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HPLC Method development

Strategy for Method Development

Steps in a common strategy for HPLC method development are summarized below:

1. Define method and separation goals

2. Gather sample and analyte information

3. Initial method development — “scouting” runs and getting the first chromatograms

4. Method fine-tuning and optimization

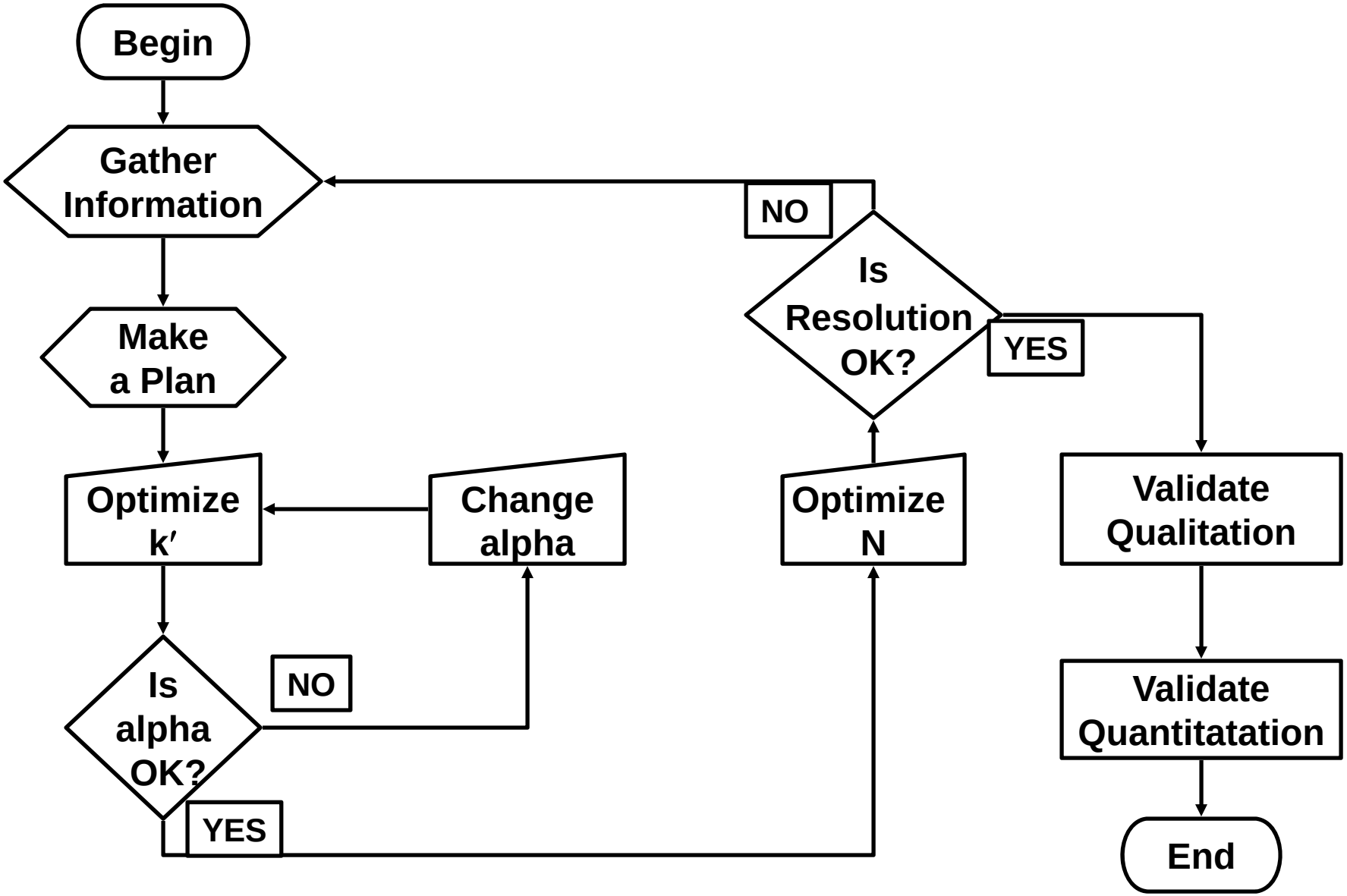
5. Method validation

HPLC Method Development Trends in Pharmaceutical Analysis

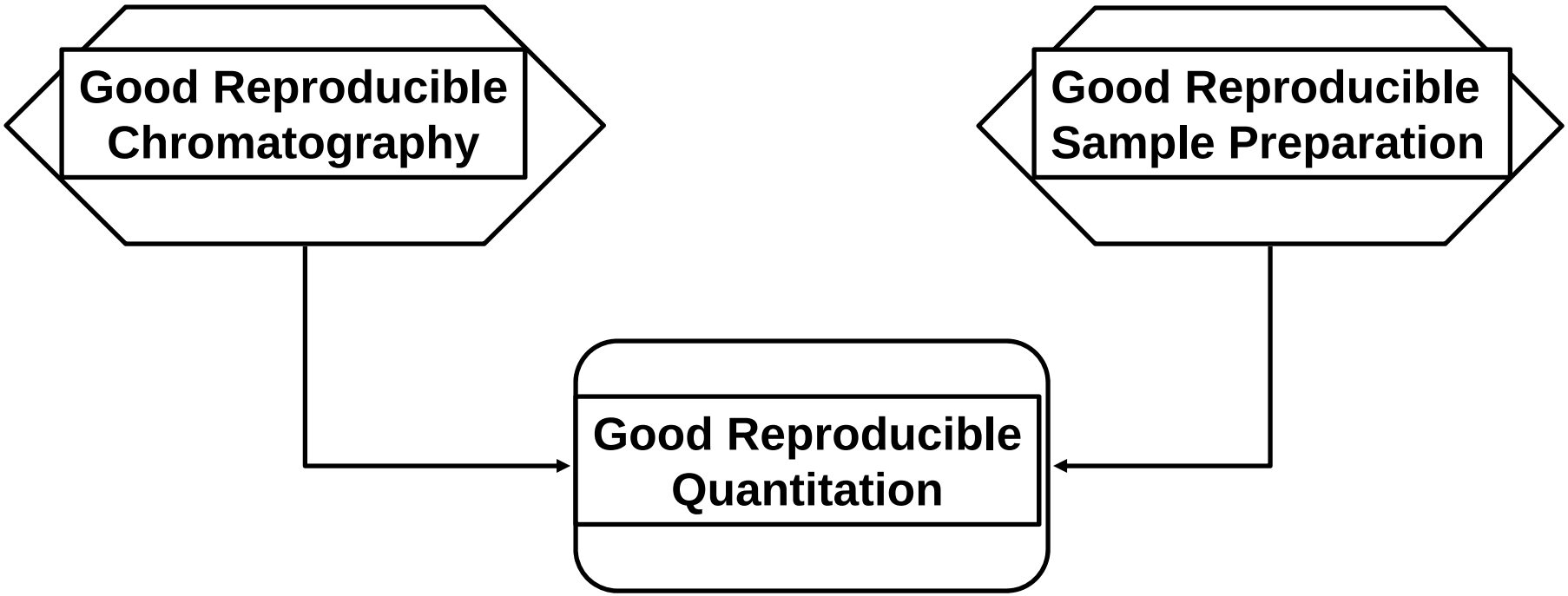
Some of the “best practices” and emergent trends in pharmaceutical method development are highlighted here:

- Modern instrumental trends for method development include:
  - — Multi-solvent pumps, photodiode array detectors (PDA), modeling software, automated development systems, and multi-column selector valves
- Trends toward MS-compatible, gradient methods for impurity testing
- Composite methods (combined assay/impurity) during early development
- Phase-appropriate method development and validation
- Use of secondary orthogonal methods to ensure separation of all impurities and unexpected unknowns
- A single method for products of all different strengths and formulations
- LC/MS/MS methods for trace analysis (e.g., impurity profiling)

Methods Development Strategy



Quantitation by HPLC



INITIAL HPLC METHOD DEVELOPMENT

During initial method development, a set of initial conditions (detector, column, mobile phase) is selected to obtain the first “scouting” chromatograms of the sample.

In most cases, these are based on reversed-phase separations on a C18 column with UV detection.

A decision on developing either an isocratic or a gradient method should be made at this point.

Initial Detector Selection

For analytes having reasonable UV absorbance, the UV/Vis detector (or the PDA detector) is the clear choice. UV/Vis detectors are reliable, sensitive, easy-to-use, and very precise.

For nonchromophoric analytes (with low or no absorbance in UV-Vis range), the choice is limited to refractive index (RI) detector or evaporative light scattering detector (ELSD) for gradient analysis.

Mass spectrometry (MS) is a possible choice for “ionizable” analytes. Although MS (or MS/MS) is the standard detector for bioanalytical assays and drug discovery screening, its use for routine assays of drug substances and products is still limited due to its high cost and lower precision.

Other detection options include conductivity detection for ionic species and electrochemical detection for neuroactive species in biochemical research.

Selection of Chromatographic Mode

Sample type	Analytes type	Common mode
Macromolecules MW > 2,000 Organics (MW < 2,000)	Organic polymers Bio-molecules Polar Medium Polarity Non Polar Ions, ionizable compounds	GPC SEC, RPC, IEC, HILIC, HIC RPC, NP, HILIC RPC RPC, NARP, NP RPC (ion suppression), RPC-IP, IEC, HILIC
Preparative	All	NP, RPC, GPC, IEC

Initial Selection of HPLC Column

Although there is a wide choice of RPC-bonded phases on the market, a silica- based C18 or C8 column remains a good starting point because of its high efficiency and stability.

Additional guidelines for initial column selection follow:

- Select columns packed with 3- or 5-µm high-purity silica-bonded phases.

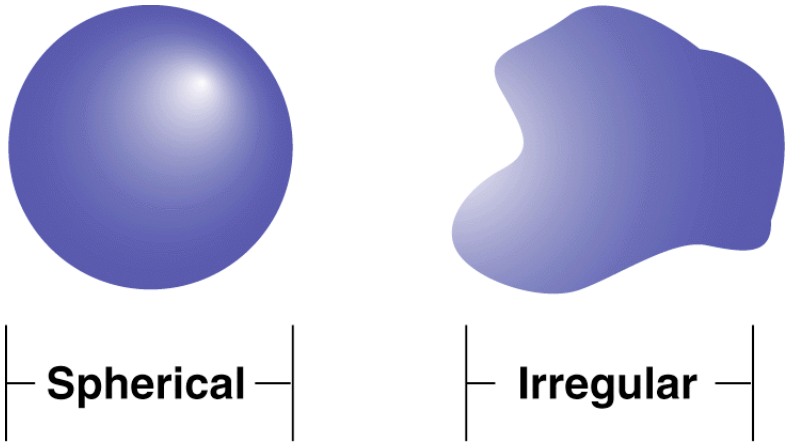
- Three-micrometer packing is probably preferable due to its faster analysis since shorter column length can be used.

- The following column dimensions are suggested:
  - 50–100mm × 4.6 mm i.d. for simple samples (e.g., assays of the main component)
  - 100–150mm × 3.0–4.6mm i.d. for purity testing or multi-component testing of complex samples
  - 20–150mm × 2.0mm columns for LC/MS

Particle Shape

Effect on chromatography

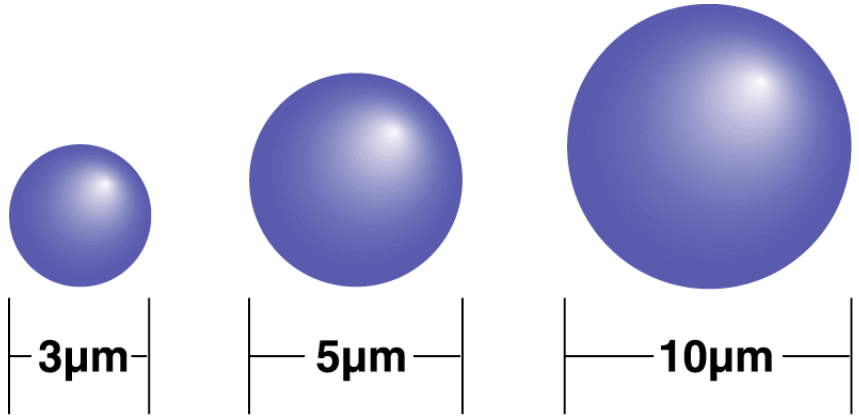
Spherical particles offer reduced back pressures and longer column life when using viscous mobile phases like 50:50 MeOH:H_2O.



Particle Size

Effect on chromatography

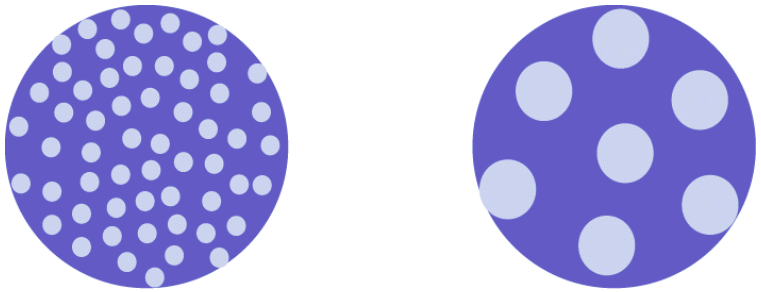
- Smaller particles offer higher efficiency, but also cause higher backpressure.
- Choose 3µm particles for resolving complex, multi-component samples. Otherwise, choose 5 or 10µm packing.



Surface Area

Effect on chromatography

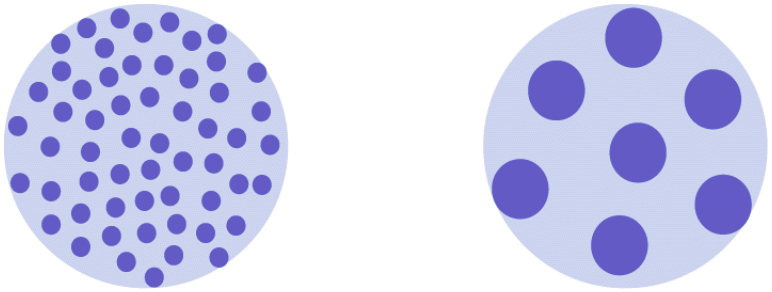
- High surface area generally provides greater retention, capacity and resolution for separating complex, multi-component samples. Low surface area packings generally equilibrate quickly, especially important in gradient analyses.



Pore Size

Effect on chromatography

- Larger pores allow larger solute molecules to be retained longer through maximum exposure to the surface area of the particles. Choose a pore size of 150Å or less for sample MW $\leq$ 2000. Choose a pore size of 300Å or greater for sample MW > 2000.



Summary of Factors Optimized During RPLC Method Development

Stage/approaches	Factor	Suggestions and comments
Initial development		
Sequential isocratic steps or scouting gradients	Column	Modern columns packed with C8 or C18 phases of 3- or 5-μm high purity-silica support
	Detection wavelength	Use PDA to evaluate λmax of all analytes. Select λmax of main component or far-UV wavelength (200–230nm).
	Mobile phase	MPA: Water for neutral analytes or acidified at pH 2–4 for acidic or basic analytes MPB: MeOH or CAN

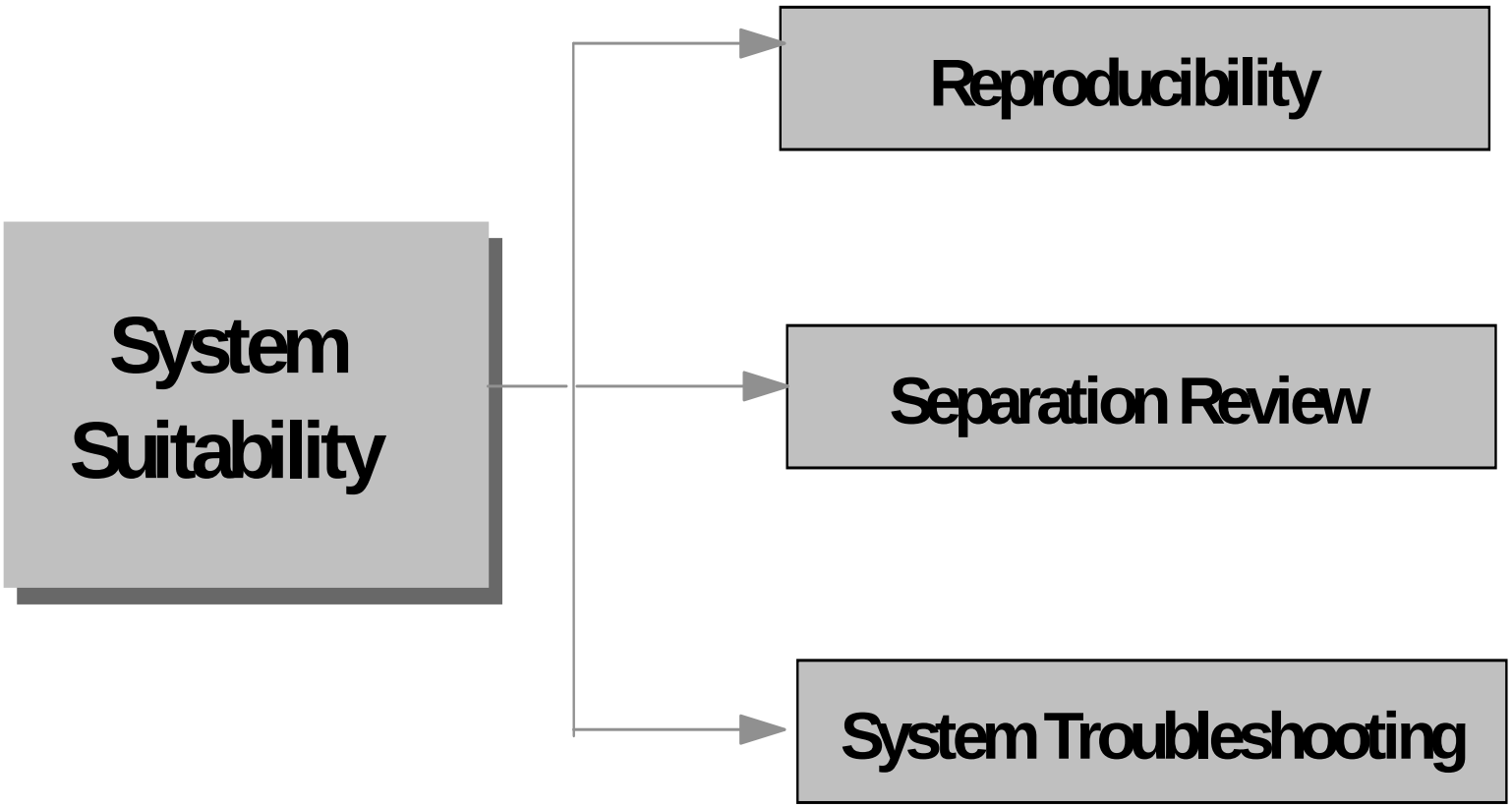
Summary of Factors Optimized During RPLC Method Development

Stage/approaches	Factor	Suggestions and comments
Method development and optimization		
Isocratic or gradient methods	Mobile phase and operating conditions	Evaluate method with impurity "cocktail" and placebo. Modify solvent type, pH, T, and tG to improve resolution

Summary of Factors Optimized During RPLC Method Development

Stage/approaches	Factor	Suggestions and comments
Method fine-tuning and finalization		
Isocratic or gradient method	Column	Evaluate method with different bonded phases Optimize column dimension. (DryLab recommended)
	Detector and sample	Finalize detection wavelength and sample concentration or injection volume

System Suitability



System Suitability

- System Suitability
  - The checking of a system, before or during analysis of unknowns, to insure system performance.
  - Plate Count, Tailing, Resolution
  - Determination of reproducibility (%RSD)
- System Suitability "Sample"
  - A mixture of main components and expected by-products utilized to determine system suitability

System Suitability

System suitability:

- Separation
  - Trending of separation criteria such as resolution, tailing and plate count, to insure the system is within specification.
- Reproducibility
  - Summary of standard analyses to insure the system is within specified reproducibility guidelines.