How to optimize your chromatography

Factors influencing Speed and Resolution

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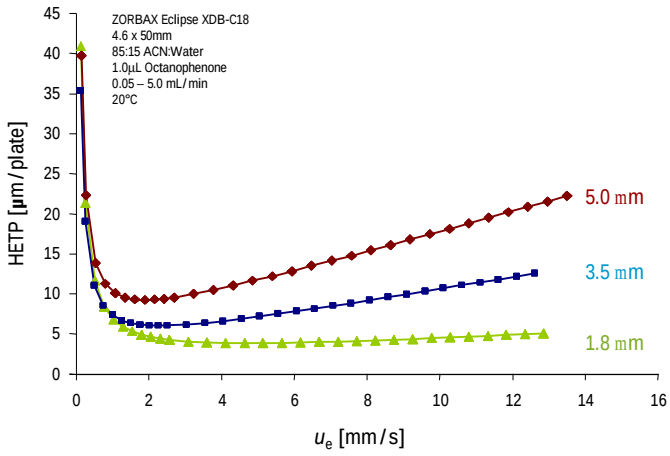
Factors to improve

- Speed
  - Run-time
  - Cycle-time
- Resolution
- Costs
  - Labor costs
  - Instrument costs
  - Solvent consumption
  - Waste production

The Effect of Particle Size

Van-Deemter curve for different particle sizes



Particle	H_{min}
5.0 µm	9.3 µm
3.5 µm	6.0 µm
1.8 µm	3.8 µm

The Effect of Particle Size

Reducing the particle size will yield a…

- **à** decreased H_{min} (better efficiency)
- **à** at higher u (higher speed)
- **à** with decreased slope (less loss of efficiency when increasing u)

The Effect of Particle Size

Development of stationary phases

Year	Particle size		Plates / 50 mm
1969		100 µm	170
1973		57 µm (pellicular)	350
1975		10 µm	2,000
1985		5.0 µm	4,000
1992		3.5 µm	7,500
2003		< 1.8 µm	12,000

The Effect of Particle Size

The big drawback of using smaller particles:

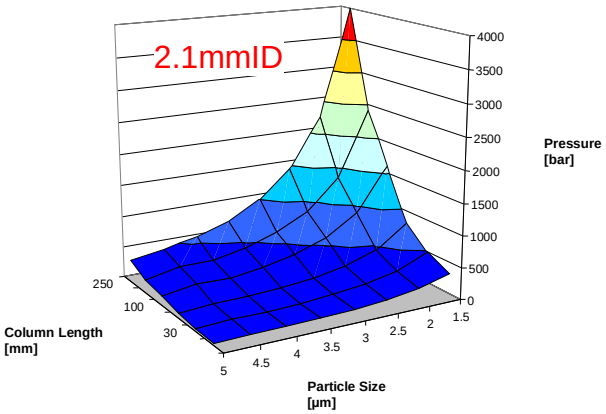
Pressure increases with the square of $1/d_p$

$$\Delta p \propto \frac{4 \cdot F \cdot L_c \cdot h}{p d_c^2 \cdot d_p^2}$$

F = flow, L_c = column length, h = viscosity, d_c = column diameter

The Effect of Particle Size

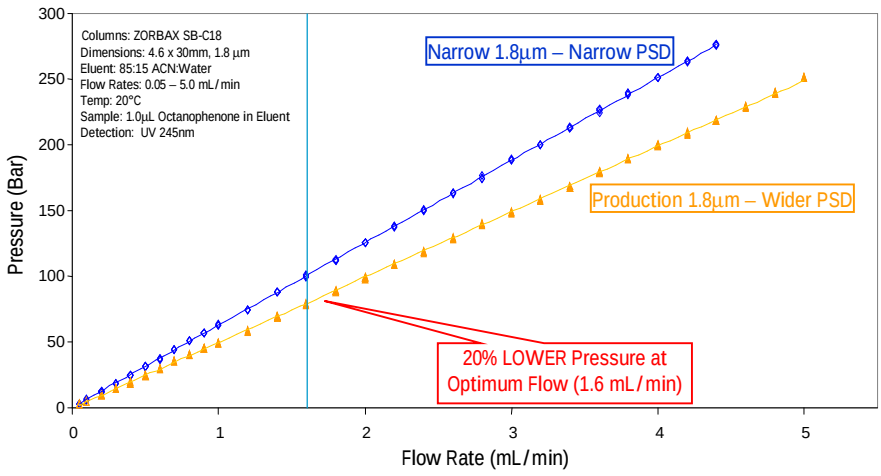
Pressure dependence of length, diameter and particle size:



Flow = 1 mL/min, viscosity = 0.75 cP

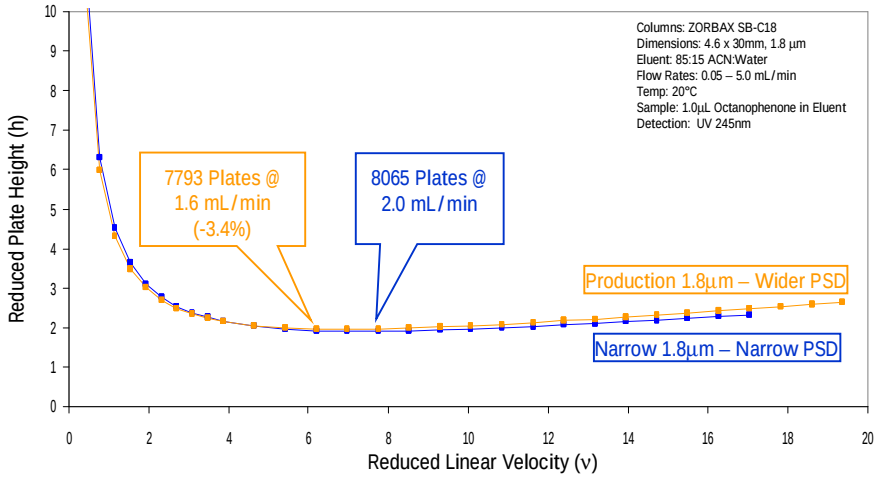
The Effect of Particle Size

Tricks to overcome this – optimize the particle size distribution (PSD)



The Effect of Particle Size

Tricks to overcome this – optimize the particle size distribution (PSD)



How to use smaller particle sizes to improve existing method?

- *Use a shorter column:*
  Compounds will **elute earlier** but with **preserved resolution** (higher efficiency N of smaller particles).
  Also, the linear velocity can be increased more than with larger particles because lower loss of efficiency with increasing velocity of smaller particles.
- *Keep column length constant:*
  Resolution R_S will increase with same retention time.

$$N \propto \frac{1}{d_p} \qquad R_S \propto \sqrt{N}$$

How to increase the speed of a conventional method?

Increasing speed

- *Length scaling – to preserve efficiency adjust the particle size*

$$N = \frac{L_{c1}}{d_{p1}} = \frac{L_{c2}}{d_{p2}} \quad \Rightarrow \quad L_{c2} = \frac{L_{c1} \cdot d_{p2}}{d_{p1}}$$

Example – present method running on a 250 x 4.6mm, 5.0µm:

$$L_{c2} = \frac{250mm \cdot 1.8 \text{ 价}}{5 \text{ 价}} = 90mm$$

So, use the closest available size – a 100 x 4.6mm, 1.8µm column

Increasing speed

- *Length scaling – adjust the time*

$$\frac{L_{c2}}{L_{c1}} = \frac{t_2}{t_1} \quad \Rightarrow \quad t_2 = \frac{L_{c2} \cdot t_1}{L_{c1}}$$

Example – scaling a 30min-method from a 250 mm to 100 mm column:

$$t_2 = \frac{100mm \cdot 30\,\text{min}}{250mm} = 12\,\text{min}$$

Increasing speed

- *If wanted, scale the diameter, too – but, adjust the flow:*

$$\left(\frac{d_{c2}}{d_{c1}}\right)^2 = \frac{F_2}{F_1} \quad \Rightarrow \quad F_2 = \left(\frac{d_{c2}}{d_{c1}}\right)^2 \cdot F_1$$

Note: With a given max. flow of a LC system a higher linear velocity can only be achieved with a smaller column ID!

Example – scaling a 1.4ml/min method from 4.6 mmID to 3 mmID:

$$F_2 = \left(\frac{3.0mm}{4.6mm}\right)^2 \cdot 1.4\text{ml/ min} = 0.595\text{ml/min}$$

Increasing speed

- *Optimizing the linear velocity (flow)*

  Smaller particles have an increased $\boldsymbol{u_{opt}}$; so, flow should be increased

$$\frac{F_1}{F_2} = \frac{t_2}{t_1} = const. \quad \Rightarrow \quad t_2 = \frac{F_1 \cdot t_1}{F_2}$$

Example – increasing flow from 1.4ml/min to 3.6ml/min:

$$t_2 = \frac{1.4\text{ml/min} \cdot 12\,\text{min}}{3.6\text{ml/min}} = 4.7\,\text{min}$$

Note: if the linear velocity is increased beyond the optimal velocity a reduction in efficiency must be expected!

Increasing speed

- *Optimizing the linear velocity (flow) – cont.*

  For gradient separations the gradient slope $\boldsymbol{s}$ should be preserved

$$s = \frac{\Delta\%B}{(F_1 \cdot t_{g_1})/V_{M1}} = \frac{\Delta\%B}{(F_2 \cdot t_{g2})/V_{M2}}$$

This describes the gradient slope as $D\%B$ per number of column void volumes (V_M).

Increasing speed

- *Finally, adjusting the injection volume*

To achieve same peak heights (LOD, LOQ) the injection volume needs to be scaled according to the column void volumes

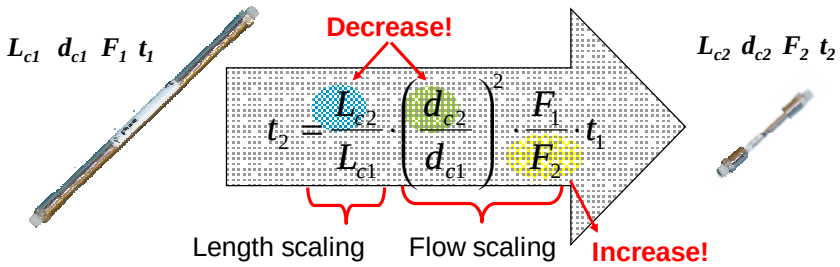
$$\frac{V_{inj2}}{V_{inj_1}} = \frac{V_{M2}}{V_{M1}}$$

Assuming an identical injection volume, with larger columns, i.e. with a bigger void volume, the peaks would dilute more, resulting in a smaller peak height and a lower detection limit.

Same sample amount but different height if the injection volume is not adjusted!

Increasing speed

- *Summary*



And of course: $N = \frac{L_{c1}}{d_{p1}} = \frac{L_{c2}}{d_{p2}} = const.$ **!!!**

$F_2 \le F_{max} = \frac{p d_{c2}^2 \cdot d_{p2}^2 \cdot p_{max}}{4 \cdot \Phi \cdot L_{c2} \cdot h}$

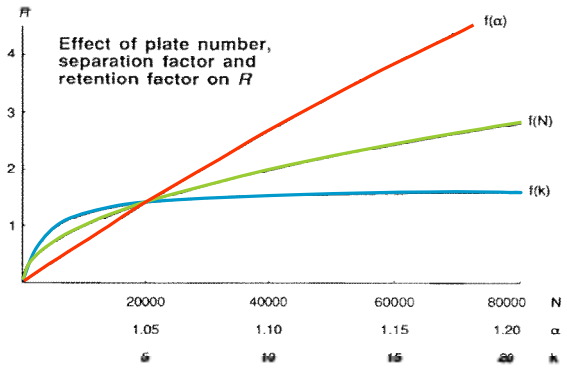
Max. flow F_{max} dependent on max. system pressure p_{max}, Column ID d_c & Length L_c, Particle size d_p, Viscosity h and the flow resitance factor F of the column

How to increase the resolution of a conventional method?

Increasing Resolution

- *The resolution equation:*

$$R_s = \frac{\sqrt{N}}{4} \cdot \left[\frac{\alpha - 1}{\alpha}\right] \cdot \left[\frac{k_2}{k_2 + 1}\right]$$



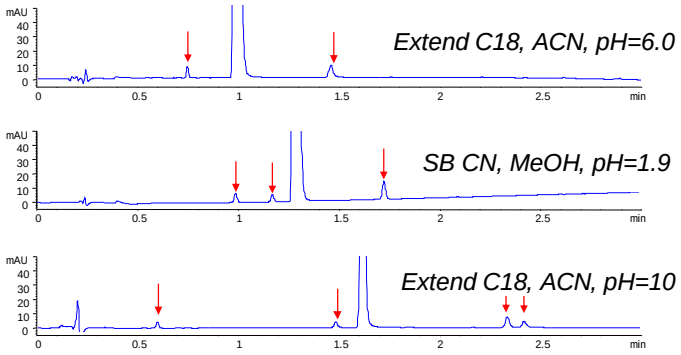
Increasing Resolution

Selectivity (α) *helps best but:*

- Is difficult to predict (experience helps, model retention)
- Requires, software supported, optimization for separation of multi-component mixtures (*ChromSword, DryLab*)

Example:

Same sample, analyzed with different conditions but always same temperature and 5-95%B gradient in the same time.



Increasing Resolution

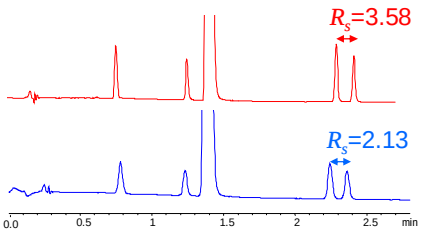
High ***plate number (N)*** *by decreasing the particle size of a column provides:*

- Sharp and narrow peaks
- Better detection (better S/N)
- Peak capacity to resolve complex samples

But, resolution increases only with the **square root of the plate number** and plate number increase is **limited by experimental conditions** (analysis time, pressure)!

Example:

Same sample, analyzed on a 4.6mm x 50mm column but with different d_p.



1.8 µm

5.0 µm

Increasing Resolution

*Increasing the **retention factor (k)***

is very helpful between 5 and 10 but beyond that only off insignificant improvement.

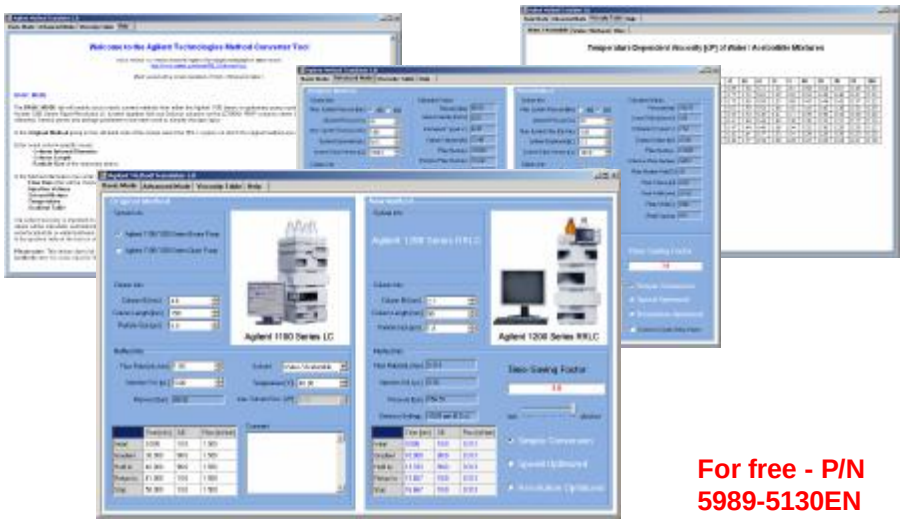
In gradient separations k can be influenced by the gradient slope, *i.e.* flow and/or gradient time.

$$k^* = \frac{t_g \cdot F}{1.15 \cdot S \cdot \Delta\%B \cdot V_M}$$

(average k for gradient separation)

So, by just increasing the flow in a gradient separation k becomes bigger and the resolution will improve!

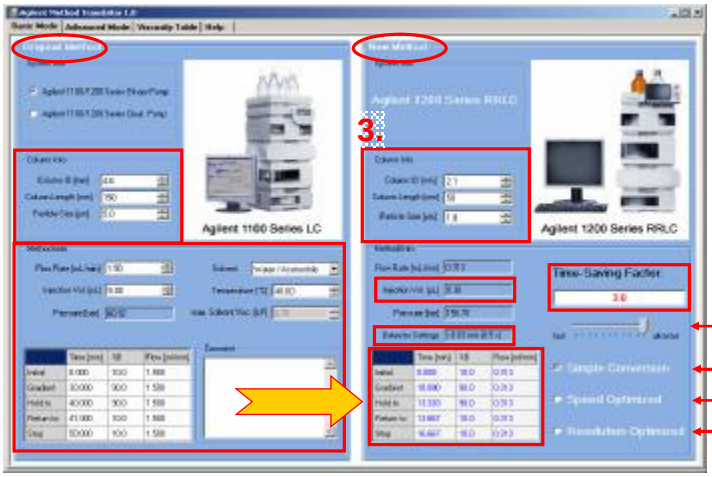
All scaling calculations to transfer methods to sub-2µm particles are done by the Agilent MethodTranslator



For free - P/N 5989-5130EN

Features of the Agilent MethodTranslator

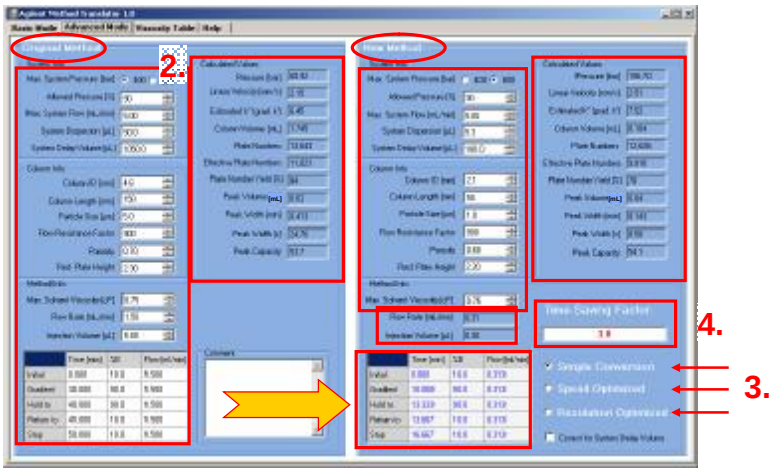
Basic mode with certain pre-set parameters:



Enter the parameters of your existing method and the parameters of the desired column you would like to convert to.

Features of the Agilent MethodTranslator

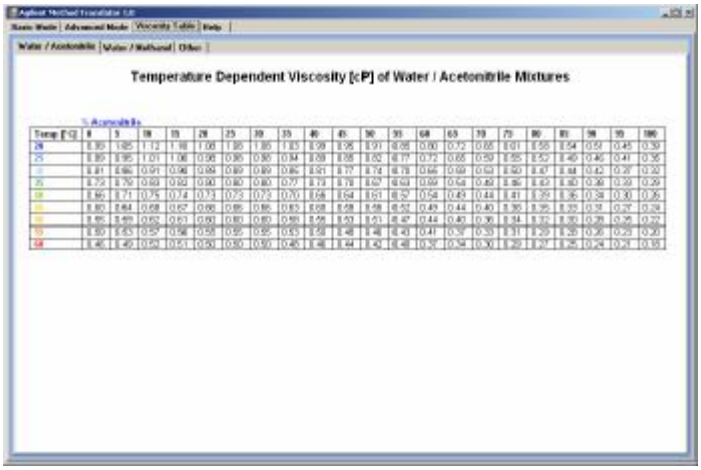
Advanced mode – all calculation parameters in your hands:



More to enter but much more information returned.

Features of the Agilent MethodTranslator

Viscosity Tables

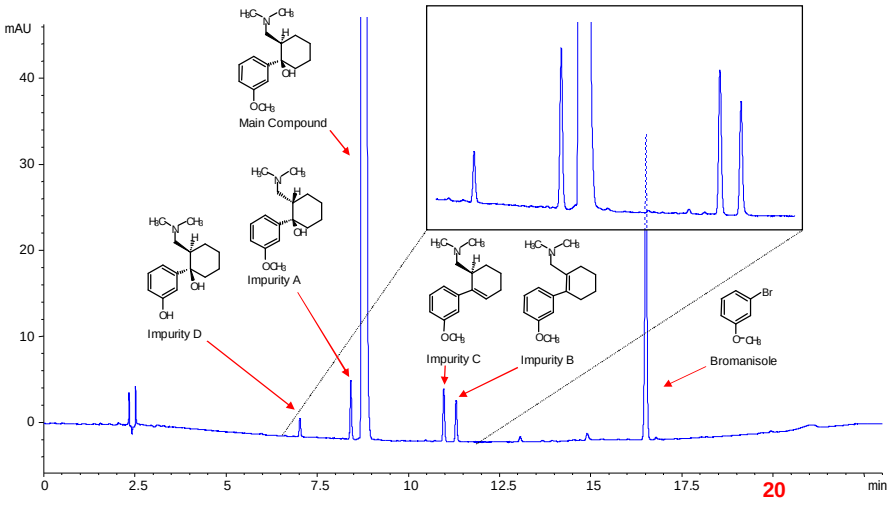


Temperature and composition dependent for H_2O/ACN and H_2O/MeOH

Does it work?

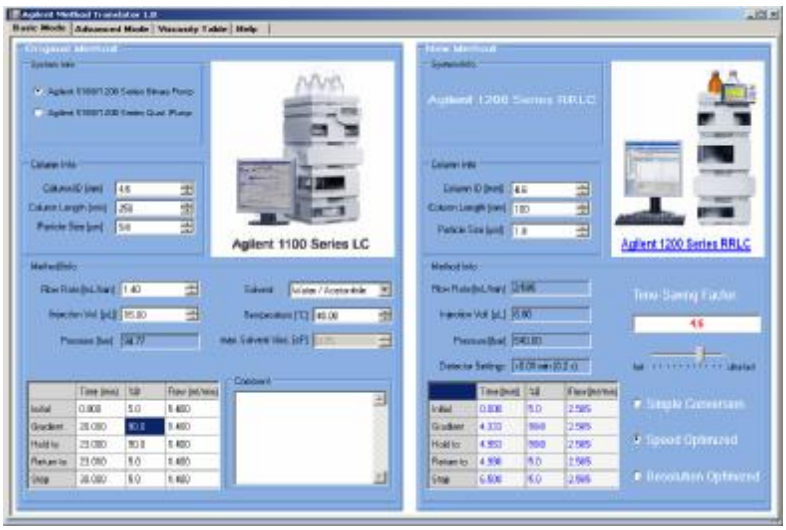
Does it work? - Example

Analysis of impurities of an active pharmaceutical ingredient by conventional HPLC (4.6mmID x 250mm, 5.0µm):

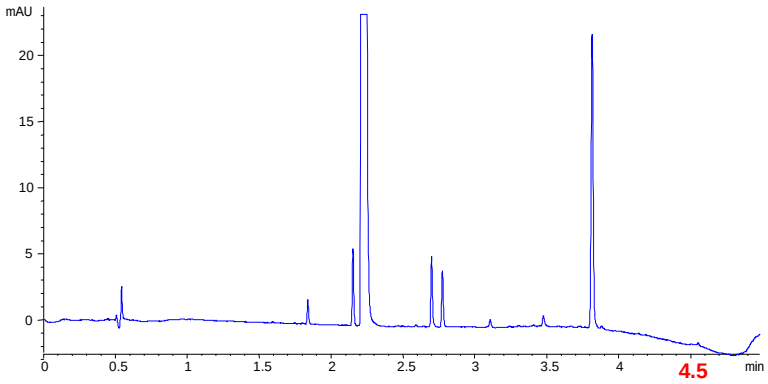


Does it work?

Converting to a 4.6 mmID x 100 mm, RRHT column:



Does it work?



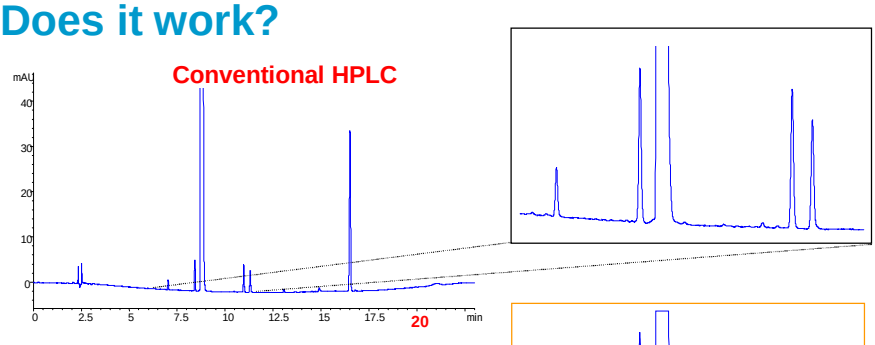
4.6 mmID x 100 mm, 1.8µm Zorbax SB C18

Speed Optimized

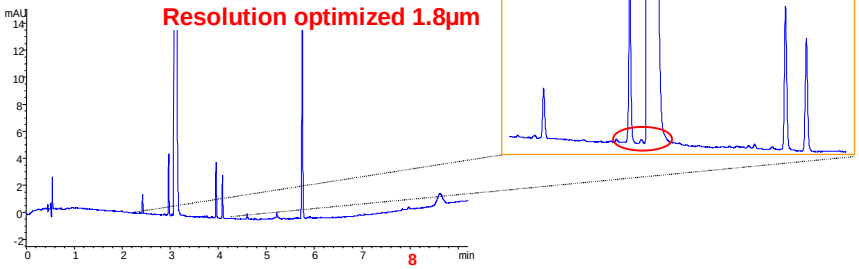
0.00 min	5%	B
4.33 min	90%	B
4.98 min	90%	B
4.99 min	5%	B
6.5 min	5%	B

Does it work?

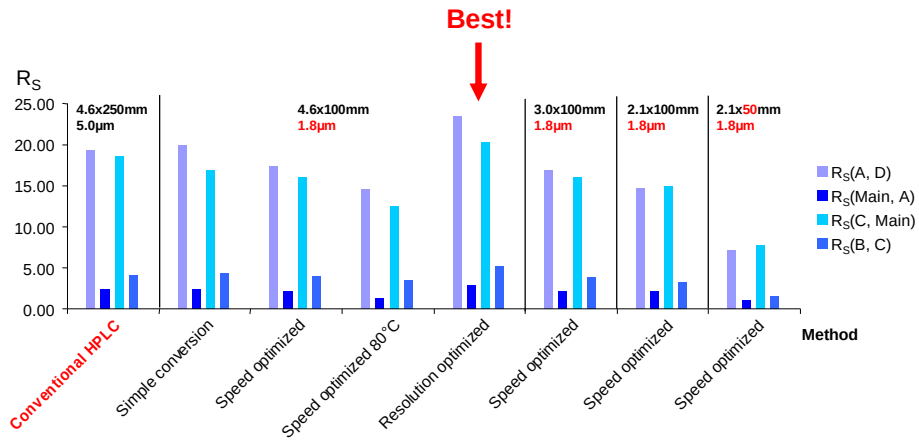
Conventional HPLC



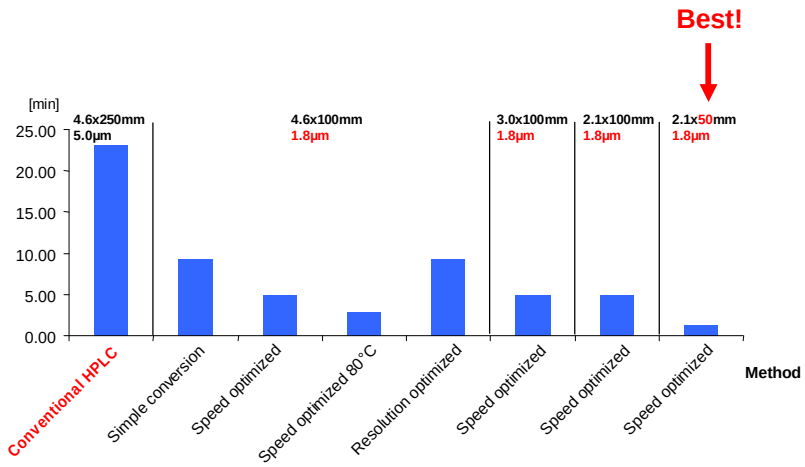
Resolution optimized 1.8µm



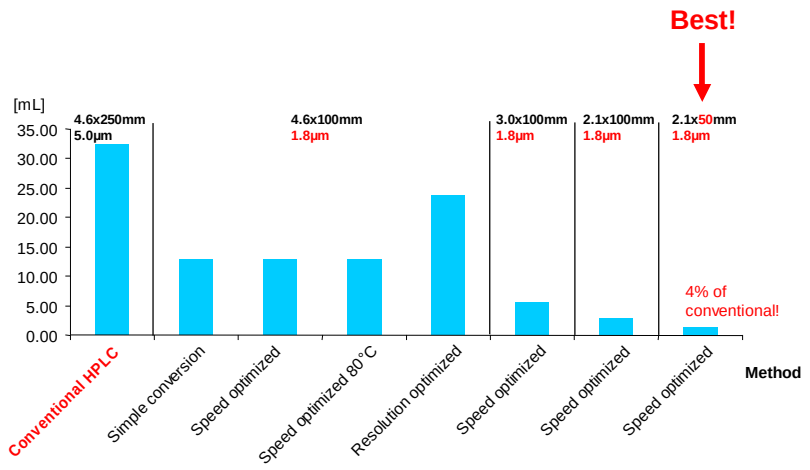
Does it work? - Resolution



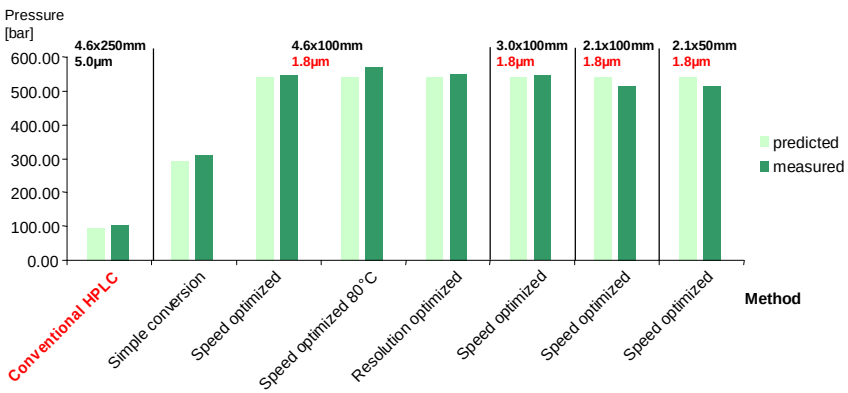
Does it work? – Run Time



Does it work? – Solvent Consumption



Does it work? - Pressure Prediction of the MethodTranslator*



*) calculated with the standard setting of the basic mode. Note: at very high flow rates larger deviations can be expected as the backpressure of capillaries are not taken into account.

The MethodTranslator - What about reality?

- ***You have to take additional backpressure of capillaries into account.***
  Smaller particles **à** *higher Efficiencies* **à** *higher demands on dead volume, BUT: backpressure induced by the column is proportional to* d_p^2 *but induced by capillaries proportional to* d_c^4
- ***Real columns have a distribution of the particle size.***
  We have a higher amount of larger particles around the mean of 1.8µm, other manufactures have a higher amount of fines around their mean particle size.
- ***What is the real temperature of the column?***
  You have to consider energy lost by transmission but energy gain from friction! So, what is the true temperature of the column, the true viscosity?
- ***Don‘t forget about the real measured Efficiency!***
  The Efficiency of the column is deminished (sometimes dramatically) by extracolumn volumes which cause bandspreading, injection volumes (not always an ideal solute can be used) and by the detection (flow cell, acquisition rate). Depending on original method and new method these contributions can be different!

What is the MethodTranslator and what is it not?

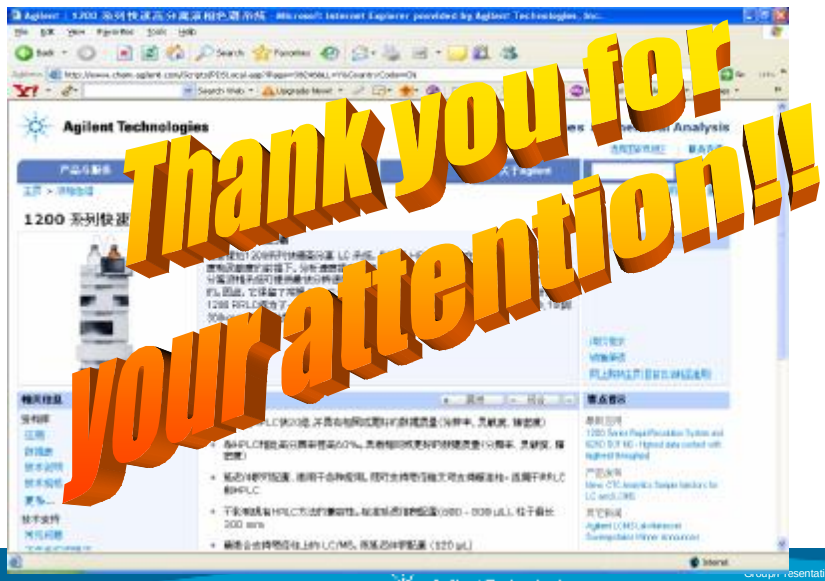
The MethodTranslator is a tool to help you in converting established methods to smaller particle size columns and utilize their advantages. It gives you a fairly good starting point, but...

...the MethodTranslator is not a method development software like ChromSword or DryLab!

Summary

- *Sub-2µm particle columns allow to get either shorter analysis times and/or higher resolutions*
- *Time and solvent savings can be significant*
- *Transfer is very easy – the Agilent MethodTranslator is a tool to support you*
- *Nowadays, methods using sub-2µm columns are as robust as conventional HPLC methods*

For more information visit:
www.agilent.com/chem/1200rr

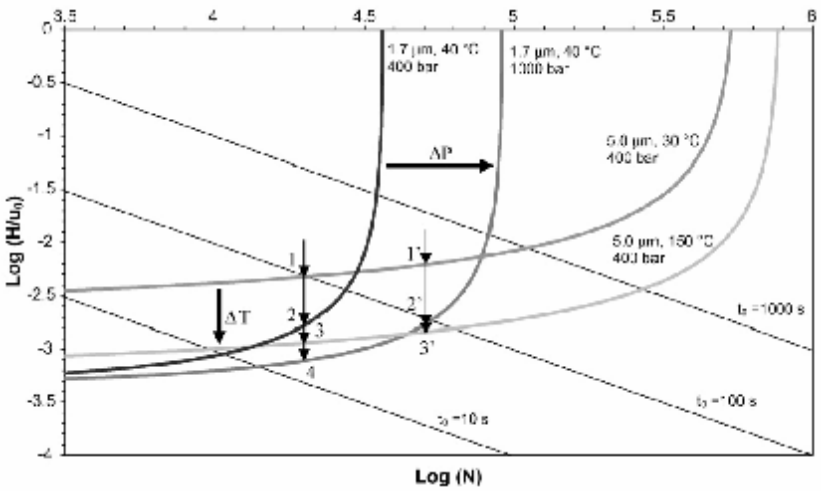


Backup

Method	ID [mm]	Length [mm]	Particle Size [µm]	Flow [mL/min]	Run Time [min]	Solvent consumption [mL]	Solvent cons. vs. Conv.	Rs (A, D)	Rs (Main, A)	Rs (C, Main)	Rs (B, C)	predicted pressure *) [bar]	measured pressure [bar]	Deviation of predicted pressure
Conventional HPLC	4.6	250	5.0	1.400	23.00	32.20	100%	19.32	2.49	18.50	4.19	94.77	103.50	8.43%
4.6 Simple conversion	4.6	100	1.8	1.400	9.20	12.88	40%	19.83	2.30	16.88	4.32	292.50	309.00	5.34%
4.6 Speed optimized	4.6	100	1.8	2.585	4.98	12.87	40%	17.29	2.12	15.99	3.88	540.00	548.00	1.46%
*4.6 Speed optimized 80° C **)*	*4.6*	*100*	*1.8*	*4.500*	*2.86*	*12.87*	*40%*	*14.53*	*1.19*	*12.50*	*3.41*	*540.00*	*569.00*	*5.10%*
4.6 Resolution optimized	4.6	100	1.8	2.585	9.20	23.78	74%	23.37	**2.83**	20.22	5.08	540.00	549.00	1.64%
3.0 Speed optimized	3.0	100	1.8	1.100	4.98	5.48	17%	16.77	2.12	16.13	3.76	540.00	547.00	1.28%
2.1 Speed optimized	2.1	100	1.8	0.539	4.98	2.68	8%	14.62	2.07	14.83	3.28	540.00	514.00	5.06%
*2.1 Speed optimized **)*	*2.1*	***50***	*1.8*	*1.077*	*1.25*	*1.35*	*4%*	*7.18*	*1.09*	*7.71*	*1.43*	*540.00*	*515.00*	*4.85%*

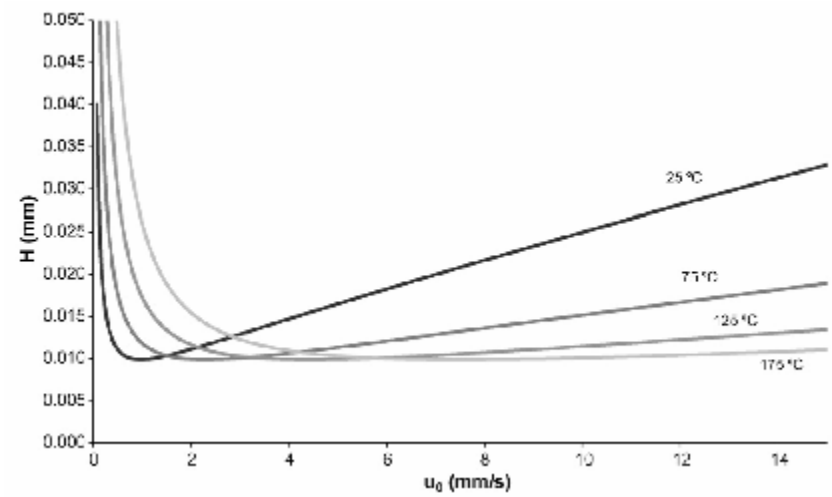
*) calculated with the standard setting of the basic mode. Note: at very high flow rates larger deviations can be expected as the backpressure of capillaries are not taken into account.
**) Note: the resolution for the critical pair is below the usually accepted limit of 2 for such an analysis.

Backup



Comparison of experimental kinetic plots for high temperature LC (5µm, 30 and 150 ◦C) and high pressure UPLC (1.7µm, 40 ◦C). Maximum pressures: 400 and 1000 bar (UPLC), 400 bar (LC). Values for φ^0, A–C, η and D^m are listed in Table 5. Columns and mobile phases: Blaze C18 200 (150mm×4.6 mm, 5µm with 40/60 acetonitrile/water and Acquity BEH C18 (100mm×2.1 mm, 1.7µm) with 30/70 acetonitrile/water.

Backup



Theoretical plate-height curves for 5m particles, illustrating the effect of temperature on plate height and linear velocity. The plots were calculated with Eq. (12) assuming values of: *A* = 0.66, *B* = 3.00 and *C*= 0.05. Diffusion coefficients for phenol in 40/60 acetonitrile/water were calculated at each temperature according to Wilke-Chang and Eqs. (11) and (13) [30,19]: 1×10−9 m2/s (25 ◦C), 2.4×10−9 m2/s (75 ◦C), 4.5×10−9 m2/s (125 ◦C), 7.7×10−9 m2/s (175 ◦C).