

HPLC Method Development for LC

This course will focus on the practical solutions for the tough chromatographic separation and sample clean-up of polar compounds, in particular the small molecules and some oligomers of biological compounds. The course will discuss the mechanism and practical respects of a number of phases used in the HPLC and SPE for polar compounds, including the reverse phases, normal phases, ion-exchange phases and HILIC phases. Based on the understanding of the mechanism and properties of the phases, one may further learn from the course how to select a right HPLC column, a right sample preparation method, as well as how to develop systematically a method for both sample clean-up and HPLC separation in LC or LC-MS applications. A number of new sample preparation methods and new column chemistries will be introduced for the improved separation of polar compounds. A mix-phase strategy will be presented as a tool for a quick separation optimization of complex mixtures, and a tool for retaining polar ionic compounds by HPLC while compatible for LC-MS. The advantages and disadvantages of different SPE methods will also be discussed in term of the convenience, throughputs, effectiveness and robustness, especially for polar compounds.

Instructor:

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1. Introduction

- 1.1 Major issues and technical difficulties related to the separation of polar compounds
- 1.2 Historical technology advances in the chromatographic separation, in particular related to the improvement for polar compounds
- 1.3 Uniqueness of polar compounds and main strategies

2. Chemistry and mechanism of different phases in HPLC and SPE

- 2.1. Mechanism of HPLC in RP, NP, IE and HILIC mode
- 2.2. Chemistry and properties of the HPLC columns on the market
- 2.3. New column chemistry for the separation of polar compounds
- 2.3. Mechanism of SPE in different methodologies
- 2.4. Balanced effort in cleanness and recovery

3. Multi-impurity Adsorption Sample Preparation (MAS) method for polar analytes

- 3.1. Method mechanism and format
- 3.2. Advantages and disadvantages
- 3.3. Application examples
- 3.4. Method development protocols
- 3.5. Practical consideration and trouble shooting

4. Method selection and optimization

- 4.1. How to choose a right HPLC column
- 4.2 Optimization of HPLC separation
 - 4.2.1 improvement of retention
 - 4.2.2 improvement of resolution
 - 4.2.3 improvement of peakshape
 - 4.2.4 automated column switch
 - 4.2.5 optimizing flow and particle size
- 4.3 Compatibility of HPLC method with MS requirements
- 4.4 How to choose a right method for sample preparation
- 4.5. Optimization of the sample preparation method

- 4.5.1. conditioning
- 4.5.2. sample preparation and loading
- 4.5.3. rinsing
- 4.5.4. eluting
- 4.5.5. recovery and consistency
- 4.5.6. method validation

5. Mix-phase strategy for the optimization of the separation

- 5.1. Mix-phase HPLC columns
- 5.2. Mechanism to alter the selectivity by mix-phase
- 5.3. Optimizing the complex separation by Mix-phase
- 5.4. Simultaneous analysis of polar and non-polar compounds by mix-phase
- 5.5. Application examples

6. Case Studies

- 6.1 Cases presented by instructors
- 6.2. Cases given by attendees

7. Questions and Answers