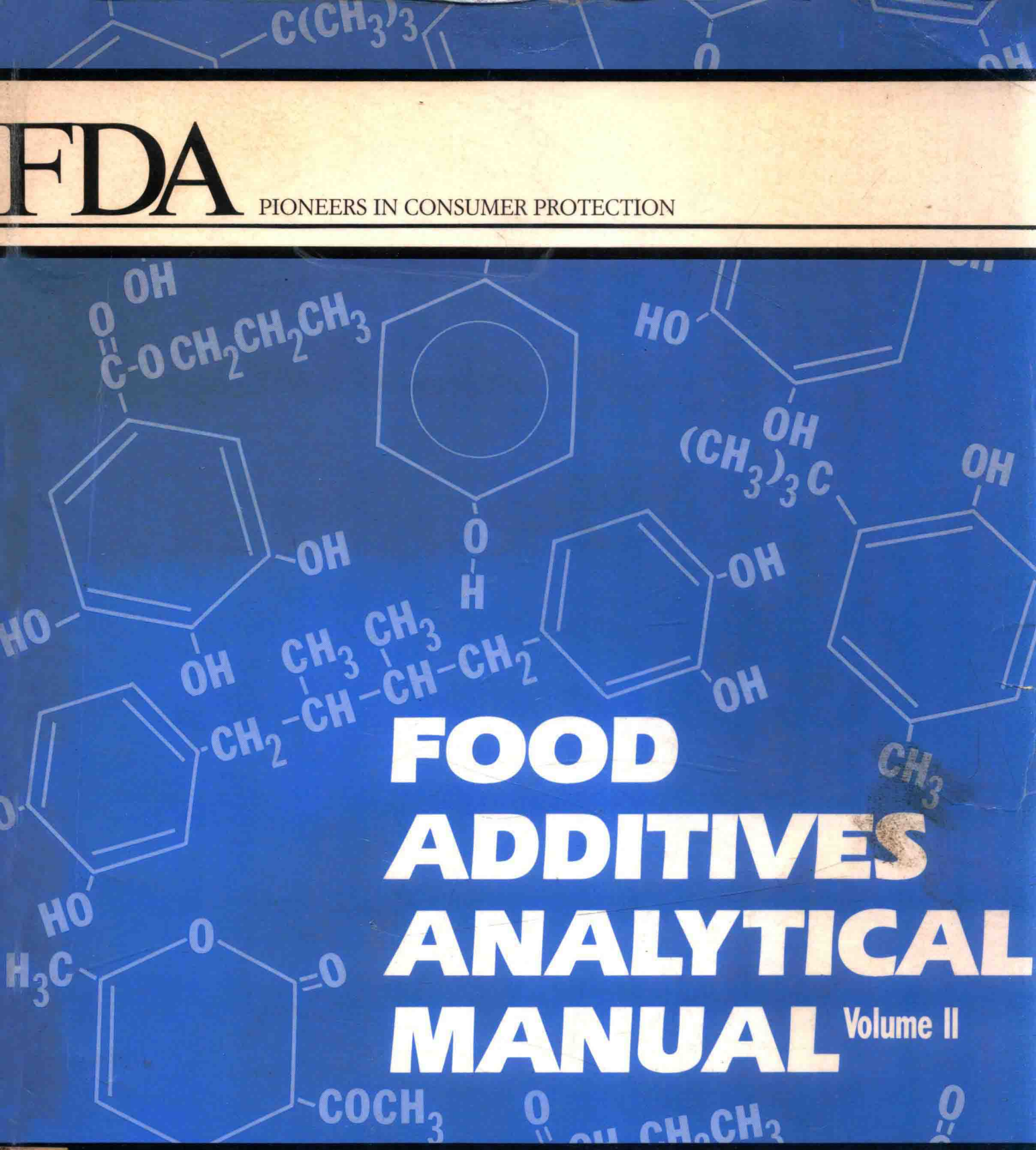
FDA

PIONEERS IN CONSUMER PROTECTION



FOOD ADDITIVES ANALYTICAL MANUAL

Volume II

PUBLISHED BY THE ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS

FOOD ADDITIVES ANALYTICAL MANUAL

VOLUME II

A Collection of Analytical Methods for
Selected Food Additives

EDITOR

T. FAZIO

U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Physical Sciences
Washington, DC

TECHNICAL EDITOR

J. SHERMA

AOAC Scientific Coordinator
Lafayette College
Department of Chemistry
Easton, PA

1987
U. S. Food and Drug Administration

Published and Distributed by

Association of Official Analytical Chemists
1111 North 19th Street, Suite 210
Arlington, VA 22209, USA

Notice to Users

Any information or data concerning the methods in this manual are welcomed. Please submit comments in writing to the editor:

T. Fazio
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Physical Sciences, HFF-400
200 C Street, SW
Washington, DC 20204

ISBN 0-935584-34-X

ACKNOWLEDGMENTS

The Editor gratefully acknowledges the invaluable assistance to the technical and editorial review provided by

J. Modderman
C. Warner

PREFACE

This edition is Volume II of the Food Additives Analytical Manual (FAAM) by the U.S. Food and Drug Administration (FDA) and is intended as with Volume I (1983, AOAC) to provide analysts with methodology needed to determine compliance with food additive regulations. The Manual contains procedural monographs describing analytical methods that have been evaluated by FDA or found to operate satisfactorily in at least two laboratories.

FAAM was originally designed, published, and supported by scientific personnel of the Executive Director for Regional Operations (EDRO) for use by District Laboratory analysts. Analytical methods were drawn from Food Additive Petitions and New Animal Drug Applications. EDRO published two editions of FAAM, in 1965 and 1973. In 1974/5, EDRO found that they did not have the resources necessary to provide a new edition of FAAM. The Division of Chemistry and Physics of the Bureau of Foods (now Center for Food Safety and Applied Nutrition (CFSAN)) assumed responsibility for preparation of FAAM, with AOAC as the prime contractor. These efforts culminated in the publication of Volume I. A continuation of these efforts and responsibilities by the Office of Physical Sciences, CFSAN, has resulted in the publication of this edition, Volume II, of FAAM.

Like FAAM-Volume I, this edition contains no methods for pesticides. These may be found in the U.S. FDA Pesticide Analytical Manual. FAAM is limited to direct food additives, indirect food additives, and contaminants likely to be present because of the use of food additives or exposure to other chemicals in food processing.

Format of the Monographs

The monographs (as in Volume I), which are titled according to the name found in the regulation, consist of three main sections: Introductory Notes, Methods, and References. For a given chemical substance, more than one method may be presented. Also, some multicomponent methods are described. Where appropriate, the structural formula, Chemical Abstracts name, Chemical Abstracts Service (CAS) Registry Number, empirical formula, molecular (formula) weight, and alternative names are listed for the chemical substance.

Under the Federal Food, Drug, and Cosmetic Act, FDA has the authority to establish and enforce regulatory limits on the amounts of chemical substances directly or indirectly added to foods. FDA also has the authority to prohibit the addition of certain chemicals to foods. In both of these instances, reliable and practical methods are required to determine the quantity of a given substance in a sample. This Manual is intended to supply these methods.

Some of the methods in this Manual have been subjected to collaborative study; however, the editors expect that the methods, skillfully performed, will provide reliable results to guide further survey or regulatory work. In the event that the results are required for legal proceedings, the analyst must

demonstrate that the method is performing properly through the use of controls and recovery studies. For regulatory procedures, a check analysis, preferably by a method based on different principles, should be performed by a second analyst to document the validity of the data.

Analytical Quality Assurance

The validity of results must be clearly demonstrable through the documented application of quality assurance practices. This is especially true because the determinations could be used as evidence in a court of law. This Manual is intended for use by experienced analysts who maintain good quality control in their laboratories. Quality control comprises the laboratory practices, carried out by analysts, to assure the accuracy and precision of their analytical results. This topic has been discussed by Horwitz in the following papers that should be required reading for all analysts:

1. "Good Laboratory Practices in Analytical Chemistry." Horwitz, W. (1978) Anal. Chem. 50, 521A-524A.

2. "Quality Assurance in the Analysis of Foods for Trace Constituents." Horwitz, W., Kamps, L. R., and Boyer, K. W. (1980) J. Assoc. Off. Anal. Chem. 63, 1344-1354.

3. "Evaluation of Analytical Methods Used for Regulation of Foods and Drugs." Horwitz, W. (1982) Anal. Chem. 54, 67A-76A.

These papers discuss recordkeeping, sample homogeneity, calibrations, reference standards, calculations, laboratory housekeeping, the statistics of interlaboratory studies, and practical analytical quality control.

INTRODUCTION

A food additive is defined as "... any substance, the intended use of, which results or may reasonably be expected to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristics of any food (including any substance intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food; and including any source of radiation intended for any such use) ..." Food, Drug, and Cosmetic Act, as amended: Sec. 201(a). Excluded from this definition are pesticide chemicals on raw agricultural commodities, color additives, and new drugs intended for use in food-producing animals. Also exempt are substances which were GRAS (generally recognized as safe) by scientific experts or sanctioned by the U.S. Department of Agriculture prior to the enactment of the Food Additives Amendment of January 1, 1958.

Direct Food Additives

Direct additives are regulated in Parts 172, 173, 179, 180, 182, 184, and 189 of Title 21, CFR (Code of Federal Regulations). Parts 172, 173, and portions of 179 and 180 cover direct additives regulated after enactment of the Food Additives Amendment to the Food, Drug, and Cosmetic Act. Part 172 covers food additives permitted for direct addition to food for human consumption, including food preservatives; coatings, films, and related substances; special dietary and nutritional additives; flavoring agents and related substances; gums, chewing gum bases, and related substances; other specific usage additives; and multipurpose additives. Part 173 covers secondary direct food additives permitted in food for human consumption, including polymer substances for food treatment; enzyme preparations and microorganisms; solvents, lubricants, and release agents; and specific usage additives, such as boiler water additives. Part 179 regulates irradiation in the production, processing, and handling of food. Part 180 covers food additives permitted in food or in contact with food on an interim basis pending additional study; substances listed include acrylonitrile copolymers, mannitol, brominated vegetable oil, and saccharin and its salts. Part 182 covers multiple purpose food substances, anticaking agents, chemical preservatives, emulsifying agents, nutrients and dietary supplements, sequestrants, and stabilizers that are GRAS. Part 184 lists substances affirmed as GRAS. Portions of Part 182 and all of Part 184 pertain to ingredients directly added to foods. Part 189 covers substances previously allowed as direct additives (Subpart B) or as indirect additives through food-contact surfaces (Subpart C), but which are now prohibited from use in foods.

Generally Recognized As Safe

Many of the GRAS additives are regulated "at levels not to exceed good manufacturing practice." This statement may be followed by an upper limit on the use level(s) considered by the Agency in its Safety Review of GRAS Ingredients. The limits indicate our understanding of current uses of the ingredient at the time the regulation was promulgated. The limits are not legal tolerances. Methods may be given in the monographs of GRAS ingredients

limited only by current good manufacturing practice (GMP); however, the regulatory analyst should note that there are no official limitations on these additives.

Indirect Food Additives

Food additives that enter food by migrating out of food packaging and food processing equipment are called indirect food additives. Unlike direct food additives, they provide no functional effect in the food. Indirect additives usually consist of such chemicals as residual monomers, solvents, plasticizers, stabilizers, antioxidants, slip agents, processing oils, etc.

CFR, Title 21, Parts 174-78, 179, 180, 182, 186, and 189, covers the regulation of these additives. Some of the indirect additives are considered GRAS, some have prior sanction status because they were in use before the passage of the 1958 Food Additives Amendment, and the rest are covered by specific regulations.

Migration tests are carried out to support petitions for the regulation of indirect additives. Food simulating solvents are generally used in these tests rather than foods themselves. This greatly simplifies the analytical procedures required to measure migration. The most commonly used food simulating solvents are:

1. distilled water for foods above pH 5.0
2. 3% acetic acid for foods pH 5.0 or below
3. 8-50% aqueous ethanol for foods of corresponding alcohol content
4. n-heptane for fatty foods

Many of the methods for indirect additives use these simulating solvents.

DIRECTORY OF SOURCES OF APPARATUS AND REAGENTS

(All references to commercial apparatus and chemicals are for descriptive purposes only and do not constitute endorsement or recommendation of a product or source by the U.S. Food and Drug Administration; equivalent products may be substituted.)

Abbott Laboratories, 14th St and Sheridan Rd, North Chicago, IL 60064

Ace Scientific Supply Co., Inc., 40-A Cotters Ln, East Brunswick, NJ 08816

Aldrich Chemical Co., Inc., 940 W St. Paul Ave, Milwaukee, WI 53201

Alltech Associates, Inc., 2051 Waukegan Rd, Deerfield, IL 60015

Altex Scientific (Division of Beckman Instruments, Inc.), 2350 Camino Ramon, P.O. Box 5101, San Ramon, CA 94583

American Hospital Supply Corp., Scientific Products Division, 1430 Waukegan Rd, McGaw, IL 60085

American Scientific Products, 1430 Waukegan Rd, McGaw Park, IL 60085

Amersham International plc, Amersham Place, Little Chalfont, Bucks HP7 9NA, United Kingdom

Analabs (Foxboro Analabs), 80 Republic Dr, North Haven, CT 06473

Applied Science Laboratories, Alltech Associates, Inc., 2051 Waukegan Rd, Deerfield, IL 60015

Atlas Chemical Division, ICI Americas, Inc., Wilmington, DE 19810

J. T. Baker Chemical Co., 222 Red School Ln, Phillipsburg, NJ 08865

Beckman Instruments, Inc., Altex Scientific Division, 2350 Camino Ramon, P.O. Box 5101, San Ramon, CA 94583

Becton-Dickinson Labware, 1915 Williams Dr, Oxnard, CA 93030

Best Foods, CPC International Inc., 1120 Commerce Ave, Union, NJ 07083

Blue M, A Unit of General Signal, 138th and Chatham Sts, Blue Island, IL 60406

Boekel Industries, Inc., 509 Vine St, Philadelphia, PA 19106

Branson Ultrasonics Corp., Parrott Dr, Shelton, CT 06484

Brinkmann Instruments, Inc., Cantiague Rd, Westbury, NY 11590

Brownlee Labs, Inc., 2045 Martin Ave, Santa Clara, CA 95050

Buchler Instruments, Inc., 1327 16th St, Fort Lee, NJ 07024

Burdick & Jackson Laboratories, Inc., 1953 S Harvey St, Muskegon, MI 49442

Calbiochem-Biochemicals/Behring Diagnostics, 10933 N Torrey Pines Rd, La Jolla, CA 92037

Directory of Apparatus and Reagents (continued)

Camag Scientific, Inc., P.O. Box 563, Wrightsville Beach, NC 28480

Cary; Varian Instrument Group, 220 Humboldt Ct, Sunnyvale, CA 94089

Chromanetics Corp., 7900 Cessna Ave, Gaithersburg, MD 20879

Digital Equipment Corp., One Iron Way, Marlboro, MA 01752

Dow Corning Corp., Box 994, Midland, MI 48686-0994

E. I. du Pont de Nemours & Co., 1007 Market St, Wilmington, DE 19898

Dynapol, Palo Alto, CA

Eastman Chemical Co., P.O. Box 431, Kingsport, TN 37662

Eastman Kodak Co., 343 State St, Rochester, NY 14650

EM Science, Division of EM Industries, Inc., 111 Woodcrest Rd, Cherry Hill, NJ 08034-0395

Emery Industries, Inc., P.O. Box 628, Mauldin, SC 29662

Finnigan MAT, 355 River Oaks Pkwy, San Jose, CA 95134-1991

Fisher Scientific Co., 711 Forbes Ave, Pittsburgh, PA 15219

Fluka AG, CH-9470 Buchs, Switzerland

The Foxboro Co., Bristol Park, Foxboro, MA 02035

Gelman Sciences Inc., 600 South Wagner Rd, Ann Arbor, MI 48106

Glyco Chemicals, Inc., Williamsport, PA 17701

Gohlke (Finnigan Corp.), 355 River Oaks Pkwy, Sun Jose, CA 95134

Goodyear Tire & Rubber Co., Chemical Division, 1144 E Market St, Akron, OH 44316

Hamilton Co., 4970 Energy Way, Reno, NV 89502

Hewlett-Packard Co., Mail Stop 2083, 3000 Hanover St, Palo Alto, CA 94304

Hodag Chemical Co., 7247 North Central Park Ave, Skokie, IL 60077

IBM Instruments, Inc., P.O. Box 3332, Danbury, CT 06813

Directory of Apparatus and Reagents (continued)

ICI Americas, Inc., Atlas Chemical Division, Wilmington, DE 19810

International Products Corp., P.O. Box 118, Trenton, NJ 08601

Kontes, Inc., Spruce St, P.O. Box 729, Vineland, NJ 08360

Kratos Analytical, 170 Williams Dr, Ramsey, NJ 07446

Launders, Frary, and Clark Co., New Britain, CT

Arthur D. Little, Inc., Acorn Park, Cambridge, MA 02140

Matheson Gas Products, Inc., P.O. Box 158, Secaucus, NJ 07094

MD Industries/Gas Products, 2460 Boulevard of the Generals, P.O. Box 945, Valley Forge, PA 19482

Millipore Corp., Ashby Rd, Bedford, MA 01730

Milton Roy Co., Analytical Products Division, 820 Linden Ave, Rochester, NY 14625

Morgan Scientific Corp., North Strong Division, Rockville, MD 20805

National Appliance Co., 10855 SW Greenburg Rd, Portland, OR 97223

National Bureau of Standards, Office of Standard Reference Materials, Room 222, Bldg B-311, Gaithersburg, MD 20899

Neslab Instruments, Inc., P.O. Box 1178, Portsmouth, NH 03801

Nu-Check Prep, Inc., P.O. Box 172, Elysian, MN 56028

Packaging Industries, Inc., Airport Rd, Hyannis, MA 02601

Packard Instrument Co., Inc., 2200 Warrenville Rd, Downers Grove, IL 60515

Perkin-Elmer Corp., Instrument Division, Main Ave, Norwalk, CT 06856

Pierce Chemical Co., P.O. Box 117, Rockford, IL 61105

Polysciences Inc., 400 Valley Rd, Warrington, PA 18976

Precision Sampling Corp., P.O. Box 15119, Baton Rouge, LA 70815

Procter and Gamble, 6071 Center Hill Rd, Cincinnati, OH 45224

Publicker Industries, Inc. 777 W Putnam Ave, Greenwich, CT 06830

PVO International, Inc., 416 Division St, Boonton, NJ 07005

Directory of Apparatus and Reagents (continued)

Rheodyne, Inc., P.O. Box 996, Cotati, CA 94928

Rucker, Melville, NY 11747

Schluerberg-Kurdle Co. (Esskay), 3800 E Baltimore Ave, Baltimore, MD 21203

Schoeffel, distributed by Kratos Analytical, Division of Spectros, 170 Williams Dr, Ramsey, NJ 07446

The Separations Group, 17434 Mojave St, P.O. Box 867, Hesperia, CA 92345

Shamrock Glass Co., 201 E Tenth St, Marcus Hook, PA 19061

Shimadzu Scientific Instruments, Inc., 7102 Riverwood Dr, Columbia, MD 21046

Ivan Sorvall, Inc., (du Pont Sorvall), Pecks Ln, Newtown, CT 06478

Spectra-Physics, Autolab Division, 3333 N First St, San Jose, CA 95134-1995

Spectrum Scientific Corp., 2401 Ogletown Rd, Newark, DE 19711

Supelco, Inc., Supelco Park, Bellefonte, PA 16823-0048

The Superior Electric Co., 383 Middle St, Bristol, CT 06010

Tektronix, Inc., P.O. Box 500, Y3-314, Beaverton, OR 97077

Thermedics Inc., 470 Wildwood St, Woburn, MA 01888-1799

Thermo Electron Instruments, Inc., 108 South St, Hopkinton, MA 01748

Tracor Instruments, Division of Tracor Inc., 6500 Tracor Ln, Building 27-7, Austin, TX 78725

U.S. Phamacopeial Convention, Inc., 12601 Twinbrook Pkwy, Rockville, MD 20852

UVP, Inc. (Ultra-Violet Products), 5100 Walnut Grove Ave, San Gabriel, CA 91778

Valco Instruments Co., Inc., P.O. Box 55603, Houston, TX 77255

Varian Instrument Group, 220 Humboldt Ct, Sunnyvale, CA 94089

Waters Chromatography, Division of Millipore, 34 Maple St, Milford, MA 01757

ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
ASTM	American Society for Testing and Materials
AUFS	absorbance unit full scale
CFR	Code of Federal Regulations
CI	chemical ionization
CV	coefficient of variation
EC	electron capture
EI	electron impact
FDA	Food and Drug Administration
FDCA	Food, Drug, and Cosmetic Act
FID	flame ionization detection (detector)
FSD	full-scale deflection
GC	gas chromatography
GC/MS	gas chromatography/mass spectrometry
GMP	Good Manufacturing Practice
GRAS	Generally Recognized As Safe
HED	Hall electroconductivity detection (detector)
IR	infrared
KD	Kuderna-Danish
LC	liquid chromatography
MID	multiple ion detection (detector)
MS	mass spectrometry
MWD	molecular weight distribution
NMR	nuclear magnetic resonance
N/P	nitrogen/phosphorus
PID	photoionization detection (detector)
RSD	relative standard deviation
RI	refractive index
RT	retention time
SD	standard deviation
SEC	size exclusion chromatography
SIM	single ion monitoring
SRM	Standard Reference Material
TEA	thermal energy analyzer
TLC	thin layer chromatography
USDA	U.S. Department of Agriculture
UV	ultraviolet

CONTENTS

$CH_2=CHCN$

Chemical Abstracts name: 2-propenenitrile
Chemical Abstracts Registry Number: 107-13-1
Empirical formula: C_3H_3N
Molecular weight: 53.06
Alternative names: vinyl cyanide; cyanoethylene

INTRODUCTORY NOTES

Acrylonitrile (AN) copolymers are regulated in 21 CFR 175.300 (resinous and polymeric coatings); 176.170 (components of paper and paperboard in contact with aqueous and fatty foods); 177.1010 (acrylic and modified acrylic plastics, semirigid and rigid); 177.1020 (AN/butadiene/styrene copolymer); 177.1030 (AN/butadiene/styrene/methyl methacrylate copolymer); 177.1040 (AN/styrene copolymer); 177.1050 (AN/styrene copolymer modified with butadiene/styrene elastomer); 177.2600 (rubber articles intended for repeated us); and 180.22 (AN copolymers). AN copolymer bottles made with AN as the principal monomer may not be used as beverage containers unless such copolymers comply with 177.1040.

21 CFR 177.1030 states that the finished food-contact article shall yield not more than 0.0025 mg/in.^{2} of AN monomer when exposed to distilled water, 3% acetic acid, and n-heptane at 190°F for 2 hours, cooled to 120°F (80-90 minutes), and maintained at 120°F for 10 days.

21 CFR 180.22 states that in the case of single-use articles having a volume to surface area ratio of less than 10 ml per square inch of food-contact surface, the finished food-contact article shall yield not more than 0.3 ppm calculated on the basis of the volume of the container when extracted to equilibrium at 120°F with food-simulating solvents appropriate to the intended use conditions.

METHODS

The Food Additives Analytical Manual, Volume I (FAAM I), contains methods for the distillation-gas chromatographic (GC) determination of acrylonitrile in food-simulating solvents with gas chromatographic/mass spectrometric (GC/MS) confirmation (pages 9-17), headspace-GC determination in polymer solutions (pages 18-26), and headspace-GC determination in 3% acetic acid (pages 27-32).

I. Manual Headspace-GSC Determination in 3% Acetic Acid

A. Scope:

Acrylonitrile (AN) is determined in 3% acetic acid, the solvent used to simulate carbonated beverages.

B. Principle:

Acrylonitrile residues in the food-simulating solvent acetic acid are determined by manual headspace-GC analysis. A detector signal amplifier and an ionic compound (Na_2SO_4) to enhance the AN concentration in the headspace are used to increase the sensitivity of detection.

C. Limit of Reliable Measurement:

The detection limit of the method is in the low parts per trillion range.

D. Apparatus:

Gas chromatograph, Perkin-Elmer Model 3920, with nitrogen/phosphorus (N/P) selective detector.

GC columns, (A) coiled glass, 6 ft (1.8 m) x 2 mm i.d., packed with 80-100 mesh Chromosorb 101; (B) coiled glass, 3 ft (0.9 m) x 2 mm i.d., packed with 100-120 mesh Chromosorb 108.

Spectrum filter and amplifier (Spectrum Scientific Corp. Model 1021A).

Headspace sample vials, 23 ml, with 12 x 20 mm finished tops and aluminum seals (Shamrock Glass Co.) and fitted butyl and Tuf-Bond Teflon/silicone septa (Pierce Chemical Co. No. 12720).

Syringes, 100 µl (Hamilton 700 series); 2 and 5 ml Pressure-Lok series A-2 gas syringes (Precision Sampling Corp.).

Oven, forced air, Stable-Therm constant temperature cabinet (Blue M Electric).

E. Reagents and Solutions:

Acetic acid, 3% aqueous solution. Prepare fresh weekly using Baker Analyzed reagent grade glacial acetic acid.

Acrylonitrile (AN), 99% pure (Polysciences, Inc.). CAUTION: AN is a teratogen and carcinogen. Use safety precautions.

AN stock standard solution, ca 2000 ppm in 3% acetic acid. In a hood, transfer 20 ml of 3% acetic acid into a tared headspace vial. Cap and

weigh to the nearest 0.1 mg. Transfer 50 µl of AN into the vial by using a 100 µl syringe. Cap the vial with a fitted butyl septum, reweigh, and seal. Calculate the exact AN concentration.

AN intermediate standard solution, ca 10 ppm. Add 0.1 ml of stock standard solution to 20 ml of 3% acetic acid.

AN working standard solution, ca 0.05 ppm. Add 0.1 ml of intermediate standard solution to 20 ml of 3% acetic acid. Seal the vial with a Teflon-faced septum. The solution is stable for 2 weeks if kept sealed and at room temperature.

F. Analysis:

1. Preparation of Headspace Standards and Samples:

a. Standards:

Transfer 4.5 g of anhydrous Na_2SO_4 to a headspace vial. Pipet 15 ml of 3% acetic acid into the vial. Add the appropriate volume of intermediate working standard solution to the vial by using a 100 µl syringe. Quickly seal the vial with a Teflon-faced septum. Transfer the vial to a forced air oven set at 90°C. Periodically shake the vial until the salt dissolves (ca 1.5 hours).

b. Samples:

Add a 15 ml sample to a headspace vial containing 4.5 g of anhydrous Na_2SO_4. Seal the vial with a Teflon-faced septum and prepare for analysis as described in (a) for headspace standards.

2. Isolation Procedures -- Analysis of Headspace Solutions:

Analyze samples and standards in the same manner. After complete dissolution of the salt, allow 30 minutes for complete phase equilibrium to occur. With a syringe previously heated in the same oven, pierce the vial septum, draw up to the full volume of the syringe once, expel, and then draw up the desired headspace volume. Let the needle remain in the vial headspace for at least 1 minute in the closed oven. Quickly close the syringe valve and remove the syringe.

3. Determinative Procedures:

a. GC Operating Conditions:

Injector temperature: 175°C
Oven temperature: column A, 115°C; column B, 125°C
Interface temperature: 215°C