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A general spray reagent for phenolic compounds on thin-layer plates

A number of spray reagents for the detection of phenolic compounds on Silica Gel G thin-layer plates are known¹. Phosphomolybdic acid detects lipids in addition to phenols and their ethers. The Folin-Ciocalteu reagent is very sensitive and gives blue spots with easily oxidisable substances in addition to phenols. The ferric chloride-ferricyanide reagent is unsuitable since the reacted reagent cannot be washed from the water-sensitive Silica Gel G plates, and a blue background colour rapidly develops and does not give any reaction with monohydric phenols. Other spray reagents such as diazotized sulphanilic acid, benzidine, *p*-nitroaniline, Fast Blue Salt B, 2,6-dibromoquinone chloroimide, 4-nitrophenyldiazonium fluoroborate and diazotized sulphanilamide undergo reaction with only those compounds which are capable of coupling. Vanillin/hydrochloric acid detects only catechins and *p*-toluenesulphanilic acid is used for the detection of flavonoids.

It was therefore considered necessary to find a suitable sensitive spray reagent

TABLE I

DETECTION OF PHENOLIC COMPOUNDS (2 μ g LEVEL) BY SPRAYING WITH A SOLUTION OF SODIUM COBALTINITRITE IN ACETIC ACID

<i>Compound</i>	<i>Colour observed</i>
Ferulic acid	Reddish brown
<i>p</i> -Coumaric acid	Reddish brown
Sinapic acid	Reddish brown
Caffeic acid	Reddish brown
Methyl gallate	Reddish brown
Catechol	Reddish brown
Gallie acid	Dark brown
Ellagic acid	Brownish black
Pyrogallol	Brownish black
α -Naphthol	Light brown
Gentisic acid	Light brown
Thymol	Light brown
<i>p</i> -Quinol	Yellow
Hesperidine	Yellow
Vanillin	Yellow
β -Naphthol	Yellow
2,4-Dinitrophenol	Yellow
Vanillic acid	Yellow
Salicylic acid ^a	Yellow
Pieric acid	Yellow
<i>p</i> -Hydroxybenzoic acid ^a	Yellow
Quercetin	Yellow
<i>p</i> -Hydroxybenzaldehyde ^a	Yellow
Phloroglucinol	Yellowish brown
3,3',4-tri- <i>O</i> -methylgallagic acid	Yellowish brown
Guaiacol	Bluish white
Thiosalicylic acid ^a	Grey
<i>p</i> -hydroxy/biphenyl	Reddish yellow
Protocatechuic acid	Red

^a Colour produced after 15 min.

which could detect various types of phenolic compounds. Compounds which have a free position *ortho* to a phenolic hydroxyl group (a prerequisite which is met by most phenolic compounds) can be converted by nitrous acid into nitrosophenols, which are detected by coloured cobalt(III) chelate compounds². Both these reactions are achieved by spraying with a solution of sodium cobaltinitrite in acetic acid. This reagent is capable of detecting different types of phenolic compounds as listed in Table I. The colours produced are reported at 2 μg concentration, although the reagent appears to be sensitive at concentrations down to 0.5 μg for many of the phenolic compounds. Yellow to brown spots were observed against a light blue background after heating the developed plates for 5 min at 105°.

A deactivation effect was observed when a carbonyl group was present in the position *ortho/para* to the single phenolic hydroxyl group. Thus, salicylic acid, *p*-hydroxybenzoic acid and *p*-hydroxybenzaldehyde did not produce the colour immediately.

Preparation of the reagent

Sodium nitrite (4.4 g) was dissolved in 10 ml of water and a solution was added containing 2.6 g of cobalt nitrate dissolved in 2 ml of glacial acetic acid and made up to 10 ml with water. The solution obtained was diluted with an equal volume of glacial acetic acid and further diluted with an equal volume of water.

*Department of Chemistry and Biochemistry,
Punjab Agricultural University,
Ludhiana (India)*

I. S. BHATIA
K. L. BAJAJ
A. K. VERMA
JOGINDER SINGH

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