Method Development: a Guide to Basics

This procedure describes the basic principles of developing a method. It includes sections on:

Method definition

A method is a set of experimental conditions designed to create a good analysis of a particular sample.

Developing a method

Method development encompasses many stages and can take months to complete, depending on the complexity and goals of the method. The process usually includes the following steps:

1. *Understand the sample*. What are its composition and properties - particularly the properties likely to be affected by the analysis? Table 1 shows some of the sample properties that you need to know before developing a method.

Table 1: Things to know about the sample

Sample property	Details
Matrix	What compounds other than the analyte are in the sample?
Concentration	• How much of the compound is present in the sample? • What is the concentration range (low or high)?
Quantity	How many compounds are present in the sample?
Chemical/physical properties	• pK_a values • molecular size and weight • electrical charge • sample solubility • sample volatility • stability and toxicity • hydrophobicity/polarity • chemical/biological reactivity • UV spectra

2. *Define the method goals* (Table 2). What do you want to get from the sample (i.e., why are you analyzing it)?

   NOTE: *This step is often overlooked but is critical to success.*

Table 2: Method goals

Method goal	Details
Detect	Is the compound present?
Quantitate	How much of the compound is present?
Identify	What is the compound?
Characterize	What are the compound properties?
Purify/isolate	Do you want to collect the compound for further use?

3. *Determine the analysis requirements* (see Table 3). These are the variables associated with the method goals. What do you need to do or know in order to reach your method goals?

Table 3: Analysis requirements

Method goal	Analysis requirement
Detection	• What detection technique can be used to analyze the sample? Is it UV absorbing, can you ionize it, does it have observable thermal characteristics, etc.
Quantitation	• How will you quantify (e.g., internal standard, external standard, absolute detection)? • What is your concentration range or sample amount (low or high)? • How many samples do you need? • What levels of accuracy and precision are required?
Identification	• How will you identify the compound (e.g., what detection technique will you use)? • How will you determine purity (e.g., UV spectral purity, percent area)?
Characterization	What properties or property levels do you need to determine?
Purification/isolation	• Do you want to isolate purified material? • Do you need to recover 100% of your sample?
Sample matrix	Is there more than one sample matrix before analysis? Will the sample matrix interfere with your analysis?
Properties	Does he analysis technique allow you to determine sample properties?

4. *Conduct research* to determine if the analysis has been performed before. Previously developed methods with quantitation and sample matrices that are close to your requirements can form a starting point for your method. Resources to consult include:
   a. Internet
   b. United States Pharmocopeia (USP)
   c. FDA requirements
   d. EPA requirements
   e. USDA methods
   f. Colleagues
   g. Professional/technical journals and meetings
   h. Corporate application notes
5. *Select the analysis technique* (see Table 4). What type of analysis will provide the information identified in the previous steps?

Table 4: Analysis techniques

Analysis technique[a]	Capabilities
Mass spectrometry (MS)	Measures mass-to-charge ratio of charged particles
Liquid chromatography (LC)	Separates samples in solution based on physical properties such as polarity, ionic strength, and molecular size.
Liquid chromatography-mass spectrometry (LC-MS)	Combines the physical separation capabilities of LC with the mass analysis capabilities of MS
Thermal analysis	Analyzes materials according to the way they change with temperature

a. Offered by Waters Corporation.

6. *Determine initial conditions*. For example, for LC you would select a detector, column, and mobile phase.
7. *Prepare the sample.* For example, for LC select the sample solvent and the proper sample preparation procedures.
8. *Develop the method*, using one of the following approaches:
   a. stepwise incremental (one-factor-at-a-time) approach based on results from previous experiment
   b. systematic screening protocol, in which you evaluate factors such as stationary phases, solvents, and pH, and column chemistry to fine-tune selectivity and retention and thereby enhance resolution.
9. *Select a standardization technique*, if required, such as an internal or external standard. For example, a PDA detector can be used to investigate the linearity of the active pharmaceutical ingredient and related substances in the proposed concentration range.

10. *Check overall performance* of the analysis technique. Performance requirements can include variables such as accuracy, precision, reproducibility, linearity, limits of detection, limits of quantitation.
11. *Verify method optimization and robustness*. Use an experimental design approach to determine the experimental factors that have significant impact on the method:
    a. HPLC conditions: % organic, pH, flow rate, temperature, wavelength, column age.
    b. MS conditions: ionization conditions, mass separation conditions
    c. Sample preparation: e.g., % organic, pH, shaking/sonication, sample size, sample age.
    d. Calculation/standardization: e.g., integration, wavelength, standard concentration, response factor correction.
12. *Validate the method.* The goal is to demonstrate that results from the method performance will not be significantly impacted by slight variations of the method conditions.

Troubleshooting method problems

An incorrect method can lead to poor analysis results, which appears as:

- LC: baseline problems, retention, peaks, sensitivity results
- MS: sensitivity, mass accuracy/mass measure, mass resolution
- Thermal: sensitivity results, inaccurate results

For more information on adjusting method conditions to solve these and other analysis problems, go to Troubleshooting Information in the Portal and select the troubleshooting guide relevant to your system.

References

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