
VITAMIN ANALYSIS for the HEALTH and FOOD SCIENCES

Ronald R. Eitenmiller
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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.

Ronald R. Eitenmiller
W. O. Landen, Jr.

Authors

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Abbreviations

- Acetic acid—HAC
Acetone—A
Acetonitrile—MeCN
Acyl—Carrier protein—ACP
Ammonium acetate—NH₄OAC
Angstrom—Å
Aquocobalamin—H₂OCbl
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 electrophoresis—CE
Centimeter—cm
Cholecalciferol—Vitamin D₂
Chloroform—CHCl₃
Coefficient of variation—CV
Competitive protein binding assays—CPBA
Cyanocobalamin—Cbl
- Dehydroascorbic Acid—DHAA
5′—Deoxyadenosyl cobalamin—AdoCbl
Detection limit—DL
2,6-Dichloroindophenol—DCIP
Dichloromethane—CH₂Cl₂
Diethyl ether—ET₂O
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 D₂
- 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—HCl
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— mM
 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 HCl—PN-HCl
 Pyridoxine-5'-phosphate—PNP
 Pyridoxine-glucoside—PN-glucoside
 4-Pyridoxic acid—4-PA

 Quantitation limit—QL

Racemic—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-phase extraction—SPE
 Specific extinction coefficient— $E_{1\text{cm}}^{1\%}$
 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

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Fat-Soluble Vitamins
