

Synthetic mixtures of equal amounts of D-galactose, D-glucose, D-mannose, D-xylose, and L-rhamnose in 10, 30, and 50  $\mu\text{g}$  amounts of each were separated readily by four developments, with intermittent drying (hair drier, about 10 min), with ethyl acetate-pyridine-water (2:1:2, v/v);  $R_F$  0.90, 1.00, 1.10, 1.30, 1.55, respectively (Fig. 1). A qualitative analysis of this complex mixture could be accomplished by a double development; for a quantitative determination, four developments afforded a satisfactory separation in 5 h; a similar separation by paper chromatography requires at least 24 h. A mixture of equal parts of D-galactose and D-glucose was determined with a 3 % error for D-galactose and 6 % for D-glucose when the concentration of each sugar per spot was maintained within the range 10 to 60  $\mu\text{g}$ .

#### Acknowledgement

Acknowledgement is made to support from the Corn Industries Research Foundation.

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Received October 29th, 1965

*J. Chromatog.*, 22 (1966) 474-476

### Separation of polyphenyls by thin-layer chromatography

Recent interest in polyphenyls as reactor coolants has necessitated both qualitative and quantitative techniques for the separation of the isomers. DENTI *et al.*<sup>1</sup> have shown that sulfonation products of polyphenyls can be separated by paper chromatography. GEISS *et al.*<sup>2</sup> obtained a separation of the polyphenyl isomers by thin-layer chromatography (TLC) but their technique was complicated and reproducibility was difficult to achieve.

The purpose of this paper is to report the separation of the polyphenyl isomers by TLC using a single developing solvent system.

#### Experimental

**Materials.** Desaga TLC apparatus and tanks were used. Aluminum oxide G according to STAHL was used to coat glass plates 20  $\times$  20  $\times$  0.4 cm. Solvents were reagent grade. Polyphenyl isomers and azobenzene were obtained from Eastman

*J. Chromatog.*, 22 (1966) 476-478

Kodak Co., Rochester, N.Y. HB-40, a partially hydrogenated terphenyl mixture, was supplied by Monsanto Chemical Co., St. Louis, Missouri.

*Preparation of plates.* 50 g of aluminum oxide G were suspended in 100 ml of distilled water and applied as a layer 250  $\mu$  thick to the glass plates with a Desaga spreader. The plates were air dried for at least 2 h, then activated 0.5 h at 120° and stored in a desiccator over silica gel and phosphorus pentoxide until use. Just before use each plate was activated again for 0.5 h at 120° and cooled in the desiccator for 20 min.

*Preparation of the solvent.* The developing solvent was prepared by mixing cyclohexane and benzene in the volume ratio of 98:2. The solvent was stored in the refrigerator until use. The developing tanks were lined with filter paper. 400 ml of solvent and a beaker containing about 100 g of phosphorus pentoxide were placed in the tank and the system was allowed to equilibrate overnight at room temperature.

*Chromatographic technique.* The separations were carried out by the ascending technique. Solutions of azobenzene, biphenyl, *o*-, *m*- and *p*-terphenyl in carbon tetrachloride were prepared and spotted 1.5 cm from the lower edge of the plate. 10  $\mu$ l containing 5  $\mu$ g of compound was the amount spotted. A synthetic mixture of biphenyl and the terphenyl isomers in carbon tetrachloride was prepared. 10  $\mu$ l containing 5  $\mu$ g of each component was spotted. HB-40 in carbon tetrachloride was applied at 20  $\mu$ g per 10  $\mu$ l spot. Azobenzene was used as a reference dye. After spotting the plate was introduced into the developing tank on to a rack above the level of the solvent and allowed to equilibrate for 0.5 h, after which it was lowered into the solvent and run to a distance of 10 cm. The developing time was about 28 min.

After development the plates were air dried for 5 min at room temperature. Higher temperatures or longer drying times caused the sublimation of the biphenyl off the plate. Detection of the spots was by visible light (for azobenzene), ultraviolet light, iodine vapours and for some plates by oxidation with potassium permanganate in sulphuric acid. The oxidation method gave the best results and consisted of a spray of 0.5 g  $\text{KMnO}_4$  in 15 ml conc.  $\text{H}_2\text{SO}_4$ . The limit of detection using this method was 0.1  $\mu$ g/spot for all the isomers. With time the biphenyl spot would fade due to sublimation.

### Results

The mean  $R_F$  values (centre of spot) were determined from six independent runs and are given in Table I. The absolute  $R_F$  values were somewhat variable be-

TABLE I

$R_F$  VALUES OF THE POLYPHENYLS

Compound	Mean $R_F \pm \sigma$	Adjusted $R_F \pm \sigma$
Biphenyl	0.53 $\pm$ 0.02	0.53 $\pm$ 0.02
<i>o</i> -Terphenyl	0.43 $\pm$ 0.03	0.43 $\pm$ 0.01
<i>m</i> -Terphenyl	0.33 $\pm$ 0.03	0.33 $\pm$ 0.01
<i>p</i> -Terphenyl	0.26 $\pm$ 0.02	0.26 $\pm$ 0.01
HB-40 spot A	0.86 $\pm$ 0.04	0.86 $\pm$ 0.02
HB-40 spot B	0.76 $\pm$ 0.04	0.77 $\pm$ 0.01
HB-40 spot C	0.56 $\pm$ 0.02	0.56 $\pm$ 0.02
HB-40 spot D	0.46 $\pm$ 0.03	0.47 $\pm$ 0.01

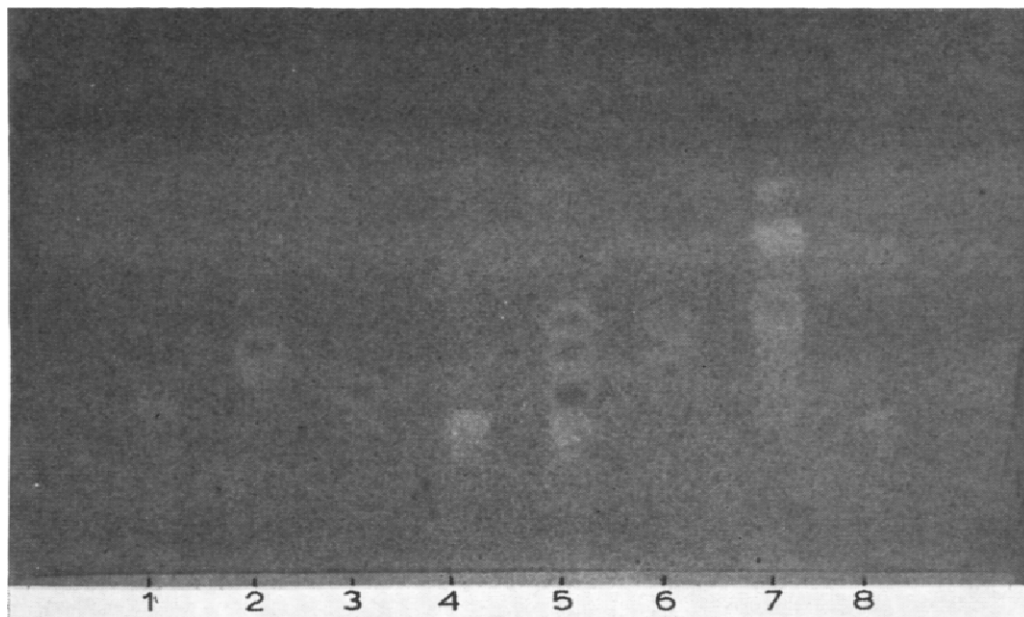


Fig. 1. Polyphenyl isomers on aluminum oxide G layer,  $250 \mu$  thick, activated for 30 min at  $120^\circ$ . Solvent: cyclohexane-benzene (98:2). Distance travelled: 10 cm (28 min). Spray:  $\text{KMnO}_4$  in  $\text{H}_2\text{SO}_4$ . 1 = Azobenzene; 2 = *o*-terphenyl; 3 = *m*-terphenyl; 4 = *p*-terphenyl; 5 = mixture; 6 = biphenyl; 7 = HB-40; 8 = azobenzene.

tween plates but, if activation differences were accounted for using a predetermined mean  $R_F$  value (0.31) for azobenzene, the variations were reduced as indicated by the adjusted  $R_F$  values of Table I. Partial hydrogenation of the terphenyl isomers increases their mobility on TLC plates. Fig. 1 is a photograph of a typical TLC separation.

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Received October 25th, 1965

*J. Chromatog.*, 22 (1966) 476-478

### Plastic sheet thin-layer chromatography in a round tank

The recently developed thin-layer chromatographic sheets with flexible plastic backs can be used in a regular rectangular chromatographic tank or in a special developing apparatus such as the "Eastman Chromatogram" sheet and developing apparatus\*. However, it is possible to combine both methods by the use of a spiral

\* Supplied by Distillation Products Industries, Rochester, New York.

*J. Chromatog.*, 22 (1966) 478-479