HPLC and UHPLC

A Helpful Guide To Modernizing Your Old HPLC Methods

Modernizing older or compendial methods can help increase your lab's productivity and lower your cost and carbon footprint. This guide gives you an overview of what method parameters to consider changing, as defined by USP 621.

1. HPLC to UHPLC

Switching from HPLC to UHPLC offers you better resolution and faster run times.

2. Column particle size

Using columns with smaller particles (<2 µm fully porous particles) can improve your separation efficiency and speed at the cost of increasing backpressure. Superficially porous particles offer you "sub-2 µm efficiency" without the same increase in backpressure.

3. Column length and inner diameter

Moving to shorter columns can increase your sample throughput with trade-offs in resolution, while narrower ID columns increase sensitivity and decrease sample and solvent consumption. To maintain resolution, you can use shorter columns with smaller particles.

4. LC system backpressure

Pressure scales linearly with flow rate and is inversely proportional to the column inner diameter squared (halving column diameter increases backpressure 4x). Ensure your instrument can meet the pressure demands of your new method.

5. Column temperature

Increasing column temperature decreases mobile phase viscosity (backpressure), which can increase separation efficiency and decrease your analysis time, in addition to supporting smaller particles and narrower columns.

6. Flow rates

When changing column particle size and/or ID, you'll want to adjust the flow rate to achieve optimal separation efficiency.

7. Injection volume

When decreasing column diameter, you must alter injection volume accordingly because volume overloading can broaden peaks and lower resolution.

8. Detector settings

Changing from HPLC to UHPLC (smaller particles and column diameter) reduces peak volume and width, so you'll need to adjust the detector data collection rate and time constant to maintain ~30 data points per peak.

9. Extra column volumes

The influence of extra column volumes on your separation efficiency becomes more prominent as column volume decreases. Use suitable capillary dimensions and low-volume connections to maximize the benefits of adopting UHPLC methods.

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