VITAMIN ANALYSIS for the HEALTH and FOOD SCIENCES

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Preface

During our careers at the University of Georgia and with the U.S. Food and Drug Administration, we have been privileged to experience and participate in the rapid advance of vitamin assay methodology. From the early 1970s, our field progressed from reliance on techniques developed decades earlier to a degree of sophistication that few of us foresaw. Indeed, we clearly remember the effort required to obtain valid data on the vitamin content of the food supply using microbiological, thin-layer and open-column chromatographic, and spectrophotometric assays. We know that scientists investigating the vitamins in clinical samples had similar experiences. A great deal of discipline was required on the part of experienced analysts and graduate students to produce analytical values which, for the most part, have withstood the test of time when compared to values obtained with current, much improved, procedures.

Challenges we firmly appreciate are training of students and analysts in the proper application of the best vitamin assay methods, and the frequently required efforts to improve, develop, or adapt existing methods to meet specific analytical needs. This book was written as a source for these activities.

Our discussion on each vitamin includes a review section aimed at providing individuals who have not studied the vitamins in-depth an appreciation of the uniqueness of the vitamin and how it participates in metabolism. This section has purposefully been kept brief. However, the references provided can be highly useful for those seeking additional information.

We strongly feel that individuals involved in the analysis of any analyte must have a basic understanding of the chemistry of the compound. Therefore, chemistry and nomenclature of each vitamin is discussed. The information might be considered inadequate by the research biochemist or analytical chemist involved in basic studies on a specific vitamin; however, our goal was to produce a usable source for analysts at the bench and not to write a multi-volume treatise. Extensive information is given in tabular form on spectral properties. Such data is routinely required on a day-to-day basis at the bench. Additionally, stability properties of the vitamins are discussed. Stability considerations can be easily disregarded or forgotten during routine analysis or during method development if the chemist is not thoroughly aware of the specific properties of the vitamin that affect stability. If this happens, extensive efforts can be negated when the oversight is recognized.

In the method section of each chapter, our purpose was two-fold. First, we felt that attention needed to be given to commonly used and available handbook, compendium, and regulatory methods. These accepted procedures are in use world-wide and, from a regulatory standpoint, maintain significant status. A summary table is provided covering many of these sources. Additionally, several of the AOAC International Methods are discussed in detail. Secondly, our primary objective was to give an interpretive review of the development of advanced methods of vitamin analysis in sufficient detail to be valuable as a methodology guide.

When analysis programs are being initiated, much effort can be saved in making correct decisions about analytical approach if the analyst has a thorough grasp of research leading to available methods. For the vitamins for which high-performance liquid chromatography (HPLC) is the best approach, detailed tables are presented describing historically significant method development advances that led the way to current methods as well as significant publications which appeared in the 1990s. References used for tabular information are provided separately from text references to ease the reader's ability to quickly find the cited bibliographic information. It is evident for each vitamin that the efforts of a few research groups have driven the field to its current capability to accurately assay the vitamin. We hope that our discussion has given the credit where it is due. At the same time, the literature is voluminous and expanding extremely rapidly. It was impossible to cite all

efforts playing a role in vitamin assay. If this book can lead those endeavoring to initiate a vitamin analysis program to the right groups of investigators producing the current advances, then, we have accomplished our purpose to provide a usable source for today's vitamin chemist.

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Dr. Ronald R. Eitenmiller is Professor of Food Biochemistry, Department of Food Science and Technology, University of Georgia. He has published widely on food composition, methods of vitamin analysis, and improvement of nutritional quality. Dr. Eitenmiller is a Fellow of the Institute of Food Technologists. He is Science Advisor to the U.S. Food and Drug Administration at the Southeastern Regional Laboratory, Atlanta Center for Nutrient Analysis. He has consulted in numerous countries on fortification, nutritional quality, and methods of vitamin analysis. Dr. Eitenmiller currently serves as Chair of the Expert Panel on Food Safety and Nutrition, Institute of Food Technologists. He is a member of the FANSA Task Force on Vitamin E.

W. O. Landen, Jr., is a research scientist at the Department of Food Science and Technology, Food Process Research and Development Laboratory, University of Georgia, Athens, Georgia. Current research centers on the improvement of methods for fat- and water-soluble vitamins in foods. He has published widely throughout his career. Mr. Landen retired from the U.S. Food and Drug Administration in 1995 after 33 years service to the FDA. He was a Research Supervisory Chemist with the Atlanta Center for Nutrient Analysis. His work with FDA was recognized by ten outstanding or special performance awards. He currently serves on the AOAC International Methods Committee on Food and Nutrition. Past service to AOAC International included membership on the AOAC-NLEA Task Force on Nutrient Analysis Methods.

Abbreviations

Acetic acid—HAC
Acetone—A
Acetonitrile—MeCN
Acyl—Carrier protein—ACP
Ammonium acetate—NH_4OAC
Angstrom—Å
Aquocobalamin—H_2OCbl
Association of Official Analytical Chemists—AOAC
Ascorbic acid—AA
Atlanta Center for Nutrient Analysis—ACNA

Benzenesulfonyl chloride—BSC
Butylated hydroxyanisole—BHA
Butylated hydroxytoluene—BHT
tert-Butylammonium hydroxide—TBAH
tert-Butyl methyl ether—TBME

Capillary electrophorosis—CE
Centimeter—cm
Cholecalciferol—Vitamin D_2
Chloroform—$CHCl_3$
Coefficient of variation—CV
Competitive protein bending assays—CPBA
Cyanocobalamin—Cbl

Dehydroascorbic Acid—DHAA
5′—Deoxyadenosyl cobalamin—AdoCbl
Detection limit—DL
2,6-Dichloroindophenol—DCIP
Dichloromethane—CH_2Cl_2
Diethyl ether—ET_2O
Diisopropylether—DIPE
Dimethylformamide—DMF
Dimethylsulfoxide—DMSO
2,4-Dinitrophenylhydrazine—DNPH
Dithiothreitol—DTT
Dodecyltrimethyl ammonium chloride—DTMAC

Electrochemical—EC
Electron impact—EI
Emission—Em
Enzyme-linked immunosorbent assay—ELISA
Enzyme protein binding assay—EPBA
Ergocalciferol—Vitamin D_2
Ethyl Acetate—EtOAC
Ethyl Alcohol—EtOH
Evaporative light scattering detector—ELSD
Excitation—Ex

Flame ionization detection—FID
Flavin adenine dinucleotide—FAD
Flavin mononucleotide—FMN
Fluorescein isothiocyanate—FITC
Formiminoglutamic acid—FIGLU

Gas liquid chromatography—GLC
Gram—g

Heptane—HEP
Hexadecyl trimethylammonium bromide—HDTMAB
Hexane—HEX
High performance-Gel permeation Chromatography—HP-GPC
High performance liquid chromatography—HPLC
Hours—h
Hydrochloric acid—HCI
Hydroxycobalamin—OHCbl

Internal Standard—IS
International Unit—IU
Isoascorbic acid—IAA
Isooctane—iOCT
Isopropyl alcohol—IPA

Kilogram—kg

Liter—L
Liquid chromatography—LC

Mass spectrometry—MS
Menadione dimethyl pyrimidinol bisulfate—MPB
Menadione sodium bisulfate—MSB
Menadione sodium bisulfate complex—MSBC
Metaphosphoric acid—MPA
Methanol—MeOH
Methylcobalamin—MeCbl
Methyl-*tert*-butyl ether—MTBE

Microliter—μL
Microgram—μg
Micrometer—μm
Milliliter—mL
Millimeter—mm
Millimole—mmol
Millimolar—m*M*
Millivolt—mV
Molar—*M*
Molar Absorptivity—ϵ

Nanometer—nm
Nanomole—nmol
National Institute of Standards and Technology—NIST
Niacin equivalent—NE
Nicotinamide adenine dinucleotide—NAD
Nicotinamide adenine dinucleotide Phosphate—NADP
Nitrocobalamin—NO_2Cbl
N-Methylnicotinamide—NMN
Normal—N
Normal Phase—NP
Nutritional Labeling and Education Act of 1990—NLEA

Octyldecylsilica—ODS
Orthophenyldiamine—OPD

P-Aminobenzoyl-glutamic acid—PABG
Parts per billion—PPB
Photodiode array—PDA
Picogram—pg
Platinum—Pt
2,2,5,7,8—Pentamethyl-6-hydroxy chroman—PMC
Petroleum ether—PE
O-Phenylenediamine—OPD
Polyunsaturated fatty acid—PUFA
Prothrombin time—PT
Pyridoxamine—PM
Pyridoxamine-5′-Phosphate—PMP
Pyridoxal—PL
Pyridoxal-5′-phosphate—PLP
Pyridoxine (pyridoxol)—PN
Pyridoxine HC1—PN-HC1
Pyridoxine-5′-phosphate—PNP
Pyridoxine-glucoside—PN-glucoside
4-Pyridoxic acid—4-PA

Quantitation limit—QL

Racmic—RAC
Radial compression module—RCM
Radioimmunoassay—RIA
Radioreceptor assay—RRA
Recommended Dietary Allowance—RDA
Reference Daily Intake—RDI
Relative standard deviation—RSD
Retinol equivalent—RE
Reversed-phase—RP

Sodium acetate—NaOAC
Solid-phrase extraction—SPE
Specific extinction coefficient—$E^{1\%}_{1\ cm}$
Sulfitocobalamin—SO_3Cbl
Sulfosalicylic acid—SSA

Tetrabutylammonium phosphate—TBAP
Tetrabutylammonium hydrogen sulfate—TBAHS
Tetrabutylammonium bromide—TBAB
Tetrahydrofuran—THF
Tetraoctylammonium bromide—TOAB
Thiachrome monophosphate—ThcMP
Thiachrome pyrophosphate—ThcPP
Thiachrome triphosphate—ThcTP
Thiamin monophosphate—TMP
Thiamin pyrophosphate—TPP
Thiamin triphosphate—TTP
Thin layer chromatography—TLC
Thymidylate—dTMP
α-Tocopherol—α-T
α-Tocotrienol—α-T3
β-Tocopherol—β-T
β-Tocotrienol—β-T3
γ-Tocopherol—γ-T
γ-Tocotrienol—γ-T3
δ-Tocopherol—δ-T
δ-Tocotrienol—δ-T3
Toluene—T
Tricarboxylic acid cycle—TCA cycle
Trichloroacetic acid—TCA
Triethylamine—TEA
Trimethyl-silyl—TMS

Ultraviolet—UV
United States Pharmacopeial Convention—USP

Volts—V

Wavelength—λ

Contents

Fat-Soluble Vitamins