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Selective detection of o-dihydroxybenzenes and other phenolic compounds on cellulose thin-layer plates

The ability to detect classes of phenols separated by thin-layer chromatography by means of selective spray reagents has obvious utility in the analysis of unknown mixtures. This report describes a scheme for the successive visualization of catechols, other types of phenols, and *o*-hydroxybenzoic acids on thin-layer plates.

SCHROEDER[1] described a double spray system consisting of phloroglucinol followed by alkali for detecting *o*-dihydroxybenzenes on filter paper at a detection limit of about 1 μg for a 1 μl sample, and he provided a review of selective detection methods for phenolic compounds. In the course of a study of the validity of the DOTY[2] colorimetric assay for isoproterenol in a pharmaceutical aerosol as a stability assay method, it was found[3] that the DOTY reagent provides a very sensitive and specific means for detecting catechols on thin-layer plates. The characteristic purple color was found to be easily detectable at a level of 0.3 μg of isoproterenol or epinephrine after chromatographic development. The general applicability of the procedure was tested with a variety of *o*-dihydroxybenzenes and related phenolic compounds. Visualization of catechols on thin-layer plates with the DOTY reagent does not interfere with subsequent detection of other types of phenols on the same chromatograms with other reagents. Coupled with R_F data, the scheme reported here provides a powerful tool for characterization of mixtures of phenolic compounds.

Procedure

The test samples were spotted in 1 μl volumes of 1 mg/ml methanol solutions. The phenols were laboratory grades used without further purification. They are identified in Tables I and II. The samples were spotted 3 cm from the bottom of 20 × 20 cm Analtech Uniplates®, consisting of a 0.25 mm layer of microcrystalline cellulose on glass without binder or phosphor. Separate development systems were employed for the phenolic amines and the other phenols, *viz.*

(1) For phenolic amines, a mixture of 2-propanol and 0.1 *N* hydrochloric acid (5:1) was used. The plates were developed to nearly the upper edge of the plate in the solvent system and air-dried before use in order to remove an apparent second front across the plates which otherwise resulted in artifactual spots. The chromatograms were developed to about 15 cm from the starting line.

(2) A benzene–methanol–acetic acid system (45:8:4) was used for the development of phenols without amine functions.

The chromatography chambers were not lined with filter paper in either system. The developed chromatograms were successively sprayed with DOTY reagent, a saturated aqueous solution of *p*-nitrobenzenediazonium fluoborate, and 2% ferric chloride in butanol followed by 0.1 *N* hydrochloric acid. The plates were air-dried between applications of reagents.

The DOTY reagent was prepared daily by combining 1 volume of *ferro-citrate solution* with 10 volumes of *buffer solution*, prepared according to the following directions:

Ferro-citrate solution: Dissolve 1.5 g ferrous sulfate in 200 ml of water containing

1.0 ml of *N* hydrochloric acid and 1 g of sodium bisulfite. Dissolve 500 mg of sodium citrate in 10 ml of this solution.

Buffer solution: Add 42 g of sodium bicarbonate and 50 g of potassium bicarbonate to about 180 ml of water. Add 37.5 g of glycine and 17 ml of concentrated ammonia solution to another 180 ml of water. Mix the two, and dilute to 500 ml with water.

Results and discussion

All compounds with the *o*-dihydroxybenzene structure were easily detected as purple spots at the 1 μg level with the DOTY reagent, while none of the non-catechols gave any color at all. The DOTY reaction has been shown to be stoichiometric[4], thus the absolute detection limit should be a function of the molecular weight of the catechol as well as the area of the spot and the visual acuity of the observer. DOTY[2] noted that salicylic acid gives an atypical color with the reagent; however, the resultant amber color has a detection limit at much higher concentration than used in these experiments, thus no interference was observed. The catechols and catecholamines are listed in Table I. The chromatographic systems used do not provide complete

TABLE I

CHROMATOGRAPHIC RESULTS WITH *o*-DIHYDROXYBENZENE DERIVATIVES

Compound	*System*	R_F *value*
Epinephrine	1	0.22
Isoproterenol	1	0.49
Norepinephrine	1	0.19
3,4-Dihydroxyphenylalanine (DOPA)	1	0.32
Catechol	2	0.84
Protocatechuic acid	2	0.27
Pyrogallol	2	0.27
Tannic acid	2	0.00

TABLE II

CHROMATOGRAPHIC RESULTS WITH PHENOLIC COMPOUNDS

Phenol	*Substituents*	*System*	*Colours*		R_F *value*
			Azo	$FeCl_3$	
Phenylephrine	*m*-(2-Methylamino-1-hydroxy)-ethyl-	1	pi	—	0.36
Metaraminol	*m*-(2-Amino-1-hydroxypropyl)-	1	pi	—	0.40
Hydroquinone	*p*-Hydroxy-	2	pi	—	0.44
Phenol	—	2	pi	—	0.94
Resorcinol	*m*-Hydroxy-	2	or	—	0.59
5-Methylresorcinol	3-Hydroxy-5-methyl-	2	or	—	0.59
Guaiacol	*o*-Methoxy-	2	pur	—	1.0
2,4-Xylenol	2,4-Dimethyl-	2	or	—	1.0
β-Resorcylic acid	2-Carboxy-5-hydroxy-	2	or	—	0.70
Gentisic acid	2-Carboxy-3-hydroxy-	2	pi	—	0.51
Salicylic acid	2-Carboxy-	2	—	or	0.87
2-Hydroxy-*p*-anisic acid	2-Carboxy-5-methoxy-	2	—	or	0.90

separations; they were chosen because the majority of the test compounds moved in them.

The non-catechol phenolic compounds, with the exception of salicylic acid and 2-hydroxyanisic acid, were easily visualized by overspraying the plates with the diazo reagent, appearing as orange, pink, or purple spots. Previous treatment with the DOTY reagent did not interfere, and, since it provided a pH of about 8.5, conferred a nearly optimum environment for azo dye formation. Use of the diazonium reagent did not affect the color of the previously visualized catechol spots. The two *o*-hydroxybenzoic acids which failed to react with either reagent were detected as orange spots by overspraying with ferric chloride and acid. Here, however, the 1 μg level was close to the detection limit. Although gentisic acid and β-resorcylic acid are also *o*-hydroxybenzoic acids, they formed azo dyes. Unlike salicylic acid and 2-hydroxyanisic acid, these compounds have an unchelated phenol function which can contribute electrons to the *ortho* and *para* positions of the ring. Table II provides a summary of the results of overspraying the plates.

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1 H. A. SCHROEDER, *J. Chromatog.*, 30 (1967) 537.
2 J. R. DOTY, *Anal. Chem.*, 20 (1948) 1166.
3 L. CHAFETZ, H. SCHRIFTMAN AND A. I. KAY, *J. Pharm. Sci.*, 57 (1968) 710.
4 M. M. TUCKERMAN, *J. Pharm. Sci.*, 51 (1962) 700.

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