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Method Transfer Best Practice

Dr. Frank Steiner
Scientific Advisor

Co-authored by Dr. Susanne Fabel, Dr. Alexandra Manka, Michael Menz, Sabrina Patzelt, Dr. Markus Martin

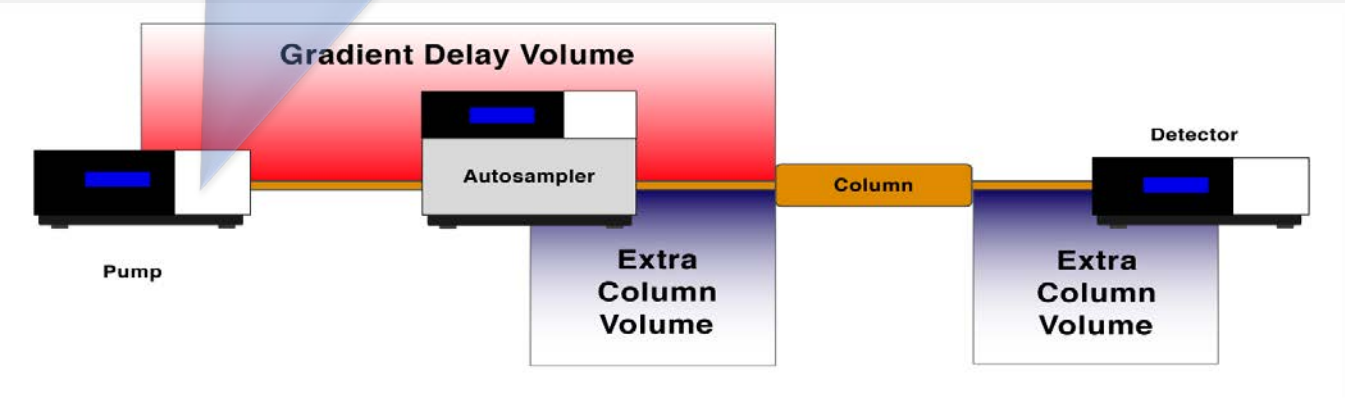
Agenda

- Important considerations for method transfer
- Direct UltiMate 3000 ➔ Vanquish method transfer by instrument similarity
- Method transfer by exchange of gradient mixer
- Unique hardware features of Vanquish systems for method transfer
  - Adaptable GDV by autosampler metering device
  - Eluent pre-heating and column thermostatting modes
- What is the best practice?

Important considerations for method transfer

Impact on Method Transfer – Instrument Properties and Settings

Pump
- Gradient generation principle
- Gradient delay volume (GDV)
- Solvent mixing characteristics



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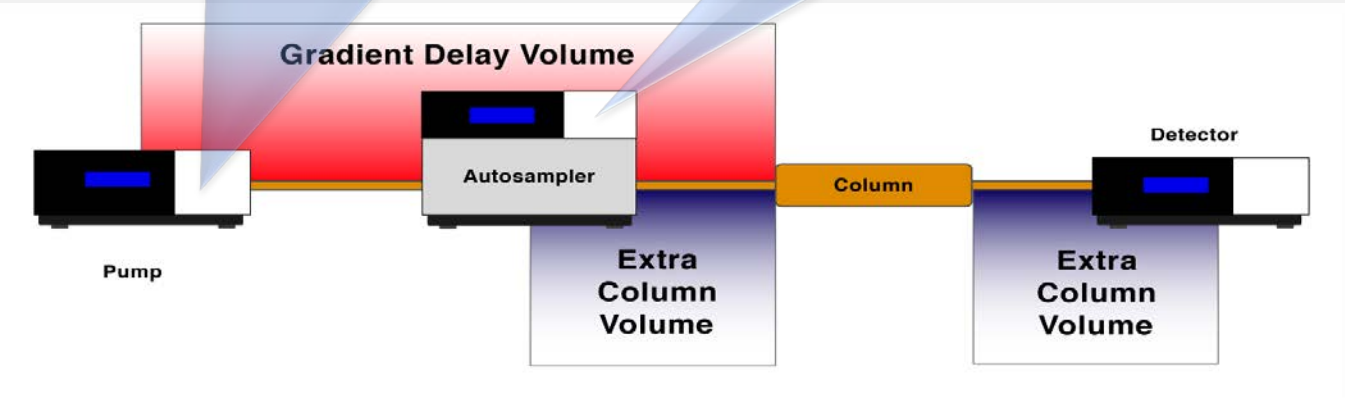
Autosampler
- Contribution to GDV
- Contribution gradient shape (flush out)



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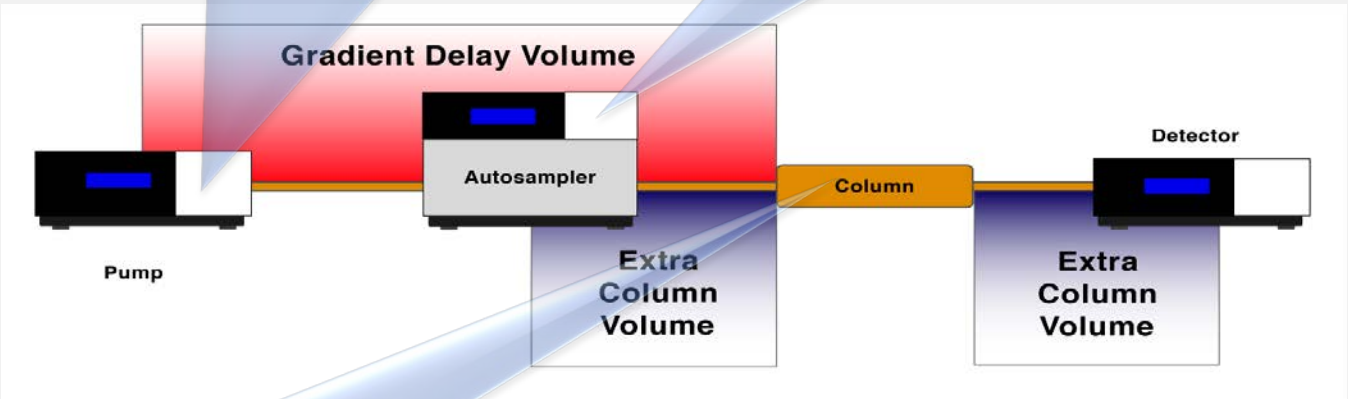

Column thermostat
- Working principle (still air vs. forced air)
- Eluent pre-heater design and working principle

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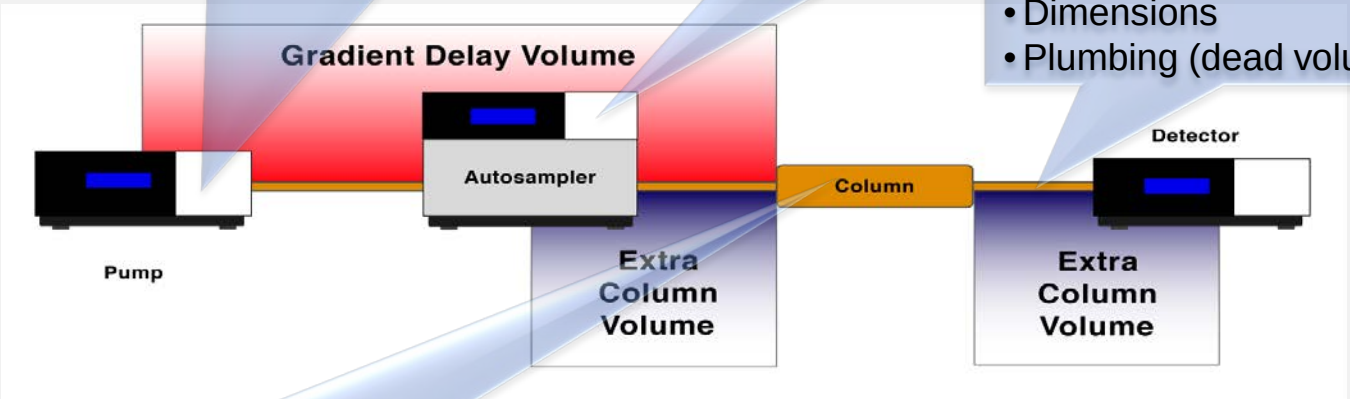
System connection tubing
- Dimensions
- Plumbing (dead volumes)



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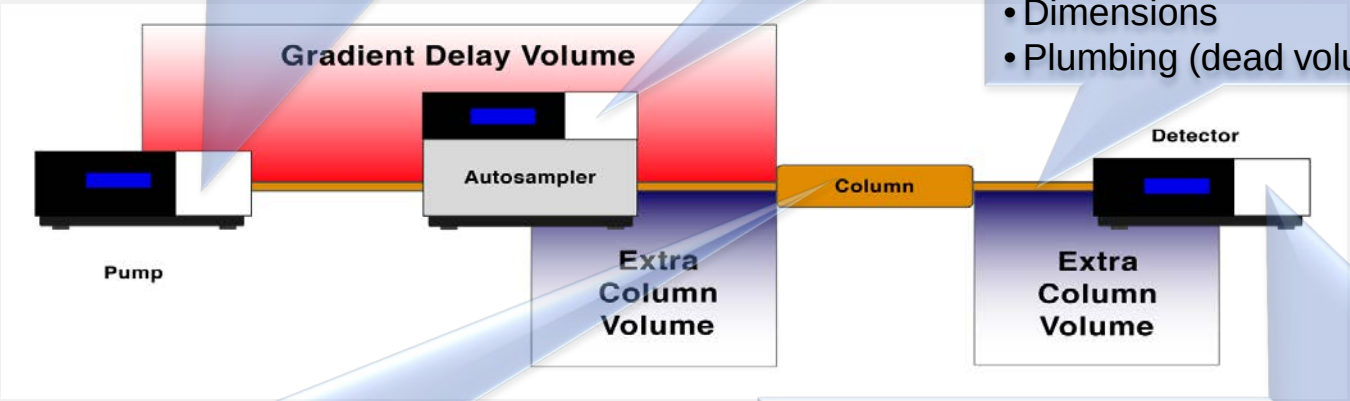
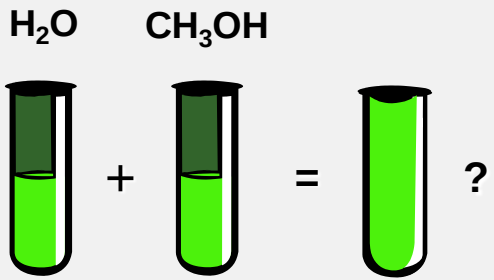


Column thermostat
- Working principle (still air vs. forced air)
- Eluent pre-heater design and working principle

Detector
- Flow cell design and dimensions
- Wavelength settings (bandwidth, reference)
- Data collection rate and filter constant

Gradient Generation and Mixing Volume



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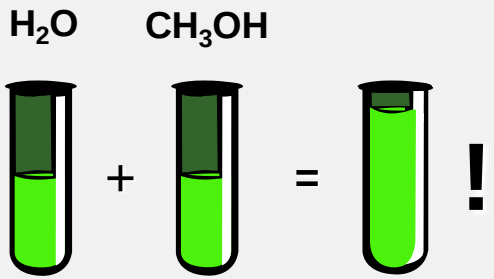

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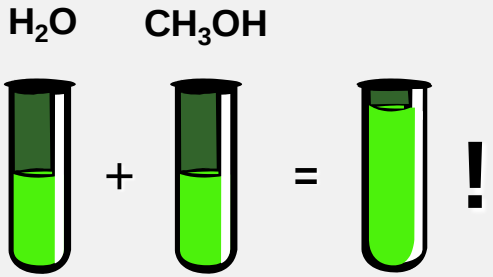

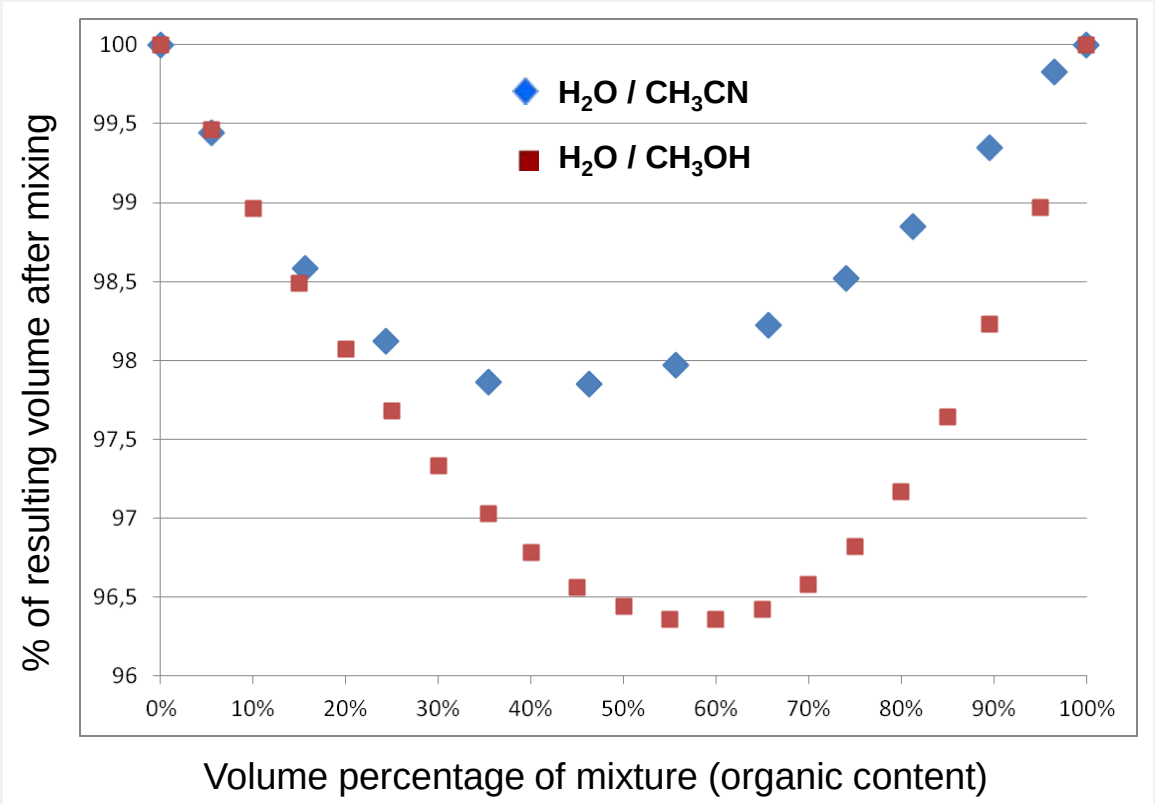

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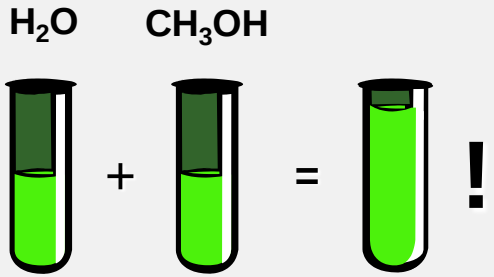
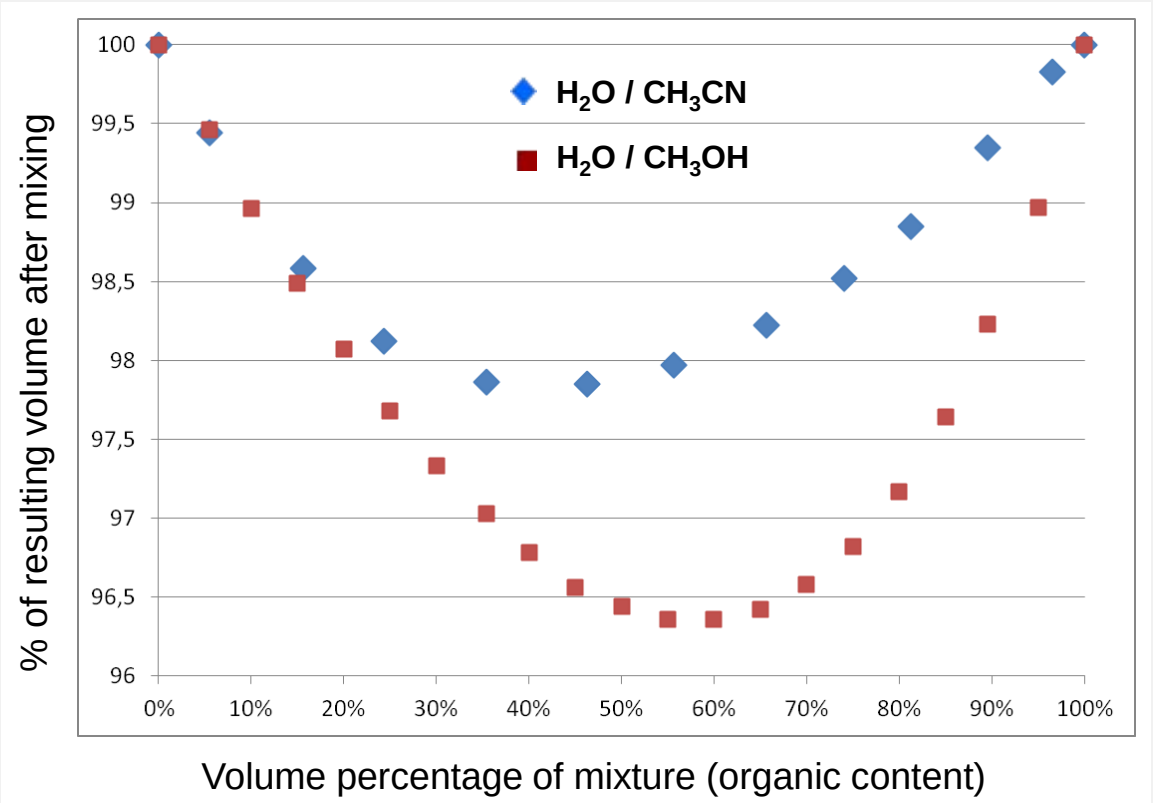


- Volume contraction has influence on gradient generation



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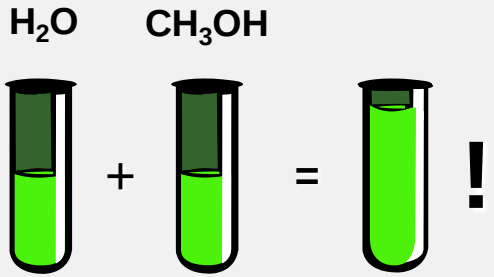
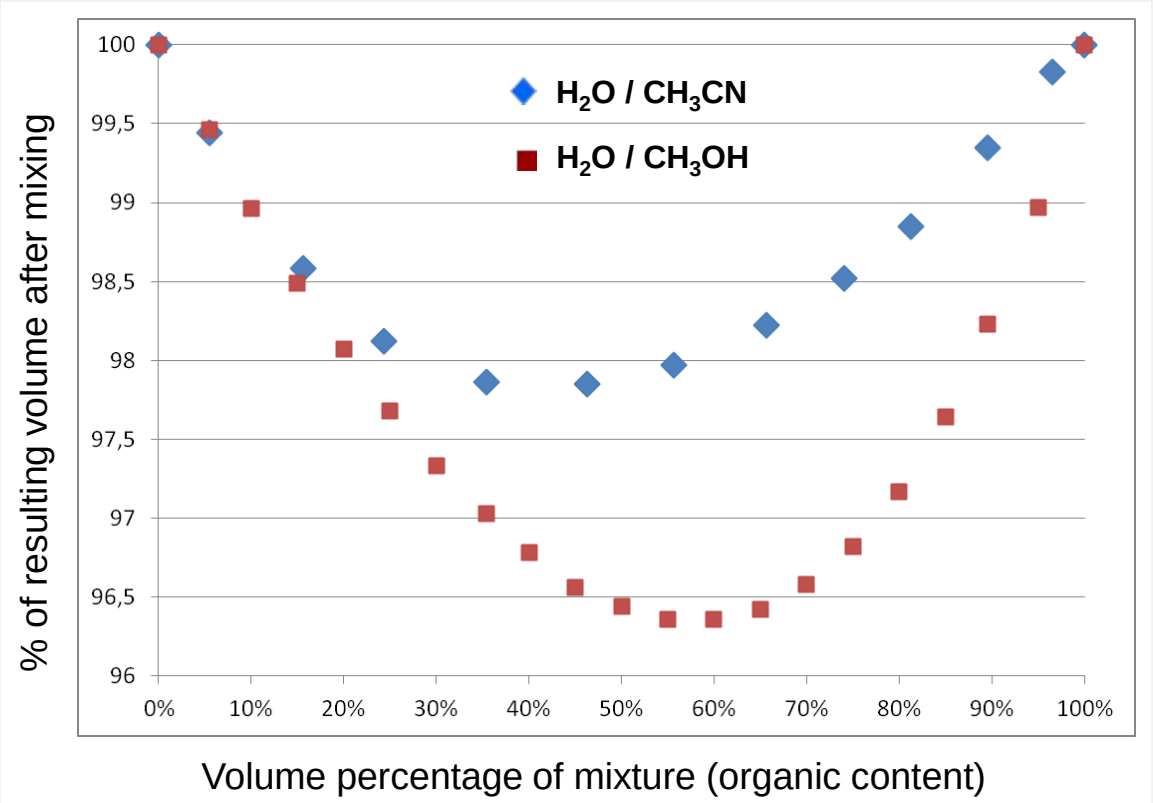
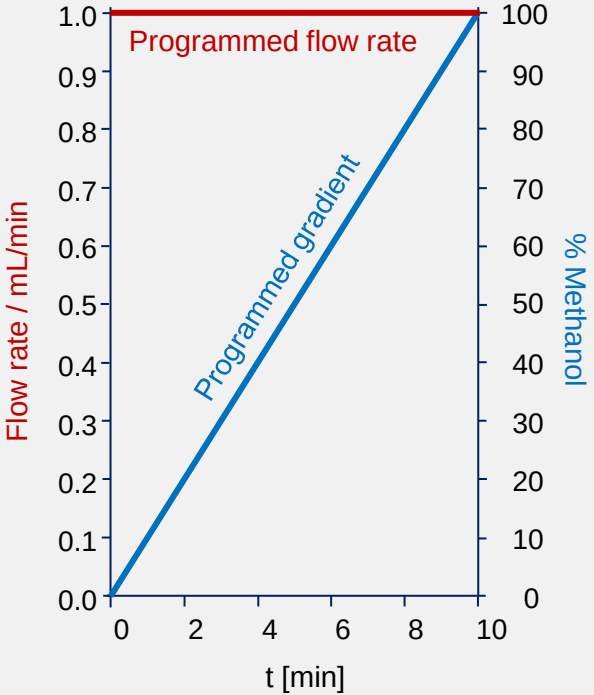
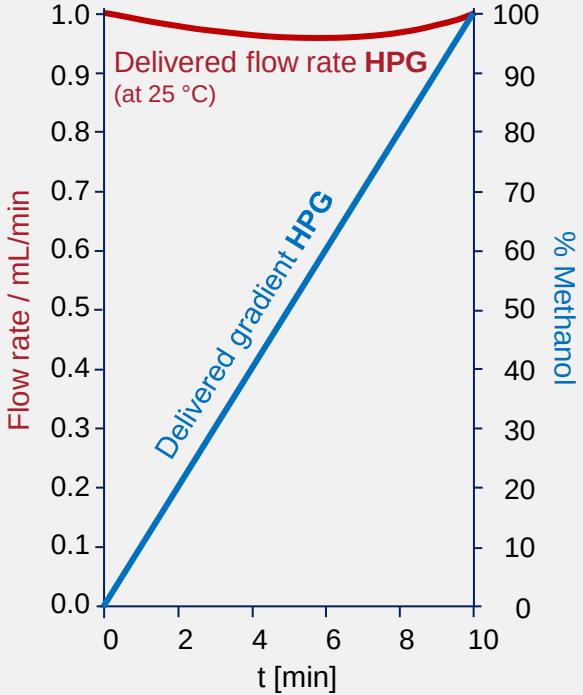


- Volume contraction has influence on gradient generation
- Effect will differ between HPG and LPG pumps, also in isocratic dial-a-mix mode



Mixing Volume Effects with HPG and LPG Instruments





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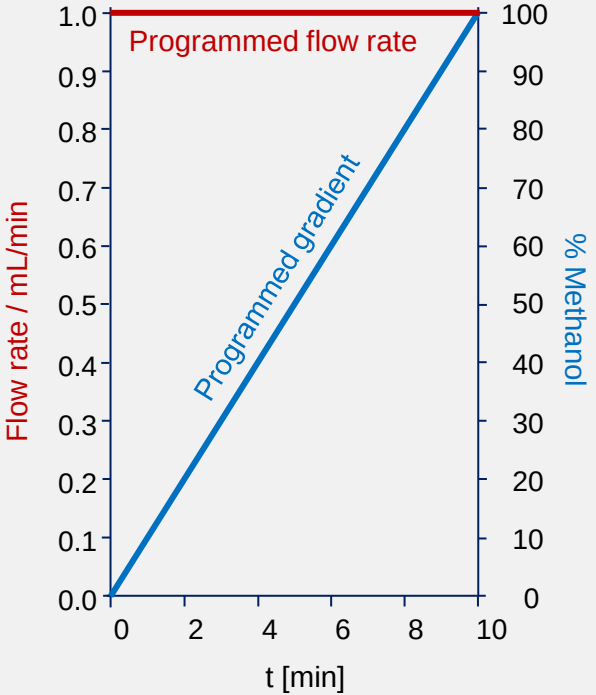

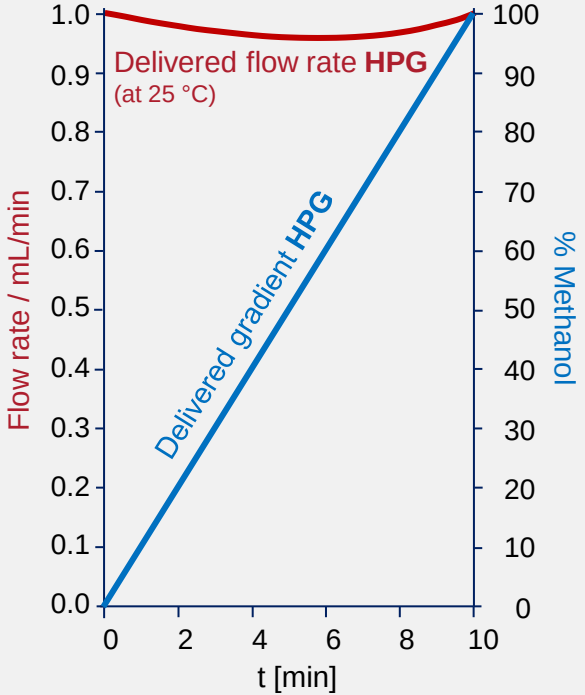

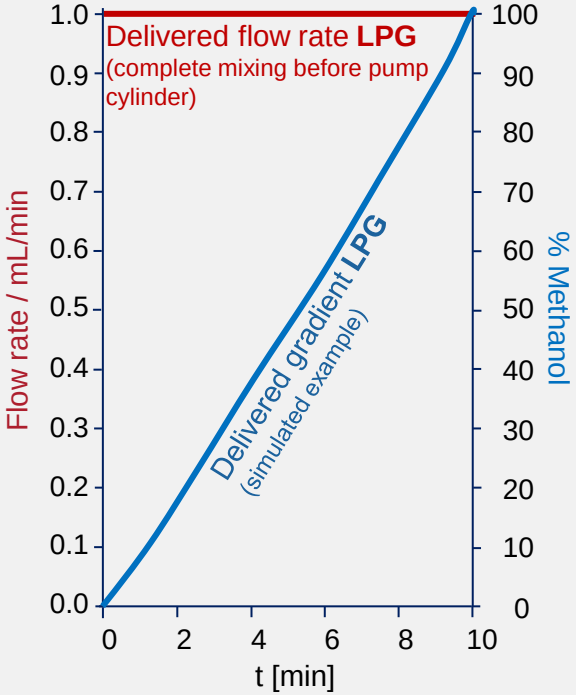

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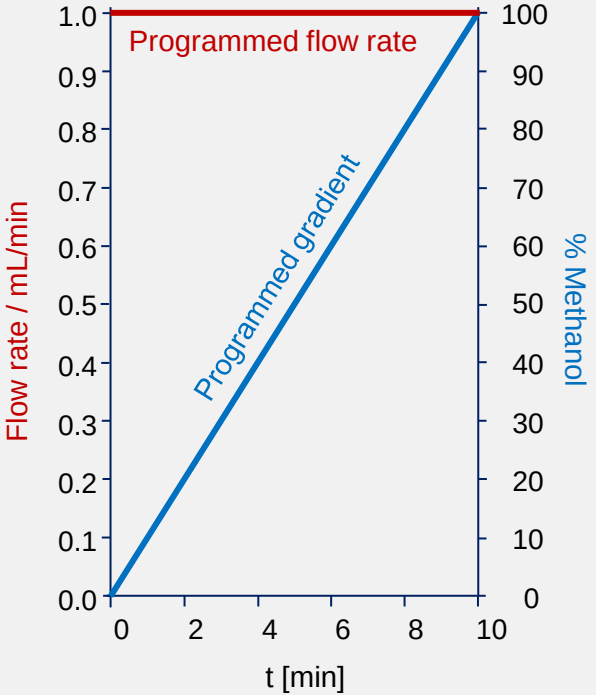
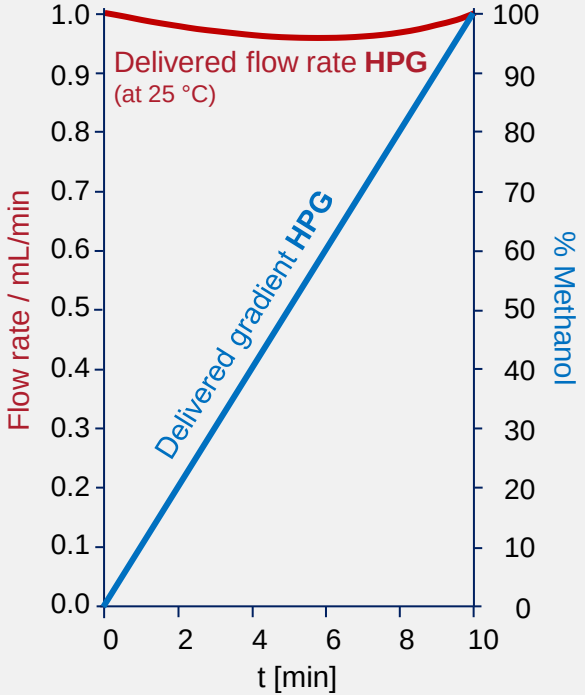
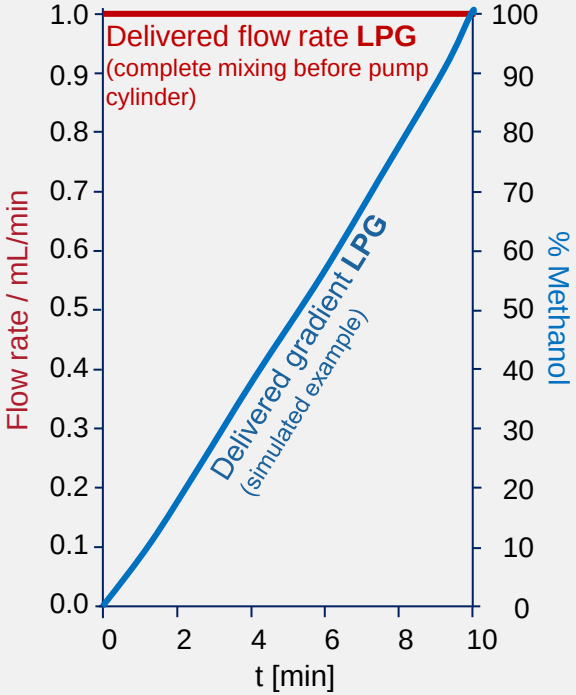


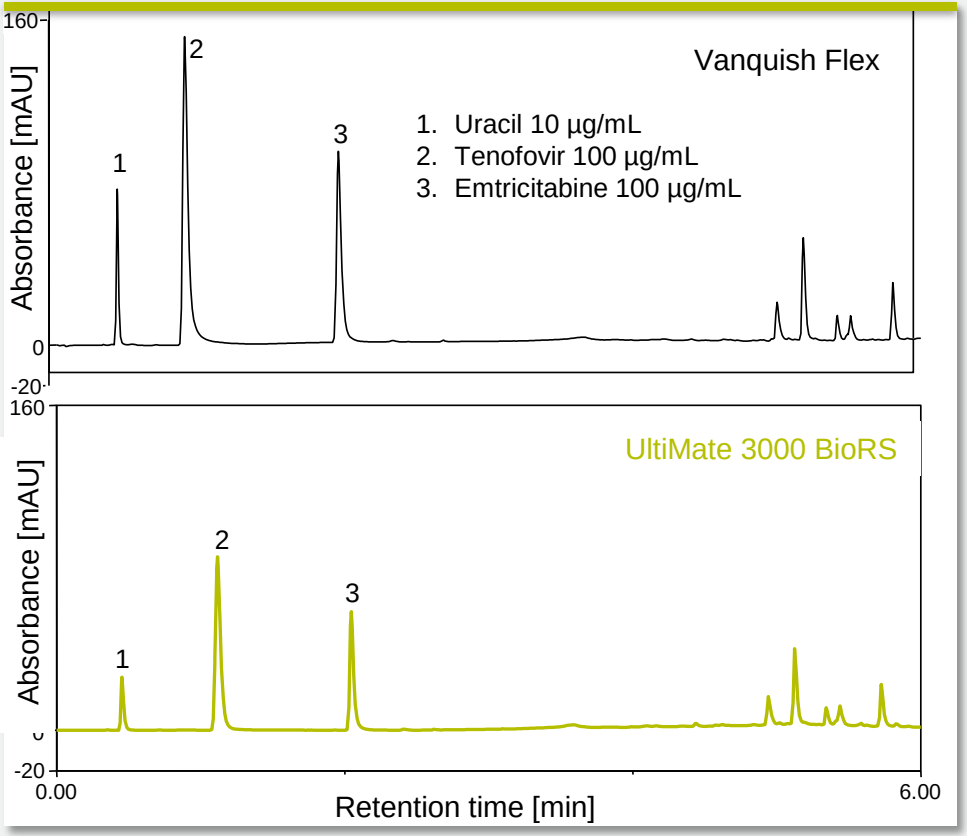




- Method transfer from HPG to HPG straightforward (if GDV matches)
- Method transfer from LPG to LPG more challenging (mixing properties must also match)
- **Better refrain from transfer between LPG and HPG** (in applications with critical resolution)

UltiMate 3000 ➔ Vanquish Flex Method transfer by instrument similarity

Method Transfer: UltiMate 3000 BioRS ➔Vanquish Flex (HIV Medication Example)



Column:	Accucore aQ, 2.6 µm, 2.1 × 100 mm
System:	Vanquish Flex System with DAD (10 mm light path)
	BioRS System, VWD with 2.5 µL flow cell (7 mm light path)
Mobile Phase:	A - 25 mM ammonium acetate buffer, pH 3.8 with acetic acid
	B - Methanol
	C - Acetonitrile
	D - Water
Flow rate:	0.600 mL/min

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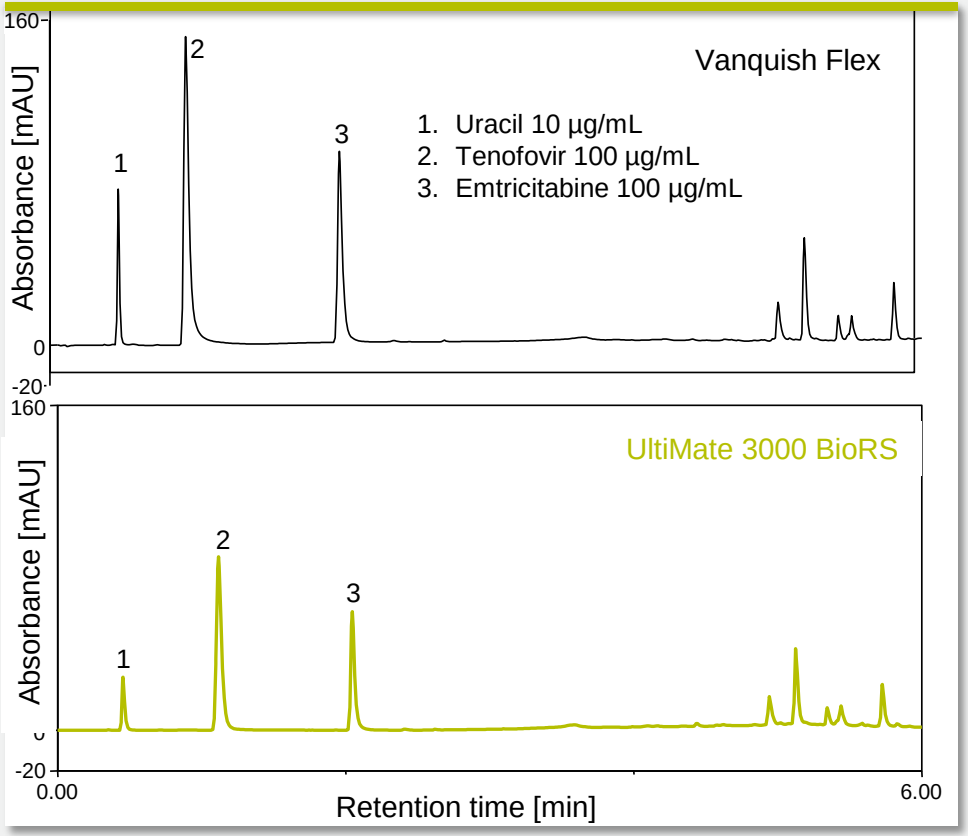
Method Transfer: UltiMate 3000 BioRS ➔Vanquish Flex (HIV Medication Example)



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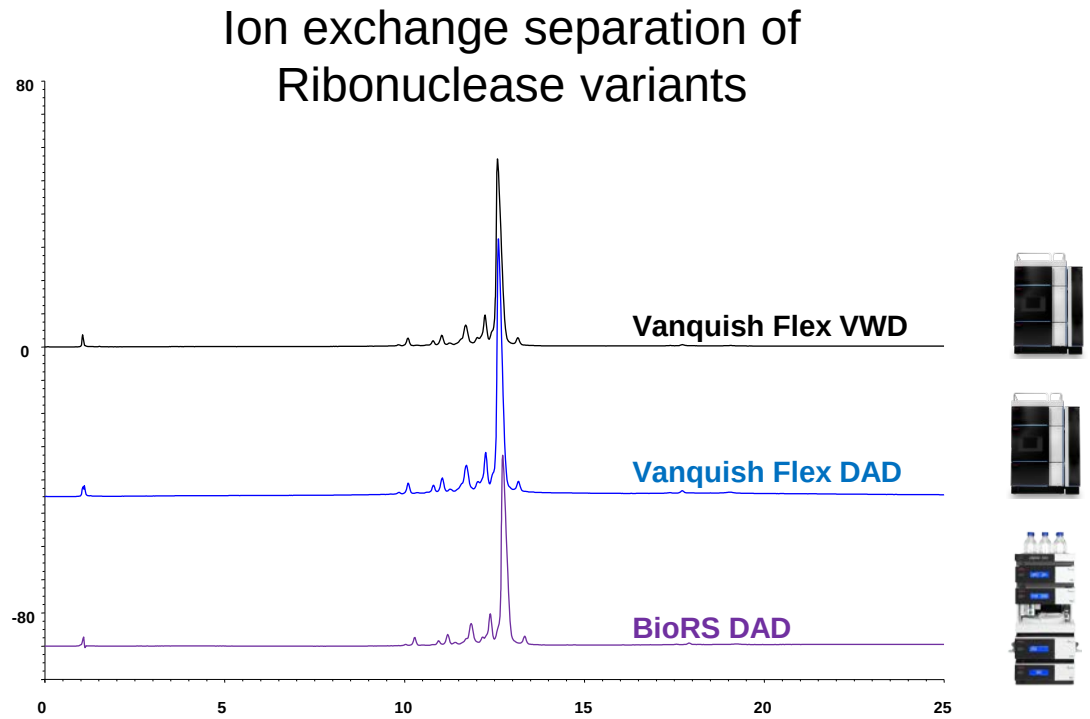
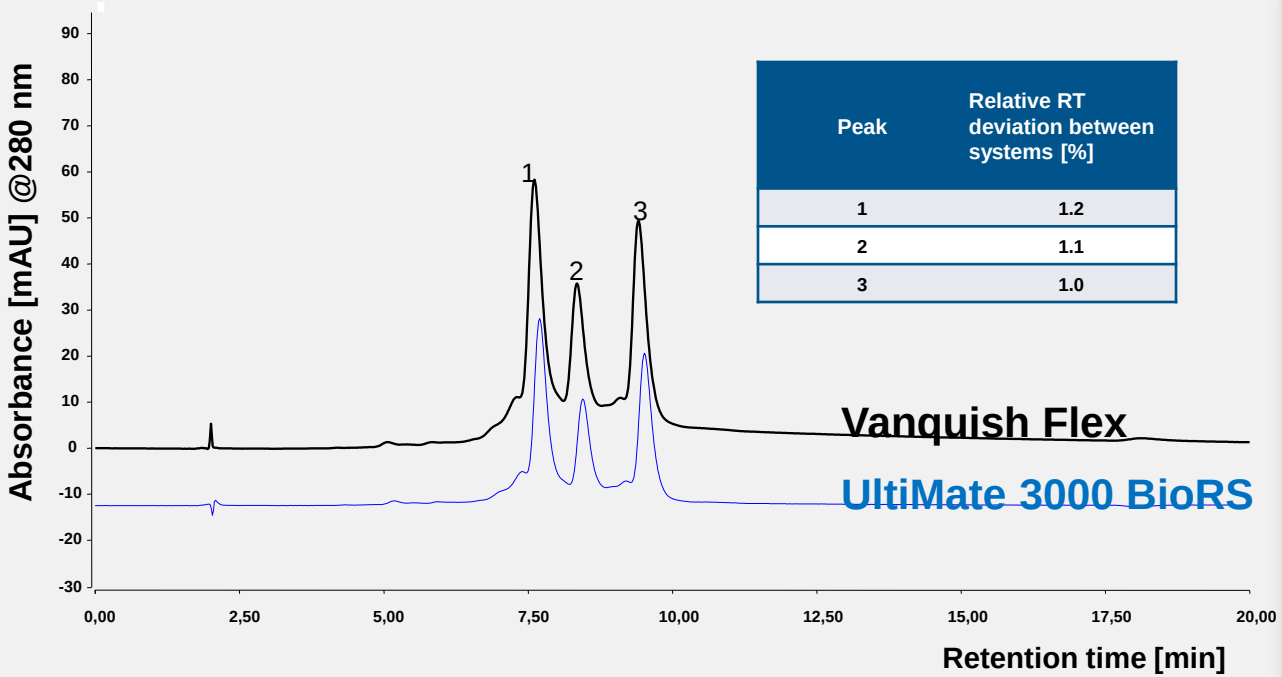
Vanquish Flex enables easy method transfer from UltiMate 3000 systems.

Method Transfer – Deamidation of Ribonuclease A



- Seamless method transfer from UltiMate 3000 BioRS to Vanquish Flex already with identical tubing and mixer (both for **DAD and VWD**)
- Improved Sensitivity with Vanquish DAD
- Increased peak resolution on Vanquish Flex with VWD

Method Transfer – Antibody Charge Variant Analysis



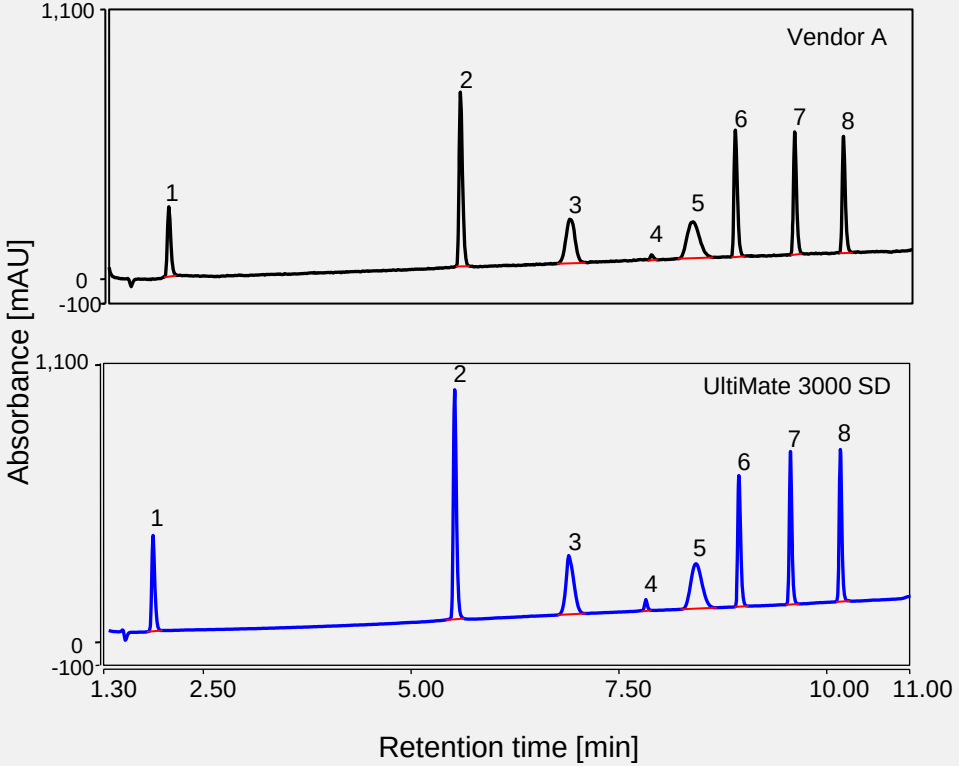
Peak	Relative RT deviation between systems [%]
1	1.2
2	1.1
3	1.0

- Method transfer between UltiMate 3000 BioRS and Vanquish Flex
- Improved sensitivity with Vanquish DAD
- Identical methods were applied and successful method transfer was achieved by recreating the same hardware conditions on both instrument platforms

MabPac SCX-10; 4x250mm, 10 µm, **A:** 20mM MES, 60mM NaCl, pH 5.7 **B:** 20mM MES, 300mM NaCl, pH 5.7
Gradient: 15-45% B in 15 min, 30 °C; 0.5mL min^{-1}; UV detection@280nm; Injection volume 4 µL

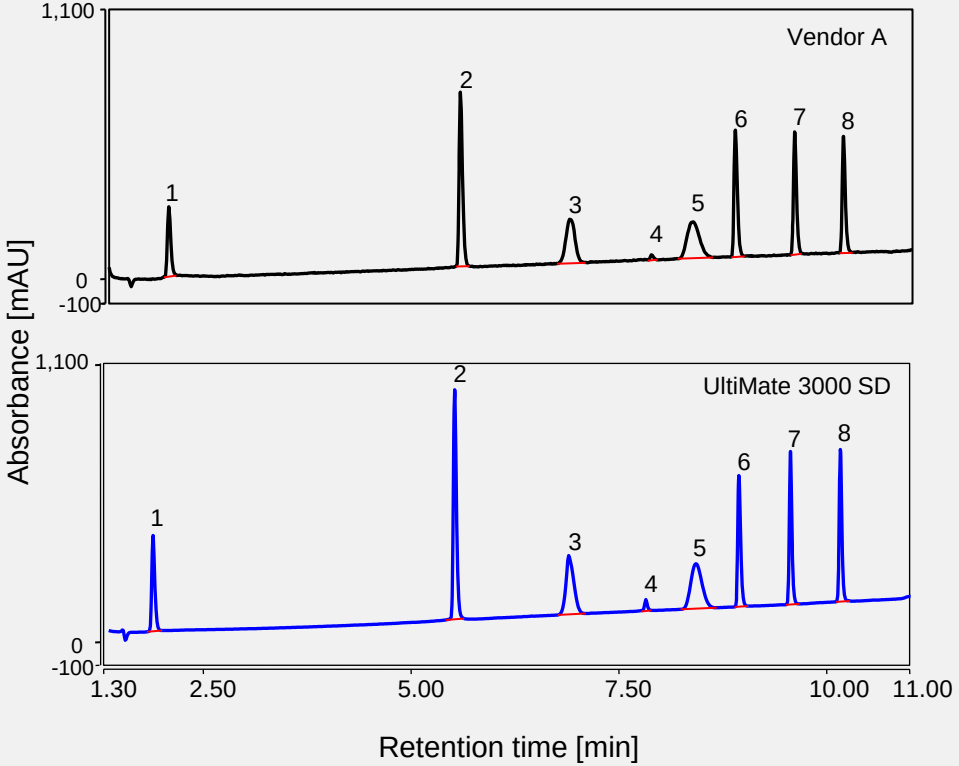
Method transfer from other vendor’s instrument by modification of gradient mixer

Transfer of a Heart Treatment Drug Analysis from Other Vendor to UltiMate 3000



Column:	Thermo Scientific™ Accucore™ XL, C18, 4.6 × 150 mm, 4 µm, P/N 74104-154630
Mobile Phases:	A: Water + 0.1% FA B: Methanol + 0.07% FA
Gradient:	0-10.0 min from 10 to 80% B, 10.0 -11.5 min 80% B, 11.5-12.0 min 80 to 10% ,12.0-17.0 min 10% B
Flow rate:	1.200 mL/min
Temperature:	50 ºC still air mode
Injection:	25 µL
Detection:	214 nm data collection rate: 10 Hz
Sample:	1. Hydrochlorthiazide 10 µg/mL 2. Chlorthalidone 20 µg/mL 3. Enalapril 60 µg/mL 4. Impurity 5. Ramipril 60 µg/mL 6. Telmisartan 20 µg/mL 7. Azilsartan 20 µg/mL 8. Valsartan 20 µg/mL

Transfer of a Heart Treatment Drug Analysis from Other Vendor to UltiMate 3000



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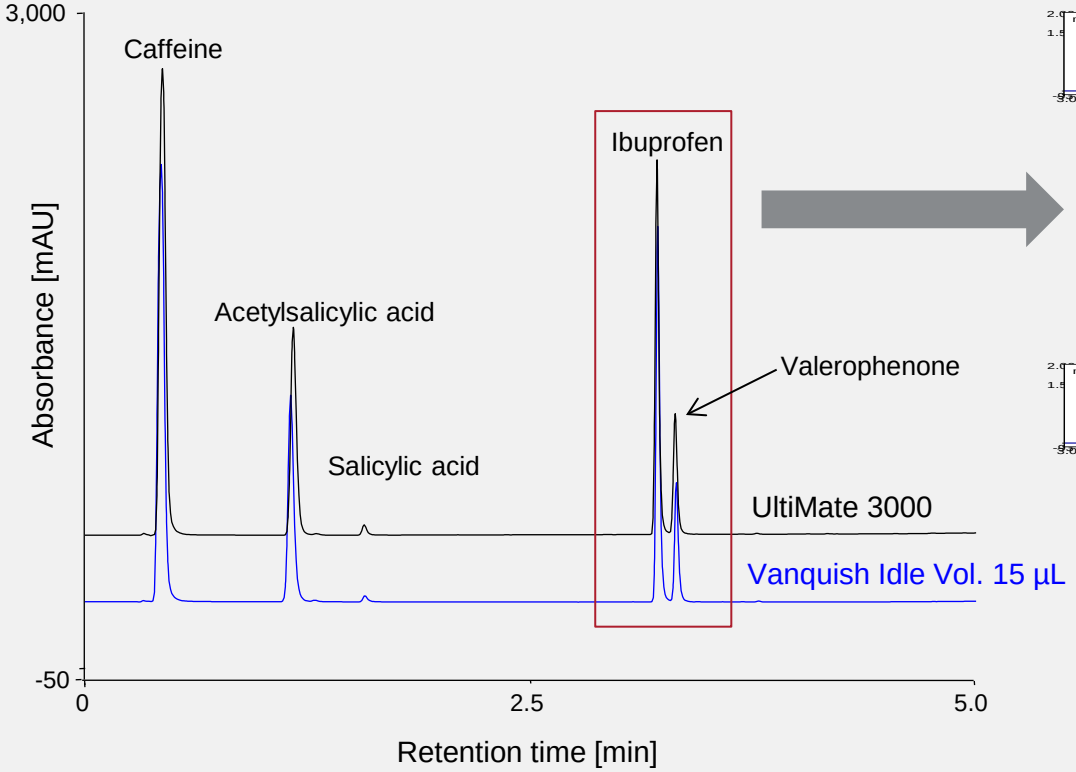
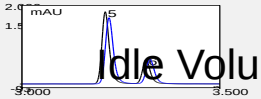
750 µL mixer instead of the default 350 µL mixer to mimic GDV of the other vendor's HPLC

Unique hardware features of Vanquish systems for method transfer

Transferring the Analysis of Pain Killer from UltiMate 3000 to Vanquish

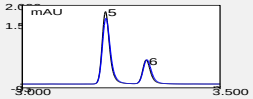




Idle Volume:
25 µL (default)

Δ=0.43 s deviation
or
Δ=0.22 % relative deviation

Δ=0.43 s deviation
or
Δ=0.21 % relative deviation



Idle Volume:
15 µL

Δ=0.06 s deviation
or
Δ=0.03 % relative deviation

Δ=0.09 s deviation
or
Δ=0.05 % relative deviation

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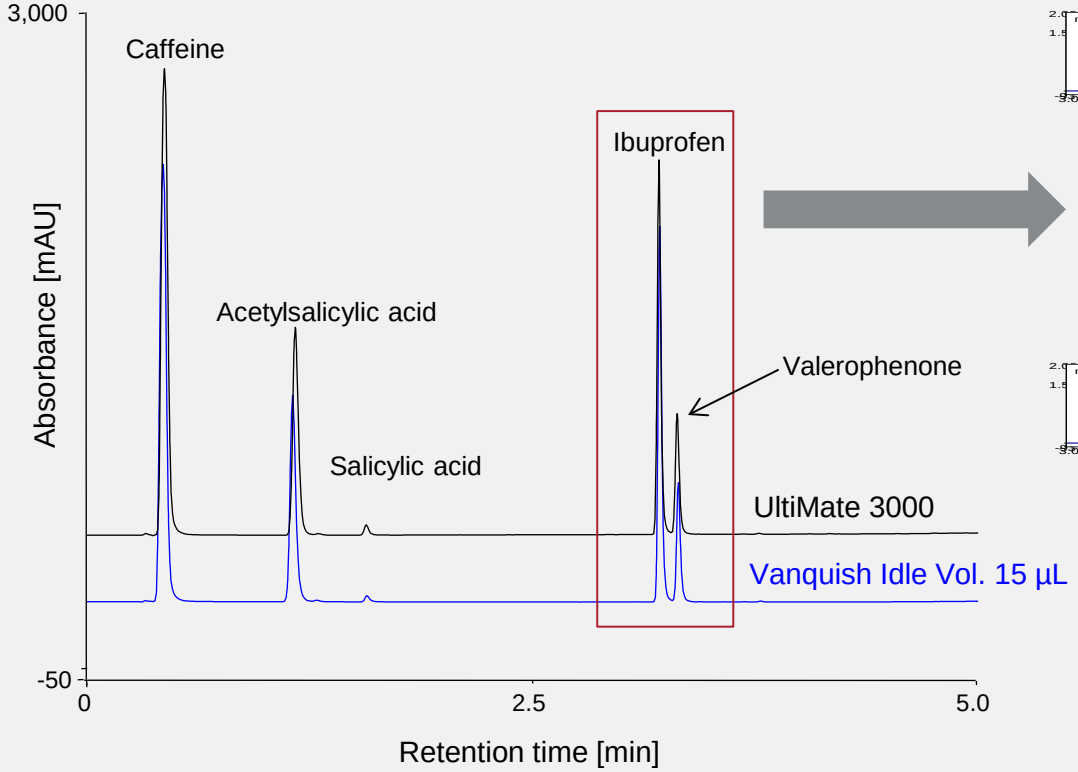
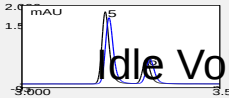
Transferring the Analysis of Pain Killer from UltiMate 3000 to Vanquish





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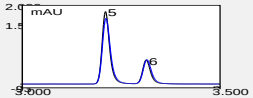

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Fine-tuning of GDV by the autosampler metering device makes the perfect match!

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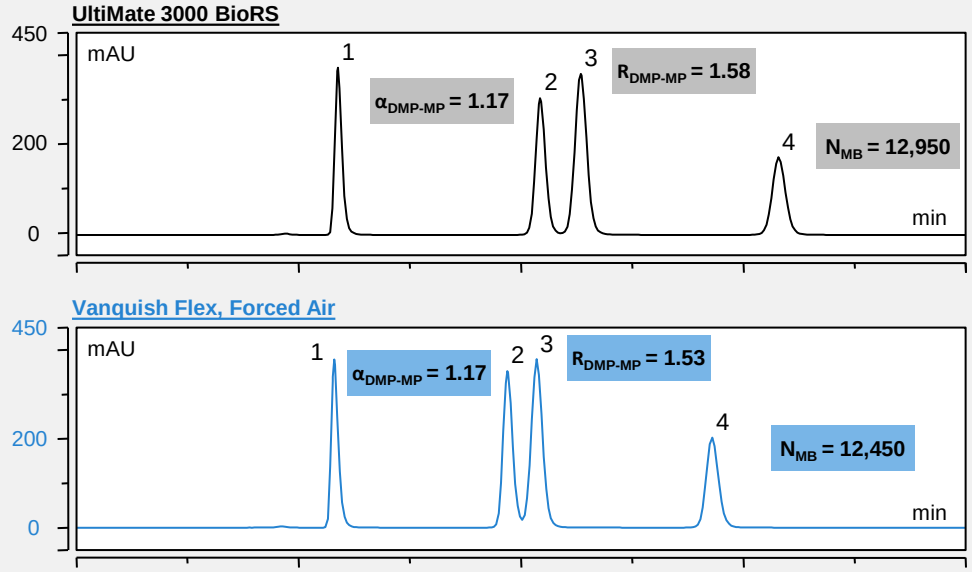
Column Thermostatting Effects – Analysis of Preservatives



Column:	Acclaim RSLC PA2, Polar Advantage II, 2.1 x 150 mm, 2.2 µm
Mobile Phase:	isocratic 20 mM phosphate buffer pH 7/methanol (35/65, v/v, dial-a-mix)
Flow rate:	0.55 mL/min, resulting in 760 bar back pressure
Temperature:	40 ºC
Injection:	1 µL
Detection:	UV, 2.5 µL flow cell, 254 nm, 50 Hz (VWD)
Sample:	1. Uracil, 2. Dimethyl phthalate (DMP), 3. Methyl parabene (MP), 4. Methyl benzoate (MB)

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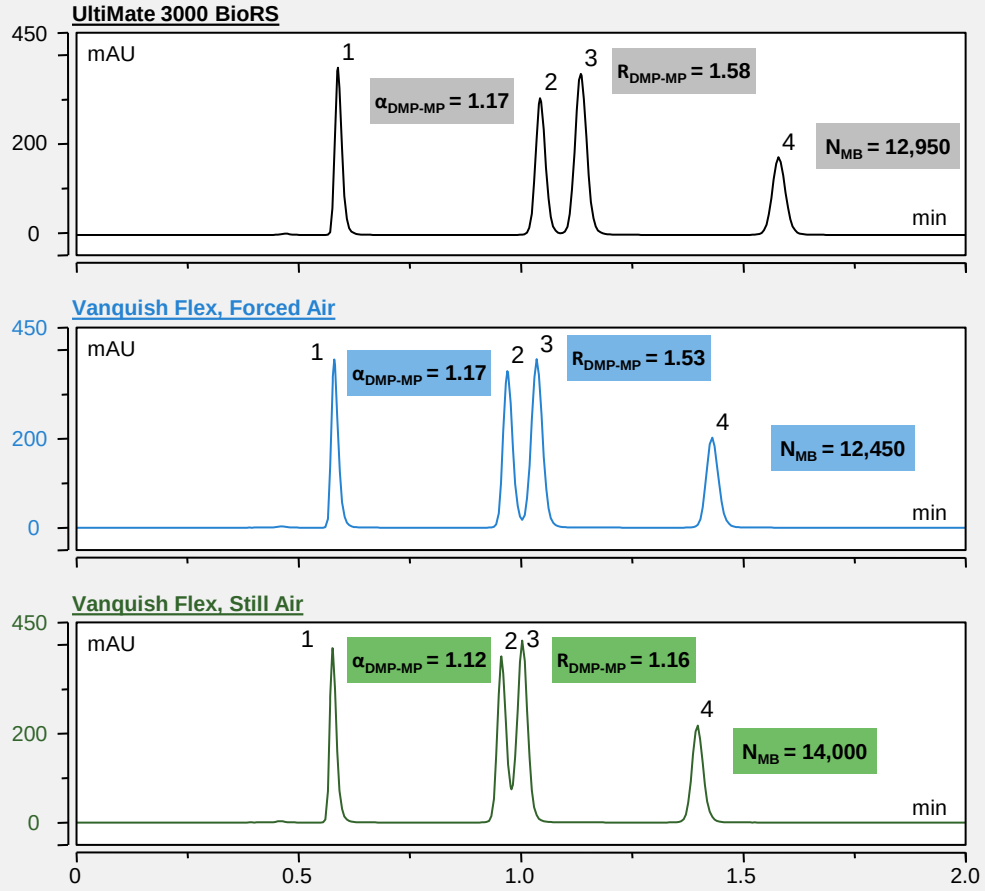

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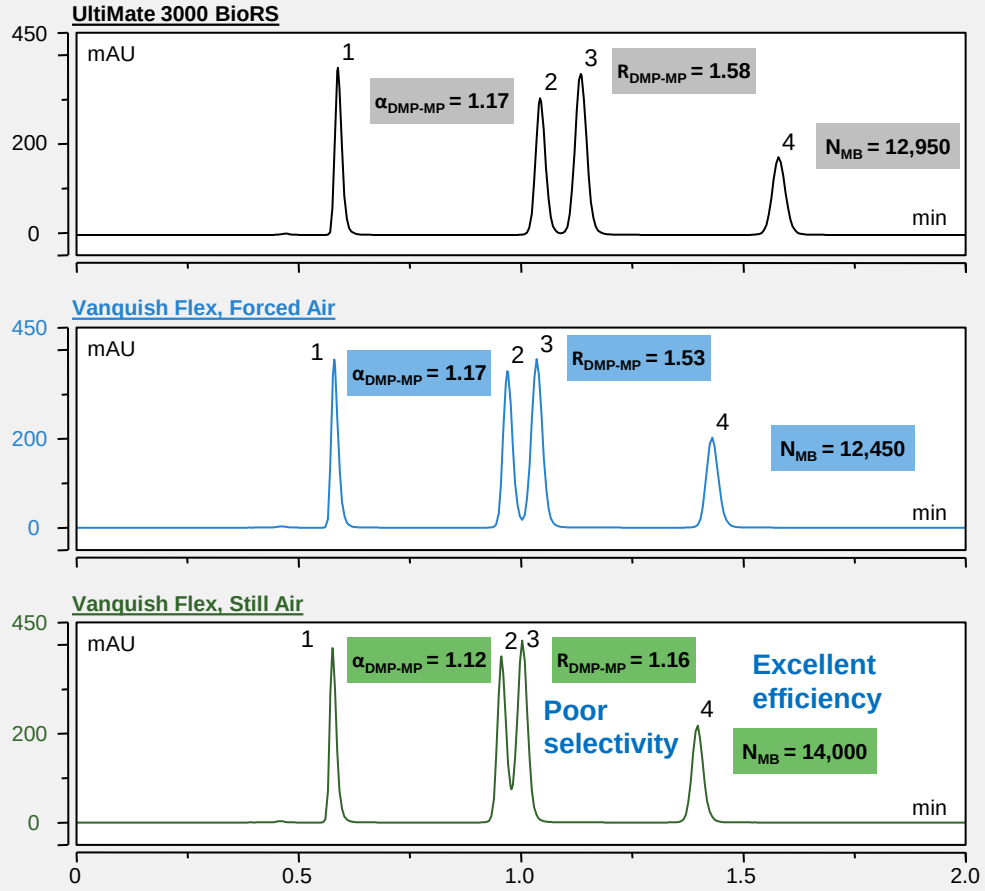

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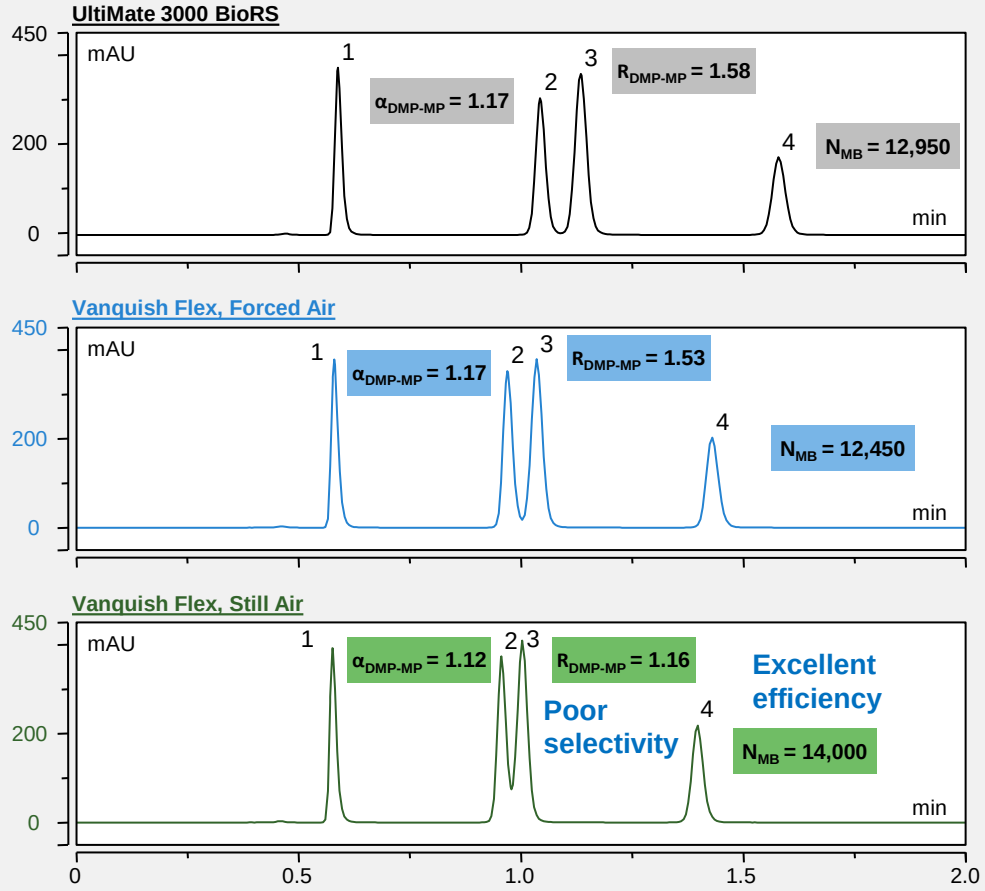

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Retention and selectivity very sensitive to temperature with this application

Minor changes in eluent pre-heating and frictional heat dissipation can matter

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Setting the Eluent Pre-heater Temperature Different to the Column Compartment

Correlation of retention factors of origin UltiMate 3000 BioRS system (◆ Forced Air principle) and target Vanquish *Flex* system (◆ Still Air mode) by reducing incoming eluent temperature enabled by the active pre-heater

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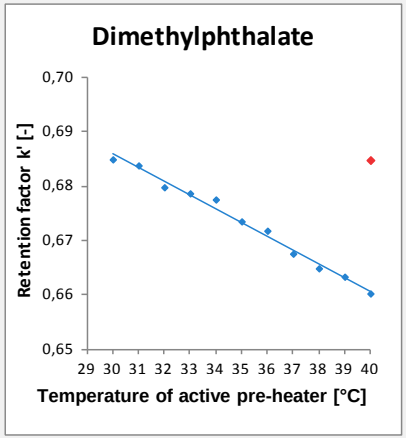

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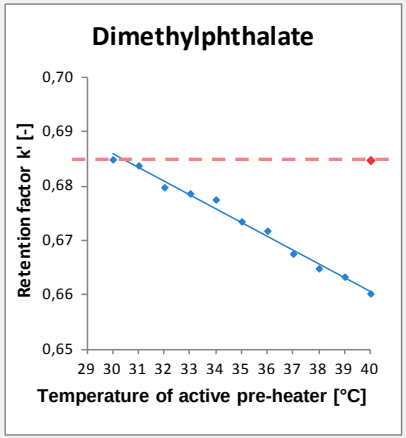

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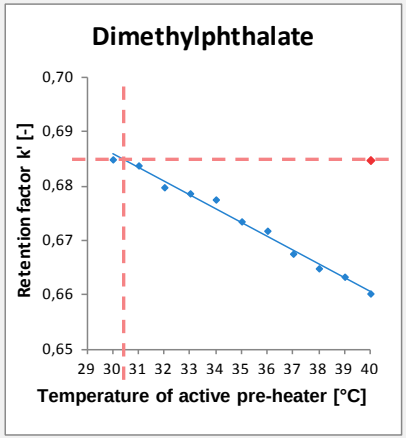

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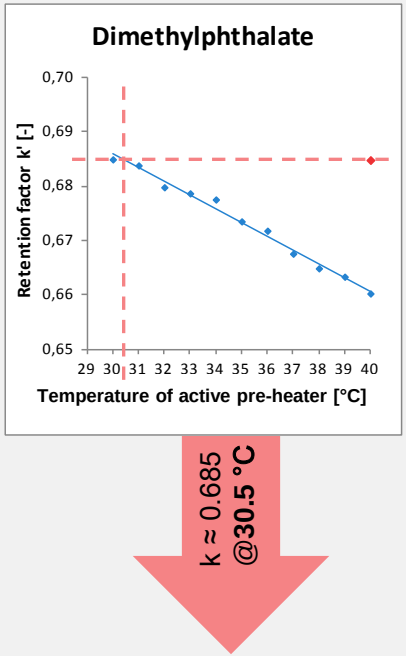

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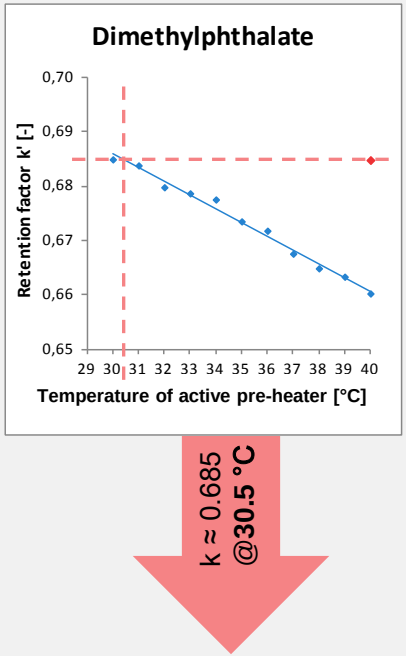

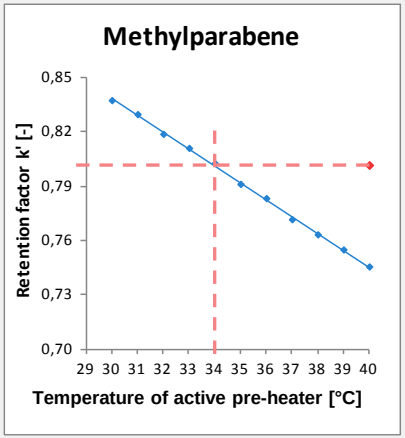

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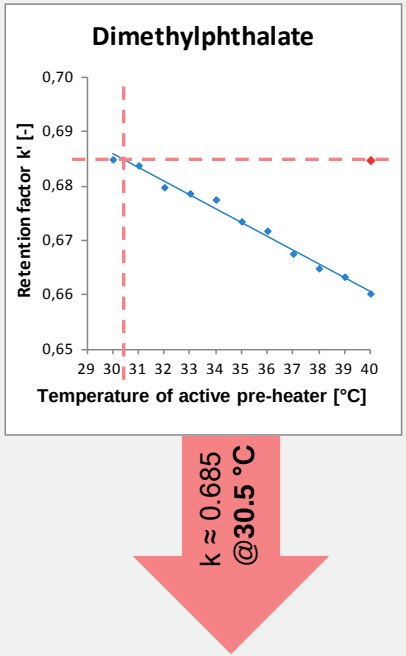

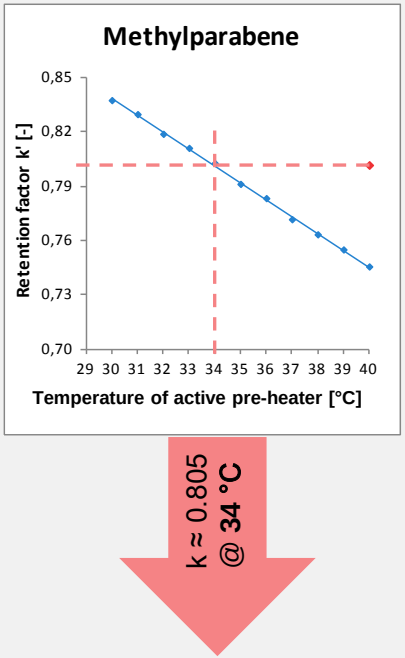

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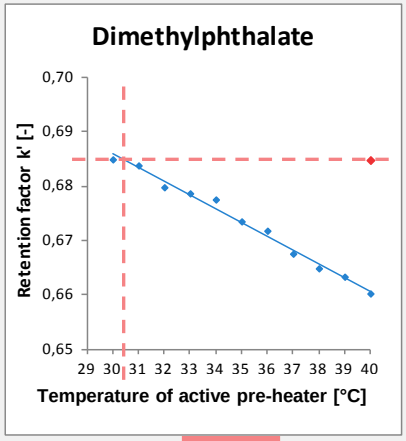
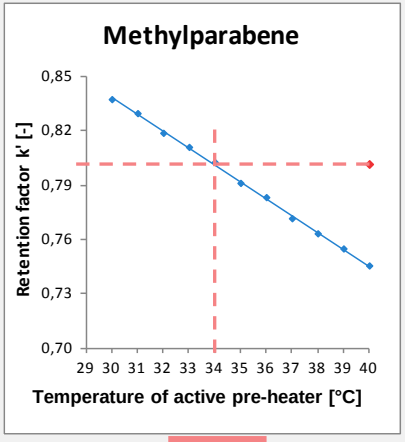
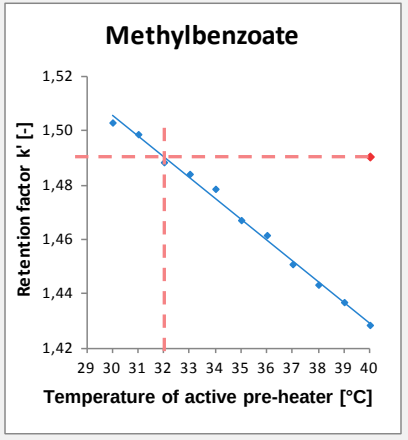


k ≈ 0.685 **@30.5 °C**



k ≈ 0.805 **@ 34 °C**



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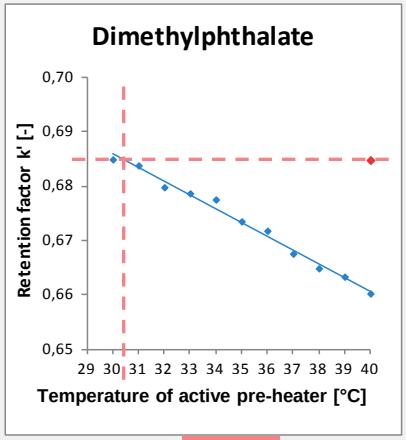

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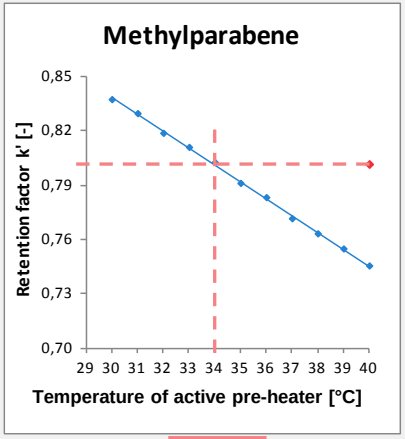

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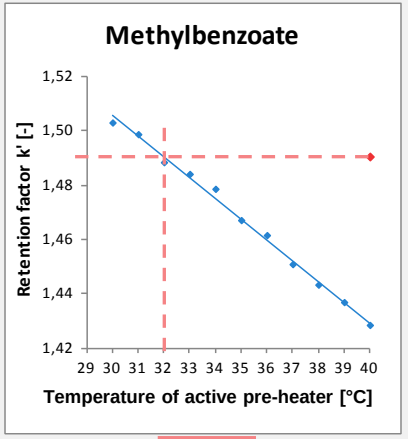

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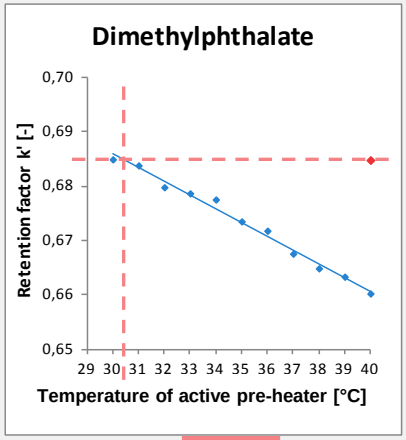

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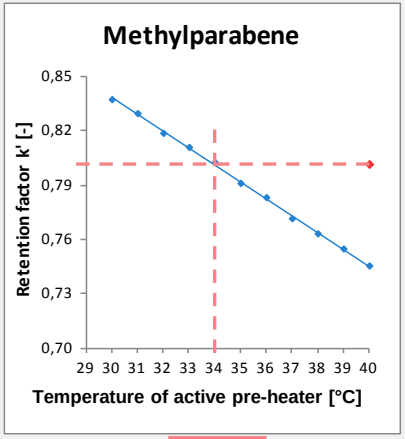

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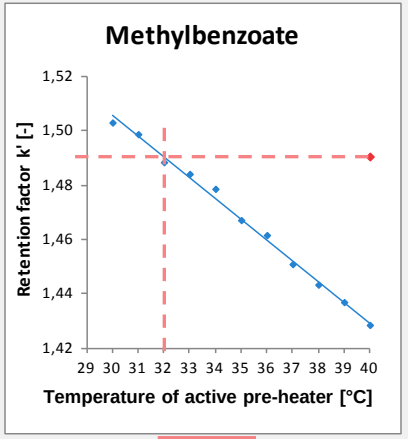

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In compromise an averaged incoming eluent temperature of 32 °C should deliver best matching retention factors for all 3 substances in Still Air mode

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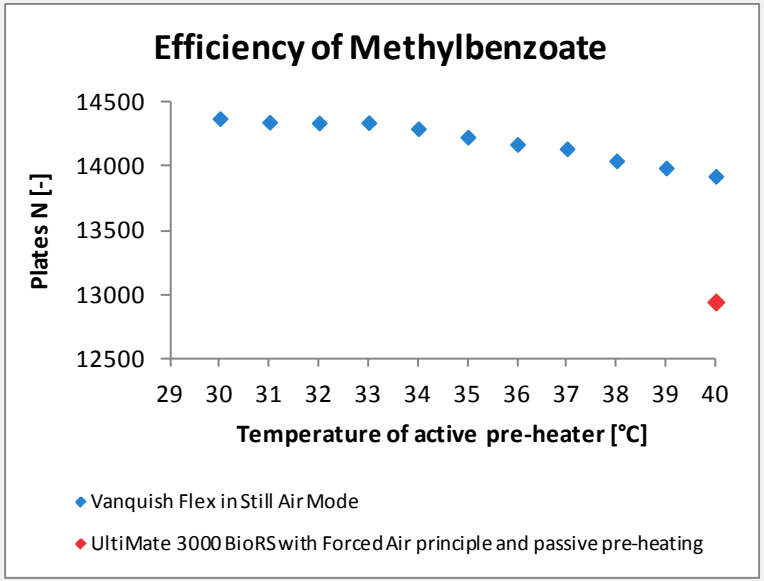

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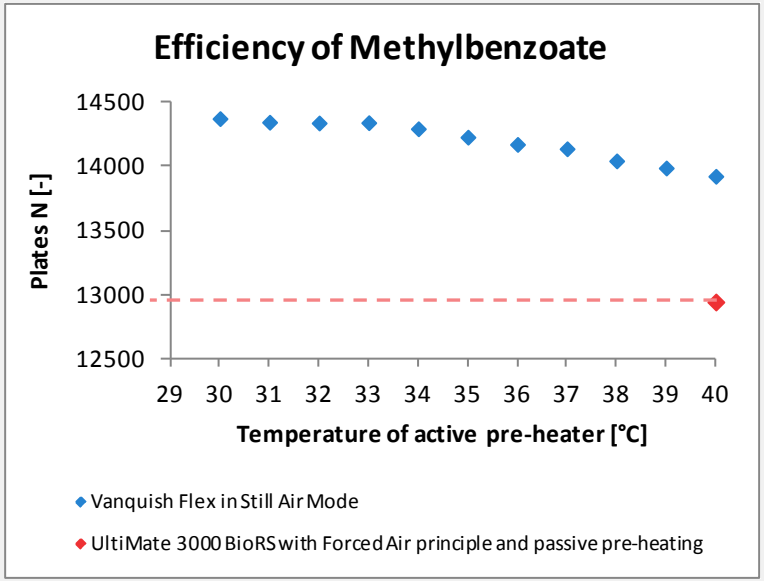

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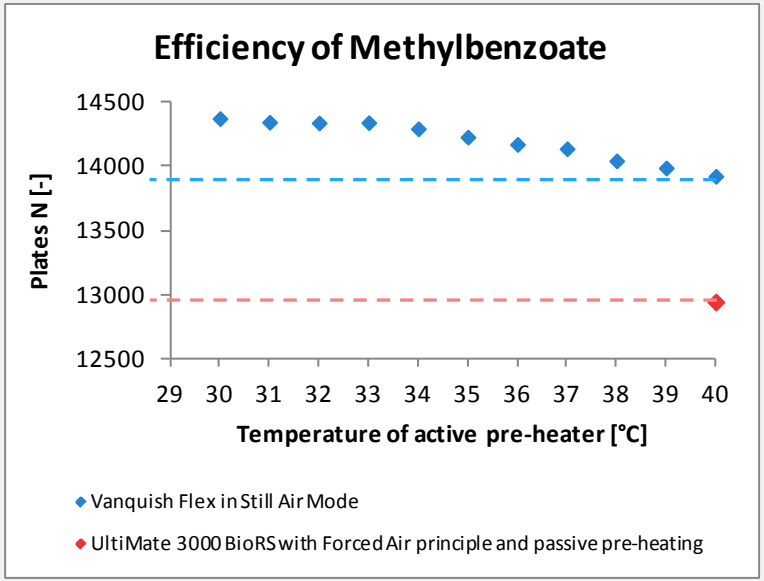

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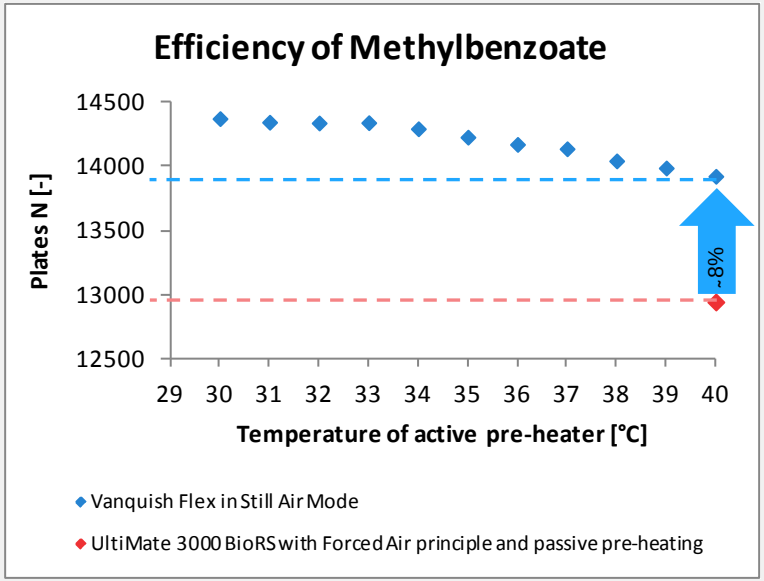

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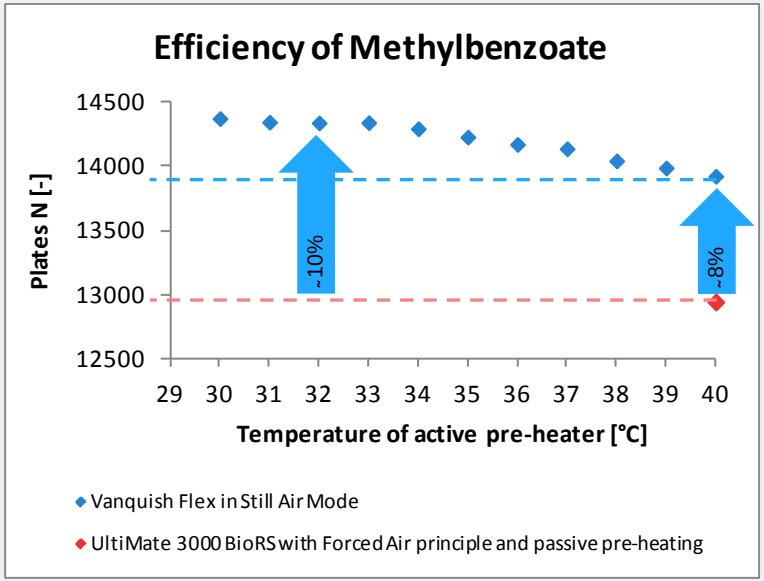

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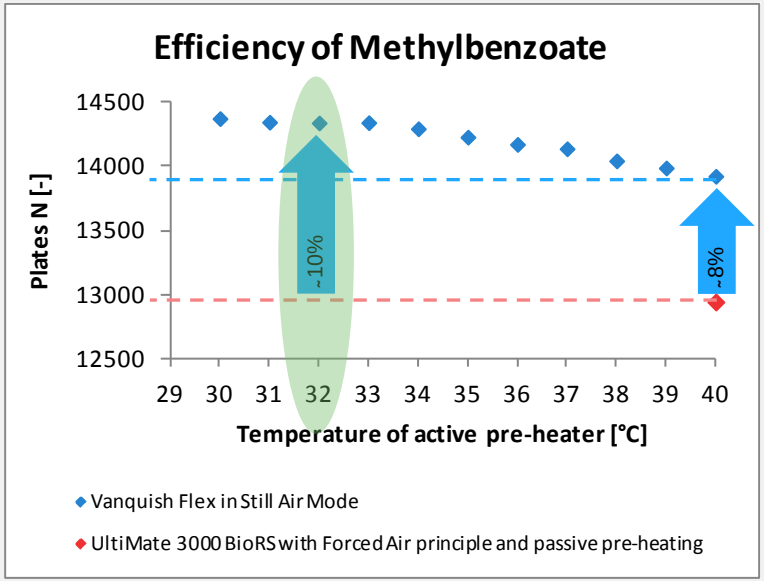

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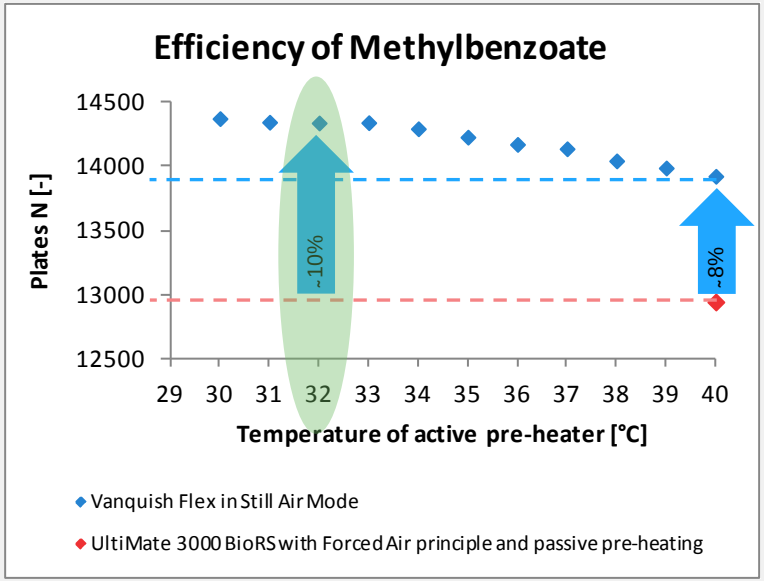

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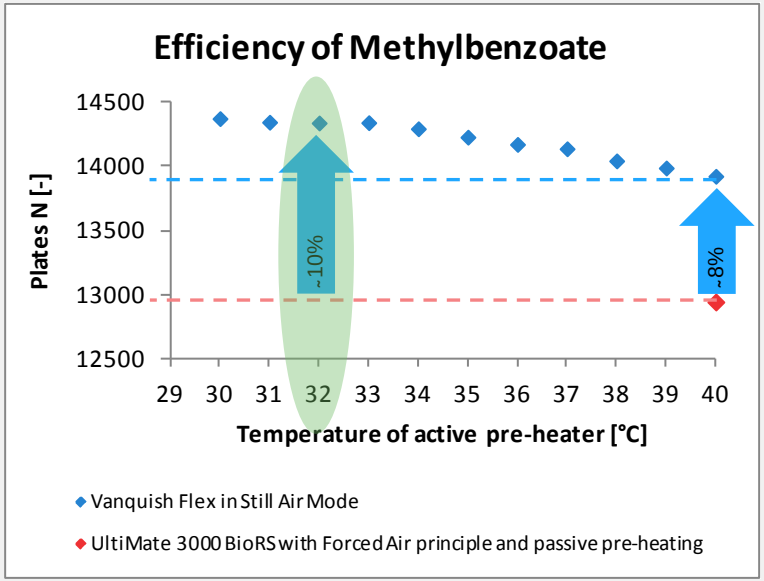

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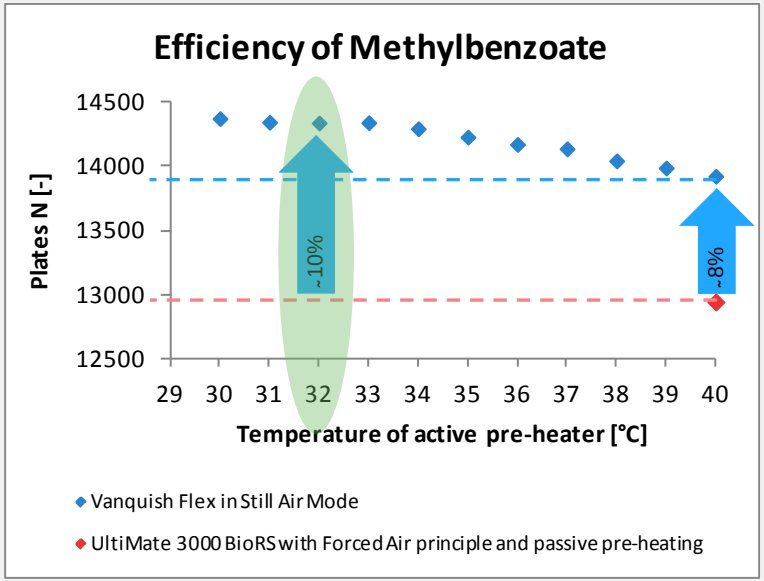

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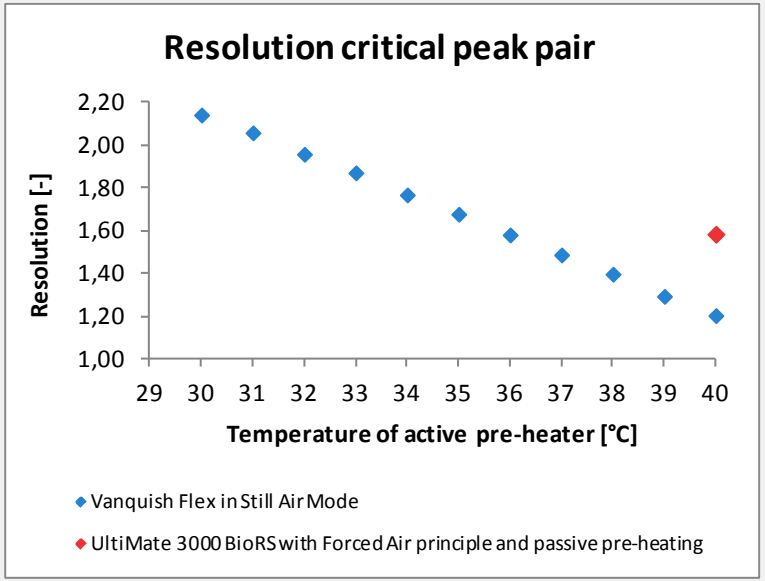

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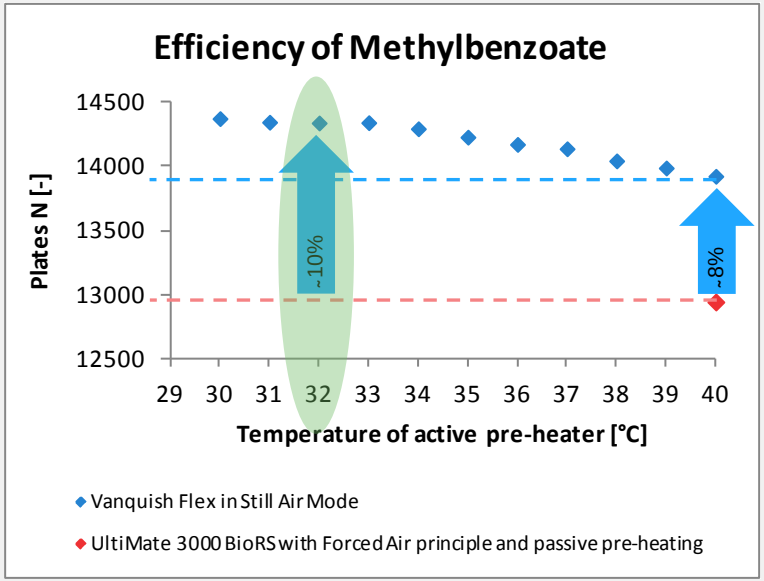

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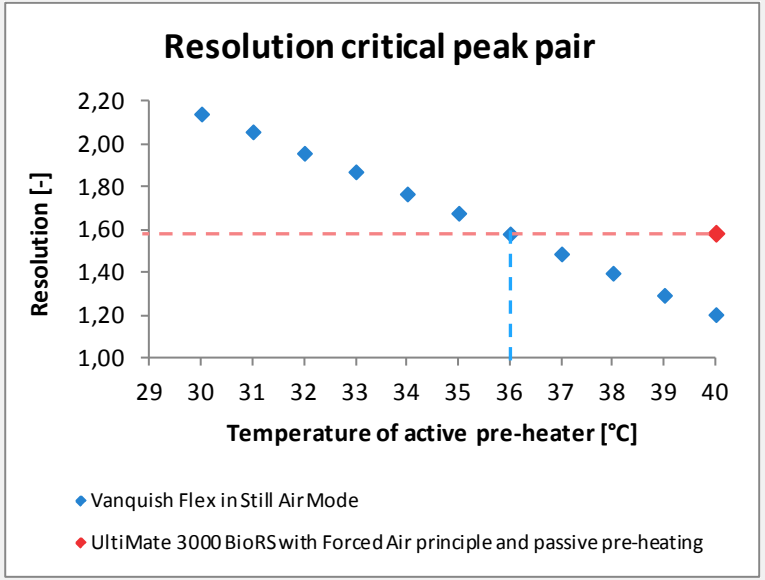

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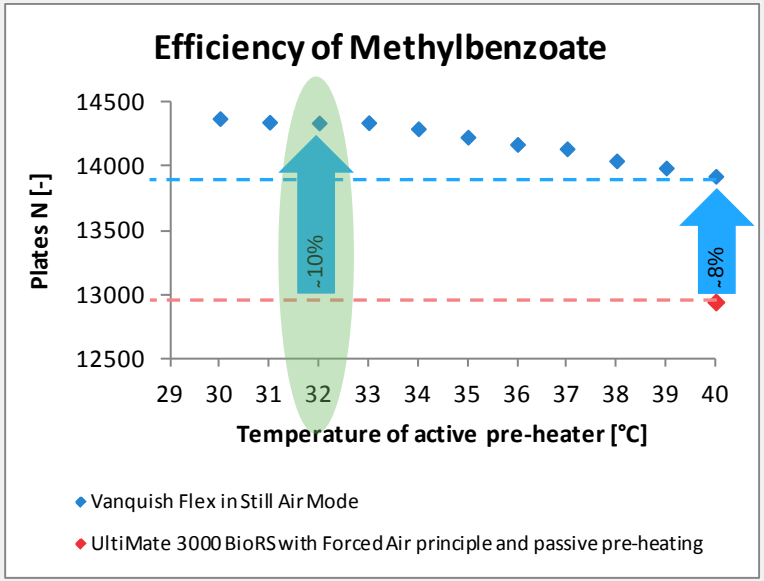

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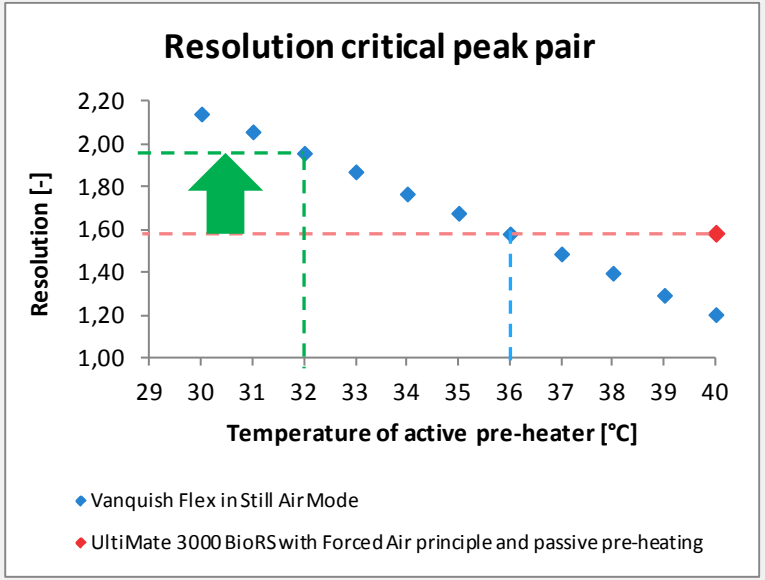

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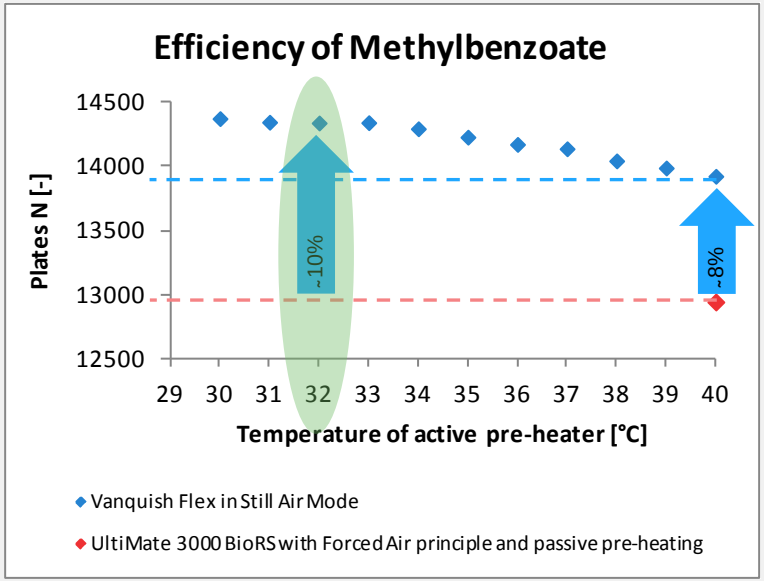

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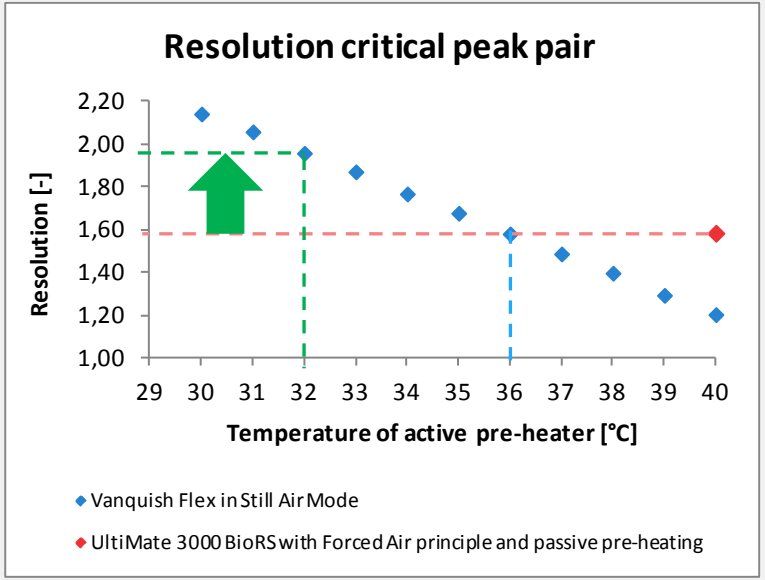
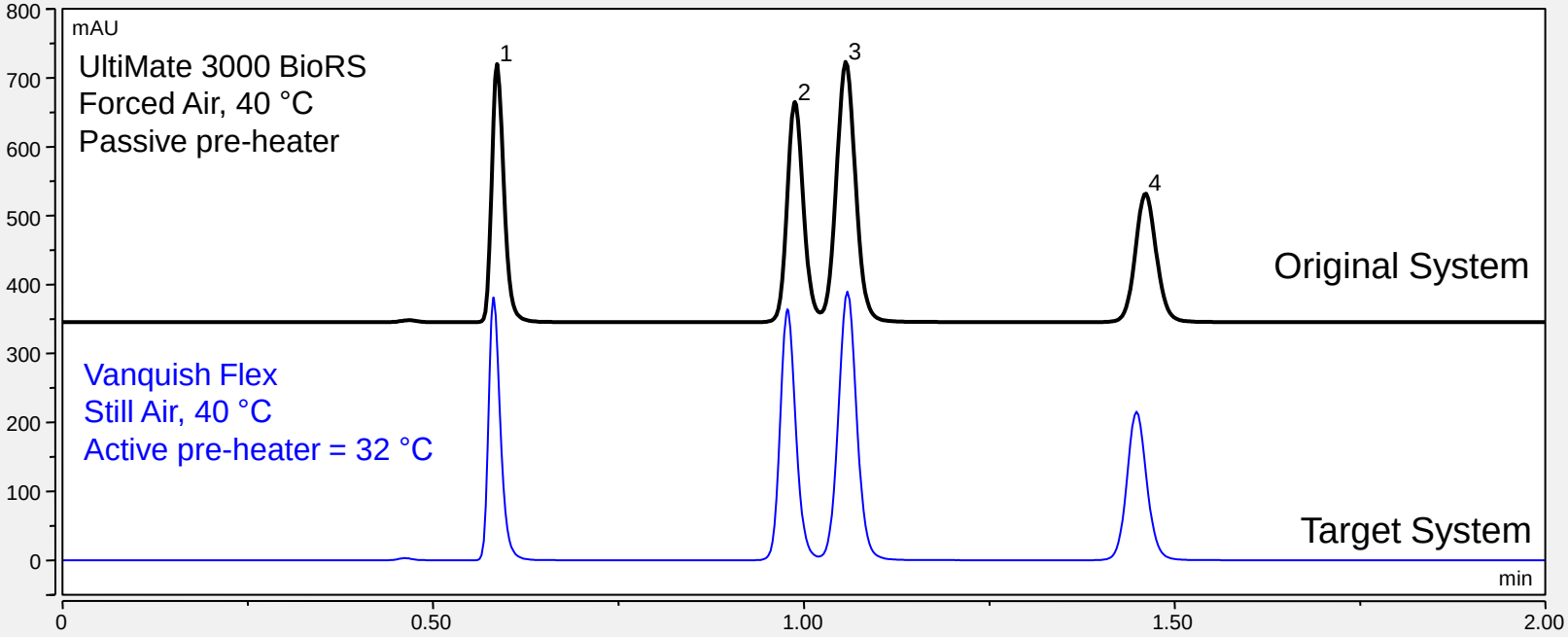


Still Air mode + reduced pre-heater temperature deliver better efficiency + better resolution

Method Transfer with Improved Resolution by Unique Thermosttating Features



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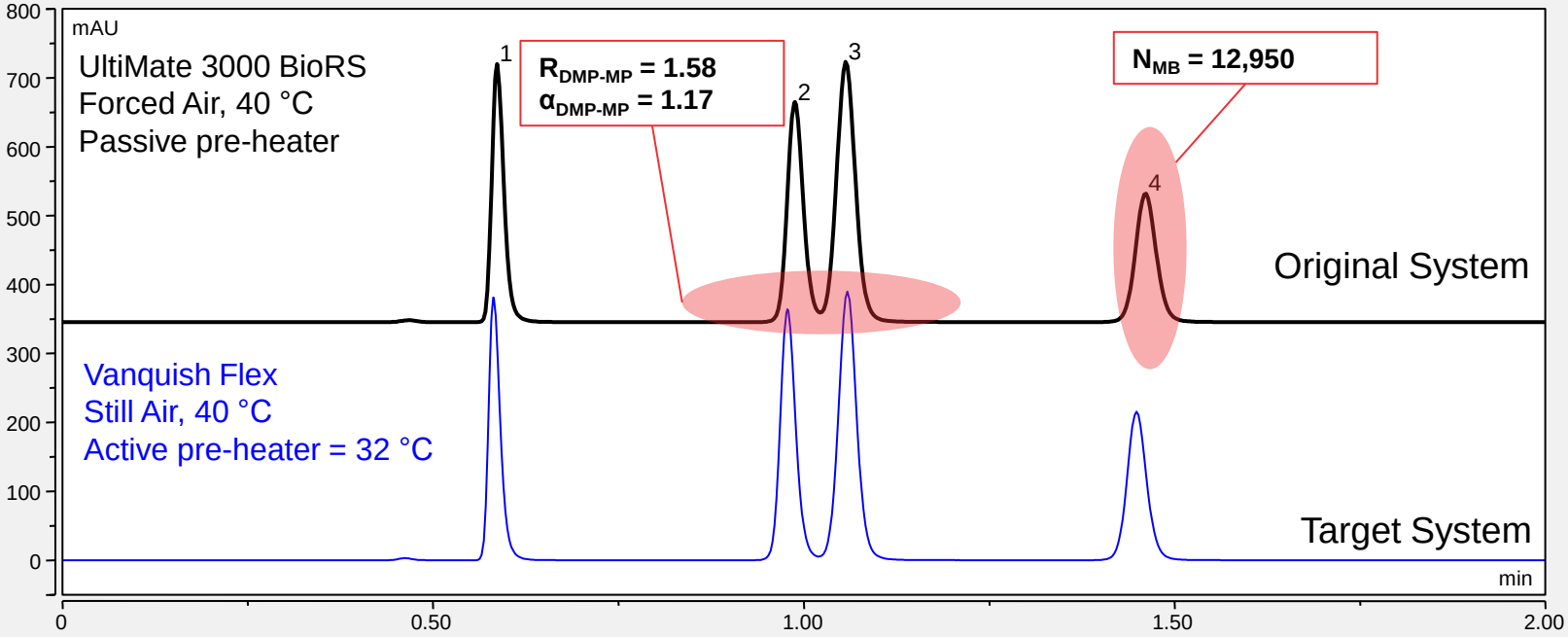

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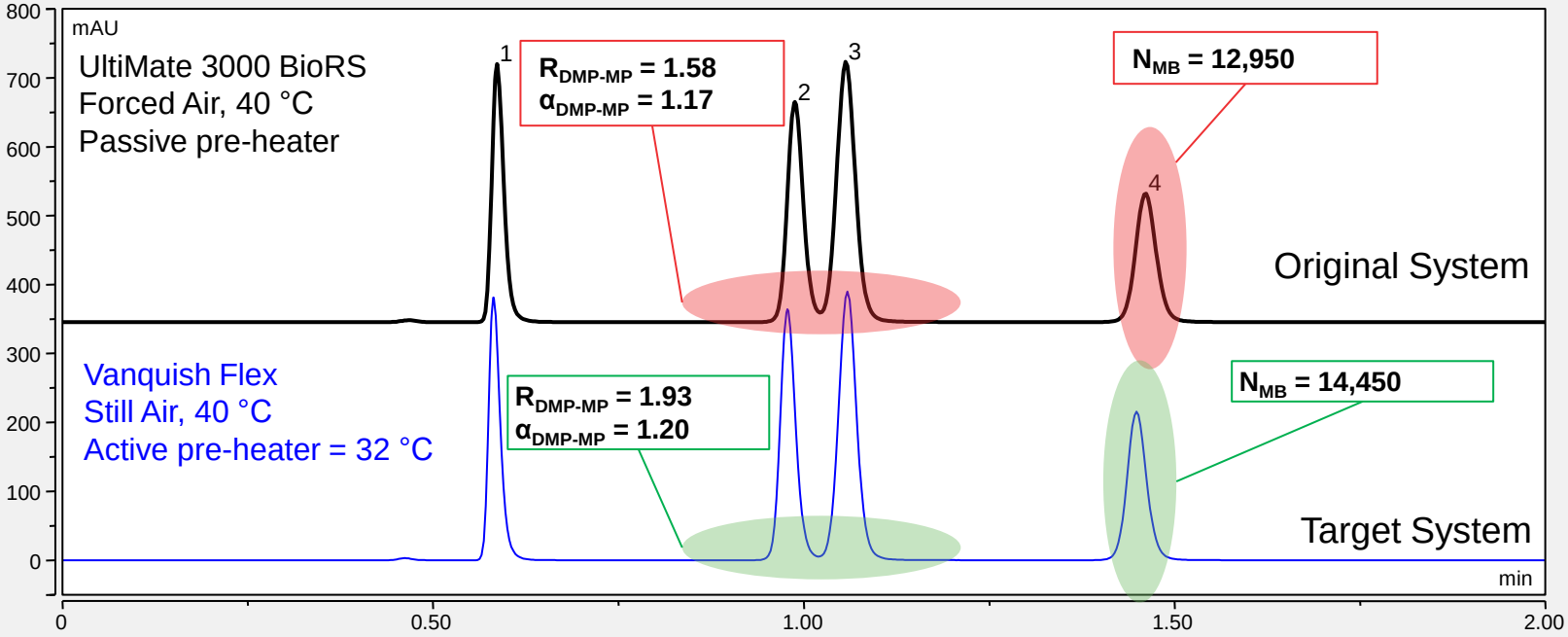

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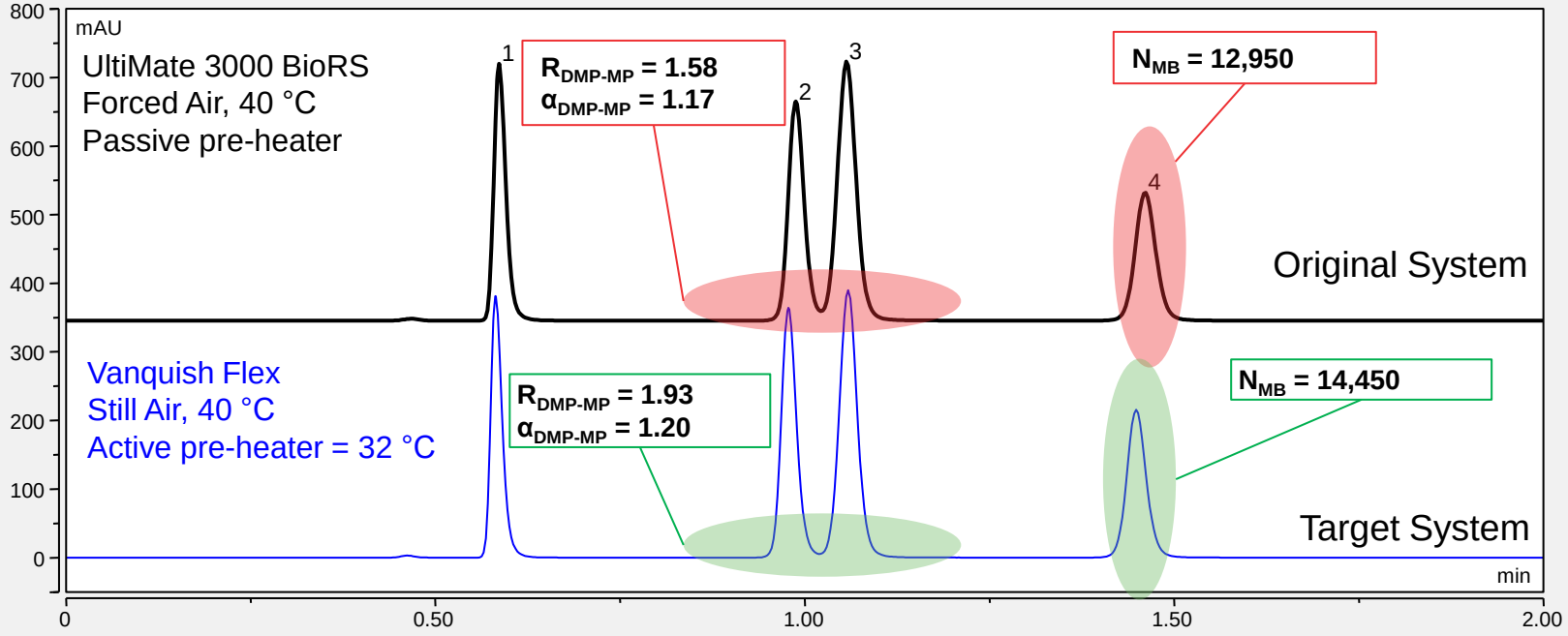


Match of retention factors and *improved* resolution

Successful method transfer with increased performance (resolution and plates) through unique thermostatting features of Vanquish platform

Method transfer

- **Gradient delay volume (GDV) adaption**
  - Adjustable metering device idle volume
  - Mixer and Viper capillaries
  - Delayed injection

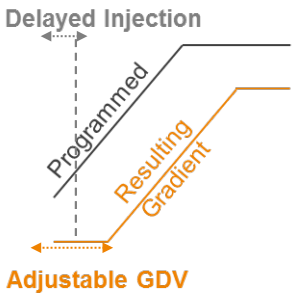

Chromeleon

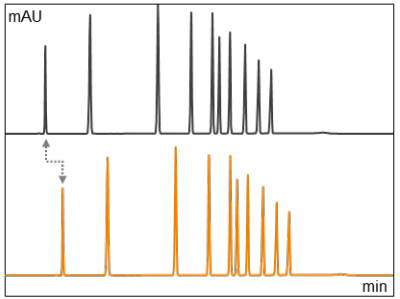

Pump H and F

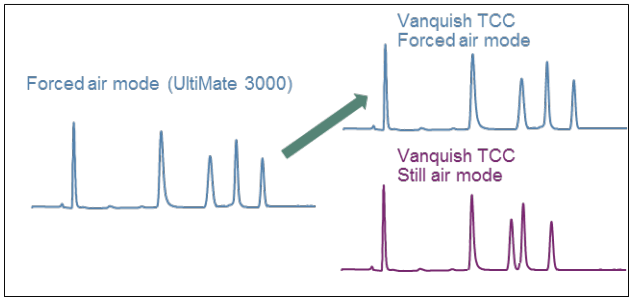

Sampler HT and FT

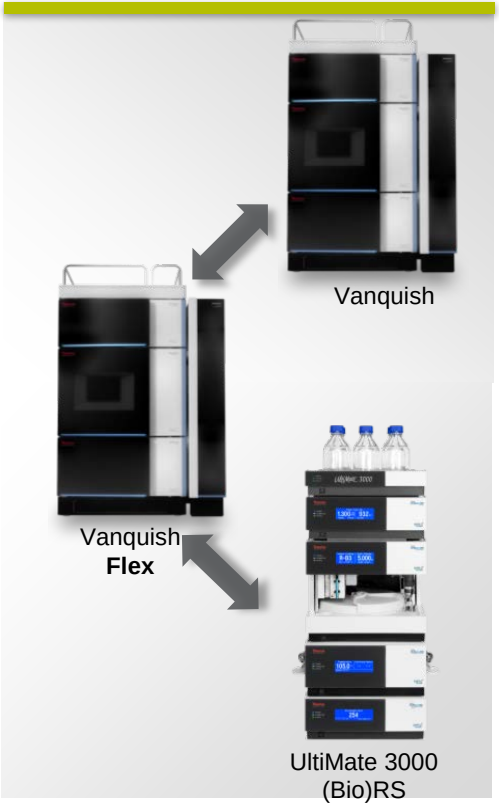
- **Match the thermostatting mode**
  - Forced and still air mode give different results
  - Mimic thermal conditions used in existing systems
  - UltiMate 3000 TCC or column compartments of competitors


Column Comp. H









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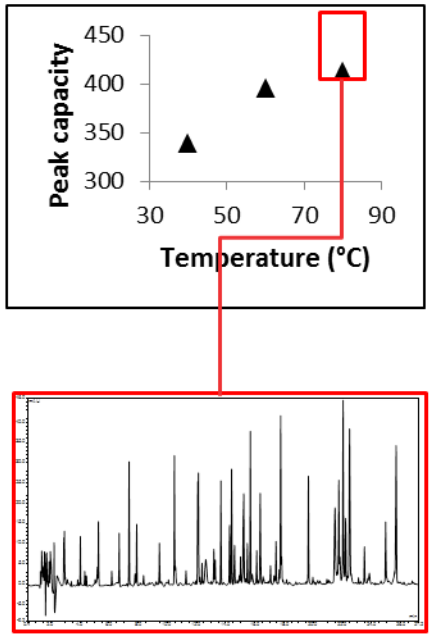
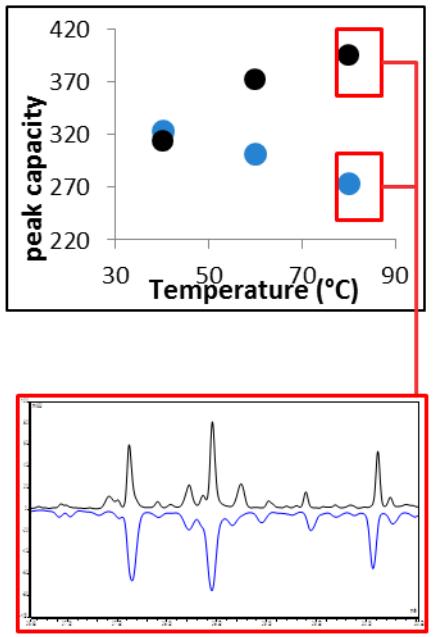
Increasing peak capacity by smart temperature control

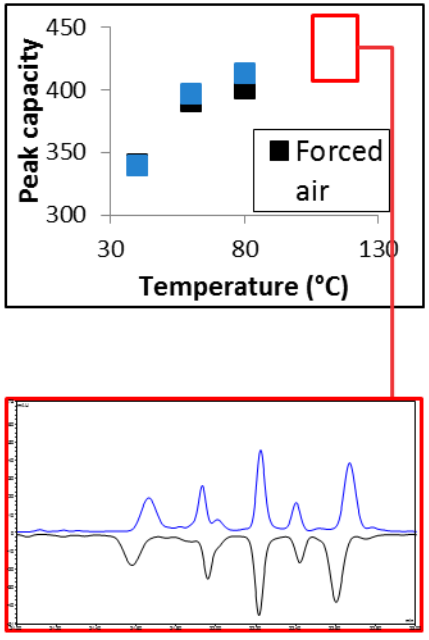
Column temperature



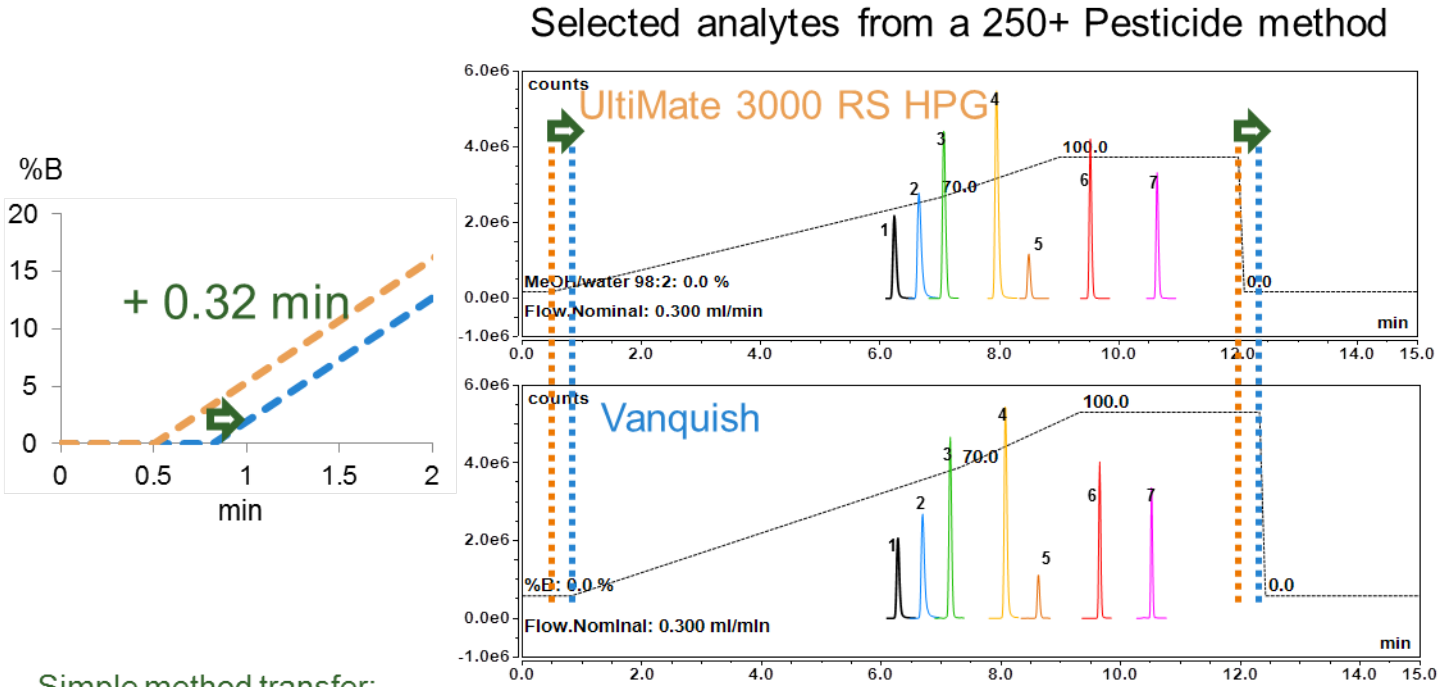
Mobile phase pre-heating



Column heating mode



Method transfer from UltiMate 3000 RS HPG to Vanquish



Simple method transfer:

Adding 0.32 min to the initial isocratic hold to account for the lower Vanquish GDV

Result:

Time shift between the two UHPLC methods at average 5 s over all 250+ pesticides.

What is the best practice?

Method Transfer Best Practice Summary

- Consider changes of prescribed method parameters vs. hardware adaptations
- Consider physico-chemical effects of gradient generation principle (HPG vs. LPG)
- Consider the advantage of seamless GDV adaptation by the adjustable metering device in Vanquish autosamplers
- Consider the influence of column and eluent thermostatting specifics and the unique thermostatting features that the Vanquish systems offer