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Note

Detection of thiols on thin-layer chromatograms with 3,5-di-tert.-butyl-1,2-benzoquinone–iron(III) chloride

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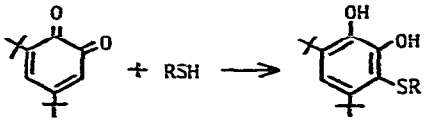
5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB) has been used for selective detection of thiols on chromatograms as yellow spots[1]. The colour is that of the thiophenolate anion of 2-nitro-5-mercaptobenzoate formed by a disulphide exchange reaction[1] and therefore the reagent gives the same colour with different thiols.

o-Quinones are known to react readily with thiols to give catechol thioethers[2]. Thus, reaction of 3,5-di-*tert.*-butyl-1,2-benzoquinone (I) affords 6-thioether derivatives of 3,5-di-*tert.*-butylcatechol (II)*. Catechols can form coloured chelates with iron(III) ion. We report here that thiols can be detected with high sensitivity and specificity by spraying chromatograms with quinone I followed by iron(III) chloride solution and that cysteine and glutathione (reduced) can be differentiated from each other by their characteristic colours.



I II

EXPERIMENTAL

Reagents

Quinone solution. A 0.1% solution of 3,5-di-*tert.*-butyl-1,2-benzoquinone (Aldrich, Milwaukee, Wisc., U.S.A.) in 2-butanone was prepared.

Iron(III) chloride solution. A 1.6% solution of iron(III) chloride hexahydrate in ethanol was prepared.

Procedure

Appropriate amounts of compounds were spotted on pre-coated cellulose thin-layer plates (E. Merck, Darmstadt, G.F.R.) of length 10 cm and the chro-

* The reaction of cysteine with quinone I gave 3-*S*-cysteinyl-4,6-di-*tert.*-butylcatechol, for which correct elemental analyses were obtained.

matogram was developed in 1-butanol–acetic acid–water (12:3:5) until the solvent front had migrated 7–8 cm past the origin. After being dried, the chromatogram was sprayed with the quinone solution and subsequently after 1 min at room temperature, with the iron(III) chloride solution.

RESULTS AND DISCUSSION

Table I summarizes the results. Most of the thiols tested gave a positive reaction to the reagent, giving different colours. Amounts of cysteine, cysteamine, glutathione and 2-mercaptopurine of 2 nmole were easily detected on a yellow background, which gradually darkened.

TABLE I

SENSITIVITIES AND COLOURS OF THIOLS AND OTHER COMPOUNDS ON THIN-LAYER CHROMATOGRAMS OBTAINED WITH QUINONE–IRON(III) CHLORIDE SPRAY REAGENT

Compound	*Sensitivity**	*Colour*
Cysteine	2:+, 5:++	Dark green
Cysteamine	2:+, 5:++	Dark green
Glutathione (reduced)	2:+, 5:++	Pinkish orange
Ergothioneine	5:±, 10:+	Dark green
2-Mercaptopurine	2:+, 5:++	Grey
6-Mercaptopurine	20:—, 50:±/+	
Cysteinesulphinic acid	20:+, 50:++	Orange**
Cysteic acid	100:—	
Cystine	100:—	
Methionine	20:—, 50:±	
3,4-Dihydroxyphenylalanine	2:+, 5:++	Dark green**
Tryptophan	20:—/±, 50:±	Orange
Histidine	50:—, 100:—/±	
Tyrosine	100:—	
Lysine	50:—, 100:—/±	
Arginine	50:—, 100:—/±	

* Numbers are amounts (nanomoles) of compounds spotted; —, not detected; ±, faint; +, weak but easily detectable; ++, clear.

** The same colours were also produced with iron(III) chloride only.

Catechols such as 3,4-dihydroxyphenylalanine can be easily distinguished from thiols by their positive test with iron(III) chloride. Although compounds with nucleophilic functional groups such as indoles and thioethers were detectable at levels of 50–100 nmole, their spots were dull and faint, and were thereby distinguishable from those of thiols. However, if these compounds were allowed to react with the quinone for 10 min, their sensitivities to iron(III) chloride increased several-fold. Therefore, in order to achieve a higher specificity for thiols, it is essential to spray the chromatograms with iron(III) chloride exactly 1 min after spraying with the quinone.

Under similar conditions, the DTNB reagent detected cysteine and glutathione with sensitivities comparable to those obtained in the present method, but it did not react with ergothioneine and 2-mercaptopurine, probably owing to their olefinic

character[1]. The marked difference between the reactivities of 2-mercaptopurine and 6-mercaptopurine indicates that the sensitivity of the quinine–iron(III) chloride reagent to heterocyclic thiols is also dependent on the thiol–thione tautomerism.

In conclusion, the quinone–iron(III) chloride reagent is a selective and sensitive spray reagent for detection of thiols on thin-layer chromatograms. An advantage of this reagent over other reagents for thiols and other sulphur-containing compounds such as DTNB, platinum(IV) iodide[3] and sodium nitroprusside[3] is that it can differentiate between cysteine and glutathione, which are difficult to separate from each other on thin-layer chromatograms.

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