HPLC METHOD DEVELOPMENT FOR LC/MS COURSE: TRADITIONAL APPROACHES AND EMERGING TRENDS OUTLINE

1. Introduction

- 1.1. Brief History of Coupling HPLC and Mass Spectrometry
  - 1.1.1. Early Pitfalls of LC/MS/MS without Good HPLC Separations
  - 1.1.2. Why is a separation important with the selectivity of MS?
    - 1.1.2.1. Ionization effects in the source of the MS
    - 1.1.2.2. Separation of labile metabolites from parent
    - 1.1.2.3. Separation of isobaric drugs or metabolites

2. Interfacing HPLC with Atmospheric Pressure Ionization

- 2.1. Electrospray Ionization (ESI)
  - 2.1.1. Optimum Operating Conditions
    - 2.1.1.1. Apparent Concentration Sensitivity
- 2.2. Atmospheric Pressure Chemical Ionization (APCI)
  - 2.2.1. Optimum Operating Conditions

3. **Developing HPLC Methods for MS Detection-Traditional Approaches and Emerging Trends**

- 3.1. Define final intended use for method
  - 3.1.1. HPLC/MS Methods in Drug Discovery
  - 3.1.2. HPLC/MS Methods in Drug Development
  - 3.1.3. Qualitative or Quantitative Analysis
- 3.2. Obtain basic chemical information on analytes
  - 3.2.1. Define ionizable groups on the molecules
  - 3.2.2. Classify polarity of the drug
  - 3.2.3. Determine molecular weight of the molecule
- 3.3. Obtain basic information on biological matrices to be analyzed
  - 3.3.1. Matrix contains protein (e.g. plasma or serum)
  - 3.3.2. Matrix does not contain protein (e.g. bile or urine)
  - 3.3.3. Tissues
- 3.4. HPLC Column Chemistry Choice
  - 3.4.1. Reversed-Phase Columns
  - 3.4.2. Normal-Phase Columns
  - 3.4.3. Other Columns
    - 3.4.3.1. Ion-Exchange Columns
    - 3.4.3.2. HILIC Columns
    - 3.4.3.3. Monolithic Columns
    - 3.4.3.4. Polar Embedded Phases
    - 3.4.3.5. Columns for use at high pH and high temperature

3.4.3.6. Sub 2 micron, “UPLC” type separations

3.4.4. On-Line Extractions

3.5. HPLC Column Size Choice

3.5.1. Optimum Length of Column

3.5.2. Optimum Diameter of Column

3.5.2.1. Flow splitting

3.6. Buffer and Solvent Choice for LC/MS

3.6.1. Buffers and solvents for LC/MS

3.6.2. Ion-pair reagents and LC/MS

3.6.3. How to optimize MS signal by choosing the correct additive and solvent

3.7. Choice of Mode of HPLC Separation

3.7.1. Gradient

3.7.2. Isocratic

3.8. Optimization of column temperature for LC/MS

3.9. Column Switching Applications

4. HPLC/MS Methods: Experiments and Results

4.1. Where to start for a typical HPLC/MS method

4.2. What is adequate retention and peak shape?

4.2.1. Calculating retention factors (k’) with LC/MS

4.2.2. Calculating chromatographic resolution (R_s)

4.3. Desired Results and What to Expect

4.3.1. Analysis Time

4.3.2. Accuracy and Precision

4.3.3. Peak shape

4.3.4. Resolution

5. Typical Problems, Solutions and Troubleshooting HPLC for MS Detection

5.1. Matrix Interferences and Ion-Suppression in LC/MS

5.1.1. How to determine the occurrence of ion-suppression

5.1.2. Possible ion-suppression effects in study samples-effects not detected in control samples

5.1.3. How to minimize ion-suppression

5.2. The Transfer and Improvement of HPLC Methods

5.2.1. How to adapt a non-LC/MS method to an LC/MS

5.2.2. Importance of HPLC system dwell volume on transfer and improvement of methods

5.3. How to improve analysis time without sacrificing quality data

5.4. How to improve chromatographic peak shape

5.5. How to improve chromatographic retention

5.6. Simultaneous HPLC/MS analysis of molecules with different properties

5.6.1. Cocktail Analysis

5.6.2. Simultaneous Analysis of Drugs and Metabolites

5.6.3. Analysis of Prodrug and Parent

5.7. LC/MS Survival Kit-Tips and Tricks for What you really need to know

5.7.1. Maximizing MS "uptime" with good HPLC practices

5.7.2. Identifying when something goes wrong

5.7.3. Troubleshooting the problem – Is the problem the HPLC or the MS?

6. Group Problem Solving-Case Studies in LC/MS Method Development

6.1. Dissecting some case studies for specific problems encountered by attendees

6.2. Method development schemes for course attendees own molecules

7. Questions and Answers