

was attached (either by a piece of Scotch brand tape or a rubber band) to the drip-tip of the chromatographic column with the precaution that the drops of eluent from the column did not impinge directly on the thermistor (prematurely tripping the buzzer relay).

The normally open relay contacts of the detector were used, in our case, to energize a buzzer. This relay can be employed in a variety of other ways. For example, it may be used as an eluent control by tripping a solenoid-operated plunger valve affixed to the chromatographic column (replacing the stopcock), and thus shutting off the flow of eluent when the desired volume has been collected. In another manner, this device can be positioned to the top of a chromatographic column. It will then warn the operator when the liquid level approaches the stage where the column would go "dry".

With such an easily constructed, inexpensive, and portable instrument, chromatographic operations may become as sophisticated as the operator desires.

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### **Combination of thin-layer chromatography and the ring-oven method for the semi-quantitative estimation of some herbicides**

The importance of the herbicides for the pre- and post-emergence control of weeds has increased in recent years. At present, many of these compounds are officially approved for such use. With the wider application of pesticides in general, the problem of residue determination grows.

The analytical problem presented by pesticide residues in food and other materials has been briefly defined<sup>1-4</sup> and is essentially unchanged. In order to study the relative retention of pesticides (herbicides) in samples of this nature, various methods are required for their microdetermination. MAJOR<sup>5</sup> proposed a paper chromatographic method. ABBOTT *et al.*<sup>6</sup> used a paper chromatographic determination using reflectance densitometry for quantitative measurement, and also thin-layer chromatography<sup>7,8</sup>. The application of the ring-oven technique to herbicide analysis has also been described<sup>9</sup>. Advances in gas chromatographic methods have lately included pesticide residue analysis though the application of gas chromatography to the analysis of these residues was initially confined to chlorinated hydrocarbons and related compounds; however, herbicides are now included<sup>10-12</sup>.

The combination of thin-layer chromatography and the ring-oven technique on a semi-quantitative basis was thought to offer a simple, rapid and inexpensive

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procedure for detection and determination of minute amounts of some herbicides. The method described in this paper has been found satisfactory in these respects.

### Experimental

Five herbicides were studied; they represent different herbicide classes (except two bipyridylum salts) and are listed in Table I.

TABLE I

COMMON AND CHEMICAL NAMES OF THE HERBICIDES USED

Common name	Chemical name
Amiben	3-Amino-2,5-dichlorobenzoic acid
CDAA (Radox)	2-Chloro-N,N-diallylacetamide
CIPC	Isopropyl-N-(3-chlorophenyl)carbamate
Diquat	6,7-Dihydropyrido[1,2-a:2',1'-c] = pyrazidinium salt
Paraquat	1,1'-Dimethyl-4,4'-bipyridinium salt

*Thin-layer chromatographic separation of herbicides.* Thin-layer chromatography has been widely used as method for separation and detection in the study of herbicide residues.

Preliminary experiments were aimed at discovering a suitable procedure for obtaining reasonable resolution of the herbicides (*i.e.* separatory-systems), and more sensitive general spraying-reagents for the detection of these compounds on chromatograms.

Chromatoplates of Silica Gel G were prepared (layer thickness: 250  $\mu$ ) and dried before use in an oven set at 105°. A micro-pipette was used to transfer solutions of the herbicides on to the chromatoplates (Amiben: 0.05%; CDAA: 0.1%; CIPC: 0.1%; Diquat: 0.05%; Paraquat: 0.1%) which were developed by an ascending technique for 45 min or longer (depending on the solvent systems). The plates were allowed to dry and sprayed with suitable spray-reagents.

TABLE II

 $R_F$  VALUES OF SOME HERBICIDES IN DIFFERENT SOLVENT SYSTEMS

Compound	$R_F$ values ( $\times 100$ )											
	1*	2	3	4	5	6	7	8	9	10	11	12
Amiben	0	0	0	0	0	0	0	64	52	—	76	65
CDAA	49	72	74	23	43	69	3	66	86	72	77	72
CIPC	75	81	78	45	58	71	32	82	89	—	—	—
Diquat	0	0	0	0	0	0	0	0	0	25	22	10
Paraquat	0	0	0	0	0	0	0	0	0	26	19	11

\* Solvent systems: 1 = chloroform; 2 = chloroform-acetone (9:1, v/v); 3 = chloroform-acetone (8:2, v/v); 4 = hexane-acetone (9:1, v/v); 5 = hexane-acetone (8:2, v/v); 6 = dioxan; 7 = benzene; 8 = benzene-acetic acid glac. (5:1, v/v); 9 = isopropanol-25%  $\text{NH}_3$ -water (8:1:1, v/v); 10 = isopropanol-HCl conc.-water (5:1:1, v/v); 11 = ethanol-HCl conc.-water (6:1:3, v/v); 12 = methanol-HCl conc.-water (6:1:3, v/v).

Table II shows the effect of different developing systems on the thin-layer chromatographic separation of the herbicides.

A combination of  $\text{NaNO}_2$  and N-1-naphthyl-ethylenediaminedihydrochloride has been successfully used for detecting Amiben spots<sup>13</sup>. Aqueous 0.5%  $\text{KMnO}_4$  was used for CDAA and also for Amiben visualisation, a combination of 0.5%  $\text{AgNO}_3$  (acidified with 2 ml of  $\text{HNO}_3$ ) and 0.5%  $\text{KMnO}_4$  was used for CIPC and methanolic  $\text{NaOH}$  (followed by heating in a stream of warm air) for Diquat and Paraquat visualisation. Table III lists the observed practical sensitivities obtained on developed chromatoplates for each of the compounds studied.

*Semi-quantitative determination of separated herbicides using the ring-oven technique.* The herbicides were determined individually with the Weisz ring-oven by spotting aliquots of the stock solutions of the appropriate herbicide on to the 5.5 cm diameter filter paper (Whatman No. 1) by means of a 10  $\mu\text{l}$  micro-pipette. The substances were then washed into ring-zones as follows: Amiben with 0.5 ml of water, CDAA and CIPC with 0.5 ml of acetone, Diquat and Paraquat with 0.5 ml of 3 N HCl. The paper was dried in a stream of warm air (except when acetone is used).

The spray-reagents mentioned above were also used successfully for the detection of the ring-zones; the practical sensitivities being listed in Table III.

The method used for the semi-quantitative determination has already been reported<sup>9,14</sup>. The amount of herbicide ( $C_a$ ) present can be expressed as:

$$C_a = C_s \frac{V_s}{V_a}$$

where  $C_s$  is the concentration of the standard solution,  $V_a$  the volume of sample solution and  $V_s$  the volume of standard solution used in the matching ring.

*Combination of the thin-layer chromatography and ring-oven techniques.* From a survey of the literature it is apparent that thin-layer chromatography has rapidly developed into a precise technique of great value to the pesticide residue analyst, but the need to find, identify and determine a very small amount of an organic compound in the presence of large quantities of natural materials can be extremely difficult and sometimes quantitative evaluation of chromatograms, based upon the measurement of spot area, will be impossible. The final determination step and conclusions can easily be carried out in such cases by means of a combination of thin-layer chromatography and the ring-oven technique.

TABLE III

THE SENSITIVITY OF HERBICIDES TO SPRAY-REAGENTS

Compound	Sensitivity ( $\mu\text{g}$ )	
	TLC*	ROT**
Amiben	0.25	0.10
CDAA	0.75	0.75
CIPC	0.50	1.00
Diquat	0.50	0.50
Paraquat	1.00	1.00

\* Thin-layer chromatography.

\*\* Ring-oven technique.

After thin-layer chromatography of the herbicide solutions, the separated herbicides were scraped from the chromatoplates quantitatively into a funnel (porosity G 4), mounted above the oven. Each batch of scrapings was washed with 1 ml of the appropriate solvent on to the 5.5 cm diameter filter paper. The substances were then washed into ring-zones and detected as explained above. Stable red rings for Amiben (over 30 days) were obtained on a white background; stable brown rings for Diquat and bluish-green rings for Paraquat were obtained on a white background. Unstable yellow rings (20 min) on a pinkish background were obtained for CDAA and CIPC.

#### Conclusions

The intrinsic inexpensiveness of the apparatus and the simplicity, speed and versatility of the technique described make it auspicious for the diagnostic, semi-quantitative and preparative studies of herbicides and their residues in contaminated samples.

Minute amounts of herbicides could be isolated and detected on the thin-layer chromatoplates, and quantitatively transferred to the ring-oven for determination.

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