Conversion of Std LC Methods to RRLC of Pharmaceutical Analysis (Incl. USP methods, a case study for USP)

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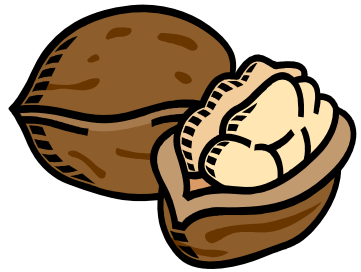
In a Nut Shell

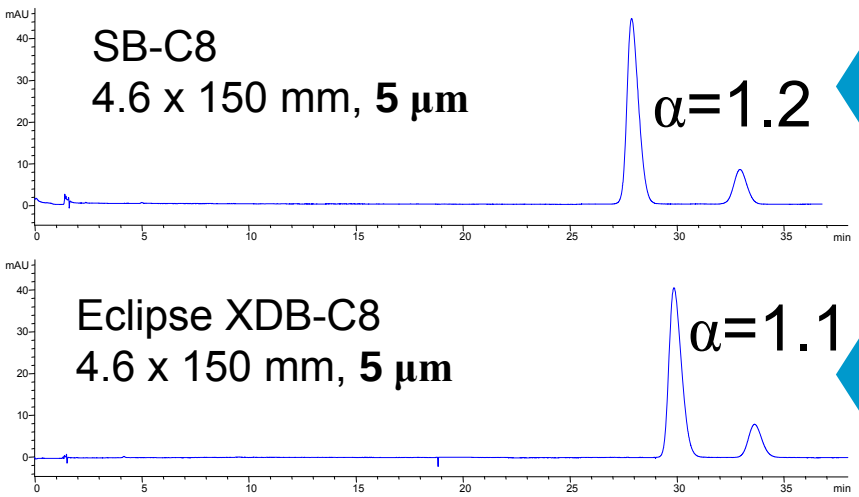


Standard LC methods, including USP methods, originally developed with 4.6 mm I.D., 5 µm columns can be easily converted to high throughput methods by substituting the original column with a 4.6 mm, 1.8µm, Rapid Resolution High Throughput (RRHT) column, or a 3.5 µm, Rapid Resolution (RR) column.

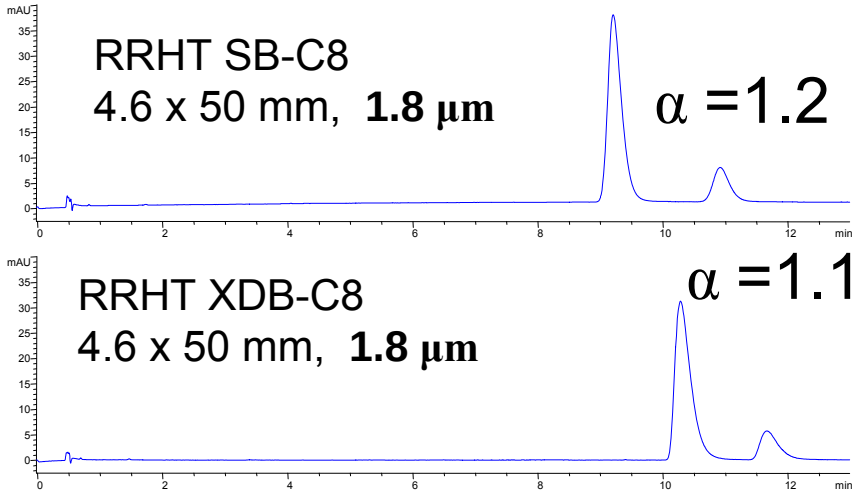
Take a look at Fast Validation of improved RRHT USP assay for ibuprofen oral suspension.

Reproducibility of Selectivity (α) Between Particle Sizes

Bonded Phases on 5 µm particles



Bonded Phases on 1.8 µm particles

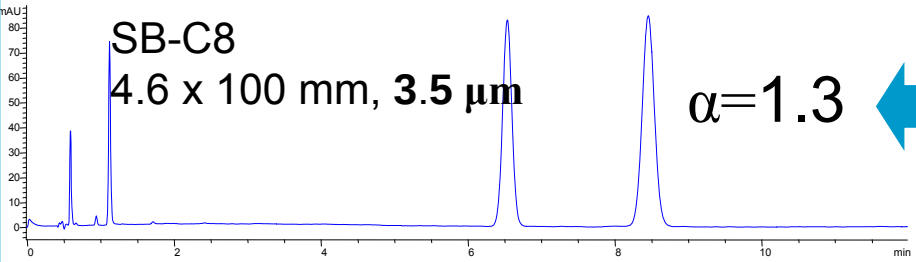


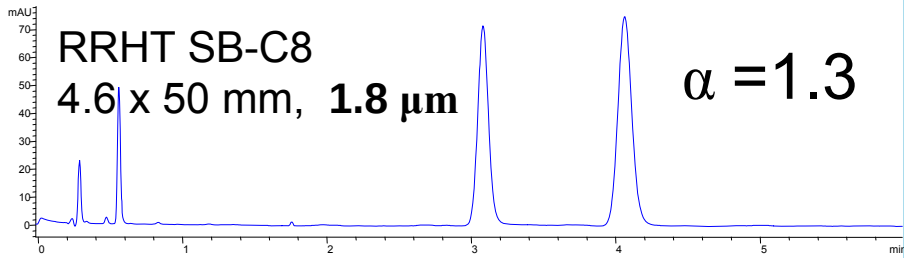
USP Method for Doxepin HCl

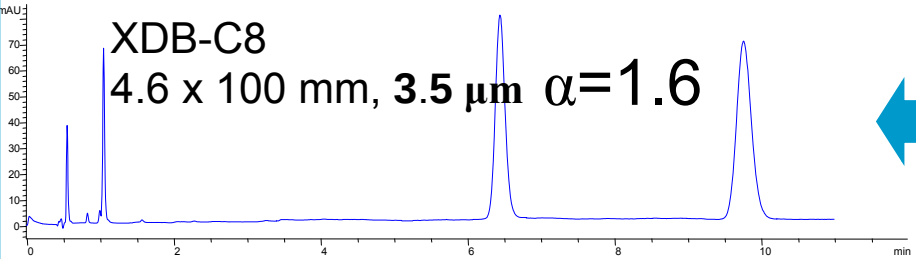
Mobile phase: (70:30) 200mM NaH_3PO_4 pH 2.5 :MeOH
Flow =1 mL/min. isocratic
Temperature. : 50 ˚C
LC: Agilent 1100
Sample: doxepin HCl, 0.1 mg/mL (82% E-isomer)

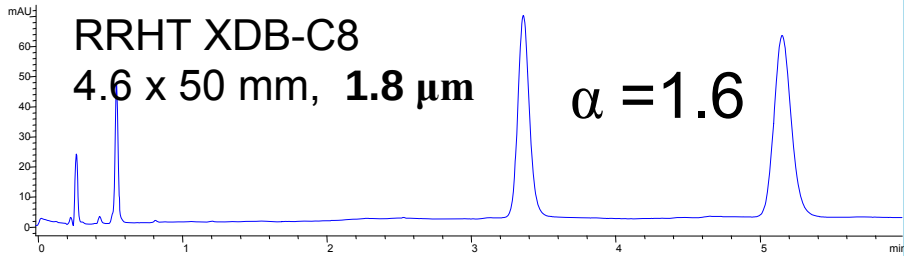
Reproducibility of Selectivity (α) Between Particle Sizes

Bonded Phases on 3.5 µm particles **Bonded Phases on 1.8 µm particles**





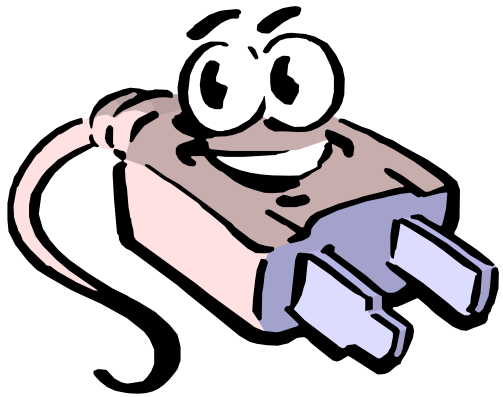


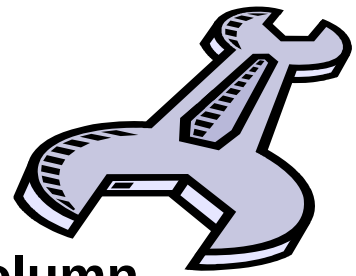


USP Method for ibuprofen oral suspension
Mobile phase: (63:37) water :acetonitrile + 1.8 mL H_3PO_4
Flow = 2.0 mL/min. isocratic
Temp. : ambient
LC: Agilent 1100
Sample: childrens ibuprofen oral suspension,
with benzophenone as internal std.
prepared as described in USP

Converting Isocratic Methods to RR or RRHT

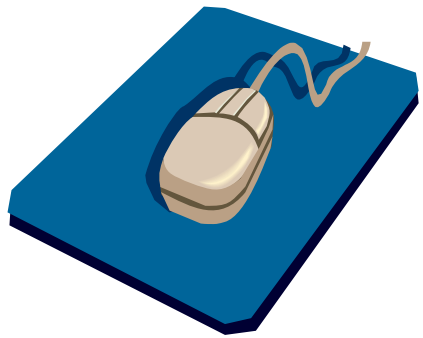
Plug & Play





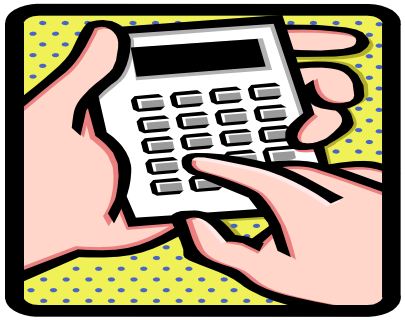
Step 1. Replace 4.6 x 150 mm, 5 μm column with an RR (3.5um) or RRHT (1.8um) column.

Step 2. Run pre-existing method.



Converting Gradient Methods to RR (3.5um) or RRHT (1.8um)

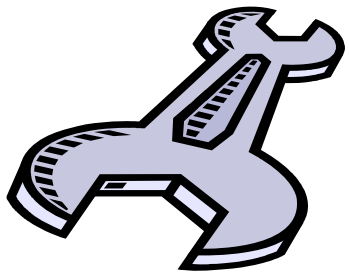
Step 1. Do the Math



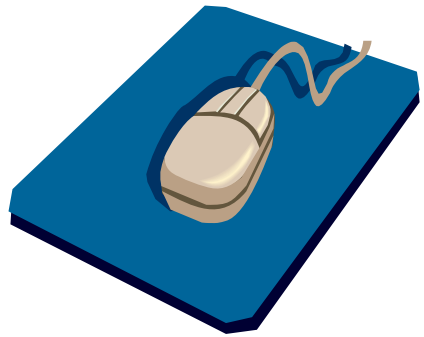
Calculate a new gradient time proportional to the change in column length: $t_{G2} = t_{G1} \times (L_2/L_1)$

- **t_{G1} = Gradient time with original column**
- **t_{G2} = Gradient time with column 2**
- **L_1 = Length of original column**
- **L_2 = Length of column 2**

Step 2. Change original column to a RR (3.5um) or RRHT (1.8um)column.



Step 3. Run new method.



How conversion works for time

Run Time or Gradient segment Time Adjustment

$$\text{Time}_{\text{col.1}} \times \left(\frac{\text{Length}_{\text{column2}}}{\text{Length}_{\text{column1}}} \right) = \text{Time}_{\text{col. 2}}$$

$$\text{i.e.} \quad 25min. \times \left(\frac{150mm}{250mm} \right) = 15min.$$

***assumes flow is proportional for columns 1 and 2**

How Conversion Works For Flow

Flow modification, for columns of different diameters

$$\mathrm{Flow}_{\mathrm{col.1}} \times \left(\frac{\mathrm{Diam}_{\mathrm{column2}}}{\mathrm{Diam}_{\mathrm{column1}}} \right)^2 = \mathrm{Flow}_{\mathrm{col.\ 2}}$$

$$\text{i.e.}\quad 1.0 ml/min \times \left(\frac{2.1 mm}{4.6 mm} \right)^2 = 0.21 ml/min$$

Conversion for Injection Volume

Keep Injection volume proportional to column volume

$$\text{Inj.Vol}_{\text{col.1}} \times \left(\frac{\text{Volume}_{\text{column2}}}{\text{Volume}_{\text{column1}}} \right) = \text{Inj.Vol}_{\text{col. 2}}$$

Zorbax column volume = 3.14 x r^2 x L x 0.6 (r and L in cm)

$$\text{i.e.} \quad 20\mu l_{\text{col.1}} \times \left(\frac{0.4ml_{\text{column2}}}{2.0ml_{\text{column1}}} \right) = 4\mu l_{\text{col. 2}}$$

Gradient Slope vs. Time and Flow

Gradient slope effects k* (“k star”) – the gradient term for the isocratic capacity factor

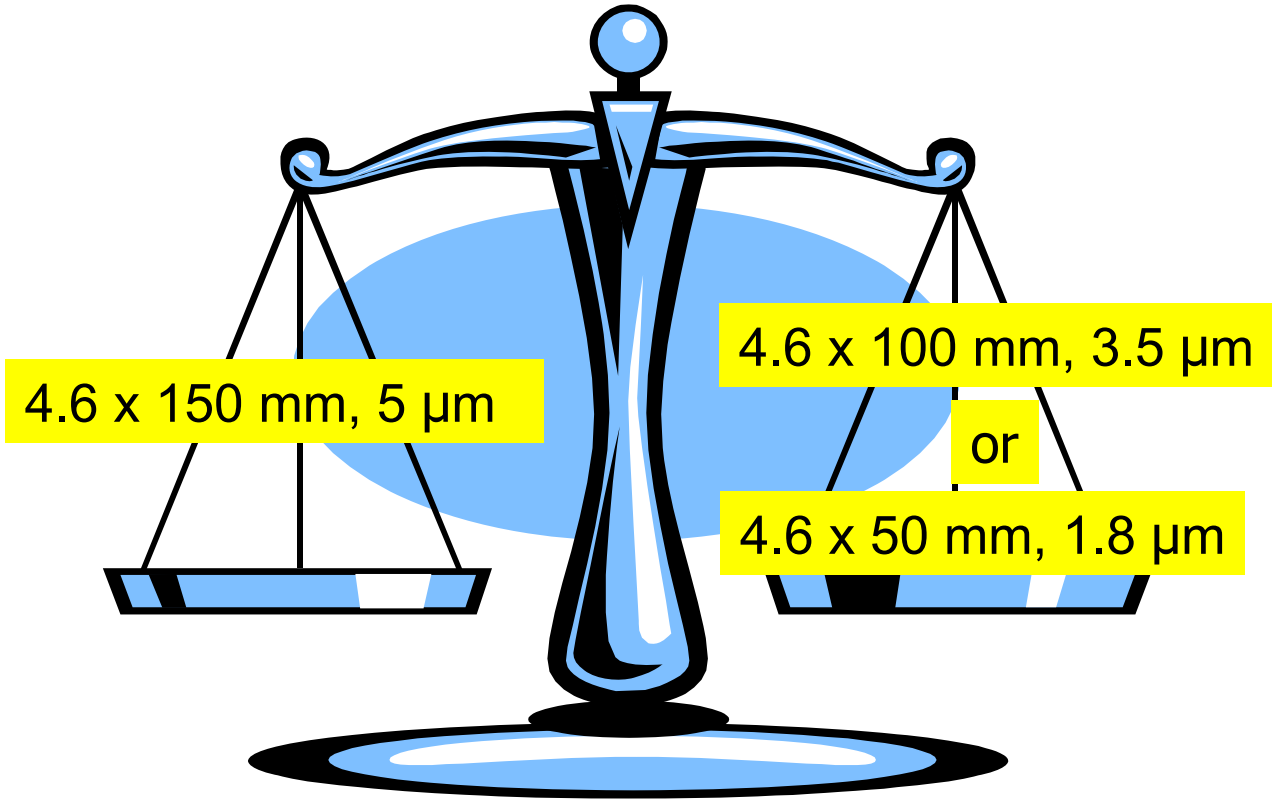
Increasing k’ or k* generally increases resolution. This can be done by decreasing solvent strength or decreasing gradient slope, respectively

$$\%\,\text{GradientSlope} = \left(\frac{(\text{End}\% - \text{Start}\%)}{\#\,\text{ColumnVolumes}} \right)$$

$$\text{i.e. } 8\% = \left(\frac{(100\% - 20\%)}{10\ \text{col. volumes}} \right)$$

column volumes = (flow x gradient time) / column volume
Zorbax column volume = 3.14 x r² x L x 0.6 (r and L in cm)

Why Plug-&-Play Works:
Column Efficiency and Resolution is Maintained:
Particle Size Decreases Proportionally to Column Length.



1.8 and 3.5 µm particles have more efficiency (N/meter) than 5 µm particles. Smaller particles packed in shorter columns allow rapid analysis, while maintaining resolution.

Transferring an Isocratic Method to a Shorter Column, Same Internal Diameter

Questions to Ask

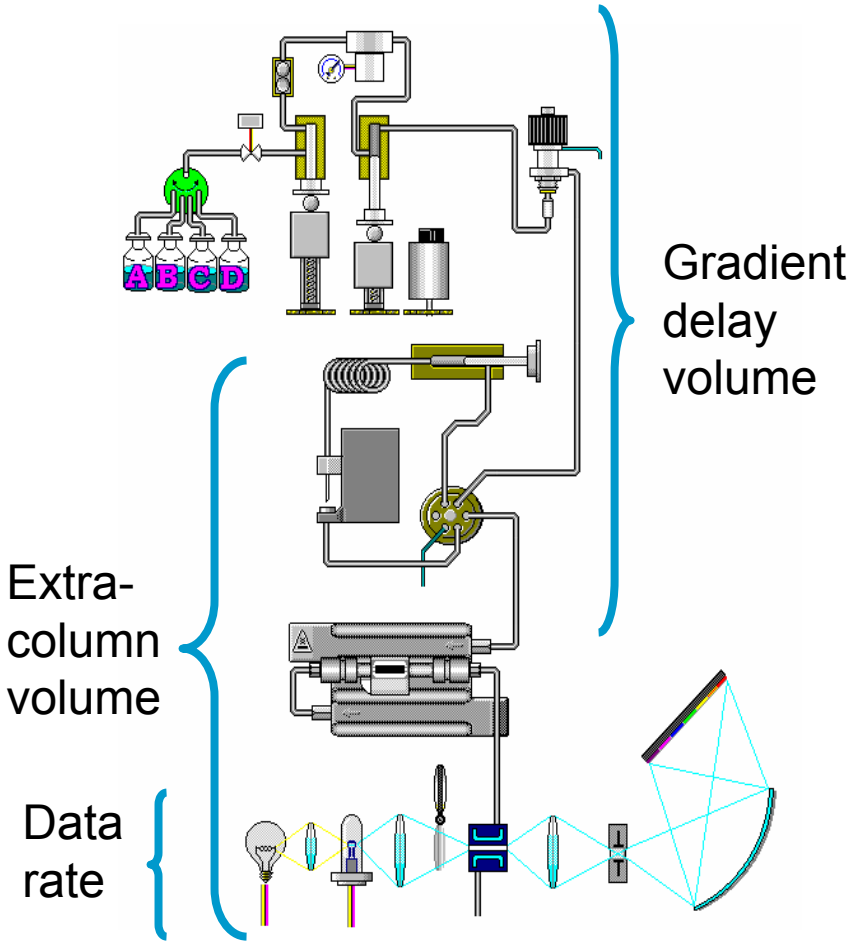
- What is the resolution for the critical pair(s) in current method?
- What is the mobile phase composition and backpressure for the current method on current instrument?
- What flow rate is used for current and will be used for the smaller column?
- Will you need smaller flow cell and smaller i.d. tubing for the shorter column to get maximum efficiency?

Calculate the following for the shorter column:

- Theoretical plates: current column and new column f (length, d_p)
- Resolution: current Rs x SQRT($N_{smaller}/N_{larger}$)
- Injection Volume: adjust by column volume ratio
- Flow Rate: adjust by square of i.d. ratio
- Expected pressure: f (temperature, solvent comp., viscosity, flow rate, length, d_p)
- Expected RT for last peak: k values should match first

Instrument Considerations:
Can the HPLC in your lab today handle RRLC ?

- **Things to consider on your system:**
  - **Gradient delay volume** – effects column re-equilibration and gradient profile
  - **Extra-column volume** – effects peak dispersion and peak width for Isocratic separations
  - **Data Rate** – match to expected peak widths-If rate is too slow sensitivity and peak detection suffer in Iso and Gradient separations



Some Considerations When Transferring Methods to Small(er) Volume Column

Isocratic Separations

Sample load (V_{inj}, [analyte])

Sample solvent strength

Extracolumn volume

- Injection volume
- Tubing volume
- Flow cell volume

Injector precision

- Can vary with V_{inj}

Gradient Separations

Same as Isocratic Separations plus…

Delay Volume

- Same instrument (different pressures)
- Different instrument (for example, Capillary 1100 vs. Binary 1100)

Gradient Time

- Adjust relative to equation for gradient retention
- Keep k* constant

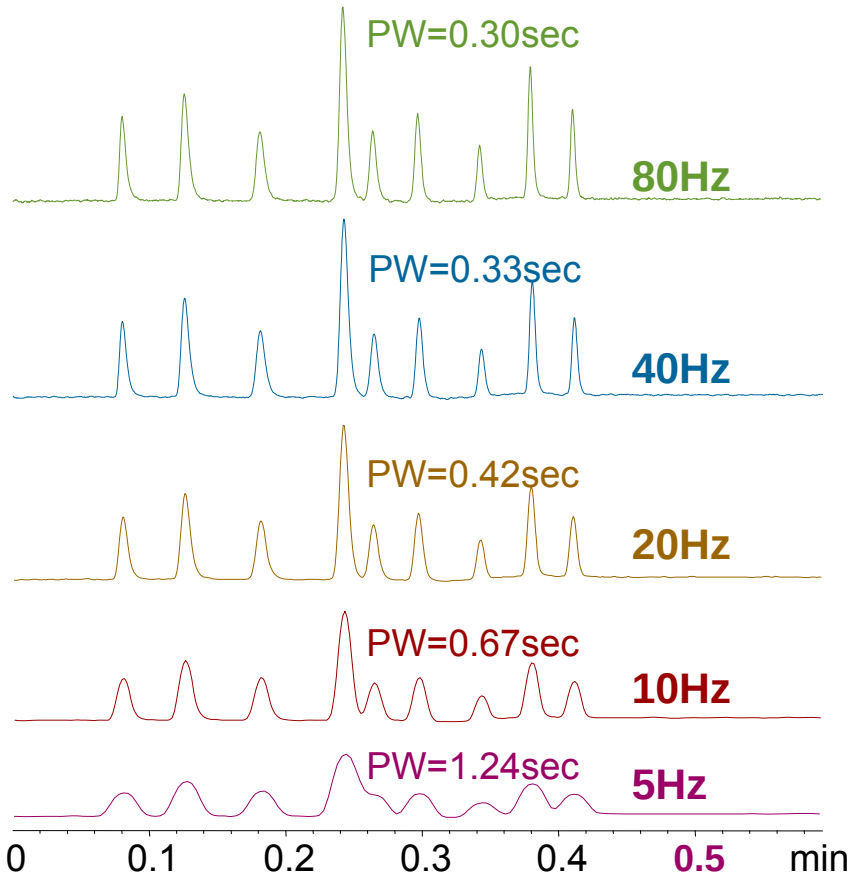
Gradient Delay Time

- Gradient delay time must be same as for larger column separation
- Ratio of gradient volume/column volume must be same as for larger column

Column Equilibration Time (Post Time)

High Speed LC with RRHT Columns

Match Detector Speed to Peak Width for Optimum Detection



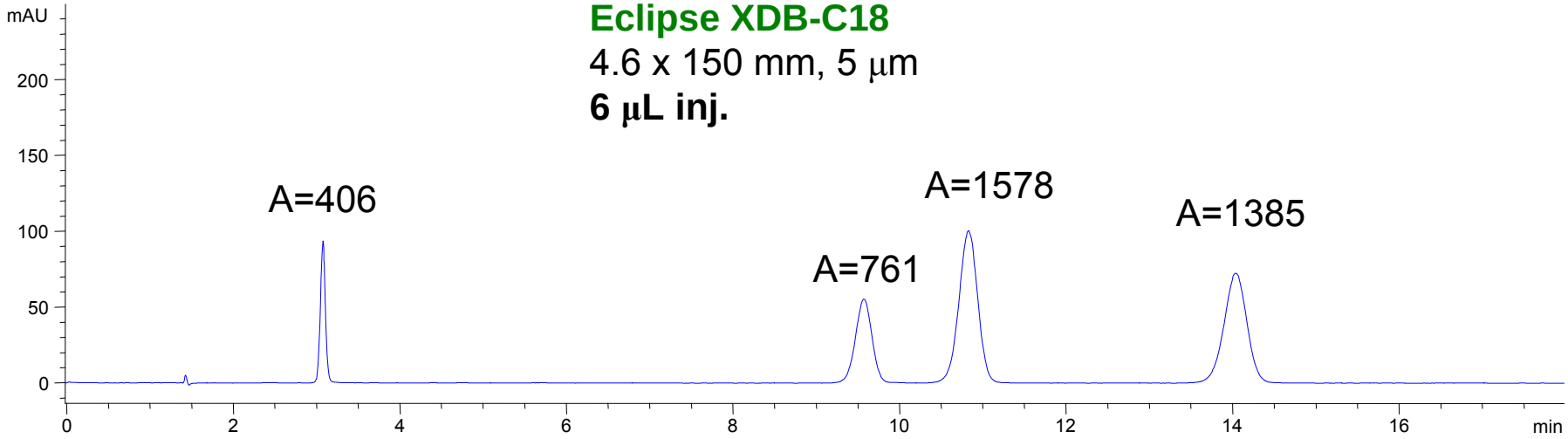
80Hz versus 10Hz (20Hz) Data Rate

- Peak Width: – 55% (– 30%)
- Resolution: + 90% (+ 30%)
- Peak Capacity: + 120% (+ 40%)
- App. Column Eff.: + 260% (+ 70%)

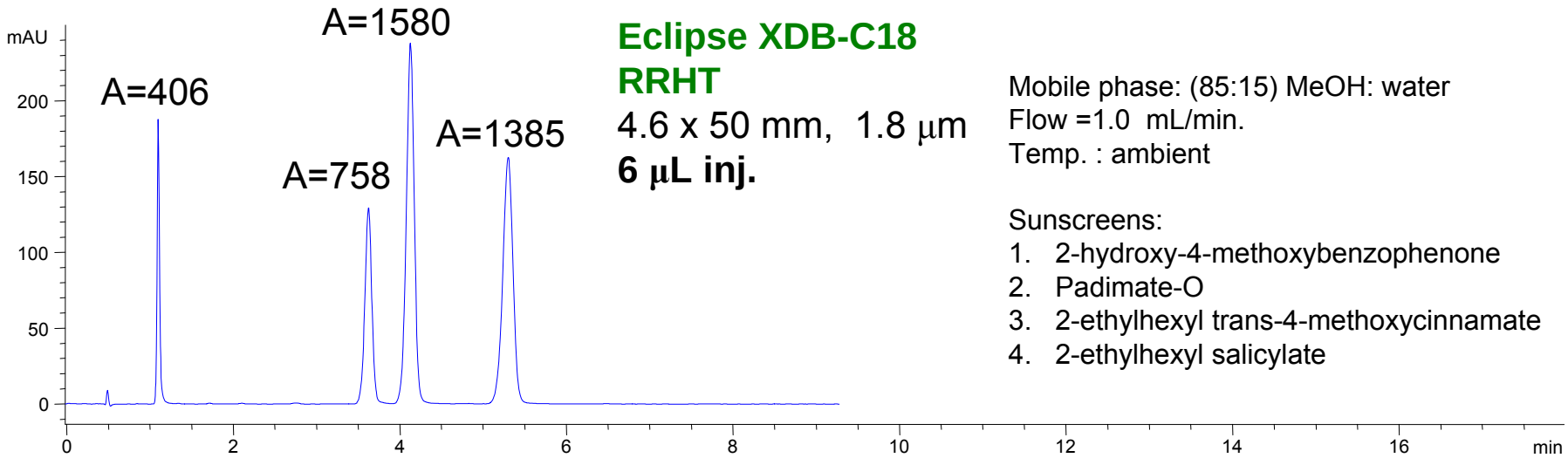
Data Rate	Peak Width	Resolution	Peak Capacity
80 Hz	0.300	2.25	60
40 Hz	0.329	2.05	55
20 Hz	0.416	1.71	45
10 Hz	0.666	1.17	29
5 Hz	1.236	0.67	16

Sample: Phenones Test Mix
Column: Zorbax SB-C18, 4.6x30, 1.8um
Gradient:: 50-100%ACN in 0.3min
Flow Rate: 5ml/min

Another Benefit is Increased Sensitivity (Signal/Noise) Comparing Same Injection Volume: 4.6 x 150 mm, 5 µm vs. 4.6 x 50 mm, 1.8 µm RRHT



Eclipse XDB-C18
4.6 x 150 mm, 5 µm
6 µL inj.



Eclipse XDB-C18
RRHT
4.6 x 50 mm, 1.8 µm
6 µL inj.

Mobile phase: (85:15) MeOH: water
Flow =1.0 mL/min.
Temp. : ambient

Sunscreens:
1. 2-hydroxy-4-methoxybenzophenone
2. Padimate-O
3. 2-ethylhexyl trans-4-methoxycinnamate
4. 2-ethylhexyl salicylate

Bottom Line

- Smaller particle sized columns (1.8 –3.5 µm) combined with shorter lengths (50, 100 mm) of the same diameter, provide similar good chromatographic results as their longer, 5µm sized counterparts, in a fraction of the time. Column efficiency and resolution are maintained due to particle size decreasing proportionally to column length.
- Gradient methods also can be converted to RR and RRHT separations, gradient time must be recalculated proportionally to the column length.
- Another benefit of using RR and RRHT columns is higher sensitivity.
- RR and RRHT columns are available in many configurations. They are ideally suited to easily and quickly improve lab productivity and economy.
- **Most Important Reason – $$$$ Shorter columns with smaller particles can reduce analysis costs significantly!**

Fast Re-Validation of USP Assay

Ibuprofen Oral Suspension

Adjustments vs. Modifications

- **If adjustments to operating conditions are needed, each of the following is the maximum specification that can be considered.**
- **All adjustments falling outside the maximum specifications will be considered as method modifications and will be subject to the method modification protocol.**

ORA Laboratory Procedure, Food and Drug Administration, Document No. ORA-LAB.5.4.5; Version No.:1.2, p. 10, Attachment A – Modification Criteria

HPLC Method Parameters That Can Be Varied

Mobile Phase

The pH of the mobile phase: +/- 0.2 pH units

Concentration of the buffer salts: +/- 10% (buffer pH must remain same +/- 0.2 pH units)

Ratio of the solvents in the mobile phase: +/- 30% relative or +/- 2% absolute, whichever is larger, but no change can exceed 10% (based on mobile phase component of 50% or less) nor can the final concentration of any component be reduced to zero.

Source: ORA Laboratory Procedure, FDA, Document ORA-LAB.5.4.5; Version No.:1.2, Attachment A

HPLC Method Parameters That Can Be Varied

Column

Column length: +/- 70% (e.g., 250 mm columns may be substituted over the range 75 – 425 mm)

Column inner diameter: +/- 50% (if method calls for 3.9 mm id, 3.0, 4.0, or 4.6 mm can be substituted)

Particle size: may be reduced up to 50%
(3 or 3.5 µm particles can be used instead of 5 µm:
1.8um can be used instead of 3 or 3.5um particles.)

Column temperature: +/- 20°C

Source: ORA Laboratory Procedure, FDA, Document ORA-LAB.5.4.5; Version No.:1.2, Attachment A

001820S1.PPT

HPLC Method Parameters That Can Be Varied

System

Flow Rate: +/- 50%

Injection Volume – Increase up to 2x – maintain peak shape, resolution, retention time, etc.

– Decrease as much as will maintain acceptable precision and sensitivity

USP Data Requirements for Method Validation

Parameter	Bulk Drug	Impurities Degradates	Product Performance
Precision	Yes	Yes	Yes
Accuracy	Yes	Yes	Maybe
Limit of Detection	No	No	Maybe
Limit of Quantitation	No	Yes	Maybe
Specificity/Selectivity	Yes	Yes	Maybe
Range	Yes	Yes	Maybe
Linearity	Yes	Yes	Maybe
Ruggedness	Yes	Yes	Yes

HPLC Method Parameters That Can Be Varied

Mobile Phase

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Concentration of the buffer salts: ± 10%
(buffer pH must remain same ± 0.2 pH units)

Ratio of the solvents in the mobile phase: +/- 30% relative or +/- 2% absolute, whichever is larger, but no change can exceed 10% (based on mobile phase component of 50% or less) nor can the final concentration of any component be reduced to zero.

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Column inner diameter: ± 50% (if method calls for 3.9 mm id, 3.0, 4.0, or 4.6 mm can be substituted)

Particle size: may be **reduced up to 50%** (e.g., 3 or 3.5 µm particles can be used instead of 5 µm; 1.8 µm can be used instead of 3 or 3.5 µm particles.)

Changes in particle size greater than 50% requires revalidation

Column temperature: ± 20°C

Source: ORA Laboratory Procedure, FDA, Document ORA-LAB.5.4.5; Version No.:1.2, Attachment A

HPLC Method Parameters That Can Be Varied

System

Flow Rate: ± 50%

Injection Volume

- **May Increase up to 2x**
  - Must **maintain peak shape, resolution, retention time, etc.**
- **May decrease** as much as desired while maintaining acceptable precision and sensitivity (meet system suitability)

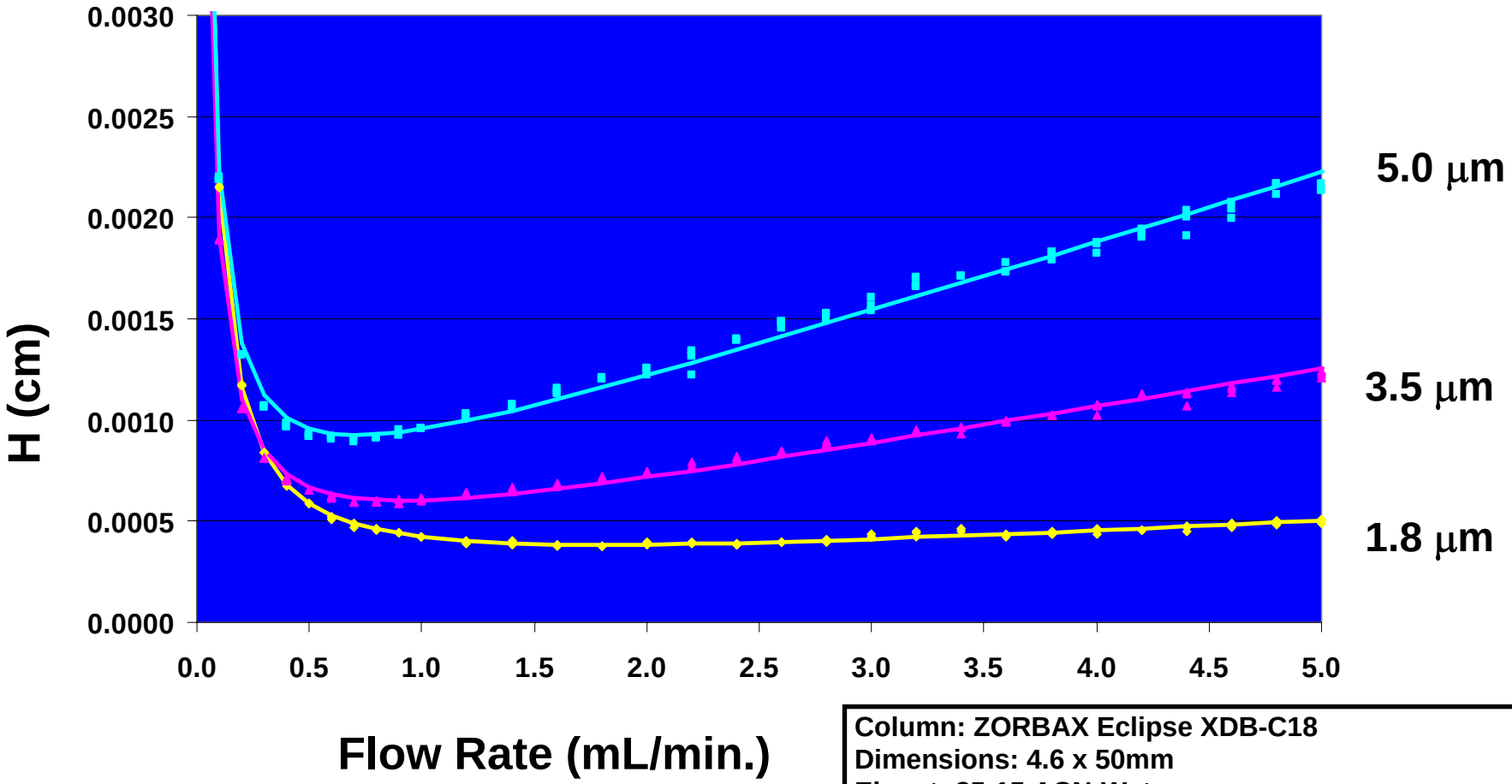
USP Assay
Ibuprofen Oral Suspension

Mobile Phase:	**37% Acetonitrile** **63% Water (0.6 mL/L Phosphoric acid)** **Column: 4.6 x 150 mm, 5 um, L7 (C8)**
Flow Rate:	**2 mL/min**
Injection Volume:	**5 uL**
Detection:	**UV 220 nm**
System Suitability:	**Ibuprofen and Benzophenone (ISTD) Solution**
Specifications:	**R_s > 1.5 b/w IBU & ISDT, T_f < 2.0**

Modifications for Faster Analysis Ibuprofen Oral Suspension

Mobile Phase:	**38% Acetonitrile: 62% Water with 0.6 mL/L H3PO4**
Column:	**4.6 x 50 mm, 1.8um, L7 (C8)**
Flow Rate:	**2 mL/min**
Injection Volume:	**1.7 uL**
Detection:	**UV 220 nm**
System Suitability:	**Ibuprofen and Benzophenone (ISTD) Solution**
Specifications:	**R_s > 1.5 b/w IBU & ISDT, T_f < 2.0**

Van Deemter plots show minimal shift to higher flow rates for smaller particle sizes.



Column: ZORBAX Eclipse XDB-C18
Dimensions: 4.6 x 50mm
Eluent: 85:15 ACN:Water
Temp: 20°C
Sample: 1.0µL Octanophenone in Eluent

Recommendations

- **Select a Rapid Resolution High Throughput L7 column to minimize analysis time and maintain resolution.**
- **Use a proper buffer and make the pH adjustment to the aqueous portion alone, but keep the mobile phase as similar as possible to maintain expected chromatographic behavior.**

Method Validation Requirements

Robustness

Linearity

Accuracy

Precision

Range

Ruggedness

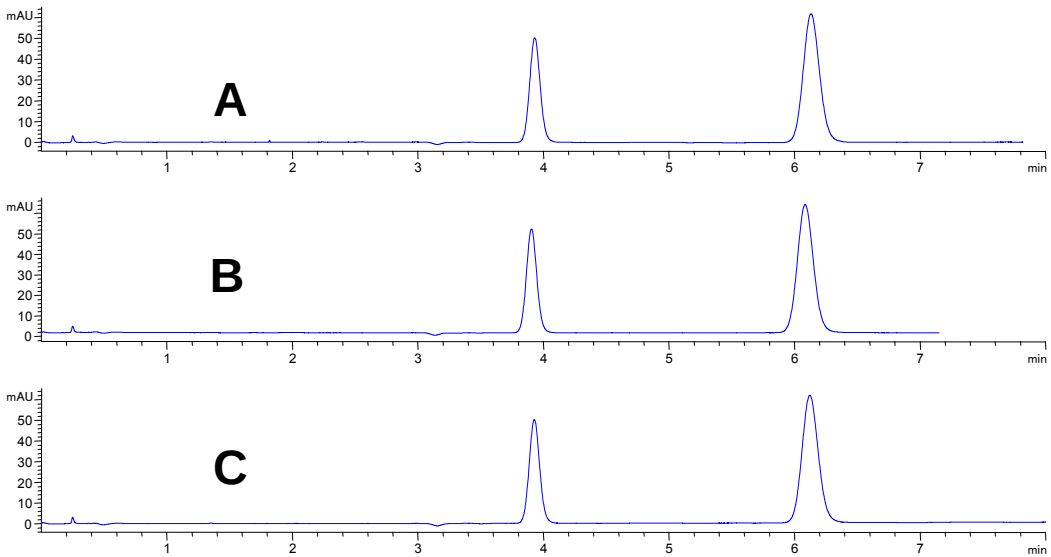
Robustness Testing

- **Use Modified Ibuprofen USP Assay method as example**
- **Vary Key Method Parameters - meet or exceed method adjustment recommendations**
- **Use System Suitability Mixture ibuprofen/benzophenone and focus on behavior of**

    1. **pH**
    2. **% Organic**
    3. **Column Lot**
    4. **Temperature**

Robustness- pH

Column: XDB-C8, 4.6 x 50 mm, 1.8 µm Mobile Phase: 68% 0.01M H3PO4 : 32% ACN Flow Rate: 2.0 mL/min
Temperature: 25C Sample: 1. Benzophenone 1 mg/mL 2. Ibuprofen 1.2 mg/mL Injection Volume: 1.7 µL

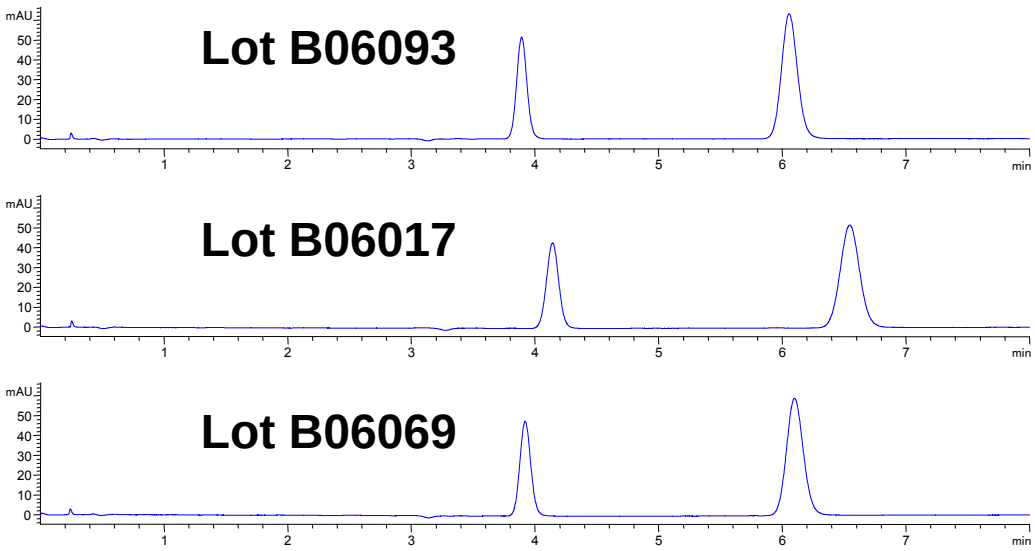


	mL acid	α	T_f
A	**0.4**	**1.60**	**1.09**
B	**0.7**	**1.60**	**1.08**
C	**0.8**	**1.60**	**1.09**

- Tested 3 pHs-mobile phase variation
- Monitor for changes in selectivity (α) and peak shape (T_f) of ibuprofen

Robustness- Column Lot

Column: XDB-C8, 4.6 x 50 mm, 1.8 μm Mobile Phase: 68% 0.01M H3PO4 : 32% ACN Flow Rate: 2.0 mL/min
Temperature: 25C Sample: 1. Benzophenone mg/mL 2. Ibuprofen .005 mg/mL Injection Volume: 1.7 μL



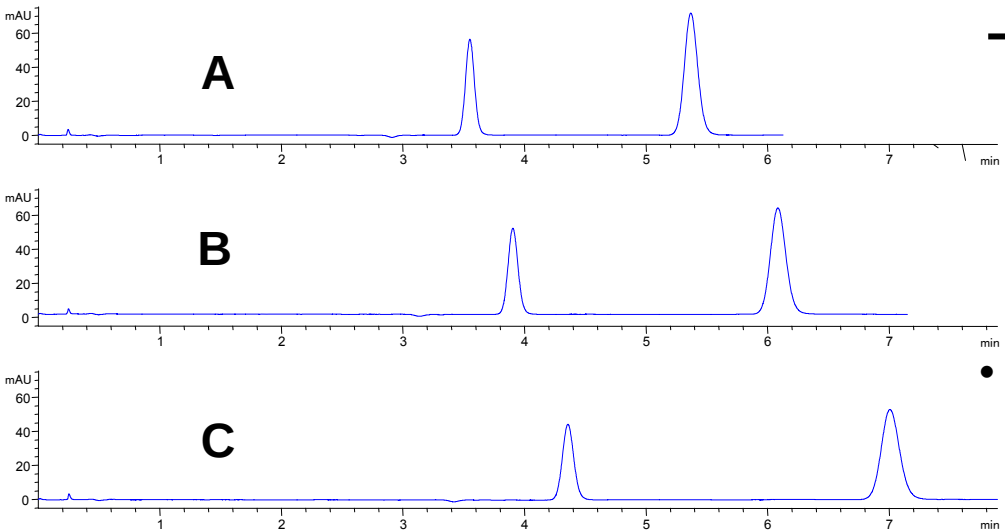
- Compare three current lots of material for consistency of retention (k) and selectivity (α).

Three Lot Summary

	Mean	SD	RSD
k (bp)	15	0.55	3.7%
k (ibu)	24	1.10	4.6%
α	1.60	0.02	1.1%

Robustness- % Organic

Column: XDB-C8, 4.6 x 50 mm, 1.8 µm Mobile Phase: X% 0.01M H3PO4 :Y% ACN Flow Rate: 2.0 mL/min
Temperature: 25C Sample: 1. Benzophenone mg/mL 2. Ibuprofen mg/mL Injection Volume: 1.7 µL

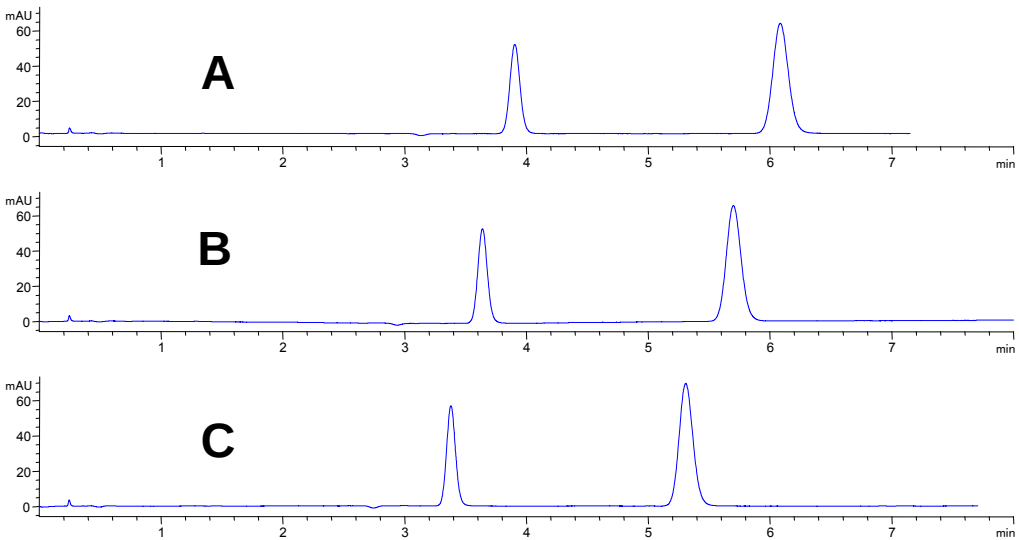


	% ACN	R_t	k	R_s
A	39	5.4	20.5	10.6
B	38	6.1	23.4	11.3
C	37	7.0	27.1	11.7

- **Expect retention, selectivity and resolution to change with change in % organic.**
- **Determine which % organic meets needs (adequate retention, resolve matrix components. most robust) without wasting time.**

Robustness- Temperature

Column: XDB-C8, 4.6 x 50 mm, 1.8 µm Mobile Phase: 68% 0.01M H3PO4 : 32% ACN Flow Rate: 2.0 mL/min
Temperature: see below Sample: 1. Benzophenone mg/mL 2. Ibuprofen .005 mg/mL Injection Volume: 1.7 µL

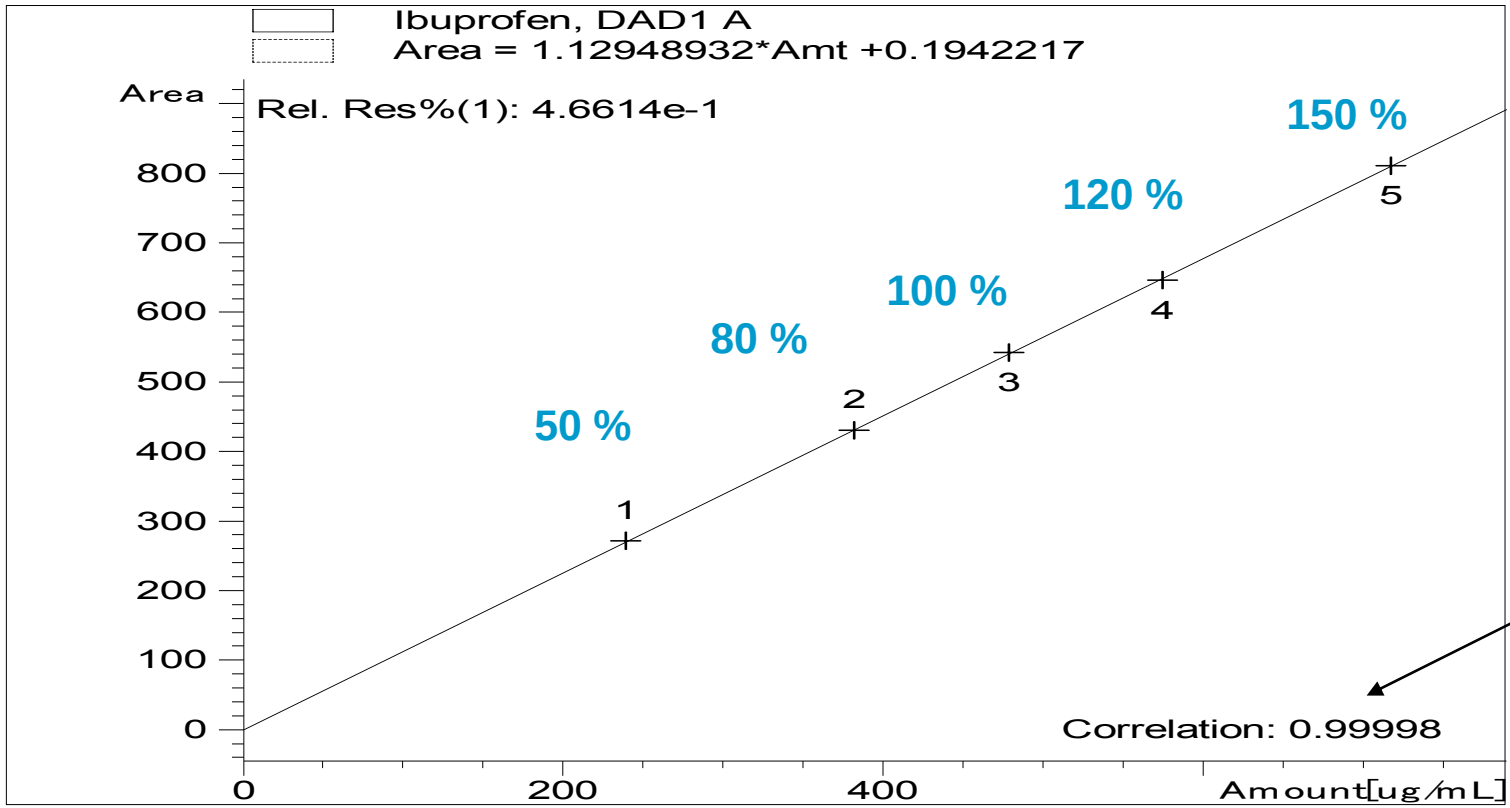


	°C	α	T_f
A	**25**	**1.60**	**1.08**
B	**30**	**1.61**	**1.09**
C	**35**	**1.62**	**1.10**

- Tested 3 temperatures – Room Temperature, 30°C and 35°C
- Monitor for changes in selectivity (α) and peak shape (T_f) of ibuprofen

Linearity

How? Regression analysis of test results vs. analyte concentration. For the "bulk substance" type of samples we must cover a range of 80 - 120% of the expected concentration.



Accuracy

How? Calculate % recovery of known amounts added to samples – above and below expected levels. We tested the ICH* recommended 3 replicates at 3 different levels – one above and two below.

Results

Level	Accuracy
120%	100.6 +/- 0.7%
100%	100.5 +/- 0.7%
80%	99.7 +/- 0.9%

* ICH - International Conference on Harmonization

Precision/Repeatability

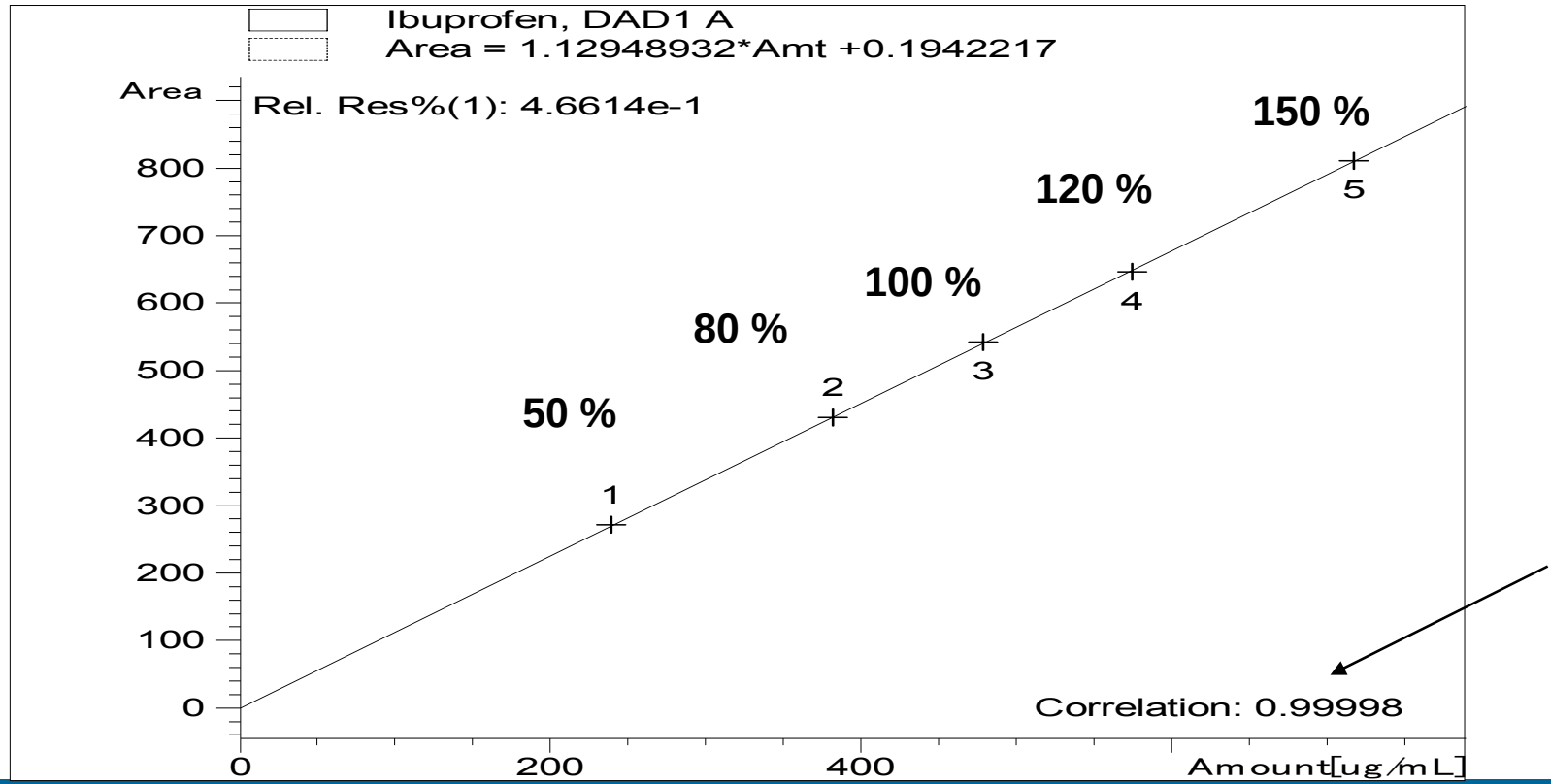
How? Calculate (relative) standard deviation of a sufficient number of sample aliquots. This can be from three levels three repetitions or 6 determinations at 100%.

Results

Level	SD	RSD
150%	0.14	0.2%
100%	0.74	0.7%
50%	0.63	0.8%

Range

How? Verify acceptable precision, accuracy, and linearity at the ends of the range and within the range. Our tested range went up to the 150% level. Therefore we need to verify linearity, accuracy, and precision at this level, in addition to those done previously.



Ruggedness/Reproducibility

How? Multiple chemists in multiple labs run samples. Results should be reproducible and can be compared to method precision.

Result – Samples were run in 3 labs by 3 chemists on 3 different instruments.

Level	Chemist 1 Accuracy/RSD	Chemist 2 Accuracy/RSD	Chemist 3 Accuracy/RSD
80%	98.6 +/- 0.3%	99.2 +/- 0.5%	99.7 +/- 0.9%
100%	100.1 +/- 0.8%	99.5 +/- 0.2%	100.5 +/- 0.7%
120%	98.2 +/- 0.7%	100.5 +/- 0.2%	100.6 +/- 0.7%
Overall	99.0 +/- 0.6%	99.7 +/- 0.3%	100.3 +/- 0.8%
Method	99.7 +/- 0.6%		

Determining System Suitability Specifications

- **What type of variation do you see normally and how much leeway do you want?**
- **What makes accurate chromatographic results possible?**
- **Try to account for column degradation and insufficiently tested methods.**
- **Use statistical calculations, if possible, for setting sys. suit. criteria for resolution, tailing factor, repeatability.**
- **Do not use absolute retention time or retention factor (k) for analytes**

System Suitability

Setting System Suitability Specifications:

- **Tailing Factor < 2.5 (allows for higher sample load)**
- **Resolution > 2.0 (allows for method variation and column aging)**
- **RSD of replicate injections < 2.0% (checks system performance)**

Rapid Resolution Columns Can Reduce Costs by up to 75% - Cost Summary (Cost/Analysis)

Cost	4.6 x 250 mm, 5 um	4.6 x 75 mm, 3.5 um	4.6 x 50 mm, 1.8 um
Column	$500	$450	$450
Labor ($30/hr)	$6.00 (12 min)	$1.80 (3.6 min)	$1.50 (2.5 min)
Solvent ($27/l)	$0.24 (8.8 mL)	$0.07 (2.6 mL)	$0.03 (1.7 mL)
Disposal ($2/l)	$0.02	< $0.01	< $0.01
Total	$6.26	$1.87	$1.54

- Short columns can reduce analysis costs by 70 - 75%

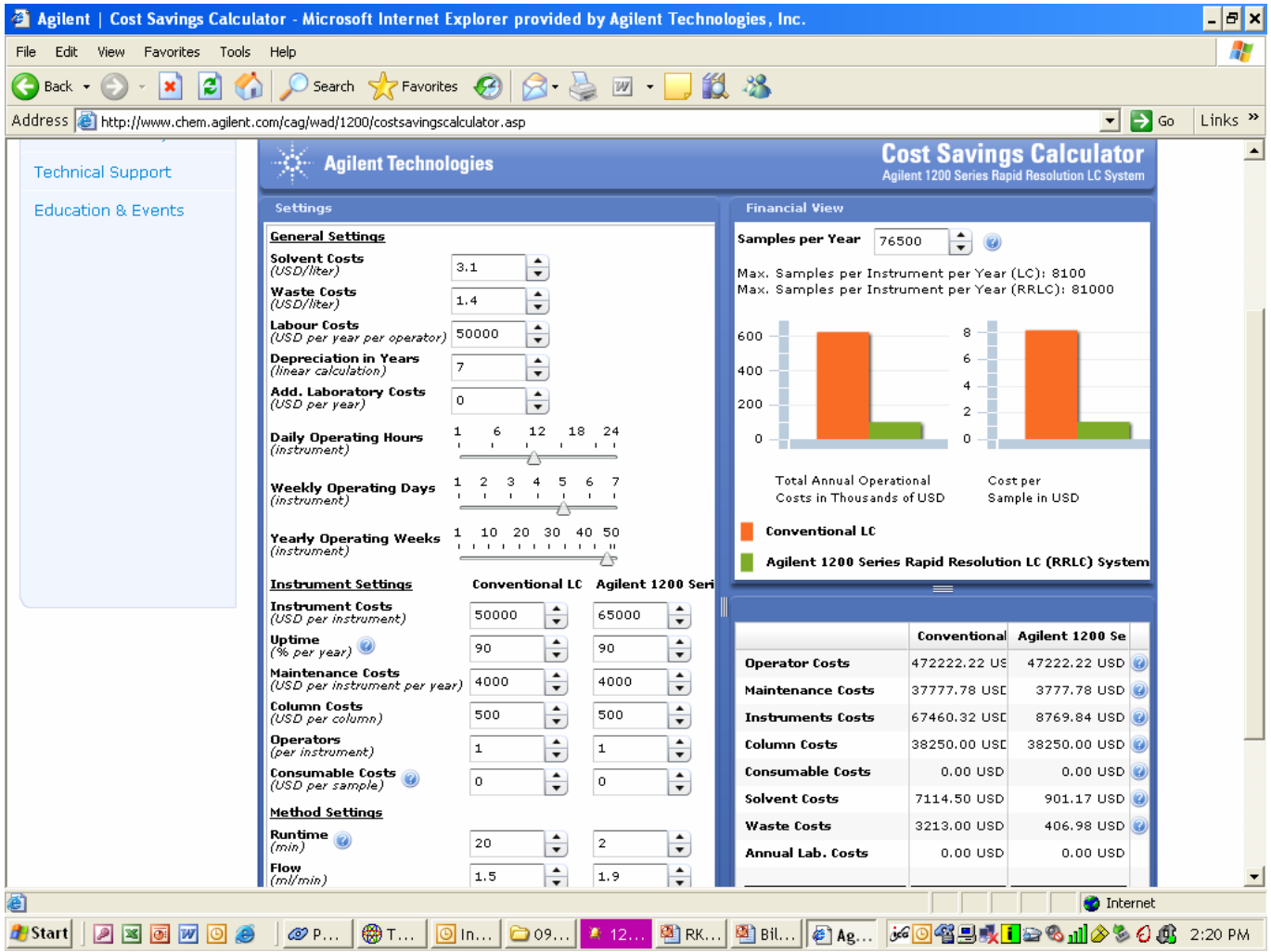
Costs Associated with Updating a Method (Cost/Analysis + Revalidation)

Cost	4.6 x 250 mm, 5 um	4.6 x 50 mm, 1.8 um	4.6 x 30 mm, 1.8 um
Column	$500	$450	$400
Labor ($30/hr)	$6.00 (12 min)	$1.35 (2.7 min)	$0.85 (1.7 min)
Solvent ($27/l)	$0.32 (12 mL)	$0.07 (2.7 mL)	$0.03 (1.7 mL)
Disposal ($2/l)	$0.02	< $0.01	< $0.01
Total for 1000 samples	$6840	$1870	$1280
Update (2 weeks $48/hour)	$0	$3850	$3850
Total	$6840	$5720	$5130

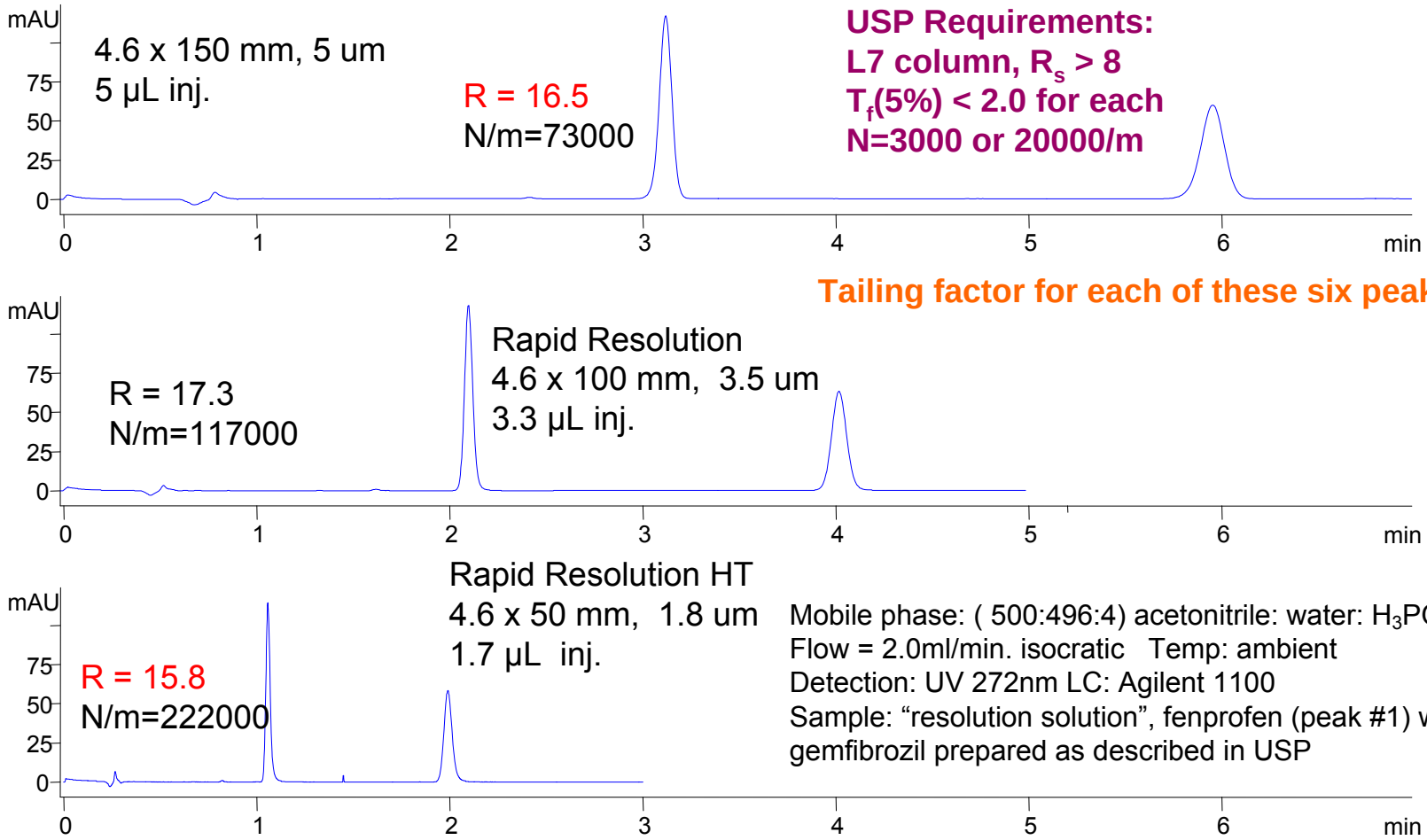
- ***IF*** **more than 900 samples will be analyzed, updating a method to a fast analysis is cost effective.**

Cost Savings Calculator

http://www.chem.agilent.com/cag/wad/1200/costsavingscalculator.asp



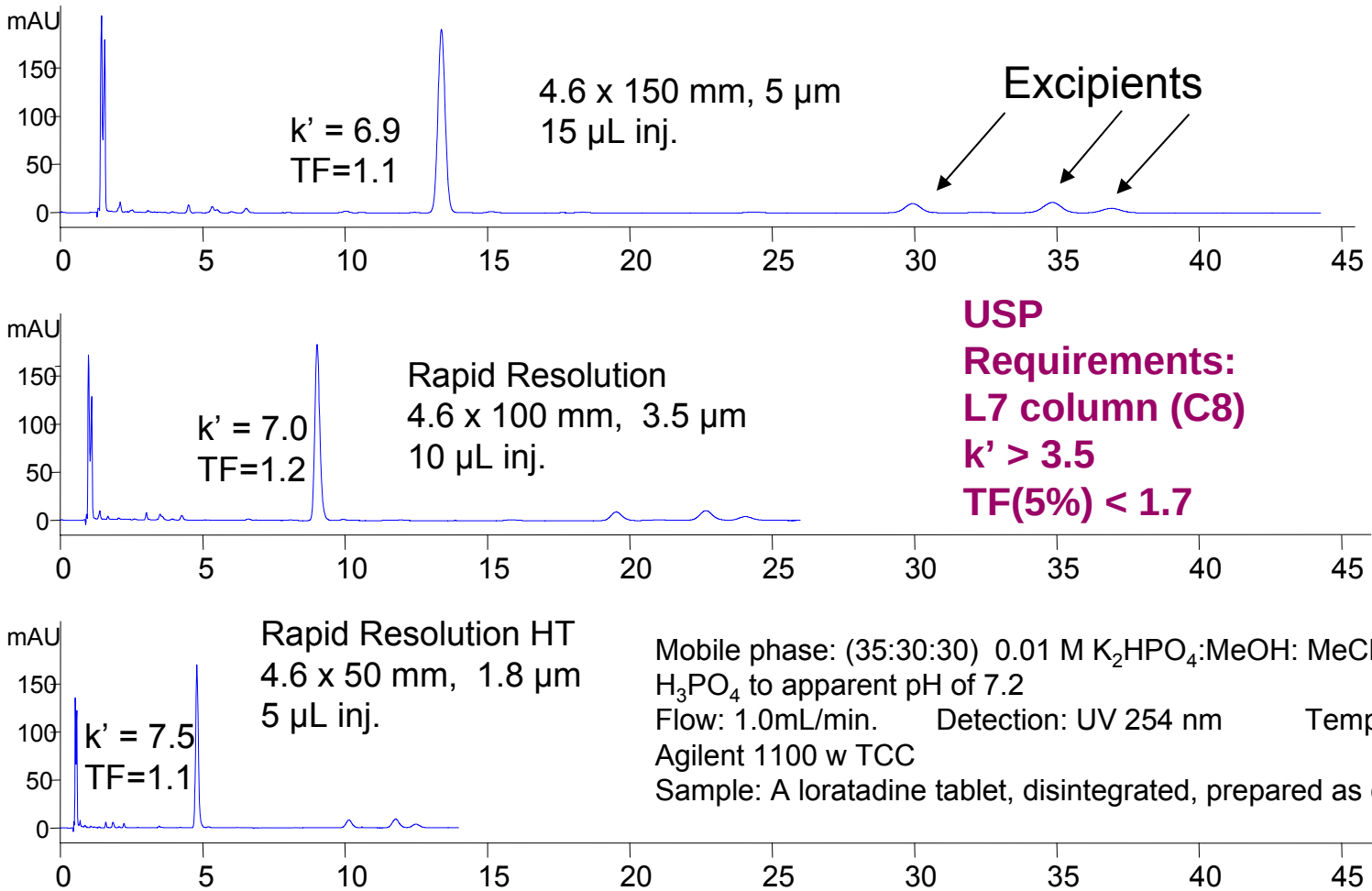
Update USP Assay for Fenprofen Calcium
(non-steroidal anti-inflammatory (NSAID))



- **High resolution and exceptional efficiency maintained for low cost updating to fast LC methods**

Updating Methods: Maintain Retention

USP Assay for Loratadine Tablets on Eclipse XDB-C8



USP Requirements:
L7 column (C8)
k' > 3.5
TF(5%) < 1.7

Mobile phase: (35:30:30) 0.01 M K_2HPO_4:MeOH: MeCN adjust with H_3PO_4 to apparent pH of 7.2
Flow: 1.0mL/min. Detection: UV 254 nm Temp.: 30ºC LC: Agilent 1100 w TCC
Sample: A loratadine tablet, disintegrated, prepared as described in USP

- **No extra time costs when bonded phase performance matches with different particle size columns**

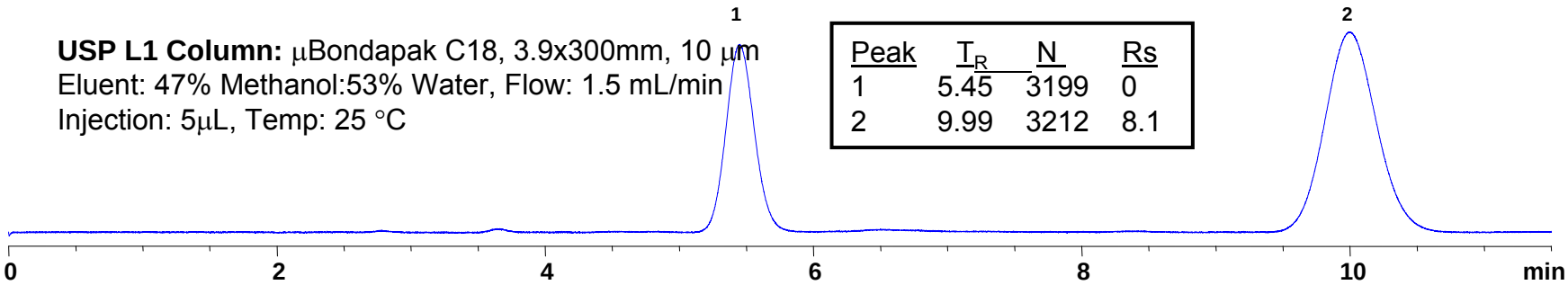
Updating Existing Methods with Rapid Resolution HT Columns

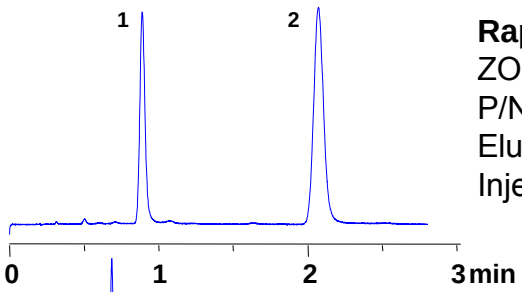
USP Analysis of Triamcinolone

Sample: 1. Triamcinolone - 0.2 µg/µL, 2. Hydrocortisone - 0.3 µg/µL
Minimum Resolution Required = 3.0

USP L1 Column: µBondapak C18, 3.9x300mm, 10 µm
Eluent: 47% Methanol:53% Water, Flow: 1.5 mL/min
Injection: 5µL, Temp: 25 °C

Peak	T_R	N	Rs
1	5.45	3199	0
2	9.99	3212	8.1

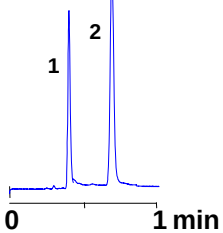




Rapid Resolution HT Column (L1)
ZORBAX Eclipse XDB-C18: 4.6x30mm, 1.8µm
P/N 923975-902
Eluent: 47% Methanol:53% Water, Flow: 1.5 mL/min
Injection: 1µL, Temp: 25 °C

Peak	T_R	N	Rs
1	0.89	3256	0
2	2.07	4851	11.8

This is a 90% change in length and 16% decrease in i.d.



Rapid Resolution HT Column (L1)
ZORBAX Eclipse XDB-C18: 4.6x30mm, 1.8µm
P/N 923975-902
Eluent: 60% Methanol:40% Water, Flow: 1.5 mL/min
Injection: 1µL, Temp: 25 °C

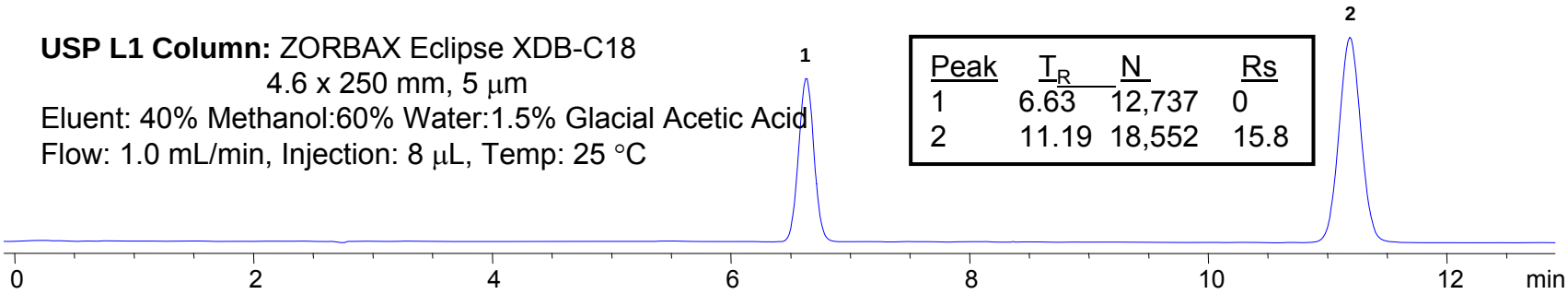
Peak	T_R	N	Rs
1	0.40	2991	0
2	0.69	4025	6.9

Update Existing Methods with Rapid Resolution HT Columns

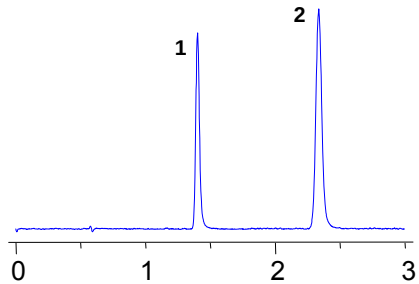
USP Analysis of Guaifenesin

Sample: 1. Guaifenesin – 0.04 µg/µL, 2. Benzoic Acid – 0.10 µg/µL

USP L1 Column: ZORBAX Eclipse XDB-C18
4.6 x 250 mm, 5 µm
Eluent: 40% Methanol:60% Water:1.5% Glacial Acetic Acid
Flow: 1.0 mL/min, Injection: 8 µL, Temp: 25 °C

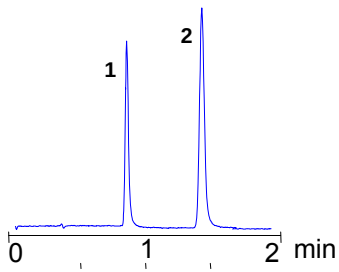


Peak	T_R	N	Rs
1	6.63	12,737	0
2	11.19	18,552	15.8



Rapid Resolution HT Column (L1)
ZORBAX Eclipse XDB-C18, 4.6x50mm, 1.8 µm
Eluent: 40% Methanol:60% Water:1.5% Glacial Acetic Acid
Flow: 1.0 mL/min, Injection: 2 µL, Temp: 25 °C

Peak	T_R	N	Rs
1	1.40	11,421	0
2	2.33	12,909	12.3



Rapid Resolution HT Column (L1)
ZORBAX Eclipse XDB-C18, 4.6x30mm, 1.8 µm
Eluent: 40% Methanol:60% Water:1.5% Glacial Acetic Acid
Flow: 1.0 mL/min, Injection: 2 µL, Temp: 25 °C

Peak	T_R	N	Rs
1	0.85	5,855	0
2	1.43	7,300	8.6

Conclusions

New suggested guidelines may make it easier to determine what is a method “adjustment” to meet system suitability.

When needed method modifications exceed “adjustments” then method validation is required.

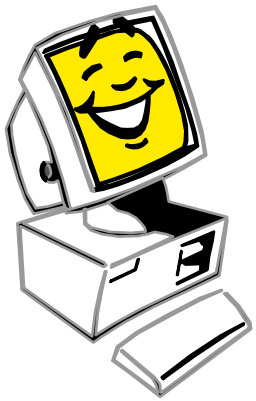
Method validation requires experimentation to verify that a method will meet the required analytical needs.

Agilent Technical Support

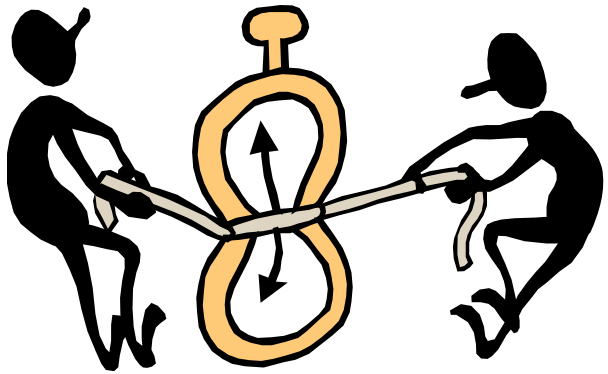
LC or GC Column Support

800-227-9770 (phone: US & Canada)

Select option 4, then option 1 for GC or option 2 for LC.



www.agilent.com/chem



The End – Thank You!

Agilent LC Column Tech Support: 800-227-9770 #4, #2

References

1. "System Suitability Test in Regulatory Liquid and Gas Chromatographic Methods: Adjustments Versus Modifications", William B. Furman, John G. Dorsey, and Lloyd R. Snyder, Pharmaceutical Technology, June 1998 p. 58-64

2. "Guidance for Industry; Analytical Procedures and Methods Validation: Chemistry, Manufacturing, and Controls Documentation", Draft Guidance, August 2000. View at http://www.fda.gov/cber/guidelines.htm

3. Mobile-Phase Buffers, Part I — The Interpretation of pH in Partially Aqueous Mobile Phases, LC GC North America, Volume 20, Number 11, November 2002, pp 1028ff.

4. Retention of ionisable compounds on high-performance liquid chromatography,
   XV. Estimation of the pH variation of aqueous buffers with the change of the acetonitrile fraction of the mobile phase, Journal of Chromatography A, 1059 (2004) 33–42

Over100 Configurations of Rapid Resolution & Rapid Resolution High Throughput Columns Available

Bonded Phases

ZORBAX Eclipse Plus-C8
ZORBAX Eclipse Plus-C18

ZORBAX StableBond-C18
ZORBAX StableBond-C8
ZORBAX StableBond-Phenyl
ZORBAX StableBond-CN
ZORBAX StableBond-AQ

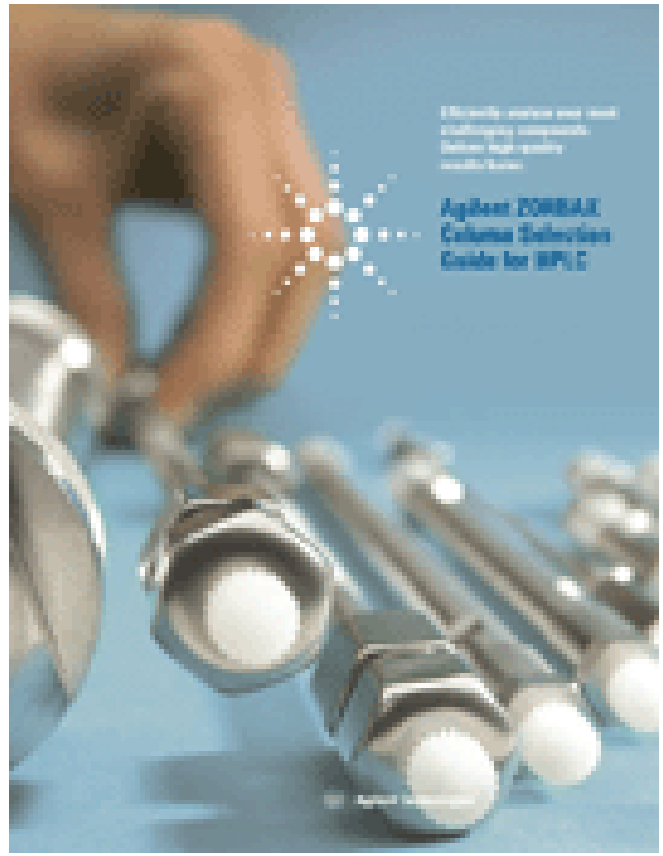
ZORBAX Eclipse XDB-C8
ZORBAX Eclipse XDB-C18

ZORBAX Rx-SIL

Diameters (mm)
2.1, 3.0, 4.6

Particle Sizes (µm)
1.8, 3.5

Lengths (mm)
30, 50, 100, 150



Separation Ruggedness
Buffer Preparation

Dissolve salt in organic-free water in 1- or 2-L beaker. Use appropriate volume to leave room for pH adjustment solution. Equilibrate solution to room temperature for maximum accuracy.

Calibrate pH meter. Use 2-level calibration and bracket desired pH. Use appropriate audit solution to monitor statistical control (for example, potassium hydrogen tartrate, saturated solution, pH = 3.56).

Adjust salt solution to desired pH. Minimize amount of time electrode spends in buffer solution (contamination). Avoid overshoot and readjustment (ionic strength differences can arise).

Transfer pH-adjusted buffer solution quantitatively to volumetric flask, dilute to volume, and mix.

Filter through 0.45 µm filter. Discard first 50 – 100 mL filtrate. Rinse solvent reservoir with small volume of filtrate and discard. Fill reservoir with remaining filtrate or prepare premix with organic modifier.

- Agilent Solvent Filtration Kit, 250-mL reservoir, 1000-mL flask, p/n 3150-0577
- Nylon filter membranes, 47 mm, 0.45 µm pore size, p/n 9301-0895

Using Buffers Successfully
Initial Column and System Equilibration

In an appropriate vessel, test highest % organic/buffer ratio to verify that buffer will not precipitate. With stirring, add organic to buffer first, not vice versa.

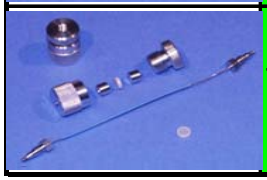
Equilibrate column with, in order:

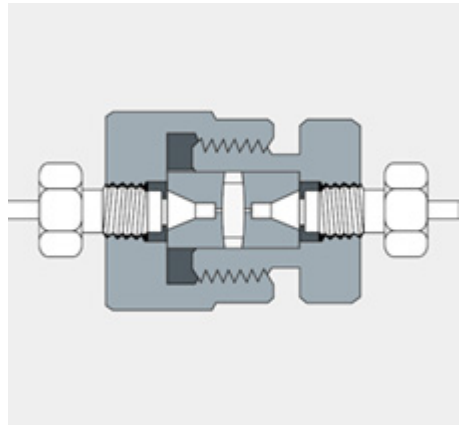
- **100% organic modifier (if brand new)**
- **mobile phase minus buffer**
- **buffered mobile phase containing highest % organic modifier (gradient high end)**
- **buffered mobile phase containing lowest % organic modifier (gradient low end).**

Inject standard or sample several times until RTs stable, or for gradient methods, precede former with 1 or 2 blank gradients.

In-Line Filters Provide Good Insurance Against System OverPressure

NEW RRLC In-line filter and fitting – max 600 bar

	Description	Part number	Porosity	Frit diameter	Flow rate	Part number Replacement Frits
	**RRLC In-line filter, 2.1 mm, max 600 bar**	**5067-1551**	0.2 µm	2.1 mm	<1 mL/min	**5067-1555 (10/pk)**
	**RRLC In-line filter, 3.0 & 4.6 mm, max 600 bar**	**5067-1553**	0.2 µm	4.6 mm	1 - 5 mL/min	**5067-1562 (10/pk)**



Protect RRHT columns with efficient in-line filter with 0.2 µm pore size frits

Determining the Dwell Volume of Your system

Replace column with short piece of HPLC stainless steel tubing

Prepare mobile phase components

A. water	- UV-transparent
B. water with 0.2% acetone	- UV-absorbing

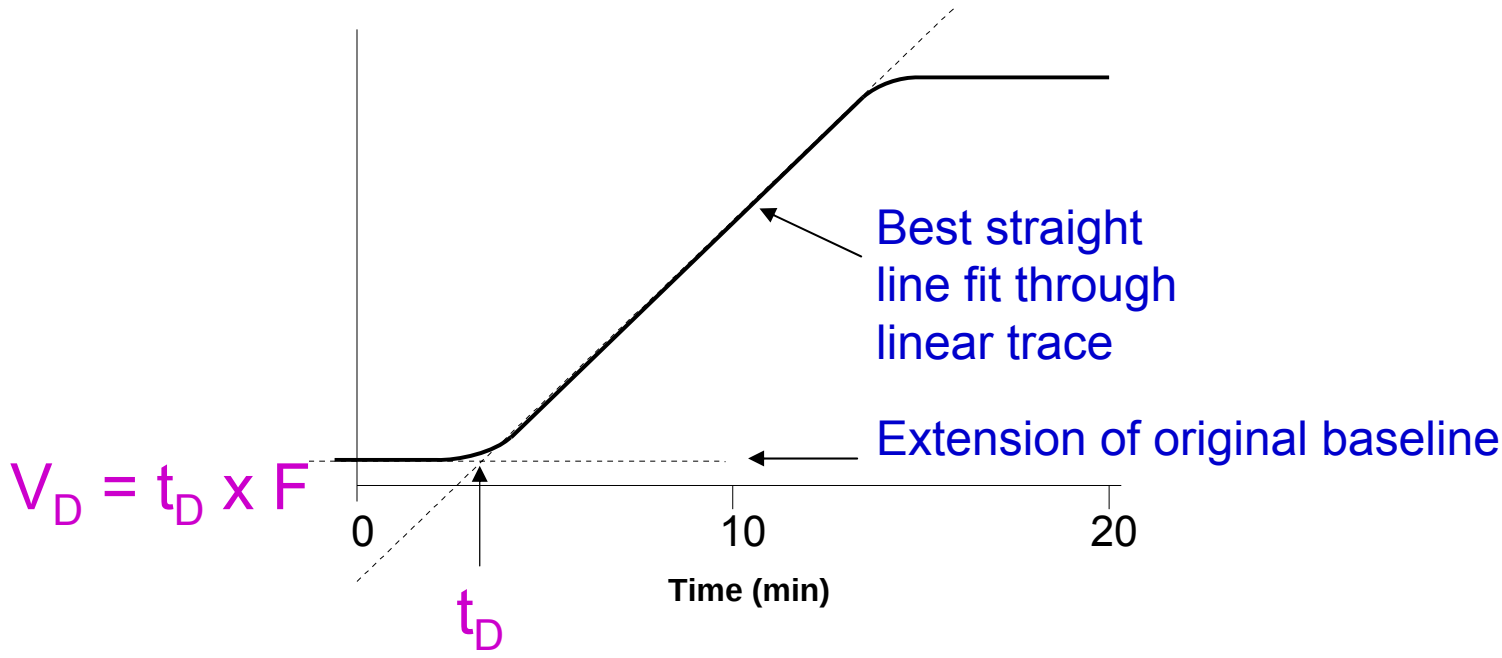
Monitor at 265 nm

Adjust attenuation such that both 100% A and 100% B are on scale

Run gradient profile 0 - 100% B/10 min at 1.0 mL/min

Record

Measuring Dwell Volume (V_D)



- Intersection of the two lines identifies dwell time (t_D)
- Dwell volume is equal to product of the flow rate and the dwell time.