

# Contents

Preface

v

## **1. High Performance Liquid Chromatography (HPLC) 1**

- 1.1 Introduction to chromatography 1
- 1.2 Classification of chromatographic methods 2
- 1.3 Separation techniques in chromatography 2
- 1.4 High performance liquid chromatography (HPLC) 4
- 1.5 Principle of HPLC 5
- 1.6 Theory of HPLC 8
- 1.7 Instrumentation of HPLC 24
  - i. Mobile phase reservoirs 26
  - ii. Pumps 30
  - iii. Mixing unit, gradient controller and solvent degassing 35
  - iv. Injector (manual or auto-injectors) 37
  - v. Columns (guard-columns, pre-columns, analytical columns, etc.) 40
  - vi. Detectors 48
  - vii. Recorder or data system 68
- 1.8 Applications of HPLC 70

## **2. Method Developments in HPLC 80**

- 2.1 Introduction 80
- 2.2 Column selection 81
- 2.3 Detector selection 85
- 2.4 Sample preparation 86
- 2.5 Eluent survey 87
- 2.6 Survey evaluation 88
- 2.7 Optimizing the separation 92
- 2.8 Qualitative analysis 99
- 2.9 Quantitative analysis 101
- 2.10 Preparative separations 106
- 2.11 Summary 113

## **3. Analytical Methods Validation and their Importance 114**

- 3.1 Accuracy 115
- 3.2 Precision 116
- 3.3 Specificity 118
- 3.4 Detection limit 119
- 3.5 Quantitation limit 121
- 3.6 Linearity and range 123
- 3.7 Ruggedness 124
- 3.8 Robustness 125

- 3.9 System suitability 126
- 3.10 Glossary 127

## **4. Troubleshooting in HPLC** **129**

- 4.1 Introduction 129
- 4.2 Abnormal pressure 132
- 4.3 Leaks 133
- 4.4 Problems with the chromatogram 135
- 4.5 Problems with the injector 139
- 4.6 Problems detected by smell, sight or sound 140
- 4.7 Key problem areas and preventive maintenance 140
- 4.8 Vacuum degasser troubleshooting 141
- 4.9 Column cooler/heater troubleshooting 142
- 4.10 Troubleshooting detector 143

## **5 High Performance thin Layer Chromatography (HPTLC)** **145**

- 5.1 Introduction 146
- 5.2 Features of HPTLC 147
- 5.3 Technical aspects of HPTLC–HPLC 148
- 5.4 Various steps involved in HPTLC 149
  - I. Sample application 149
    - A. Selection of chromatographic layer/HPTLC plates 149
      - a. Sorbents used in HPTLC plates (pre-coated plates) 150
      - b. Plate size 151
      - c. Layer thickness 151
      - d. Particle size of the sorbents 152
    - B. Pre-washing of pre-coated plates 152
      - a. Ascending method 152
      - b. Dipping method 152
      - c. Continuous method 153
    - C. Activation of pre-coated plates 153
    - D. Sample and standard preparation 153
      - a. Application of the sample and standard solution 154
      - b. Advantages of application of sample as band 154
  - II. Chromatogram development 155
    - A. Selection of mobile phase 155
    - B. Pre-conditioning (chamber saturation) 157
    - C. Chromatographic development and drying 158
      - a. Chromatographic development 158
      - b. Drying 158
  - III. Densitometric chromatographic evaluation 159
    - A. TLC scanner 159
    - B. Detection and visualization 159
      - i. Qualitative detection 160
      - ii. Quantitative detection 160
  - IV. Photo documentation in TLC/HPTLC 161
  - V. Validation of analytical method 164
- 5.5 Comparison of HPLC and HPTLC 166
- 5.6 Comparison of HPTLC and TLC 167
- 5.7 Applications of HPTLC 167

**6. HPTLC Method Development and Method Validation 180**

- 6.1 Introduction 180
- 6.2 Method development in HPTLC 181
- 6.3 HPTLC method validation 181
- 6.4 Methodology for HPTLC analysis 184
  - A. Stationary phase 184
  - B. Mobile phase 184
  - C. Layer pre-washing 185
  - D. Preparation and selection of HPTLC plates 185
  - E. Sample preparation 186
  - F. Application of sample 186
  - G. Development of optimum mobile phase 188
  - H. Solvent polarity 189
    - I. Chromatographic development 189
  - J. Densitometric chromatogram evaluation (scanning) 199
  - K. Quantitative estimation 199
    - i. Estimation of sennoside B from capsule 199
    - ii. Estimation of glycyrrhizin from capsule 201
    - iii. Simultaneous estimation of sennoside and glycyrrhizin 203

**Questions and Answers 205**

- General 205
- Column and column lifetime 207
- Variable retention times 210
- Drifting retention times 211
- Column-to-column and batch-to-batch reproducibility 212
- Sample preparation problems 212
- Gradient system 213
- Degassing 213
- Sources of peak tailing 214
- Reverse phase and normal phase chromatography 215
- System volume, dead volume, dwell volume 216
- Transfer of gradient methods 217
- Clogged system 217
- Column backpressure 217
- Peak area fluctuations 217
- Unsolved questions based on HPLC 218

**Experimental 219**

- Glossary of HPLC Terms* 231
- Suggested Reading* 237
- Index* 241