
Contents

1. Method Choice and Development	1
Introduction, 1	
Choice of a Method, 4	
Method Development, 13	
Summary, 14	
Literature Cited, 14	
2. Biological Assays	17
General Considerations, 17	
Animal Assays, 19	
Human Assays, 21	
Basic Features in Biological Assays, 23	
A Typical Animal Assay—Niacin, 28	
A Typical Human Assay—Niacin, 36	
Literaturz Cited. 39	
3. Microbiological Assays	43
General Considerations, 43	
Methodology, 45	
Equipment, 50	
Reagents, 51	
Procedures, 58	
Literature Cited, 62	

4. Chromatographic Assay of Vitamins	65
General Considerations,	65
Methods Available,	84
Analytical Methodology: Simultaneous Analysis of Vitamin A and Vitamin E,	84
Simultaneous Analysis of Niacin, Niacinamide, Pyridoxine, Thiamin, and Riboflavin,	87
Literature Cited,	93
5. Automated Vitamin Analysis	95
General Considerations,	95
Methods Available,	96
Determination of Ascorbic Acid and Dehydroascorbic Acid (Total Vitamin C),	98
<i>2,4-Dinitrophenylhydrazine Determination of Vitamin C in Foods with Concentrations Larger Than 10 mg/100 g,</i>	98
<i>2,4-Dinitrophenylhydrazine Determination of Vitamin C in Foods with Expected Concentrations of Less than 10 mg/100 g,</i>	103
<i>Fluorometric Determination of Ascorbic Acid and Dehydroascorbic Acid (Total Vitamin C),</i>	105
Determination of Riboflavin,	108
<i>Fluorometric Determination of Riboflavin (Light Destruction Method),</i>	108
<i>Fluorometric Determination of Riboflavin (Hydrosulfite Destruction Method),</i>	112
Determination of Thiamin,	115
Colorimetric Determination of Niacin and Niacinamide,	118
Determination of Vitamin A,	121
Automated Microbiological Vitamin Assays,	126
Literature Cited,	131
6. Sampling for Vitamin Analyses	135
General Considerations.	135
The General Problem,	136
Statistics Applied to Sampling,	139
Applications to Various Types of Products,	145
Literature Cited.	150
7. Vitamin A	153
General Considerations,	153
Analytical Methodology,	161

<i>Colorimetric Method.</i>	161
<i>Ultraviolet Absorption Method</i>	169
<i>Fluorometric Method,</i>	171
<i>High-Performance Liquid Chromatography,</i>	175
<i>Other Vitamin A Methods,</i>	179
Application of Methods,	180
Literature Cited.	180
8. Carotenes	185
General Considerations,	185
Methods Available,	194
Analytical Methodology,	198
<i>Open-Column Chromatography,</i>	198
<i>Alumina Column Chrotnatography for Blood/Plasma,</i>	208
<i>Thin-Layer Chromatography,</i>	209
<i>High-Performance Liquid Chromatography,</i>	210
Literature Cited,	217
9. Vitamin D	221
General Considerations,	221
Methods Available,	224
Analytical Methodology,	229
<i>Colorimetric Method,</i>	229
<i>High-Performance Liquid Chromatography,</i>	239
Literature Cited,	251
10. Vitamin E	255
General Considerations,	255
Methods Available,	261
Analytical Methodology,	266
<i>Colorimetric Procedure for Biological Fluids,</i>	266
<i>Colorimetric Procedure—Thin-layer and Oxidative Chromatography,</i>	267
<i>Gas-Liquid Chromatography—Pharmaceutical Preparation,</i>	
276	
Literature Cited,	280
11. Vitamin K	285
General Considerations,	285
Methods Available,	292
Analytical Methodology,	293
<i>Reduction-Oxidation Method,</i>	293
<i>Ethylcyanoacetate Method for Water-Soluble Menadione Derivatives,</i>	295

<i>2,4-Dinitrophenylhydrazine Method for Menadione,</i>	297
<i>Modified 2,4-Dinitrophenylhydrazine Method for Combined Forms of Menadione.</i>	299
<i>Method for Whole Blood Prothrombin Clotting Time,</i>	300
<i>Method for Whole Blood Clotting Time,</i>	300
Literature Cited,	301
12. Vitamin C (L-Ascorbic and Dehydro-L-Ascorbic Acids)	303
General Considerations,	303
Methods Available,	305
Analytical Methodology—Sampling and Extraction,	321
<i>Determination of Ascorbic Acid and Total Vitamin C with 2,4-Dinitrophenylhydrazine,</i>	323
<i>Differential Determination of d-Isoascorbic Acid and L-Ascorbic Acid with 2,4-Dinitrophenylhydrazine,</i>	329
<i>2,6-Dichloroindophenol Titration Method in Absence of Interfering Substances,</i>	330
<i>2,6-Dichloroindophenol Titration of Ascorbic Acid in Presence of Ferrous and Stannous Salts,</i>	334
<i>2,6-Dichloroindophenol Titration of Ascorbic Acid Utilizing Blanks with Formaldehyde Condensation of Ascorbic Acid,</i>	336
<i>Fluorometric Determination of Total Vitamin C (Ascorbic and Dehydroascorbic Acids) with o-Phenylenediamine,</i>	338
Literature Cited,	341
13. Thiamin	349
General Considerations,	349
Methods Available,	351
Analytical Methodology,	352
<i>Thiochrome Method,</i>	352
Literature Cited,	361
14. Riboflavin	365
General Considerations,	365
Methods Available,	367
Analytical Methodology,	368
<i>Microbiological Method,</i>	368
<i>Fluorometric Method,</i>	375
Literature Cited,	380
15. Niacin	385
General Considerations,	385
Methods Available,	387

CONTENTS

xv

Analytical Methodology, 389	
<i>Micmbiological Method for Niacin and Niacinamide,</i> 389	
<i>Colorimetric Method for Niacin and Niacinamide.</i> 393	
Literature Cited, 397	
16. Pantothenic Acid	399
General Considerations, 399	
Methods Available, 403	
Analytical Methodology, 405	
<i>Sample Preparation,</i> 405	
<i>Microbiological Method,</i> 407	
<i>Radioimmunoassay,</i> 410	
<i>Partial Purification of Pantetheinase.</i> 411	
Literature Cited, 413	
17. Vitamin B₆	417
General Considerations, 417	
Methods Available, 418	
Analytical Methodology, 420	
<i>Microbiological Method,</i> 420	
<i>High-Performance Liquid Chromatography Method,</i> 428	
Literature Cited, 441	
18. Folacin	445
MICROBIOLOGICAL AND ANIMAL ASSAYS, 445	
General Considerations, 445	
Methods Available, 450	
Analytical Methodology, 452	
<i>Micmbiological Method,</i> 452	
<i>Animal Assays,</i> 462	
Literature Cited, 466	
CHROMATOGRAPHIC AND RADIOMETRIC ASSAYS, 473	
Separation of Folacin Compounds, 473	
Folacin Radioassay Procedures, 482	
Analytical Methodology, 486	
<i>Determination of Folic Acid in Fortified Cereal and Infant Formula Products by Reverse Phase HPLC,</i> 486	
<i>Determination of Total Folacin by Competitive Binding Radioassay,</i> 488	
Literature Cited, 491	
19. Vitamin B₁₂	497
General Considerations, 497	
Methods Available, 500	

Analytical Methodology,	502
<i>Extraction Procedures,</i>	502
<i>Microbiological Method, Lactobacillus leichmannii,</i>	503
<i>Microbiological Method, Ochromonas malhamensis,</i>	506
<i>Radioisotope Dilution Method,</i>	508
Literature Cited,	512
 20. Vitamin B ₁₂ and Folacin Radioassays in Blood Serum	 515
General Considerations,	515
Methods Available,	519
Analytical Methodology,	522
<i>Vitamin B₁₂ Radioassay,</i>	522
<i>Folacin Radioassay,</i>	525
Literature Cited,	531
 21. Biotin	 535
General Considerations,	535
Methods Available,	539
Analytical Methodology,	541
<i>Microbiological Method,</i>	541
Literature Cited,	549
 22. Choline	 555
General Considerations,	555
Methods Available,	560
Analytical Methodology,	564
<i>Reineckate Method for Total Choline,</i>	564
<i>Fluorometric Method for Acetylcholine,</i>	567
Literature Cited,	571
Abbreviations	575
Manufacturers and Suppliers	579
Index	583