Shaking the slurry during its make-up probably prevents any structure formation during the hydration of the calcium sulfate. During the drying step this structure formation then takes place in such a manner as to produce a more water-resistant coating. Plates which have been coated using a slurry, and then thoroughly air-dried, lose only the surface layer of the coating when rinsed in a stream of cold water. Most of the silica gel adheres to the glass and must be removed by rubbing. Coatings prepared from the conventional 2:1 mixture are almost completely washed off the plate when treated in the same manner. The adherent layer on plates coated from slurries makes these coatings more resistant to flaking when they are developed in solvent systems containing a high percentage of water.

Not all solutions can be used to prepare satisfactory slurries by the procedure outlined. Slurries prepared with solutions 0.1 *M* in phosphate ion produced, within three days, crystalline appearing aggregates which presumably were calcium phosphate. A slurry prepared using McILVAINE's citrate–phosphate buffer, pH 3.2, was too thin to coat. A mixture prepared using the same buffer in a 2:1 ratio of it to silica gel G coated satisfactorily and did not set to a gel even if not shaken.

Silica gel H slurries are also usable over a period of time. Aside from the higher cost of silica gel H, only one disadvantage of this material has been noted. A silica gel H slurry in 0.1 *M* NaOH after one week's standing contained aggregates of silica gel which could not be dispersed by shaking. The coatings prepared using silica gel H slurries have the same mechanical properties as those prepared from silica gel G slurries. Silica gel H would have an advantage when coatings are prepared using materials, *e.g.* phosphate ion, which react with the calcium sulfate in silica gel G.

Acknowledgement

This study was supported by funds from Research Grant RG 9863, National Institute of Health, United States Health Service.

Cuyahoga County Coroner's Office, Cleveland, Ohio (U.S.A.) WINSTON W. FIKE
IRVING SUNSHINE

1 J. H. RUSSELL, *Rev. Pure Appl. Chem.*, 13 (1963) 15.
2 P. DAUVILLIER, *J. Chromatog.*, 11 (1963) 405.
3 I. SUNSHINE AND W. W. FIKE, *New Engl. J. Med.*, 271 (1964) 487.

Received September 25th, 1964

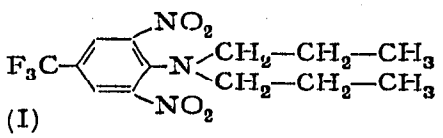
J. Chromatog., 18 (1965) 405–406

Separation of trifluralin and some related compounds by two-dimensional thin-layer chromatography

Trifluralin (I) (α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-*p*-toluidine) is a selective pre-emergent herbicide for use on agronomic crops[1–3]. It is active against a great variety of broadleaf weeds and annual grasses.

Prior to starting metabolic studies of trifluralin in plant and soil systems, it was necessary to investigate the chromatographic behavior of this compound,

related compounds and possible metabolites. Two-dimensional thin-layer chromatography on Silica Gel GF separated trifluralin and 17 related substances. Optimum separations were obtained with benzene–ethylene dichloride (1:1) and *n*-hexane–methanol (98:2).



(I)

The compounds that are not colored are detected as blue absorbing spots when the Silica Gel GF plate is exposed to short wavelength U.V. radiation.

Experimental

Thin-layer plates (20 cm × 20 cm × 0.2 cm) were coated with Silica Gel GF 254 (Brinkmann Instruments, Inc., Long Neck, N.J.) as described by STAHL[4,5] using a suspension of 30 g of Silica Gel GF in 60 ml of distilled water in a 250 μ spreader. The plates were activated by drying at 110° for 60 min.

Five μg of trifluralin and each of the 17 compounds (Table I) in benzene solution were applied at a point of 3 cm from the left edge and 3 cm from the bottom of the plate.

After developing in solvent I (benzene–1,2-dichloroethylene (1:1)), the plates were dried at room temperature for 30 min, rotated 90° and developed in solvent II (*n*-hexane–methanol (98:2)). The chromatography jars were lined with filter paper to insure saturation.

Results and discussion

Fig. 1 shows the separation obtained with the compounds listed in Table I. The detection limit of the colored compounds and those that show as blue spots under short wavelength U.V. radiation is approximately 0.5 μg.

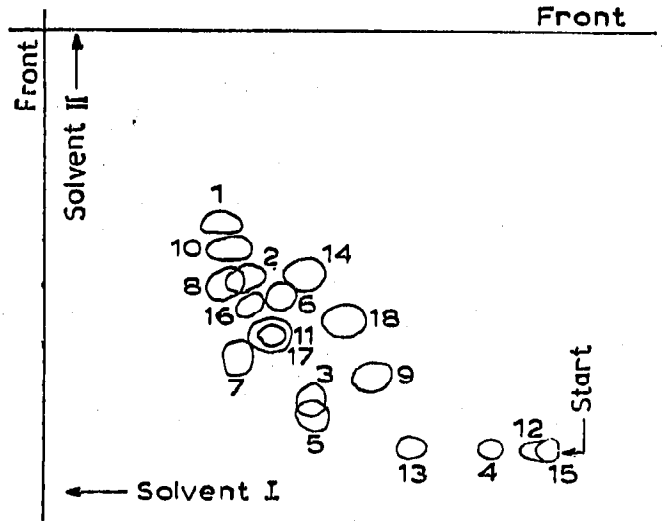


Fig. 1. Thin-layer chromatogram of trifluralin and 17 related compounds. Silica Gel GF. Solvent I = benzene–1,2-dichloroethylene (1:1); solvent II = *n*-hexane–methanol (98:2).

TABLE I

INVESTIGATION OF TRIFLURALIN AND RELATED SUBSTANCES

Number	*Name*	*Color on TLC*
1	Trifluralin	pale yellow
2	α,α,α-Trifluoro-2,6-dinitro-N-(*n*-propyl)-*p*-toluidine	intense yellow
3	2,6-Dinitro-4-trifluoromethylaniline	green yellow
4	α,α,α-Trifluoro-5-nitrotoluene-3,4-diamine	yellow
5	α,α,α-Trifluoro-5-nitro-N^4-(*n*-propyl)-toluene-3,4-diamine	yellow brown
6	2,6-Dinitro-N-(*n*-propyl)-*p*-toluidine	intense yellow
7	N^2,N^2-Di-(*n*-propyl)-3-nitro-5-trifluoromethyl-O-phenylenediamine	light yellow
8	N,N-Di-(*n*-propyl)-2,6-dinitro-*p*-toluidine	colorless
9	N^4,N^4-Di-(*n*-propyl)-α,α,α-trifluorotoluene-3,4,5-triamine	colorless
10	N,N-Di-(*n*-propyl)-2-nitro-α,α,α-trifluoro-*p*-toluidine	yellow brown
11	3,5-Dinitrobenzotrifluoride	colorless
12	2,6-Dinitro-α,α,α-trifluoro-*p*-cresol	intense yellow
13	2-Nitro-4-trifluoromethylaniline	pale yellow
14	N-(*n*-Propyl)-2-nitro-*p*-toluidine	deep brown-red
15	3,5-Dinitro-4-(di-*n*-propylamino)-benzoic acid	yellow
16	N-(*n*-Propyl)-2-nitro-α,α,α-trifluoro-*p*-toluidine	yellow
17	3,5-Dinitro-4-methoxybenzotrifluoride	colorless
18	3,5-Dinitro-4-(di-*n*-propylamino)-methylbenzoate	pale yellow

Since it is often difficult to obtain the 0.5 μg of metabolites in plant and soil samples, other methods of detection are needed. The separation of the compounds obtained with the thin-layer chromatographic system are adequate to permit dividing the chromatogram into zones which can be investigated by other techniques. In our laboratories, nanogram to picogram quantities of material have been isolated from thin-layer chromatographic plates and measured by gas–liquid chromatography. When radioactive labelled materials are used in metabolic studies, the separated zones can be eluted and counted by liquid scintillation counter.

Acknowledgements

The compounds used in this study were prepared and purified in the Chemical Research Division, Eli Lilly and Company, Indianapolis, Ind. The author is indebted to Dr. Q. F. SOPER for making available these compounds, to Mr. J. B. TEPE for interest in this work and to Mr. B. H. RAGATZ for technical assistance.

Eli Lilly and Company, Greenfield Laboratories, Greenfield, Ind. (U.S.A.) T. GOLAB

1 E. F. ALDER, W. L. WRIGHT AND Q. F. SOPER, *Proc. Northeast. Weed Control Conf.*, 15 (1961) 298.
2 E. F. ALDER AND R. B. BEVINGTON, *Proc. Northeast. Weed Control Conf.*, 16 (1962) 505.
3 S. J. PIECZARKA, W. L. WRIGHT AND E. F. ALDER, *Proc. Northeast. Weed Control Conf.*, 16 (1962) 356.
4 E. STAHL, *Chemiker Ztg.*, 82 (1958) 323.
5 E. STAHL, *Pharm. Rundschau*, 1 (1959) 1.

Received October 13th, 1964