Quality control analytical methods- Switch from HPLC to UPLC

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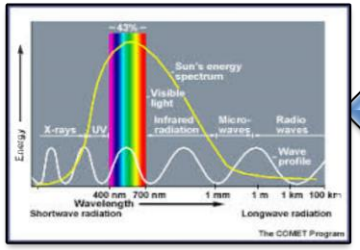
Outline of Talk

- Analytical techniques in QC
- Introduction to HPLC
- UPLC
  - Principles
  - Advantages of UPLC
  - Considerations in converting HPLC methods to UPLC
- Conclusion

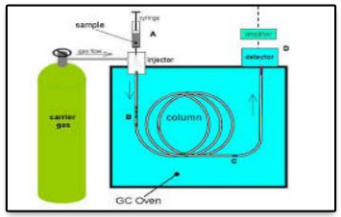
Quality control is an essential operation of the pharmaceutical industry. Drugs must be marketed as safe and therapeutically active formulations whose performance is consistent and predictable.

Various analytical techniques used in quality control of pharmaceuticals are …..

- Titrimetric techniques
- Thin layer chromatography,
- High performance thin layer chromatography
- High-performance liquid chromatography (HPLC)
- Gas chromatography
- Spectrophotometry
- Near infrared spectroscopy (NIRS)
- Fluorimetry and phosphorimetry
- Nuclear magnetic resonance spectroscopy (NMR)
- Electrochemical methods
- Hyphenated techniques



Spectrophotometry methods



Gas chromatography

HPLC

High performance liquid chromatography (HPLC) is a well known technique that has been used in laboratories worldwide from more than last 30 years.

Ultra performance LC

- A new class of separation science based on chromatography columns with very small particles introduced in 2004.
- Provides improved resolution, speed and sensitivity with no comprises
- Suitable for chromatographic applications
    - Appropriate for developing new methods
    - Appropriate for improving existing methods

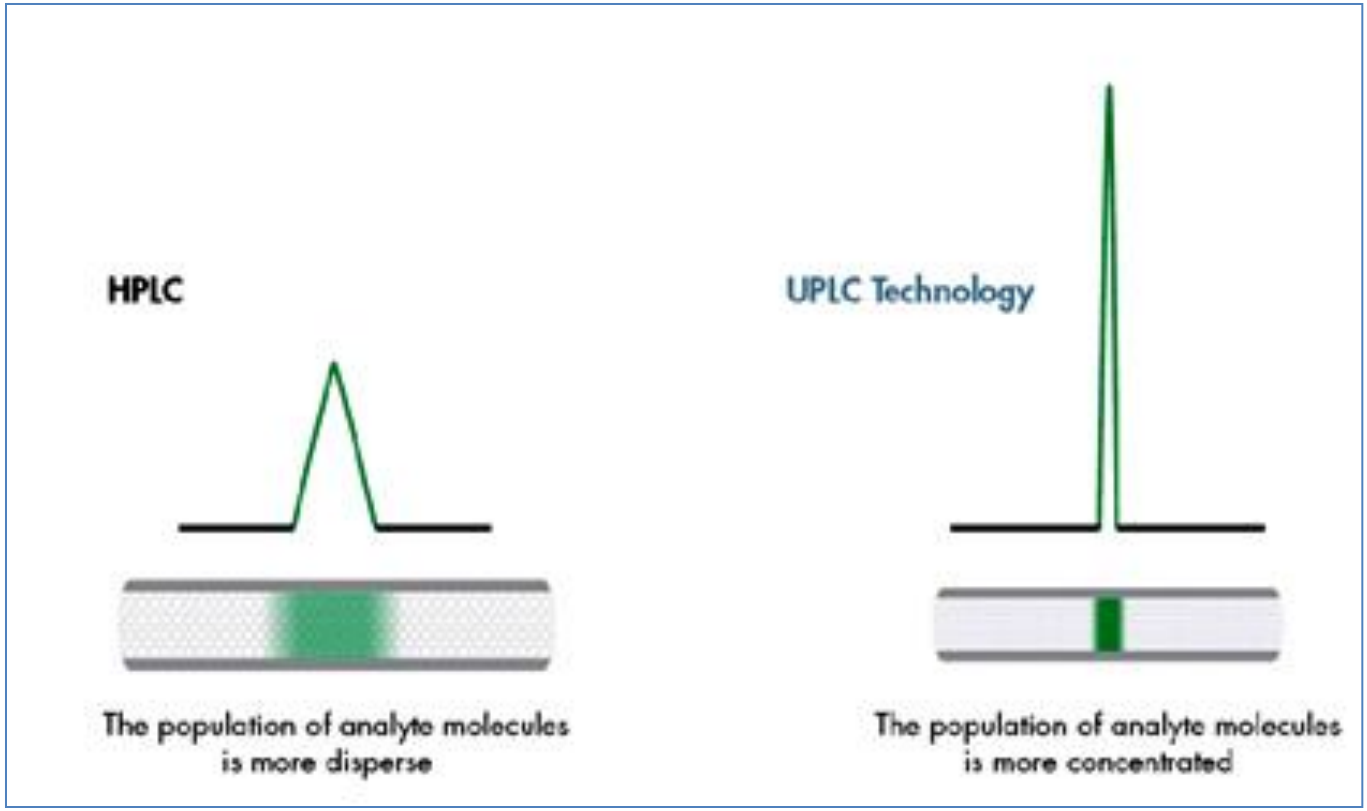
- UPLC is a specialized chromatographic method that runs faster, resolves better and uses less solvent than its cousin, HPLC.
- Researchers in biochemistry, cell and molecular biology, clinical medicine and many other areas rely on UHPLC to separate different types of molecules from a mixture—whether these molecules are proteins, peptides, metabolites, pharmaceutical compounds or other chemicals.

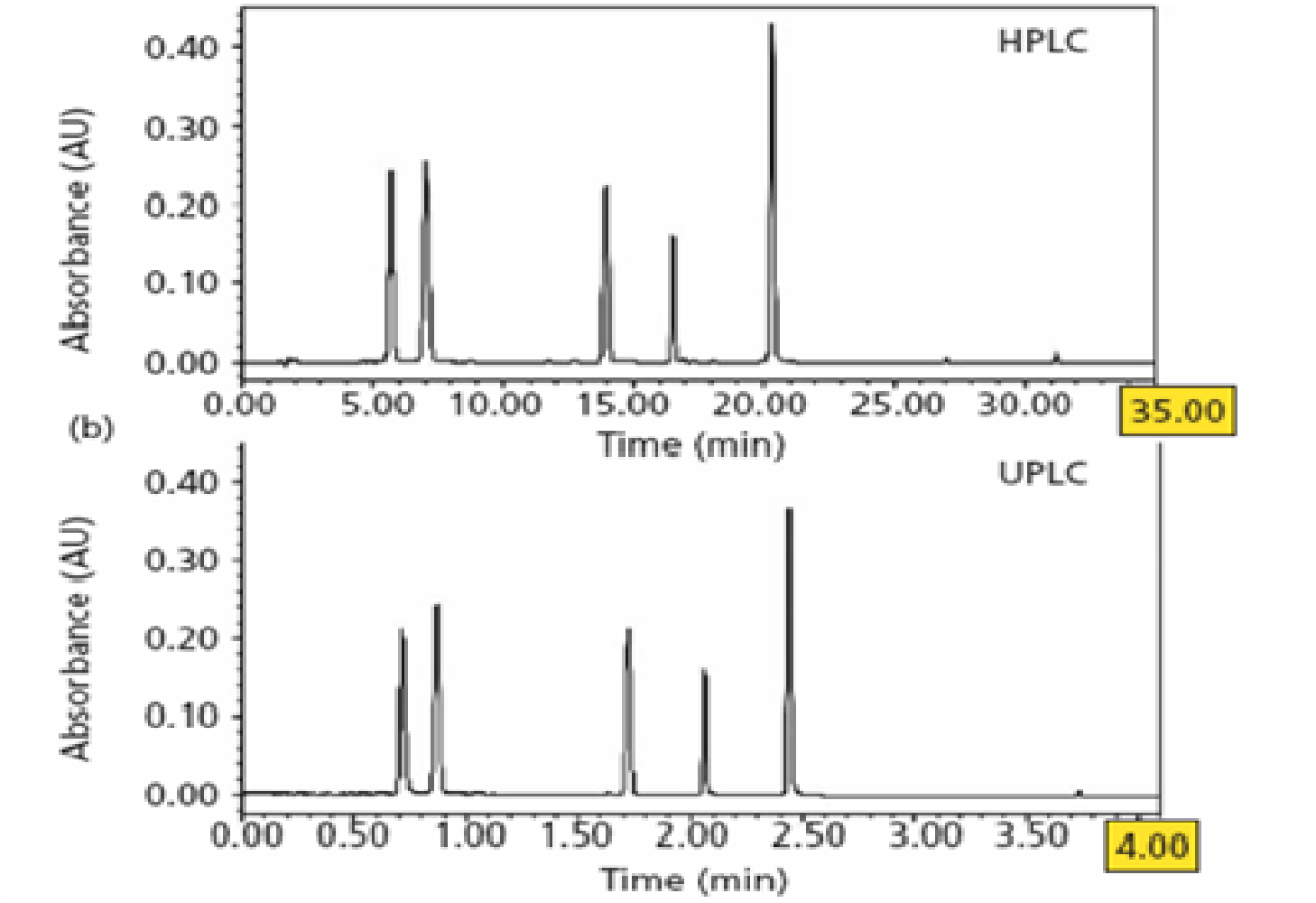
HPLC and UPLC technologies are in use today throughout QC, QA, and manufacturing facilities around the world for process development, in-process analysis, API release testing, drug product release testing, stability monitoring programs and more.

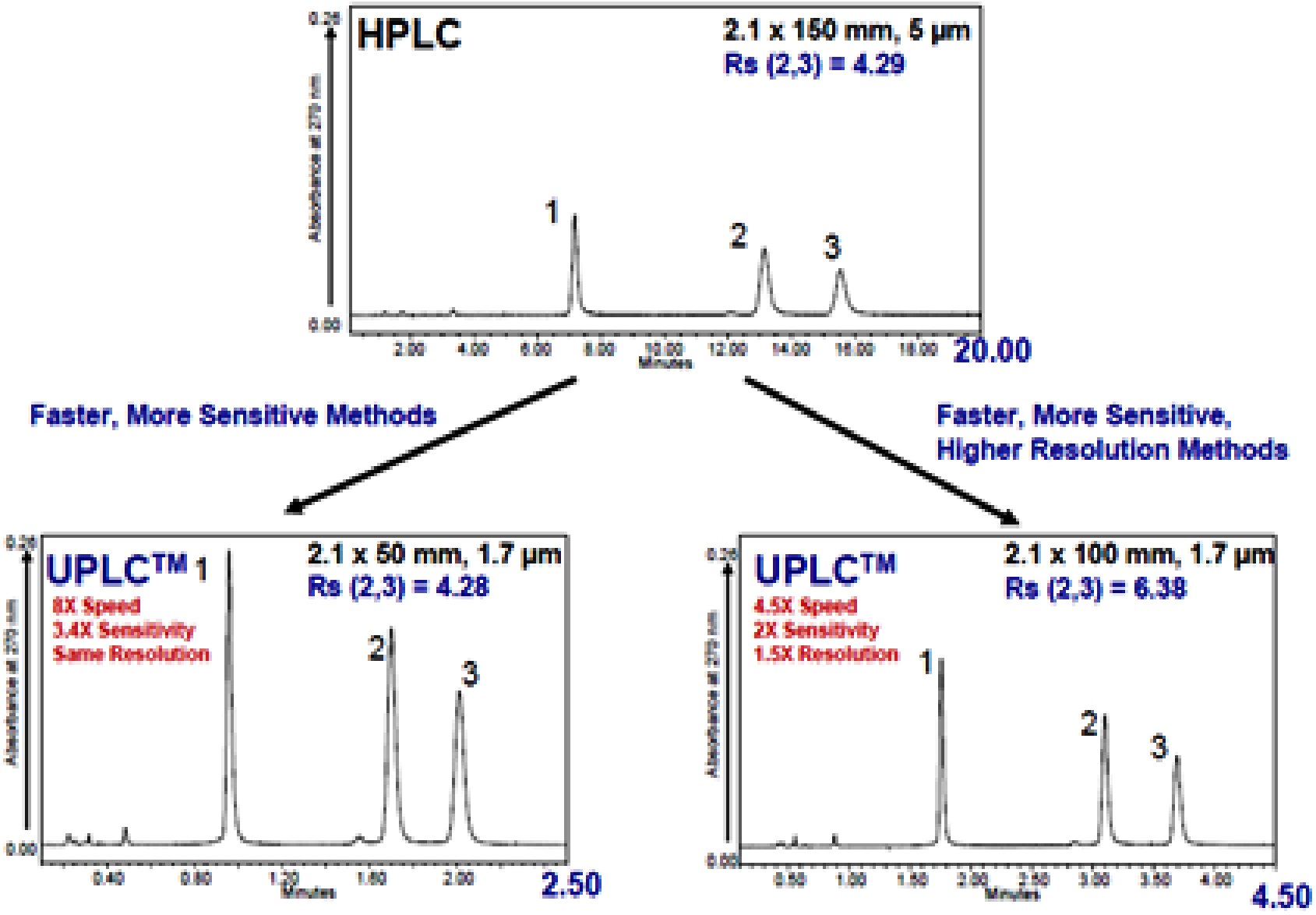
Why UPLC?

Advantages of UPLC over HPLC

- Resolution
- Run time
- Solvent consumption


HPLC
UPLC Technology
The population of analyte molecules is more disperse
The population of analyte molecules is more concentrated


HPLC
Absorbance (AU)
0.40
0.30
0.20
0.10
0.00
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
Time (min)
(b)
UPLC
Absorbance (AU)
0.40
0.30
0.20
0.10
0.00
0.00
0.50
1.00
1.50
2.00
2.50
3.00
3.50
4.00
Time (min)


HPLC
2.1 x 150 mm, 5 µm
Rs (2,3) = 4.29
1
2
3
20.00
Faster, More Sensitive Methods
Faster, More Sensitive, Higher Resolution Methods
UPLC™ 1
8X Speed
3.4X Sensitivity
Same Resolution
2.1 x 50 mm, 1.7 µm
Rs (2,3) = 4.28
2.50
UPLC™
4.5X Speed
2X Sensitivity
1.5X Resolution
2.1 x 100 mm, 1.7 µm
Rs (2,3) = 6.38
4.50

What is The KEY to UPLC® Separations?

1. Small Particles
2. High Backpressures
3. Expensive Instrumentation
4. Low Band Spreading

Why to convert HPLC to UPLC methods….

- Get faster results with more resolution

  More information

  More robust methods

  More samples analyzed per system, per scientist

Increased productivity

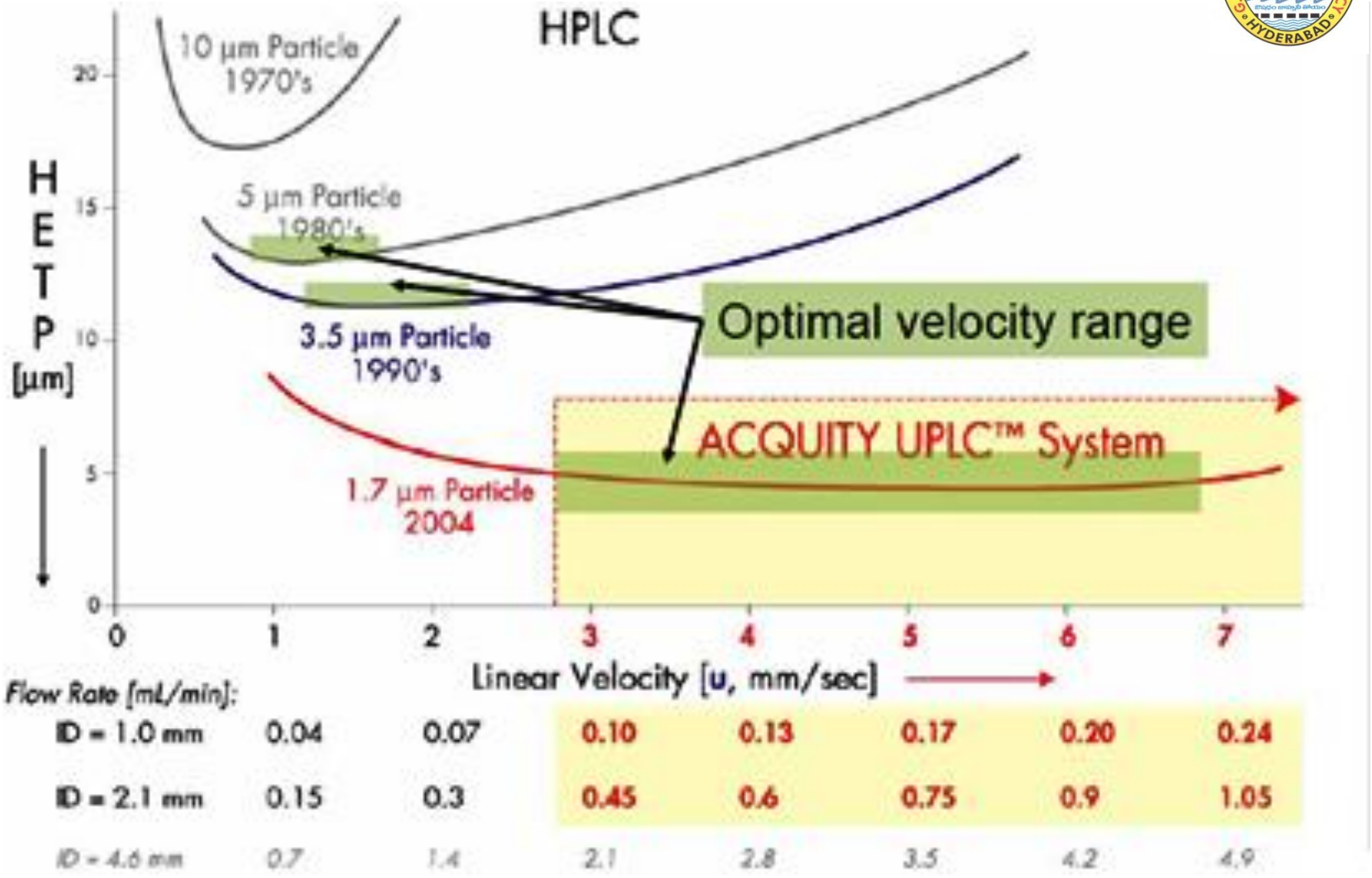
What is involved in converting from HPLC to UPLC?

- Time
- UPLC hardware
- Method conditions

Method conversion process….

- Refer to current LC method
  - Observe system volumes, solvents and detection technique
  - Define objectives for improvement
- Select column dimensions
- Scale injection volume to column dimensions
- Use gradient and flow rate scaled from current method
- Use a gradient and flow rate scaled for UPLC

Van Deempters plot



Determination of column dimensions

- Internal Diameter

  -Generally prefer 2.1 mm

  -Only use 1mm for specific reason

- Length

  - If primary goal is speed

    50 mm length to start

  -If primary goal is resolution

    100 mm length to start

HPLC 4.6 x 150mm

20µL injection/2.49mL = 0.8%

UPLC

2.1 x 50mm

20µL injection/0.19mL = 11%

Sample volume too large for smaller column volume

- Geometrically scale injection volume to column volume
- Capacity proportional to surface area and internal solvent volume
- Suggested minimum injection volume on the instrument is 0.5 – 1.0 µL
  - If calculated injection volume is too small for injection, dilute 5-10X with initial strength mobile phase
  - Typically 5 µL maximum injection volume on 2.1x50 mm

Target injection volume =

$$\text{Original injection volume} \times \frac{\text{Target Column Volume}}{\text{Original Column Volum}}$$

Scaling a **10µL** injection on 4.6 x 150mm to 2.1 x 50mm

$$\mathbf{10\mu L} \times \frac{3.14 \times 1.1^2 \times 50}{3.14 \times 2.3^2 \times 150} =$$

$$\mathbf{10\mu L} \times \frac{0.19}{2.49} = \mathbf{10\mu L} \times 0.076$$

$$= \mathbf{0.8\mu L}$$

- Adjust flow rate proportional to column diameter squared for constant linear velocity(geometrically scaled)
- Adjust gradient table to maintain the same number of column volumes of solvent through the target column
- Finally adjust the flow rate(linear velocity) for smaller particles

Scaling a **1.5mL/min** flow rate on 4.6x150mm to 2.1x50mm*

$$\text{Target Flow Rate} = \text{Original Flow Rate} \times \frac{\pi \times r^2 \text{ of Target}}{\pi \times r^2 \text{ of Original}}$$

This reduces to:

$$\text{Target Flow Rate} = \text{Original Flow Rate} \times \frac{d^2_{\text{Target}}}{d^2_{\text{Original}}}$$

So:

$$1.5\text{mL/min.} \times \frac{2.1^2}{4.6^2} = 0.31\text{mL/min.}$$

The Method Translator can help you adapt your old HPLC protocol into a newer UPLC protocol. The Cost Savings Calculator estimates the savings you may derive from using an UPLC instrument vs. a generic “conventional liquid chromatography” system.

Conclusion

UPLC Technology can provide tremendous benefits to routine testing of generic drug products, including increased sample throughput and decreased solvent consumption. This offers significant cost benefits associated with running more samples in less time, and can decrease the overall operating expenses in development and quality control laboratories.