Trace Quantitative Analysis by Mass Spectrometry

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The authors dedicate this book to all of our mentors and colleagues,
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Contents

Preface xvii

Acknowledgements xxiii

1 Measurement, Dimensions and Units 1
- 1.1 Introduction 1
- 1.2 The International System of Units (SI) 3
- 1.3 'Mass-to-Charge Ratio' in Mass Spectrometry 6
- 1.4 Achievable Precision in Measurement of SI Base Quantities 9
- 1.5 Molecular Mass Limit for Trace Quantitation by Mass Spectrometry 11
- 1.6 Summary of Key Concepts 14

2 Tools of the Trade I. The Classical Tools 17
- 2.1 Introduction 17
- 2.2 Analytical and Internal Standards: Reference Materials 18
  - 2.2.1 Analytical Standard (Reference Standard) and its Traceability 18
  - 2.2.2 Certified (Standard) Reference Materials (CRMs) 20
  - 2.2.3 Surrogate Internal Standard (SIS) 21
  - 2.2.4 Volumetric Internal Standard (VIS) 25
- 2.3 The Analytical Balance 25
  - 2.3.1 Balance Calibration 27
  - 2.3.2 Sources of Uncertainty in Weighing 27
  - 2.3.3 Weighing the Analytical Standard 31
- 2.4 Measurement and Dispensing of Volume 32
  - 2.4.1 Standard Volumetric Flasks 32
  - 2.4.2 Pipets 33
    - *2.4.2a Classical Pipets* 33
    - *2.4.2b Micropipets* 33
  - 2.4.3 Loop Injectors for High Performance Liquid Chromatography (HPLC) 35
  - 2.4.4 Syringes 38
- 2.5 Preparation of Solutions for Calibration 39
  - 2.5.1 Matrix-Free Calibration Solutions 40
  - 2.5.2 Matrix Matched Calibrators 40
  - 2.5.3 Quality Control (QC) Samples 41
    - *2.5.3a QCs in Method Development and Validation* 41
    - *2.5.3b QCs in Sample Analysis* 41
- 2.6 Introduction to Calibration Methods for Quantitative Analysis 42
  - 2.6.1 Calibration Using an External Standard 42
  - 2.6.2 Calibration for the Method of Standard Additions 44

2.6.3 Calibration Using a Surrogate Internal Standard 44
2.6.4 Curves used in Conjunction with 'Continuing Calibration Verification Standards' 46
2.7 Summary of Key Concepts 47

3 Tools of the Trade II. Theory of Chromatography 51
3.1 Introduction 51
3.2 General Principles of Chemical Separations 53
3.3 Summary of Important Concepts 55
3.4 Plate Theory of Chromatography 58
3.4.1 Elution Equation for the Plate Theory 59
3.4.2 Retention Volume and Time 61
3.4.3 The Separation Ratio (Selectivity Factor) for Two Solutes 62
3.4.4 Capacity Factor (Ratio) of a Solute 62
3.4.5 Column Efficiency and Height Equivalent of the Theoretical Plate 63
3.4.6 Chromatographic Resolution 64
3.4.7 Effective Plate Number 65
3.4.8 Maximum Sample Injection Volume for a Specific Column 65
3.4.9 Peak Capacity of a Column 66
3.4.10 Gaussian Form of the Plate Theory Elution Equation 67
3.5 Nonequilibrium Effects in Chromatography: the van Deemter Equation 69
3.5.1 Multipath Dispersion 70
3.5.2 Longitudinal Diffusion 71
3.5.3 Resistance to Mass Transfer in the Mobile and Stationary Phases 71
3.5.4 Optimization to Maximize Column Efficiency 72
3.5.5 Relationships for Estimating Optimized Conditions 74
3.5.6 Numerical Estimates for Optimized Parameters 76
3.5.7 Ultra-Small Stationary Phase Particles 77
3.5.8 Monolithic Columns 80
3.5.9 Ultra High Flow Rate Liquid Chromatography 81
3.5.10 Packed Microcolumns 81
3.5.10a The Knox Equation 84
3.5.10b Chromatographic Dilution 87
3.5.10c Flow Impedance Parameter and Separation Impedance 87
3.5.11 Gas Chromatography 88
3.5.11a Effect of Gas Compressibility on Elution Equation for Packed Columns 88
3.5.11b Open Tubular Columns and the Golay Equation 89
3.5.12 Peak Asymmetry 90
3.6 Gradient Elution 92
3.7 Capillary Electrophoresis and Capillary Electrochromatography 97
Appendix 3.1 Derivation of the Plate Theory Equation for Chromatographic Elution 102
Appendix 3.2 Transformation of the Plate Theory Elution Equation from Poisson to Gaussian Form 103
Appendix 3.3 A Brief Introduction to Snyder's Theory of Gradient Elution 104
List of Symbols Used in Chapter 3 105

4 Tools of the Trade III. Separation Practicalities 109
4.1 Introduction 109
4.2 The Analyte and the Matrix 110
4.3 Extraction and Clean-Up: Sample Preparation Methods 111
4.3.1 Liquid–Liquid Extraction (LLE) 112
4.3.1a Solid-Supported Liquid–Liquid Extraction (SLE) 114
4.3.1b Single Drop Microextraction (SDME) 114
4.3.1c Dispersive Liquid–Liquid Microextraction (DLLE) 115

4.3.1d Flow Injection Liquid–Liquid Extraction 115
4.3.1e Membrane Extraction 115
4.3.1f Protein Precipitation from Biological Fluids 117
4.3.2 Liquid Extraction of Analytes from Solid Matrices 117
4.3.2a Soxhlet Extraction 117
4.3.2b Pressurized Solvent Extraction 117
4.3.2c Sonication Assisted Liquid Extraction (SAE) 119
4.3.2d Microwave Assisted Extraction (MAE) 120
4.3.2e Supercritical Fluid Extraction (SFE) 121
4.3.3 Solid Phase Extraction from Liquids and Gases 124
4.3.3a Flash Chromatography 124
4.3.3b Purge-and-Trap Analysis for Volatile Organic Compounds 124
4.3.3c Solid Phase Extraction (SPE) 125
4.3.3d Turbulent Flow Chromatography 128
4.3.3e Molecularly Imprinted Polymers (MIPs) 130
4.3.3f Solid Phase Microextraction (SPME) 132
4.3.3g Stir-Bar Sorptive Extraction (SBSE) 133
4.4 Chromatographic Practicalities 133
4.4.1 Stationary Phases for SPE and Liquid Chromatography 133
4.4.1a Alumina and Silica Particles 134
4.4.1b Derivatization of Silica for Normal and Reverse Phase Chromatography 136
4.4.1c Ion Exchange Media 137
4.4.1d Chiral Separations 137
4.4.1e Affinity Media 143
4.4.2 Mobile Phases Used in SPE and Liquid Chromatography 144
4.4.2a Solvent Polarity and Elution Strength 145
4.4.2b Reverse Phase Chromatography 146
4.4.2c Hydrophilic Interaction Chromatography (HILIC) 146
4.4.3 Mobile and Stationary Phases for Gas Chromatography 147
4.4.3a GC Mobile Phase 147
4.4.3b Temperature Programming 149
4.4.3c GC Stationary Phases 150
4.4.4 Sample Injection Devices for Chromatography 152
4.4.4a Automated Loop Injectors for HPLC 152
4.4.4b GC Injectors 152
4.4.5 Pumps for HPLC 154
4.4.6 Capillary Electrophoresis and Electrochromatography 157
4.4.7 Micro Total Analysis Systems (Lab-on-a-Chip) 160
4.4.8 General Comments on Detectors for Chromatography 163
4.5 Summary of Key Concepts 166
Appendix 4.1 Responses of Chromatographic Detectors: Concentration vs Mass–Flux Dependence 169

5 Tools of the Trade IV. Interfaces and Ion Sources for Chromatography–Mass Spectrometry 173
5.1 Introduction 174
5.1.1 Matrix Effects 175
5.2 Ion Sources that can Require a Discrete Interface Between Chromatograph and Source 176
5.2.1 Electron Ionization and Chemical Ionization 176
5.2.1a Discrete Chromatograph-Ion Source Interfaces 180
5.2.1b Chemical Derivatization for EI and CI 182
5.2.2 Matrix Assisted Laser Desorption/Ionization (MALDI) 184
5.2.3 ‘Lab-on-a-Chip’ 195

- 5.3 Ion Sources not Requiring a Discrete Interface 196
  - 5.3.1 Flow Fast Atom Bombardment (Flow-FAB) 196
  - 5.3.2 Thermospray Ionization 197
  - 5.3.3 Atmospheric Pressure Ionization (API) 198
    - *5.3.3a Coupling of API Sources to Mass Spectrometers* 199
  - 5.3.4 Atmospheric Pressure Chemical Ionization (APCI) 203
  - 5.3.5 Atmospheric Pressure Photoionization (APPI) 206
  - 5.3.6 Electrospray Ionization (ESI) 211
    - *5.3.6a Ionization Suppression/Enhancement: Matrix Effects* 221
    - *5.3.6b ESI-MS: Concentration or Mass Flow Dependent?* 230
  - 5.3.7 Atmospheric Pressure Desorption Methods 236
- 5.4 Source–Analyzer Interfaces Based on Ion Mobility 237
- 5.5 Summary of Key Concepts 238
- 5.1 Appendix 5.1: Methods of Sample Preparation for Analysis by MALDI 242

6 Tools of the Trade V. Mass Analyzers for Quantitation: Separation of Ions by *m/z* Values 245

- 6.1 Introduction 245
- 6.2 Mass Analyzer Operation Modes and Tandem Mass Spectrometry 248
  - 6.2.1 The Selectivity–Sensitivity Compromise 249
  - 6.2.2 Tandem Mass Spectrometry (MS/MS) 251
  - 6.2.3 Figures of Merit for Mass Analyzers 255
    - *6.2.3a Accessible m/z Range* 255
    - *6.2.3b Resolving Power* 256
    - *6.2.3c Accuracy and Precision of Mass Measurement* 257
    - *6.2.3d Transmission Efficiency* 258
    - *6.2.3e Duty Cycle* 259
    - *6.2.3f Data Acquisition Rate* 259
    - *6.2.3g Dynamic Range (Range of Reliable Response)* 261
    - *6.2.3h Versatility for Tandem Mass Spectrometry* 261
    - *6.2.3i Ease of Use* 261
    - *6.2.3j Capital and Maintenance Costs* 261
- 6.3 Motion of Ions in Electric and Magnetic Fields 261
  - 6.3.1 Introduction to Interactions of Electric and Magnetic Fields with Ions 263
  - 6.3.2 Ion Optics and Lenses: Instrument Tuning 265
- 6.4 Mass Analyzers 266
  - 6.4.1 Calibration of the *m/z* Axis (‘Mass Calibration’) 266
  - 6.4.2 Quadrupole Mass Filters 267
    - *6.4.2a RF-Only Quadrupoles* 276
  - 6.4.3 Triple Quadrupole Instruments 277
  - 6.4.4 Magnetic Sector Analyzers 280
  - 6.4.5 Quadrupole Ion Traps 284
    - *6.4.5a Three-Dimensional (Paul) Traps* 285
    - *6.4.5b Two-Dimensional (Linear) Traps* 301
  - 6.4.6 The QqQ_{trap} Analyzer 309
  - 6.4.7 Time of Flight and QqTOF Analyzers 311
  - 6.4.8 FTICR and Orbitrap Analyzers 320
- 6.5 Activation and Dissociation of Ions 320
- 6.6 Vacuum Systems 326
  - 6.6.1 Pumping Speed, Conductance and Gas Flow 327
  - 6.6.2 Vacuum Pumps 329
    - *6.6.2a Rotary Vane Pumps* 329
    - *6.6.2b Diffusion Pumps* 330

6.6.2c Turbomolecular Pumps 330
6.6.2d Differential Pumping 332
6.6.3 Vacuum Gauges 334
6.6.3a Capacitance Manometer 334
6.6.3b Pirani Gauge 334
6.6.3c Thermocouple Gauge 335
6.6.3d Ionization Gauge 335
6.7 Summary of Key Concepts 336
Appendix 6.1 Interaction of Electric and Magnetic Fields with Charged Particles 339
Appendix 6.2 Leak Detection 340
Appendix 6.3 List of Symbols Used in Chapter 6 341

7 Tools of the Trade VI. Ion Detection and Data Processing 345
7.1 Introduction 345
7.1.1 Signal:Noise vs Signal:Background 347
7.1.1a Shot Noise in the Ion Beam 350
7.1.1b Data Smoothing Before Integration 352
7.1.1c Integration and Experimental Determination of Signal:Background 353
7.2 Faraday Cup Detectors 353
7.3 Electron Multipliers 354
7.3.1 Discrete Dynode Secondary Electron Multipliers 354
7.3.1a Off-Axis Conversion Dynodes 357
7.3.2 Channel Electron Multipliers 359
7.3.2a Single Channel Electron Multipliers 359
7.3.2b Channel Electron Multiplier Arrays 362
7.4 Post-Detector Electronics 365
7.4.1 Analog Signal Processing 365
7.4.2 Digital Electronics for Ion Counting Detectors 366
7.4.3 Computer-Based Data Systems 367
7.5 Summary of Key Concepts 368

8 Tools of the Trade VII: Statistics of Calibration, Measurement and Sampling 373
8.1 Introduction 374
8.1.1 Systematic and Random Errors: Accuracy and Precision 375
8.2 Univariate Data: Tools and Tests for Determining Accuracy and Precision 377
8.2.1 Mean, Median, Standard Deviation, Standard Error 377
8.2.2 Significant Figures and Propagation of Error 379
8.2.3 Normal (Gaussian) Distribution 382
8.2.4 Hypothesis Tests: False Positives and False Negatives 385
8.2.5 Student's *t*-Test and Fisher F-Test for Comparison of Means and Variances: Applications to Two Data Sets 387
8.2.6 Statistical Testing of More Than Two Data Sets: Bartlett Test and ANOVA 394
8.2.7 Multiple-Range Test and Huber–Davies Test for Outliers 398
8.3 Bivariate Data: Tools and Tests for Regression and Correlation 398
8.3.1 Correlation Analysis 401
8.3.2 Simple Linear Regression for Homoscedastic Data 401
8.3.3 Test for Goodness of Fit: Irreproducibility, Heteroscedacity and Nonlinearity 407
8.3.4 Inversion of the Calibration Equation 410
8.3.5 Weighted Linear Regression 411
8.3.6 Regression for Nonlinear Data: the Quadratic Fitting Function 415
8.3.7 Summarized Procedure to Determine Best-Fit Calibration Curve 416
8.3.8 Nonlinear Least-Squares Regression 418

8.4 Limits of Detection and Quantitation 418
  8.4.1 Limit of Detection 419
  8.4.2 Limits of Quantitation 427
8.5 Calibration and Measurement: Systematic and Random Errors 428
  8.5.1 Analyses Without Internal Standards 428
    8.5.1a External Linear Calibration With a Zero Intercept 428
    8.5.1b Method of Standard Additions 430
    8.5.1c External Linear Calibration With a Nonzero Intercept 432
    8.5.1d Systematic Errors in the Method of Standard Additions: Youden Plots 434
    8.5.1e Strategies When Analytical Standards are not Available 435
  8.5.2 Analyses Using Internal Standards 436
    8.5.2a Volumetric Internal Standards for GC Analyses 437
    8.5.2b Use of Surrogate Internal Standards 439
    8.5.2c Cross-Contributions Between Analyte and Internal Standard – a Need for Nonlinear Regression 444
8.6 Statistics of Sampling of Heterogeneous Matrices 448
8.7 Summary of Key Concepts 453
Appendix 8.1 A Brief Statistics Glossary 455
Appendix 8.2 Symbols Used in Discussion of Calibration Methods 458

9 Method Development and Fitness for Purpose 461
9.1 Introduction 461
9.2 Fitness for Purpose and Managing Uncertainty 461
9.3 Issues Between Analyst and Client: Examining What's at Stake 463
  9.3.1 Define the Analytical Problem 463
  9.3.2 Consider the Needs of all Interested Parties 463
    9.3.2a Client's Perspective vs Analyst's Perspective 463
  9.3.3 Define Uncertainty Tolerance 465
    9.3.3a Setting Targets for Quantitation 466
    9.3.3b Setting Targets for Identification 466
  9.3.4 Balancing Thoroughness vs Time and Resources 472
  9.3.5 The Analyst as Expert Witness 474
9.4 Formulating a Strategy 474
  9.4.1 Defining the Scope and Method Requirements 474
  9.4.2 Considering Prevailing Guidelines or Regulatory Requirements 476
    9.4.2a Good Laboratory Practice 476
    9.4.2b Laboratory Accreditation 478
    9.4.2c Method Development and Validation Without Established Guidance 478
  9.4.3 The Analyte 478
    9.4.3a Structures and Chemical Properties – MS Considerations 478
    9.4.3b Structures and Chemical Properties – Analytical Considerations 479
  9.4.4 The Reference Standard 479
    9.4.4a Availability and Source 479
    9.4.4b Receipt and Documentation 480
    9.4.4c Certificate of Analysis 481
    9.4.4d Assigned Purity 481
    9.4.4e Chiral Purity 482
    9.4.4f Storage and Stability 482
  9.4.5 The Surrogate Internal Standard (SIS) 482
    9.4.5a General Considerations 482
    9.4.5b Stable Isotope Internal Standards 482

- *9.4.5c Analog or Homolog Internal Standards* 484
- *9.4.5d SIS for Multi-analyte Assays* 484
- 9.4.6 The Analytical Sample 484
  - *9.4.6a Sample Availability vs Method Requirements* 485
  - *9.4.6b Documentation of Sample Receipt, Storage and Use* 485
- 9.4.7 The Control Matrix 486
  - *9.4.7a Obtaining a Suitable Control Matrix* 486
  - *9.4.7b Using the Control Matrix to Assess Selectivity* 486
  - *9.4.7c Surrogate Matrices* 489
- 9.4.8 Evaluate Analytical Options 489
  - *9.4.8a Reviewing The Literature and Previous Methodologies* 489
  - *9.4.8b Review Existing Laboratory SOPs* 490
- 9.5 Method Development 490
  - 9.5.1 Instrument Qualification, Calibration and Maintenance 490
    - *9.5.1a Equipment Qualification* 491
    - *9.5.1b Mass Spectrometer Calibration* 494
    - *9.5.1c Maintenance* 494
    - *9.5.1d Validation of Computerized Laboratory Instruments and Equipment* 494
  - 9.5.2 Instrument Optimization 496
  - 9.5.3 LC–MS/MS Optimization for MRM Analysis Using QqQ 497
    - *9.5.3a API Interface and Q_1 Optimization* 498
    - *9.5.3b Selecting Product Ions and CID Conditions* 502
    - *9.5.3c Final MRM Optimization* 503
  - 9.5.4 Preparation of Stock and Spiking Solutions 504
    - *9.5.4a Primary Stock Solutions* 504
    - *9.5.4b Sub-Stocks and Spiking Solutions* 506
    - *9.5.4c Miscellaneous Considerations* 506
  - 9.5.5 Chromatography Method Development 507
    - *9.5.5a General Considerations* 507
    - *9.5.5b Sample Throughput and Selectivity* 507
    - *9.5.5c Multi-dimensional Separations* 508
    - *9.5.5d Miscellaneous Components* 510
  - 9.5.6 Sample Preparation – Extraction and Clean-Up 511
    - *9.5.6a Evaluating Options* 512
    - *9.5.6b Use of Blanks During Method Development and Analysis* 513
    - *9.5.6c Using Spikes and ‘Recovery Samples’* 513
  - 9.5.7 Evaluating Sensitivity and Linearity in Matrix 515
- 9.6 Matrix Effects 517
  - 9.6.1 Evaluating Ion Suppression in Method Development 518
  - 9.6.2 Addressing Ion Suppression/Enhancement 519
  - 9.6.3 Interferences 520
- 9.7 Contamination and Carryover 522
  - 9.7.1 Laboratory Contamination 522
    - *9.7.1a Common Causes and Impact* 522
    - *9.7.1b Monitoring Contamination in the Laboratory* 522
  - 9.7.2 Carryover 523
    - *9.7.2a Evaluating Carryover During Method Development* 524
    - *9.7.2b Addressing Carryover in the Method* 525
- 9.8 Establishing the Final Method 527
  - 9.8.1 Curve Preparation 527
    - *9.8.1a Calibration Solutions* 527
    - *9.8.1b Matrix Matched Calibrators* 528

9.8.2 Preparation of QCs 530
9.8.3 System Suitability 531
9.8.4 Testing Method Robustness and Ruggedness 533
9.8.5 Establishing Final Stepwise Procedure 535
9.8.6 Final Method Qualification/Assessment 536

10 Method Validation and Sample Analysis in a Controlled Laboratory Environment 539
10.1 Introduction 539
10.2 Method Validation 540
10.2.1 Figures of Merit for Full Validation 540
10.2.2 Selectivity 540
10.2.3 Sensitivity, Range of Reliable Response and Linearity 542
10.2.4 Accuracy and Precision 543
10.2.5 Reproducibility 543
10.2.6 Recovery 544
10.2.7 Stability in Solution 544
10.2.8 Stability in Matrix 545
10.2.8a In-Process Stability (Room Temperature Stability) 545
10.2.8b Freeze–Thaw Stability 546
10.2.8c Long Term Storage Stability (Sample Stability) 546
10.2.9 Other Special Validation Requirements 547
10.2.9a Extract and Re-Injection Stability 547
10.2.9b Assessing Carryover and Potential for Laboratory Contamination 548
10.2.9c Incurred Sample Re-Analysis and Stability 548
10.2.9d Integrity of Dilution 549
10.2.10 Abbreviated and Partial Validation 549
10.2.11 Cross Validations 550
10.3 Conduct of the Validaton 551
10.3.1 Validation Plan or Protocol 551
10.3.2 Validation Sample Analysis 552
10.3.2a Data Review 552
10.3.2b Addressing Validation Run Failures 553
10.3.3 Documentation of Supporting Data 553
10.3.3a Assay Procedure 553
10.3.3b Standards and Stock Solutions 553
10.3.3c Preparation of Calibrators and QCs 553
10.3.3d Acceptance Criteria 553
10.3.3e Sample Analysis 554
10.3.3f Run Summary Sheets 555
10.3.3g Chromatograms 555
10.3.3h Communications 555
10.3.4 The Validation Report and Scientific Review 555
10.3.4a Reference Standard and Solutions 556
10.3.4b QCs and Calibrators 556
10.3.4c Assay Procedures 556
10.3.4d Run Acceptance Criteria 556
10.3.4e Sample Analysis Tables 556
10.3.4f Failed Runs 557
10.3.4g Deviations From Method or SOPs 558
10.3.4h Chromatograms 558
10.3.4i Amendments 558

10.4 Examples of Methods and Validations Fit for Purpose 559
  10.4.1 Bioanalytical Method Validation 559
    10.4.1a Sensitivity and the Calibration/Standard Curve 560
    10.4.1b Accuracy and Precision 561
    10.4.1c Selectivity 562
    10.4.1d Matrix Effects 562
    10.4.1e Recovery 563
    10.4.1f Reproducibility 563
    10.4.1g Stability – Overview 563
    10.4.1h Stock Solution Stability 563
    10.4.1i Freeze and Thaw Stability 564
    10.4.1j Short Term Temperature Stability 564
    10.4.1k Long Term Stability 564
    10.4.1l Incurred Sample Re-Analysis 565
  10.4.2 Risk Assessment Methods 567
  10.4.3 Enforcement Methods 567
    10.4.3a FDA Enforcement Methods for Drug Residues in Animal Food Products 568
10.5 Validated Sample Analysis 570
  10.5.1 Sample Batch Preparation and Extraction 570
  10.5.2 Sample Analysis 570
    10.5.2a Analytical Run Lists 570
    10.5.2b Instrument Set-Up and System Suitability 571
    10.5.2c Failed Runs and Re-Analysis 571
  10.5.3 Data Review 572
    10.5.3a Evaluating Chromatography and Instrument Response 572
    10.5.3b Evaluating the Curve and QCs 575
    10.5.3c Rejection of Data 576
  10.5.4 Sample Re-Assays 577
  10.5.5 Addressing Carryover During Sample Analysis 578
  10.5.6 Investigation and Corrective Action 580
10.6 Documentation 582
  10.6.1 Sample Tracking 582
  10.6.2 Sample Analytical Report 583
10.7 Traceability 583

11 Examples from the Literature 585
11.1 Introduction 585
11.2 Food Contaminants 585
  11.2.1 Acrylamide 586
  11.2.2 Paralytic Shellfish Poisons 598
11.3 Anthropogenic Pollutants in Water 605
  11.3.1 Disinfection By-Products 605
    11.3.1a MX [3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone] 607
    11.3.1b N-nitrosodialkylamines 612
  11.3.2 Multi-residue Methods for Pharmaceutical Residues 616
    11.3.2a Validated Method for Macrolide Antibiotics 617
    11.3.2b Extraction/Clean-up for Multi-residue Analysis of Compounds of Widely Different Polarity etc. 620
    11.3.2c Screening Method for Multiclass Antibiotics 621
11.4 GC–MS Analyses of Persistent Environmental Pollutants 623
  11.4.1 'Dioxin-like' Compounds 623
  11.4.2 Other Persistent Pollutants 636

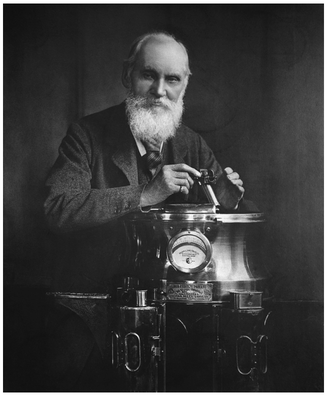
11.5 Bioanalytical Applications 637
  11.5.1 Drug Discovery Methods 638
    11.5.1a Passive Permeability Test (Caco-2 Assays) 639
    11.5.1b Drug–Drug Interaction Studies (CYP450 Assays) 641
    11.5.1c Clearance Rate Tests (Metabolic Stability) 643
    11.5.1d Pharmacokinetics and ADME Studies 646
    11.5.1e Metabolite Identification and Quantitation 648
  11.5.2 Drug Development Phase Validated Methods 654
    11.5.2a Enantiomer-Specific Analyses 655
    11.5.2b Methods for Two Concentration Ranges 660
11.6 Quantitative Proteomics 661
  11.6.1 Identification of Proteins by Mass Spectrometry 664
  11.6.2 Relative (Comparative) Quantitation 667
    11.6.2a Isotope-Coded Affinity Tag (ICAT) 669
    11.6.2b Isotope Tagging for Relative and Absolute Quantitation (ITRAQ) 669
    11.6.2c Other Chemical Labeling Methods 671
    11.6.2d Proteolytic Labeling Methods 672
    11.6.2e Culture-Based Labeling Methods 673
  11.6.3 Absolute Quantitation 675
    11.6.3a AQUA 675
    11.6.3b QCAT Peptides 676
    11.6.3c Stable Isotope Standards and Capture by Anti-Peptide Antibodies (SISCAPA) 677
11.7 Analysis of Endogenous Analytes 680

Epilog 683

References 685

Index 709

Preface



'When you can measure what you are speaking about and express it in numbers, you know something about it; but when you cannot express it in numbers, your knowledge is of a meagre and unsatisfactory kind . . .'
William Thomson (Lord Kelvin), Lecture to the Institution of Civil Engineers, 3 May 1883.

The discipline devoted to careful measurement of specific properties of the universe around us is known as *metrology*. The famous statement by William Thomson, quoted above, summarizes the importance of quantitative measurements for the testing of scientific hypotheses; indeed, without such quantitative testing, it is fair to say that hypotheses can not be regarded as scientific at all. Missing from Thomson's comment, however, is a mention of the importance of careful evaluation of the uncertainties that are present in any quantitative measurements and the resulting degree of confidence that can be placed in them and any conclusions drawn from them. These uncertainties are just as important as the 'best' quantitative measured value itself.

This book is devoted to the science and art of *chemical metrology*, taken here to mean the quantitative measurement of amounts of specific (known) chemical compounds present at trace levels (roughly defined as one part in 10^6–10^{12}) in complex matrices. Examples are drugs and their metabolites in body fluids, pesticide residues in foodstuffs, contaminants in drinking water etc. Such measurements are extremely demanding, and involve the use of a wide range of apparatus and of experimental procedures and methods of data evaluation, all of which must be used properly if reliable estimates of chemical concentrations and their associated uncertainties are to be obtained. While this is true of any chemical analysis, the modern advances in trace-level analysis are critically dependent on developments in mass spectrometry.

For several decades before its application to chemical analysis, mass spectrometry was a major tool in fundamental physics. The invention of mass spectrometry is usually attributed to Joseph John Thomson, no relative to William Thomson (Lord Kelvin) whose picture appears above. In 1897 J.J. Thomson measured the ratio of the charge of an electron to its mass, thus confirming for the first time that this then-mysterious entity possessed properties characteristic of a particle. (It is interesting that his son G.P. Thomson later emulated his father by winning a Nobel Prize, but for demonstrating that the electron *also* possesses properties characteristic of a wave!). An account of the life and work of J.J. Thomson was published (Griffiths 1997) to commemorate the centenary of the first measurement of mass-to-charge of an elementary particle.

F.W. Aston, a student of Thomson, won a Nobel Prize for using mass spectrometry to demonstrate the existence of the isotopes of the elements (Aston 1919), and for developing a higher resolution mass spectrometer that permitted measurement of atomic masses with sufficient accuracy and precision for the first reliable estimates of so-called mass defects, i.e., deviations of actual (measured) atomic masses from those predicted from the sums of the masses of the constituent elementary particles (protons, neutrons and electrons). Later, this work was extended by K.T. Bainbridge whose measurements of mass defects (Bainbridge 1932, 1936) were of sufficient accuracy and precision to confirm for the first time the famous relationship derived by Albert Einstein concerning the equivalence of mass (in this case the mass defect) and energy (in this case the binding energy of protons and neutrons within an atomic nucleus).

The first analysis of positive ions is attributed to Wien, who used a magnetic field to separate ions of different mass-to-charge ratios (Wien 1898), but the first appreciation of the potential of the new technique in chemical analysis appears to have again resulted from the work of Thomson in his famous book *Rays of Positive Electricity and Their Application to Chemical Analysis* (Thomson 1913). The present book is intended as an introduction to the use of mass spectrometry for quantitative measurements of the amounts of specific (known) chemical compounds (so-called 'target analytes') present at trace levels in complex matrices. This modern day meaning of 'quantitative mass spectrometry' is rather different from its much more specialized historical meaning in the earliest days of application of the technique to chemistry.

In the two decades spanning about 1940–1960, the petroleum industry was the major proponent of mass spectrometry as a tool of analytical chemistry, and indeed the first few issues of *Advances in Mass Spectrometry* (essentially the proceedings of the International Conferences on Mass Spectrometry) were sponsored and published by the Petroleum Institute. Raw petroleum and its distillate fractions are incredibly complex mixtures of chemical compounds, mainly corresponding to chemical compositions $C_cH_hN_nO_oS_s$, and it is impossible to devise a complete chemical analysis of such an extremely large number of components at concentrations covering a dynamic range of many orders of magnitude. However, some knowledge of chemical composition is required by chemical engineers for optimization of the industrial processes required to produce end products with the desired properties. To this end petroleum chemists devised the concepts of compound *class*, i.e., compounds with a specified composition with respect to heteroatoms only ($N_nO_oS_s$), and compound *type*, i.e., compounds with a specified value of Z when the composition is expressed as $C_cH_{2c+Z}N_nO_oS_s$. Clearly the parameter Z is related to the degree of unsaturation. Reviews of this application of mass spectrometry have been published (Grayson 2002; Roussis 1999). The earliest methods yielded information on relative amounts of hydrocarbon compounds in a distillate, i.e. *type* analyses for the compound class with $n = o = s =$ zero. A high resolution adaptation of the original low resolution mass spectral methods was first published in 1967, and permitted determinations of 18 saturated- and aromatic-hydrocarbon types and four aromatic types containing sulfur.

Essentially, the general approach first identified specific mass-to-charge ratio (*m/z*) values in the electron ionization mass spectra that are characteristic of each compound type, and obtained a calibration based on analysis of mixtures of known composition:

$$\mathbf{S} = \mathbf{R}.\mathbf{C}$$

where **S** is a vector containing the appropriate sums of signal intensities at the *m/z* values that are characteristic for each compound type, **C** is a vector whose elements are the concentrations of these compound types, and **R** is the (square) matrix of mass spectrometric response factors determined from the calibration experiments (average response coefficients for each type are on the diagonal of the matrix and the off-diagonal elements take into account inter-type contributions to signal intensities at the characteristic *m/z* values). Quantitative analysis of an unknown thus requires inversion of the response matrix:

$$\mathbf{C} = \mathbf{R}^{-1}.\mathbf{S}$$

An example of such a type analysis for the class $C_cH_{2c+Z}S$, for both a raw petroleum feedstock and one of its products from a refinery process designed to remove the sulfur content, is shown in Figure P.1 (here the carbon number c is replaced by n). Modern developments in quantitative petroleum analysis incorporate new advances in separation science as well as in mass spectrometric technologies, especially ionization techniques and ultrahigh resolving power (Marshall 2004) and improved calibration of the response matrix (Fafet 1999).

This early and narrow interpretation of the phrase 'quantitative mass spectrometry' is now badly outdated, although petroleum analysis is still an important branch of analytical chemistry that is still being developed. However, apart from its historical importance it introduced important concepts, including calibration and response factor, that will appear throughout this book. Nowadays, quantitation by mass spectrometry generally refers to determination of target analytes (known and specified chemical species, rather than groups of compounds defined within 'classes' or 'types' as in the petroleum case), present in a complex matrix at trace levels. This book is intended as an introduction to this demanding branch of measurement science, one that is crucial for meaningful studies of a wide range of phenomena including environmental, pharmacological and biomedical studies.

The approach adopted throughout the book is to emphasize the fundamentals underlying the scientific

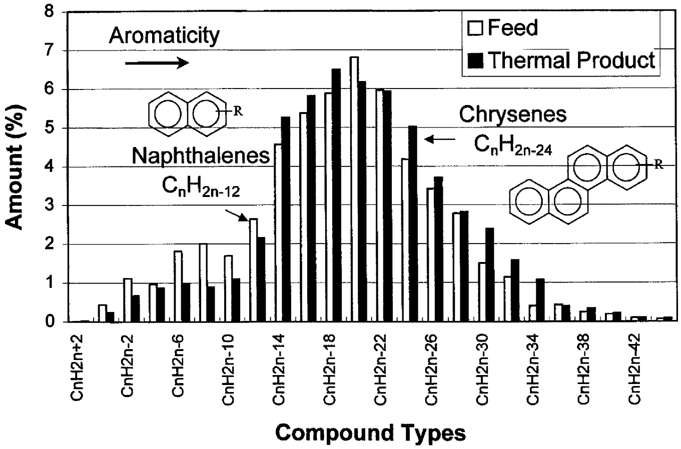


Figure P.1 Type analysis of a petroleum feedstock and its hydrocracked product for compound class $C_nH_{2n+Z}S$. Reproduced with permission from S. Roussis, *Rapid Commun. Mass Spectrom.* **13**, 1031–1051 (1999).

instruments and methodologies, illustrated by historically important developments as well as innovations that were current at the time of completing the manuscript (late summer 2007). Hopefully this will prove to be of more lasting value for the reader. However, a discussion of 'fundamentals' without any description of how 'the fundamental things apply' (see text box) to real-world problems is unlikely to be of much use to anyone, so the final chapter discusses some illustrative examples from the literature.

As mentioned above, although this book is devoted to *quantitative* analysis of specified 'target' analytes, the analyst must have a degree of confidence that the signals being measured do indeed arise from the presence of that target analyte (confirmation of analyte identity), and only from that analyte (signal purity). Therefore, even quantitative analyses inevitably involve some degree of confirmation of analyte structure and identity, and also a check for potential contributions to the recorded instrumental signals from other compounds. The degree to which such checking of analyte identity and of signal purity is necessary will vary from case to case, e.g., analysis of a synthetic pharmaceutical drug in blood plasma following a clinical dose is much less likely to require a high degree of identity confirmation than that of a chlorinated pollutant in an environmental sample. Determination of the appropriate degree of such checks is an example of application of the concept of '*Fitness for Purpose*', another major theme of this book. This principle is discussed more fully in Chapter 9.2, but will appear in several intervening chapters so a very brief introduction to the concept is presented here, based on a discussion (Bethem 2003) of its applicability to mass spectrometric analyses. The following principles are quotations from this work (Bethem 2003):

1. Ultimately it is the responsibility of the analyst to make choices, provide supporting data, and interpret results according to scientific principles and qualified judgment.
2. Analysts should use methods which are Fit for Purpose. Analysts should be able to show that their methods are Fit for Purpose.
3. Fitness for Purpose means that the uncertainty inherent in a given method is tolerable given the needs of the application area.
4. Targets for measurement uncertainty describe how accurate and precise the measurements need to be. Targets for identification confidence describe how certain one needs to be that the correct analyte has been identified.
5. Establishing method fitness consists of showing that the targets for measurement uncertainty and identification confidence have been met.

In its simplest terms, the 'Fitness for Purpose' principle corresponds to the commonsense notion that an analytical method must provide answers with sufficiently low uncertainties that the requirements of the user of the data are fully met within specified constraints of time, cost etc. On the other hand there is no point in developing, validating and using an analytical method with extremely low uncertainties in the data (high precision and accuracy) if a considerably less demanding method (generally less expensive in terms of money, time and effort) will suffice.

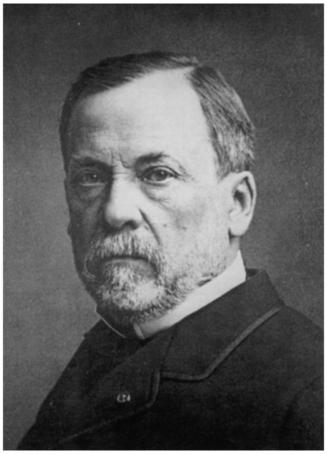
‘The Fundamental Things Apply —’

with apologies to ‘As time goes by’ by Herman Hupfeld (1931); Warner Bros. Music Corp.

This famous song was featured in the films *Casablanca* and *Sleepless in Seattle*, as well as the well-known British TV comedy series of the same name. This is a long stretch from the subject of this book, but the above quotation from the lyrics seems appropriate.

A distinction between ‘fundamental’ and ‘applied’ science is drawn by some, including (alas) by some funding agencies! One of the themes of this book is best expressed by quoting one of the giants of 19th century science:

> *‘There does not exist a category of science to which one can give the name applied science. There are science and the applications of science, bound together as the fruit of the tree’. Louis Pasteur, ‘Revue Scientifique,’ Paris, 1871.*



Louis Pasteur

It is not essential for an experimental scientist to be familiar with and understand every detail of the theoretical underpinnings of his/her laboratory work. However, to be able to properly plan an experimental investigation so that the results can be meaningfully interpreted, it *is* essential that he/she should understand the background of the relevant theory, its basic assumptions, and the limits of its applicability and the magnitude of the consequences of the approximations involved. This general theme was a guiding principle in writing this book, and hopefully this approach will ensure that the book will have a reasonably long useful lifetime. However, fundamentals without much discussion of how they apply to real-world problems in trace analytical chemistry are not of themselves very useful, and discussions of how the ‘fundamental things apply’ will appear later in the book.

A specific example of the continuity between ‘applied’ and ‘fundamental’ research, of direct relevance to the subject of this book (see Chapter 5), is provided by the direct line of development starting from an electrostatic paint sprayer designed for industrial use, through attempts by materials scientists to prepare single molecules of synthetic polymers in the gas phase to enable fundamental studies, to the eventual award of a Nobel Prize to John B. Fenn for the invention of electrospray ionization mass spectrometry and its application to biochemistry and molecular biology.

Figure P.2 shows a generalized procedure for achieving Fitness for Purpose.

This book is not intended to cover important branches of mass spectrometry that provide accurate and precise quantitative measurements of *relative* concentrations, e.g., of variations in isotopic ratios of an element by isotope ratio mass spectrometry (IRMS) and accelerator mass spectrometry (AMS). Rather, this book is mainly concerned with determinations of absolute *amount of substance* (see Chapter 1 for a definition and explanation), particularly for compounds present at trace levels in complex matrices. (The only exception is the inclusion of a brief description of methods used to determine *differences* in levels of proteins in living cells or organisms subjected to different stimuli, e.g., disease state vs normal state).

The book covers analysis of ‘small’ ($< 2000\,\mathrm{Da}$) organic molecules, in environmental and biomedical matrices. The first book exclusively devoted to this subject (Millard 1977) is now rather out of date as a result of more recent spectacular advances in mass spectrometric technology. Very recently two excellent introductions to the subject have appeared (Duncan 2006; Lavagnini 2006). The present book differs from these with regard to their respective lengths; the present book is much longer, as a result of the attempt to provide a comprehensive introduction to all the many ancillary techniques and tools that must be coordinated to provide a reliable result for a trace-level quantitative analysis by mass spectrometry. Thus, many of the present chapters discuss matters that are common to any quantitative analytical method, not only to those in which mass spectrometry is the key component providing the final analytical signal

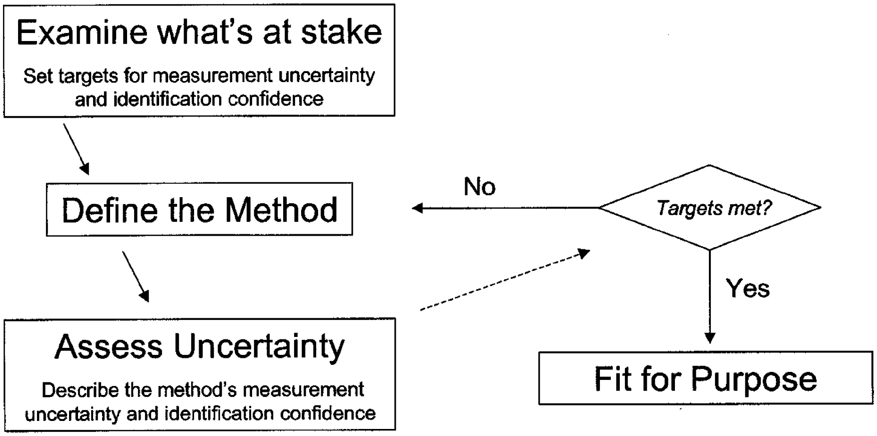

Examine what's at stake
Set targets for measurement uncertainty and identification confidence
Define the Method
Assess Uncertainty
Describe the method's measurement uncertainty and identification confidence
No
Targets met?
Yes
Fit for Purpose

Figure P.2 Outline of a general recommended process for achieving Fitness for Purpose. Reproduced from Bethem *et al.*, *J. Amer. Soc. Mass Spectrom.* **14**, 528 (2003), with permission, copyright (2003) Elsevier.

used to estimate the concentration of the target analyte. The present book is written at a level that presupposes some basic undergraduate-level knowledge of chemistry, physics, and mathematics and statistics.

This book also treats the more recent developments of quantitative analysis of specific proteins in biological systems, even though these hardly qualify as 'small molecules'. However, it does *not* cover the important aspect of analysis of trace level metals by, e.g., ICP–MS; an excellent book covering this subject has appeared recently (Nelms 2005).

It must be emphasized that any book such as this can only be regarded as a preparation for the real learning process in this demanding practical art, namely, exposure to working on real-life problems in a real laboratory. The kinds of measurements that are addressed here really do push the various technologies involved to their current limits, and 'learning by doing' is the only truly meaningful method. This principle is well illustrated by a quotation from what might be described as 'the older literature':

> *Those who are good at archery learnt from the bow and not from Yi the Archer. Those who know how to manage boats learnt from boats and not from Wo (the legendary boatman). Those who can think learned for themselves and not from the Sages.* Kuan Yin Tze, 8th century.

The dangers involved in a sole reliance on 'learning by reading', in a practical discipline like analytical chemistry, are summarized in the following advice:

> *Il ne faut pas laisser les intellectuals jouer avec les allumettes. (Don't let the intellectuals play with the matches).* Jacques Prévert, 1900–1977.

However, it is hoped that reading this book will be useful both in providing enough background information that the first exposure to the 'learning by doing' process will not seem quite so daunting, and also will provide a useful reference thereafter. The book attempts to cover a wide range of subdisciplines, and inevitably some errors of both omission and commission will remain despite extensive checking. The authors would greatly appreciate assistance from colleagues in identifying and correcting these errors.

Robert K. Boyd, Cecilia Basic, Robert A. Bethem
October 2007

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