Reagents for the detection of antioxidants on thin layers of silica

Antioxidants are used in foodstuffs and plastic materials to prevent autoxidation. Because only a few antioxidants are permitted in foodstuffs and food packaging materials, methods for their detection became necessary. Usually the antioxidants are extracted from the food or plastic with suitable solvents, followed by separation on thin layers of silica gel[1–5]. The detection and identification may be hindered by the fact that the reagents used are not specific for antioxidants: they may also produce coloured spots with other extracted substances or they may not react with all antioxidants. Most investigators therefore use a range of reagents.

In this paper some reagents are suggested and in Table I the colours obtained with reagents that produce different colours with different antioxidants are listed for a number of antioxidants. In these cases 20 μg of antioxidant were spotted and the silica-covered chromatoplate was sprayed after elution. (For solvents see refs. 3 and 5.) The colours may be different when other quantities of antioxidant are used.

Detection reagents

Reagents which can be used for the detection of antioxidants can be divided into groups as follows:

(A) Chemicals which produce colours when reduced; since antioxidants are used to prevent oxidation they are reducing substances.

(B) Substances which can be coupled to phenols (many antioxidants are substituted phenols): (1) diazo-compounds; (2) aromatic aldehydes; (3) Gibbs reagent, which forms indophenols that form coloured salts with alkali.

(C) Stable free radicals, which accept a hydrogen radical from the antioxidant.

(D) Substances that form coloured addition compounds.

Group A

(1) *Potassium permanganate.* Potassium permanganate produces yellow spots on a pink background with all oxidisable substances. It is therefore not specific for antioxidants.

It is prepared by dissolving a few grains of potassium permanganate in 10 ml acetone.

(2) *Ferric–ferricyanide.* A mixture of ferric sulphate and ferricyanide is turned into Prussian blue by reducing agents. It is specific for antioxidants but gives the same blue colour with nearly all of them. A disadvantage is that some time after spraying the whole chromatoplate turns blue.

It is prepared by mixing one volume of an 0.5 % ferric sulphate (anhydrous) solution in 1 N H_2SO_4 with one volume of an 0.2 % aqueous solution of potassium ferricyanide.

(3) *Dipyridyl.* BURTON[6] suggested a modification of type of reaction with the ferric–ferricyanide reagent by using dipyridyl, which gives a permanent white background. The spots with all antioxidants are red-brown.

One volume of an 0.5 % ferric sulphate (anhydrous) solution is mixed with one volume of an 0.5 % solution of α,α-dipyridyl in methanol.

(4) *Phosphomolybdic acid.* This reagent develops blue spots with reducing agents;

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TABLE 1

COLOURS OBTAINED WITH DIFFERENT ANTIOXIDANTS ON SILICA COVERED CHROMATOPLATES AFTER SPRAYING WITH DIFFERENT REAGENTS

Antioxidant	*Colour with reagent number*								
	2	*4*	*5*	*6*	*7*	*8*	*9*	*11*	*12*
Propyl gallate	blue	blue	yellow	red	pink	pink	purple	grey	brown
Octyl gallate	blue	blue	yellow	chocolate	pink	pink	brown	grey	brown
Dodecyl gallate	blue	blue	yellow	orange	pink	pink	grey	grey	brown
Nordihydroguaiaretic acid	blue	blue	rustbrown	brick red	purple	pink	brown	grey	chocolate
Gum guaiac	blue	blue	orange	redbrown	light purple	purple	brown	brown	chocolate
2,4,5-Trihydroxybutyrophenone	blue	blue	ochre	brown	grey	ochre	brown	brown	chocolate
Stearoyl-*p*-aminophenol	blue	blue	grey	brown	grey	—	purple	—	ochre
4,4′-Butylene-bis-(6-*tert.*-butyl-3-methylphenol)	blue	blue	brown	rustbrown	violet	purple	brown	pink	violet
4,4′-Thio-bis-(16-*tert.*-butyl-3-methylphenol)	blue	blue	brown	rustbrown	claret	red	brown	rustbrown	brown
2,2′-Dihydroxy-3,3′-dicyclohexyl-5′5-dimethyldiphenylmethane	blue	purple	light purple	pink	brown	grey	yellow	yellow	black
Dilauryl thiodipropionate	blue*	—	—	yellow	—	bluish	grey	yellow	—
Butylidene-2,2-bis-(octylthioglycolate)	blue*	—	—	yellow	—	bluish	—	yellow	—
Diphenyl-*p*-phenylenediamine	blue	blue	violet	ochre	yellow	green	grey	ochre	grey
2-Naphthyl-*p*-phenylenediamine	blue	blue	rustbrown	redbrown	green	green	pink	brownish	blue
2-*tert.*-Butyl-4-hydroxyanisole	blue	blue	brick red	violet	brown	brown	violet	—	pink
4,4′-Cyclohexylidene-bis-(2-cyclohexylphenol)	blue*	blue	orange	brown	buff	rustbrown	violet	grey	chocolate

4,4′, 4″-Tris-(6-*tert.*-butyl-*m*-cresol)-1,3,3-crotonaldehyde	blue*	blue	orange	brown	violet	violet	brown	pink	brown
2-Hydroxy-4-alkylbenzophenones	blue	—	yellow	orange	yellow	brown		yellow	yellow
2-(2′-Hydroxy-5′-alkylphenyl)-benzotriazoles	blue	—	ochre	violet	yellow	—	grey	yellow	light orange
Phenyl-α-naphthylamine	blue	blue	rustbrown	brown	yellow	greenish yellow	rustbrown	ochre with blue rim	blue
Phenyl-β-naphthylamine	blue	blue	violet	purple	yellow	yellow	blue	yellow	green
2,2′-Methylene-bis-(4-methyl-6-*tert.*-butylphenol)	blue	violet	yellow	ochre	green	red	brown	violet greenish	brown
2,2′-Thio-bis-(4-methyl-6-*tert.*-butylphenol)	blue	blue	rustbrown	brick red	brown	brown	bluish	grey	brown
2,6-Di-*tert.*-butylphenol	green	blue	yellow	yellow	purple	violet	grey	grey	brown
2,6-Di-*tert.*-butyl-4-methylphenol	blue	purple	—	yellow	purple	violet	grey**	grey	brown
2,2′-Propylene-bis-(4,4′,6,6′-*tert.*-butylphenol)	blue	blue	yellow	yellow	buff	pink	bluish grey	—	purple
1,3,5-Trimethyl-2,4,6-tris-(3,5-di-*tert.*-butyl-4-hydroxybenzyl)-benzene	bluish purple	purple	yellow	yellow	violet	blue	buff	—	red
n-Octadecyl β-(4′-hydroxy-3,5-di-*tert.*-(butylbenzene)-propionate	blue	blue	yellow	pink	violet	blue	brownish violet	—	brown
Tris-(nonyl-phenyl)-phosphite	blue	blue	buff	claret	pink	pink	purple*	—	brown
Di-octylphenylphosphite	blue	blue	buff	orange	pink	pink	blue	—	brown
2,2′-Methylene-bis-[6-(2-methylcyclohexyl)-4-methylphenol]	blue	violet	ochre	pink	purple	buff	brown	grey	brown

* Colour develops only after heating the chromatoplate.
** Mixed colour of green, brown and pink.

it is not specific for antioxidants and though the colour may differ from purple to green-blue for various substances it generally gives dark blue spots.

10 g of phosphomolybdic acid are dissolved in 90 % ethanol. The plate is heated for 10 min at 105°.

Group B(1)

(5) *Diazo-reagent.* Diazotized *p*-nitroaniline has been used for a long time for the detection of aromatic compounds; with antioxidants it forms yellow to brown spots.

800 mg *p*-nitroaniline are dissolved in a mixture of 250 ml water and 20 ml hydrochloric acid (25 %). A 5 % sodium nitrite solution is added dropwise until the solution is colourless.

(6) *Red salt.* Some typical colours are obtained with some types of antioxidant when red salt is used, *e.g.* orange spots are given by benzophenone derivatives that are used as U.V. absorbers[7] in plastics and claret spots by phosphites.

It is prepared by dissolving successively in 10 ml water 100 mg sodium acetate and 200 mg red salt A.L. (C.I. 37275).

Group B(2)

Coupling of antioxidants with aromatic aldehydes has the disadvantage that the colour is not very reproducible, but it may be useful because different bright colours can be obtained.

(7) *Anisaldehyde.* This is prepared by dissolving 500 mg *p*-methoxybenzaldehyde (anisaldehyde) in a mixture of 10 ml glacial acetic acid and 85 ml methanol and adding 5 ml concentrated sulphuric acid.

The plate is heated for 10 min at 105°.

(8) *Vanillin.* 400 mg vanillin are dissolved in a mixture of 95 ml methanol and 5 ml concentrated sulphuric acid. The plate is heated for 10 min at 105°.

Group B(3)

(9) *Gibbs reagent.* Though it is not specific for antioxidants it develops very typical colours, particularly with some antioxidants which do not give specific colours with other reagents (*e.g.* butylhydroxytoluene).

It consists of 100 mg 2,6-dichloro-*p*-benzoquinone-4-chlorimine in 100 ml ethanol. Spraying of the chromatoplate with this solution is followed by a spray of a 2 % borax solution in 50 % ethanol.

Group C

(10) *α,α-Diphenyl-β-picrylhydrazyl.* WOGGON *et al.*[4] described this reagent for the quantitative determination of antioxidants on chromatoplates: the violet-coloured stable free radical α,α-diphenyl-β-picrylhydrazyl is decolorised stoichiometrically by antioxidants. All the spots are yellow against a deep violet background.

It is prepared by dissolving 100 mg α,α-diphenyl-β-picrylhydrazyl in 100 ml 96 % ethanol.

Group D

(11) *Palladium chloride.* Palladium chloride forms addition compounds with many aromatic compounds, the colours of which may differ.

150 mg palladium chloride are dissolved in 100 ml 0.2 *N* hydrochloric acid.

(*12*) *Antimony pentachloride.* 20 ml antimony pentachloride are mixed with 80 ml carbon tetrachloride. The plate is heated for 10 min at 105°.

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The cytogenetics of *Lotus*

XII. Thin-layer chromatography in the separation of secondary phenolic compounds in *Lotus* (Leguminosae)

The successful separation of secondary phenolic compounds in *Lotus* through the use of the Shandon thin-layer chromatographic equipment and silica gel G as the coating material has recently been reported by GRANT AND WHETTER[1]. The availability of commercially prepared coated plates would eliminate the initial time needed to learn the technique required in order to obtain a satisfactory coating on the plates as well as the time required for the messy preparation of the silica gel coating on the plates. This note reports the results obtained in the separation of secondary phenolic compounds in *Lotus* using the techniques reported in the earlier paper by GRANT AND WHETTER[1] but using prepared Eastman Chromagram sheets, Type K301R with a fluorescent indicator, and the Eastman Chromagram Developing Apparatus (Eastman Organic Chemicals, Distillation Products Industries, Rochester, New York).

Preliminary tests

Samples of fresh leaves of *Lotus* were prepared by weighing out 0.08 g and leaving them in 0.5 ml of 1 % hydrochloric acid in methanol at room temperature, in the dark, overnight. The plates were prepared for development by applying an approximately 7 μl spot of sample solution with a micropipette at a distance of 2.0 cm from the base. Two spots were run for each sample. Ascending development was carried