracy of the method are comparable with those of gas–liquid chromatography. The detection limit for the herbicides under study was 0.005 mg kg^{-1}.

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