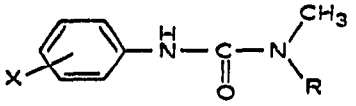
Specific detection of urea herbicide residues by separation of their amines on cellulose thin-layer plates

The group of phenylurea herbicides is constantly enlarging and at present includes the compounds listed in Table I. Residues of these substances in crop and soil samples are normally determined by the method developed by E. I. Du Pont de Nemours & Co., which consists of alkaline hydrolysis to the corresponding aromatic amines, steam distillation/extraction, and colorimetric measurement[1–3]. The procedure has been adapted to distinguish between aniline, 4-chloroaniline, and 3,4-dichloroaniline[4]. However, when analysis of samples of unknown spraying history is required, this method does not permit separation and specific detection of the various possible anilines derived from the herbicides listed in Table I.

TABLE I

CHEMICAL STRUCTURE OF PHENYLUREA HERBICIDES



Common name or code number	*X*	*R*
Fenuron	—	CH_3
Monuron	4-Cl	CH_3
Monolinuron	4-Cl	OCH_3
Buturon	4-Cl	$CH(CH_3)C \equiv CH$
Metobromuron	4-Br	OCH_3
Diuron	3,4-Cl,Cl	CH_3
Linuron	3,4-Cl,Cl	OCH_3
Neburon	3,4-Cl,Cl	C_4H_9
C-6313	3-Cl,4-Br	OCH_3
Fluometuron	3-CF_3	CH_3
Chloroxuron	4-phenoxy-(4′-Cl)	CH_3

Paper and thin-layer chromatographic methods for the separation and detection of structurally unchanged urea herbicides have been reported by MAJOR[5], HENKEL[6], and KATZ[7]. These procedures have not been found entirely satisfactory for residue analyses in crop and soil samples owing to interference by co-extractives and/or insufficient separation of some of the compounds mentioned above. In addition, they do not account for certain potential aniline-containing metabolites or conjugates, which have to be considered as residues[8].

In this note we wish to describe a rapid and sensitive chromatographic procedure which allows separation and detection of residue amounts of the different anilines, once they have been removed from the substrate according to the standard method. The compounds are diazotized and coupled with N-ethyl-1-naphthylamine and are then separated as azo-dyes on cellulose plates.

Experimental

Aniline standards. 2 mg of each aniline to be tested is dissolved in 50 ml 0.5 *N* hydrochloric acid.

Sodium acetate buffer 2 *M*. The solution contains 272 mg of $CH_3COONa \cdot 3\ H_2O$ per ml of distilled water.

Sodium nitrite. Reagent grade, 2% aqueous solution.

Sulphamic acid. Reagent grade, 10% aqueous solution.

N-Ethyl-1-naphthylamine (Fluka A.G. Buchs SG, Switzerland, or Aldrich Chemical Co., Inc., Milwaukee, Wis., U.S.A.), 1% ethanolic solution. The solution is shaken briefly with activated carbon, allowed to stand overnight and filtered. It is stored in darkness and the carbon treatment is repeated whenever the solution turns brown.

Cellulose powder for TLC, MN 300* (Macherey, Nagel & Co., 516 Düren, Germany). The adsorbent is used without a binder. TLC plates 20 × 20 cm or 10 × 20 cm are used. The thickness of the layer is 300 μ. The slurry is prepared as indicated by the manufacturer. The plates are dried for 60 min at 110° and stored in a desiccator over blue silica gel.

Solvent system. Dimethylformamide–0.5 *N* hydrochloric acid–ethanol (60:20:20).

Diazotisation and coupling

The plant and soil samples undergo alkaline hydrolysis, steam distillation, and extraction according to the standard procedure[2,3].

An aliquot of the hexane (isooctane) extract, representing 20 g of the original plant or soil material, is placed in a 100-ml separatory funnel and extracted with three 5-ml portions of 0.5 *N* hydrochloric acid. The combined acid extracts are transferred to a 50-ml Erlenmeyer flask and 1 ml of 2% sodium nitrite solution is added, mixed, and allowed to stand for 10 min. Excess nitrite is destroyed by adding 1 ml of 10% sulphamic acid solution and shaking vigorously until nitrogen generation ceases. 1 ml of the 1% ethanolic N-ethyl-1-naphthylamine solution is pipetted into the flask and the coupling is accelerated by adding 4 ml of sodium acetate buffer. The mixture is allowed to stand for 10 min and then acidified with 2 ml 5 *N* hydrochloric acid.

The dye solution is extracted with 10 ml of diethyl ether in a 50-ml separatory funnel, and the extraction is repeated once with 5 ml of the same solvent. The combined ether phases are transferred to a 25-ml Erlenmeyer flask and dried carefully with 3–4 g of anhydrous sodium sulphate. The dried solution is decanted into a 15-ml tapered test tube and the sodium sulphate rinsed with small portions of ether. The solvent is completely evaporated in a gentle stream of air and taken up in 0.3 to 0.5 ml of acetone.

For diazotisation and coupling of standards, 0.2 ml of standard solution is placed in a 50-ml Erlenmeyer flask, diluted with 15 ml of 0.5 *N* hydrochloric acid, and then treated as described above.

Thin-layer chromatography

5 to 10 μl of the acetone concentrate of standards and samples is spotted portionwise on the starting line of the cellulose plate. The plate is developed for 15 cm

* It is important that this particular type of cellulose be used. Several other types have been tested, but much less satisfactory separations were observed.

(3 h) in a saturated tank. After development the plate is removed and dried at room temperature.

Results and discussion

The R_F values of the coupled anilines are listed in Table II. Since the type of azo-dye varies with the varying substitution on the phenyl moiety, Table II also gives the colours of the spots, which serve as an additional means of identification. Fig. 1 shows a typical chromatogram of standards and of distillates of untreated and herbicide-containing cotton seeds.

The minimum quantity of anilines which may be visualized without difficulty on the plate has been determined as 0.03 to 0.04 μg. Thus, when concentrating the

TABLE II

R_F VALUES AND COLOURS OF AZO-DYES OF ANILINES ON CELLULOSE THIN-LAYER PLATES

Compound	R_F	*Colour*
Aniline	0.65–0.68	purple
4-Chloroaniline	0.45–0.48	purple
4-Bromoaniline	0.40–0.43	purple
3,4-Dichloroaniline	0.29–0.32	carmine-purple
3-Chloro-4-bromoaniline	0.25–0.28	carmine-purple
3-Trifluoromethylaniline	0.56–0.59	carmine
4-Amino-4'-chlorodiphenylether	0.14–0.17	blue

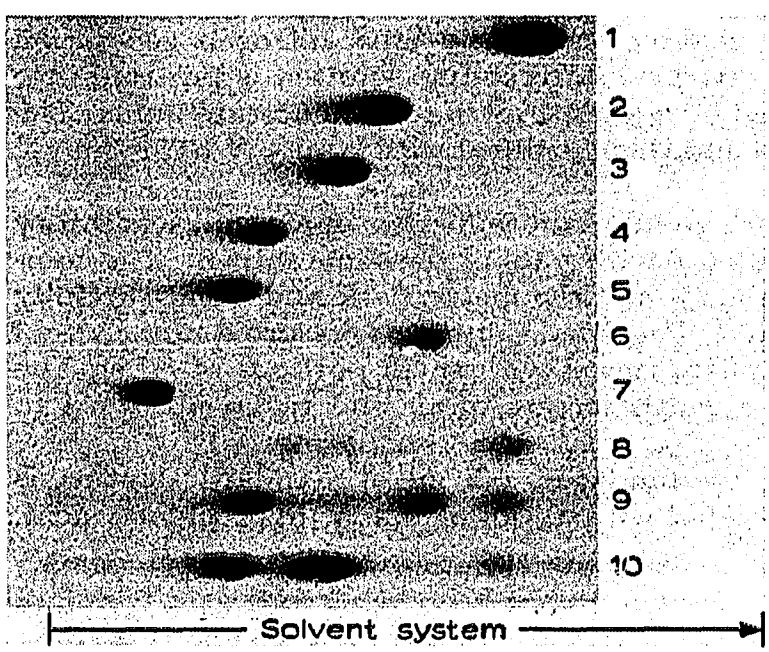


Fig. 1. TLC separation of azo-dyes of standard anilines, and of untreated and herbicide-containing distillates derived from cotton seeds. Solvent system: dimethylformamide–0.5 *N* hydrochloric acid–ethanol (60:20:20). Quantity of anilines applied: 0.3 to 0.4 μg. 1 = Aniline; 2 = 4-chloroaniline; 3 = 4-bromoaniline; 4 = 3,4-dichloroaniline; 5 = 3-chloro-4-bromoaniline; 6 = 3-trifluoromethylaniline; 7 = 4'-chloro-4-amino-diphenyl ether; 8 = cotton, blank; 9 = cotton + fluometuron + diuron; 10 = cotton + metobromuron + C-6313.

samples to 0.3 ml of acetone and spotting 10 μl of this concentrate (corresponding to approximately 0.7 g of plant or soil material), the limit of detection of the method is 0.05 to 0.1 p.p.m. in terms of the original herbicide. Blank spots produced by diazotisation and coupling of distillates of a number of untreated soil and plant samples have been observed to be weak or practically non-existent. If their R_F values should interfere with those of the anilines to be detected, they may normally be distinguished by the colours of their spots. For semi-quantitative evaluation of the plates, a series of graded standard solutions is used for comparison.

Depending on the quantity of coupled aniline present, the spots will exhibit more or less pronounced tailing (see Fig. 1). Therefore, the smallest volume possible, which will still produce visible spots, should be applied. For best results, the quantities of anilines spotted should be within the range of 0.04 to 0.4 μg.

When distinction between 4-chloroaniline and 4-bromoaniline (or 3,4-dichloroaniline and 3-chloro-4-bromoaniline) is required, it is advantageous to fortify distillates of untreated crop or soil samples with each compound, and to use these solutions as references. Provided the analyses are limited to disubstituted anilines, separations are improved by developing the plates for a second time.

It is evident that the present chromatographic procedure will distinguish only between urea herbicides having differing phenyl moieties. Compounds with identical phenyl-substituents (*e.g.* diuron, linuron and neburon) have to be separated before hydrolysis to their anilines. The application of the method described above is not limited to urea herbicides; it may be extended to phenylcarbamate- or anilide-type pesticides.

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Received September 19th, 1966