

CONTENT

1. INTRODUCTION TO H.P.L.C. OF SMALL MOLECULES	1
Introduction	1
Fundamental H.p.l.c. Equations	1
H.p.l.c. Modes of Separation	3
Chiral Separation	7
Sample Preparation Techniques	8
Guard Column	10
Column Switching	10
Column Testing	11
Column Maintenance	11
References	12
2. AMINO ACIDS AND SMALL PEPTIDES	13
Introduction	13
Derivatization	13
Practical H.p.l.c. Separation of OPT-amino Acids	15
Common Problems Encountered	19
Modifications and Extensions to the OPT Method	22
Direct H.p.l.c. of Amino Acids	23
Liquid Chromatographic-Electrochemical Detection (LCEC) of Small Peptides	24
Conclusions	26
Acknowledgements	27
References	27
3. BIOGENIC AMINES	29
Introduction	29
Basic Set-up for LCEC	32
Pre-injection Sample Preparation	34
High-performance Liquid Chromatography	36
Electrochemical Detection	41
Peak Identification, Sensitivity and Calibration	44
Future Developments	45
Acknowledgements	46
References	46
4. CARBOHYDRATES	49
Introduction	49
Detection Systems for H.p.l.c. of Carbohydrates	50
Stationary and Mobile Phases for H.p.l.c. of Carbohydrates	52

Application of H.p.l.c. to Carbohydrate Compositional Analysis	57
Analytical and Preparative Separations of Oligosacchrides Condstaining Neutral and Sugars	59
H.p.l.c. for the Analytical and Preparative Separation of Anionic Oligosaccharides	65
5. LIPIDS	69
Introduction	69
H.p.l.c. of Fatty Acids	69
H.p.l.c. of Prostaglandins	79
H.p.l.c. of Triglycerides	87
H.p.l.c. of Phospholipids	94
6. BILE ACIDS	103
Introduction	103
H.p.l.c. of Bile Acids in Bile	104
H.p.l.c. of Bile Acids in Serum	104
Clinical Studies	105
References	116
7. STEROIDS	117
Introduction	117
Biochemistry of Steroids	117
Role of H.p.l.c. in Steroid Analysis	121
Analysis of Steroids Using H.p.l.c.	122
Preparation of Smples	126
Applications	130
Conclusion	155
Acknowledgements	155
References	155
8. VITAMINS	157
Introduction	157
Vitamin A and Provitamin Carotenoids	158
Vitamin D	165
Vitamin E	173
Vitamin K	180
Vitamin C	190
Thiamin (Vitamin B1)	194
Riboflavin (Vitamin V2)	199
Nicotinic Acid and Nicotinamide (Niacin)	201
Vitamin B6	206
Folic Acid (Folate, Folacin)	211
Acknowledgements	217
References	218

9. NUCLEOTIDES, NUCLEOSIDES AND BASES	221
Introduction	221
The Structure and Physical Properties of Bases and Related Compounds	222
Separation of Nucleic Acid Compounds	222
Detection of Nucleic Acid Components	238
Extraction Procedures	243
Isolation of Nucleosides from Biological Materials	249
Identification of Peaks	250
High Specificity Analyses	252
Future Directions	258
References	259
10. PORPHYRINS	261
Introduction	261
Extraction of Porphyrins from Biological Materials	263
Separation of Porphyrins as Esters	270
Separation of Porphyrins as Free Acids	276
Assays of Haem Biosynthetic Enzymes	295
Acknowledgements	303
References	303
11. BILE PIGMENTS	305
Introduction	305
Structure and Physio-chemical Properties of Bile Pigments	306
H.p.l.c. of Bile Pigments	310
Clinical Significance of the Serum Bile Pigments Detected by H.p.l.c.	321
References	322