

VITAMINS IN FOODS

Analysis, Bioavailability, and Stability

GEORGE F.M. BALL



Taylor & Francis
Taylor & Francis Group
Boca Raton London New York

A CRC title, part of the Taylor & Francis imprint, a member of the Taylor & Francis Group, the academic division of T&F Informa plc.

About the Author

George Ball has accumulated many years of commercial and research laboratory experience in pharmaceutical analysis, clinical analysis, biochemical analysis, and food analysis. He has contributed to original research publications relating to biochemistry (platelet function) and endocrinology (control of ovulation) and is the author of several books and book chapters on vitamins. He received the B.Sc. honors degree in agricultural sciences from the University of Nottingham, UK in 1975.

Preface

Optimal vitamin status is a prerequisite for good health, and government-approved food fortification strategies are deemed necessary to ensure adequate intake of certain vitamins. Knowledge about vitamin bioavailability from food is essential for the estimation of dietary requirements. Equally important is knowledge of a vitamin's stability toward post-harvest handling of food, processing, storage, and preparation for consumption. To acquire this knowledge, one must learn about vitamin chemistry and how the vitamin is absorbed and metabolized. Successful research into vitamin bioavailability and stability is entirely dependent on the development and validation of suitable analytical methods. Vitamin bioavailability from food is subject to many variables imposed by food constituents and the preparation of food. Great progress has been made over the past decade, largely attributable to innovative analytical methodology, but there are many inconsistencies and the continuation of a multipronged research effort from independent laboratories must be encouraged to achieve solid and vital data.

I would like to acknowledge the expertise and diligence of Lynn Saliba at the British Library.

George F. M. Ball

Contents

Part I Properties of Vitamins

Chapter 1. Nutritional Aspects of Vitamins	
1.1 Definition and Classification of Vitamins	3
1.2 Nutritional Vitamin Deficiency	4
1.3 Vitamin Requirements	4
1.4 Vitamin Enhancement of Foods	5
1.5 Stability of Vitamins	6
1.5.1 Water Activity and Lipid Oxidation	6
1.5.2 First-Order Kinetics	7
1.5.3 Effects of Food Processing on Vitamin Retention.....	9
1.5.3.1 Dehydration	9
1.5.3.2 Blanching	10
1.5.3.3 Canning	11
1.5.3.4 Pasteurization and Ultra-High-Temperature Processing	11
1.5.3.5 Microwave Heating	12
1.5.3.6 Hydrothermal Processes (Flaking, Puffing, and Extrusion)	13
1.5.3.7 Freezing	14
1.5.3.8 Irradiation	14
1.5.3.9 High Hydrostatic Pressure Treatment	15
1.5.3.10 Curing and Smoking	16
1.5.4 Milling	17
1.5.5 Effects of Food Storage on Vitamin Retention	17
1.5.6 Effects of Domestic Cooking on Vitamin Retention	17
References	18

Chapter 2. Intestinal Absorption and Bioavailability of Vitamins: Introduction

2.1 General Principles of Solute Translocation	23
2.2 Intestinal Absorption	24
2.2.1 The Villus	24
2.2.2 The Luminal Environment	25
2.2.3 Adaptive Regulation of Intestinal Nutrient Transport	25

2.2.3.1	Nonspecific Anatomical Adaptations to Changing Metabolic Requirements and Food Deprivation	25
2.2.3.2	Dietary Regulation of Intestinal Nutrient Carriers	26
2.2.4	Digestion, Absorption, and Transport of Dietary Fat	27
2.2.5	Transport of Glucose and Fructose: A Model for the Absorption of Some Water-Soluble Vitamins	28
2.2.6	Effects of Dietary Fiber on Absorption of Nutrients	30
2.3	Bioavailability.....	32
2.3.1	General Concepts	32
2.3.2	Methods for Estimating Vitamin Bioavailability in Human Subjects	33
2.3.2.1	Plasma Response	33
2.3.2.2	Urinary Excretion	34
2.3.2.3	Oral-Fecal Balance Studies and the Determination of Prececal Digestibility	34
2.3.2.4	Use of Stable Isotopes	35
	References	36

Chapter 3. Vitamin A: Retinoids and the Provitamin A Carotenoids

3.1	Background.....	39
3.2	Chemical Structure, Biopotency, and Physicochemical Properties.....	40
3.2.1	Structure and Biopotency	40
3.2.1.1	Retinol	40
3.2.1.2	Provitamin A Carotenoids	41
3.2.2	Physicochemical Properties	43
3.2.2.1	Appearance and Solubility	43
3.2.2.2	Stability in Nonaqueous Solution	45
3.2.2.2.1	Retinoids.....	45
3.2.2.2.2	Carotenoids.....	45
3.3	Vitamin A in Foods	45
3.3.1	Occurrence	45
3.3.1.1	Vitamin A	46
3.3.1.2	Provitamin A Carotenoids	47
3.3.2	Stability.....	48
3.3.2.1	Introduction	48
3.3.2.2	Vitamin A in Milk	51
3.3.2.3	Supplemental Vitamin A in Corn Flakes and Rice	56

3.3.2.4	Provitamin A Carotenoids	57
3.3.3	Vitamin A Equivalency	60
3.3.4	Applicability of Analytical Techniques	61
3.4	Intestinal Absorption, Metabolism, and Transport	61
3.4.1	Absorption	62
3.4.2	Metabolic Events Within the Enterocyte	63
3.4.2.1	Esterification of Retinol	63
3.4.2.2	Conversion of Provitamin Carotenoids to Retinoids	63
3.4.3	Liver Uptake of Chylomicron Remnats and Storage of Vitamin A	66
3.4.4	Plasma Transport of Retinol and Carotenoids	66
3.4.5	Tissue Uptake and Metabolism of Retinol	67
3.5	Bioavailability.....	67
3.5.1	Introduction	67
3.5.2	<i>In vivo</i> Methods of Assessing β -Carotene Bioavailability	68
3.5.2.1	Use of Radioisotopes in Cannulated Patients	68
3.5.2.2	Animal Models	69
3.5.2.3	Serum, Plasma, or Chylomicron Responses not Involving Isotopic Tracers	69
3.5.2.4	Methods Involving Stable Isotopes	71
3.5.3	<i>In vitro</i> Methods of Assessing β -Carotene Bioaccessibility and Bioavailability	74
3.5.3.1	<i>In vitro</i> Digestion Methods to Assess β -Carotene Bioaccessibility	74
3.5.3.2	<i>In vitro</i> Studies of β -Carotene Absorption Using Caco-2 Cells	76
3.5.4	Host-Related Factors Affecting the Bioavailability of β -Carotene	77
3.5.5	Dietary Factors Affecting the Bioavailability of β -Carotene	77
3.5.5.1	Location of Carotenoids in the Plant Source	77
3.5.5.2	Food Matrix	78
3.5.5.3	Dietary Protein	80
3.5.5.4	Dietary Fat and Energy	81
3.5.5.5	Dietary Fiber	86
3.5.5.6	Plant Sterols	87
3.5.6	Conclusions	88
3.6	β -Carotene Supplementation.....	88
3.6.1	Effect of Vegetable Consumption on Vitamin A Status in Populations at Risk of Vitamin A Deficiency	88

3.6.2 Effects of β -Carotene Supplementation on Breastmilk Carotenoids	90
References	92

Chapter 4. Vitamin D

4.1 Background	107
4.2 Chemical Structure, Biopotency, and Physicochemical Properties	108
4.2.1 Structure and Biopotency	108
4.2.2 Physicochemical Properties	108
4.2.2.1 Appearance and Solubility	108
4.2.2.2 Stability in Nonaqueous Solution	109
4.3 Vitamin D in Foods	110
4.3.1 Occurrence	110
4.3.2 Stability	112
4.3.3 Expression of Dietary Values	113
4.3.4 Applicability of Analytical Techniques	113
4.4 Intestinal Absorption, Transport, and Metabolism	114
4.5 Bioavailability.....	115
References	116

Chapter 5. Vitamin E

5.1 Background	119
5.2 Chemical Structure, Biopotency, and Physicochemical Properties.....	120
5.2.1 Structure	120
5.2.2 Biopotency	121
5.2.3 Physicochemical Properties	122
5.2.3.1 Appearance and Solubility	122
5.2.3.2 Stability in Nonaqueous Solution	122
5.2.3.3 <i>In Vitro</i> Antioxidant Activity	122
5.3 Vitamin E in Foods	123
5.3.1 Occurrence	123
5.3.2 Stability	126
5.3.3 Expression of Dietary Values	128
5.3.4 Applicability of Analytical Techniques	128
5.4 Intestinal Absorption and Transport.....	128
5.4.1 Absorption	129
5.4.2 Plasma Transport and Distribution	129
5.4.3 Preferential Secretion of 2 <i>R</i> - α -Tocopherol Stereoisomers by the Liver	129
5.4.4 Storage	130
5.5 Bioavailability.....	130
5.5.1 Efficiency of Vitamin E Absorption	130

5.5.2	Effects of Polyunsaturated Fats on Vitamin E Absorption	131
5.5.3	Effects of Dietary Fiber on Vitamin E Absorption	131
5.5.4	Effect of Plant Sterols on Vitamin E Bioavailability	132
5.6	Vitamin E Requirements.....	132
	References	132

Chapter 6. Vitamin K

6.1	Background.....	137
6.2	Chemical Structure, Biopotency, and Physicochemical Properties.....	138
6.2.1	Structure and Biopotency	138
6.2.2	Physicochemical Properties	139
6.2.2.1	Appearance and Solubility	139
6.2.2.2	Stability in Nonaqueous Solution	139
6.3	Vitamin K in Foods.....	139
6.3.1	Occurrence	139
6.3.2	Stability	141
6.3.2.1	Effects of Hydrogenation	142
6.3.3	Applicability of Analytical Techniques	142
6.4	Intestinal Absorption and Transport.....	143
6.4.1	Absorption and Transport of Dietary Vitamin K	143
6.4.2	Bacterially Synthesized Menaquinones as a Possible Endogenous Source of Vitamin K	143
6.5	Bioavailability.....	145
	References	146

Chapter 7. Thiamin (Vitamin B₁)

7.1	Background.....	149
7.2	Chemical Structure, Biopotency, and Physicochemical Properties.....	149
7.2.1	Structure and Potency	149
7.2.2	Physicochemical Properties	150
7.2.2.1	Appearance and Solubility	150
7.2.2.2	Stability in Aqueous Solution	150
7.3	Thiamin in Foods	151
7.3.1	Occurrence	151
7.3.2	Stability	152
7.3.3	Applicability of Analytical Techniques	154
7.4	Intestinal Absorption	154
7.5	Bioavailability.....	155
7.5.1	Bioavailability of Thiamin in Foods	155
7.5.2	Antithiamin Factors	156

7.5.2.1	Thiaminases	156
7.5.2.2	Polyphenols	157
7.5.3	Effects of Alcohol	159
7.5.4	Effects of Dietary Fiber	160
References		160

Chapter 8. Flavins: Riboflavin, FMN, and FAD (Vitamin B₂)

8.1	Background.....	165
8.2	Chemical Structure, Biopotency, and Physicochemical Properties	165
8.2.1	Structure and Potency	165
8.2.2	Physicochemical Properties	167
8.2.2.1	Appearance and Solubility	167
8.2.2.2	Stability in Aqueous Solution	167
8.3	Vitamin B ₂ in Foods	168
8.3.1	Occurrence	168
8.3.2	Stability	169
8.3.3	Applicability of Analytical Techniques	171
8.4	Intestinal Absorption.....	171
8.4.1	Absorption of Dietary Vitamin B ₂	171
8.4.2	Absorption of Bacterially Synthesized Vitamin B ₂ in the Large Intestine	172
8.5	Bioavailability.....	173
References		173

Chapter 9. Niacin

9.1	Background.....	177
9.2	Chemical Structure, Biopotency, and Physicochemical Properties.....	178
9.2.1	Structure and Potency	178
9.2.2	Physicochemical Properties	179
9.2.2.1	Appearance, Solubility, and Other Properties	179
9.2.2.2	Stability in Aqueous Solution	179
9.3	Niacin in Foods	179
9.3.1	Occurrence	179
9.3.2	Stability	181
9.3.3	Applicability of Analytical Techniques	182
9.4	Intestinal Absorption.....	183
9.5	Bioavailability.....	183
9.5.1	Niacin	183
9.5.2	Tryptophan	187
References		187

Chapter 10. Vitamin B₆

10.1	Background	189
10.2	Chemical Structure, Biopotency, and Physicochemical Properties	190
10.2.1	Structure and Potency	190
10.2.2	Physicochemical Properties	191
10.2.2.1	Appearance and Solubility	191
10.2.2.2	Stability in Aqueous Solution	192
10.3	Vitamin B ₆ in Foods	193
10.3.1	Occurrence	193
10.3.2	Stability	194
10.3.3	Applicability of Analytical Techniques	197
10.4	Intestinal Absorption	198
10.5	Bioavailability	199
10.5.1	Bioavailability of Vitamin B ₆ in Foods	199
10.5.2	Effects of Alcohol	201
10.5.3	Effects of Dietary Fiber	201
10.5.4	Glycosylated Forms of Vitamin B ₆	202
	References	205

Chapter 11. Pantothenic Acid

11.1	Background	211
11.2	Chemical Structure, Biopotency, and Physicochemical Properties	211
11.2.1	Structure and Potency	211
11.2.2	Physicochemical Properties	212
11.2.2.1	Appearance and Solubility	212
11.2.2.2	Stability in Aqueous Solution	213
11.3	Pantothenic Acid in Foods	213
11.3.1	Occurrence	213
11.3.2	Stability	213
11.3.3	Applicability of Analytical Techniques	216
11.4	Intestinal Absorption	216
11.4.1	Digestion and Absorption of Dietary Pantothenic Acid	216
11.4.2	Absorption of Bacterially Synthesized Pantothenic Acid in the Large Intestine	217
11.5	Bioavailability	217
	References	218

Chapter 12. Biotin

12.1	Background	221
12.2	Chemical Structure, Biopotency, and Physicochemical Properties	221

12.2.1	Structure and Potency	221
12.2.2	Physicochemical Properties	222
12.2.2.1	Appearance and Solubility	222
12.2.2.2	Stability in Aqueous Solution	223
12.3	Biotin in Foods.....	223
12.3.1	Occurrence	223
12.3.2	Stability	223
12.3.3	Applicability of Analytical Techniques	225
12.4	Intestinal Absorption	226
12.4.1	Digestion and Absorption of Dietary Biotin	226
12.4.2	Absorption of Bacterially Synthesized Biotin in the Large Intestine	227
12.5	Bioavailability.....	228
	References	229

Chapter 13. Folate

13.1	Background.....	231
13.2	Chemical Structure, Biopotency, and Physicochemical Properties	232
13.2.1	Structure and Potency	232
13.2.2	Physicochemical Properties	233
13.2.2.1	Appearance, Solubility, and Ionic Characteristics	233
13.2.2.2	Stability in Aqueous Solution	234
13.3	Folate in Foods.....	236
13.3.1	Occurrence	236
13.3.2	Stability	238
13.3.3	Applicability of Analytical Techniques	244
13.4	Absorption, Transport, and Metabolism	245
13.4.1	Deconjugation of Polyglutamyl Folate	245
13.4.2	Absorption of Dietary Folate	246
13.4.3	Influence of Folate-Binding Protein on the Absorption of Folate from Milk	247
13.4.4	Adaptive Regulation of Folate Absorption	249
13.4.5	Salvage of Dietary 5-Methyl-5,6-DHF	249
13.4.6	Absorption of Bacterially Synthesized Folate in the Large Intestine	250
13.4.7	Plasma Transport and Intracellular Metabolism	251
13.4.8	Folate Homeostasis	251
13.5	Bioavailability.....	252
13.5.1	Introduction	252
13.5.2	Methods for Assessing Folate Bioavailability	252
13.5.2.1	Plasma Response	252

13.5.2.2	Stable-Isotopic Methods	253
13.5.2.3	Use of Ileostomy Subjects	255
13.5.3	Inherent Bioavailability of Monoglutamyl and Polyglutamyl Folates	256
13.5.4	Bioavailability of Naturally Occurring Folate in Fruits and Vegetables	257
13.5.5	Bioavailability of Folate in Milk	258
13.5.6	Effects of Soluble Food Components on Folate Bioavailability	260
13.5.7	Effects of Dietary Fiber on Folate Bioavailability	262
13.5.8	Bioavailability of Folate in Fortified Foods	263
13.5.9	Effects of Alcohol on Folate Status	264
References		264

Chapter 14. Vitamin B₁₂ (Cobalamins)

14.1	Background.....	275
14.2	Chemical Structure, Biopotency, and Physicochemical Properties	276
14.2.1	Structure and Potency	276
14.2.2	Physicochemical Properties	277
14.2.2.1	Appearance and Solubility	277
14.2.2.2	Stability in Aqueous Solution	278
14.3	Vitamin B ₁₂ in Foods	278
14.3.1	Occurrence	278
14.3.2	Stability	279
14.3.3	Applicability of Analytical Techniques	281
14.4	Absorption and Conservation.....	281
14.4.1	Digestion and Absorption of Dietary Vitamin B ₁₂	282
14.4.2	Conservation of Vitamin B ₁₂	283
14.5	Bioavailability.....	283
14.5.1	Efficiency of Absorption	283
14.5.2	Bioavailability Studies	284
14.5.2.1	Effects of Dietary Fiber	284
14.5.2.2	Effects of Alcohol	284
14.5.2.3	Effects of Smoking	285
References		285

Chapter 15. Vitamin C

15.1	Background.....	289
15.2	Chemical Structure, Biopotency, and Physicochemical Properties	290
15.2.1	Structure and Potency	290

15.2.2	Physicochemical Properties	291
15.2.2.1	Solubility and Other Properties	291
15.2.2.2	Stability in Aqueous Solution	292
15.3	Vitamin C in Foods.....	292
15.3.1	Occurrence	292
15.3.2	Stability	294
15.3.3	Applicability of Analytical Techniques	299
15.4	Intestinal Absorption.....	300
15.4.1	General Principles	300
15.4.2	Transport Mechanisms	301
15.4.2.1	Ascorbic Acid	301
15.4.2.2	Dehydroascorbic Acid	302
15.4.3	Efficiency of Ascorbate Absorption in Humans	302
15.5	Bioavailability.....	303
15.5.1	Bioavailability of Vitamin C in Foods	303
15.5.2	Effects of Dietary Fiber	304
15.5.3	Effects of Alcohol	305
	References	305

Part II Analysis of Vitamins

Chapter 16. Analytical Considerations

16.1	Bioassays	311
16.2	<i>In Vitro</i> Analytical Techniques	312
16.3	Analytical Approach	312
16.4	Preparation of Sample Extracts for Analysis	313
16.4.1	Extraction	314
16.4.2	Cleanup	314
16.5	Method Evaluation.....	314
16.5.1	Measurement Value and Uncertainty	314
16.5.2	Quality Assurance	316
16.5.3	Food Reference Materials	316
16.5.4	Method Validation	318
	References	320

Chapter 17. Extraction Techniques for the Water-Soluble Vitamins

17.1	Vitamin B ₁	321
17.2	Vitamin B ₂	322
17.3	Niacin	323
17.4	Vitamin B ₆	326

17.5	Pantothenic Acid	328
17.6	Biotin	328
17.7	Folate	329
17.8	Vitamin B ₁₂	331
17.9	Vitamin C	332
	References	333

Chapter 18. Microbiological Methods for the Determination of the B-Group Vitamins

18.1	Introduction	339
18.2	General Principles	339
18.2.1	Turbidimetric Methods	339
18.2.2	Methods Based on the Measurement of Metabolic Carbon Dioxide	341
18.3	Conventional Turbidimetric Method Using Test Tubes	342
18.3.1	Summary	342
18.3.2	Laboratory Facilities and Cleaning of Glassware	343
18.3.3	Media	344
18.3.4	General Assay Procedure	344
18.3.4.1	Maintenance of Stock Cultures	345
18.3.4.2	Preparation of the Inoculum Culture	346
18.3.4.3	Preparation of the Assay (Basal) Medium	347
18.3.4.4	Extraction of the Vitamin from the Test Material	348
18.3.4.5	Setting Up the Assay	348
18.3.4.6	Quantification	349
18.3.5	Partial Automation of the Assay Procedure	350
18.4	Turbidimetric Method Using Microtiter Plates	350
18.5	Assays of Individual B-Group Vitamins	351
18.5.1	Vitamin B ₁	351
18.5.2	Vitamin B ₂	352
18.5.3	Niacin	354
18.5.3.1	Determination of Total Niacin	354
18.5.3.2	Determination of Bound Nicotinic Acid	355
18.5.3.3	Determination of Added Nicotinic Acid	356
18.5.4	Vitamin B ₆	356
18.5.5	Pantothenic Acid	359
18.5.6	Biotin	360
18.5.7	Folate	360
18.5.8	Vitamin B ₁₂	361
	References	363

Chapter 19. Physicochemical Analytical Techniques (Excluding HPLC)

19.1	AOAC Titrimeetric Method for Vitamin C	369
19.2	Direct Spectrophotometric Determination of Vitamin C	372
19.3	Colorimetric Methods for Niacin and Vitamin C	373
	19.3.1 Determination of Niacin by the König Reaction (AOAC Method)	373
	19.3.2 Colorimetric Methods for Vitamin C	374
19.4	Fluorometric Methods for Thiamin, Riboflavin, Vitamin B ₆ , and Vitamin C	375
	19.4.1 Thiamin (AOAC Method)	375
	19.4.2 Riboflavin (AOAC Method)	375
	19.4.3 Vitamin B ₆	376
	19.4.4 Vitamin C (AOAC Method)	377
19.5	Enzymatic Methods for Nicotinic Acid and Ascorbic Acid	378
	19.5.1 Nicotinic Acid	378
	19.5.2 Ascorbic Acid	379
19.6	Continuous-Flow Analysis	380
	19.6.1 Segmented-Flow Methods	380
	19.6.2 Flow-Injection Analysis	380
	19.6.3 Applications to Food Analysis	381
	19.6.3.1 Fat-Soluble Vitamins	381
	19.6.3.2 Thiamin	381
	19.6.3.3 Riboflavin	382
	19.6.3.4 Thiamin and Riboflavin Simultaneously	382
	19.6.3.5 Niacin	382
	19.6.3.6 Vitamin C	383
19.7	Gas Chromatography	385
	19.7.1 Principle	385
	19.7.2 Column Technology	385
	19.7.3 Detectors	386
	19.7.4 Derivatization Techniques	386
	19.7.5 Quantification	387
	19.7.6 Applications to Food Analysis	387
	19.7.6.1 Vitamin E	387
	19.7.6.2 Thiamin	388
	19.7.6.3 Niacin	388
	19.7.6.4 Vitamin B ₆	388
	19.7.6.5 Pantothenic Acid	389
19.8	Supercritical Fluid Chromatography	390
	19.8.1 Principle	390
	19.8.2 Instrumentation	391

19.8.3	Columns	393
19.8.4	Applications to Food Analysis	394
19.9	Capillary Electrophoresis	394
19.9.1	Principle	394
19.9.2	Capillary Zone Electrophoresis	396
19.9.3	Micellar Electrokinetic Capillary Chromatography ...	396
19.9.4	Operational Aspects	397
19.9.5	Applications to Food Analysis	399
19.9.5.1	Thiamin	399
19.9.5.2	Riboflavin, FMN, and FAD	399
19.9.5.3	Niacin	406
19.9.5.4	Vitamin C	408
	References	409

Chapter 20. Determination of the Fat-Soluble Vitamins by HPLC

20.1	Nature of the Sample.....	419
20.2	Extraction Procedures	419
20.2.1	Alkaline Hydrolysis (Saponification)	419
20.2.1.1	Vitamin A	421
20.2.1.2	Carotenoids	422
20.2.1.3	Vitamin D	422
20.2.1.4	Vitamin E	423
20.2.2	Alcoholysis	424
20.2.3	Enzymatic Hydrolysis	424
20.2.4	Direct Solvent Extraction	425
20.2.4.1	Vitamin A and Carotene	426
20.2.4.2	Carotenoids	427
20.2.4.3	Vitamin D	427
20.2.4.4	Vitamin E	428
20.2.4.5	Vitamin K	428
20.2.5	Matrix Solid-Phase Dispersion	429
20.2.6	Supercritical Fluid Extraction.....	430
20.2.6.1	Principle	430
20.2.6.2	Instrumentation	430
20.2.6.3	Applications	431
20.3	Cleanup Procedures.....	435
20.3.1	Precipitation of Sterols	436
20.3.2	Open-Column Chromatography	436
20.3.2.1	Magnesia	436
20.3.2.2	Alumina	436
20.3.2.3	Silica Gel	437
20.3.3	Solid-Phase Extraction	437
20.3.3.1	General Considerations	437
20.3.3.2	Application in Vitamin D Determinations ..	438

20.4	HPLC Systems	439
20.4.1	Principle	439
20.4.2	Explanations of Chromatographic Terms	440
20.4.2.1	Retention	440
20.4.2.2	Separation	440
20.4.2.3	Resolution	441
20.4.2.4	Efficiency	442
20.4.3	The Column	442
20.4.4	Chromatographic Modes	444
20.4.4.1	Normal-Phase Chromatography	444
20.4.4.1.1	Adsorption Chromatography	444
20.4.4.1.2	Polar Bonded-Phase Chromatography	447
20.4.4.2	Reversed-Phase Chromatography	448
20.4.4.3	Two-Dimensional HPLC	452
20.4.5	Detection Systems	453
20.4.5.1	Introduction	453
20.4.5.2	Absorbance Detection	454
20.4.5.3	Fluorescence Detection	455
20.4.5.4	Electrochemical Detection	456
20.4.5.5	Mass Spectrometry	457
20.5	Applications of HPLC	457
20.5.1	Vitamin A	457
20.5.1.1	Detection	457
20.5.1.2	Quantification	463
20.5.1.3	Normal-Phase Separations	465
20.5.1.4	Reversed-Phase Separations	466
20.5.2	Provitamin A Carotenoids	469
20.5.2.1	Sources of Variation in the Methodology	469
20.5.2.2	Detection	469
20.5.2.3	Potential Problems with the Chromatography	472
20.5.2.4	Normal-Phase Separations	474
20.5.2.5	Reversed-Phase Separations	475
20.5.2.5.1	C_{18} -Bonded Phases	475
20.5.2.5.2	C_{30} -Bonded Phases	486
20.5.3	Vitamin D	489
20.5.3.1	Detection	489
20.5.3.2	Quantification	489
20.5.3.3	Cleanup Procedures	499
20.5.3.4	Normal-Phase Separations	500
20.5.3.5	Reversed-Phase Separations	500

20.5.4	Vitamin E	505
20.5.4.1	Detection	506
20.5.4.2	Quantification	510
20.5.4.3	Normal-Phase Separations	511
20.5.4.4	Reversed-Phase Separations	526
20.5.5	Vitamin K	527
20.5.5.1	Detection	528
20.5.5.2	Normal-Phase Separations	540
20.5.5.3	Reversed-Phase Separations	541
20.5.6	Simultaneous Determination of Two or Three Vitamins	546
20.5.6.1	Normal-Phase Separations	549
20.5.6.2	Reversed-Phase Separations	562
	References	567

Chapter 21. Determination of the Water-Soluble Vitamins by HPLC

21.1	HPLC Systems	585
21.1.1	The Column	585
21.1.2	Chromatographic Modes	585
21.1.2.1	Ion Exchange Chromatography	585
21.1.2.2	Ion Exclusion Chromatography	587
21.1.2.3	Reversed-Phase Chromatography	588
21.1.2.4	Reversed-Phase Ion-Pair Chromatography	589
21.1.3	Derivatization	591
21.2	Applications of HPLC	592
21.2.1	Thiamin	592
21.2.1.1	Detection	592
21.2.1.2	Methodology	594
21.2.2	Vitamin B ₂	598
21.2.2.1	Detection	598
21.2.2.2	Methodology	600
21.2.3	Niacin	612
21.2.3.1	Detection	612
21.2.3.2	Methodology	612
21.2.4	Vitamin B ₆	624
21.2.4.1	General Considerations	624
21.2.4.2	Detection	625
21.2.4.3	Methodology	626
21.2.5	Pantothenic Acid	638
21.2.5.1	Detection	638
21.2.5.2	Applications	639

21.2.6	Biotin	645
21.2.6.1	Detection	645
21.2.6.2	Application	646
21.2.7	Folate	646
21.2.7.1	General Considerations	646
21.2.7.2	Cleanup Procedures	648
21.2.7.3	Detection	650
21.2.7.4	Methodology	653
21.2.8	Vitamin B ₁₂	676
21.2.9	Vitamin C	677
21.2.9.1	Detection	677
21.2.9.2	Methodology	682
21.2.10	Multiple Vitamin Analyses	701
21.2.10.1	Thiamin and Riboflavin	701
21.2.10.2	Riboflavin and Pyridoxine	715
21.2.10.3	Nicotinamide and Pyridoxine	715
21.2.10.4	Three or More Vitamins	716
	References	720

Chapter 22. Biospecific Methods for Some of the B-Group Vitamins

22.1	Introduction	735
22.2	Immunoassays	735
22.2.1	The Immunological Reaction	735
22.2.2	Radioimmunoassay	737
22.2.2.1	Principle	737
22.2.2.2	Determination of Pantothenic Acid	737
22.2.3	Enzyme-Linked Immunosorbent Assay	738
22.2.3.1	Principle	738
22.2.3.2	Determination of Pantothenic Acid	740
22.2.3.3	Determination of Vitamin B ₆	741
22.3	Protein-Binding Assays.....	741
22.3.1	Radiolabeled Protein-Binding Assays	741
22.3.1.1	Principle	741
22.3.1.2	Determination of Biotin	743
22.3.1.3	Determination of Folate	743
22.3.1.4	Determination of Vitamin B ₁₂	745
22.3.2	Enzyme-Labeled Protein-Binding Assays	747
22.3.2.1	General Procedure	747
22.3.2.2	Determination of Biotin	747
22.3.2.3	Determination of Folate	747
22.3.2.4	Determination of Vitamin B ₁₂	748
22.4	Biomolecular Interaction Analysis	749
22.4.1	Principle	749

22.4.2	Biosensor-Based Immunoassay for Supplemental Biotin and Folate	750
22.4.3	Biosensor-Based Protein-Binding Assay for Supplemental and Endogenous Vitamin B ₁₂	751
References		752
Chapter 23. Summarized Appraisal of Analytical Techniques		
23.1	Microbiological Assays	757
23.2	High-Performance Liquid Chromatography	758
23.2.1	Introduction	758
23.2.2	Fat-Soluble Vitamins	759
23.2.2.1	Vitamin A	759
23.2.2.2	Carotenoids	759
23.2.2.3	Vitamin D	760
23.2.2.4	Vitamin E	760
23.2.2.5	Vitamin K	760
23.2.3	Water-Soluble Vitamins	761
23.2.3.1	Thiamin and Flavins	761
23.2.3.2	Niacin	762
23.2.3.3	Vitamin B ₆	762
23.2.3.4	Pantothenic Acid	763
23.2.3.5	Biotin	763
23.2.3.6	Folate	763
23.2.3.7	Vitamin C	763
23.3	Supercritical Fluid Chromatography	764
23.4	Capillary Electrophoresis	764
23.5	Flow-Injection Analysis.....	765
23.6	Biospecific Methods	765
23.7	Evaluation of Vitamin Bioavailability From Food Analysis Data	767
23.7.1	Fat-Soluble Vitamins	767
23.7.2	Water-Soluble Vitamins	767
23.7.2.1	Thiamin	767
23.7.2.2	Vitamin B ₂	768
23.7.2.3	Niacin	768
23.7.2.4	Vitamin B ₆	769
23.7.2.5	Pantothenic Acid	769
23.7.2.6	Biotin	769
23.7.2.7	Folate	769
23.7.2.8	Vitamin B ₁₂	770
23.7.2.9	Vitamin C	770
References		770
Index		777