

**INTERNATIONAL UNION OF
PURE AND APPLIED CHEMISTRY**

APPLIED CHEMISTRY DIVISION

FOOD SECTION

**SURVEY OF ANALYTICAL
METHODS AVAILABLE FOR THE
ESTIMATION OF SOME FOOD
ADDITIVES IN FOOD**

**LONDON
BUTTERWORTHS**

APPLIED CHEMISTRY DIVISION
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ADDITIVES IN FOOD**

Chemical problems which require study in relation to the use of food additives fall into two main groups:

- (1) tests for identification, specification and analytical methods required to establish the purity of food additives to ensure that they conform to appropriate regulatory requirements:
- (2) analytical methods for the measurement of food additives in treated food.

When in 1965 the programme of the Food Additive Commission (now the Food Additives and Contaminants Commission) of the Food Section was under consideration, the FAO/WHO Expert Committee on Food Additives had already published a series of Reports which set out specifications, together with tests for identity and analytical methods for ensuring the conformity to these specifications for a large number of food additives then in use¹⁻⁵. A number of similar reports have appeared since⁶⁻¹¹.

The Commission therefore decided to study the analytical methods available for the estimation of food additives in treated food. An extensive list of additives was examined and from this the following were selected for study: ammonium persulphate, ascorbic acid and ascorbates, benzoic acid and benzoates, butylated hydroxyanisole, butylated hydroxytoluene, ethylene and propylene oxides, gallates, hydroxybenzoic acid and its esters, nordihydroguaiaretic acid, potassium bromate, propionates, sorbic acid, sorbitol, sulphur dioxide and sulphites, tartaric acid and tartrates and the tocopherols. The first step was to ascertain which of these were permitted for food use in the various member countries; and, if permitted, what analytical methods for food were used. It soon appeared that there were not, as a rule, any standard or official analytical methods. Where analysis of food was considered desirable, the choice of the method was often left to the individual analyst. The Chairman of the Section the late Dr Alastair Frazer therefore invited the Laboratory of the Government Chemist in London to suggest a method of analysis suitable for each of these additives in the foods which were likely to be treated. The methods suggested were then circulated to all other member countries, who were asked to suggest any alternative methods that they might use.

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At the same time the present secretary of the Section Dr A. J. Collings undertook an extensive review of the literature. From these various sources the following monographs were compiled. The references have been checked by Professor J. Reith and Professor W. Souci.

February 1970

H. EGAN
Chairman, Food Section

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AMMONIUM PERSULPHATE

Chemical name: Ammonium persulphate.

Food additive use: As a maturing agent for flour.

Physical properties: A colourless crystalline solid, soluble in water.

Analytical methods: Ammonium persulphate is an oxidizing agent and this property is used for its analysis.

(1) Titrimetric methods

(i) *Iron(II) sulphate method*—Excess standard iron(II) sulphate solution is added to the sample containing the ammonium persulphate and allowed to stand for one hour. After this time the excess iron(II) sulphate is estimated by titration with standard potassium permanganate¹.

(ii) *Potassium iodide method*—An aqueous solution of potassium iodide is added to a suspension of the sample in dilute sulphuric acid. The liberated iodine is titrated with standard sodium thiosulphate^{2, 3}.

Benzoyl peroxide and potassium bromate interfere with these methods.

(2) Other methods

(i) *Fluorescence*—Ammonium persulphate in flour and dough can be estimated by its property to re-oxidize leuco-fluorescein to the intensely fluorescent fluorescein⁴.

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ESTIMATION OF SOME FOOD ADDITIVES IN FOOD
ASCORBIC ACID AND ASCORBATES

Chemical name: Ascorbic acid; L-ascorbic acid; 3-keto-L-gulofuranolactone.
Synonym: Vitamin C.
Food additive use: As an antioxidant in emulsions of oils and fats and as an antibrowning agent in unprocessed cut fruits and fruit juices.

Physical properties: Colourless white solid m.pt 190°–192° (decomp) soluble in water, slightly soluble in ethanol.

Analytical methods: Ascorbic acid is very easily oxidized to dehydroascorbic acid and in some methods care must be taken to keep oxidation down to a minimum. However, in analytical methods where the first stage is the oxidation of ascorbic acid, prevention of oxidation in the clean-up procedure is unnecessary. Many analytical methods are suitable for use directly on the sample under examination without any clean-up.

(1) Clean-up methods

(i) *Oxalic acid method*—Ascorbic acid is present in fruits, fruit juices and vegetables, and is readily extracted by homogenization of the tissues in aqueous oxalic acid, followed by filtration^{1,2}.

(ii) *Phosphoric acid method*—The tissue is ground with a mixture of tin(II) chloride in phosphoric acid or aqueous phosphoric acid. The extract contains ascorbic acid and dehydroascorbic acid^{3,4}.

(2) Chromatographic separation methods

(i) *Paper chromatography*—Several solvent systems have been reported. Phenol/sodium citrate/potassium dihydrogen phosphate; aqueous oxalic acid saturated with butanol/acetonitrile/acetone/ten per cent metaphosphoric acid. This method of separation is subject to errors due to oxidation in the drying stage. These may be reduced by drying the paper by vacuum high-frequency induction heating, or a correlation factor should be used^{1,5,6,7}.

(ii) *Thin layer chromatography*—This method has been used in the clean-up of derivatives of ascorbic acid prior to the final analytical stage^{8,9,10}.

(iii) *Column chromatography*—This technique has been used in the clean-up of the 2,4-dinitrophenyl hydrazine derivatives of dehydroascorbic acid formed following the oxidation of ascorbic acid¹¹.

(iv) *Detection of spots on paper chromatograms*—

(a) *Phosphomolybdic acid reagent*. Ascorbic acid is a reducing agent and will reduce phosphomolybdic acid to the heteropolymolybdenum blue complex^{11,12}.

(b) *2,6-Dichlorophenolindophenol method*. This again depends upon the reducing properties of ascorbic acid. The reagent is decolorized by the ascorbic acid, which is itself oxidized to dehydroascorbic acid^{12,13,14,15,16}.

(c) *2,4-Dinitrophenyl hydrazine derivative*. The derivative of dehydroascorbic

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acid is intensely coloured and spraying with sulphuric acid increases sensitivity^{3, 11, 17, 18}.

(v) *Elution of spots from chromatograms*—Ascorbic acid and dehydroascorbic acid may be extracted with aqueous oxalic acid. The 2,4-dinitrophenyl hydrazine derivative of dehydroascorbic acid may be extracted with 85 per cent sulphuric acid^{1, 3, 9}.

(vi) *Gas chromatography*—Ascorbic acid can be determined by the gas chromatography of the trimethyl silyl derivative³⁸.

(3) Colorimetric methods

(i) *2,6-Dichlorophenolindophenol method*—This method depends on the oxidation of ascorbic acid to dehydroascorbic acid by 2,6-dichlorophenolindophenol, which is reduced with a consequent colour change^{12, 13, 14, 15, 16}.

(ii) *2,4-Dinitrophenyl hydrazine method*—The oxidation product of ascorbic acid—dehydroascorbic acid condenses with 2,4-dinitrophenyl hydrazine to give a coloured compound which can be analysed spectrophotometrically. The ascorbic acid is oxidized by bromine prior to the analysis. Dehydroascorbic acid may be analysed in the presence of ascorbic acid by omitting the oxidation stage and adding thiourea before the addition of the 2,4-dinitrophenyl hydrazine reagent. For milk most interfering substances can be removed by precipitation with acetic acid/metaphosphoric acid solution^{3, 11, 17, 18, 19, 20, 21}.

(iii) *Phosphomolybdic acid reagent*—Ascorbic acid reduces phosphomolybdic acid to the intensely coloured molybdenum blue. This is a sensitive method for all reducing agents^{22, 23, 24}.

(iv) *Diazotized 4-chloro-2-nitroaniline method*—This method depends upon the coupling reaction between ascorbic acid and the reagent¹².

(v) *Diazotized 4-methoxy-2-nitroaniline method*—Another coupling reaction of ascorbic acid which gives an intensely coloured product^{25, 26, 27}.

(vi) *2,2-Biphenyl-1-picrylhydrazyl method*—This reagent is a stable free radical which is decolorized by ascorbic acid and several other food additives with anti-oxidant properties²⁸.

(4) Titrimetric methods

(i) *Iodine/thiosulphate method*—This method is based on the reduction of iodine to iodide by ascorbic acid. Standard iodine solution is added to the sample and the excess is back-titrated with either standard thiosulphate or *N*-bromosuccinimide. Free sulphur dioxide can be masked with glycolaldehyde^{29, 30, 31, 32, 33, 34}.

(ii) *Vanadometric method*—This method depends upon the reduction of ammonium vanadate by ascorbic acid. Excess vanadate is back-titrated with standard ammonium iron(II) sulphate³⁵.

(iii) *2,6-Dichlorophenolindophenol method*—Ascorbic acid reduces 2,6-dichlorophenolindophenol with a change of colour. At the end point the indophenol is no longer decolorized. This method can be used without prior clean-up of the sample. However, in the presence of intensely coloured impurities the end point is not clear. This may be overcome by the addition

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of ether to the titration system and at the end-point, when there is unchanged indophenol present, the ether layer becomes coloured. Traces of iron(II) and tin(II) can be prevented from interfering by buffering in acetate solution, oxidation by air and chelation with EDTA before titration^{12, 14, 15, 36, 37}.

(iv) *Mercurimetric titration*—Ascorbic acid reduces mercury(II) chloride to the insoluble mercury(I) chloride. The precipitate may be analysed by adding potassium iodide and titrating the liberated iodine with thiosulphate. Alternatively, the excess mercury(II) chloride may be titrated with EDTA using Eriochrome Black T as the indicator. This method is suitable for coloured samples^{36, 39, 40}.

(v) *Complexometric titration*—Ascorbic acid is reacted with copper acetate. The precipitate thus obtained is dissolved in nitric acid and the copper titrated with EDTA using PAN as indicator⁴¹.

(5) Polarographic method

Ascorbic acid is determined from the anodic wave produced in a phosphate buffer at pH 6.8, or in 0.01M oxalic acid/diphthalate buffer at pH 4.5. Dehydroascorbic acid is determined by the increase in wave height following reduction to ascorbic acid^{2, 42, 43, 44, 45, 46}.

(6) Other methods

(i) *Fluorescence*—The ascorbic acid is oxidized to dehydroascorbic acid with indophenol solution. The solution is separated by paper chromatography and the dried paper washed with *o*-phenylene diamine. The chromatogram is developed a second time to separate the coupled product from excess reagent. The vitamin C is determined from its fluorescence photographically^{47, 48, 49}.

(ii) *Turbidimetric determination*—This method is based on the capacity of ascorbic acid to reduce silver nitrate to silver. The limit of detection is 1 µg/ml⁵⁰.

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BENZOIC ACID AND BENZOATES

Chemical name: Benzoic acid; benzenecarboxylic acid.

Food additive use: As a preservative.

Physical properties: Colourless crystalline solid m.pt 122° soluble in hot water, ethanol and chloroform.

The u.v. absorption $E_{1\text{cm}}^{1\%} = 814.6$ at λ_{max} , 230 nm solvent water.

ANALYTICAL METHODS**(1) Clean-up methods**

(i) *Extraction*—Benzoic acid is readily isolated from most food by extraction of an acidified sample with diethyl ether or cyclohexane. With certain foods an emulsion may form, but this can often be broken by the addition of petroleum ether or sodium chloride^{1, 2, 3}.

(ii) *Steam distillation*—Benzoic acid can be quantitatively isolated from the acidified sample of food by steam distillation⁴.

(2) Chromatographic methods

(i) *Paper chromatography*—Several solvent systems have been reported for the separation of benzoic acid from other additives; suitable systems include butanol/ammonia/water/ethanol and cyclohexanol/ammonium carbonate^{1, 2, 5, 6, 7, 8, 9}.

(ii) *Thin layer chromatography*—Benzoic acid may be isolated from the extract or obtained as a distillate from food. A suitable solvent system is hexane/acetic acid using Kieselgel, silica gel or GF 254 as absorbent⁴.

(iii) *Detection of spots*—

(a) *Mohler reaction*. Benzoic acid is treated with potassium nitrate in acid solution. The resulting dinitrobenzoic acid is reduced with sodium or ammonium sulphide to the nitro-amino- or diamino-benzoic acid, which has a vivid reddish brown colour^{2, 10}.

(b) *Iron(III) hydroxylamine reaction*. Benzoic acid is treated with thionyl chloride and hydroxylamine to form a hydroxamic acid which gives a colour with iron(III) ions¹¹.

(c) *Fluorescence*. Benzoic acid fluoresces when irradiated with ultra-violet light. Thus the spot may be detected by a non-destructive method. The benzoic acid can then be eluted for quantitative assay.

Fluorescence can be enhanced by spraying the paper after chromatography with 0.05 per cent fluorescein in 50 per cent aqueous methanol⁸.

(iv) *Gas chromatography*—The extracted benzoic acid is methylated and the methyl ester identified by gas chromatography^{12, 13, 14, 15, 16, 17}.

(v) *Ion exchange*—Benzoic acid can be separated from other aromatic compounds by absorption on to an ion exchange resin¹⁸.

(3) Spectrophotometric method

(i) *Ultra-violet*—Benzoic acid can be estimated by its ultra-violet absorption at 228 nm in acid solution. Alternatively, it can be oxidized with chromic acid and the product determined by its absorption at 272 nm^{18, 19, 20, 21, 22, 23}.

(4) Titrimetric method

If the clean-up method yields only benzoic acid and no other acidic substance, then it may be estimated by titration against standard base²⁴.

(5) Polarographic method

The benzoic acid is treated with 1:1 sulphuric acid, nitric acid at 100°. After the addition of water and gelatin solution the benzoic acid derivation is determined polarographically²⁷.

(6) Other methods

(i) *Electrophoresis*—Benzoic acid can be distinguished from other food additives by its electrophoretic migration. Buffer systems reported have been dimethylformamide/pyridine/acetic acid/water or butan-1-ol/ammonia/water^{5, 6}.

(ii) *Microbiological assay*—Benzoic acid inhibits the growth of certain micro-organisms, but the inhibition is pH dependent. This has been used for distinguishing benzoic acid from other additives^{24, 25, 26}.

(iii) *Sublimation*—Benzoic acid may be separated from saccharin by sublimation²⁸.

(iv) *Distinction between benzoic acid and sorbic acid*—Sorbic acid is readily brominated, whereas benzoic acid is not. Thus the relative retention on the paper chromatogram in sorbic acid is significantly changed by bromination, whereas that for benzoic acid is not. Their ultra-violet absorption spectra are also different^{19, 20, 29}.

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BENZOYL PEROXIDE

- Chemical name:** Benzoyl peroxide.
- Food additive use:** Bleaching agent for flour and milk for the production of cheese. The residue in flour is benzoic acid.
- Physical properties:** A colourless solid; m.pt 106°; slightly soluble in water ethanol, soluble in ether, acetone and benzene. The u.v. absorption $E_{1\text{cm}}^{1\%} = 1111$ at λ_{max} . 230 nm solvent cyclohexane.

ANALYTICAL METHODS

Benzoyl peroxide is an oxidizing agent. The methods of analysis either make use of its oxidizing properties or the benzoyl peroxide is reduced to benzoic acid, which is then assayed.

(1) *Reduction to benzoic acid*—An ethereal suspension of the food sample is reduced with iron/aqueous hydrochloric acid. The benzoic acid formed is estimated¹.

(2) *Titrimetric method*—An aqueous solution of potassium iodide is added to the acetone extract containing the benzoyl peroxide. The liberated iodine is immediately titrated against standard thiosulphate².

(3) *Polarographic method*—This is a specific and very sensitive method for benzoyl peroxide⁴.

(4) *Colour test*—If benzoyl peroxide is present in flour, a blue-green colour is produced when a few drops of one per cent alcoholic 4,4'-diaminodiphenylamine solution are added³.

(5) *Other methods*—(i) *Leucomethylene blue method*. Benzoyl peroxide can be estimated by using it to oxidize leucomethylene blue to the intensely coloured methylene blue⁵.

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BUTYLATED HYDROXYANISOLE

Chemical name: A mixture of 3- and 5-tertiarybutyl 4-hydroxyanisole (BHA). All commercial samples contain about 94 per cent of the 3-isomer.

Food additive use: As an antioxidant in fats.

Physical properties: A white waxy solid insoluble in water, but freely soluble in methanol and ether; m.pt not less than 48°.

334 at λ_{\max} . 228 nm.

The u.v. absorption $E_{1\text{cm}}^{1\%} = 205$ at λ_{\max} . 291 nm.

solvent 80 per cent ethanol.

ANALYTICAL METHODS**(1) Clean-up method**

BHA may be readily extracted from fats by extraction with methanol, ether or aqueous ethanol. It may be further purified by steam distillation. For gas chromatographic techniques BHA may be extracted from some products with carbon disulphide^{1, 2, 3, 4, 5}.

(2) Chromatographic methods

(i) *Paper chromatography*—This is useful for the detection of BHA and other antioxidants in food. Several solvent systems have been reported which are suitable for the detection of several different antioxidants simultaneously^{6, 7}.

(ii) *Thin layer chromatography*—BHA, together with several other antioxidants, can be analysed using thin layer chromatography. A suitable solvent system is petroleum ether/benzene/acetic acid (1 : 2 : 1) using Kieselgel HG as absorbent^{4, 8, 9}.

(iii) *Gas chromatography*—BHA has been analysed by gas chromatographic techniques. This is a rapid and sensitive method. Under certain conditions both BHT and BHA may be determined simultaneously in the same sample^{2, 5, 10, 11, 12, 13}.

(3) Spectrophotometric methods

(i) *Ultra-violet*—BHA has a characteristic ultra-violet absorption and this is used for its analysis. By measurement of the absorption at 270 nm, 290 nm and 310 nm, any error due to interference from fat may be corrected¹⁴.

(ii) *Infra-red*—BHA as a solution in carbon disulphide shows a characteristic absorption band at 11.42 μ which may be used for analysis¹⁵.

(4) Colorimetric methods

(i) *Reaction with 2,6-dichloroquinone-4-chloroimide*—This reagent is fairly specific for BHA, but certain components from smoked foods also give a colour with this reagent^{1, 15, 16, 17, 18}.

(ii) *Sulphanilic acid method*—BHA couples with diazotized sulphanilic acid to give a purple colour in the presence of sodium hydroxide^{4, 19}.

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(iii) *2,2'-Bipyridyl reagent*—BHA reduces iron(III) ions to iron(II) which give a colour with the 2,2'-bipyridyl reagent¹.

(iv) *Phosphomolybdic acid*—BHA together with other antioxidants reduces phosphomolybdic acid to molybdenum blue^{3, 4, 16, 20}.

(v) *Nitroso method*—BHA is nitrosated directly in the oil and the nitroso product isolated by a combination of solvent extraction and liquid chromatographic techniques. The product is measured spectrophotometrically at 480 nm²¹.

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BUTYLATED HYDROXYTOLUENE

Chemical name: 3,5-ditertiarybutyl 4-hydroxytoluene (BHT).

Food additive use: As an antioxidant in fats.

Physical properties: White solid; m.pt 70°; soluble in ether, insoluble in water.

The u.v. absorption $E_{1\text{cm}}^{1\%} = \frac{83}{\text{solvent 80 per cent ethanol.}} \text{ at } \lambda_{\text{max.}} 278 \text{ nm.}$

ANALYTICAL METHODS**(1) Clean-up method**

BHT is freely soluble in ether and may be isolated efficiently from food by simple extraction in a Soxhlet apparatus. The extract may be further cleaned up by removal of the solvent followed by low temperature crystallization from methanol to remove excess fat, or by column chromatography. The final stage in the clean-up procedure is usually steam distillation^{1,2,3,4}.

(2) Chromatographic methods

(i) *Thin layer chromatography*—Thin-layer systems have been reported for the detection and estimation of BHT and other antioxidants. Suitable solvent systems are acetic acid/chloroform (1:2) using silica gel, methanol/carbon tetrachloride (3:7) using polyamide powder or chloroform alone or petroleum ether/benzene/acetic acid (1:2:1) using Kieselgel HF.

(ii) *Column chromatography*—BHT can be separated from fat by column chromatography on silica gel. Using benzene/petroleum ether (1:1) only BHT is eluted. Florisil can also be used as the absorbant^{18,23}.

(iii) *Gas chromatographic method*—Several different gas chromatographic systems have been reported for BHT. In general they are more rapid than chemical detection methods and extremely sensitive. Many of the reported systems are suitable for BHT and BHA^{9,10,11,12,13,14,15,16,17}.

(3) Spectrophotometric method

(i) *Infra-red*—BHT has a characteristic infra-red absorption spectrum. It contains a strong absorption band at 12.85 μ and this has been used as a method of analysis. It is not as sensitive as the colorimetric methods, but it is specific¹⁹.

(4) Colorimetric methods

(i) *2,2'-Bipyridyl reagent*—BHT reduces iron(III) ions to iron(II) which can be estimated by reaction with 2,2'-bipyridyl reagent. The complex derived from BHT takes 16 hours to develop^{3,4}.

(ii) *o-Dianisidine reagent*—BHT couples with diazotized *o*-dianisidine to give a coloured product. The test is not entirely specific for BHT^{4,20}.

(iii) *Phosphomolybdic acid*—BHT will reduce phosphomolybdic acid to molybdenum blue^{3,4,21}.

(iv) *Nitroso method*—BHT is nitrosated in the product and the nitroso

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derivative isolated by a combination of solvent extraction and liquid chromatographic methods. It is estimated spectrophotometrically at 480 nm²².

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ETHYLENE AND PROPYLENE OXIDES

- Chemical name:** Ethylene oxide. oxirane.
Propylene oxide.
- Food additive use:** As a fumigant.
- Physical properties:** Ethylene oxide: A colourless gas b.pt 13° at 780 mm.
Soluble in water and organic solvents.
Propylene oxide: A colourless liquid b.pt 35° 760 mm.

ANALYTICAL METHODS

(1) Clean-up method

The compounds can be extracted from food with a mixture of acetone/water (5:1)¹.

(2) Chromatographic methods

(i) *Gas chromatography*—Propylene and ethylene oxides are very volatile and suitable for analysis by gas chromatography. Several methods have been reported^{1, 2, 3, 4, 5}.

(3) Spectrophotometric method

(i) *Infra-red*—Ethylene and propylene oxides have characteristic infra-red spectra. The absorption bands have been used for analysis⁶.

(4) Titrimetric method

The respective oxide is heated with pyridine chloride in a pressure vessel. The acid produced is titrated against standard alkali⁷.

(5) Polarographic method

The oxides are converted to aldehydes by hydration in the presence of sulphuric acid to glycols followed by oxidation with periodate. The estimation is performed in 0.1N lithium hydroxide supporting electrolyte⁸.

(6) Colour tests

A number of colour tests have been developed for the spot testing of ethylene oxide. They are based on water-soluble salts which react with ethylene oxide to give products with higher pH which alters the colour of an incorporated indicator¹⁰.

(7) Other methods

(i) *Chlorohydrin method*—This method has been standardized for use in the determination of ethylene oxide in grain⁹.

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GALLATES PROPYL, OCTYL, DODECYL

Chemical name: Propyl gallate; *n*-propyl ester of 3,4,5-trihydroxybenzoic acid.

Octyl gallate; *n*-octyl ester of 3,4,5-trihydroxybenzoic acid.

Dodecyl gallate; *n*-dodecyl (or lauryl) ester of 3,4,5-trihydroxybenzoic acid.

Food additive use: As antioxidants in fats.

Physical properties: Propyl gallate: creamy white solid m.pt 147°. Slightly soluble in water, very soluble in ethanol.

The u.v. absorption $E_{1\text{cm}}^{1\%} = 497$ at $\lambda_{\text{max.}} 275.5$ nm.
solvent 80 per cent ethanol.

Octyl gallate: white to creamy white solid; insoluble in water, soluble in ethanol; m.pt about 101°.

The u.v. absorption $E_{1\text{cm}}^{1\%} = 373$ at $\lambda_{\text{max.}} 276$ nm.
solvent 80 per cent ethanol.

Dodecyl gallate: white waxy solid, m.pt about 96°; insoluble in water, soluble in petrol, methanol and fats.

The u.v. absorption $E_{1\text{cm}}^{1\%} = 370$ at $\lambda_{\text{max.}} 276$ nm.
solvent 80 per cent ethanol.

ANALYTICAL METHODS

(1) Clean-up method

(i) Propyl gallate is water-soluble and may be selectively extracted by water from a petrol extract of the food. The remaining gallates and other antioxidants may be extracted from the pre-treated solution with absolute methanol, or other solvents^{1, 2, 3, 4}.

(ii) Gallates together with several other antioxidants can be isolated by vacuum sublimation⁵.

(2) Chromatographic methods

(i) *Paper chromatography*—Several solvent systems have been developed for the separating of gallates and other antioxidants; carbon tetrachloride/methanol (4:1); petroleum ether/benzene/acetic acid (2:2:1), are suitable systems^{6, 7}.

(ii) *Thin layer chromatography*—The solvent system Shell Sol A/propanol/acetic acid/water (45:6:3:6) using ten per cent acetylated cellulose is suitable for gallate analysis^{8, 9}.

(iii) *Gas chromatography*—Propyl gallate can be estimated by gas chromatography together with other antioxidants⁵.

(3) Spectrophotometric method

(i) *Ultra-violet*—Gallates have absorption maximum at 276 nm and this may be used for their assay^{11,13}.

(4) Colorimetric methods

(i) *Phosphomolybdic acid*—Gallates, like other antioxidants, give a blue colour with phosphomolybdic acid².

(ii) *Iron(II) ion reaction*—Gallates give a coloured product with iron(II) ions which is specific for gallates^{1,2,3,6}. The propyl, octyl and dodecyl gallates can be separately determined¹.

(iii) *2,2'-Bipyridyl reagent*—This is a non-specific test for gallates, but is useful as a spray in thin layer and paper chromatography³.

(iv) *2,2'-Diphenyl-1-picryl hydrazyl method*—Propyl gallate gives a coloured product when reacted with 2,2'-diphenyl-1-picryl hydrazyl¹⁰.

(5) Other methods

(i) *Weisz ring oven method*—Dodecyl gallate can be estimated together with other antioxidants using the Weisz ring oven technique¹².

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HYDROXY BENZOATES METHYL, ETHYL OR PROPYL

- Chemical names:** Methyl *p*-hydroxybenzoate (methyl ester of *p*-hydroxybenzoic acid). Ethyl *p*-hydroxybenzoate (ethyl ester of *p*-hydroxybenzoic acid). Propyl *p*-hydroxybenzoate, (*n*-propyl ester of *p*-hydroxybenzoic acid).
- Food additive use:** As antimicrobial preservatives.
- Physical properties:** *Methyl p-hydroxybenzoate*: a white crystalline solid, m.pt 131°, slightly soluble in water, soluble in ethanol.
The u.v. absorption $E_{1\text{cm}}^{1\%} = 893$ at $\lambda_{\text{max.}} 252$ nm.
solvent ethanol.
- Ethyl p-hydroxybenzoate*: a crystalline white solid, m.pt 116°, soluble in ethanol.
The u.v. absorption $E_{1\text{cm}}^{1\%} = 860$ at $\lambda_{\text{max.}} 252$ nm.
solvent ethanol.
- Propyl p-hydroxybenzoate*: a crystalline white solid m.pt 96°, soluble in ethanol, insoluble in water.
The u.v. absorption $E_{1\text{cm}}^{1\%} = 840$ at $\lambda_{\text{max.}} 252$ nm.
solvent ethanol.

ANALYTICAL METHODS

(1) Clean-up methods

(i) *Extraction*—Alkyl *p*-hydroxybenzoate esters and *p*-hydroxybenzoic acid are readily extracted from an acidified sample with ether or chloroform^{1, 2, 3}.

(ii) *Steam distillation*—Alkyl *p*-hydroxybenzoate esters can be isolated from food by steam distillation^{4, 5}.

(2) Chromatographic methods

(i) *Paper chromatography*—Several solvent systems have been reported for the separation of hydroxybenzoates from each other and from food. Systems include cyclohexane/ammonium carbonate buffer; or butanol/ammonia/water (7:2:1) or (5:2:1)^{3, 6, 7, 8}.

(ii) *Thin layer chromatography*—Using a standardized solvent Shell Sol A/propanol/acetic acid/formic acid (45:6:3:6) and ten per cent acetylated cellulose/Kieselgel (8:2) as absorbent, alkyl *p*-hydroxybenzoates may be separated^{4, 9, 10, 11, 12}.

(iii) *Detection of spots*—Alkyl *p*-hydroxybenzoates fluoresce when irradiated with ultra-violet light. This is useful for the detection of spots on chromatograms. The fluorescence can be intensified by spraying the chromatogram with silver nitrate and quinine/hydrochloric acid^{7, 9}.

(iv) *Gas chromatography*—Ethyl hydroxybenzoate may be analysed by gas chromatography either directly or as the trimethyl silyl derivative^{4, 13, 14, 15}.

(v) *Ion exchange*—Benzoic acid and *p*-hydroxybenzoic acid may be

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resolved in Dowex 2 × 8 acetate form 200–300 mesh ion exchange by gradient elution using acetic acid/methanol mixtures^{16,17}.

(3) Spectrophotometric method

(i) *Ultra-violet*—Alkyl *p*-hydroxybenzoates may be assayed by their ultra-violet absorption^{3,4,9,17,18,19}.

(4) Colour tests

Alkyl *p*-hydroxybenzoates give a red colour with Millon's reagent (mercury dissolved in nitric acid)^{1,21}.

(5) Other methods

(i) *Electrophoresis*—Esters of *p*-hydroxybenzoic acid show electrophoretic mobility. Buffer systems used include dimethylformamide/pyridine/acetic acid/water pH 5.0 and butanol/ammonia/water^{6,20}.

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NORDIHYDROGUAIARETIC ACID

Chemical name: Nordihydroguaiaretic acid (NDGA); β -dimethyl- γ -bis-(3,4-dihydroxyphenyl)butane; 4,4'-(2,3-demethyl-tetramethylene)dipyrocatechol.

Food additive use: As an antioxidant in oils and foods.

Physical properties: NDGA is a white to grey crystalline solid, soluble in ethanol, insoluble in water.

ANALYTICAL METHODS**(1) Clean-up method**

The food is extracted with hexane or petrol and the NDGA is removed from this extract with 80 per cent ethanol; other antioxidants are extracted together with NDGA^{1, 2, 3}.

(2) Chromatographic methods

(i) *Paper chromatography*—Several methods have been reported: petroleum ether/benzene/acetic acid (2:2:1) or by 2-dimensional chromatography using benzene and acetonitrile, respectively^{4, 5}.

(ii) *Thin layer chromatography*—Several solvent systems have been reported: acetic acid/chloroform (1:2) using silica gel or methanol/carbon tetrachloride (3:7) using polyamide powder layer; using Keisegel HF chloroform can be used alone^{3, 6}.

(iii) *Detection methods*—

(a) NDGA may be detected by spraying the chromatogram with silver nitrate solution, which is subsequently reduced to give a black spot⁵.

(b) Similarly, NDGA can be detected by its ability to reduce iron(III) chloride⁷.

(c) NDGA gives a colour reaction with 2,6-dichloroquinone chloro-imide, which may be used for its detection⁷.

(d) *2,2'-Bipyridyl reagent*. This reagent reacts with NDGA and with most other antioxidants, but NDGA gives a colour within one minute, whereas with other antioxidants the colour takes longer to develop¹.

(3) Spectrophotometric method

(i) *Ultra-violet*—NDGA exhibits strong absorption in the ultra-violet and this may be used for its assay⁸.

(4) Titrimetric method

NDGA may be estimated by titration with cerium(IV) sulphate, the end point being detected potentiometrically or by use of a redox indicator^{1, 9}.

(5) Other methods

(i) *Weisz ring oven method*—NDGA may be estimated using the Weisz ring

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oven technique. Several other antioxidants can also be determined with this method¹⁰.

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POTASSIUM BROMATE

- Chemical name:** Potassium bromate.
- Food additive use:** As a maturing agent in flour or antimicrobial agent in cheese.
- Physical properties:** A colourless crystalline solid; soluble in water, slightly soluble in ethanol.

ANALYTICAL METHODS**(1) Clean-up methods**

When flour is treated with bromate the residue found in the bread is bromide. The residue can be extracted from the food with water or the whole sample ashed prior to analysis.

(3) Titrimeric methods

(i) *Iodometric method*—Potassium bromate oxidizes iodide ions to iodine which can be estimated by titration with thiosulphate^{2, 3, 4, 5, 6}.

(ii) *Hypovanadous method*—Potassium bromate can be estimated by redox titration against hypovanadous salts⁷.

(iii) *Bromatometry*—Potassium bromate is reduced with arsenic(III) oxide and the excess reagent back-titrated with potassium bromate⁸.

(3) Other methods

(i) *Rosaniline method*—The bread or other bakery product is wet ashed with potassium permanganate, nitric acid and silver nitrate. The resulting silver chloride and silver bromide are reduced and the bromide ions oxidized to free bromine. Fuchsin is brominated to give rosaniline which is estimated¹.

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PROPIONATES

- Chemical name:** Salts of propionic acid or of propanoic acid.
Food additive use: As mould inhibitors in many foodstuffs.
Physical properties of parent acid: Propionic acid is a colourless liquid b.pt 141° at 760 mm; freely soluble in water and in ethanol.

ANALYTICAL METHODS**(1) Clean-up methods**

Propionic acid can be steam distilled from the acidified sample of food. The distillate contains a number of volatile acids, but the specificity can be improved by collecting the distillate in a suspension of barium carbonate, followed by removal of the insoluble barium salts. The propionic acid can be recovered from the soluble salts by steam distillation after acidification^{1, 2, 3, 4, 5}.

(2) Chromatographic methods

(i) *Paper chromatography*—Propionic acid may be identified by paper chromatography; acetone/*t*-butanol, *n*-butanol/ammonia are suitable solvent systems^{1, 2, 6}.

(ii) *Column chromatography*—Propionic acid can be separated from other organic acids by chromatography on propylene glycol supported on silicic acid and eluted with butanol/petroleum ether^{1, 7}.

(iii) *Detection of spots*—Propionic acid may be detected by spraying the chromatogram with methyl red/bromothymol blue reagent².

(iv) *Gas chromatography*—Propionic acid is readily detected and analysed by gas chromatography^{1, 8, 9, 10, 11, 12}.

(v) *Ion exchange*—Propionic acid can be determined by chromatography on an ion exchange resin in the presence of a known amount of isovaleric acid which can be added to act as an internal standard^{1, 13}.

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SORBIC ACID

Chemical name: Sorbic acid; *trans*-2,4-hexadienoic acid.

Food additive use: As a preservative.

Physical properties: A white crystalline solid m.pt 133°; soluble in ethanol and chloroform.

The u.v. absorption $E_{1\text{cm}}^{1\%} = \frac{2250}{\lambda_{\text{max.}}}$ 254 nm.
solvent ethanol.

ANALYTICAL METHODS

(1) Clean-up methods

Sorbic acid may be steam distilled at 760 or 100 mm pressure from the acidified food sample. It can be isolated by extraction of the acidified distillate with ether or methylene chloride. It can be purified by sublimation^{1, 2, 3, 4, 5, 6, 7, 8, 9, 10}.

(2) Chromatographic methods

(i) *Paper chromatography*—Sorbic acid may be identified and analysed by paper chromatography. Suitable solvent systems are cyclohexane (saturated with ammonium carbonate) or *n*-butanol/ammonia/water (5:2:3). Prior bromination of the sorbic acid may be necessary to differentiate from benzoic acid^{11, 12, 13, 14, 15, 16, 17, 18}.

(ii) *Detection of spots*—Sorbic acid may be detected by its fluorescence under ultra-violet light; alternatively, the fluorescence can be intensified by treatment of the chromatogram with silver nitrate/quinoline¹⁶.

(iii) *Gas chromatography*—Sorbic acid can be identified and analysed by gas chromatography. The residue is mixed with methanol/sulphuric acid and the resultant methyl ester chromatographed^{6, 7, 19, 20, 21, 22, 23}.

(3) Spectrophotometric methods

(i) *Ultra-violet*—Sorbic acid exhibits ultra-violet absorption with a maximum at 255 nm in ethanol, which can be used for sorbic acid analysis. An indication of the background absorption can be made by measuring the optical density at 280 nm^{8, 10, 16, 24, 25, 26, 27, 28, 29, 30}.

(ii) *Infra-red*—Sorbic acid shows a characteristic infra-red absorption with strong bands at 1690 cm⁻¹ and 1000 cm⁻¹ (carbon tetrachloride) suitable for analysis^{31, 32}.

(4) Titrimetric methods

(i) *Bromination methods*—Sorbic acid is readily brominated. Bromine is added to the sample and the unreacted bromine back titrated³.

(ii) *Sodium perchlorate method*—Sorbic acid is reacted with aqueous sodium perchlorate and the excess back titrated with standard potassium iodide³⁹.

(5) Colorimetric methods

(i) *Thiobarbituric acid*—Sorbic acid is oxidized with potassium dichromate to give malonic dialdehyde, which, on heating with thiobarbituric acid, yields a red dye^{1, 2, 26, 33, 34, 35, 36, 17}.

(ii) *Resorcinol*—Sorbic acid is heated with resorcinol/sulphuric acid to give a product which is intense yellow in alkaline solution³⁷.

(iii) *Other colour tests*—Sorbic acid gives a colour when heated at 100° with *p*-hydroxybenzaldehyde, vanillin, or ethyl vanillin³⁸.

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SORBITOL

- Chemical name:** 1,2,3,4,5,6-hexanehexol.
Synonyms: *d*-Glucitol, *d*-sorbitol, *d*-sorbit.
Food additive use: As a substitute for glucose in foods.
Physical properties: Crystalline white solid, m.pt 165°; soluble in water, slightly soluble in ethanol.

ANALYTICAL METHODS**(1) Clean up method**

Sorbitol is readily isolated from a food sample by extraction with water.

(2) Chromatographic methods

(i) *Paper chromatography*—Sorbitol may be identified and analysed by paper chromatographic methods; suitable solvent systems include ethyl acetate/acetic acid/water (6:3:2) and butanol/ethanol/water^{1,2,3}.

(ii) *Thin layer chromatography*—Sorbitol can be determined in wine following its separation by thin layer chromatography: a suitable solvent system is butan-1-ol/acetone/water (4:5:1) or propan-2-ol/ethyl acetate/water/glacial acetic acid (27:3.5:1:0.5)^{4,5}.

(iii) *Column chromatography*—Sorbitol may be chromatographed on a column of Fuller's earth or siliceous earth^{6,8,9}.

(iv) *Detection of spots*—

(a) *Permanganate*. Sorbitol spots can be detected by the reduction of permanganate^{4,6}.

(b) *Sodium periodate*. Sorbitol and other polyols react with sodium periodate and this can be used for its detection on paper or thin layer chromatograms⁴.

(c) *4,5-Dichlorofluorescein method*. Sorbitol can be detected on chromatograms by spraying with 4,5-dichlorofluorescein, lead tetraacetate. The spots show up as bright yellow areas when irradiated with ultra-violet light^{3,7}.

(v) *Gas chromatography*—Sorbitol can be estimated by gas chromatography of the trimethyl silyl derivatives^{10,11,12}.

(3) Titrimetric methods

Sorbitol is reacted with sodium periodate and the excess determined iodometrically¹⁷.

(4) Colorimetric methods

(i) *Komarowsky reaction*—Sorbitol gives a colour when heated with a cyclic aldehyde, thiourea and concentrated sulphuric acid. This can be used for its estimation¹⁹.

(5) Other methods

(i) *Optical rotation method*—Ammonium molybdate causes a specific change in the optical rotation shown by sorbitol. This is widely used for sorbitol analysis^{13,14}.

(ii) *Gravimetric method*—Sorbitol condenses with benzaldehyde to give a benzyldine derivative. This reaction has been used for sorbitol determination^{6,15}.

(iii) *o-Chlorobenzaldehyde method*—Interfering sugars may be removed by fermentation and then the sorbitol precipitated by condensation with *o*-chlorobenzaldehyde. The precipitate of *o*-trichlorobenzalsorbitol can be identified by its melting point, = 217°, or that of the hexa-acetylsorbitol derivative (m.pt 97–99), which is given by reaction of *o*-trichlorobenzalsorbitol with acetic anhydride¹⁶.

(iv) *Polarimetric method*—Sorbitol can be determined polarimetrically as its molybdate complex. Sugars which interfere with the periodate method can be tolerated¹⁸.

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SULPHUR DIOXIDE AND SULPHITES

Chemical names: Sulphur dioxide; sodium sulphite; sodium hydrogen sulphite; sodium metabisulphite.

Food additive use: As a preservative.

Physical properties: Sulphur dioxide is a gas. The salts of sulphurous acid are crystalline solids which liberate sulphur dioxide upon acidification.

ANALYTICAL METHODS

(1) Clean-up methods

(i) *Steam distillation*—Sulphur dioxide can be steam distilled under nitrogen from an acidified sample of the food to be analysed. Methanol can be added to reduce the boiling point^{1,2}.

(ii) *Micro diffusion*—Sulphur dioxide can be isolated from alcoholic beverages by placing the sample, acidified with the phosphoric acid, in a Conway micro diffusion cell³.

(2) Chromatographic method

(i) *Gas chromatography*—Sulphur dioxide can be identified by gas chromatography on a column of Teflon—coated with squalene or dinonyl phthalate⁴.

(3) Titrimetric methods

(i) *Direct titration*—The steam distillate can be titrated directly against standard base^{1,2}.

(ii) *Oxidation with chloramine T*—Chloramine T can be used as an oxidizing agent in the amperometric titration of sulphur dioxide⁵.

(iii) *Iodometric method*—The steam distillate is added to standard iodine and the excess iodine back-titrated with standard thiosulphate^{6,7,8}.

(4) Polarographic method

Sulphur dioxide can be analysed by polarography¹⁵.

(5) Colorimetric method

Sulphur dioxide reacts with *p*-rosaniline to give a coloured product suitable for quantitative analysis. This method has been automated^{12,16,17,18,19}.

(6) Other methods

(i) *Gravimetric method*—

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- (a) *Barium sulphate method*. The sulphur dioxide is oxidized to sulphate and estimated as barium sulphate. The barium sulphate can be estimated by weight and/or compleximetric titration^{2, 9, 10, 11, 12, 13}.
- (b) *Mercury(II) iodide method*. The sulphur dioxide reduces copper(II) to copper(I) which is precipitated as the mercuri-iodide¹⁴.
- (ii) *Reduction to sulphide*—Sulphur dioxide is reduced to sulphide and estimated as lead sulphide⁹.

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TARTARIC ACID AND TARTRATES

- Chemical name:** Tartaric acid; 2,3-dihydroxysuccinic acid.
Food additive use: As a synergist with antioxidants and as an acidulant.
Physical properties: A colourless white solid, m.pt 160°–170°; soluble in water, slightly soluble in ether.

ANALYTICAL METHODS

(1) Clean-up method

Tartaric acid is extractable with water from the sample under test.

(2) Chromatographic methods

(i) *Paper or thin layer chromatography*—Tartaric acid can be identified and estimated by paper or thin layer chromatographic methods. A suitable solvent system for paper or thin layer cellulose is ethyl acetate/acetic acid/water (3 : 1 : 1)^{1, 2, 3, 4}.

(ii) *Gas chromatography*—Tartaric acid may be estimated by the gas chromatography of its methyl ester^{2, 5}.

(iii) *Ion exchange*—Further clean-up may be achieved by anion exchange techniques. The resins may be used in the acetate form^{6, 7}.

(3) Polarographic method

Potassium antimony tartrate forms two polarographic waves which are suitable for its estimation^{12, 13}.

(4) Colorimetric methods

(i) *Ammonium vanadate*—Tartaric acid gives a colour reaction with ammonium vanadate. Interference can be compensated for by using a blank consisting of the sample solution in which the tartaric acid had been destroyed by periodate^{14, 15}.

(ii) *Ammonium iron(II) sulphate*—Tartaric acid can be estimated by its colour reaction with ammonium iron(II) sulphate⁸.

(5) Other methods

(i) *Periodate method*—Tartaric acid is cleaved by periodate to give glyoxylic acid, which can be estimated by several colorimetric methods^{16, 17}.

(ii) *Crystallographic method*—Calcium carbonate is added to the sample and observed microscopically after 2–3 hours. If tartaric acid is present, crystals of calcium tartrate form¹⁸.

(iii) *Gravimetric method*—The tartaric acid in the eluate from the anion exchange column or wine itself can be precipitated as potassium hydrogen tartrate and this can be estimated by weight or by titration against standard base^{7, 8, 9, 10, 11}.

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TOCOPHEROLS

Chemical name: DL-tocopherol; D-tocopherol, 2,5,7,8-tetramethyl-2-(4',8',12'-trimethyl decyl)-6-chromanol.

Food additive use: As an antioxidant. The synthetic form is DL.

The u.v. absorption $E_{1\text{cm}}^{1\%} = 71-76$ at $\lambda_{\text{max}} 292$ nm.
solvent ethanol.

Physical properties: A yellow viscous liquid; insoluble in water; soluble in ethanol; oxidizes readily in air.

MIXED TOCOPHEROL CONCENTRATE

Synonym: Vitamin E concentrate.

Food additive use: As an antioxidant.

Definition: Mixed tocopherols concentrate contains not less than 34 per cent total tocopherols. Of the total tocopherols present, not less than 50 per cent consists of DL-tocopherol.

Physical properties: A brownish to red viscous liquid; insoluble in water, but soluble in ethanol; oxidizes in air.

ANALYTICAL METHODS**(1) Clean-up method**

Tocopherols are readily extracted into acetone/petroleum ether mixture or with diethyl ether. In many methods it is necessary to carry out a preliminary saponification stage^{1,2,3}.

(2) Chromatographic methods

(i) *Paper chromatography*—Tocopherols can be identified and isolated by paper chromatographic techniques. Paper, part-treated with squalene using isopropanol/water (85:15), is one of several suitable systems^{4,5,6,7,8,9}.

(ii) *Thin layer chromatography*—Several thin-layer systems are available for the separation and identification of tocopherols. Suitable solvent systems for silica gel are ethyl acetate/petroleum ether (7.5 per cent); chloroform/benzene, or petroleum ether/diethyl ether (5:1)^{3,9}.

(iii) *Detection of spots*—Tocopherol spots can be identified by the irradiation of the chromatograph with ultra-violet light. The sensitivity can be improved by its preliminary treatment with fluorescein^{10,6,4}.

(iv) *Column chromatography*— α and γ tocopherols can be separated by selective elution with ether-hexane from an alumina column^{4,11}.

(v) *Gas chromatography*—The tocopherol extract is treated with trimethyl silyl chloride and the silyl ether estimated by gas chromatography¹².

(3) Spectrophotometric method

(i) *Ultra-violet*—Tocopherols exhibit characteristic ultra-violet absorption. This has been used for analysis¹³.

(4) Polarographic method

The tocopherols are saponified and oxidized with cerium(IV) sulphate to the tocopheryl quinones which are determined polarographically²⁰.

(5) Colorimetric methods

(i) *2,2'-Bipyridyl*—Tocopherols are reducing agents capable of reducing iron(III) ions to iron(II). Iron(III) chloride is added to the sample and the iron(II) ions thus formed estimated by the 2,2'-bipyridyl method. This method has been automated^{13, 14, 15, 16, 17, 18, 19}.

(ii) *4,7-Diphenyl-1, 10-phenanthroline method*—This method is based upon the reduction of iron(III) to iron(II) by tocopherol. The iron(II) produced is estimated by reaction with 4,7-diphenyl-1, 10-phenanthroline⁸.

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