A method for detecting free phenol, aniline, indole and quinone derivatives in paper chromatography

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Abstract

Sodium nitroprusside reaction with phenols, anilnes, indoles and quinones under alkaline conditions can be used to detect these compounds on paper chromatograms.

1. Introduction

Various reagents are available for the detection of phenolic compounds, anilines, quinones and indole derivatives. Diazonium salts[1], tertazotized benzidine[2, 3], antimony pentachloride[4], phosphomolybdic acid[5] and ceric ammonium nitrate[6] are widely used to detect phenols and anilines, while Ehrlich's reagent[7] and magnesium acetate[8] are used for the detection of indole and quinone derivatives, respectively. We have found that sodium nitroprusside, a commonly used reagent for the detection of acetone and its derivatives[9] react with phenolic compounds, anilines, indoles and quinone derivatives. This reaction can be arranged as a detection method for the above compounds in paper chromatography.

2. Materials and methods

10–25 μg samples of each compound in appropriate solvent were spotted on Whatman No. 3 chromatographic papers and air-dried. The paper sheets were sprayed at first with a freshly prepared 15% solution of sodium nitroprusside and then with 2N ammonium hydroxide. Various colors appeared were immediately recorded. The papers were then heated for about 5–10 min at 110° C and the stable colors obtained were noted. Inclusion of hydroxylamine hydrochloride with nitroprusside reagent increased the sensitivity and intensity of the colors.

3. Results and discussion

The nitroprusside reaction is specific for phenols, anilines, quinones and indole derivatives. Aliphatic alcohols, amines, saturated cycloalcohols, carboxylic acids, sugars and aldehydes did not react with nitroprusside, while acetone and its derivatives which

are known to react[8] gave violet colored spots. The colors developed by various polyhydroyphenols are summarized in Table I. These compounds developed deep colored

Table I

Color reaction of sodium nitroprusside and ammonium hydroxide detection reagent with polyhydroxy phenols

Compound	Color produced after sparying	Stable color obtained after heating
Catechol	blue	black
Protocatechuic acid	violet	dark blue black
Pyrocatechuic acid	violet	dark blue green
Homoprotocatechuic acid	pink	deep brown
4-Methylcatechol	pink	dark brown
Protocatechualdehyde	green	brown
2, 3-Dihydroxy-*p*-toluic acid	pink	deep green
3, 4-Dihydroxymandelic acid	pink	bluish grey
Dopa	brown	dark brown
Dopamine	grey	bluish grey
Epinephrine	brown	dark brown
Chlorogenic acid	green	brown
Isochlorogenic acid	...	brown
Caffeic acid	light blue	dark grey
Dihydrocaffeic acid	pink	brown
Resorcinol	bluish green	green
Orcinol	light orange	yellowish green
2, 4-Dihydroxybenzoic acid	pink	dark blue
2, 4-Dihydroxybenzaldehyde	blue	blue
Quinol	light brown	brown
Gentisic acid	light pink	bluish violet
Homogentisic acid	green	deep brown
Homogentisic acid lactone	violet	grey
Hydroxyquinol	grey	black
Pyrogallol	brown	dark brown
Phloroglucinol	brown	bluish green
Gallic acid	green	black
Methyl gallate	light brown	grey
Phloroglucinol carboxylic acid	brown	grey
2, 4, 6-Trihydroxybenzaldehyde	pink	blue
3, 4-Dihydroxy-5-methoxy-benzaldehyde	brown	brown
1, 3-Dihydroxynaphthalene	brown	brown

spots and can be easily distinguished from monohydroxyphenols which produced blue, green and pink colored spots (Table II). Apart from the phenolic compounds, mandelic

Table II

Color reaction of sodium nitroprusside and ammonium hydroxide detection reagent with phenolic compounds

Compound	Color produced after spraying	Stable color obtained after heating
Vanillin	grey	silky green
Vanillic acid	blue	green
Ferulic acid	bluish grey	pink
Isoferulic acid	light grey	bluish grey
Syringic acid	pale blue	green
Sinapic acid	bright blue	bright pink
Vanilmandelic acid	grey	violet
Homovanillic acid	light blue	bluish violet
Vanillalcohol	violet	blue
Chromotropic acid	pink	bluish green
4-Hydroxyphenylacetaldehyde	grey	light blue
o-Tyrosine	...	bluish green
m-Tyrosine	...	yellowish green
p-Tyrosine	...	yellowish green
Octopamine	...	bluish green
1-Naphthol	blue	bluish green
2-Naphthol	blue	bluish grey
Phloretin	...	pale grey
4-Hydroxybiphenyl	...	pale brown
4-Hydroxyphenylpyruvic acid	bright red	bluish green
3-Hydroxybenzylalcohol	...	pale blue
Guaiacol	pale blue	light grey
4-Hydroxyphenylpropionic acid	...	pale blue
o-Coumaric acid	blue	pink
m-Coumaric acid	...	light blue
p-Coumaric acid	...	light blue
4-Hydroxyphenyllacetic acid	blue	bluish green
3-Hydroxybenzaldehyde	...	light blue
Salicylaldehyde	...	light blue
3-Hydroxymandelic acid	bluish green	sky blue
4-Hydroxymandelic acid	light blue	blue
2-Hydroxyphenylacetic acid	...	bluish green
3-Hydroxyphenylacetic acid	...	bluish green
4-Hydroxyphenylacetic acid	...	bluish green
Salicylic acid	...	bright blue
3-Hydroxybenzoic acid	light blue	blue
4-Hydroxybenzoic acid	pale blue	blue
4-Hydroxybenzoylformic acid	yellowish brown	orange

acid, benzoylformic acid, phenylpyruvic acid, acetophenone, phenylacetaldehyde, 4-methoxybenzoylformic acid, 2-phenyllactic acid, phenylalanine, dicoumarol, 3-phenyllactic acid, isonitrosoacetophenone and 2-nitrobenzoic acid produced light blue to pink colored spots. The following phenolic compounds, however, did not react: Phenol, *o*-cresol, *m*-cresol, *p*-cresol, thymol, *p*-chloro-*m*-cresol, 4-hydroxybenzaldehyde, 4-hydroxybenzylalcohol, isovanillic acid, 2-nitrophenol, 3-nitrophenol, 4-nitrophenol and 3, 5-dinitrosalicylic acid.

The color reactions of various aniline derivatives with sodium nitroprusside are given in Table III. These compounds generally produced bright green, violet and brown coloured spots. However, *m*-toluidine, sulfanilic acid and 4-nitroaniline did

Table III

Color reaction of sodium nitroprusside and ammonium hydroxide detection reagent with aniline derivatives

Compound	Color produced after spraying	Stable color obtained after heating
Anthranilic acid	pale brown	reddish brown
3-Aminobenzoic acid	...	bluish grey
4-Aminobenzoic acid	...	brown
3-Hydroxyanthranilic acid	brown	brown
4-Aminosalicylic acid	...	light brown
Aniline	...	light brown
2-Aminophenol	brown	yellowish brown
3-Aminophenol	green	bright silky green
m-Toluidine	...	...
p-Toluidine	pink	violet
Methylanthranilate	pink	brown
Ethylanthranilate	light violet	brown
DL-Kynurenine	...	green
Sulfanilic acid	...	...
4-Nitroaniline	...	...
4-Dimethylaminobenzaldehyde	...	...
4-Dimethylaminobenzonitrile	...	...
Diphenylamine	...	light brown
o-Phenylenediamine	green	dark green
m-Phenylenediamine	green	dark green
p-Phenylenediamine	green	dark green
Benzidine	bluish green	dark green

not react with the reagent. The reaction is also applicable to indole and quinone derivatives (Table IV).

Table IV

Color reaction of sodium nitroprusside and ammonium hydroxide detection reagent with the derivatives of indole and qunione

Compound	Color produced after spraying	Stable color obtained after heating
A. *Indole derivatives*		
Indole	brilliant blue	blue
Indoleacetic acid	light red	blue
Indolepropionic acid	...	light blue
Indoxylacetate	violet	violet
Tryptophol	light red	blue
Tryptophan	yellowish green	yellowish green
B. *Quinone derivatives*		
Naphthoquinone	blue	violet
Diphenylenedioxide-2, 3-quinone	light grey	violet
Isophenoxazine	light brown	yellow
Cinnabarinic acid	yellow	bright pink
2, 6-Dichloro-*p*-benzoquinone-4-chloroimide	grey	silky gree·.
Manadione	...	yellow

The following compounds did not produce colored spots (in concentrations up to 50 µg) : Benzoic acid, phenylacetic acid, benzene, toluene, coumarin, *p*-toluic acid, 2-fluorobenzoic acid, 3-fluorobenzoic acid, 4-fluorobenzoic acid, 4-chlorobenzoic acid, cinnamic acid, phenylpropionic acid, benzaldehyde, cinnamaldehyde, phenylpropionaldehyde, phenoxyacetic acid, 4-nitrobenzoic acid, 3-nitrobenzoic acid, 4-nitrophenyl acetic acid, 4-fluorophenylacetic acid, 2-nitrobenzaldehyde. phenethanol, benzylalcohol, 2-4-dichlorophenoxyacetic acid, 2, 4, 5-trichlorophenoxyacetic acid, 2, 3-dimethoxybenzaldehyde, 2, 4-dimethoxybenzaldehyde, 2, 5-dimethoxy-benzaldehyde, anisic acid, 3, 4-dimethoxycinnamic acid, phenylacrylic acid, *p*-toluimanilide, 2-methoxybenzoic acid, 3-methoxybenzoic acid, phthalic acid, phthalic anhydride, isonitrosopropiophenone, shikimic acid, quinic acid, 4-hydroxycoumarin, naphthoxyacetate, naphthylacetic acid, anisole, nicotinic acid, nicotinamide, quinoline, 4-chloromercuribenzoate, trichloroacetic acid, allantoin, citric acid, NAD, NADP, NADH. NADPH, ethylacetate, chloroethylacetate, formic acid, acetic acid, acetaldehyde, isopropanol, *n*-propanol, ethanol, butanol, cyclohexane, pyridine, collidine, sulfanilamide, benzylamine, Tris, valine, iso-

leucine, leucine, asparagine, aspartic acid, glycine, lysine, citrulline, ornithine, thiamine, thiourea and urea.

This method can be used to distinguish vanillic acid from isovanillic acid, 2-nitrobenzoic acid from 3- and 4-nitrobenzoic acids, isonitrosoacetophenone from isonitrosopropiophenone, phenylacetaldehyde from benzaldehyde and phenylpropionaldehyde, mandelic acid from phenylacetic acid and 4-hydroxybenzaldehyde and 4-hydroxybenzoic acid from the other isomers.

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