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New Legislation and Official Literature Issued During 1977

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Lists are given of some of the documents of 1977 relating to legislation likely to be encountered in Public Analysts' laboratories. Reference is also made to some of the reports issued in 1977.

The word of regret about the apparent passing of the MAFF Food Standards Division's *LAA/Bulletins* which appeared in last year's review was premature. The bulletins, issued against the reference FS 4812, are marked, *for use by Food and Drugs authorities, Port Health authorities and Public Analysts only.* There is also a statement on them that they will normally be issued twice a year. In fact, they seem now to appear around April, once a year. *LAA/Bulletin/9* was dated April 1977 and was in Public Analysts' hands in May. Number nine was quite the best summary of the food legislation of 1976 to have appeared anywhere, and it is a pity that the inhibiting notice about restricted availability continues to appear. Its removal and a little patience in awaiting its publication, would make this present review, to a large extent, unnecessary.

Rather easily confused with the *LAA/Bulletin* series are the MAFF *Information Bulletins for Public Analysts.* Those appear monthly, and are concerned with EEC Methods of Analysis. Unfortunately, across the country generally, local authority staffing offices seem inclined to allow staff expansion anywhere other than in laboratories, which means that much of the Ministry's effort in producing the *Information Bulletins* is wasted, because it is possible with existing laboratory facilities to check only a selected few of the methods of analysis which are being described.

In 1977 MAFF *Information Bulletins* numbers 15 to 26 were issued, their contents being essentially methods of analysis in the areas indicated below:

Bulletin number	Topics
15	Insoluble substances in instant coffee.
16	Chlorides in food. Preservatives. Metallic contaminants (mercury, lead, arsenic, cadmium, copper, zinc). Sodium and potassium. Weibull-Stoldt Fat Method. Sampling.
17	Cocoa and chocolate.
18	Milk and milk products.
19	Composition of honey.
20	Sugar products.
21	Natural mineral waters.
23	Further notes on honey.
24	Sugar methods (including the Luff Schoorl Method).
25	Purity of food additives.
26	Vinyl chloride monomer in foodstuffs.

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Two very good summaries of proposed food legislation appeared in the *Proceedings* of the Analytical Division of the Chemical Society for 1977. The writer's name did not appear, but both articles had an authoritative appearance. The present position of U.K. legislation appeared on page 237 of the September issue. and the EEC legislation is discussed on page 314 of the November issue.

The *MAFF Joint Announcements* in 1977 dealt with the following matters.

No. 4 Re-appointment of Professor A. G. Ward, C.B.E., as Chairman of the Food Standards Committee.

No. 80 Announcing the publication of a Food Standards Committee Report on Beer (in which OG is thought worth indicating by up to five X marks, and the amount of malted barley might be indicated).

No. 102 Proposed Anti-oxidants in Food Regulations.

No. 128 Announcing regulations making changes in the use of Preservatives in Food (implementing EEC Directives 76/462/EEC and 76/463/EEC).

No. 132 Announcing regulations to limit the amount of erucic acid in edible oils and fats.

No. 184 Announcing new regulations controlling the composition, description and labelling of condensed milk and dried milk products.

No. 187 Announcing regulations to control the composition, description and labelling of fruit juices and concentrates.

No. 192 Announcing a Food Additives and Contaminants Committee report on the use of sulphur dioxide, caustic soda and monosodium glutamate instead of artificial colouring in canned peas.

No. 211 Stating that the EEC Council of Members had adopted a Directive on the composition and labelling of extracts containing coffee and those also containing chicory.

No. 263 Announcing that a Food Standards Committee Report on the Use of Fructose in Foods for Diabetics had recommended no change for the time being, but inviting submissions in connection with a forthcoming review.

No. 271 Delaying the change in declaring food energy values from calories to kilojoules.

No. 278 Appointments to the Food Standards Committee.

No. 298 Requesting comment in connection with a review of food sweetening agents.

No. 355 Announcing that, in the summer of 1978, a consultative document will be issued to precede the drafting of a new Food and Drugs Act.

No. 408 Explaining that in a second part of the Food Standards Committee Review of Food Labelling the committee recommends that exceptions to the general policy of labelling with full lists of ingredients should cease and that a revised list of generic terms should be made before harmonisation with European law is discussed.

New legislation in 1977 dealt with the following matters.

New Legislation Affecting Milk

The Milk and Dairies (General) (Amendment) Regulations 1977 (and circular FSH 2/77), *SI 1977 No. 171.* Removed a legal obstruction to the importation of bottles or cartons of cream in retail packs.

The Milk (Great Britain) (Amendment) Order 1977, SI 1977 No. 859. Increased the retail prices for milk by a penny a pint from 22 May 1977.

The Milk (Great Britain) (Amendment) (No. 2) Order 1977, SI 1977 No. 1441. Increased from 2 October 1977 the price of Channel Islands Milk by ½p per pint.

The Brucellosis Melitensis (Amendment) Order 1977, SI 1977 No. 945. The Brucellosis (Area Eradication) (England and Wales) (Amendment) Order 1977, SI 1977 No. 949. The Brucellosis (England and Wales) (Amendment) Order 1977, SI 1977 No. 1751. The Milk (Special Designation) Regulations 1977, SI 1977 No. 1033. Revoked former regulations and restored them with the provision that the designation *untreated* shall be permitted, but only when the milk so described shall have come from a herd accredited as free from brucellosis.

Circular FSH 6/77. Guideline Figure for the Fat Content of Standardised Whole Milk Imported into the UK During the Milk Year Beginning 1 April 1977 (EEC Regulation No. 540/77). 3·77 per cent. fat (in fact Regulation (EEC) 648/77 made the 1977/78 Milk Year commence on 1 May 1977).

The Association of County Councils in 1977 considered a request from the National Institute for Research in Dairying, for county councils to participate in a project with the British Standards Institution to confirm a recognised standard freezing point for milk, to be used in calculating the amount of adulteration when there had been an addition of extraneous water. In April they hesitated, but in July they recommended that counties approached should participate in the project.

New Legislation Affecting Food

In reply to questions in Parliament, Mr Hattersley stated that from February 1974 to October 1977 food prices had risen by 90·6 per cent., and general retail prices had risen by 83·4 per cent.

The U.K. food legislation of 1977 was as follows.

The Preservatives in Food (Amendment) Regulations 1977, SI 1977 No. 645. The regulations added another salt of propionic acid to preservatives for use in bread, Christmas puddings and flour confectionery, and made changes in purity criteria for some other food preservatives to bring them in line with Directive 76/463/EEC.

The Erucic Acid in Food Regulations 1977, SI 1977 No. 691. The regulations provided for a limit of 10 per cent. erucic acid in the total fatty acids in the oil or fat of food from 1 July 1977 to 1 July 1979, and thereafter for the limit to be reduced to 5 per cent. Erucic Acid is defined as *cis-docos-13-enoic* acid, which makes its chemical estimation complicated. Why only the *cis* acid should be considered harmful is not too clear.

The Fruit Juices and Fruit Nectars Regulations 1977, SI 1977 No. 927. The regulations control the composition, description and labelling of fruit juices including concentrated and dried juices, and an unfermented mixture of fruit, water and sugar which will be known as Fruit Nectar. There are prohibitions on medicinal claims and limits for permitted additives such as Vitamin C, sugar, acidulants, volatile flavouring oils, dimethyl polysiloxane and preservatives.

The Condensed Milk and Dried Milk Regulations 1977, SI 1977 No. 928. The regulations control the composition, description and labelling of certain condensed and dried milk products, making changes to harmonise with EEC practice. Thus, one now finds that whereas in Britain, Unsweetened Evaporated Milk was less concentrated than Condensed Milk, it is now Condensed Milk which is less concentrated than Evaporated Milk. In retail sales, only the products to which the reserved descriptions listed in Schedule 1 to the regulations apply may be sold (i.e. products preserved by heat treatment or with sugar). Schedule 2 to the regulations lists permitted additives.

Other Food Matters

The Sugar Board (Dissolution) Order 1977, SI 1977 No. 224. Acting under powers in the Agriculture (Miscellaneous Provisions) Act 1976 the MAFF issued this Statutory Instrument, which reads: "The Sugar Board established by section 1 of the Sugar Act 1956 is hereby dissolved". This follows the U.K. adoption of the EEC sugar arrangements in 1973 and the expiry of the Commonwealth Sugar Agreement in 1975. The Agriculture (Miscellaneous Provisions) Act 1976 (Commencement No. 1) Order came into force on 14 February 1977.

The Poultry Meat (Slaughterhouse Checks on the Absorption of Water) Regulations 1977, SI 1977 No. 1854. Imposed upon Food and Drugs Authorities an obligation to ensure that the steps taken at places of slaughter to prevent excessive inclusion of water in poultry carcasses are properly carried out.

Circular FSH 8/77. Poultry Meat Hygiene. The circular clarifies arrangements to overcome difficulties which arise as a result of the Poultry Meat (Hygiene) Regulations 1976.

MAFF letter ref. FSC 122 (20 April 1977) Water Content of Poultry Meat. Confirmed that the Annex III method involving the grinding of whole frozen chicken carcasses will be the method adopted in forthcoming regulations.

MAFF letter ref. FS 5616 and Codex Alimentarius paper CX/SB 77/9 referring to standards for soups. The paper set out a point of view in favour of considering no detailed standards for soups for a period of at least five years.

Institute of Trading Standards/UK Association of Frozen Food Producers Code of Practice on Reformed Meat (Code 3). "Packs containing Slices and Steaks made from reformed meat should clearly refer to the fact that the meat has been processed by including the words "Chopped and shaped" in and as part of the appropriate designation (other words, such as "and formed" may be used if desirable)".

MAFF letter ref. ADF 99 (enclosing Draft Circular letter to Enforcement Authorities concerning purity criteria for diacetyl tartaric acid esters of mono- and di-glycerides of fatty acids as prescribed in Schedule 1, Part II of the Emulsifiers and Stabilisers in Food Regulations 1975). Raises the acid value to 130.

MAFF letter ref. ADF 187. ORANGE G and CHOCOLATE BROWN FB. These colours are to be withdrawn from 1 January 1978. YELLOW 2G is probably to be deleted by the end of 1979. RED 2G is under review. Discussions are under way for the continued use of titanium dioxide and vegetable carbon.

MAFF letter ref. ADF 283A. Saccharin. Gives reasons for the U.K. delaying prohibiting use of saccharin (following Canadian questions into its safety) until further work has been carried out.

MAFF letter ref. FSC 330. Xylitol. Italian Chewing Gum on sale in Britain is thought to contain Xylitol, a carbohydrate which has been advertised in *Food Manufacture Ingredients Guide* as a sweetener but is considered by the Ministry to be a humectant now being evaluated by the Food Additives and Contaminants Committee and meanwhile prohibited by the Miscellaneous Additives in Food Regulations 1974. A discussion of xylitol appeared in the September and December issues of *Process Biochemistry* 1977.

MAFF letter ref. FS 4884. Cocoa and Chocolate Products. Referred to the need for a tolerant attitude to manufacturers caught by the two dates which had been put forward for changes in weight marking of certain products, and clarified the intention in Regulation 4 of the Cocoa and Chocolate Product Regulations 1976, which prohibits the use of reserved descriptions and then makes exceptions to the prohibition. The second and third of those relate to ingredients and to products which are clearly not chocolate products. Because of Regulation 11 of the Labelling of Food Regulations "Chocolate Sponge" might strictly need to be called "Chocolate Flavour Sponge" but current usage and the LAJAC Code of Practice might make "Chocolate Sponge" an acceptable designation.

MAFF letter ref. FS 6573. Demerara Sugar. Stated that discussions between the Ministry, the trade and ITSA had resulted in the general agreement (a) that "Demerara Sugar" be regarded as an indication of a type of sugar and not as an appropriate designation, (b) in addition to "Demerara Sugar" the prepacked Article should bear an appropriate designation and (c) the following designations as appropriate would be acceptable:

(i) white sugar and cane molasses;

(ii) raw cane sugar and cane molasses;

(iii) raw cane sugar;

with lists of ingredients as necessary.

MAFF letter ref. FSC 39 C. Mineral Hydrocarbons on Dried Fruit. The specifications for the purity of the mineral oil used on fruit are, according to SI 1966 No. 1073 those of the B.P. The B.P. however, gives very different tolerances for liquid paraffin and semi-liquid paraffin. Although the trade uses only liquid paraffin, a new test method, namely that of the American

Society for Testing of Materials, ASTM D 2269–73, will soon be specified with a maximum absorbance of 0·1.

MAFF letter referring to an EEC Proposal relating to materials containing vinyl chloride monomer and which are intended to come into contact with food. The proposed limits are as follows:

Maximum VC in vinyl chloride homopolymers	1 mg/kg
Maximum VC in vinyl chloride co-polymers used for containers for liquids	1 mg/kg
Maximum VC in vinyl chloride co-polymers for all other purposes	5 mg/kg
Maximum level of VC in food	0·05 mg/kg

MAFF letter ref. FS 5933. Milk Content of Ice-cream. Although Milk-solids-other-than-fat is not defined in the Ice-cream Regulations, the intention is that the proportions of lactose, protein and mineral matter should be those found in natural milk, and should not be interpreted to include whey or lactose ingredients.

MAFF letters ref. FS 6566. The Interpretation Act 1889. Questions on how the Interpretation Act 1889 should apply in regulations about foodstuffs arise from time to time, and local authority legal departments have been among those which have ruled that one cannot read the Meat Pie and Sausage Roll Regulations, 1967 for example, in such a way that Meat Pie (in the singular) may be interpreted as including the plural. In two letters from the MAFF Food Standards Division Branch B however, it has been stated that the Interpretation Act applies to all Acts and Regulations passed since 1850. There is a specific statement in Section 2 (2) of the Meat Pie and Sausage Roll Regulations that the Interpretation Act shall apply, which means that multiple groups of pies may be taken as "a sample". In the Fish and Meat Spreadable Product Regulations, the fact that Fish can be read as plural may mean that the Interpretation Act could operate to allow named fish (plural such as Salmon and Shrimp) to make up not less than 70 per cent. of a spread, rather than spreads being made from a single species, as the regulations are usually interpreted to require.

MAFF Circular FSH 14/77. Water Content of Poultry Meat. An explanation of how Regulation (EEC) 2867/76 is to be implemented.

Fertilisers and Feeding Stuffs Legislation

The reprinting of the whole of the Milk (Special Designation) Regulations for the sake of one change made to Untreated Milk leads one to wonder why an updated reprinting of the regulations connected with Fertilisers and Feeding Stuffs could not have been undertaken, so that enforcement bodies might be spared the present doubts about whether one has found the relevant sections of the legislation. The new material to appear in 1977 was as follows.

The Fertilisers and Feeding Stuffs (Amendment) Regulations 1977, SI 1977 No. 115. The Regulations make changes in the definition of "preservative" and substitute the words "permitted preservative" for "preservative" etc., in the principal regulations. Four schedules list permitted antioxidants, permitted emulsifiers, stabilisers and binders, increased allowances of Vitamin D in poultry feeds, and permitted preservatives.

MAFF letter ref. DW 272. Warned that the official method for the determination of Vitamin A is unreliable.

MAFF letter ref. DW 117. Enclosed document *Feed 1 Guide to the Method of Analysis Contained in Schedule 5 of the Fertilisers and Feeding Stuffs (Amendment) Regulations 1976.* The *Guide* contained comments on parts of the EEC procedures considered to be too unsatisfactory for inclusion in present U.K. directions; it included advice on means by which satisfactory results might be obtained when using the new procedures, and it suggested sources of supply for certain reagents. The warnings involved aspects of the prescribed method for the determination of moisture, problems connected with the determination of oil, a U.K. insistence on subjecting British Chemists to the hazards of using mercury catalysts instead of the EEC safer, copper ones, and doubts about the methods for the determination of urea, biuret and uric acid. There was a warning that the prescribed EEC method of calibration in the prescribed phosphorus determination procedure needs scrupulous attention to detail, and there was a frank statement that the EEC methods for the determinations of fibre and of sugar are inferior to the older U.K. methods. Too much indicator has been prescribed in the water soluble chlorides determination, and the Laboratory of the Government Chemist has expressed reservations about the ashing procedure. There is a warning that the method of analysis for Vitamin A requires skilled operators who have made themselves conversant with the method, and some likely sources of error are listed. For Vitamin B, additional manipulation, as suggested in *Analyst*, 1951, p. 127, is recommended, and the method for Vitamin K is stated to be unreliable. The method for hydrocyanic acid is said to be too insensitive to check the limits which have been prescribed, and similarly an alternative method for volatile mustard oil is being sought. On the subject of the method of analysis for aflatoxins the *Guide* seems to suggest that more scrupulous handling precautions than most local authority laboratories can provide may be necessary.

The Fertilisers Regulations 1977, SI 1977 No. 1489. The regulations supersede (insofar as they apply to fertilisers) Regulations 1, 6, 8, 9, 10, 11, 12, 13 and 14 and Part I of Schedule 2, Part I of Schedule 4 and Part I of Schedule 5 of the Fertilisers and Feeding Stuffs Regulations 1973 and Regulation 1 2 (4), (5) and (6) and 3 (1), and Schedule 7 of the Fertilisers and Feeding Stuffs (Amendment) Regulations 1976. The Regulations apply to a wider range of materials than formerly, and the names applied to fertilisers become more specific. The limits of variation allowed tend to be stricter, and will apply only in a negative direction. There will no longer be any offsetting of excesses against deficiencies, and phosphorus, potassium and magnesium will need to be declared both as oxide and as the element. The information required to be given in Statutory Statements will have to be given within 14 days of delivery. The problem with all these scraps of paper is not insurmountable, but one may appreciate the extent of the problem, if it is remarked that one needs to turn to the 1973 Regulations for definitions of feeding stuffs, to the 1977 Regulations for definitions of fertilisers, back to the 1973 Regulations for the maximum amounts of trace elements allowed in feeding stuffs, but to the 1977 Amendment Regulations for the changes in vitamins, to the 1973 Regulations for when copper becomes a medicinal additive, or to the new Schedule I in the 1976 Amendment Regulations for the amount below which no declaration of copper is necessary: and in the background, one has all the present doubt about which tests are effective and whether finding no preservative or antioxidant that has been declared means that the quantity used is small, and whether finding excess silica means contamination with sand, or permitted use of bentonite.

The Medicines (Animal Feeding Stuffs) (Enforcement) (Amendment) Regulations 1977, SI 1977 No. 1584. The regulations correct misprints in the Medicines (Animal Feeding Stuffs) (Enforcement) Regulations 1976 and change the form of the wording in the certificate of analysis forms required by the regulations.

MAFF letter. ref. DR 232 (enclosing a letter dated 7 June 1976 which analysts had not received) lists changes in the Product Licence for NITROVIN so that there was a prohibition on the mixing of the material with antibiotics after 31 December 1976.

The Compendium of Medicinal Feed Additives. This was updated during 1977 to include new medicaments such as AVOTAN.

New Medicines Legislation

The following items deserve attention.

The Medicines (Certificate of Analysis) Regulations 1977, SI 1977 No. 1399. The regulations revoke the Medicines (Certificates of Analysis) Regulations 1976, and prescribe new forms of certificate on which results of analysis should be given.

THE B.P. 1973 ADDENDUM 1977

This Addendum, effective from 1 December 1977, amends the B.P. 1973 and the B.P. Addendum 1975, and is published on the recommendation of the Medicines Committee in accordance with Section 99 (b) of the Medicines Act.

The 57 additions include the following:

Anthelmintics:	bephenium hydroxynaphthoate, levanisole hydrochloride and thiabendazole.
Antibacterials:	furazolidone and nitrofurazone.
Antiemetic:	methochlopramide hydrochloride.
Antihistamine:	trimeprazine hydrochloride.
Hypotensive:	debrisoquine sulphate.
Migraine relief:	methsergide mallate.
Narcotic analgesic:	fentamyl citrate.

Nine monographs are transferred to the Addendum from the B.P.C. to give effect to the requirements of the European Pharmacopoeia, while 73 monographs of the B.P. 1973 and the Addendum 1975 are withdrawn in favour of the European Monographs.

The test for uniformity of weight of single dose preparations described in the European Pharmacopoeia has been adopted and applies to capsules, powders for injectable preparations, non-sugar-coated or non-enteric-coated tablets and suppositories. The European Pharmacopoeia requirements for uncoated tablets also applies to both uncoated and film coated tablets of the B.P. The term "citric acid" now refers to the anhydrous material and the material previously designated Citric Acid is now referred to as Citric Acid monohydrate; this is also in line with the European Pharmacopoeia requirements.

The new monograph for Digoxin Tablets includes a more reliable assay, a test for uniformity of content which is applicable to lower strength tablets, and an amplification of the dissolution rate.

High pressure liquid chromatography is used in the standardisation of Idoxurine eye drops (eye drops being included for the first time).

There are important changes in the use of infra-red spectroscopy for identification purposes. Comparison of identity is now made with a reference spectrum rather than by use of a reference substance for many substances, and it is hoped that all infra-red identification will be made by the use of a series of reference spectra as they become available. A single class of substances, known as "British Pharmacopoeia Chemical Reference Substances, suitable for assay and test purposes", is now available, and has replaced the two classes known as "British Pharmacopoeia Authentic Specimens" previously used for this purpose, and the more highly purified materials known as "British Chemical Reference Substances". The "British Pharmacopoeia Chemical Reference Substances" will be withdrawn in favour of the "European Pharmacopoeia Chemical Reference Substances" as these become available.

Amendments. A series of amendments to the B.P. 1973 and the Addendum 1977, also effective from 1 December 1977, was published by the DHSS in November 1977; these were mainly concerned with additional determinations of declared contents of sealed containers for injection purposes.

The British Pharmacopoeia (Veterinary) 1977. The first edition has also been published on the recommendation of the Medicines Commission in accordance with Section 99 (6) of the Medicines Act. There is now only one official compendium in the U.K. for medicinal substances, and this is the B.P., one part being for human medicines and one part for veterinary medicines. In general, the same analytical standards are applicable for articles that can be used for both human and for veterinary purposes. Less stringent standards regarding impurities for mass external application or for materials which are used as feed additives can be allowed. Special attention has been paid to the detection and limitation of impurities that may occur during manufacture or during storage, and allowances for these increases have been made. It is, however, up to manufacturers to maintain standards more exacting than the minimum laid down by the Pharmacopoeia. High pressure liquid chromatography is used in the assay of the synthetic prostaglandins, cloprostenol sodium and fluprostenol sodium. The appendices include a general test for antibiotics in milk using triphenyltetrazolium chloride; this test which is non-specific, and is standardised using sodium benzyl penicillin, is used to differentiate between the intra-mammary injections used for lactating animals and the slow release injections used for non-lactating animals.

Martindale. The Extra Pharmacopoeia. 27th Edition. This new edition, published during 1977, has over 9000 more entries than the 26th Edition, and the international coverage has been considerably extended.

The Medicines (Bal Jivan Chamcho Prohibition) Order 1977, SI 1977 No. 172. The order continued until 9 May 1977 the prohibition in the Medicines (Bal Jivan Chamcho Prohibition) Order 1976.

The Medicines (Bal Jivan Chamcho Prohibition) (No. 2) Order 1977, SI No. 670. This order continued the prohibition in the former orders.

The Medicines (Renewal Applications for Licences and Certificates) (Amendment) Regulations 1977, SI 1977 No. 180.

The Medicines (Labelling) Amendment Regulations 1977, SI 1977 No. 996 (supplied with a correction slip which was in error). The regulations postponed by six months the date of commencement given in SI 1976 No. 1726, and require medicines to be labelled, "Keep out of the reach of children". Warning marks for some medicines, including aspirin, are required, together with markings (except on doctor's prescribed packs) which show that some medicines are "Prescription only" medicines and products to be sold only through a pharmacy. A full account of Medicine Labelling appeared in the *Pharmaceutical Journal* for 16 July 1977 (p. 55).

The Medicines (Medicines Act 1968 Amendment) Regulations 1977, SI 1977 No. 1050. The regulations provide for certain EEC obligations relating to proprietary medicines to be implemented.

The Medicines (Pharmacy and General Sale) (Appointed Day) Order 1977, SI 1977 No. 2126. After 1 February 1978 any drugs not on a General Sale list might only be sold from a Pharmacy.

The Medicines (Prescription Only) Order 1977, SI 1977 No. 2127. Essentially a list of drugs which may only be supplied against a prescription. It should be read with Schedule 2 of the Misuse of Drugs Act. It incidentally replaced the Hexachlorophane Prohibition Order 1973 and the Phenacetin Prohibition Order 1974.

The Medicines Act 1968 (Commencement No. 7) Order 1977, SI 1977 No. 2128. Fixed 1 February 1978 as the day on which Section 1 subsection (2) and subsection 3 (b) of the Food and Drugs Act 1978 were removed and replaced by Sections 63 and 64 of the Medicines Act 1968, prohibiting additions or abstractions with regard to drugs. By virtue of Section 12 (1) of the Poisons Act 1972 the Order also brought that Act into force to replace the parts of the Pharmacy and Poisons Act 1933 which related to non-medicinal poisons. The Pharmacy and Medicines Act and certain other legislation were also repealed.

The Medicines (Retail Sale or Supply of Herbal Remedies) Order 1977, *SI 1977 No. 2130.* Limits the exemption given for herbal remedies in Section 56 of the Medicines Act.

The Medicines (Prohibition of Non-Medicinal Antimicrobial Substances) Order 1977, SI 1977 No. 2131. Essentially takes the place of Part II of the Therapeutic Substances Act.

The Medicines (Sale or Supply) (Miscellaneous Provisions) Regulations 1977, SI 1977 No. 2132. Provided for the enforcement of the provisions of Sections 53, 54 and 66 of the Act by local authorities and the Pharmaceutical Society. These provisions related to restrictions on sale of drugs not on the General Sale list, and to supervision of premises. No order or regulations appeared to have been made under Section 108 of the Act for the enforcement of the Sections 63 and 64 which replaced the old legislation in the Food and Drugs Act 1955 however.

The Medicines (Pharmacy and General Sale—Exemption) Order 1977, SI 1977 No. 2133. Provide in accordance with Section 52 of the Medicines Act for certain categories of persons to be supplied with drugs not on the General Sale list.

The Medicines (Exemptions from Restrictions on the Retail Sale or Supply of Veterinary Drugs) Order 1977, SI 1977 No. 2167. Lists certain drugs exempt from restrictions when intended for use on animals.

The Medicines (Labelling) Amendment (No. 2) Regulations 1977, SI 1977 No. 2168. Made provisions for modified labelling of drugs referred to in SI 1977 No. 2167.

The Misuse of Drugs Act 1971 (Modification) Order 1977, SI 1977 No. 1243. This order brings under control certain tryptamine drugs and phenthylamine drugs.

The Misuse of Drugs (Designation) Order 1977, SI 1977 No. 1379. Redefines cannabis and makes changes in the list of drugs for which no medicinal use is likely.

The Misuse of Drugs (Amendment) Regulations 1977, SI 1977 No. 1380. Modify the Misuse of Drugs Regulations 1973 (as amended) to take account of the changes in the Modification Order.

Environmental Matters

The items in this section are merely shown in the form of a list.

The Control of Atmospheric Pollution (Exempted Premises) Regulation 1977, SI 1977 No. 18.

D of E Circular 2/77. Control of Pollution Act 1974. Part IV. Pollution of Atmosphere (lists the types of emissions likely to occur from different industries).

The Land Drainage (Election of Internal Drainage Boards) (Amendment) Regulations 1977, SI 1977 No. 366.

D of E Seventeenth Progress Report of the Standing Technical Committee on Synthetic Detergents.

Building Research Establishment Digest No. 139. Control of Lichens, Moulds and Similar Growths.

Health and Safety Commission CONSULTATIVE DOCUMENT: Hazardous Substances (Conveyance by Road) Tank Labelling Regulations.

The Control of Pollution (Licensing of Waste Disposal) (Amendment) Regulations 1977, SI 1977 No. 1185.

D of E Circular 79/77. Control of Pollution Act 1974 Part 1 (Waste on Land) Licensing Amendments.

Consumer Protection. The Aerosol Dispensers (EEC Requirements) Regulation 1977, SI 1977 No. 1140 (prohibits vinyl chloride monomer and allows some indulgence on weight marking and declarations of origin).

House of Lords Session 1976–1977. 16th Report, R/1628/76. Commission Interim Report on some Persistent Organo-chlorine Compounds.

The Dutch Elm Disease (Local Authorities) Order 1977, SI 1977 No. 1074 (lists all the local authorities to which previous orders applied).

The Dutch Elm Disease (Restriction on Movement of Elms) Order 1977, SI 1977 No. 1075.

Plant Health. The Beet Cyst Nematode Order 1977, SI 1977 No. 988.

Draft CEN Standard BSI doc (76/60813 DC) Para. 4.5. The Toys (Safety) Regulations. Recommendation that printing inks on paper and board be tested for metals on a basis of metal present in the paper or board. (Method of extraction given in Institute of Trading Standards Administration Confidential Bulletin No. 60, 4 March 1977.)

Weights and Measures

Weights and Measures Act 1976. Includes in Section 12 a provision to waive Food and Drugs Act standards, etc., in time of food shortages.

The Bread Prices No. 2 Order (as amended), *SI 1976 No. 2128, SI 1977 No. 360 and SI 1977 No. 1166.*

The Weights and Measures Act 1963 (Honey) Order 1977, SI 1977 No. 558.

The Measuring Container Bottles (EEC Requirements) Regulation 1977, SI 1977 No. 932.

The Weights and Measures Act 1963 (Cocoa and Chocolate Products) Order 1977, SI 1977 No. 1332.

The Weights and Measures Act 1963 (Sugar) Amendment Order 1977, SI 1977 No. 1333.

The Weights and Measures Act 1963 (Cheese) Order 1977, SI 1977 No. 1335.

The Price Marketing (Meat) Order 1977, SI 1977 No. 1412.

The Weights and Measures (Marketing of Goods and Abbreviations of Units) (Amendment) Regulations 1977, SI 1977 No. 1683.

The Alcoholometers and Alcohol Hydrometers (EEC Requirements) Regulations 1977, SI 1977 No. 1753.

Bulletins—Consumer Safety, issued by Consumer Safety Unit of Department of Prices and Consumer Protection for TSO's and EHO's.

No. 1 Advertised the Government Laboratory's CHAIS (Consumer Hazards Analytical Information Service).

No. 2 Referred to the EEC Directive on Packaging and Labelling of Dangerous substances and summarised an EEC Directive on Cosmetic Products

No. 3 Referred to imitation cigarettes and Bal Jivan Chamcho Baby Tonic.

No. 4 Mentioned childrens' harmonicas.

No. 5 Referred to styrene vapour released from polystyrene toys.

No. 6 Advertised the Government Laboratory and offered information about cosmetic analyses.

No. 7 Contained January to June Index.

No. 8 Suggested preferential sampling of toys with yellow and green pigmented paints.

No. 9 Mentions caustic soda hair straighteners.

Health and Safety

The Health and Safety at Work Etc., Act 1974 (Commencement No. 4) Order 1977, SI 1977 No. 294.

Other Reports, etc.

The Council of the European Communities adopted the two following Directives:

Dietary Foods. Directive 77/94/EEC (see Official Journal L 26/77).

Coffee Extracts and Chicory Extracts Directive 77/436/EEC (see Official Journal L 172/77).

DRAFT EUROPEAN STANDARD FOR NATURAL MINERAL WATERS

Limits	milligrammes per litre
Copper	1
Manganese	2
Zinc	5
Borate (as H_3BO_3)	30
Organic matter (by $KMnO_4$)	3
Arsenic	0·05
Barium	1
Cadmium	0·01
Chromium VI	0·05
Lead	0·05
Mercury	0·001
Selenium	0·01
Fluoride	2
Nitrate (as NO_3)	45
Sulphide	0·05
Radium 226	30 pCi per litre
Cyanide (CN)	0·01
Nitrites (NO_2)	0·005

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J. Assoc. Publ. Analysts, 1978, **16**, 17–24

The Determination of Butylated Hydroxyanisole (B.H.A.), Butylated Hydroxytoluene (B.H.T.) and Individual Gallate Esters in Fats and Oils by High Performance Liquid Chromatography

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The method describes a simple extraction of fats and oils followed by the simultaneous determination of five antioxidants by high performance liquid chromatography. A separate extraction procedure for the quantitative confirmation of B.H.T. is also proposed.

The "Antioxidants in Food Regulations 1974", SI 1974 No. 1120, define propyl, odtyl and dodecyl gallate, B.H.A. and B.H.T., among others, as being permitted antioxidants. In Schedule 3 to these Regulations, the maximum permitted level of antioxidant in anhydrous oils and fats is listed at 100 p.p.m. for propyl gallate or octyl gallate or dodecyl gallate or any mixture thereof and 200 p.p.m. for B.H.A. or B.H.T. or any mixture thereof. There are a considerable number of publications[1–20] covering the determination of one or more of the above mentioned antioxidants.

Extraction techniques include steam distillation,[1] solvent distillation,[2] solvent extraction,[3–16] and vacuum sublimation[20]. Detection has been accomplished by colorimetry,[1] thin layer chromatography (T.L.C.),[6,10,11,12,15,16] spectrophotometry,[8,9,18,19] polarography,[17] paper chromatography,[13,14] gas chromatography (G.C.)[2,3,4,5,20] and high performance liquid chromatography (H.P.L.C.)[7].

When the analyses of fats and oils for antioxidants are contemplated, problems may arise with the need for removal of potentially interfering co-extractives. Steam distillation provides a clean extract although time consuming when routine analyses are required and it may give rise to low recoveries[7]. Solvent distillation alone is not suitable for individual determination of gallates[2] and solvent extraction techniques prior to T.L.C. or G.C. require extensive clean-up.

The proposed method describes a simple extraction of the melted sample with methanol followed by removal of excess fat prior to the simultaneous analysis of the five antioxidants with the aid of an internal standard by gradient elution H.P.L.C.

A modification of a method by Phipps[7] is employed for the quantitative determination of B.H.T.

Experimental

APPARATUS

Two Waters Associates M 6000A HPLC pumps.

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† Now with Waters Associates Limited, 324 Chester Road, Hartford, Northwich, Cheshire CW8 2AH.

Waters Associates U6K sample injection system.

Waters Associates M 660 solvent programmer.

Applied Chromatography Services 750-21 variable wavelength u.v. monitor, operating range 200–600 nm.

Servoscribe RE 571.20 pen recorder.

Water Associates 10 μmu Bondapak C_{18} 30 cm × 4 mm i.d. reverse phase column.

Precision Sampling Corporation B-110 pressure lok 25 μl syringe.

M.S.E. "Multex" centrifuge.

Jobling type 319 vacuum rotary evaporator.

Waters Associates organic clarification kit comprising a 0·5 μm filter.

REAGENTS

1. *Distilled water.*
2. *Methanol* (Rathburn glass distilled grade).
3. *Acetic acid glacial* (Fisons AR), a molar solution prepared in distilled water.
4. *N-Heptane* (Fisons SLR).
5. *Dimethyl sulphoxide* (DMSO) (BDH AR).
6. *Petroleum ether (B.P. 30–40°C)* (Fisons low in aromatics).
7. *Anhydrous sodium sulphate* (BDH AR) heated at 450°C overnight to remove organics.
8. *2-Ethoxyethanol* (Fisons SLR).
9. *Methanol:* M acetic acid in distilled water (80:20).
10. *3:4-Xylenol* (Fisons SLR).

PREPARATION OF SOLUTIONS

(1) An internal standard comprising 100 p.p.m. of 3:4-xylenol in methanol.

(2) A mixture of B.H.A., B.H.T., octyl gallate, dodecyl gallate each at 100 p.p.m. and propyl gallate at 50 p.p.m. in methanol.

(3) A standard solution made up of equal volumes of (1) and (2) above.

(4) A 100 p.p.m. B.H.T. standard in methanol is prepared for the quantitative confirmation of B.H.T.

COLUMN OPERATING CONDITIONS A

For simultaneous antioxidant analysis:

Mode	Gradient elution.
Mobile phases	M acetic acid in distilled water in pump A, methanol in pump B.
Gradient	The programme consists of a linear gradient from 55 per cent. methanol to 85 per cent. methanol in 15 minutes and a 10 minute hold at 85 per cent. methanol. To return to initial conditions a reverse programme of 10 minutes is used.
Flow rate	1·5 ml/min: this produces a back pressure of approximately 3500 lb/in² at 55 per cent. methanol.

Detector	U.v. at 280 nm, 0·1 absorbance units full scale. Figure 1 shows a chromatogram obtained from a 25 μl injection of the standard solution.
Chart speed	300 cm/hour.

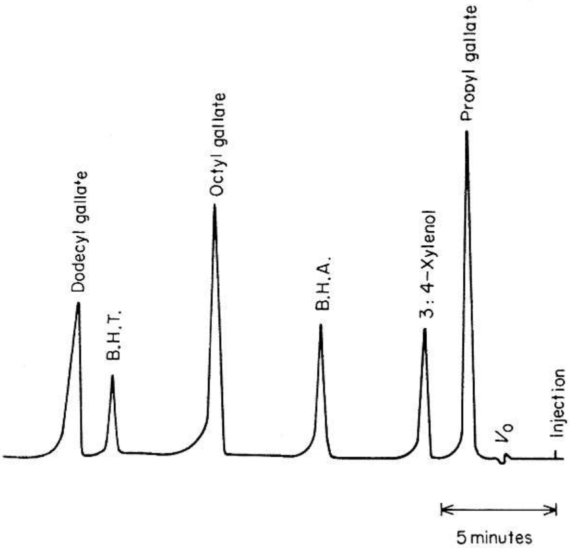

Fig. 1. HPLC trace from a 25 μl injection of standard solution.

COLUMN OPERATING CONDITIONS B

For quantitative confirmation of B.H.T.:

Mode	Isocratic elution.
Mobile phase	80 per cent. methanol/20 per cent. M acetic acid.
Flow rate	1·5 ml/min: this produces a back pressure of approximately 2750 lb/in^2.
Detector	U.v. at 280 nm, 0·1 absorbance units full scale. Figure 2 shows a chromatogram obtained from a 25 μl injection of the B.H.T. standard.
Chart speed	300 cm/hour.

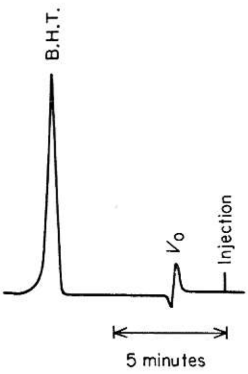

Fig. 2. HPLC trace from a 25 μl injection of standard solution of B.H.T..

METHOD

Weigh approximately 20 gm of sample into a stoppered centrifuge tube of 70 ml capacity and warm in a water bath at 40–50°C until the sample has melted. Add 20 ml of methanol by pipette and shake vigorously for 2 minutes, allow to settle and centrifuge for 10 minutes at 3000 rev./min. Transfer to a deep freeze for a few hours to aid the solidification of any excess fat from the methanol layer, then decant and filter the methanol extract through an organic clarification kit or suitable alternative. Dilute a portion of this filtrate with an equal volume of internal standard solution and inject 25 μl on to the column using operating condition A. Following the reverse programme and re-establishment of equilibrium at the initial conditions (shown by the return to a steady back pressure) make a 25 μl injection of the standard solution for evaluation purposes. Retention times are shown in Table I.

TABLE I

RETENTION DATA FOR THE FIVE ANTIOXIDANTS UNDER COLUMN OPERATING CONDITION A

Compound	Retention time, *minutes*	Retention time relative to 3:4-xylenol
Non-retained peak (V_o)	2·3	0·39
Propyl gallate	4·0	0·68
3:4-xylenol	5·9	1·00
B.H.A.	10·3	1·75
Octyl gallate	14·9	2·53
B.H.T.	19·5	3·31
Dodecyl gallate	21·1	3·56

If B.H.T. is identified from its retention time and quantitative confirmation is required, the following method is proposed.

Weigh accurately approximately 4 gm of sample, dissolve in 20 ml of *n*-heptane and extract four times with 25 ml portions of D.M.S.O. Add 200 ml of distilled water to the combined D.M.S.O. extracts, allow to cool and extract with two 75 ml portions of 30–40°C petroleum ether. Filter the combined petroleum ether through anhydrous sodium sulphate, add 0·5 ml of 2-ethoxyethanol and rotary-evaporate until all the petroleum ether has been removed. Transfer the remaining liquid to a 5 ml volumetric flask and make up to the mark with methanol, rinsing the rotary evaporator flask in the process. Filter as in the initial extraction procedure and inject 25 μl on to the column using operating condition B. After a suitable time has elapsed (see Table II) inject 25 μl of the B.H.T. standard onto the column.

TABLE II

RETENTION TIME OF B.H.T. UNDER COLUMN OPERATING CONDITION B

Compound	Retention time, *minutes*
Non-retained peak (V_o)	2·3
B.H.T.	7·6

Results and Discussion

The chromatogram of the standard solution is shown in Figure 1 whilst the retention data are given in Table I. Quantitative assessment of any antioxidant found in a sample is determined from the ratio of the peak heights of the antioxidant and internal standard for both the standard solution and the sample.

Calibration graphs prepared by plotting the amount of antioxidant against the ratio of the peak height for the antioxidant to the peak height for the internal standard are linear over the range of 25–250 p.p.m. for all the antioxidants. The concentration of antioxidant in a sample is thus determined from the following equation:

$$\text{concentration of antioxidant in sample in p.p.m.} = \frac{20}{S} \times \frac{\mathrm{A_{sam}}}{\mathrm{I_{sam}}} \div \frac{\mathrm{A_{std}}}{\mathrm{I_{std}}} \times X,$$

where S = weight of sample taken in gm,
$\mathrm{A_{sam}}$ = peak height measurement of antioxidant in the sample,
$\mathrm{I_{sam}}$ = peak height measurement of internal standard in the sample,
$\mathrm{A_{std}}$ = peak height measurement of antioxidant in the standard,
$\mathrm{I_{std}}$ = peak height measurement of internal standard in the standard,
X = concentration of antioxidant in the standard in p.p.m. before dilution with the internal standard.

The reproducibility of retention data and peak height ratios under gradient elution conditions have been found to be within ± 1 per cent. during a working day. Day to day variations can, however, exceed this figure progably because of changes in ambient temperature affecting the efficiency of the column.

Recoveries from standard additions of antioxidants to fats and oils are listed in Table III.

TABLE III
RECOVERY OF ANTIOXIDANTS ADDED TO FATS AND OILS

Antioxidant	Level of addition, *p.p.m.*	Sample type and recovery, *per cent.*				Mean recovery with standard deviation, *per cent.*
		Lard	Fat	Dripping	Oil	
Propyl gallate	50	108	107	104	104	106 ± 3
	100	102	105	102	106	104 ± 2
	200	106	106	106	105	106 ± ½
Octyl gallate	50	102	101	95·5	94	98 ± 4
	100	99	104	103	103	102 ± 3
	200	96	98	95	96	96 ± 1
Dodecyl gallate	50	91	93	85	96	91 ± 5
	100	91	92	90	92	91 ± 3
	200	90	88	89	91	89 ± 1
B.H.A.	50	72	68	70	63	68 ± 5
	100	68	70	71	68	69 ± 1
	200	72	71	70	67	70 ± 2
B.H.T.	50	40	35·5	34	28	34 ± 4
	100	39	33	38	33	36 ± 3
	200	37	34	37	32	35 ± 2

The low recoveries for B.H.T. necessitate the adoption of an extraction procedure specifically for B.H.T. when an accurate quantitative figure is needed. Using this procedure, Figure 3 shows the chromatogram relevant to a sample of lard spiked with B.H.T. at the 100 p.p.m. level with peaks for co-extracted material well separated from the B.H.T. peak. Calculation of the amount of B.H.T. in a sample is by comparison with a known standard of B.H.T.

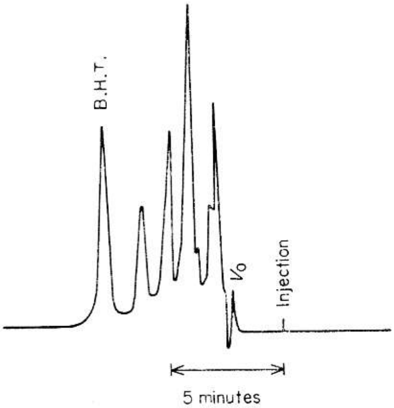

Fig. 3. Trace from a 25 μl injection of a lard extract spiked with 100 p.p.m. of B.H.T.

$$\text{Concentration of B.H.T. in sample in p.p.m.} = \frac{\mathrm{H_{sam}}}{\mathrm{H_{std}}} \times \frac{5}{S} \times X,$$

where $\mathrm{H_{sam}}$ = peak height measurement of B.H.T. in sample,
$\mathrm{H_{std}}$ = peak height measurement of B.H.T. in standard,
S = weight of sample taken in gm,
X = concentration of B.H.T. standard in p.p.m.

The use of isocratic conditions in the determination of B.H.T. provides greater precision than is afforded by the use of gradient elution and an internal standard was not found to be necessary with the U6K injector. Recoveries from this procedure listed in Table IV are in agreement with the recoveries found by Phipps[7]. The limits of detection for the antioxidants in fats and oils using column operating condition A are 10 p.p.m. for the gallates and 20 p.p.m. for B.H.A. and B.H.T. These are by no means the ultimate limits of detection but are set to minimise interference from organic contamination present in the

TABLE IV

RECOVERY OF B.H.T. ADDED TO FATS AND OILS

Level of addition, *p.p.m.*	Recovery, *per cent.* Phipps method [7]	Recovery, *per cent.* Proposed method
100	97 average	100
200		98

distilled water and baseline drift caused by the change in solvent composition during the gradient programme. Figure 4 shows a typical sample chromatogram using these operating conditions.

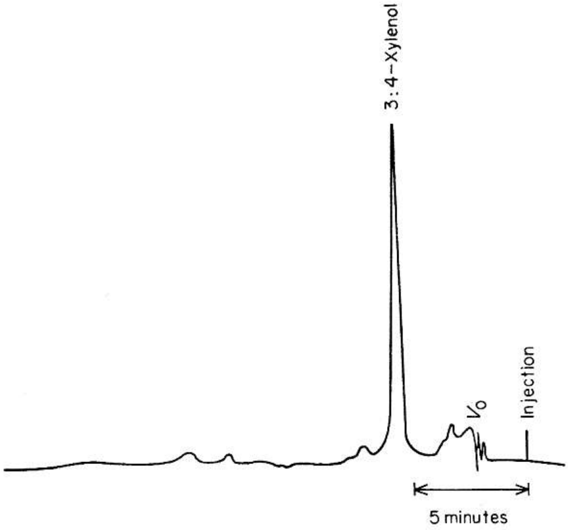

Fig. 4. Trace from a 25 μl injection of an extract of dripping.

To obtain greater sensitivity, organic contaminants may be removed from the distilled water by passing it through the column and collecting as it elutes. The contaminants are retained on the column and are removed by flushing the column with approximately 30 ml of methanol at a flow rate of 1·5 ml/min.

Excessive baseline drift at high detector sensitivities can give rise to difficulties of measurement. To overcome this, isocratic elution at a suitable solvent composition may prove successful where measurement of an individual peak is

TABLE V

ANTIOXIDANT FOUND IN SUBMITTED SAMPLES

	Antioxidant found, *p.p.m.*		
Sample type	B.H.T.	B.H.A.	Dodecyl gallate
American lard	35		
American lard	77		
American lard	44		
American lard	41		
English lard	39		
Refined lard	42		19
Lard	52		13
Lard	105		
Lard	32		
Lard	35		
Lard	100		
Lard			18
Refined dripping	20	62	

All the above samples bore labels declaring the presence of permitted antioxidants.

required. Contamination from repeated sample injections is not a problem, as any build-up of fat on the column is simply removed at the end of a working day by flushing with several column volumes of methanol.

Forty-three samples comprising lards, fats, drippings and vegetable oils have so far been analysed. Thirteen of those were found to contain antioxidant. The results are listed in Table V.

Conclusion

The use of HPLC permits the rapid, simultaneous determination of antioxidants in fats and oils without the need for extensive sample clean-up so characteristic of many other techniques. Any accumulation of fat on the column is easily removed, enabling routine assays to be carried out without any deterioration in column performance.

The author thanks Mr P. V. Nicholas for his considerable help in the preparation of this paper and Mr A. J. Harrison, Chief Scientific Adviser, for his permission to publish this paper.

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The Determination of Biphenyl and 2-Hydroxybiphenyl in Citrus Fruits

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A critical evaluation is made of the methods of analysis for biphenyl and 2-hydroxybiphenyl in citrus fruits as given in the recent "Preservatives in Food Regulations" 1974 and 1975. Recommendations for improvements in the statutory methods are made and an alternative G.L.C. procedure for both compounds is proposed. Typical levels found in samples of fruit and the recoveries obtained using both methods are given.

Biphenyl and 2-hydroxybiphenyl are used as fungicides in the post-harvest treatment of citrus fruits to prevent deterioration during transport and storage. The limits given in the "Preservatives in Food Regulations" 1974 and 1975 (Statutory Instruments No. 1119 and 1487) for residual levels are:

biphenyl : 70 mg/kg,
2-hydroxybiphenyl : 12 mg/kg.

Following the receipt by the Ministry of Agriculture, Fisheries and Food of some criticisms of the methods of analysis for these compounds (Schedule 5, Parts I, II and III), a thorough evaluation of the methods was undertaken at the Ministry's request. The methods are as described in an EEC directive[1] and have been translated into the Statutory Instruments (SIs) as required under the Treaty of Accession.

THE QUALITATIVE METHOD (SCHEDULE 5, PART I)

This was tested on several different samples, both spiked and unspiked, and was found to work satisfactorily; confirmation was obtained of the differing sensitivity of the method towards the two compounds; i.e. limits of detection of 5 mg/kg for biphenyl and 1 mg/kg for 2-hydroxybiphenyl.

THE CLEVENGER-TYPE SEPARATOR (SCHEDULE 6)

A description of this piece of apparatus was given in the EEC directive[1] and was put into the Statutory Instrument which came into force on 1 August 1974. It was not found possible to purchase the apparatus ready made from any of the major laboratory suppliers, and when it was constructed according to the dimensions given, the general appearance was considerably different from that in the diagram shown in the Statutory Instrument (Figure 1). Another piece was constructed following the same general appearance and size but disregarding the individual dimensions, and yet a third of our own design but following the same basic principles and overall size. It was found that the

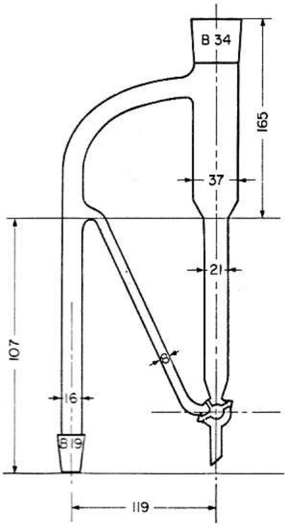

Fig. 1. Diagram of a modified Clevenger-type separator. Reproduced from Statutory Instrument 1975 No. 1487. Dimensions in millimetres.

recoveries of biphenyl and 2-hydroxybiphenyl were satisfactory using all three pieces of apparatus. It would appear, therefore, that minor deviations from the stated dimensions are not critical. In order to satisfy the need for a ready made apparatus an extensive search was made of the catalogues of the main laboratory suppliers. It revealed that a Type 2 receiver, 12·5 ml, as specified in BS 756: 1952, Dean and Stark apparatus, was the nearest item obtainable ready made and this could be purchased from most of the supply houses. This would only need the insertion of a three-way tap at the bottom of the graduated arm to be very similar in appearance and dimensions to the apparatus described in the Statutory Instrument. Such a modification could be easily made by a competent glass blower.

THE QUANTITATIVE METHOD FOR BIPHENYL (SCHEDULE 5, PART II)

In this method it was found that the two hour distillation period was satisfactory but that the analysis of the cyclohexane extract was time consuming and that very close attention to detail, particularly at the T.L.C. stage, was necessary before satisfactory results could be obtained. Table I shows the variation in absorbance of three standards each spotted on to three plates. Each group of three gives a reasonable straight line plot but the difference between each group is quite noticeable. Table II shows the variation of absorbance with the total time between spotting and measurement. Thus, the importance of a minimum but carefully controlled period of exposure is clearly demonstrated. The recoveries obtained using this method are shown as part of Table III. The specified method gave a limit of detection of 5 mg/kg. However, it was found that by viewing the developed plates under u.v. light instead of scraping off the spots this limit of detection could be lowered, in a semi-quantitative manner, to 1 mg/kg.

TABLE I

VARIATION IN ABSORBANCE OF STANDARD SOLUTIONS OF BIPHENYL AFTER T.L.C.

	Absorbance at 248 nm, 10 mm cell *vs.* blank		
μg BP/10 ml ethanol	(a)	(b)	(c)
30	0·32	0·275	0·295
50	0·52	0·42	0·495
70	0·74	0·62	0·675

TABLE II

VARIATION IN ABSORBANCE OF STANDARD SOLUTIONS OF BIPHENYL WITH TIME OF EXPOSURE ON T.L.C. PLATES

Time of exposure	Absorbance at 248 nm, 10 mm cell *vs.* blank
0–10 minutes	0·710
30 minutes	0·670
2 hours	0·655
3 hours	0·625
20 hours	0·040

TABLE III

RECOVERIES OF FUNGICIDES FROM CITRUS FRUITS

		2-Hydroxybiphenyl			Biphenyl		
Fruit	Method	Added, *mg*	Found, *mg*	Recovery, *per cent.*	Added, *mg*	Found, *mg*	Recovery, *per cent.*
Oranges 1	SI	—	(0·3)†	96	—	(4·8)	92
		2·3	2·5		5·0	9·2	
Oranges 2	G.L.C.	—	(0·3)	87	—	(18·4)	93
		3·0	2·9		15·0	32·3	
Oranges 3	SI	—	(0·1)	90	—	(6·5)	86
		1·0	1·0		8·0	13·4	
Grapefruit 1	SI	—	(0·1)	85	—	(4·7)	85
		2·0	1·8		10·0	13·2	
Grapefruit 2	G.L.C.	—	(0·2)	90	—	(0·3)	94
		3·0	2·9		15·0	14·4	
Lemons 1	SI	—	(0·1)	90	—	(<0·2)	98
		2·0	1·9		10·0	9·9	
Lemons 2	G.L.C.	—	(0·3)	90	—	(0·2)	99
		3·0	3·0		15·0	15·1	

† Figures in parentheses indicate natural values.

THE QUANTITATIVE METHOD FOR 2-HYDROXYBIPHENYL (PART III)

The chief problem in this method lies with the interpretation that might be put on the distillation instructions. Our findings were that if the instructions given were followed, namely—"heating gently . . ., without allowing the purée to boil or foam"—then no 2-hydroxybiphenyl at all distilled over. It was necessary to boil the mixture moderately vigorously so that one drop distilled over every 3–5 seconds for a total period of six hours, before satisfactory recoveries were obtained. Using this distillation procedure and the specified spectrophotometric method with 4-aminophenazone, the overall recovery rate was confirmed to be about 80–90 per cent. Individual results are given in Table III. The limit of detection was governed by the value of the blank, which was equivalent to 0·7 mg/kg for 2-hydroxybiphenyl. Thus, the limit of detection is normally taken to be 1 mg/kg.

In view of the above difficulties with the statutory methods, a single G.L.C. method for both compounds was developed.

GLC PROCEDURE FOR BOTH BIPHENYL AND 2-HYDROXYBIPHENYL

A number of methods for the determination of these compounds by G.L.C. have been put forward in recent years. Morries[2] used a simple cold extraction of the comminuted fruit but gave no details of recoveries whilst his quoted limit of detection for 2-hydroxybiphenyl of 5 mg/kg is in our opinion too high when compared with the permitted level of 12 mg/kg. Beernaert[3] used steam distillation for the separation stage, extracted the distillate with petroleum ether and then separated the two compounds from other components using gas chromatography with temperature programming. More recently, Westöö and Anderson[4] have used a differently modified Clevenger trap[5] for the steam distillation clean-up stage, with separate chromatographic columns for the determination of the two compounds. If an accurate result for low levels of 2-hydroxybiphenyl was required a further clean-up step was incorporated.

The method proposed here uses the modified Clevenger trap given in SIs 1119 and 1487 for the steam distillation step. This is considerably different in size and shape from that proposed by Westöö,[5] and a single gas chromatographic column for the determination of both compounds using heptadecane as an internal standard. If, despite the fact that the amount of 2-hydroxybiphenyl found in the sample is well below the statutory limit, an accurate assessment of the amount present is required then it may be necessary to use the clean-up procedure recommended by Westöö and Anderson[4].

Method

REAGENTS

1. *Sulphuric acid*, 50 per cent. v/v.
2. *Cyclohexane*, spectrosol grade.
3. *Heptadecane*, A.R. grade—dissolve 500 mg in cyclohexane and make up to 50 ml in a volumetric flask. 1 ml ≡ 10 mg of heptadecane.
4. *2-Hydroxybiphenyl*, A.R. grade—dissolve 500 mg in cyclohexane and make up to 50 ml in a volumetric flask. 1 ml ≡ 10 mg of 2-hydroxybiphenyl.
5. *Biphenyl*, A.R. grade—dissolve 500 mg in cyclohexane and make up to 50 ml in a volumetric flask. 1 ml ≡ 10 mg of biphenyl.

APPARATUS

Food-mixer—Kenwood, or similar with liquidiser attachment.

Coffee grinder.

Distillation apparatus: 1 litre round-bottom flask fitted with a modified Clevenger separator (see Figure 1) and a water jacket condenser.

Heating mantle.

Vacuum evaporator with 50 ml flasks.

Gas chromatograph: Becker model 420 fitted with a flame ionisation detector and connected to a Servoscribe chart recorder, model RE541.

Column: glass, 2 m × 4·0 mm i.d., packed with 3 per cent. OV-17 on Gas Chrom. Q.

OPERATING CONDITIONS

Injector temperature: 210°C.

Column temperature—programme: 130°C for 10 min,
130–160°C at 10°C per min,
160°C for 22 min.

Detector temperature: 225°C.

Carrier gas flow rate: 30 ml nitrogen/min.

Detector gas pressures: hydrogen and air set according to manufacturer's instructions.

PROCEDURE

Separate the flesh and the peel from at least 2 kg of fruit. Cut the peel into small pieces and grind in a coffee grinder. Homogenise the flesh in a liquidiser. Combine the portions and mix well. Take 300 g of recombined fruit pulp and add 300 ml of water. Homogenise at low speed in a mixer and pour into the 1 litre round-bottom flask, rinsing the homogeniser with 50 ml water and adding this to the flask. Add an anti-foam tablet, a few anti-bumping granules and 15 ml of 50 per cent. sulphuric acid. Assemble the distillation apparatus and place 15 ml of water and 20 ml of cyclohexane in the side arm of the separator. Boil the mixture gently for 20 min and then more vigorously for the remainder of 2 hours such that one drop distils every 3–5 seconds. At the end of this time allow the mixture to cool. Run the water from the Clevenger separator into a 100 ml separating funnel and the cyclohexane into a 25 ml volumetric flask. Rinse the Clevenger separator with 4 ml of cyclohexane and add this to the water in the separating funnel. Shake well, allow the layers to separate, discard the water and add the cyclohexane to that in the 25 ml volumetric flask. Make up to volume with cyclohexane, add a little anhydrous sodium sulphate and mix well (this is extract "a"). Set up the separator again with a fresh portion of 20 ml of cyclohexane and repeat the two hour distillation and subsequent separation as above (this is extract "b").

PREPARATION OF STANDARD GRAPHS

Prepare five standard solutions (A–E) containing the indicated amounts of fungicide and internal standard:

		A	B	C	D	E
Volume (ml) of biphenyl solution	10 mg/ml	0·4	0·8	1·2	1·6	2·0
Volume (ml) of 2-hydroxybiphenyl	10 mg/ml	0·1	0·2	0·3	0·4	0·5
Volume (ml) of heptadecane	10 mg/ml	1·0	1·0	1·0	1·0	1·0

Make each up to 5 ml with cyclohexane.

Inject 1·5 μl of each standard and record the chromatogram. Measure the areas (height × width at half height) of the peaks. Plot the area ratio of fungicide to internal standard against mg fungicide per 10 mg of heptadecane for each compound.

DETERMINATION OF BIPHENYL

To 10 ml of extract "a" add 0·4 ml of heptadecane solution, 10 mg/ml. Inject 1·5 μl and record the chromatogram. Measure the peak areas and calculate the ratio of peak area of biphenyl to peak area of heptadecane. From the standard graph read off the biphenyl content, allowing for the amount of heptadecane added. If necessary the solution may be reduced in volume under vacuum but because of the volatility of biphenyl the volume should not be taken below 2 ml.

DETERMINATION OF 2-HYDROXYBIPHENYL

Combine 10 ml of extract "a" and 10 ml of extract "b" and add 0·1 ml of heptadecane solution, 10 mg/ml. Evaporate the solution under vacuum but without rotating the flask until the volume is reduced to a few ml. Inject 1·5 μl and record the chromatogram. Measure the peak areas and calculate the ratio of peak area of 2-hydroxybiphenyl to peak area of heptadecane. From the standard graph read off the 2-hydroxybiphenyl content allowing for the amount of heptadecane added. The attenuation of the instrument should be adjusted so that the heptadecane peak is at least 40 per cent. of maximum. The biphenyl peak may then be off scale. Care should be taken to identify correctly the 2-hydroxybiphenyl peak particularly if it is small; natural substances present in citrus fruit sometimes give peaks corresponding to 0·5–1·0 mg/kg of 2-hydroxybiphenyl at the same retention time. If the main interest is in whether the fruit complies with the legal requirements this is unlikely to be serious but if an absolute value of 2-hydroxybiphenyl in the sample is required then the clean-up procedure, according to Westöö and Anderson,[4] is employed.

Results and Discussion

Samples of oranges, lemons and grapefruits were examined for both fungicides by the quantitative methods given in the SI and also by the G.L.C. procedure.

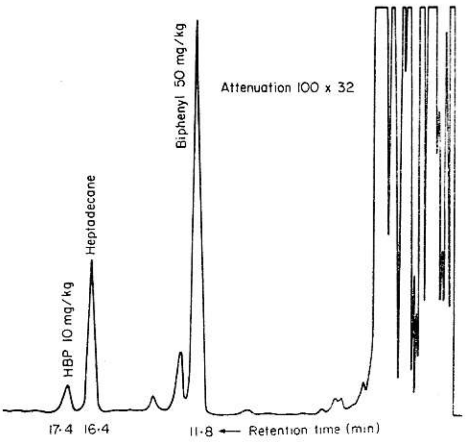

Fig. 2. Determination of biphenyl in oranges.

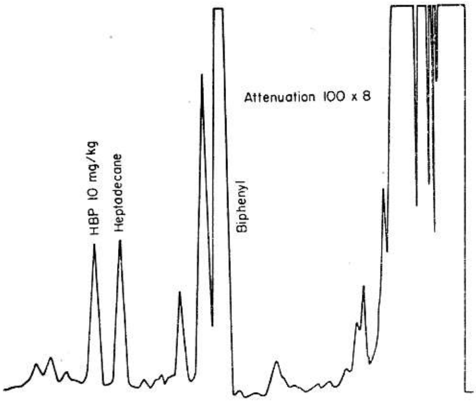

Fig. 3. Determination of 2-hydroxybiphenyl in oranges.

The recoveries obtained by a comparison of spiked and unspiked samples are given in Table III whilst the residues found in other samples using both methods are given in Table IV. The chromatograms shown in Figures 2 and 3 are those

TABLE IV

RESIDUES OF FUNGICIDES IN CITRUS FRUITS

		2-Hydroxybiphenyl, *mg/kg*		Biphenyl, *mg/kg*	
Fruit		By SI	By G.L.C.	By SI	By G.L.C.
Oranges	1	<1	0·5	35·4	39·0
	2	2·6	not determined	6·9	5·9
	3			5·7	6·2
Grapefruit	1	<1	0·3	5·3	5·5
	2			4·1	3·8
Lemons	1	<1	0·3	<0·2	0·2
	2			<0·2	0·2

of two 300 g portions from the same original sample of oranges spiked with 15 mg of biphenyl (50 mg/kg) and 3 mg of 2-hydroxybiphenyl (10 mg/kg) and then treated by the G.L.C. procedures given above. Figures 4(a) and (b) show the chromatograms of unspiked orange samples, with and without the clean-up step recommended for accurate low level determinations. After clean-up it can be seen that most of the spurious, and possibly interfering, peaks have been eliminated. Figure 4(c) shows the chromatogram of a sample of oranges, which was spiked with 3 mg/kg of 2-hydroxybiphenyl, and for which this clean-up procedure was used.

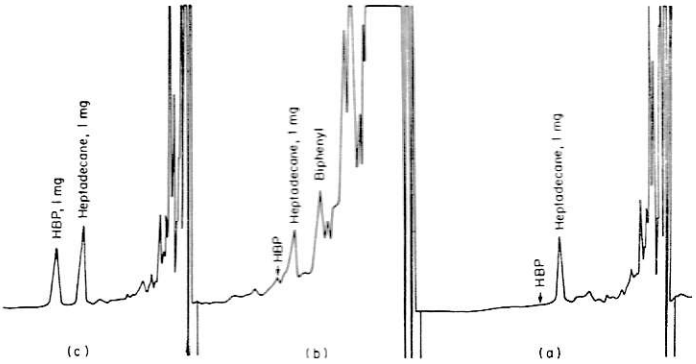

Fig. 4. Effect of clean-up on extracts of 2-hydroxybiphenyl from oranges. (a) Unspiked, after clean-up; (b) unspiked, before clean-up; (c) spiked, after clean-up.

Conclusion

A summary of our findings is that for accuracy the G.L.C. method is preferred for biphenyl and the Statutory Instrument method for 2-hydroxybiphenyl. However, for uniformity, simplicity and speed the G.L.C. method is preferred for both; the accuracy of this method for the low level determination of 2-hydroxybiphenyl can be improved by the incorporation of an additional clean-up stage.

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