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# Optimizing Older UHPLC Systems for Use with New, Smaller ID Columns

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Analytical Solutions to Analytical Problems

# Background/Introduction

- The original UHPLC systems, now more than 15 years old, were designed for use with the smaller columns and particles that were just being developed during that period. At that time, the most common small-bore columns were 2.1 mm internal diameter with lengths of 50 – 100 mm. Many successful applications were developed with this combination of instrument and column technologies.
- Interest in even smaller-bore columns (e.g., 1.0 mm ID) has grown, in recent years, to offer even more solvent savings and an improvement in sensitivity (peak height) if injection volumes are held constant. However, these columns have proven to be challenging for most users to achieve expected results due to extra-column effects on many UHPLC instruments. The recent introduction of 1.5 mm ID columns has heightened interest in small-bore columns because their dimensions may allow use with an older UHPLC, if the instrument can be modified to reduce extra-column volume.

# Theory/Purpose

- These operating parameters are easily within the range of even the original UHPLC systems.  In contrast, the microbore (1 mm ID) columns require operating conditions that are much more challenging for a typical UHPLC system.

# Reference Column Conditions

- Recommended operating conditions can be calculated from a reference column configuration

| Type | Diameter, mm | Length, mm | Particle Size, um | Flow, mL/min. | Inj. Vol., uL | Void Volume, mL | Void Time, min. | N | $V_P$ , uL (k=2) |
|---|---|---|---|---|---|---|---|---|---|
| FPP | 4.6 | 250 | 5.0 | 1.00 | 20 | 2.70 | 2.70 | 25000 | 205 |
| SPP | 4.6 | 250 | 5.0 | 1.00 | 20 | 2.08 | 2.08 | 25000 | 157 |

# Calculated Conditions

- Calculated Operating Conditions for Modern Columns on an Original UHPLC

| Type | Diameter, mm | Length, mm | Particle Size, um | Flow, mL/min. | Inj. Vol., uL | Void Volume, mL | Void Time, min. | N | $V_P$ , uL (k=2) |
|---|---|---|---|---|---|---|---|---|---|
| FPP | 4.6 | 100 | 3 | 1.67 | 8.00 | 1.080 | 0.648 | 16667 | 100.4 |
| | 3.0 | 100 | 3 | 0.71 | 3.40 | 0.459 | 0.648 | 16667 | 42.7 |
| | 2.1 | 100 | 3 | 0.35 | 1.67 | 0.225 | 0.648 | 16667 | 20.9 |
| | 1.5 | 100 | 3 | 0.18 | 0.85 | 0.115 | 0.648 | 16667 | 10.7 |
| | 1.0 | 100 | 3 | 0.08 | 0.38 | 0.051 | 0.648 | 16667 | 4.7 |
| SPP | 4.6 | 100 | 2.7 | 1.85 | 8.00 | 0.831 | 0.449 | 18519 | 73.3 |
| | 3.0 | 100 | 2.7 | 0.79 | 3.40 | 0.353 | 0.449 | 18519 | 31.2 |
| | 2.1 | 100 | 2.7 | 0.39 | 1.67 | 0.173 | 0.449 | 18519 | 15.3 |
| | 1.5 | 100 | 2.7 | 0.20 | 0.85 | 0.088 | 0.449 | 18519 | 7.8 |
| | 1.0 | 100 | 2.7 | 0.09 | 0.38 | 0.039 | 0.449 | 18519 | 3.5 |

Values calculated relative to reference column to maintain constant linear velocity and other operating parameters. Assuming k = 2 and $h_r$ = 2.

Performance may be affected.

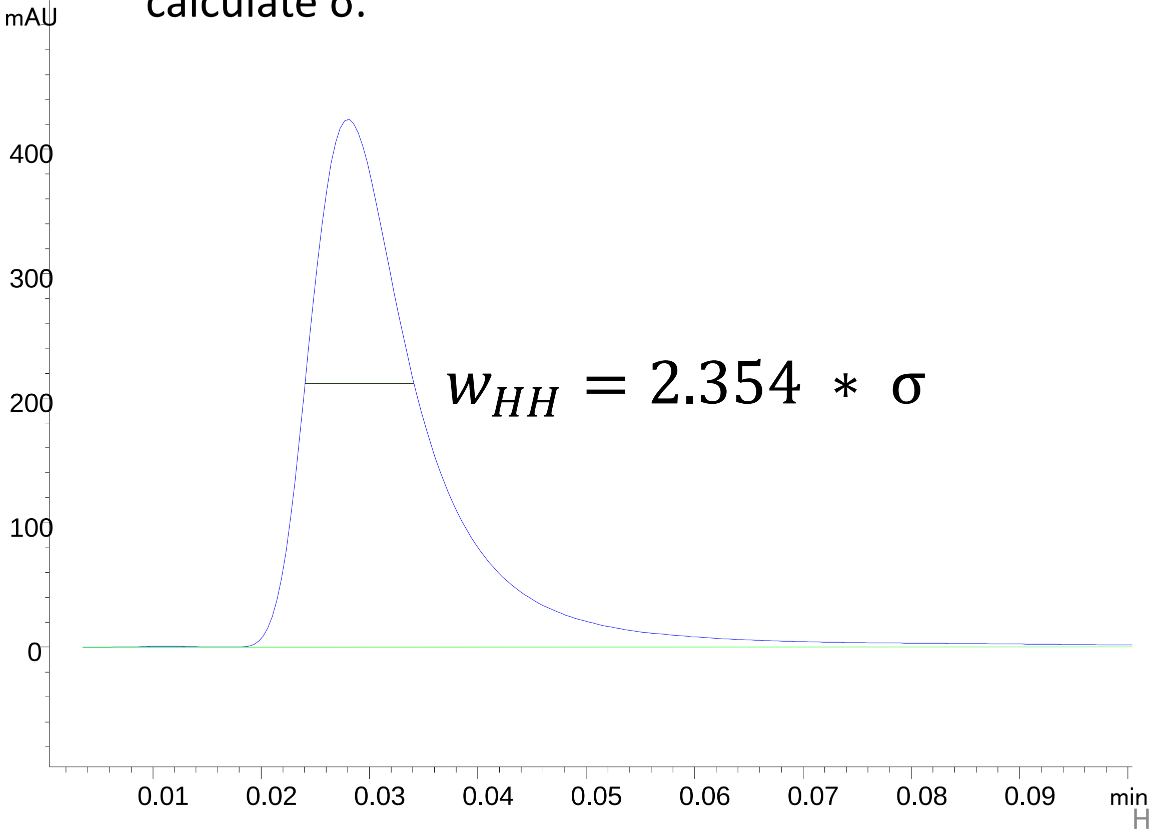
Not recommended.

Operating 1.0 mm and 1.5 mm ID columns on an older UHPLC system may result in significantly decreased performance due to the larger extra-column contributions from these instruments. The values for the 1.5 mm ID columns suggest that these UHPLC systems might provide acceptable results if the extra-column contribution could be reduced.

# Measuring Extra-Column Volumes

**Zero Dead Volume (ZDV) Approach (1)**

- Remove column and substitute ZDV union.
- Inject 1 uL of test sample at desired flow rate.
- Measure peak width at half-height (HH) and calculate σ.

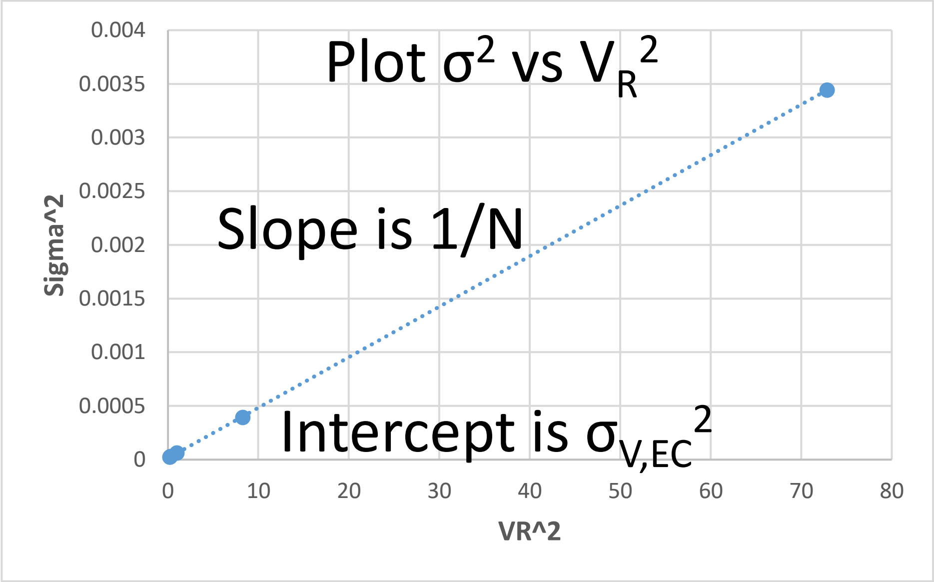



$$w_{HH} = 2.354 * \sigma$$

**Column Approach**

- Operate column under desired conditions.
- Inject test sample containing members of a homologous series.
- Measure retention volume and peak width.

$$\sigma^2_{Total} = \frac{t_R^2}{N} + \sigma^2_{t,EC} = \frac{V_R^2}{N} + \sigma^2_{V,EC}$$



# Objective of this work

- This poster summarizes our efforts to optimize a classic Agilent 1290 UHPLC system for use with 1.5 mm ID columns.  Efforts focused on finding and eliminating the source of as much extra-column volume as possible with minimal cost.
- The contribution of individual components was evaluated by removing or upgrading that component and measuring the extra-column volume before and after the change.  From these experiments an "Optimized" configuration was identified and tested.
- Standard and Optimized configurations were evaluated with 100 mm columns of different diameters using a mixture with components having retention factor values (k) between about 0.5 and 20.  Both isocratic and gradient conditions were employed.
- Only modifications to less expensive "consumable" parts were considered.

# Experimental

- Equipment
  - Agilent "Classic" 1290
    - Binary pump
    - High Performance Autosampler (ALS)
    - Inline 0.2 $\mu$m Filter
    - Column Compartment with 14-port/6-position valve
    - Diode Array with Fiber Optic Flow Cell (1 $\mu$L)

| Parameter | 3.0 X 100 mm* | 2.1 X 100 mm* | 1.5 X 100 mm* |
|---|---|---|---|
| Flow, mL/min. | 0.75 | 0.40 | 0.20 |
| Injection Volume, µL | 2.0 | 1.0 | 1.0 |
| Column Compartment | 35 °C | 35 °C | 35 °C |
| Detector | 250 nm, 20 Hz | 250 nm, 20 Hz | 250 nm, 20 Hz |
| Mobile Phase | ACN/Water (80/20) | ACN/Water (80/20) | ACN/Water (80/20) |
| Gradient | 20-100 % ACN/10 min. | 20-100 % ACN/10 min. | 20-100 % ACN/10 min. |

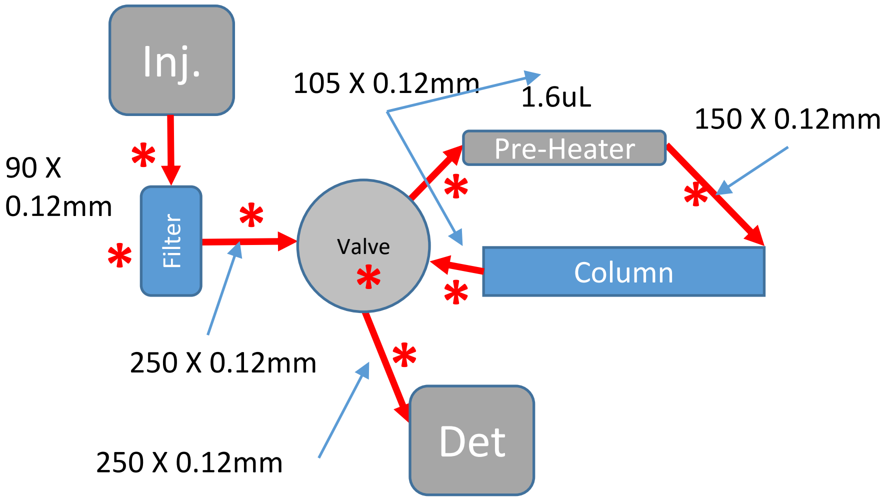
- Test Probes (~100– 200 $\mu$g/ml each)
  - Uracil (for measuring $t_M$)
  - Dimethylphthalate (DMP)
  - Diethylphthalate (DEP)
  - Dibutylphthalate (DBP)
  - Dihexylphthalate (DHP)
  - Dioctylphthalate (DOP)

* All columns are Halo C18 from AMT.

# Results

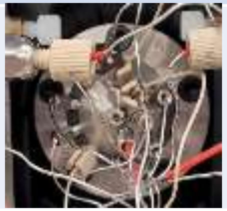
- Upgrading the Instrument Configuration

**Standard Configuration**

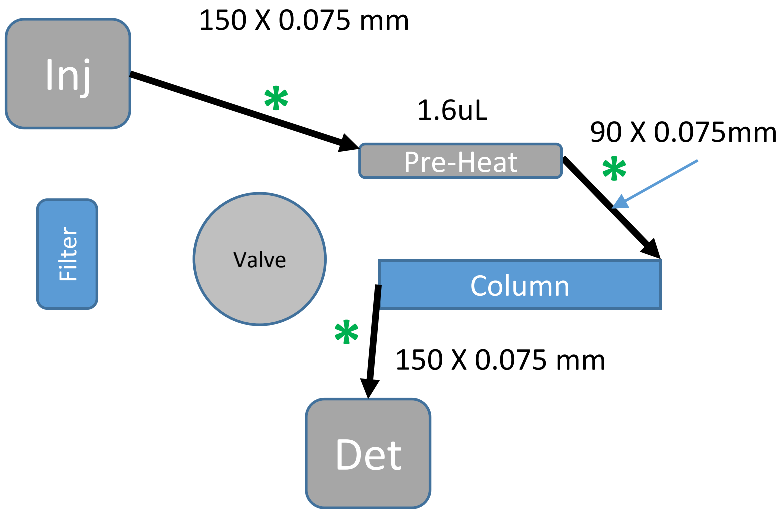



* **Candidate component for removal or upgrade to reduce dispersion.**

# Strategies for Reducing Extra-Column Dispersion

| Component | Standard Configuration | Optimized Configuration |
|---|---|---|
| Inline Filter |  | Removed |
| Selection Valve |  | Removed |
| Pre-Post Column Tubing |  |  |
| Pre-Column Heater |  | No Change |
| Column Connection (from Heater) |  |  |

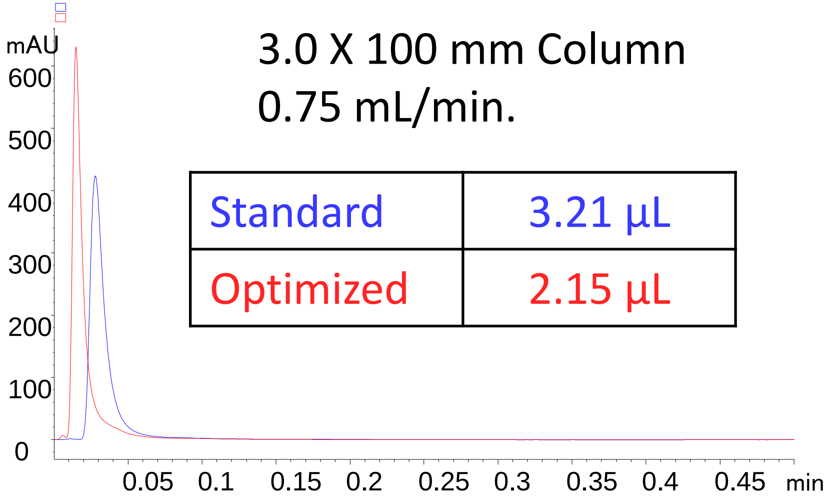
**Optimized Configuration**



# Dispersion Results (σ in μL) With No Column

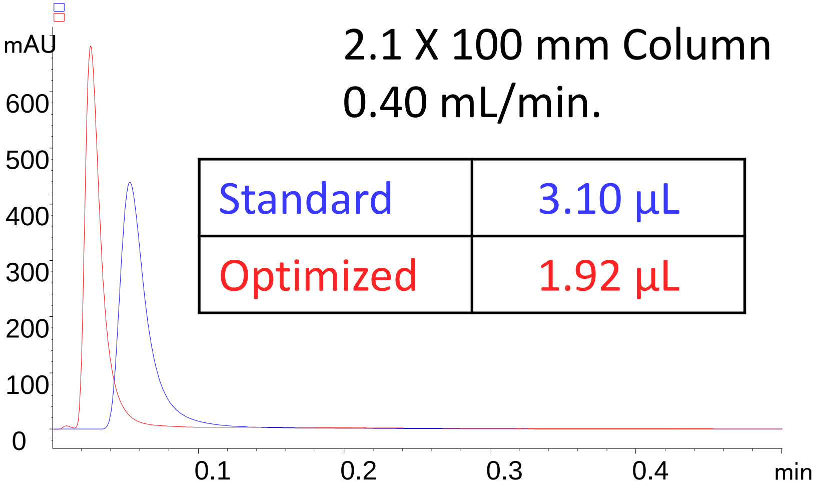
**Standard Configuration**
**Optimized Configuration**

3.0 X 100 mm Column
0.75 mL/min.



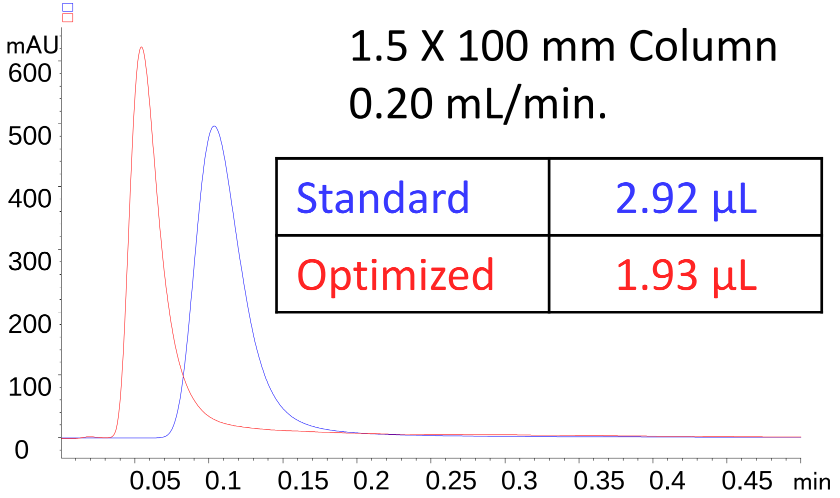

| | |
|---|---|
| Standard | 3.21 μL |
| Optimized | 2.15 μL |

2.1 X 100 mm Column
0.40 mL/min.




| | |
|---|---|
| Standard | 3.10 μL |
| Optimized | 1.92 μL |

1.5 X 100 mm Column
0.20 mL/min.

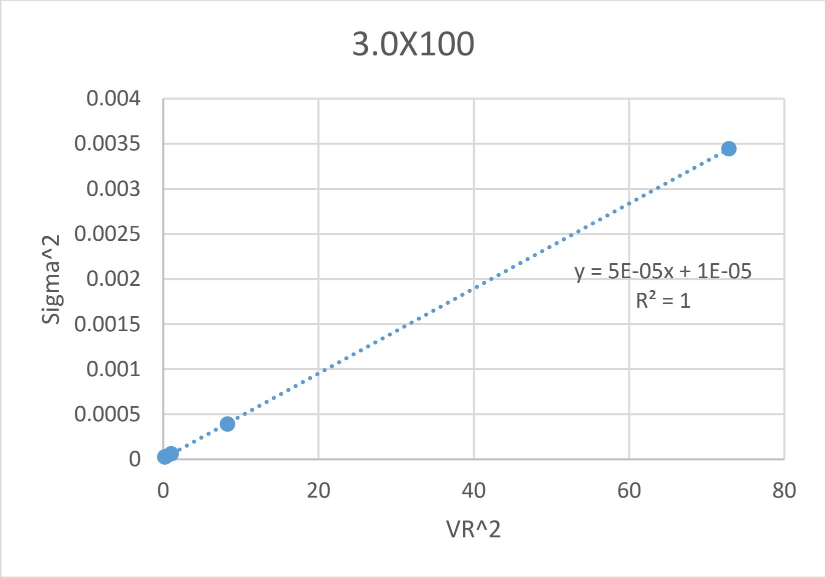


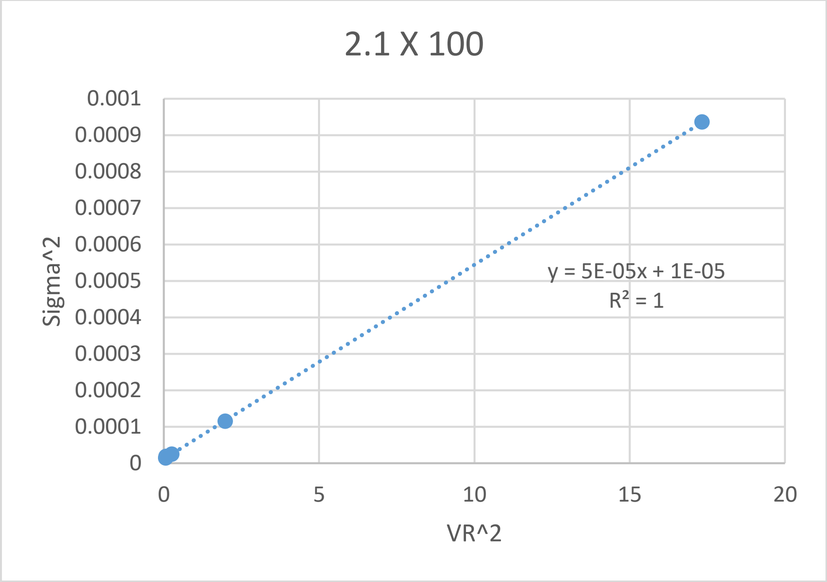

| | |
|---|---|
| Standard | 2.92 μL |
| Optimized | 1.93 μL |

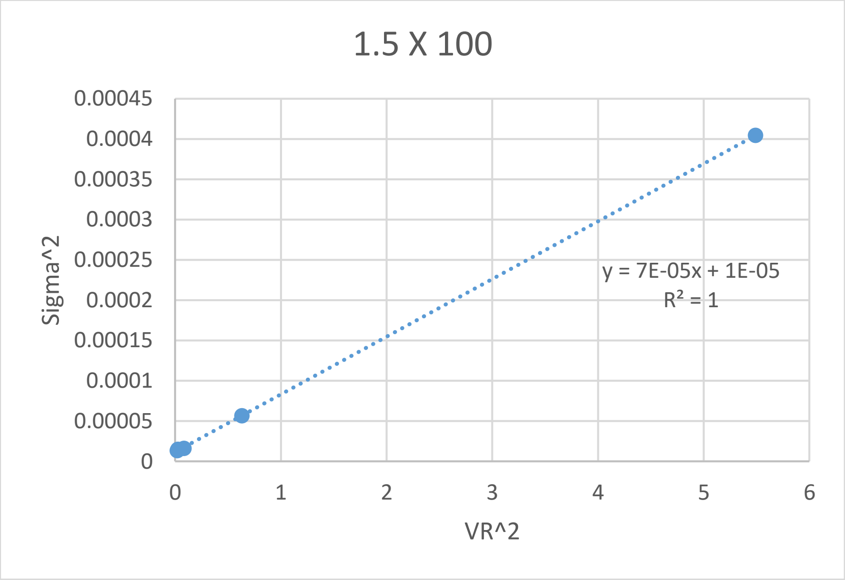
Values calculated from 1 uL injection of standard and measuring peak width at half height. Values are average of three replicate injections.

- Discussion
  - As expected, dispersion is larger at faster flows.
    - This fact is an additional benefit of the smaller-bore columns.
  - Optimization removed about 1 μL of extra-column dispersion by this method.
    - > 30 % reduction

# Dispersion Results Using Columns – Standard Configuration



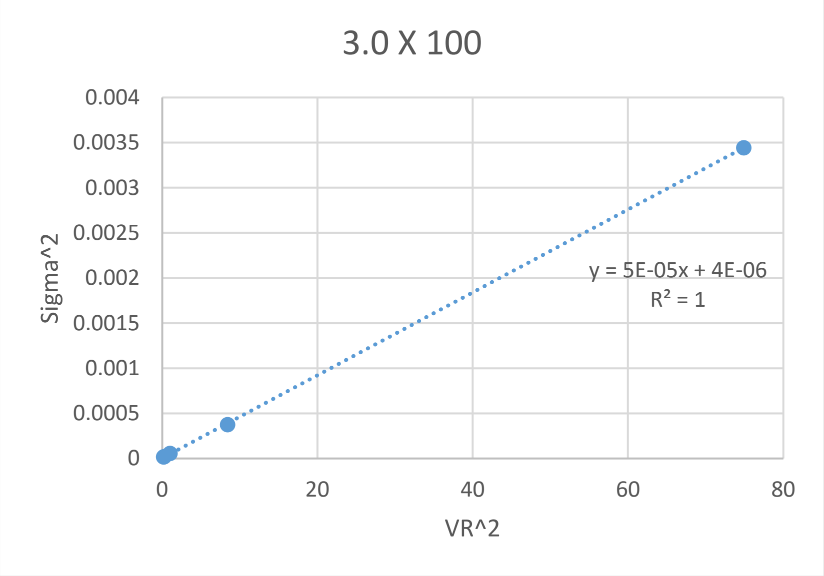



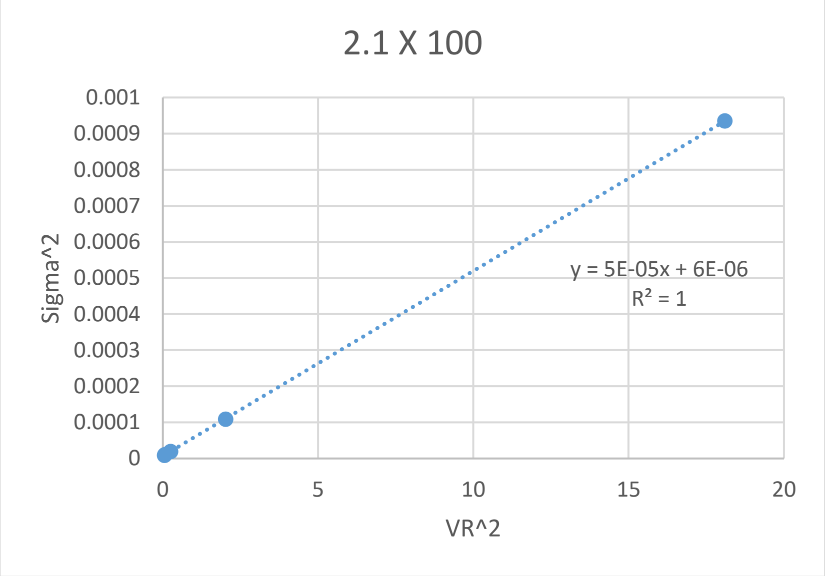

| | | | |
|---|---|---|---|
| Slope | 4.70826E-05 | N | **21239** |
| Intercept | 1.16268E-05 | **Sigma** | **3.41 uL** |
| r^2 | 0.99999 | | |

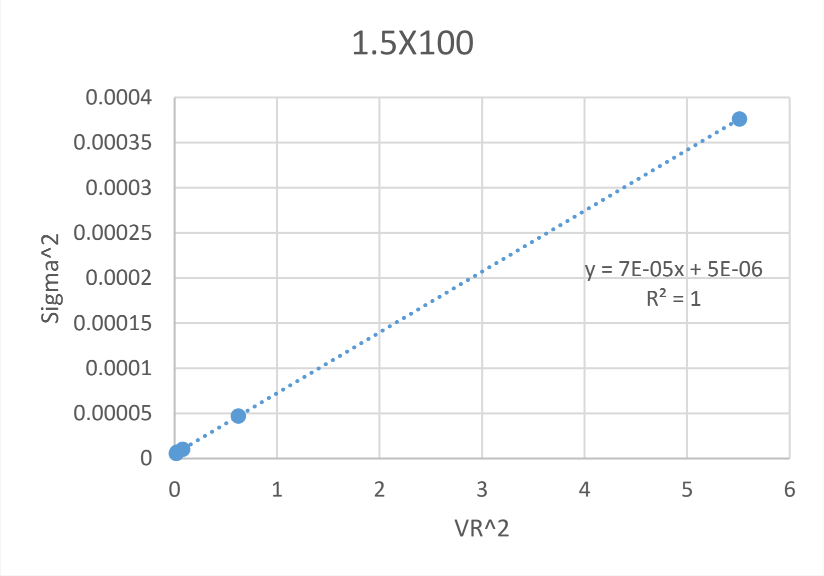
| | | | |
|---|---|---|---|
| Slope | 5.41081E-05 | **N** | **18771** |
| Intercept | 1.20651E-05 | **Sigma** | **3.46 uL** |
| r^2 | 0.99998 | | |

| | | | |
|---|---|---|---|
| Slope | 7.22817E-05 | **N** | **13980** |
| Intercept | 1.10045E-05 | **Sigma** | **3.39 uL** |
| r^2 | 0.99994 | | |

# Dispersion Results Using Columns – Optimized Configuration




| | | | | |
|---|---|---|---|---|
| Slope | 4.59E-05 | N | 21787 | |
| Intercept | 3.79E-06 | **Sigma** | **1.95** | **uL** |
| r^2 | 0.99997 | | | |

| | | | | |
|---|---|---|---|---|
| Slope | 5.14E-05 | N | 19473 | |
| Intercept | 5.99E-06 | **Sigma** | **2.45** | **uL** |
| r^2 | 1.00000 | | | |

| | | | | |
|---|---|---|---|---|
| Slope | 6.74E-05 | N | 14839 | |
| Intercept | 4.95E-06 | **Sigma** | **2.22** | **uL** |
| r^2 | 1.00000 | | | |

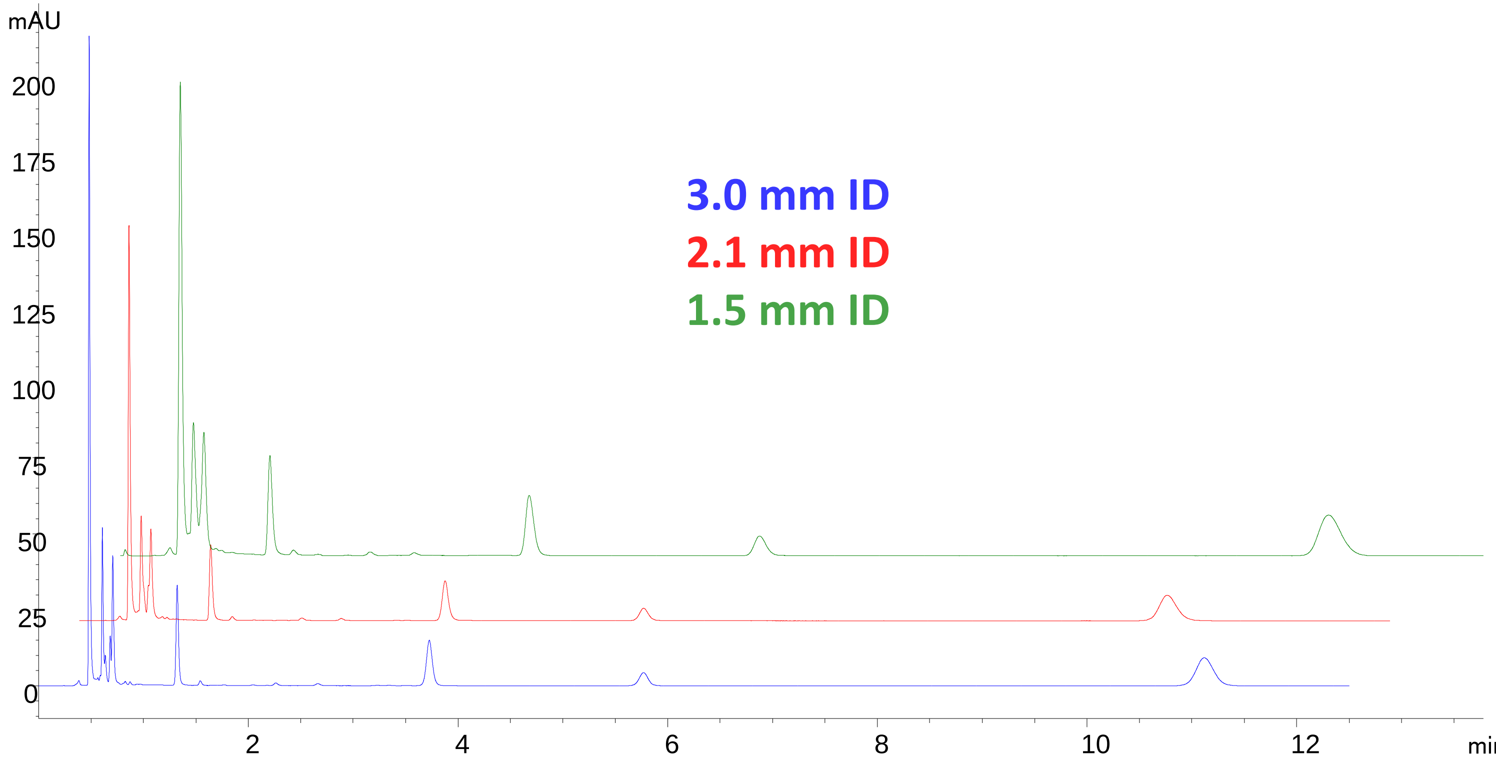
# Comparison of Dispersion Measurement Methods

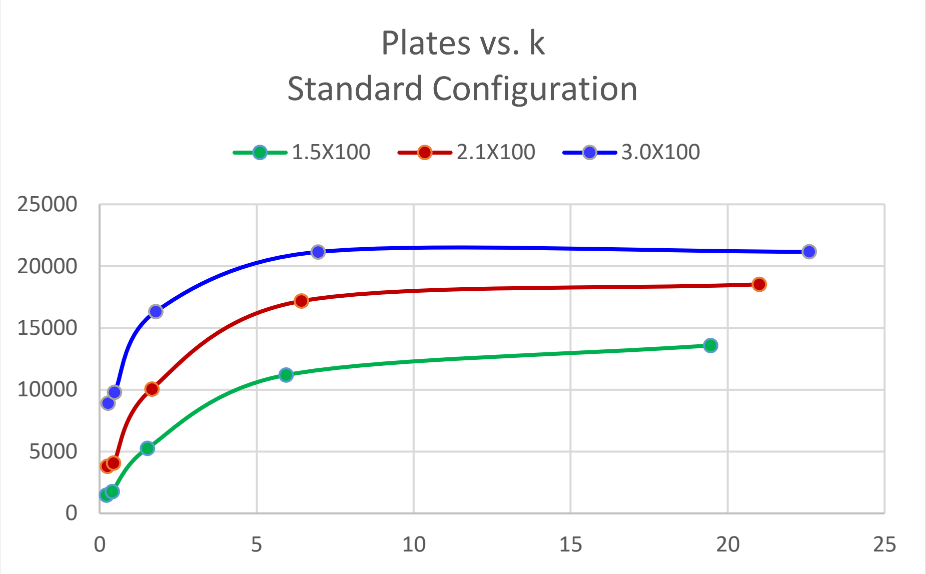
| Column | Standard Configuration | | Optimized Configuration | |
|---|---|---|---|---|
| | Union | Column | Union | Column |
| 3.0 X 100 | 3.21 | 3.41 | 2.15 | 1.95 |
| 2.1 X100 | 3.10 | 3.46 | 1.92 | 2.45 |
| 1.5 X 100 | 2.92 | 3.39 | 1.93 | 2.22 |

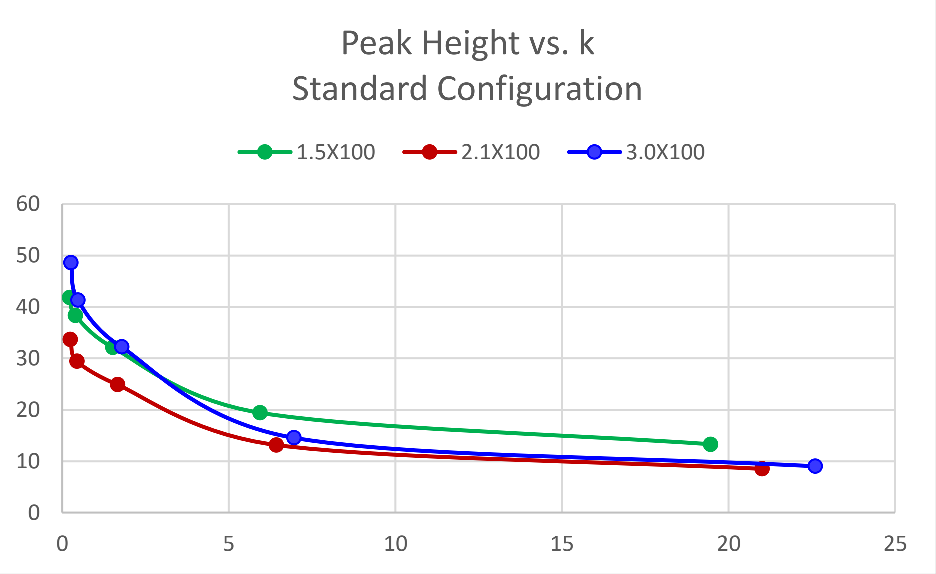
All values in uL.

The two different methods give similar results but will never be the same since they are using different assumptions and methods for the measurement process.

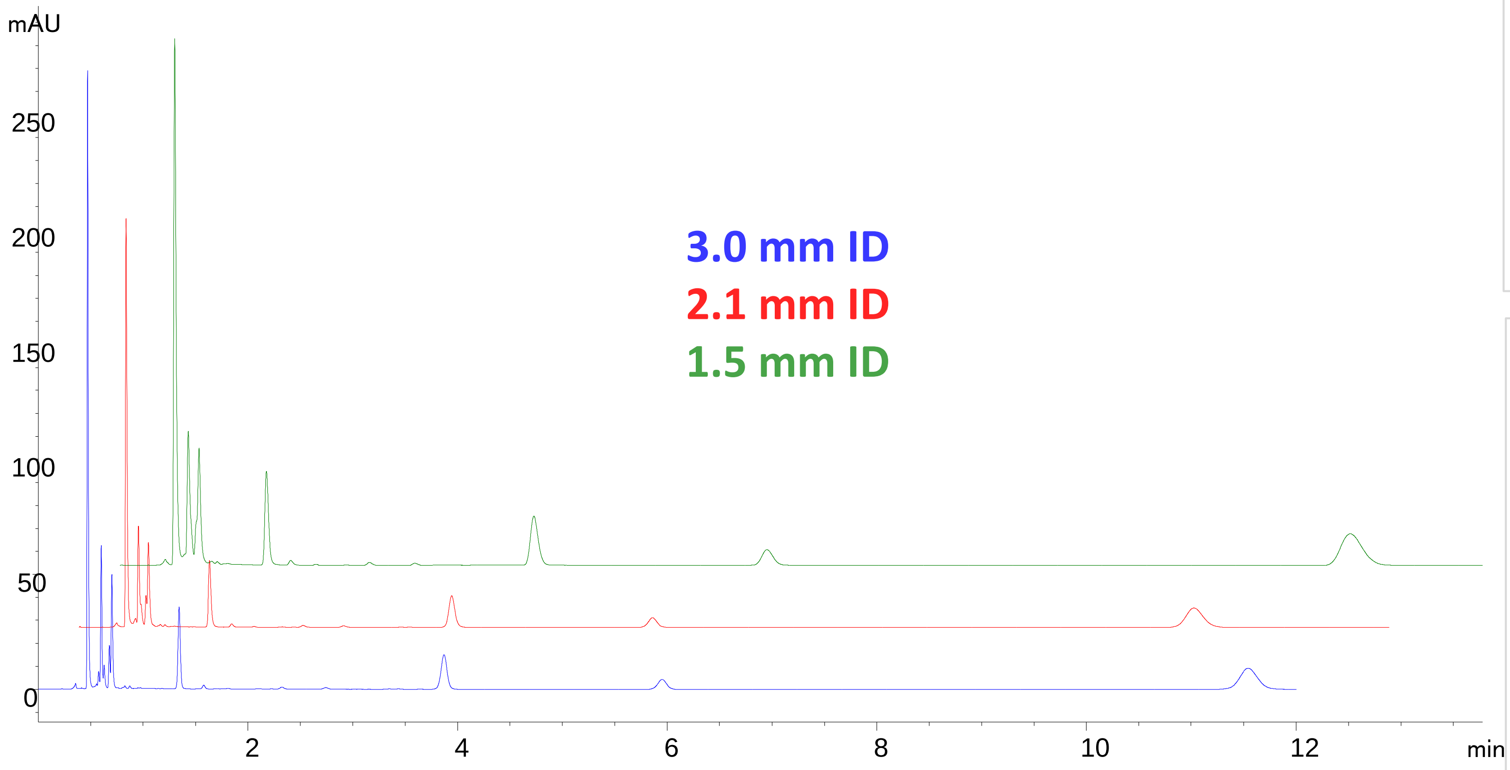
# Standard Configuration - Isocratic

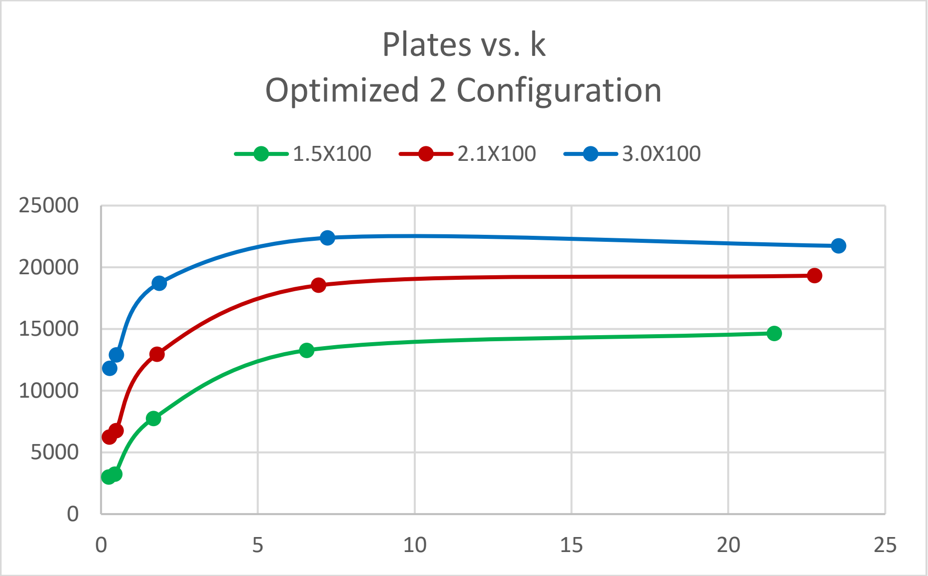


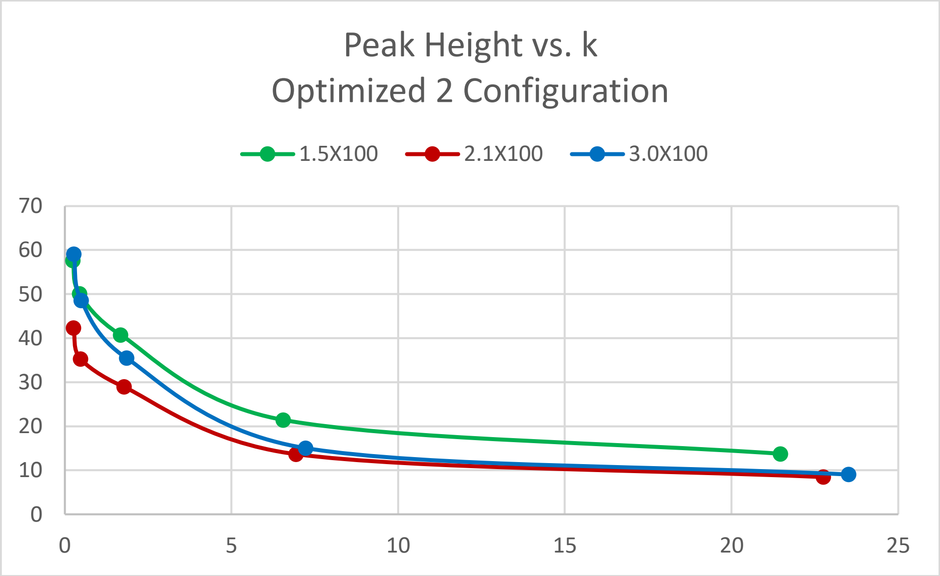



# Optimized Configuration - Isocratic

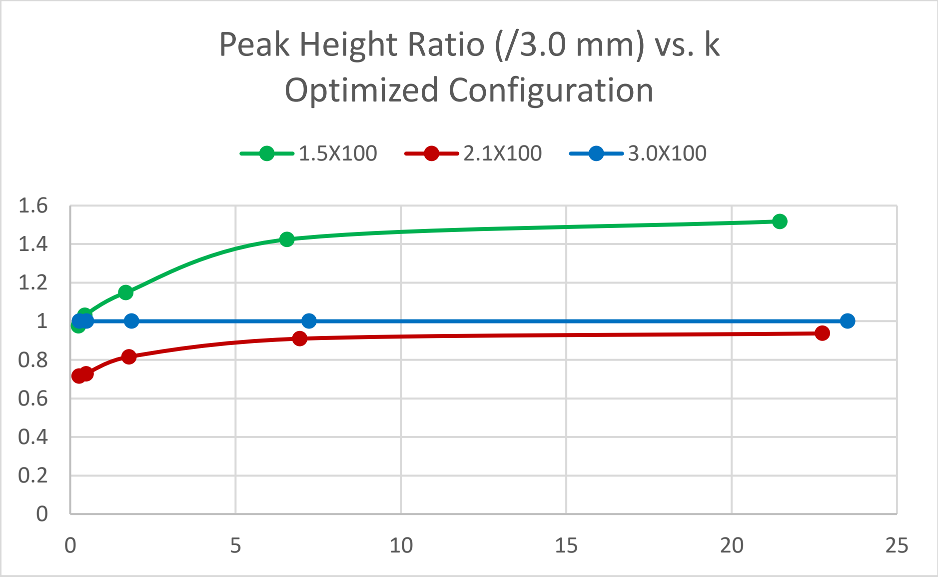


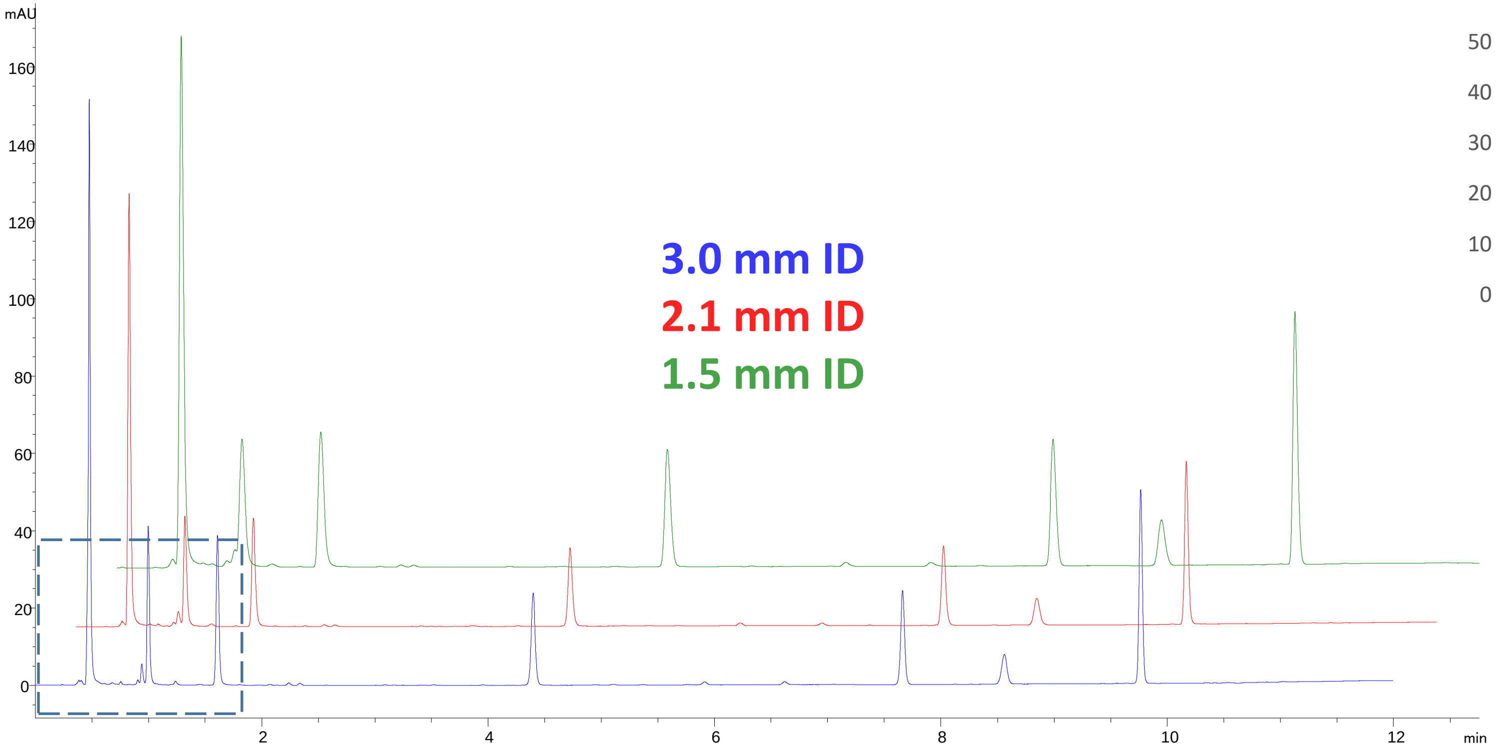



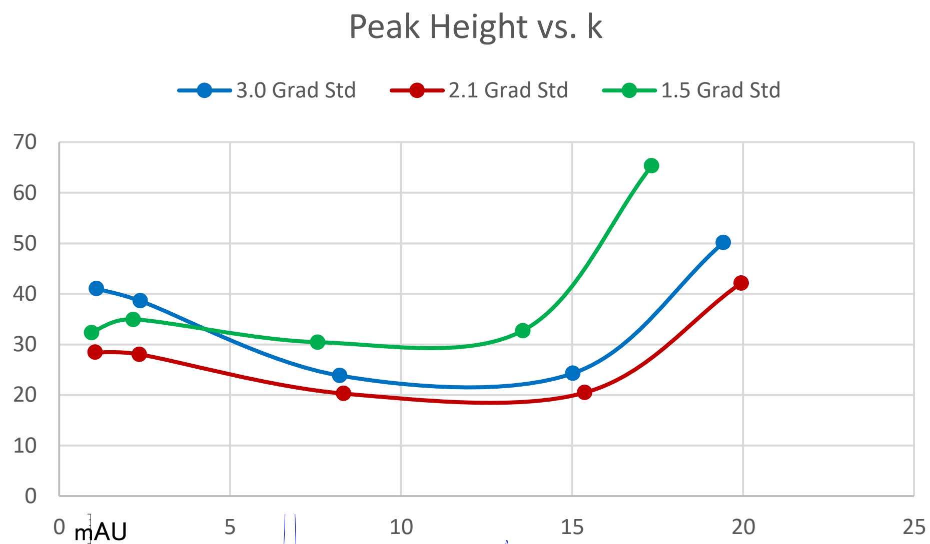
# Discussion - Isocratic

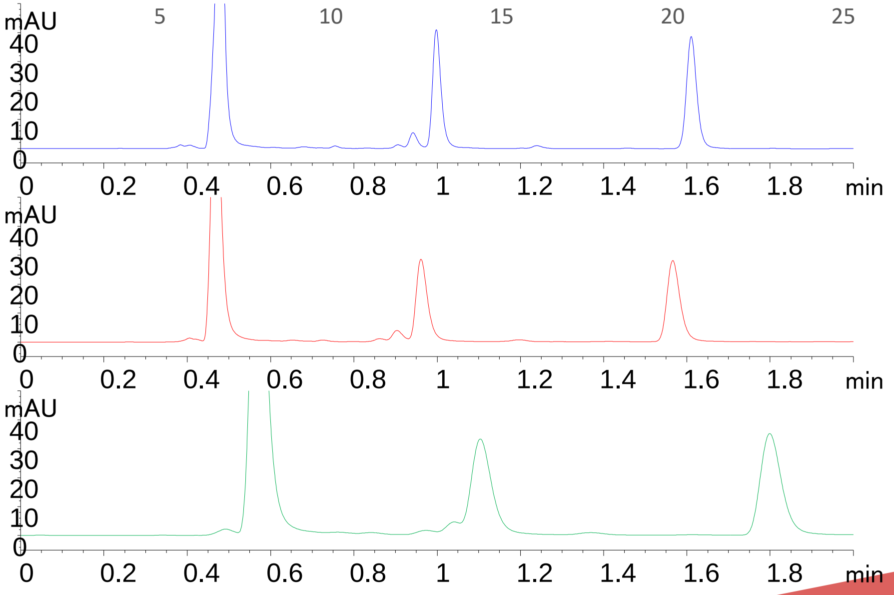
- Efficiency and resolution
  - As expected, the plate counts and resolution decrease for the smaller-bore columns as the influence of the extra-column volume becomes more significant.
  - However, the improvement in efficiency and resolution for the optimized configuration is much larger for the smaller columns.
- Peak Height
  - With standard configuration, the 3.0 mm ID column is superior to the 2.1 mm ID column for k < 5, but at larger values the two are equivalent. Even under sub-optimum configurations, the 1.5 mm ID column is superior to the other two columns when k > 5.
  - For optimized configuration, the 1.5 mm column is superior to the other columns for k > 2. The improvement is > 40 % when k > 5.



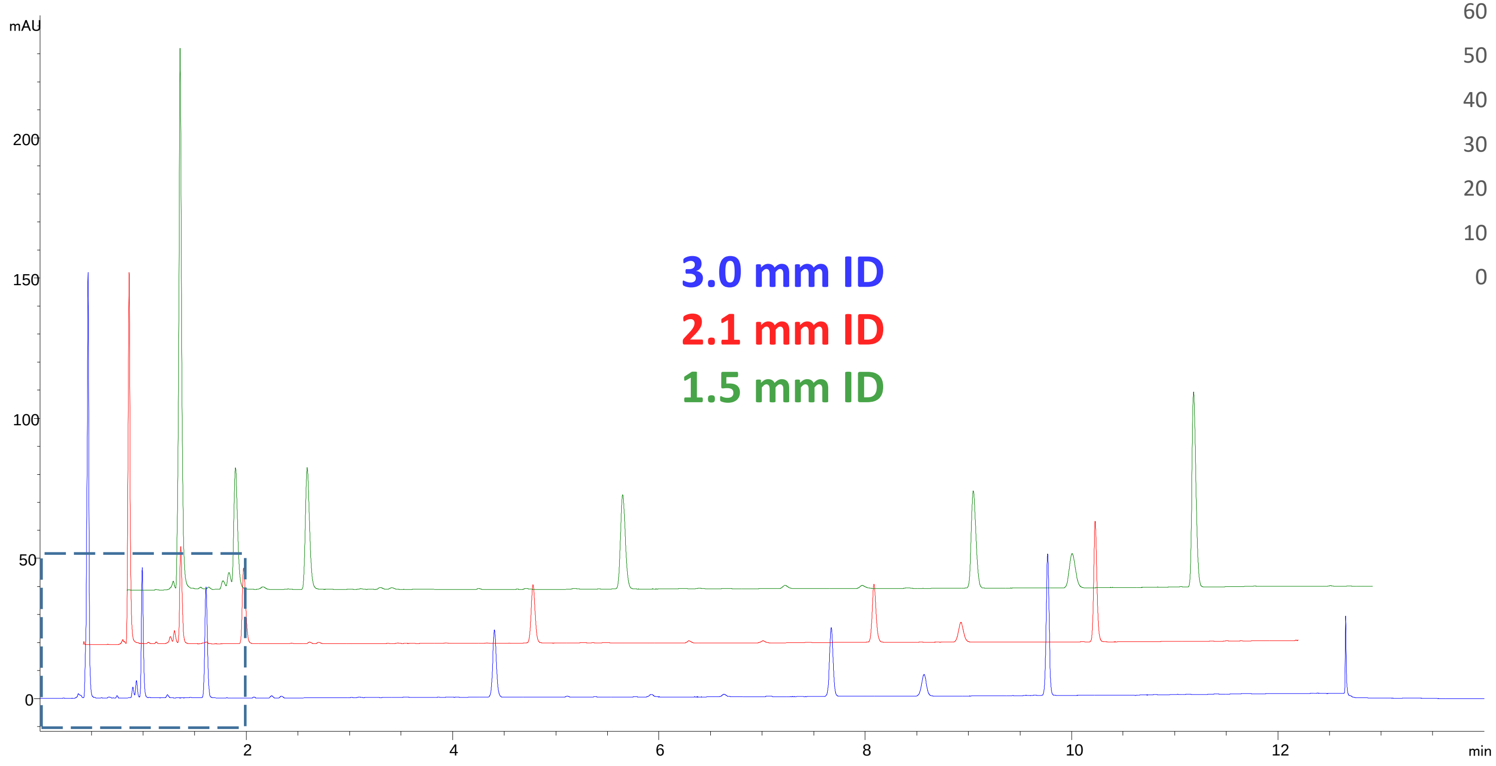
# Standard Configuration – Gradient

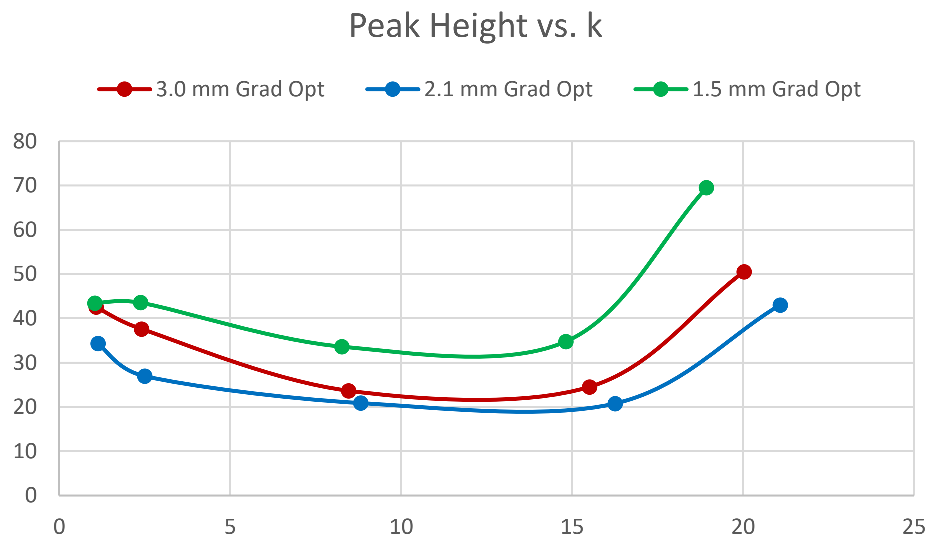


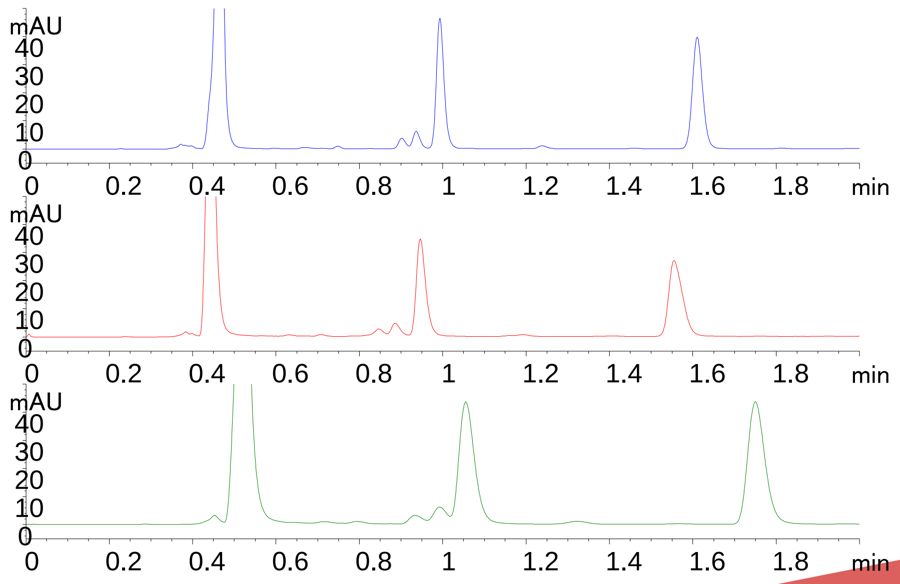



# Optimized Configuration - Gradient

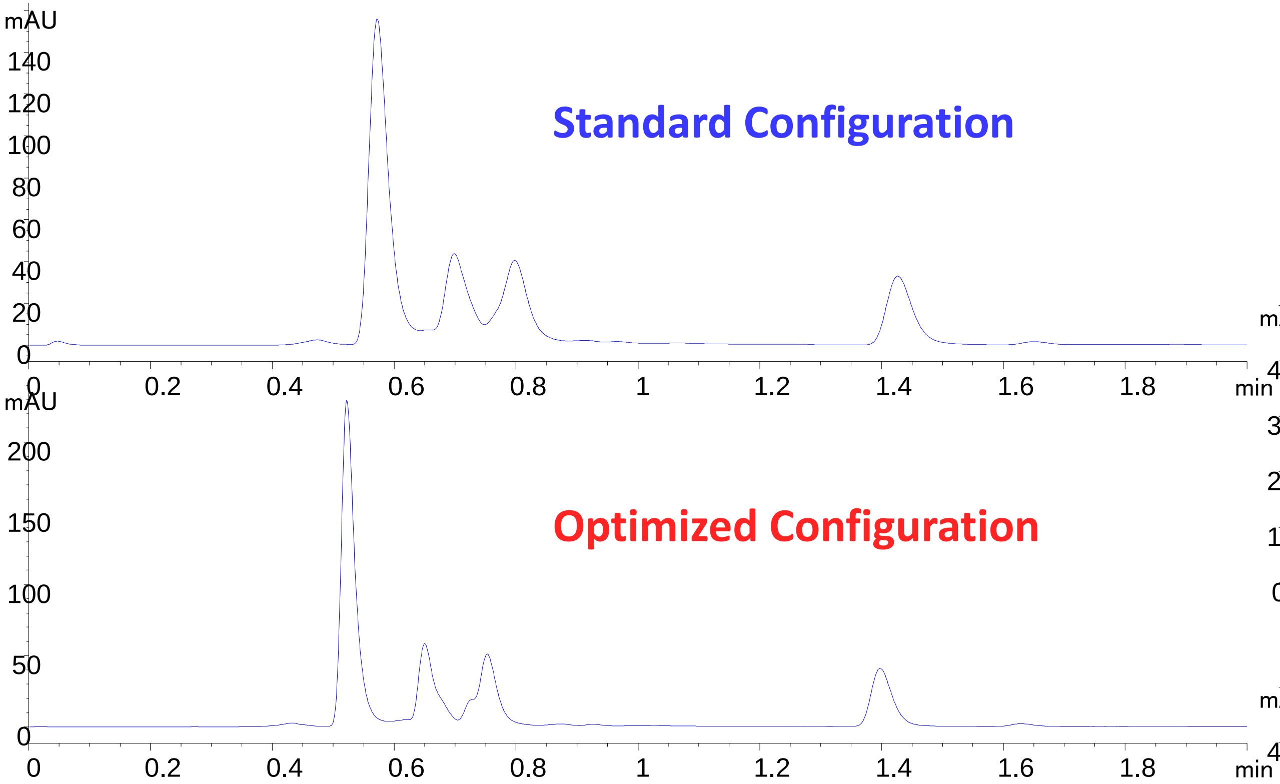


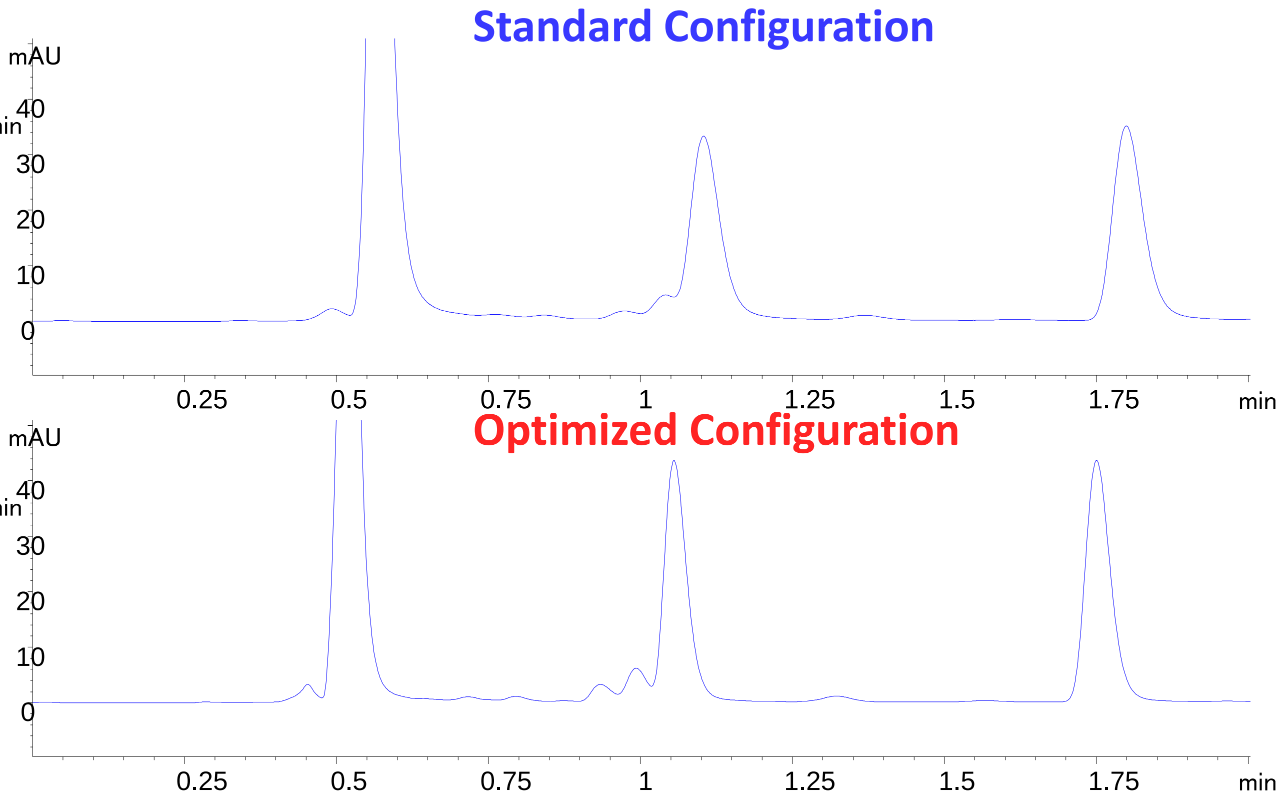



# 1.5 mm Column – Standard and Optimized

**Isocratic**




**Gradient**



# Discussion- Gradient

- Resolution
  - With standard configuration, some reduction in resolution of early eluting impurities is noted for the 1.5mm column even though pre-column EC effects have been eliminated by focusing.
  - When extra-column dispersion is reduced, resolution improves significantly more for the same impurities because post-column EC effects still exist.
- Peak Height
  - With standard extra-column dispersion, the 3.0 mm ID column outperforms the other columns, up to about k = 5.  Beyond that point, the 1.5 mm ID column always produces the largest peak heights.
  - Once the system is optimized, the 1.5 mm ID column, produces the largest peak heights under all conditions.

# Conclusion

- Without modifications to a classic 1290, a 1.5 mm ID column will produce significantly fewer theoretical plates than a matched 2.1 mm ID column due to EC effects.
- Optimization to reduce extra-column dispersion can recover some of these losses, although the smallest column still produces fewer theoretical plates.
- Despite the efficiency differences, the smallest column produces larger peak heights than either the 2.1- or 3.0-mm ID columns under most conditions, in both isocratic and gradients modes.

# Impact of this work/Outlook

- Advantages
  - Fairly simple changes to the system allow the effective use of a 1.5 mm ID column. Only consumables were changed; there were no major (i.e., expensive) hardware changes.
  - The small-bore column studied here offers solvent savings of 50 % over a 2.1mm ID column, and 75 % over a 3.0 mm ID column.
  - As an added benefit, peak heights are larger under most conditions, which is beneficial in trace analysis situations.
- Limitations
  - The optimized system does not provide pre-column filtration, so effective sample filtering is required.
  - These columns may not be appropriate for mixtures with early-eluting components or minimal resolution pairs.
  - Better results may be possible with a smaller detector flow cell, which would not be a trivial expense.

# References


- (1) D. R. Stoll, K. Broeckhoven, LCGC North America, 2021, Volume 39, 159–166.

# Acknowledgements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