QUANTITATIVE PAPER-CHROMATOGRAPHIC DETERMINATION OF PHENOLS*

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A routine paper-chromatographic method for the quantitative analysis of mixtures of phenols was needed for research in this laboratory. While such methods as comparison of spot areas and intensity have been described[1], these methods are subject to certain limitations[2]. Thus, it seemed desirable to develop a method in which the phenols would be eluted from the chromatogram and measured colorimetrically after reaction with an appropriate reagent. The following method has proven satisfactory.

METHODS AND MATERIALS

Reagents

1. Folin-Ciocalteu reagent[1].
2. 20% Na_2CO_3 solution (w/v).

Solvent systems

1. Organic phase of benzene–acetic acid–water (2:2:1, v/v/v). Irrigation time: 24 h.
2. 20% acetic acid. Irrigation time: 6 h.
3. Organic phase of ethyl acetate–acetic acid–water (2:1:2, v/v/v). Irrigation time: 6 h.

Chromatography papers

Whatman grades 1, 4, and 54.

Phenolic samples

The compounds were dissolved in 50% ethanol. 10 to 100 μg were applied with a micropipette by streaking a 2.5-inch strip on the reference line of the sheet of paper. Spots of the known compounds were applied on either side of the main band containing the unknown. The papers were then irrigated using descending technique at room temperature. Irrigation times listed are approximate times for Whatman No. 54 paper.

Elution and determination

After the chromatograms had been dried, the side strips containing the known spots were cut from the chromatogram. The spots on the side strips were located by spraying first with the Folin-Ciocalteu reagent (diluted 1:1 with H_2O) and then with Na_2CO_3

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solution. The side strips were placed alongside the center portion of the chromatogram and a rectangular area in which the unknown would be located was marked lightly with pencil. The rectangle, usually 2 × $2^1/_2$", was removed, cut into small pieces and placed in a medium porosity sintered glass crucible. The crucible was placed in a crucible holder on a filter flask containing a test tube. The paper was then washed twice with 10-ml portions of hot water and the eluate collected in the test tube. Gentle suction was applied to remove most of the water from the paper and the crucible. The eluate was transferred quantitatively to a 25-ml volumetric flask. To the flask was added 0.5 ml of Folin-Ciocalteu reagent (diluted 1:1 with water), 2 ml of 20% Na_2CO_3 solution, and enough water to bring to volume. The blue color was allowed to develop for 2 hours. The optical density was determined with a Coleman Universal Spectrophotometer at 660 mμ with a PC-5 filter. In all cases, from each irrigated sheet a rectangle of paper, the same size as that containing the sample was treated in the same manner and used as a blank.

RESULTS AND DISCUSSION

Standard curves were obtained for some representative phenols (resorcinol, gallic acid, and caffeic acid). Fig. 1 is the standard curve for gallic acid. The method is sensitive enough to determine easily 10 μg of gallic acid per 25 ml of solution.

R_F values were determined for some of the common phenols. Table I lists the R_F values for these phenols in 20% acetic acid and ethyl acetate–acetic acid–water. The mobilities of these phenols in benzene–acetic acid–water are calculated as $R_{catechol}$ values as the solvent dripped off the end of the paper.

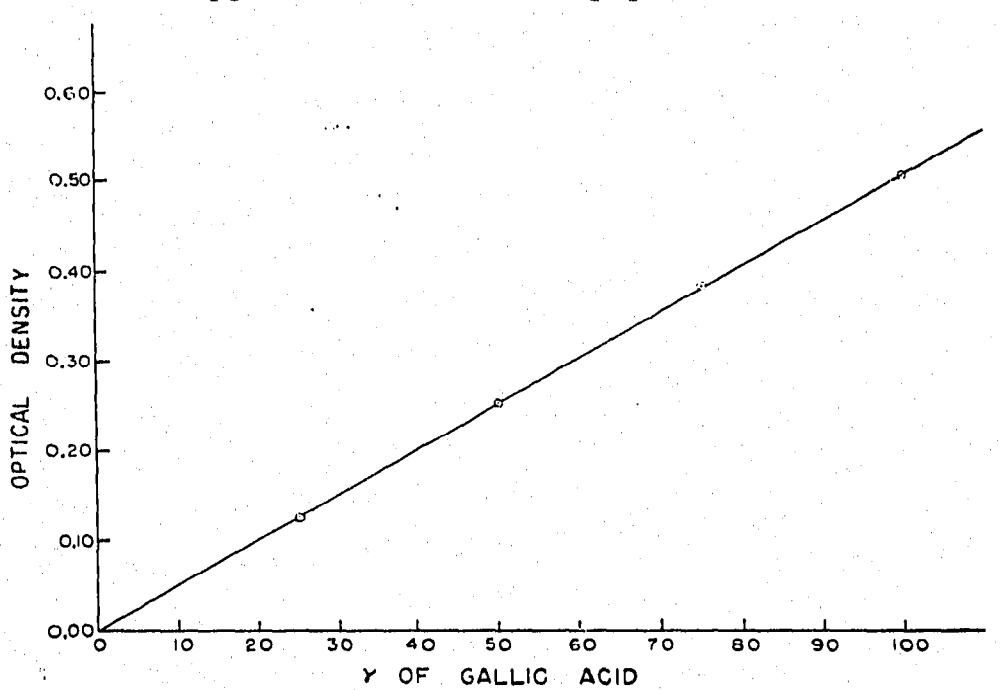

Fig. 1. Standard curve for gallic acid which was chromatographed, eluted, and reacted with Folin-Ciocalteu reagent. Whatman No. 54 paper irrigated with benzene–acetic acid–water (2:2:1). Each point is an average of 3 determinations.

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TABLE I

AVERAGE R_F AND $R_{CATECHOL}$ VALUES OF PHENOLS ON WHATMAN NO. 54 PAPER

Compound	R_F		$R_{catechol}$
	20% Acetic acid	Ethyl acetate–acetic acid–water (2: 1: 2)	Benzene–acetic acid–water (2: 2: 1)
Catechol	0.74	0.92	1.00
Resorcinol	0.70	0.91	0.30
Hydroquinone	0.71	0.91	0.28
Protocatechuic acid	0.62	0.86	0.13
2,4-Dihydroxybenzoic acid	0.63	0.94	0.58
2,5-Dihydroxybenzoic acid	0.66	0.94	0.42
Protocatechuic aldehyde	0.66	0.91	0.30
Orcinol	0.68	0.92	0.49
Phloroglucinol	0.61	0.74	0.01
Pyrogallol	0.63	0.82	0.10
Gallic acid	0.44	0.69	0.01
Caffeic acid	0.38	0.93	0.16
Chlorogenic acid	0.68	0.74	0.01

An inspection of the table shows that one solvent system may be used to separate certain compounds. Thus, orcinol and protocatechuic aldehyde can be separated with benzene–acetic acid–water but not with the other two irrigants. Even with the three irrigants it was not possible to separate the following groups: resorcinol, hydroquinone, and protocatechuic aldehyde; protocatechuic acid and pyrogallol; phloroglucinol and chlorogenic acid.

While several colorimetric methods for phenols have been reported, the procedure using the Folin-Ciocalteu reagent was chosen because it is a rapid, sensitive method applicable to phenols[1]. Although the procedure has given reproducible results, certain precautions are to be noted. The reaction is nonspecific in that other reducing substances will react with the reagent. Preliminary experiments indicated that certain chromatography papers contained impurities which might result in colored and variable blanks. S & S papers Nos. 589 and 598 were found to do so, and thus were not satisfactory.

Similarly, certain solvent systems may contain reducing substances as impurities which may remain on the paper and interfere with the quantitative method. It has been found in the present study that high and variable blanks were attained from paper that had been irrigated with butanol–acetic acid–water (4 : 1 : 5, v/v/v).

SUMMARY

A routine paper-chromatographic method for the quantitative determination of phenols is described. After elution, the phenols are measured colorimetrically using the Folin-Ciocalteu reagent.

REFERENCES

[1] H. G. BRAY AND W. V. THORPE, Analysis of Phenolic Compounds of Interest in Metabolism, in D. GLICK, *Methods of Biochemical Analysis*, Vol. I, Interscience Publ., New York, 1954, pp. 27–52.
[2] R. J. BLOCK, E. L. DURRUM AND G. ZWEIG, *Paper Chromatography and Paper Electrophoresis*, Academic Press, Inc , New York, 1955.

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