# Table of Contents

**Preface**

1. **Introduction to Pharmaceutical Analysis**
   1.1 Applications and Definitions 1
   1.2 The Life of Medicines 4
   1.3 The Quality of Medical Products 8
   1.4 Summary 11

2. **International Pharmacopoeias, Regulations and Guidelines**
   2.1 Overview of Legislation 13
   2.2 Legislation and Regulations for Industrial Production 14
   2.3 Life Time of Drugs and Drug Substances 17
   2.4 Pharmacopoeias 18
   2.5 International Harmonization 19
      2.5.1 International Conference on Harmonization 20
      2.5.2 Pharmacopoeial Discussion Group 20
   2.6 Legislation and Regulations for Pharmacy Production 20
   2.7 Summary 21

3. **Fundamental Chemical Properties, Buffers and pH**
   3.1 pH and pKₐ 23
   3.2 Partition 25
   3.3 Stereochemistry 28
   3.4 Stability Testing 29
   3.5 Summary 30

4. **Fundamentals of Pharmaceutical Analysis**
   4.1 What is a Pharmaceutical (Chemical) Analysis? 33
   4.2 How to Specify Quantities and Concentrations? 35
   4.3 Basic Laboratory Equipment 37
      4.3.1 The Analytical Balance 37
      4.3.2 Pipettes 41
      4.3.3 Volumetric Flasks 44
      4.3.4 Burettes 47
18 Sample Preparation
18.1 Why is Sample Preparation Required? 273
18.2 Main Strategies 274
18.3 Recovery and Enrichment 276
18.4 Protein Precipitation 278
18.5 Liquid–Liquid Extraction 279
  18.5.1 Fundamentals 279
  18.5.2 A Closer Look at the Theory 279
  18.5.3 Extraction Solvents 282
  18.5.4 Calculation of Recovery 283
  18.5.5 Multiple Extractions 285
  18.5.6 LLE with Back-Extraction 286
18.6 Solid–Liquid Extraction 287
18.7 Solid Phase Extraction 287
  18.7.1 Fundamentals 287
  18.7.2 The SPE Column 288
  18.7.3 Conditioning 289
  18.7.4 Equipment 290
  18.7.5 Reversed-Phase SPE 290
  18.7.6 Secondary Interactions 292
  18.7.7 Ion Exchange SPE 293
  18.7.8 Mixed-Mode SPE 295
  18.7.9 Normal-Phase SPE 297
18.8 Summary 298

19 Analytical Chemical Characteristics of Selected Drug Substances 299
19.1 Amitriptyline and Mianserin 299
19.2 Morphine and Codeine 301
19.3 Ibuprofen and Naproxen 302
19.4 Furosemide 304
19.5 Paracetamol (Acetaminophen) 306
19.6 Neutral Drugs 307

20 Quantification and Quality of Analytical Data 309
20.1 Peak Height and Peak Area 309
20.2 Calibration Methods 310
  20.2.1 External Standard Method 310
  20.2.2 Internal Standard Method 313
  20.2.3 Standard Addition 314
  20.2.4 Normalization 314
20.3 Validation
  20.3.1 Analytical Procedure
  20.3.2 Accuracy
  20.3.3 Precision
  20.3.4 Specificity
  20.3.5 Detection Limit
  20.3.6 Quantification Limit
  20.3.7 Linearity and Range
  20.3.8 Robustness
  20.3.9 Test Methods in the European Pharmacopeia
20.4 System Suitability
  20.4.1 Adjustment of Chromatographic Conditions

21 Chemical Analysis of Drug Substances
  21.1 What is a Pharmaceutical Raw Material, how is it Produced and why must it be Controlled?
  21.2 The Pharmacopoeias – the Basis for Control of Pharmaceutical Raw Materials
  21.3 Which Contaminants are Found in Raw Materials, What are the Requirements in a Maximum Content and Why?
    21.3.1 Well Defined Chemical Compounds
    21.3.2 Mixtures of Organic Compounds
  21.4 How to Check the Identity of Pharmaceutical Raw Materials
    21.4.1 Overview of the Identification Procedures
    21.4.2 Techniques used for the Identification of Well Defined Chemical Compounds
      21.4.2.1 Infrared Absorption Spectrophotometry
      21.4.2.2 Ultraviolet and Visible Absorption Spectrophotometry
      21.4.2.3 Thin-Layer Chromatography
      21.4.2.4 Melting Point
      21.4.2.5 Polarimetry
      21.4.2.6 High Performance Liquid Chromatography
      21.4.2.7 Chloride and Sulfate Identification
  21.5 How to Test for Impurities in Pharmaceutical Raw Materials
    21.5.1 Main Purity Tests for Well Defined Chemical Compounds
      21.5.1.1 Appearance of Solution
      21.5.1.2 Absorbance
      21.5.1.3 Acidity/Alkalinity
      21.5.1.4 Optical Rotation
      21.5.1.5 Related Substances
      21.5.1.6 Solvent Residues
      21.5.1.7 Foreign Anions
      21.5.1.8 Cationic Impurities
      21.5.1.9 Loss on Drying
      21.5.1.10 Determination of Water
21.5.2 Purity Tests for Raw Materials of the Type of Mixtures of Organic Compounds

21.5.2.1 Oxidizing Substances
21.5.2.2 Acid Value
21.5.2.3 Hydroxy Value
21.5.2.4 Iodine Value
21.5.2.5 Peroxide Value
21.5.2.6 Saponification Value
21.5.2.7 Unsonifiable Matter
21.5.2.8 Other Tests

21.5.3 Identification of the Raw Materials of the Type of Mixtures of Organic Compounds

21.6 How to Determine the Purity of Pharmaceutical Raw Materials

21.6.1 Acid–Base Titration in Aqueous Environment
21.6.2 Acid–Base Titration in a Non-Aqueous Environment
21.6.3 Redox Titrations
21.6.4 High Performance Liquid Chromatography
21.6.5 UV spectrophotometry

21.7 How to Control Compounds for Which no Pharmacopoeia Monograph Exists

21.8 How are Ph.Eur. and USP Updated?

22 Chemical Analysis of Final Pharmaceutical Products

22.1 Quality Control of Final Pharmaceutical Products
22.2 Monographs and Chemical Testing
22.3 Identification of the Active Pharmaceutical Ingredient
22.4 Assay of the Active Pharmaceutical Ingredient
22.5 Chemical Tests for Final Pharmaceutical Products

23 Analysis of Drugs in Biological Fluids

23.1 Introduction
23.1.1 Drug Development
23.1.2 Therapeutic Drug Monitoring
23.1.3 Forensic and Toxicological Analysis
23.1.4 Doping Control Analysis

23.2 The Biological Matrix

23.3 Bioanalytical Methods

xii Table of Contents
23.3.7 Detection 464
23.3.8 Calibration and Quantification 465
23.4 Examples 466

23.4.1 Sample Preparation

- 23.4.1.1 Sample Preparation Procedure by LLE 466
- 23.4.1.2 Comments to the Procedure 466
- 23.4.1.3 Sample Preparation Procedure by LLE and Back Extraction 467
- 23.4.1.4 Comments to the Procedure 467
- 23.4.1.5 Sample Preparation Procedure by SPE 467
- 23.4.1.6 Comments to the Procedure 468
- 23.4.1.7 Sample Preparation Procedure by Protein Precipitation 468
- 23.4.1.8 Comments to the Procedure 468

23.4.2 Quantitative Determination 468

- 23.4.2.1 Quantitative Determination of Amitriptyline in Serum by LC-MS 468
- 23.4.2.2 Comments to the Procedure 469
- 23.4.2.3 Determination of Valproic Acid in Serum by GC-MS 471
- 23.4.2.4 Comments to the Procedure 471

23.4.3 Identification 472

- 23.4.3.1 Sample Preparation Procedure for Unknown Screening by Mixed Mode Cation Exchange 472
- 23.4.3.2 Comments to the Procedure 472
- 23.4.3.3 GC-MS Procedure for Unknown Screening 473
- 23.4.3.4 Comments to the Procedure 473
- 23.4.3.5 LC-MS-MS Procedure for Unknown Screening 475
- 23.4.3.6 Comments to the Procedure 475

Index 477