Approaches Towards Method Compatibility Between HPLC and UHPLC Systems

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Introduction

Modern UHPLC instruments are optimized for fast separations in small diameter columns.

Delay volumes have been reduced by a factor of 5-10 compared to conventional HPLC instrumentation.

Due to differences in pump and mixer design differences in gradient formation exist between different types of HPLC instruments

Approaches have been evaluated to overcome these problems and a novel solution is proposed to provide method compatibility between HPLC and UHPLC.

Different Concepts of Method Transfer

From Instrument to Instrument w/o change in Method

-> see 1290 Infinity Compatibility Mode

-> available tools : **none so far**

From column dimension to column dimension (e.g. 4.6 -> 2.1, 50 mm -> 100 mm)

-> recalculate flow rates, recalculate gradient times, adjust connection capillaries and flow cells, due to delay volume results may vary, method might need revalidation

-> available tools: Agilent Method translator

From particle size to particle size (HPLC -> UHPLC)

-> is often associated with a change of instruments due to constraints in max. pressure, extra-column band broadening etc.. Some fine-tuning of method usually required due to frictional heating effects

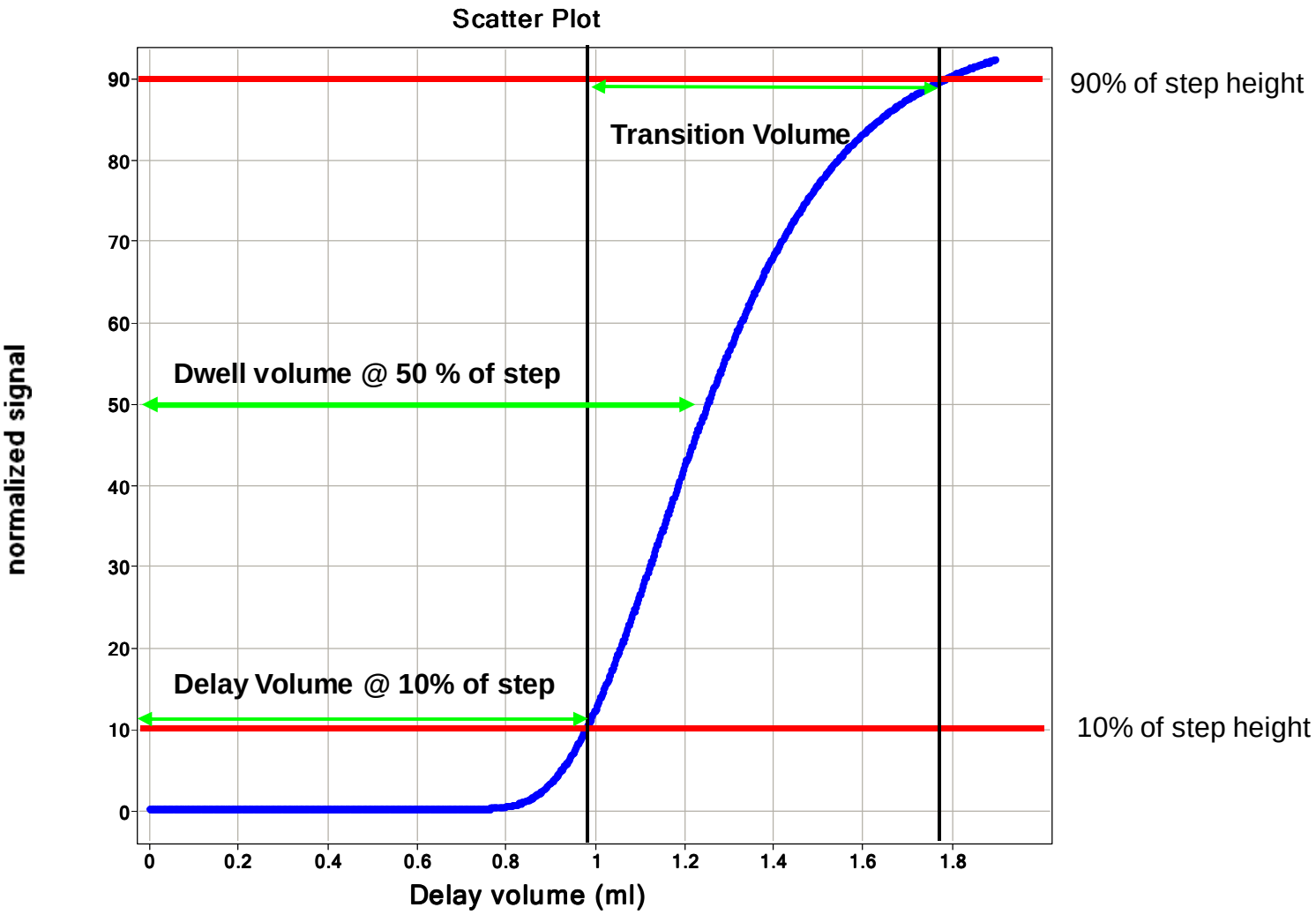
-> available tools : Agilent Method translator, Third Party Method development SW (ACD Labs, ChromSword etc.)

From one eluent type or phase chemistry to an other

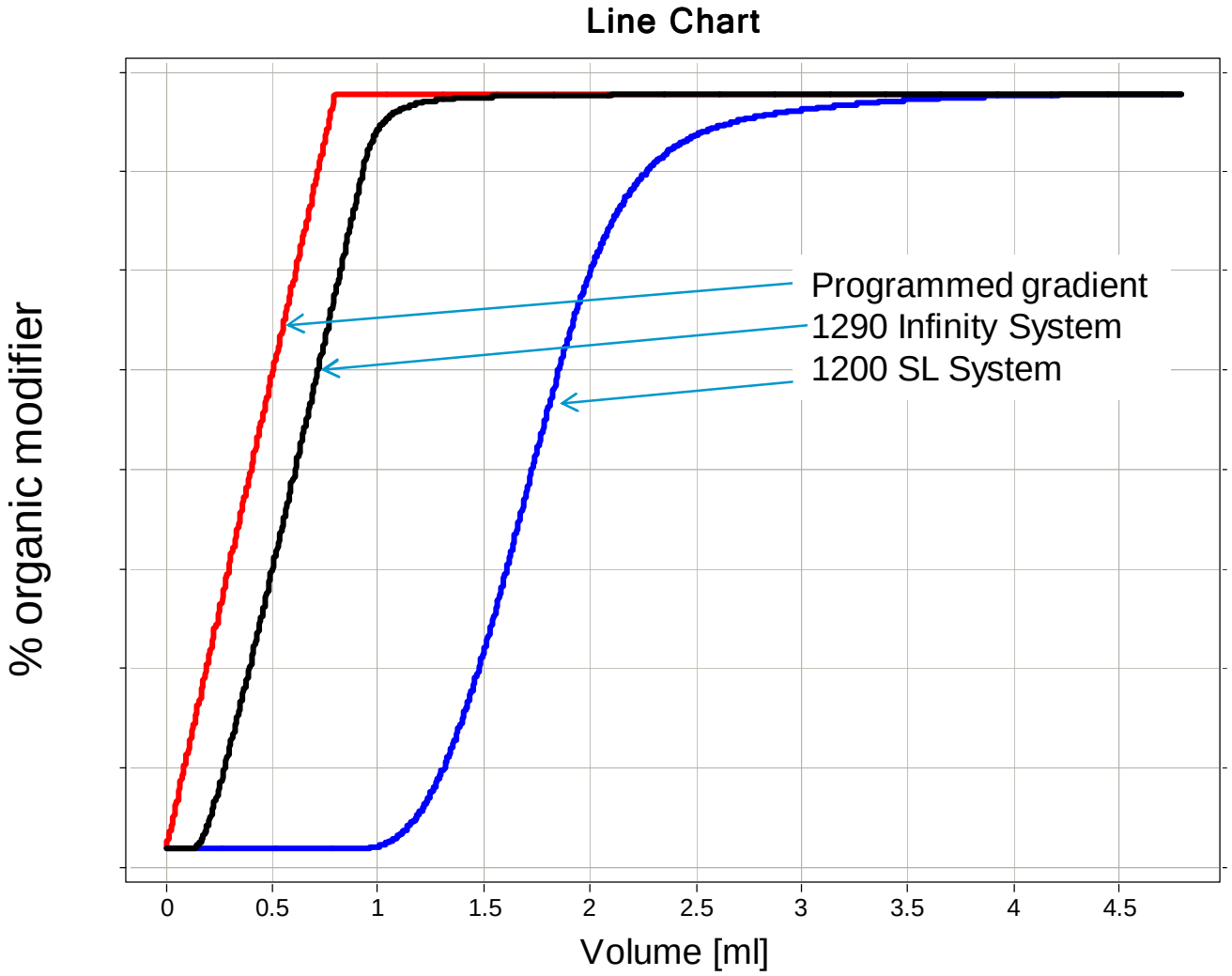
-> see Method development

-> available tools : Method development SW (ACD Labs etc.)

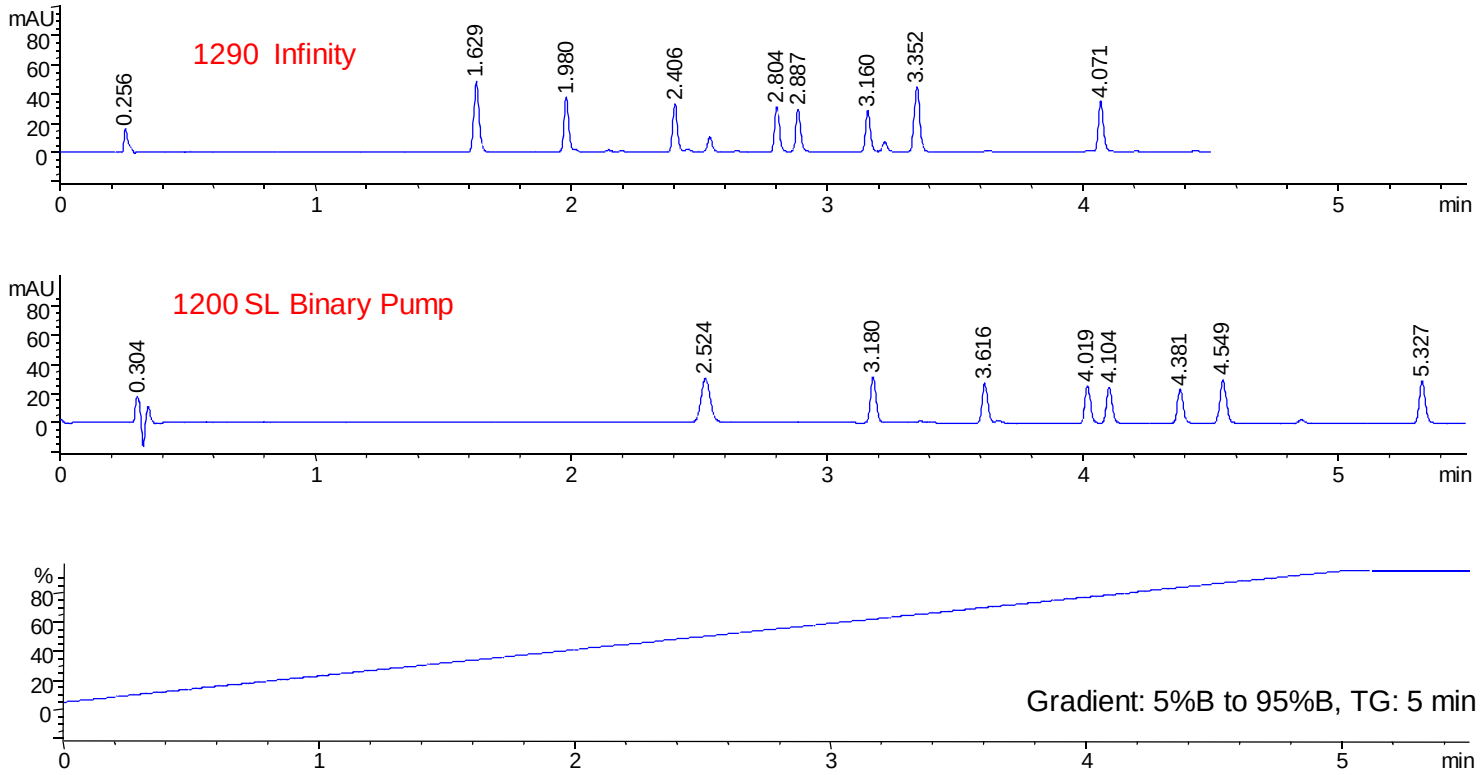
Definition of Delay / Transition volume



Difference in Delay Volume between Agilent 1200 SL and 1290 Infinity System



Comparison of Gradient Runs on Different Instruments



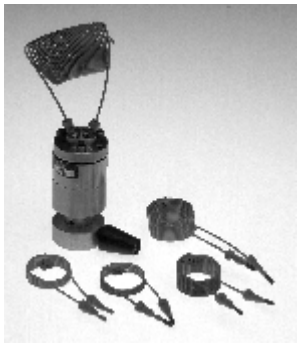
Column: Zorbax SB-C18, 2,1x50 mm, 1,8µ
Mobile phase: A = water / B = acetonotrile
Sig: 254,10nm; Ref: 360,100nm

Temperatue: 40℃
Inj.vol.: 1µl
Flow rate: 0,5 ml/min

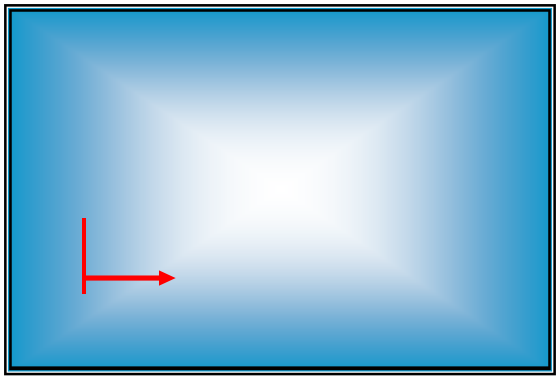
Sample: Sigma

Current Common Solutions

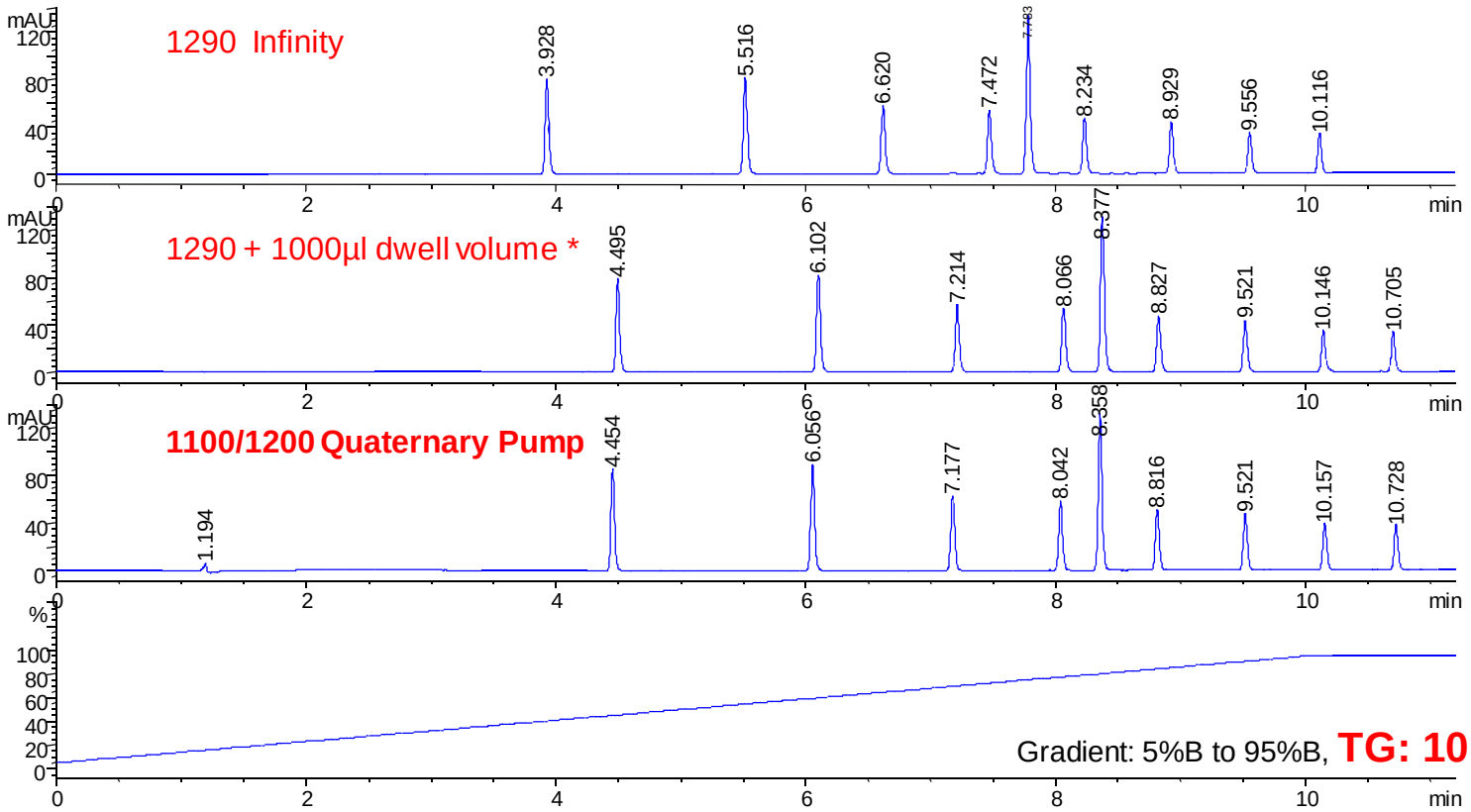
add physical Volume (plumbing solution)



Programmed isocratic hold



“Plumbing solution”: Comparison 1100/1200 Quaternary Pump vs 1290 Infinity



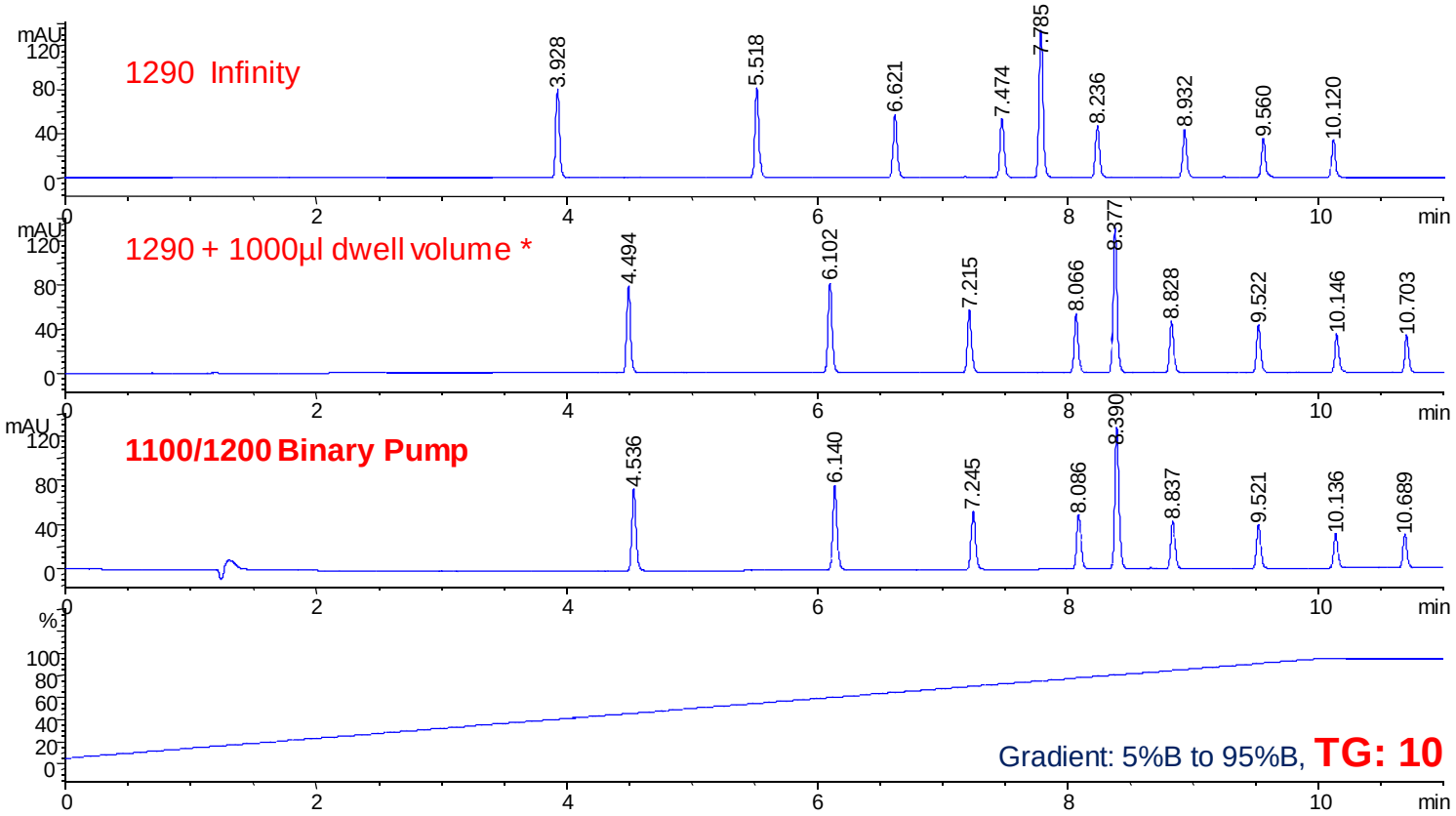
Column: Zorbax SB-C18, 4,6x150 mm, 3,5µ
Mobile phase: A = water / B = acetonotrile
Sig: 254,10nm; Ref: 360,100nm

Temperatue: 40℃
Inj.vol.: 5µl
Flow rate: 1,5 ml/min

Sample: **RRLC check out**

*experimentally obtained dwell volume value

“Plumbing solution”: Comparison 1100/1200 Binary Pump vs 1290 Infinity



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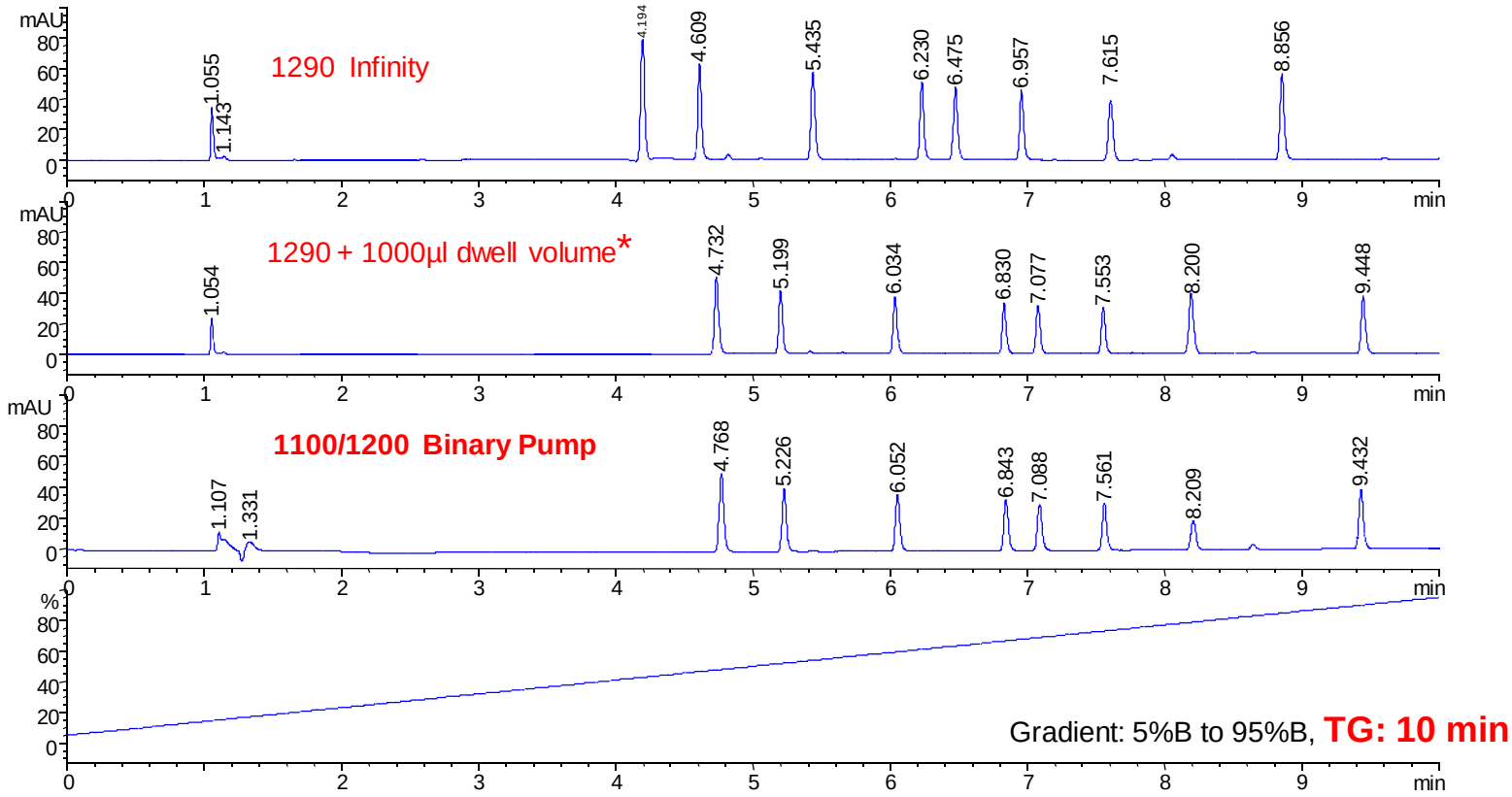
Sig: 254,10nm; Ref: 360,100nm

Temperatue: 40℃
Inj.vol.: 5µl
Flow rate: 1,5 ml/min

Sample: **RRLC check out**

*experimentally obtained dwell volume value

“Plumbing solution”: Comparison 1100/1200 Binary Pump vs 1290 Infinity



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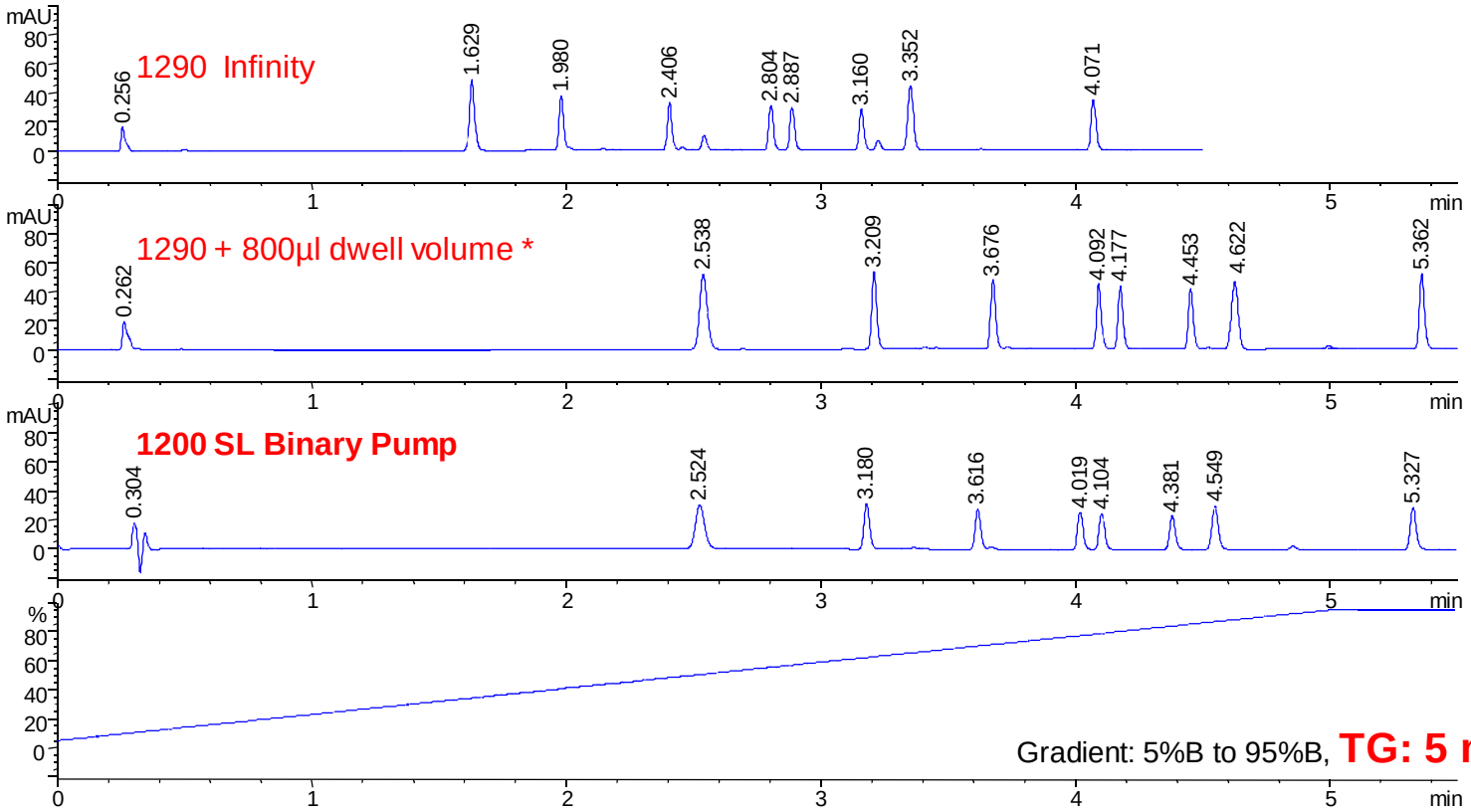
Mobile phase: A = water / B = acetonotrile
Sig: 254,10nm; Ref: 360,100nm

Temperatue: 40℃
Inj.vol.: 5µl
Flow rate: 1,5 ml/min

Sample: **Sigma**

*experimentally obtained dwell volume value

“Plumbing solution”: Comparison 1200 SL Binary Pump vs 1290 Infinity



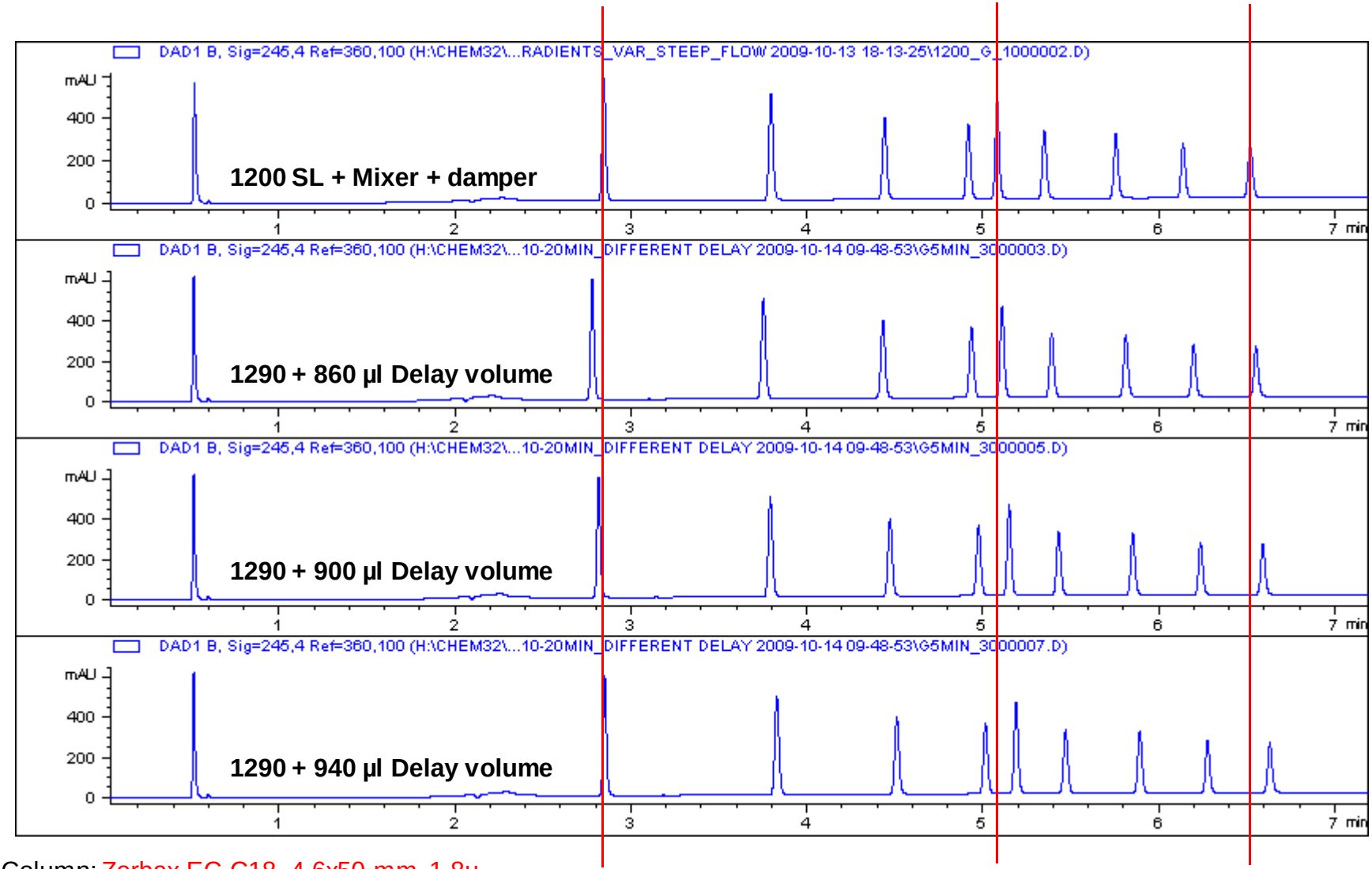
Column: Zorbax SB-C18, 2,1x50 mm, 1,8µ
Mobile phase: A = water / B = acetonotrile
Sig: 254,10nm; Ref: 360,100nm

Temperatue: 40℃
Inj.vol.: 1µl
Flow rate: 0,5 ml/min

Sample: **Sigma**

*experimentally obtained dwell volume value

Isocratic Hold: Comparison 1200 SL / 1290 Infinity
5 min Gradient @ 1 ml/min



Column: Zorbax EC-C18, 4.6x50 mm, 1,8µ
Mobile phase: A = water / B = acetonotrile
Gradient 5 – 95 % B in 5 min
Sample: RRLC checkout sample

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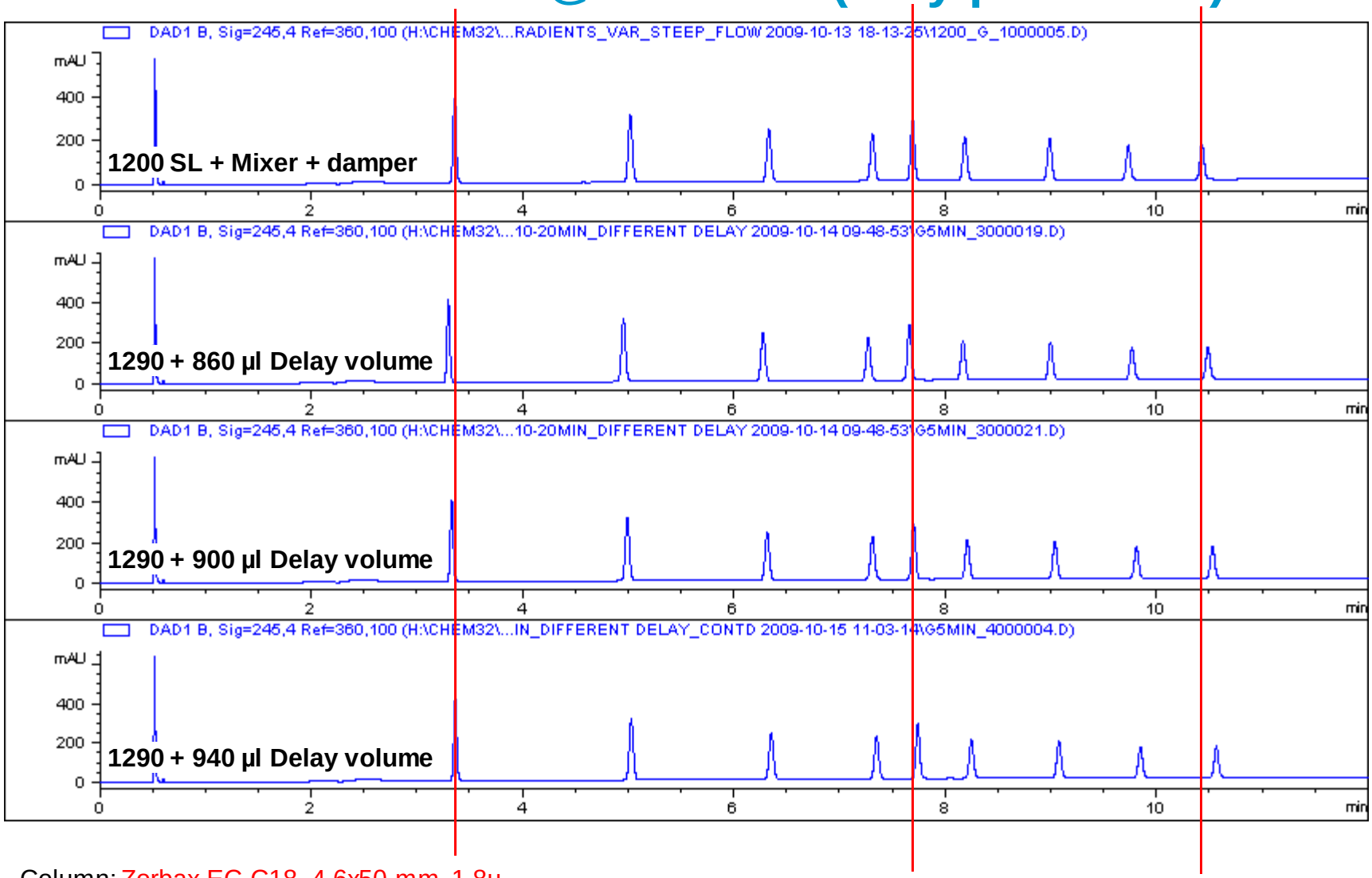
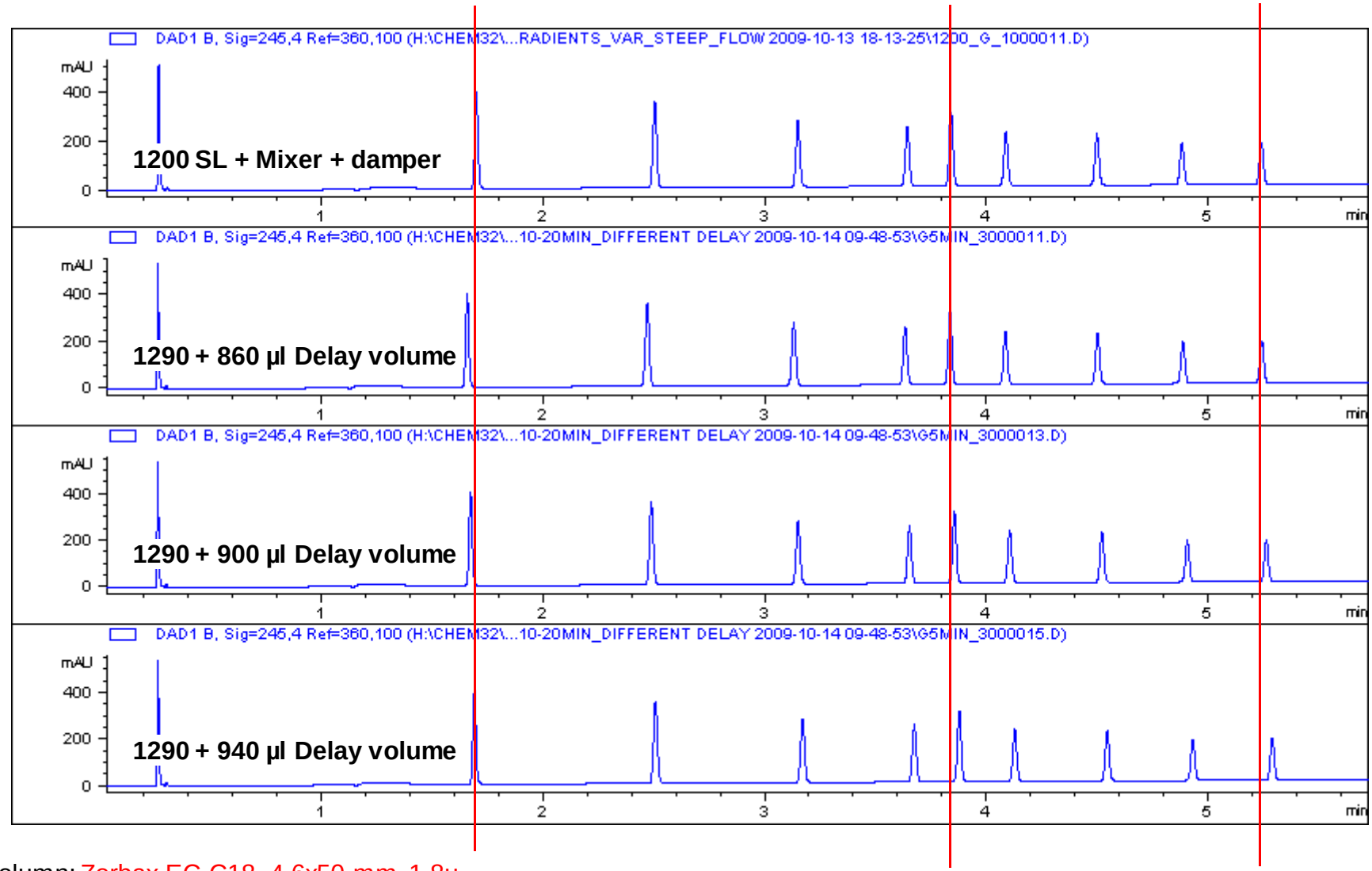
Isocratic Hold: Comparison 1200 SL / 1290 Infinity
10 min Gradient @ 1 ml/min (Alkylphenones)



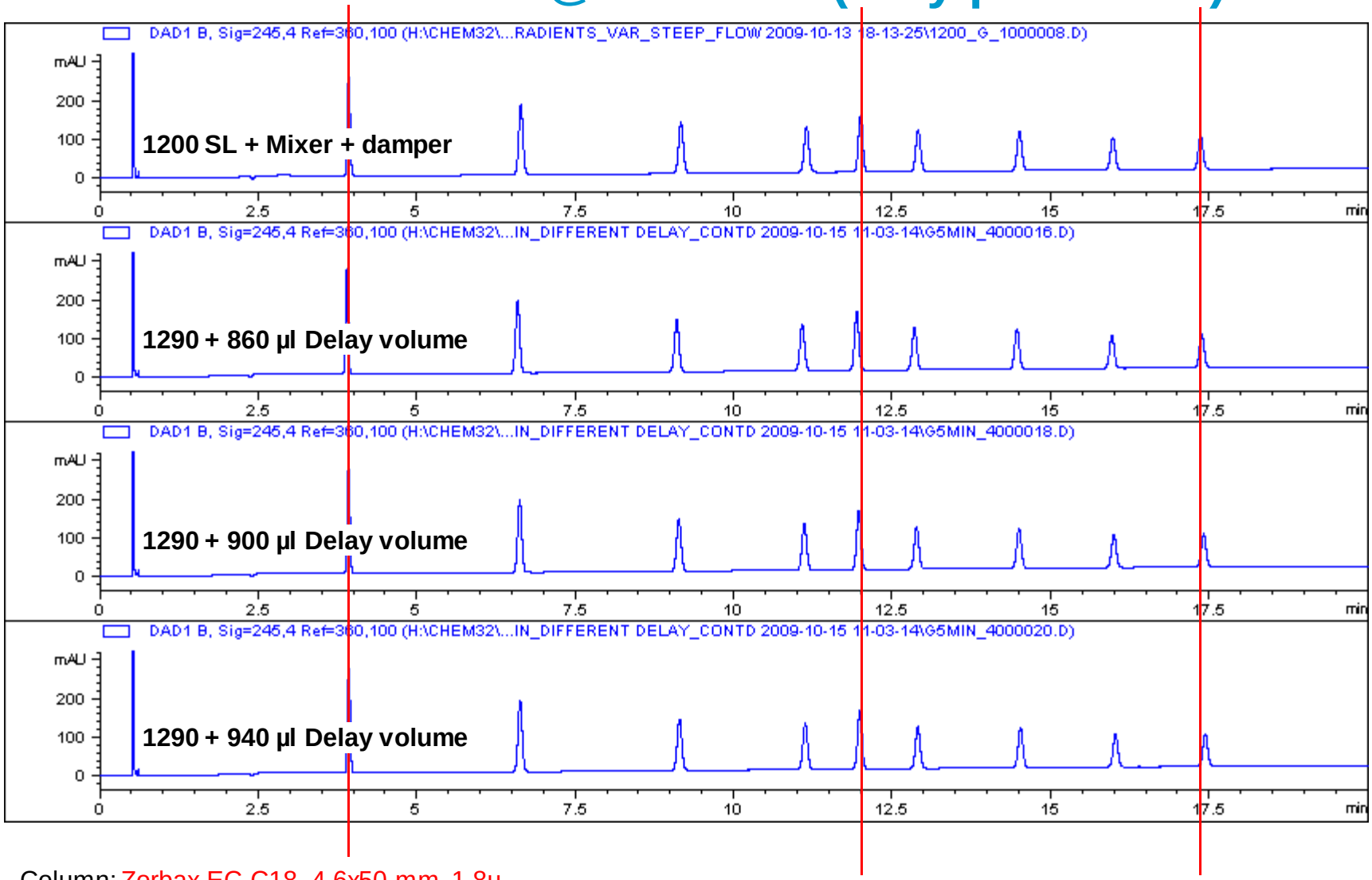
Column: Zorbax EC-C18, 4.6x50 mm, 1,8µ
Mobile phase: A = water / B = acetonotrile
Gradient 5 – 95 % B in 5 min
Sample: RRLC checkout sample

Isocratic Hold: Comparison 1200 SL / 1290 Infinity
10 min Gradient @ 2 ml/min (Alkylphenones)



Column: Zorbax EC-C18, 4.6x50 mm, 1,8µ
Mobile phase: A = water / B = acetonotrile
Gradient 5 – 95 % B in 10 min

Isocratic Hold: Comparison 1200 SL / 1290 Infinity
20 min Gradient @ 1 ml/min (Alkylphenones)



Column: Zorbax EC-C18, 4.6x50 mm, 1,8µ
Mobile phase: A = water / B = acetonotrile
Gradient 5 – 95 % B in 5 min
Sample: RRLC checkout sample

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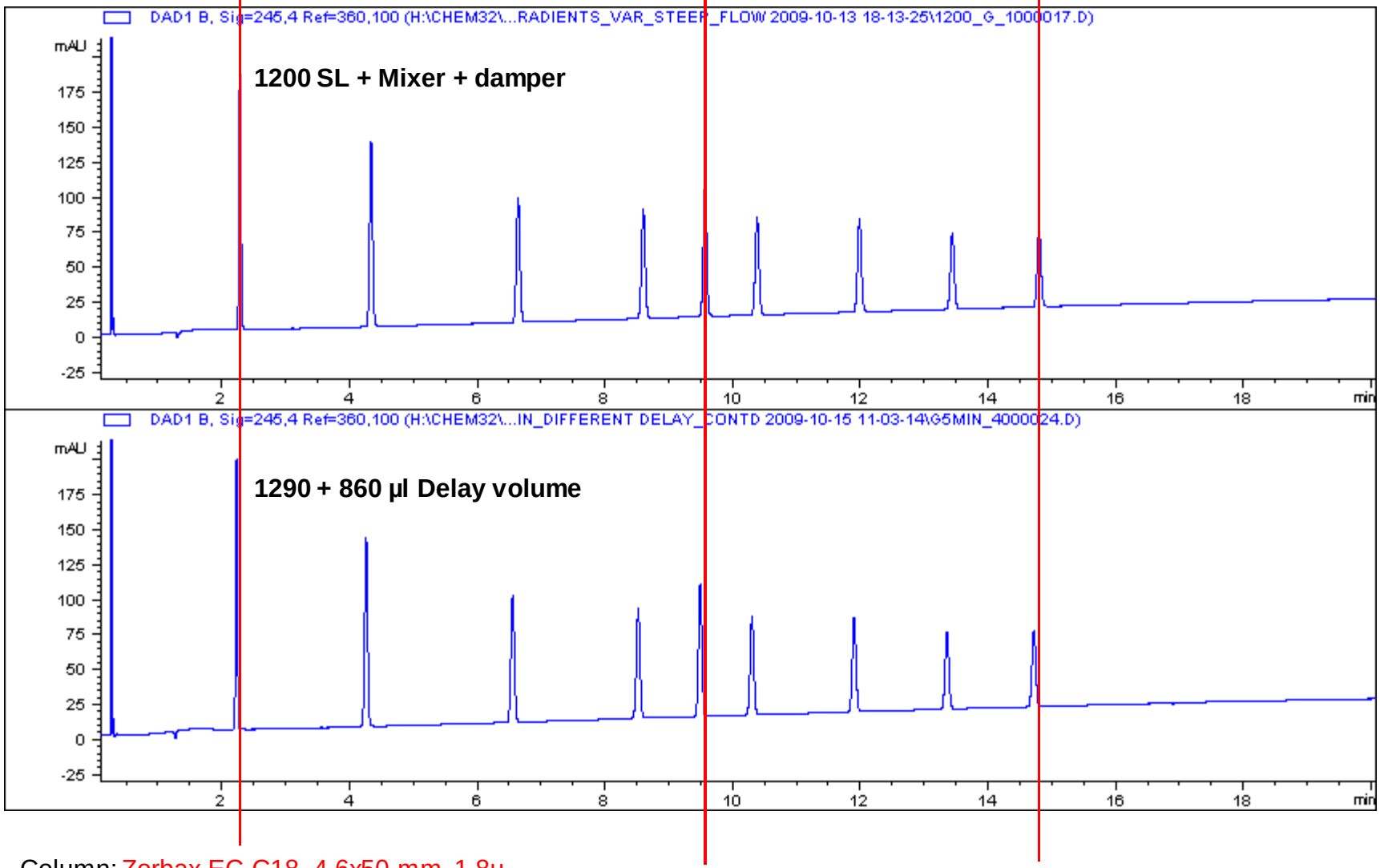
Isocratic Hold: Comparison 1200 SL / 1290 Infinity
20 min Gradient @ 2 ml/min (Alkylphenones)



Column: Zorbax EC-C18, 4.6x50 mm, 1,8µ
Mobile phase: A = water / B = acetonotrile
Gradient 5 – 95 % B in 5 min
Sample: RRLC checkout sample

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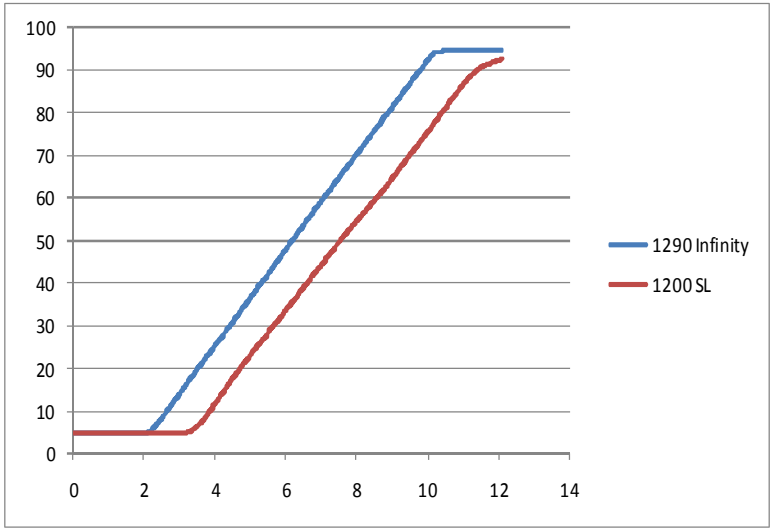
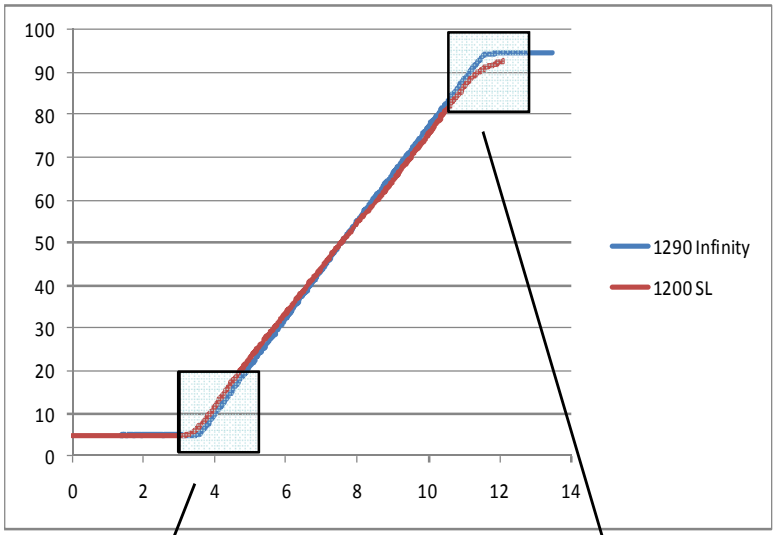
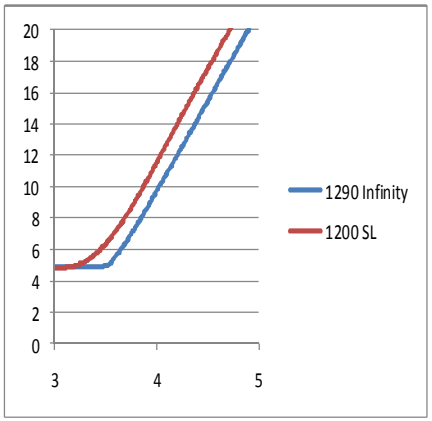
1200 SL Binary Pump vs 1290 Infinity with Programmed Isocratic Hold

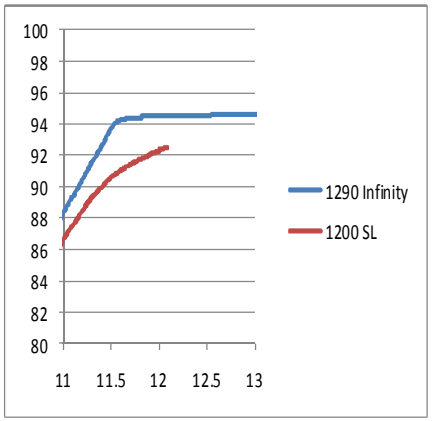
Original gradient response traces



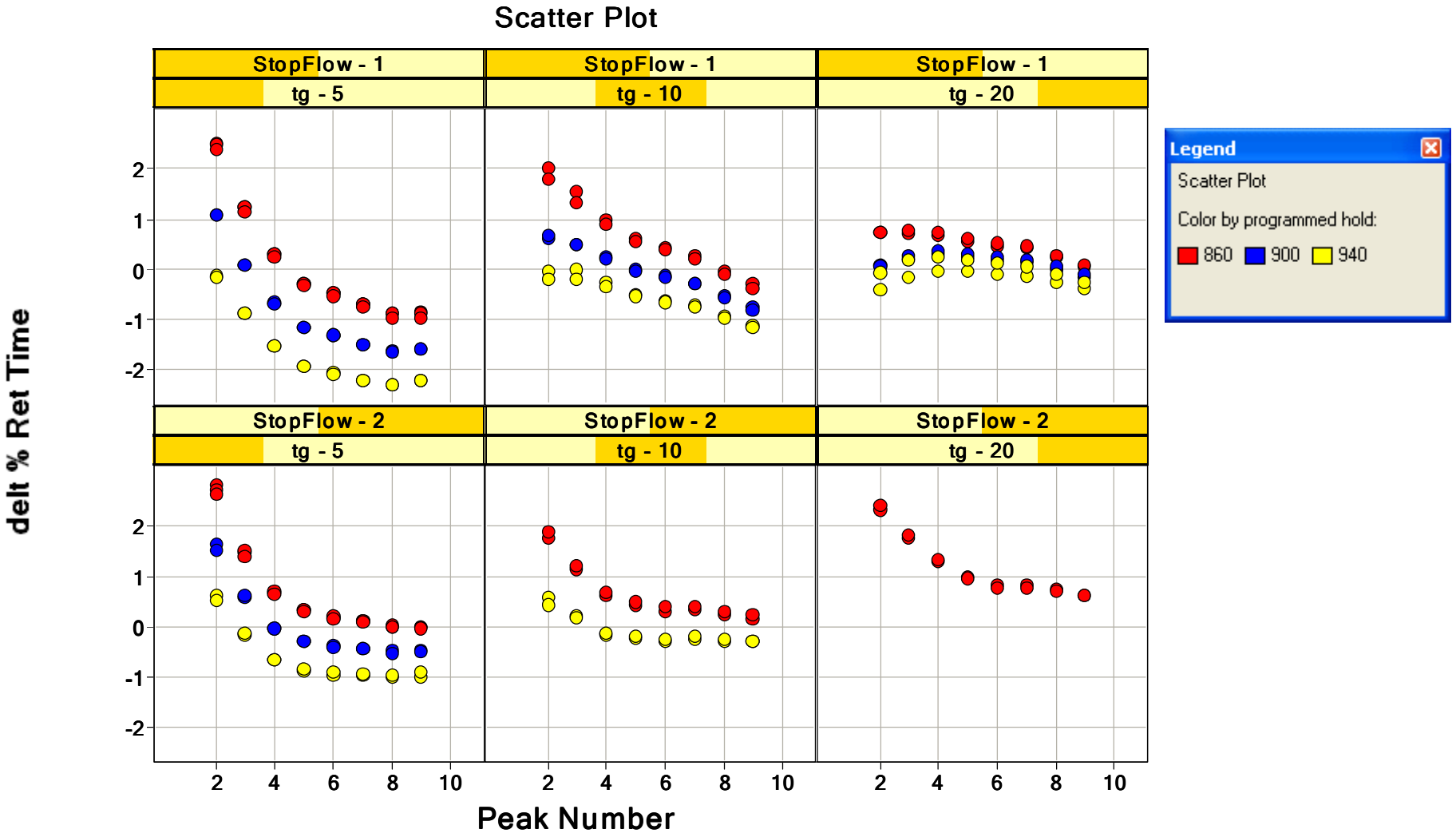
Faster gradient shifted by isocratic hold







Relative Difference in Retention Time between 1200 SL and 1290 Infinity with different programmed delays



Column: Zorbax EC-C18, 4.6x50 mm, 1,8µ
Mobile phase: A = water / B = acetonotrile
Gradient 5 – 95 % B in 5 min
Sample: RRLC checkout sample

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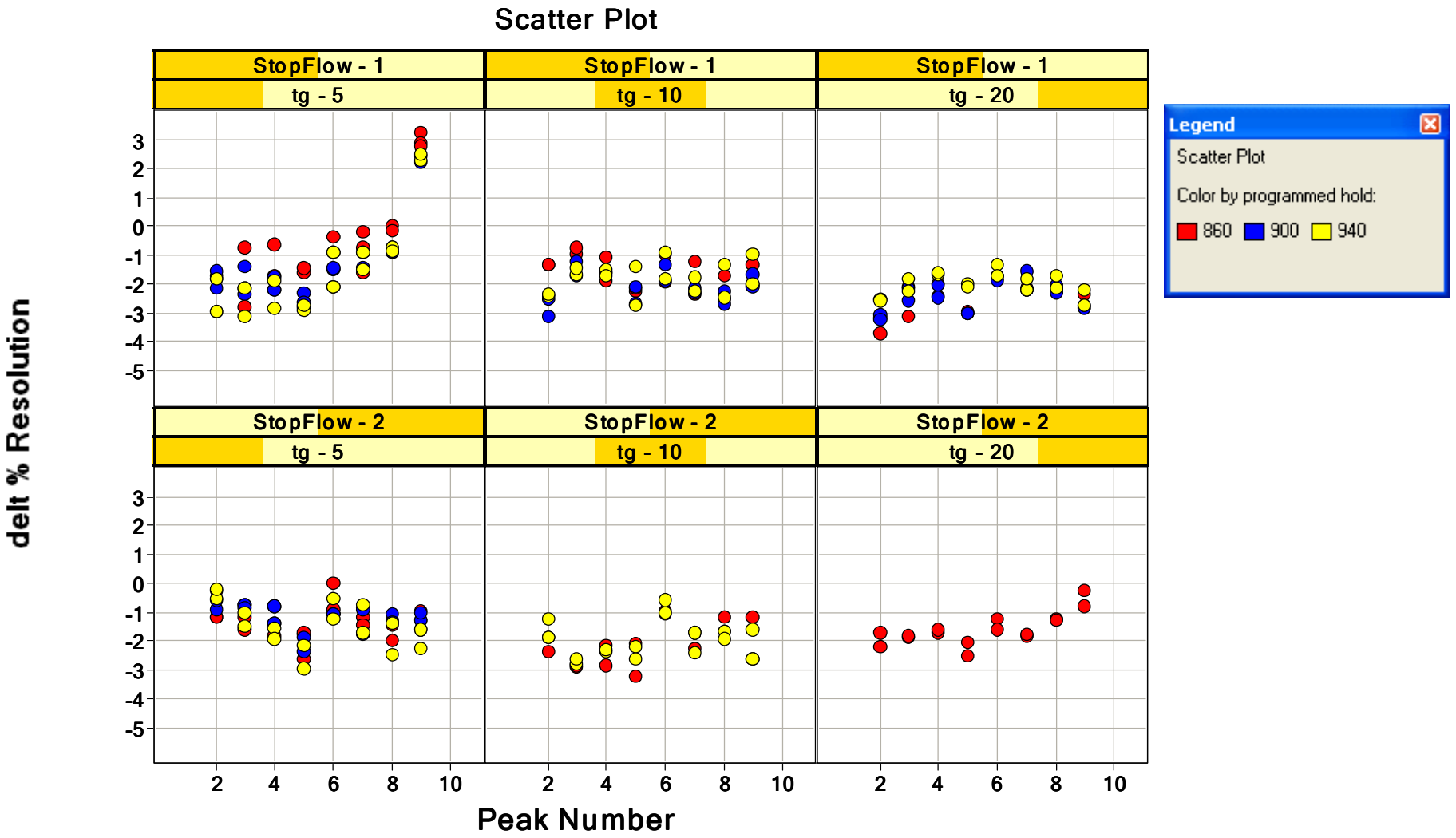
Difference in Resolution of Adjacent Peaks



Column: Zorbax EC-C18, 4.6x50 mm, 1,8µ
Mobile phase: A = water / B = acetonotrile
Gradient 5 – 95 % B in 5 min
Sample: RRLC checkout sample

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1290 Infinity Compatibility / Emulation Mode

Hypothesis:

Any instrument with a low delay volume (e.g. 1290 Infinity) can emulate an instrument with a larger delay volume.
e.g.
Agilent 1100/1200 / 1200 SL <-> 1290 Infinity w small/large Jet Weaver

Use Cases:

Backward compatibility :
Methods from larger delay volume systems can be run **unchanged** on the 1290 Infinity in emulation mode **with same results**

Method development:
1290 Infinity in emulation mode can be used to develop methods for other systems

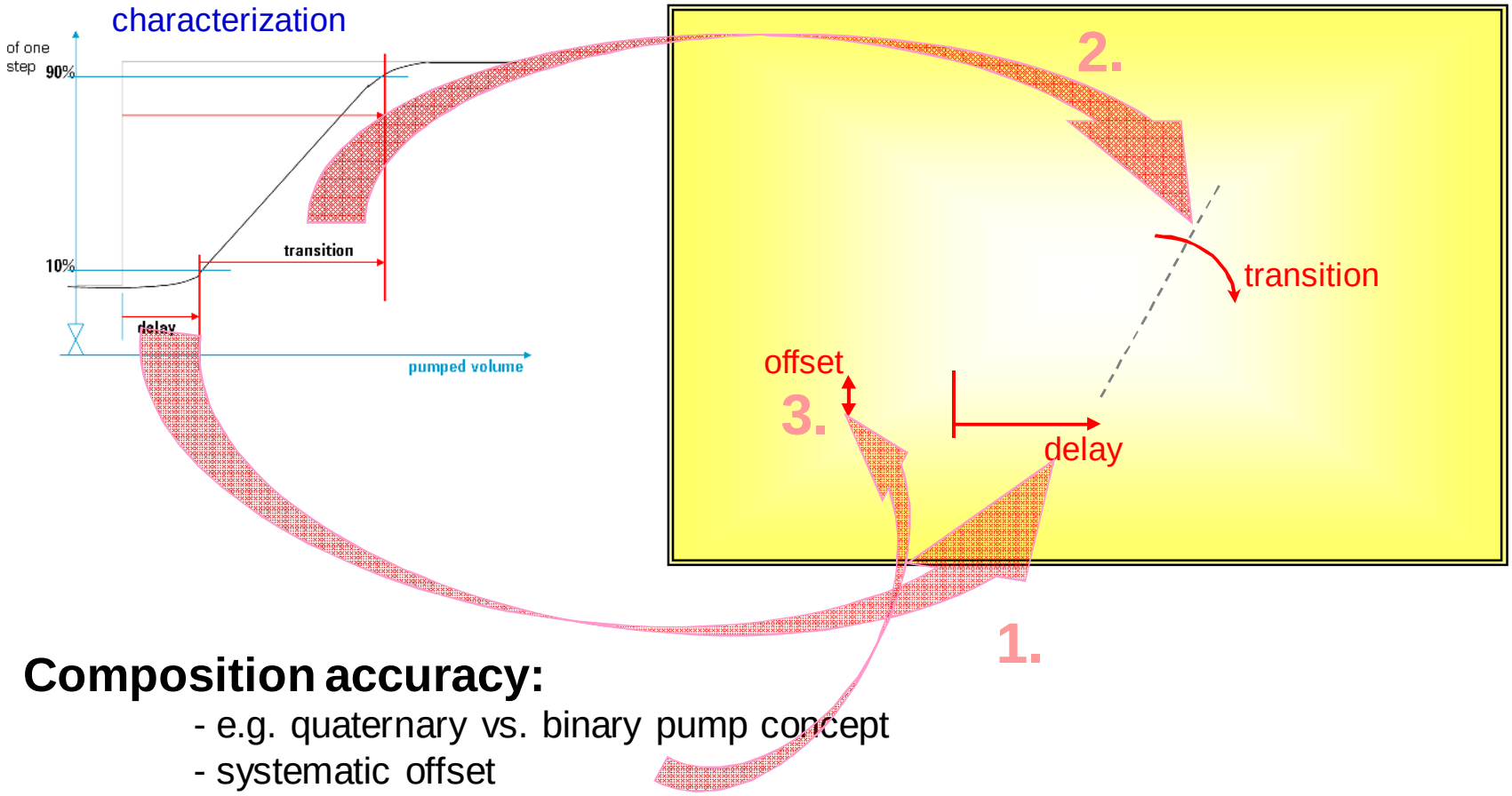
Possible implementation

Determine the transfer functions between 1290 and the system to be emulated
Implement an Algorithm that calculates gradient shape for emulation mode (FW/SW)
Internally execute a modified timetable to emulate starting system

Elements to consider

Delay and Transition volume:

- Fast gradients may be delayed AND "bent" by transition volume

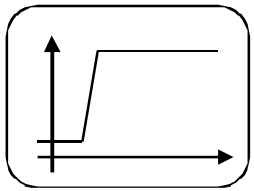


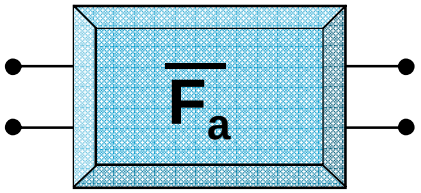
Composition accuracy:

- e.g. quaternary vs. binary pump concept
- systematic offset

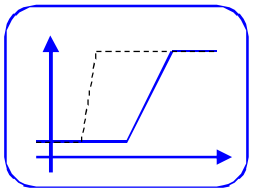
Execution Concept I

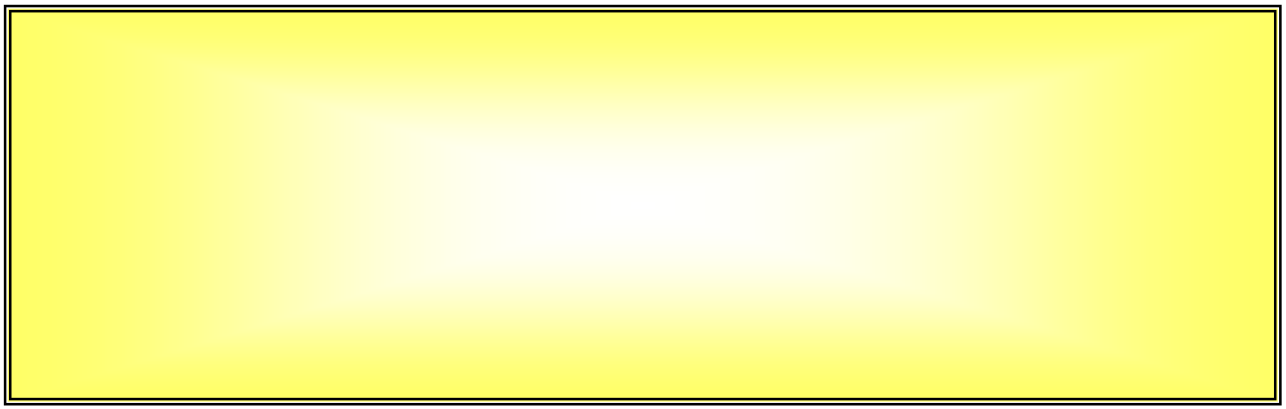
TTBL-a

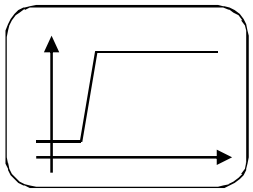


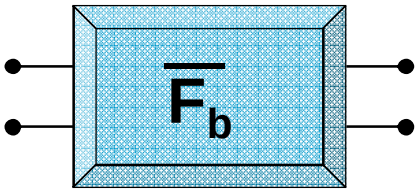


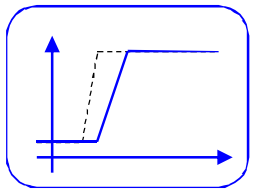
step response



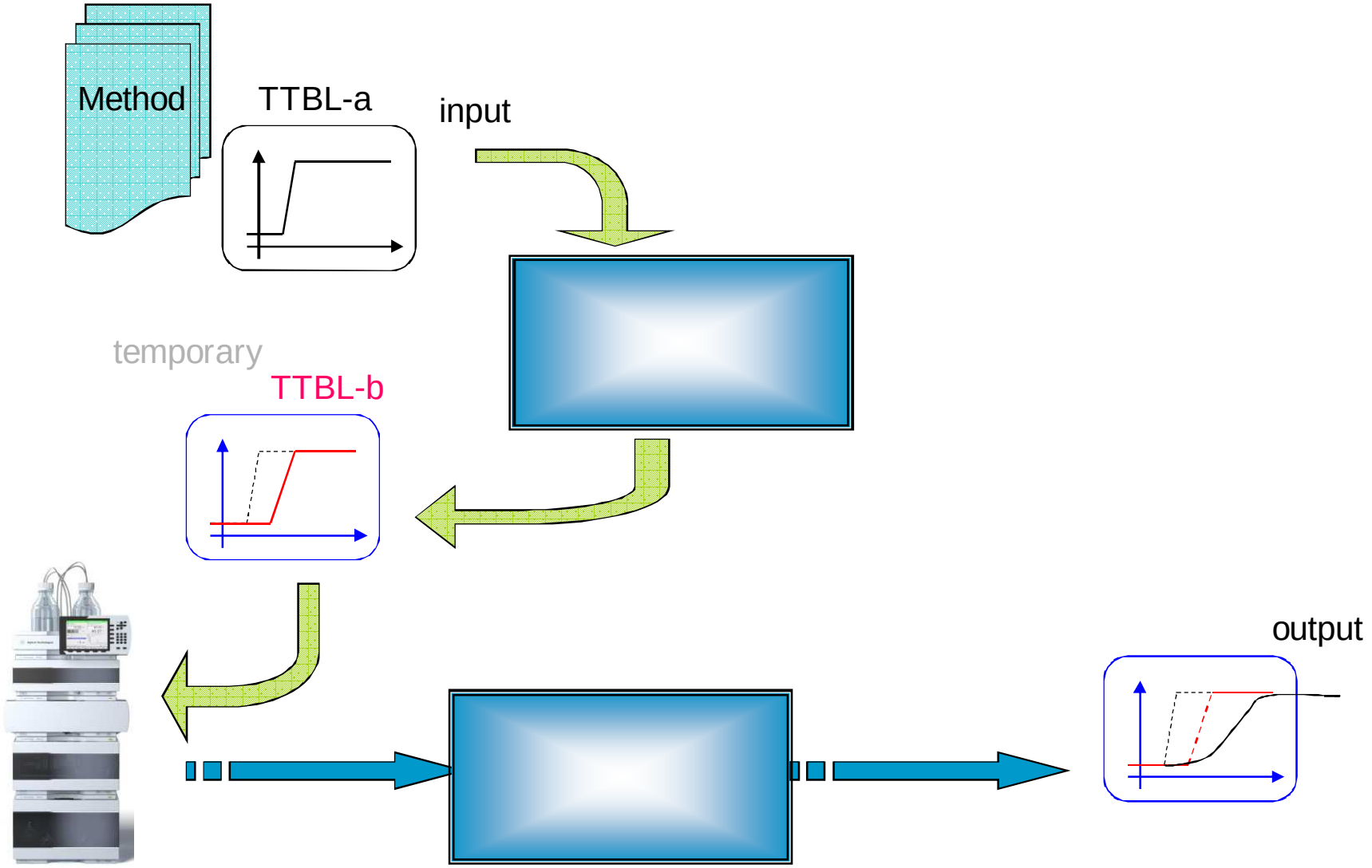




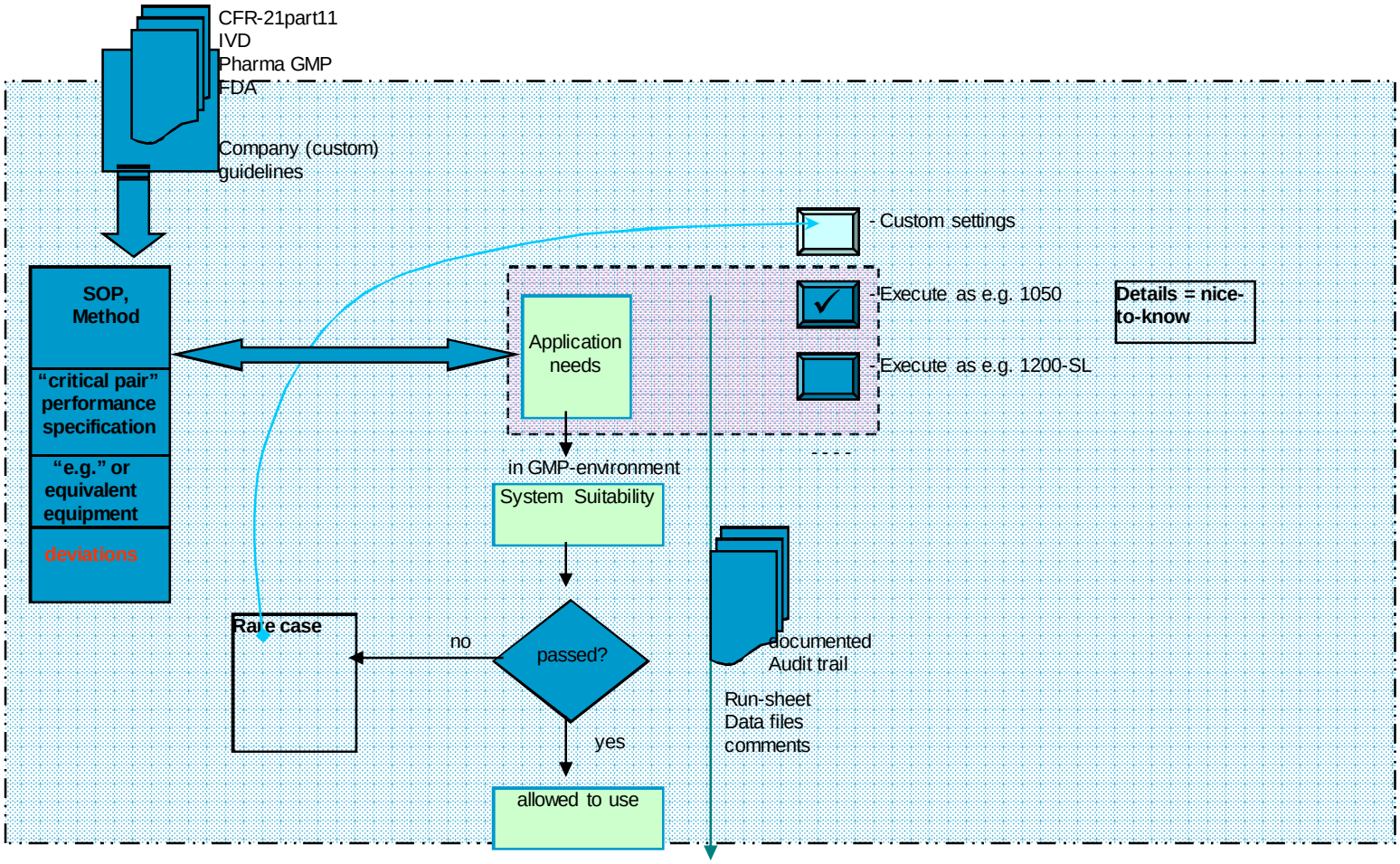




Execution concept II



Possible Workflow Schematics



Conclusions

Due to minimized delay volumes in modern UHPLC instrumention, methods developed on standard HPLC instruments will not give the same results in retention time and resolution when run on an UHPLC system

Adding physical volumes „plumbing solution“ can lead to more comparable results but is not user friendly and requires exact knowledge of the difference in system delay volumes.

Adding an isocratic hold tho the method also does not always yield the desired result as it can correct for delay in gradient start but not for gradient shape.

A concept is proposed that allows to perform a SW/FW driven emulation of a variety of instruments on the Agilent 1290 Infinity system.

Systems can be pre-characterized but also tools for characterization of unknown systems can be provided.