METHODS OF VITAMIN ASSAY

Fourth Edition

Edited by

Jorg Augustin, Ph.D.
Food Research Center
University of Idaho

Barbara P. Klein, Ph.D.
Department of Foods and Nutrition
University of Illinois

Deborah Becker, B.S.
SCI-TEK Laboratories

Paul B. Venugopal, Ph.D.
Vedal, Inc.

For the Association of Vitamin Chemists

A Wiley-Interscience Publication
JOHN WILEY & SONS
New York / Chichester / Brisbane / Toronto / Singapore

Library of Congress Cataloging in Publication Data:
Main entry under title:

Methods of vitamin assay.

"A Wiley-Interscience publication."
Rev. ed. of: Methods of vitamin assay / Association of Vitamin Chemists. 3rd ed. 1966.
Includes bibliographies and index.
1. Vitamins——Analysis. I. Augustin, Jorg. II. Association of Vitamin Chemists. Methods of vitamin assay.

RS190.V5M48 1984 641.1'8 84-7335
ISBN 0-471-86957-0

Printed in the United States of America
10 9 8 7 6 5 4 3 2

Contributors

RAYMOND BERRUTI, B.S., Heterochemical Corp., 111 East Hawthorne Avenue, Valley Stream, NY 11580.

KENNETH J. CARPENTER, PH.D., Department of Nutritional Sciences, University of California, Berkeley, CA 94720.

C. O. CHICHESTER, PH.D., Department of Food Science and Technology, University of Rhode Island, Kingston, RI 02881.

HENRY B. CHIN, PH.D., National Food Processors Association, 1950 6th Street, Berkeley, CA 94710.

NEVILLE COLMAN, M.D. PH.D., Hematology and Nutrition Laboratory, Veterans Administration Medical Center, 130 West Kingsbridge Rd., Bronx, NY 10468.

INDRAJIT D. DESAI, PH.D., Division of Human Nutrition, University of British Columbia, Vancouver, B.C., VGT 1W5 CANADA.

SELWYN DE SOUZA, PH.D., Department of Food Science, University of Georgia, Athens, GA 30601.

JONATHAN W. DEVRIES, PH.D., James Ford Bell Technical Center, General Mills, Inc., 9000 Plymouth Avenue North, Minneapolis, MN 55427.

JANET A. DUDEK, M.S., National Food Processors Association, 1401 New York Avenue, N.W., Washington, DC 20005.

DAVID C. EGBERG, PH.D., James Ford Bell Technical Center, General Mills, Inc., 9000 Plymouth Avenue North, Minneapolis, MN 55427.

RONALD R. EITENMILLER, PH.D., Department of Food Science, University of Georgia, Athens, GA 30601.

EDGAR R. ELKINS, B.S., National Food Processors Association, 1401 New York Avenue, N.W., Washington, DC 20005.

WAYNE C. ELLEFSON, M.S., Hazleton Raltech, Inc., Box 7545, Madison, WI 53707.

JESSE F. GREGORY, III, PH.D., Department of Food Science and Human Nutrition, University of Florida, Gainesville, FL 32611.

R. GAURTH HANSEN, PH.D., Department of Nutrition and Food Science, Utah State University, Logan, UT 84322.

VICTOR HERBERT, M.D., J.D., Department of Medicine, Hahnemann University School of Medicine, Philadelphia, PA 19102.

ALAN J. JOHNSON, M.S., Systems Validation, Eli Lilly & Co., 1200 South Kentucky Ave., Indianapolis, IN 46221.

PAMELA M. KEAGY, PH.D., Western Regional Research Center, U.S. Department of Agriculture, 800 Buchanan, Berkeley, CA 94710.

LAWRENCE J. MACHLIN, PH.D., Research Division, Hoffmann-LaRoche, Inc., Nutley, NJ 07110.

R. A. MOFFITT, M.S., Analytical Services, Carnation Co., 8015 Van Nuys Boulevard, Van Nuys, CA 91412.

R. J. NOEL, PH.D., Department of Biochemistry, Purdue University, West Lafayette, IN 47907.

D. B. PARRISH, PH.D., Department of Biochemistry, Kansas State University, Manhattan, KS 66506.

OMER PELLETIER, PH.D., Laboratory Center for Disease Control, Health Protection Branch, Health and Welfare Canada, Ottawa, Ontario, K1A OL2 CANADA.

MARILYN M. POLANSKY, M.S., Vitamin and Mineral Nutrition Laboratory, Human Nutrition Institute, U.S. Department of Agriculture, Building 307, Base East, Beltsville, MD 20705.

ROBERT D. REYNOLDS, PH.D., Vitamin and Mineral Nutrition Laboratory, Human Nutrition Institute, U.S. Department of Agriculture, Building 307, Beltsville, MD 20705.

J. SCHEINER, M.S., Roche Chemical Division, Hoffmann-LaRoche, Inc., Nutley, NJ 07110.

JITENDRA J. SHAH, M.S., M.B.A., SGS Control Services, Memphis, TN 38118.

KENNETH L. SIMPSON, PH.D., Department of Food Science and Technology, University of Rhode Island, Kingston, RI 02881.

LAXMAN SINGH, PH.D., Vitamins, Inc., 200 East Randolph Drive, Chicago, IL 60601.

WON O. SONG, PH.D., Department of Food Science and Human Nutrition, Michigan State University, East Lansing, MI 48824.

KENT K. STEWART, PH.D., Department of Food Science and Technology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.

J.N. THOMPSON, PH.D., Nutrition Research Division, Health and Welfare Canada, Ottawa, Ontario, K1A 0L2 CANADA.

SAMPSON C.S. TSOU, Asian Vegetable Research and Development Center, Box 42, Shanhua Tainan, Taiwan 741 REPUBLIC OF CHINA.

JOSEPH T. VANDERSLICE, PH.D., Nutrition Composition Laboratory, U.S. Department of Agriculture, Human Nutrition Research Center, Beltsville, MD 20705.

PAUL B. VENUGOPAL, PH.D., Vedal Inc., 6705 Trenton, Darien, IL 60559.

MICHAEL N. VOIGT, PH.D., Biochemistry Department, Memorial University, St. John's, Newfoundland, A1B 3X9 CANADA.

JOAN H. WALSH, PH.D., R.D., Department of Family Practice, San Joaquin General Hospital, Stockton, CA 95201.

BONITA W. WYSE, PH.D., R.D., Department of Nutrition and Food Science, Utah State University, Logan, UT 84322.

Preface

Methods of Vitamin Assay of the Association of Vitamin Chemists has become a classic in its field. The third edition, which is now out of print, was published seventeen years ago in 1966. Since then, several events occurred that have exerted a major impact on the field of vitamin analysis. The most important of these were the advent of nutritional labeling of foods and the increased interest of consumers in nutrition. These in turn, created a rash of investigations of the nutrient content, including vitamins, of foods, as well as studies of vitamin metabolism. Recently, more interest has been generated regarding the impact of vitamins, not only on nutritional status or health, but also on the effects of vitamins on disease prevention. The result has been a sharply increased demand on vitamin assay.

All these events led to two major developments with regard to vitamin analysis: (1) the increased analytical workload led to the development of automated analysis systems which allow the handling of a several-fold increase in sample numbers when compared to existing manual methods, and (2) the increased exposure of analysts and researchers to vitamin analysis made them aware of shortcomings of existing methods, leading to the development of new methods with higher sensitivity and greatly increased accuracy and precision. An example of new methodology that is emerging is high-performance liquid chromatography. This system offers the opportunity for the simultaneous determination of more than one vitamin, but even more importantly, it permits the chemical analysis of such vitamins as vitamin D in biological systems that previously could only be determined by animal assays.

The fourth edition constitutes an update to the state of the art in the field of vitamin analysis. Not only are the methods outlined in the chapters covering individual vitamins updated, but just as importantly, the new edition contains four additional chapters. Of these, three cover novel analytical systems, that is, chromatographic assays, radioimmunoassays, and automated assays. Because

today's analysts are constantly faced with new technology, and since many are engaged in methods development, the editors considered it opportune to touch on some of these problems in a separate chapter.

In the formative stages of the fourth edition, the editors, in agreement with the Association of Vitamin Chemists, decided to assign chapter responsibilities to individual contributors. We believe this approach to be the most effective way to convey expert information in the various areas of vitamin assay to the analyst. In addition, more background on each vitamin was included to provide an historical perspective of the importance of each nutrient.

The methods described in this edition under individual vitamins are, in some instances, very similar to those in the previous edition. Where necessary, they were updated; if no longer in use, they were deleted. The decision to include or exclude any method was left up to the contributors and the editors, rather than a committee, as was the case with the earlier editions. The ultimate criterion for inclusion or exclusion of a method was the current extent of its usage, signifying that the new edition covers only those in present use. In addition, an effort was made in each chapter to acquaint the reader with new analytical developments for a particular vitamin. Thus, the scope of the book has expanded from a manual of analytical procedures to a reference source for new techniques.

We have attempted to maintain the spirit of the previous edition of *Methods of Vitamin Assay* in the presentations. However, those familiar with the third edition will find some changes in format and delivery among the chapters. Among the changes is the provision of a list of abbreviations used, as well as an overall list of suppliers for special equipment and chemicals for the assay procedures. We hope that the old and new users of *Methods of Vitamin Assay* will find the fourth edition as helpful as the previous editions.

JORG AUGUSTIN
BARBARA KLEIN
DEBORAH BECKER
PAUL B. VENUGOPAL

Moscow, Idaho
Urbana, Illinois
Northbrook, Illinois
Darien, Illinois

July 1984

Contents

1 Method Choice and Development

Kent K. Stewart

INTRODUCTION

The field of vitamin determination is undergoing rapid change. No longer are analysts limited to a few slow biological assays or to chemical methods that are of limited usefulness due to their lack of sensitivity and selectivity. The analyst today is faced with a dazzling array of methods for the determination of vitamins. There are methods using liquid chromatography, gas chromatography, mass spectrometry, infrared, visible, and ultraviolet spectroscopy, enzvmes, flow injection analysis, and many others. Recently, there have been an increasing number of methods that use more than one technique, the so-called hyphenated methods, for example, the combination of gas chromatography and mass spectrometry. The problem is that the choice of the appropriate method can become very difficult since most analysts do not have the expertise to evaluate all the available techniques. It is often difficult to assess the appropriateness of any one method even if no others are available. Since most method development studies are not done under the conditions associated with the particular problems of the individual analyst, it is common that analysts will be required to do some method development or modification to solve their current problems.

Successful selection of the appropriate method, the successful development of a new method, or the successful modification of an existing method requires considerable insight into the nature of the problems and a careful use of the available resources. There are very few overviews that suggest the appropriate strategy for the selection and development of methods. Most of these are a few

lines or pages in general textbooks on analytical chemistry (see, e.g., references 1 and 2). Yet obviously such strategies are needed for those who do vitamin determinations. It is quite likely that the recent surge in new method development will continue for some years, and that the analysts of the future will be faced with an even more perplexing array of assay methods.

It was not always so. In the early days of vitamin assay, the methods used were mostly biological in nature. Growth rates or the lack of a pathological response were common assay techniques. The elucidation of the chemical structures and the metabolic pathways of the vitamins led to the discovery that given biological responses could be stimulated by several chemical compounds. As a result, the general concept was established that a given vitamin activity was associated with a number of chemically closely related compounds. The class of chemically similar compounds that elicited the same qualitative biological response has been called vitamers; for example, the vitamers of vitamin B_6 are pyridoxine, pyridoxal, pyridoxamine, and their respective phosphate esters. The discovery that sometimes even if the vitamers were present, the biological response was absent or was limited, led to the development of the concept of biological availability. Furthermore, it soon became apparent that while vitamers elicit the same qualitative biological response, often the quantitative responses differed with the animal species with the different chemical isomers of a vitamin. Measurement of a given vitamin activity with one species did not necessarily measure its activity in another. Obviously, more effective assay systems were needed. Fortunately, the potential for such systems was available.

Modern bioanalytical chemistry can be said to have started with the development of gas chromatography by Martin and James (3) and the amino acid analyzer by Spackman et al. (4). Since the invention of these powerful new techniques in the 1950s, the analytical chemistry of the vitamins has expanded explosively. There is now a large literature of new techniques for the assay of different vitamins and vitamers. No attempt to review the current literature will be made since it has been well covered by the other chapters in this book. Today many analysts use modern separation techniques and are determining the concentration of each separate vitamer in a sample. When the vitamin activity is needed, the quantity of each vitamer is multiplied by its biological potency for the species in question, and then the total activity is obtained by summing the individual activities as exemplified by the work of Slover et al. (5) with vitamin E assays. Presently, good methods are available for many of the vitamers in most matrices. Table 1.1 has an update of a recent evaluation of the state of the methods for vitamin assay in most matrices (6). The criteria for the evaluation of the methods were: If a qualified analyst used the best of the current methods, would the vitamin be accurately and precisely determined in food matrices?

The potential sources of errors in today's assays are many and varied. An idealized method can be flow charted as is shown in Figure 1.1 (7). Errors can enter at any place in the flow chart, and any error in any part of the assay can result in an incorrect final answer. If the wrong sample is taken, then no matter how careful the analyst is in performing the determination, the answer will

TABLE 1.1 State of Methodology for the Determination of Vitamins in 1982

Sufficient	Substantial	Conflicting	Fragmentary
	Niacin	Folacin	Biotin
	Riboflavin	Pantothenic acid	Choline
	Thiamin	Vitamin A	Vitamin K
	Vitamin C	Vitamin B_6[a]	
		Vitamin B_{12}[a]	
		Vitamin D	
		Vitamin E[a]	

[a]New methods look very promising.

probably be wrong. If the samples are improperly homogenized, then the aliquot taken will probably not be representative of the whole sample. If the extraction processes do not completely extract the vitamer(s), or if the vitamer(s) are partially or completely destroyed during the extraction, then the result will be too low. If the vitamers are not separated from each other or from interfering compounds, the results may be either low or high, depending upon the char-

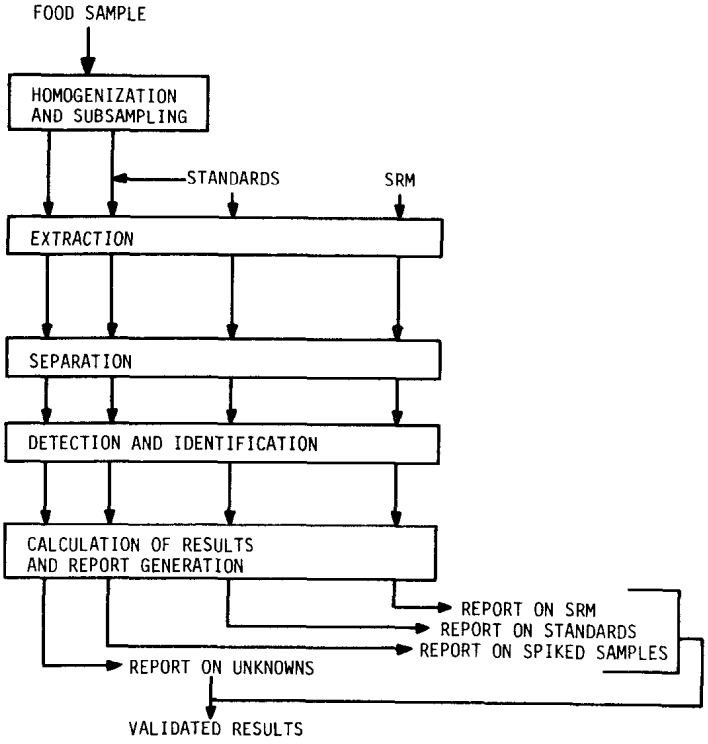

FIGURE 1.1 Flow chart for an ideal analytical method for the vitamin analysis of foods. Taken from reference 7. Reprinted by permission of Association of Official Analytical Chemists.