

VisionHT™ Media Platform — Method Transfer Guidelines

Physics of Method Transfer

When transferring your assay from one format to another there are some practical considerations that should be taken in account to maintain equivalent run conditions.

Column Length

$$L_2 = \frac{L_1 \times P_2}{P_1}$$

L = Length

P = Particle Size

$$45\text{mm} = \frac{150\text{mm} \times 1.5\mu\text{m}}{5\mu\text{m}}$$

Decreasing the particle size will increase the theoretical plate count of the column as well as the back pressure. By adjusting the column length we can maintain the separation.

Column Volume

$$F_2 = \frac{[D_2]^2 \times F_1}{[D_1]^2}$$

F = Flow

D = Diameter

$$0.208 = \frac{2.1^2 \times 1.0\text{mL/min}}{4.6^2}$$

The flow rate must be adjusted to maintain the linear velocity of the mobile phase traveling through the column, which is essential to maintain peak efficiency and decrease associated backpressure.

Sample

$$V_1 = V_2 \times \frac{D_2^2 \times L_2}{D_1^2 \times L_1}$$

V = Volume

D = Diameter

L = Length

$$0.69\mu\text{l} = 10 \times \frac{2.1^2 \times 50}{4.6^2 \times 150}$$

Decreases in column length and diameter lead to a decrease in column capacity. Decreasing the sample loading prevents column overload. Sample matrix effects will be more pronounced so that matching the sample solvent to the mobile phase is critical.

Matching Performance from Different Size Columns

When converting your methods or application to a shorter column to save time, you must also reduce the particle size to increase efficiency. To get the equivalent performance, match the efficiency from your current dimension/particle size to the shorter column with a smaller particle size.

Column Efficiency as a Function of Particle Size

Plates/m	10u/40,000	5u/80,000	3u/133,333	1.5u/266,667
Column Length	Plates/Column*	Plates/Column*	Plates/Column*	Plates/Column*
250mm	10,000	20,000	33,333	66,667
150mm	6,000	12,000	20,000	40,000
100mm	4,000	8,000	13,333	26,667
50mm	2,000	4,000	6,667	13,334
33mm	1,320	2,640	4,400	8,800
20mm	800	1,600	2,666	5,333
10mm	400	800	1,333	2,667

*Theoretical plates/m vs Theoretical plates/column, Reduced plate height of 2.5

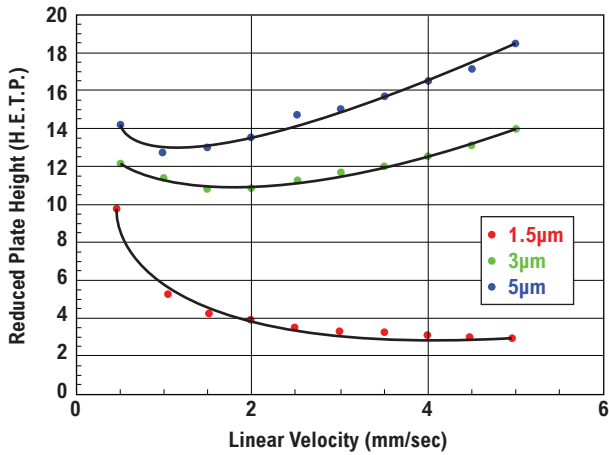
Required Column Efficiency as a Function of Selectivity (for Rs = 1.5, k' = 2)

Selectivity	Column Efficiency
1.5	324
1.3	900
1.2	2025
1.1	8100
1.05	34200

Column Combinations — Same Efficiency (for Rs = 1.9, k' = 5, and α = 1.1)

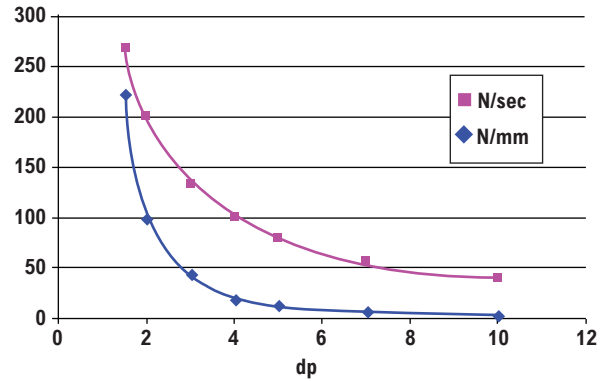
h = 2.5 Defined						
Plates/col Available	Length (mm)	i.d. (mm)	Particle Size (µm)	Run Time (k' = 6 mins)	Flow (mL/min)	Pressure Drop (Based on 1 psig)
20,000	250	4.6	5	15	1	1600
12,000	150	4.6	5	9	1	960
13,333	100	4.6	3	6	1	1778
10,000	50	4.6	2	3	1	2000
13,333	50	4.6	1.5	3	1	3556
12,000	150	4.6	5	4.5	2	1920
13,333	100	4.6	3	3	2	3556
10,000	50	4.6	2	1.5	2	4000
13,333	50	4.6	1.5	1.5	2	7111
13,333	50	4.6	1.5	1	3	10667
13,333	50	2	1.5	1	0.57	10667
13,333	50	1	1.5	1	0.14	10667

Van Deemter Comparison



Reducing particle size extends optimal linear velocities allowing faster run times without reducing efficiency.

Efficiency as a Result of Particle Size



Rapid increase in both plates / mm of column length and plates generated /second at low particle size.

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