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	
	Literature Cited, 39	
	Literature Cited, 39	
3.	Microbiological Assays	43
	General Considerations, 43	
	Methodology, 45	
	Equipment, 50	
	Reagents, 51	
	Procedures, 58	
	Literature Cited, 62	

xii CONTENTS

Analytical Methodology: Simultaneous Analysis of Vitamin A

65

4. Chromatographic Assay of Vitamins

General Considerations, 65

and Vitamin E, 84

Methods Available,

	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 2,4-Dinitrophenylhydrazine Determination of Vitamin C in Foods with Concentrations Larger Than 10 mg/100 g, 98 2,4-Dinitrophenylhydrazine Determination of Vitamin C in Foods with Expected Concentrations of Less than 10 mg/100 g, 103 Fluorometric Determination of Ascorbic Acid and Dehydroascorbic Acid (Total Vitamin C), 105 Determination of Riboflavin, 108 Fluorometric Determination of Riboflavin (Light Destruction Method), 108 Fluorometric Determination of Riboflavin (Hydrosulfite Destruction Method), 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 General Considerations, 135 The General Problem, 136 Statistics Applied to Sampling, 139	135
7.	Applications to Various Types of Products, 145 Literature Cited, 150 Vitamin A General Considerations, 153 Analytical Methodology, 161	153

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

xiv CONTENTS

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

General Considerations, 385 Methods Available, 387

	Analytical Methodology, 389 Microbiological Method for Niacin and Niacinamide, 389 Colorimetric Method for Niacin and Niacinamide, 393 Literature Cited, 397	
16.	Pantothenic Acid	399
	General Considerations, 399 Methods Available, 403 Analytical Methodology, 405 Sample Preparation, 405 Microbiological Method, 407 Radioimmunoassay, 410 Partial Purification of Pantetheinase, 411 Literature Cited, 413	
17.	Vitamin B ₆	417
	General Considerations, 417 Methods Available, 418 Analytical Methodology, 420 Microbiological Method, 420 High-Performance Liquid Chromatography Method, 428 Literature Cited, 441	
18.	Folacin	445
	MICROBIOLOGICAL AND ANIMAL ASSAYS, 445 General Considerations, 445 Methods Available, 450 Analytical Methodology, 452 Microbiological Method, 452 Animal Assays, 462 Literature Cited, 466 CHROMATOGRAPHIC AND RADIOMETRIC ASSAYS, 473 Separation of Folacin Compounds, 473 Folacin Radioassay Procedures, 482 Analytical Methodology, 486 Determination of Folic Acid in Fortified Cereal and Infant Formula Products by Reverse Phase HPLC, 486 Determination of Total Folacin by Competitive Binding Radioassay, 488 Literature Cited, 491	
19.	Vitamin B ₁₂	497
	General Considerations, 497 Methods Available, 500	

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