


Smart Method Development & Method Transfer Workflows

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Outline

- Introduction
- QbD for method development workflows
- Introducing Agilent‘s smart method development and method transfer solutions

- Demo Session

  - Instrument to Instrument (I2I) Method Transfer using ISET
  - Automated Scouting of Stationary and Mobile Phases Using Agilent 1290 Infinity II Method Development Solution

Challenges For Analytical Laboratories Today

- Demand to increase the efficiency of workflows
  - Increasing number of samples and tasks
  - Limitation in resources
  - Minimizing costs

Approaches To Increase The Efficiency

- Applying QbD in method development processes
- Automation for method development processes
- Streamline method transfer processes
- Reducing error prone manual workload
- Reducing instrument downtime

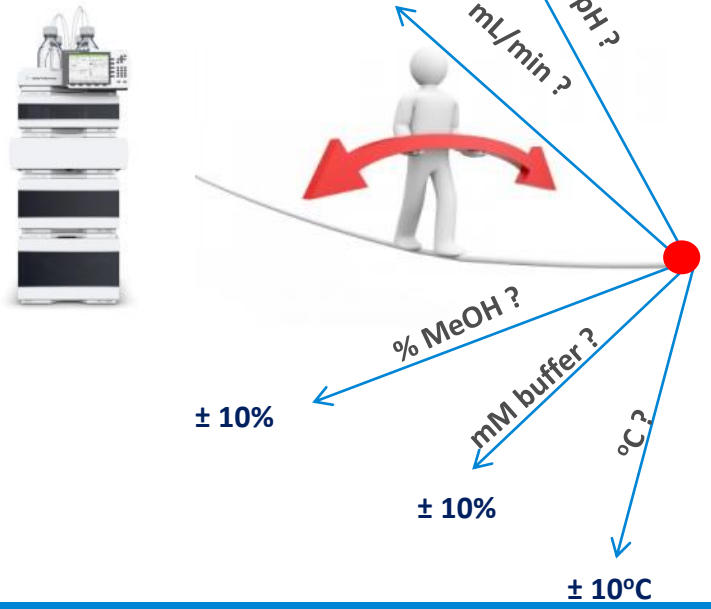
The Role Of QbD In Analytical Method Development

- Analytical method development is an important part of the drug development process
- The quality principles which have been described in the ICH guideline Q8 (R2) should be implemented to eliminate risks or failures
- http://www.fda.gov/downloads/Drugs/Guidances/ucm073507.pdf

(ICH = International Conference of Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Founded in 1990 by an FDA initiative)

Method Development & Transfer Workflows

The Classic Approach



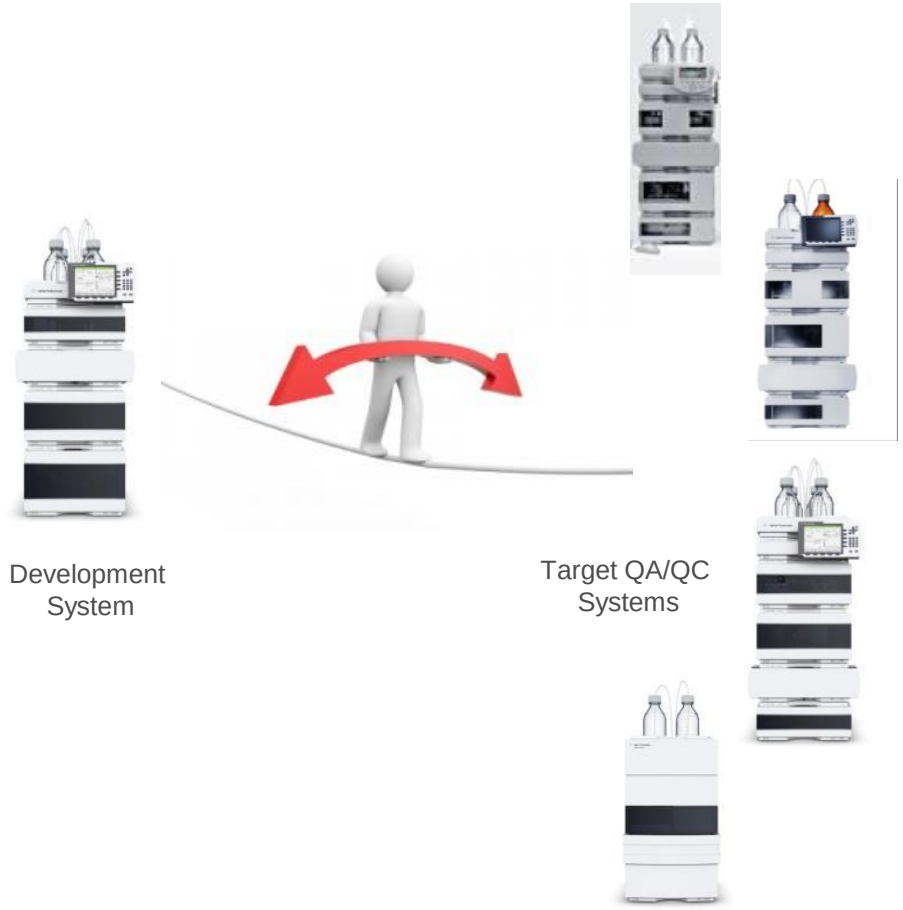
- **Parameters determined by trial- and error.**
- **Robustness tests performed by the one factor at the time approach (OFAT)**

Slope 14.5% /min MeOH
pH 7 +/- 0.2
45°C +/- 2 °C
1.0 mL/min +/- 0.1

Transfer

Transfer

Reported Failures Of Non Robust Methods



Development System

Target QA/QC Systems

- Different results on different systems
- Variability of critical method attributes (resolution, tailing, etc)
- Small changes in pH, temperature or flow rate shows a large effect
- Even buffer solutions from different operators deliver different results

QbD Based Method Development Workflow

- Define Critical Method Attributes **CMAs** (resolution, peak tailing, signal/noise, etc.)
- Define critical method parameters **CMPs** (flow, temp, pH, etc.) which affects the **CMAs**

QbD Based Method Development Workflow

- Key is the statistical „Design of Experiment“ **(DoE)** where multiple **CMPs** will be varied in each experiment
- A method is robust, as long all changes of **CMPs** are within the determined **Design Space**

3rd Party QbD Software Collaboration Partners

Turn your LC into an automated QbD method development system!

Advanced Chemistry Development ACD/Labs

AutoChrom

ChromSword Automated Method Development

ChromSwordAuto

S-Matrix

Fusion QbD

OpenLAB

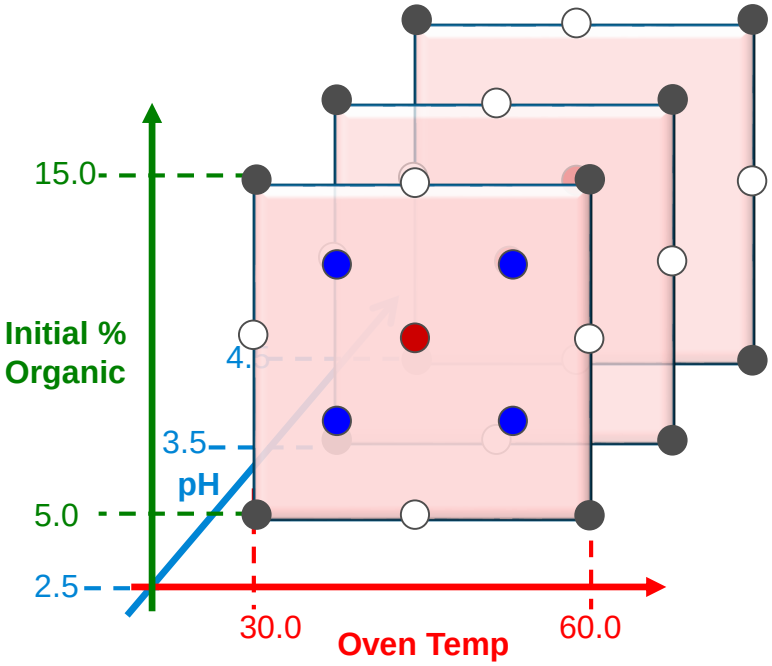
CAPTURE · ANALYZE · SHARE

Chemstation

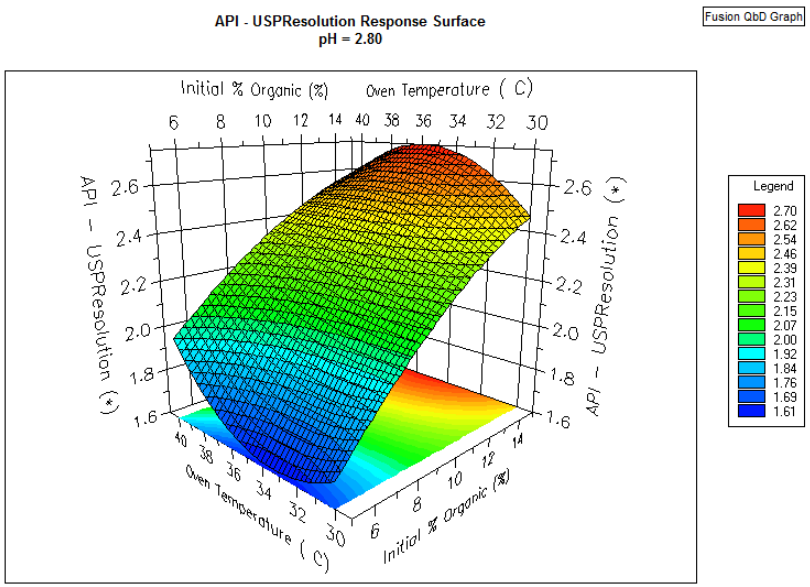
Only Agilent's Method Development Solution is supported by all three big manufactures of automated method optimization software!

Fusion QbD Software (S-Matrix)

- Statistical approach identifying the best separation conditions
- A graphic plot illustrates the position of the best conditions



Sequence of Experiments



3 Dimensional Results Plot

Robustness Testing & Establishing a Design Space (Fusion QbD Software)

List of CMPs and CMAs for robustness testing

CMV	Coded name*	Method nominal	Robust range
Pump flow rate (mL/min)	A	0.6	± 0.01
Oven temperature (°C)	B	33	± 1
pH	C	6.76	± 0.1
Buffer concentration (mM)	D	10	± 0.5
Injection volume	E	1	± 0.1

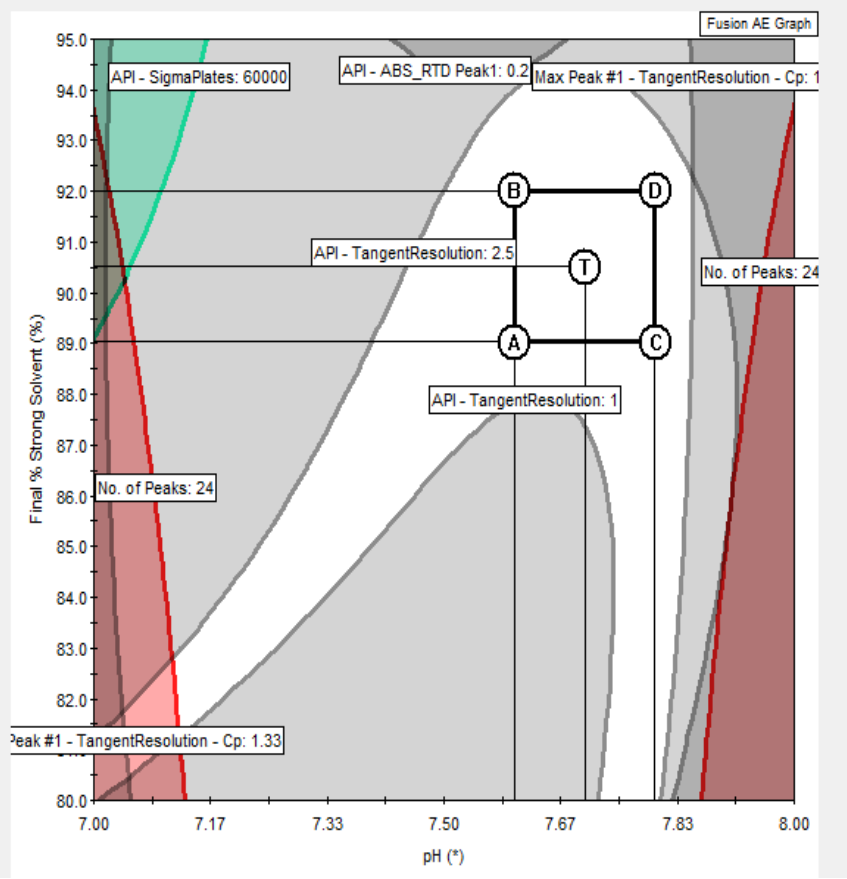
*Coded name used in models showing multiple interactions

CMA	Mean	RSD
API tangent resolution	2.7	3.3 %
API area	4,504.5	1.9 %
API RT	10.0	0.7 %
ADPK RT*	9.2	0.67 %

*Adjacent peak

* The coded names are used in robustness model displays

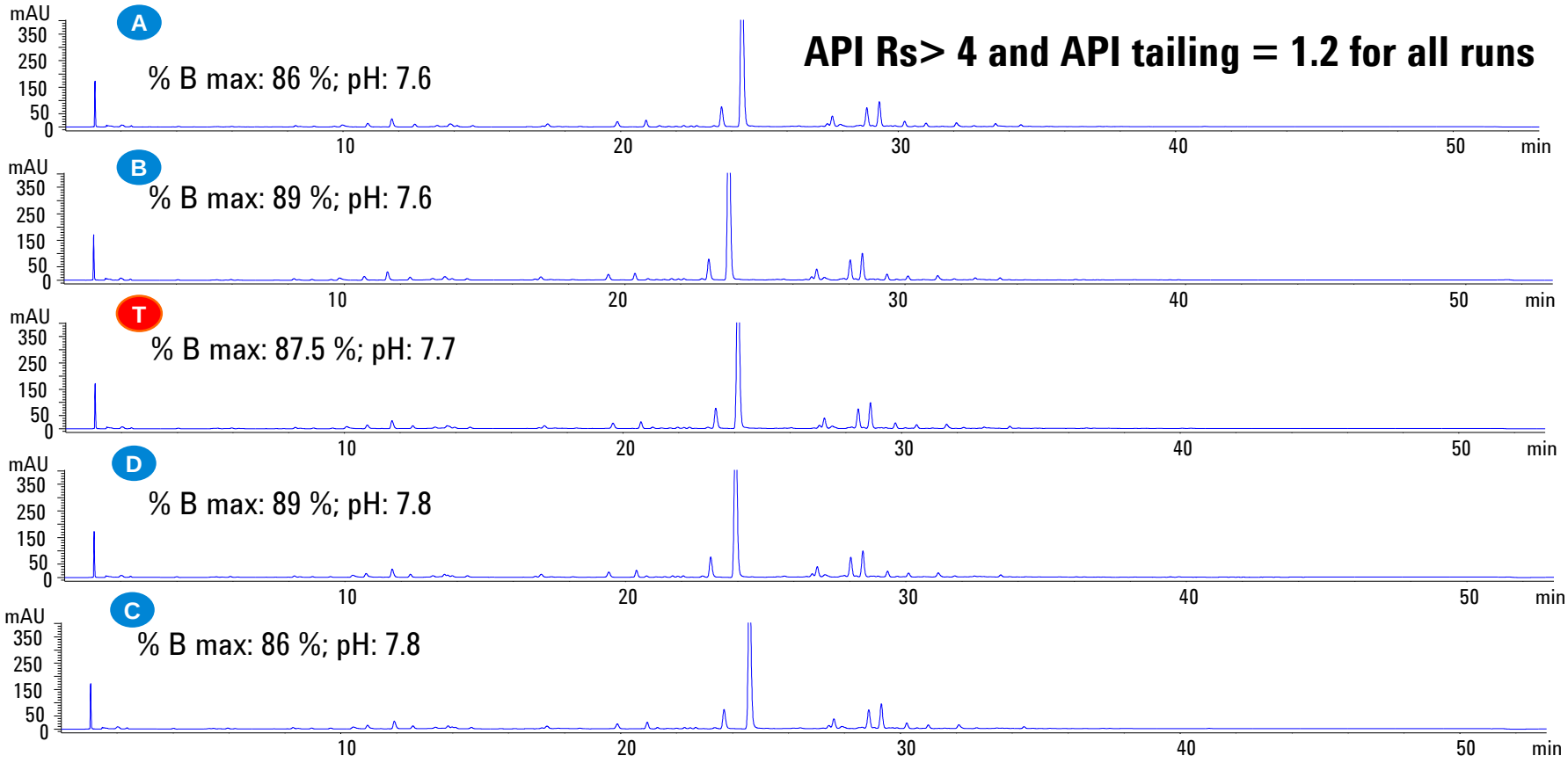
Design Space



Robustness Testing: Experimental Data

Conditions applied from center point and the four corner points of the Design Space

Critical Method Attributes remain within the limits

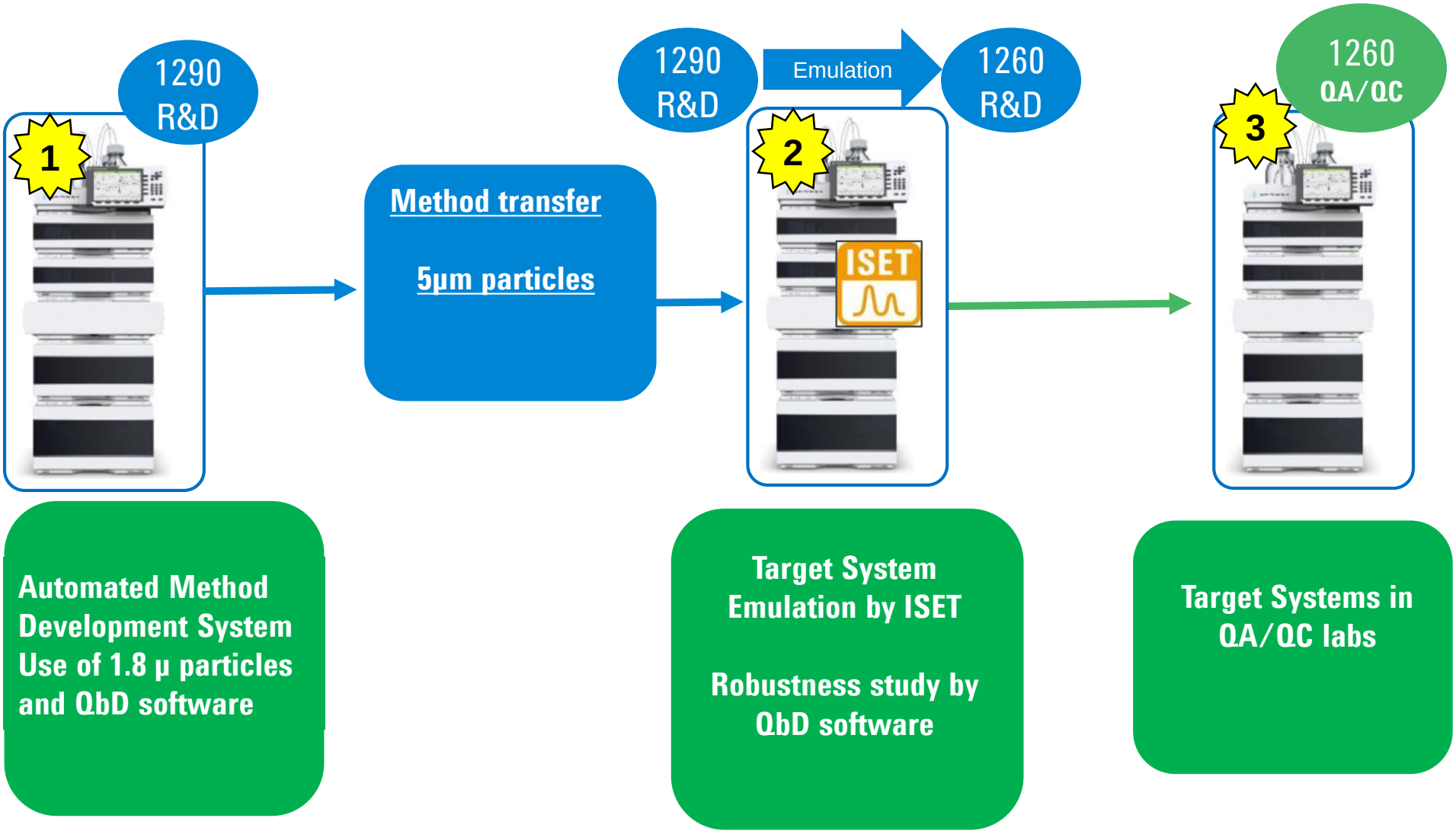


What Are The Advantages ?

- **Increase of efficiency** by avoiding unnecessary experiments during the method development process (no trial an error!)
- **DoE** is delivering more robust methods compared to the classic „one-factor-at-the-time“ (OFAT) robustness testing process
- Changes of **CMPs** to optimize processes are allowed without revalidation when staying within the defined **Design Space**

A Smart Method Development & Transfer Workflow

From UHPLC to HPLC in a nutshell



Agilent Application Note

QbD Based Method Development on an Agilent 1290 Infinity UHPLC System Combined with a Seamless Method Transfer to HPLC Using Intelligent System Emulation Technology

Application Note

Pharmaceutical QA/QC

Author

Vinayak A.K
Agilent Technologies
Bangalore, India

Abstract

In this Application Note, a method was developed on sub-2 µm particle columns and transferred to a conventional HPLC method using Quality by Design (QbD) principles. A seamless method transfer between the different chromatography systems was achieved using the Agilent Intelligent System Emulation Technology (ISET). Initial method development work on the Agilent Infinity 1290 system included automated chemistry screening of different sub-2 µm particle columns under different chromatographic conditions involving multiple combinations of eluents, flow rates, gradient slopes, and temperatures. Subsequent optimization of the best performing chemistry system was carried out using a Design of Experiments (DOE) approach to establish the ICH Design Space. The Agilent Method Translator was used for the transfer from sub-2 µm column material to conventional column material. An Agilent 1290 Infinity UHPLC system was used in combination with ISET to emulate an Agilent 1260 Infinity HPLC system. This enabled establishing a Design Space for the HPLC method. Reproducibility and resolution of the transferred method was then verified on an Agilent 1260 Infinity HPLC system. This successfully demonstrates that ISET in combination with Fusion QbD Method Development and Validation Software (S-Matrix) enables an efficient transfer of UHPLC methods to HPLC methods within the QbD approach.

Title: QbD Based Method Development on an Agilent 1290 Infinity UHPLC system Combined with a Seamless Method Transfer to HPLC Using Intelligent System Emulation Technology
Type: Application Note
Publication Number: 5991-5701EN
Pages: 8
Target segments: Pharmaceutical QA/QC
Language: English
Author: Vinayak A.K.
LitStation Availability: May 5, 2015, CPOD

Step #1: Method Development

Automated Method Development System
Use of 1.8 µ particles and QbD software

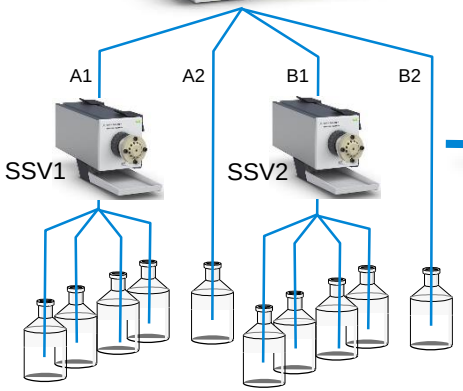
1290 Infinity II Method Development Solution

Schematics

1290 Infinity II MCT

1290 Infinity II Flexible Pump

External Solvent Selection Valves



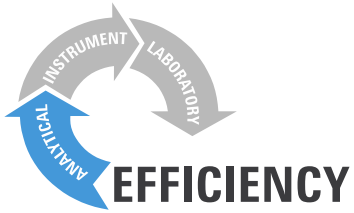
Up to 12 solvents per SSV!!!

12 + 1 x 12 + 1 = 169

8 x 169 = 1352

- 1352 different combinations of column chemistries and eluents
- A nearly infinite number of separation conditions is created by including different temperature and flow rates as variable parameters

Agilent 1290 Infinity II Method Development Solution
All Options

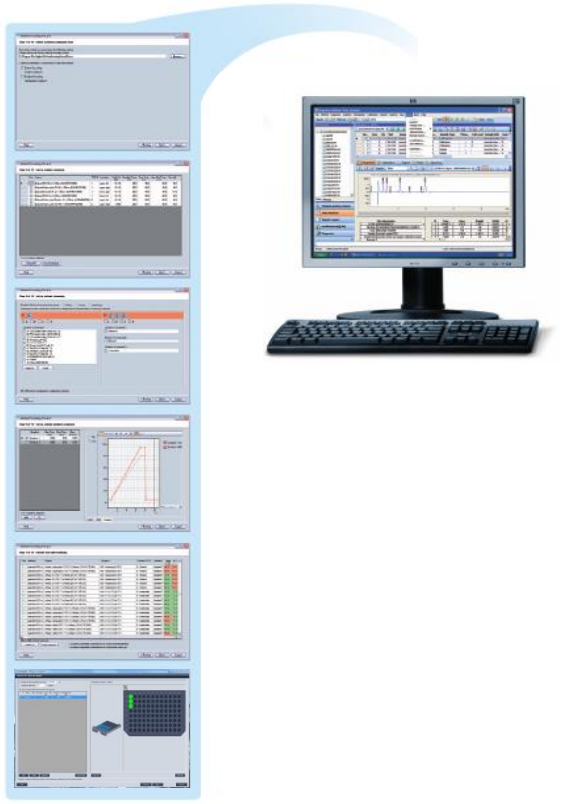


Agilent 1290 Infinity II Method Development Solution
6100 Series MSD and 1290 ELSD are optional detectors to ensure the most comprehensive compound detection

Agilent ChemStation Method Scouting Wizard

There is no easier way to set up even complex screening campaigns

- **Define project**
  Choose scouting combinations and base method.
- **Select columns**
  All installed columns are shown automatically.
- **Select solvents**
  Pump types and valves are automatically detected.
- **Define gradients**
  Select between different gradients and temperatures.
- **Review and select screening methods**
  Check for incompatible combinations.
- **Set up samples**
  Define injection volumes and number of repetitions.

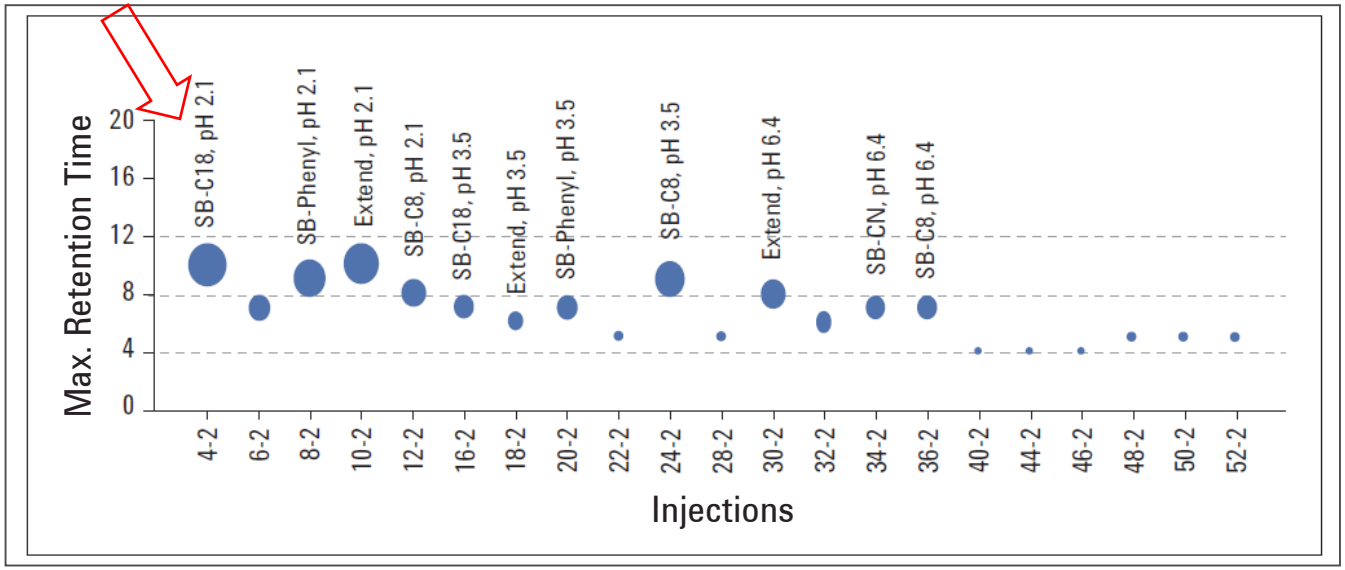


Easy and fast setup of sequences !

Agilent ChemStation Method Scouting Wizard

Ease of Use: Screening Report

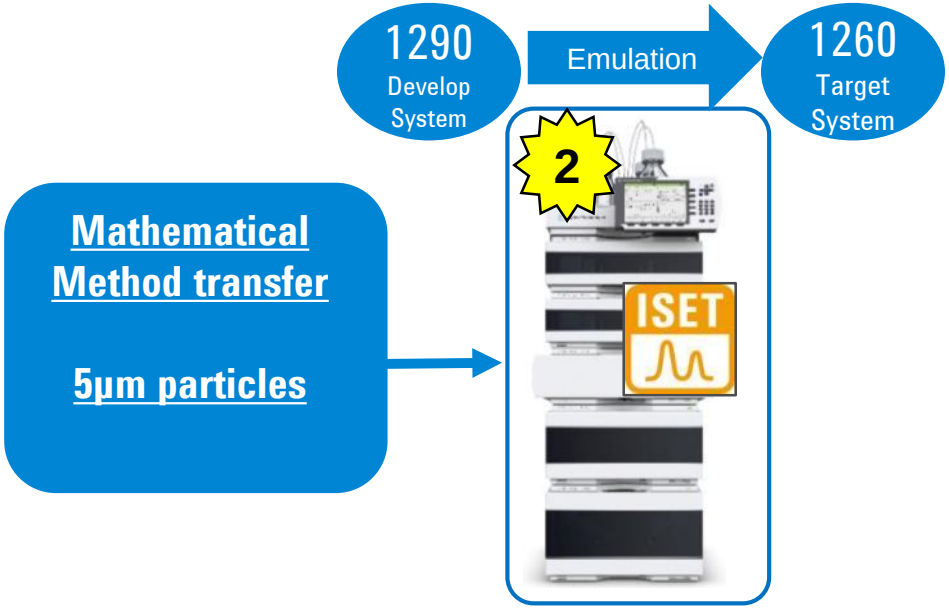
Fast screening of mobile and stationary phases with 1290 Infinity II LC –
Analysis of Degradation Products of Metoprolol



Bubble size represents the number of integrated peaks and, consequently, best mobile and stationary phase combination.

https://www.agilent.com/cs/library/applications/5991-0989EN.pdf

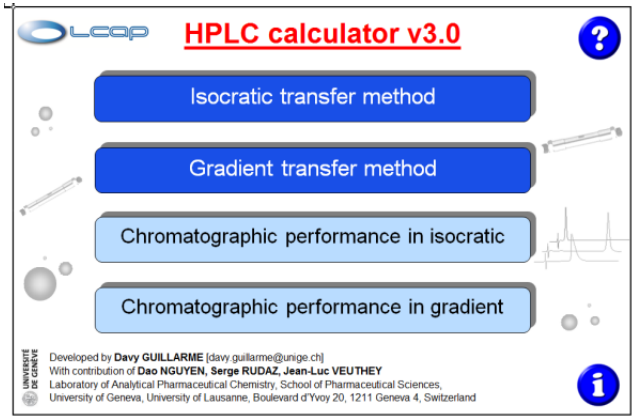
Step #2:
Method Transfer And Target System Emulation



Target System Emulation by ISET

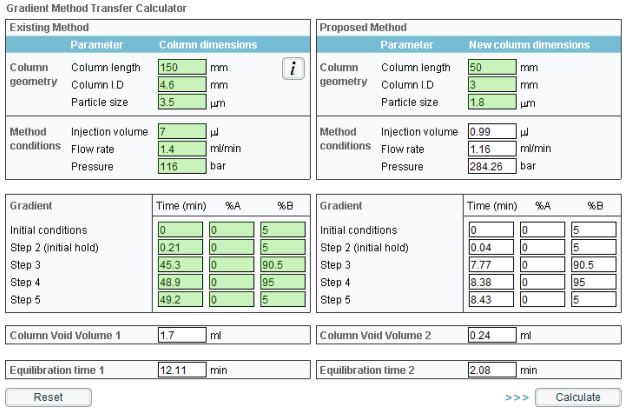
Robustness study by QbD software

Method Transfer From UHPLC To HPLC



HPLC Calculator University of Geneva

http://www.unige.ch/sciences/pharm/fanal/lcap/telechargement.htm

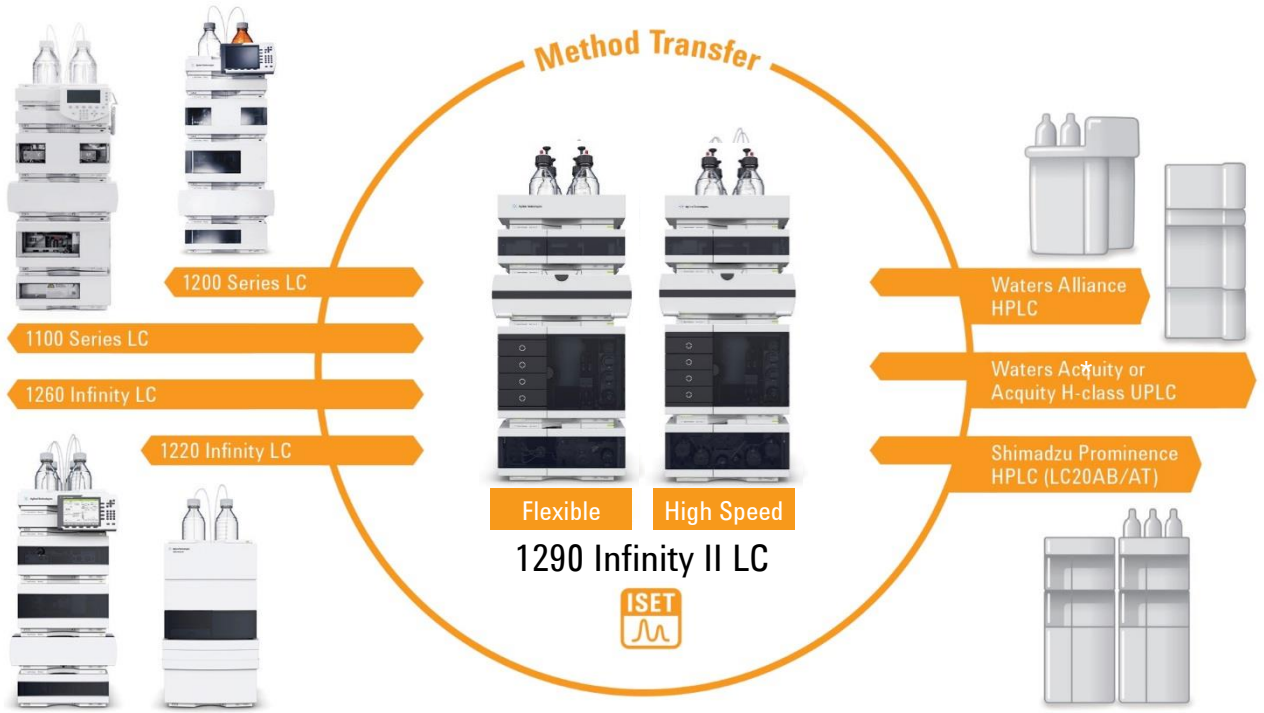


Crawford Scientific Calculator

http://www.crawfordscientific.com/HPLC_Method_Transfer_On-line.htm

Cross-System Method Transfer Solution

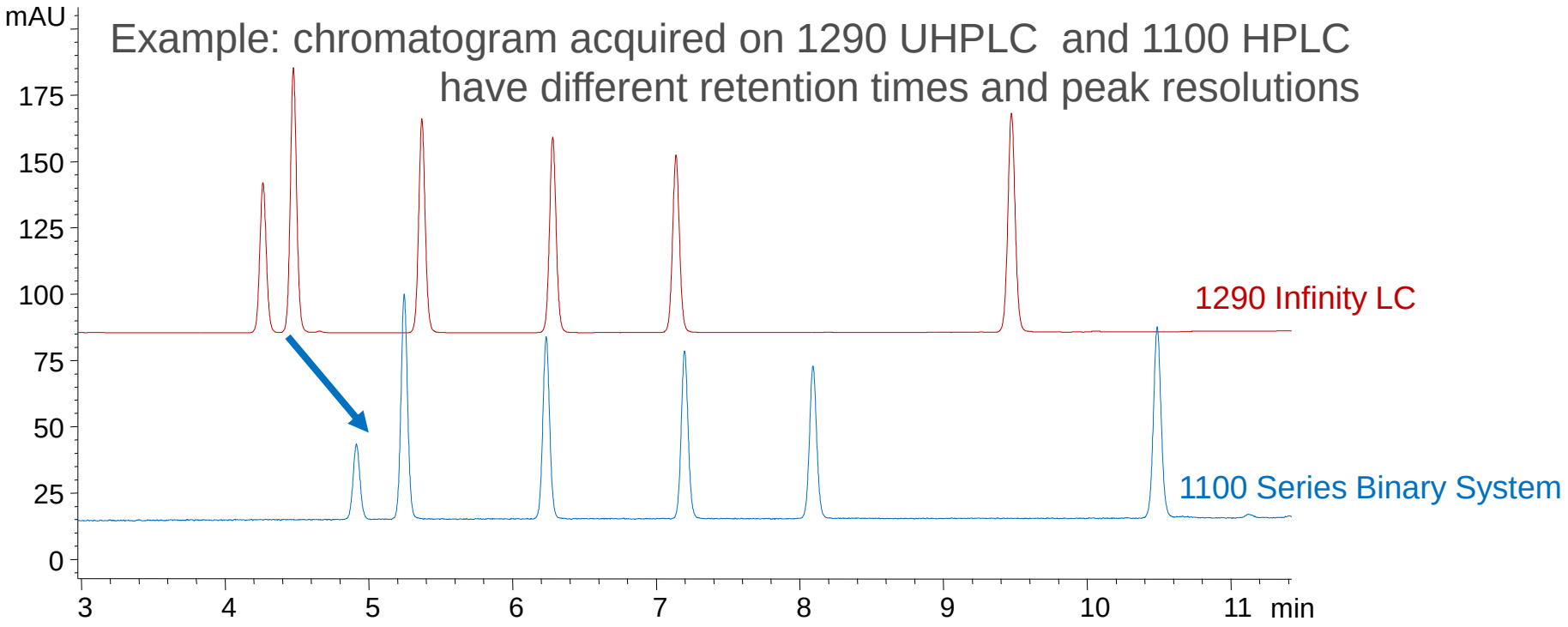
Intelligent System Emulation Technology (ISET)



*The **only** LC System in the world able to emulate other LCs without any hardware changes – just by a mouse-click!*

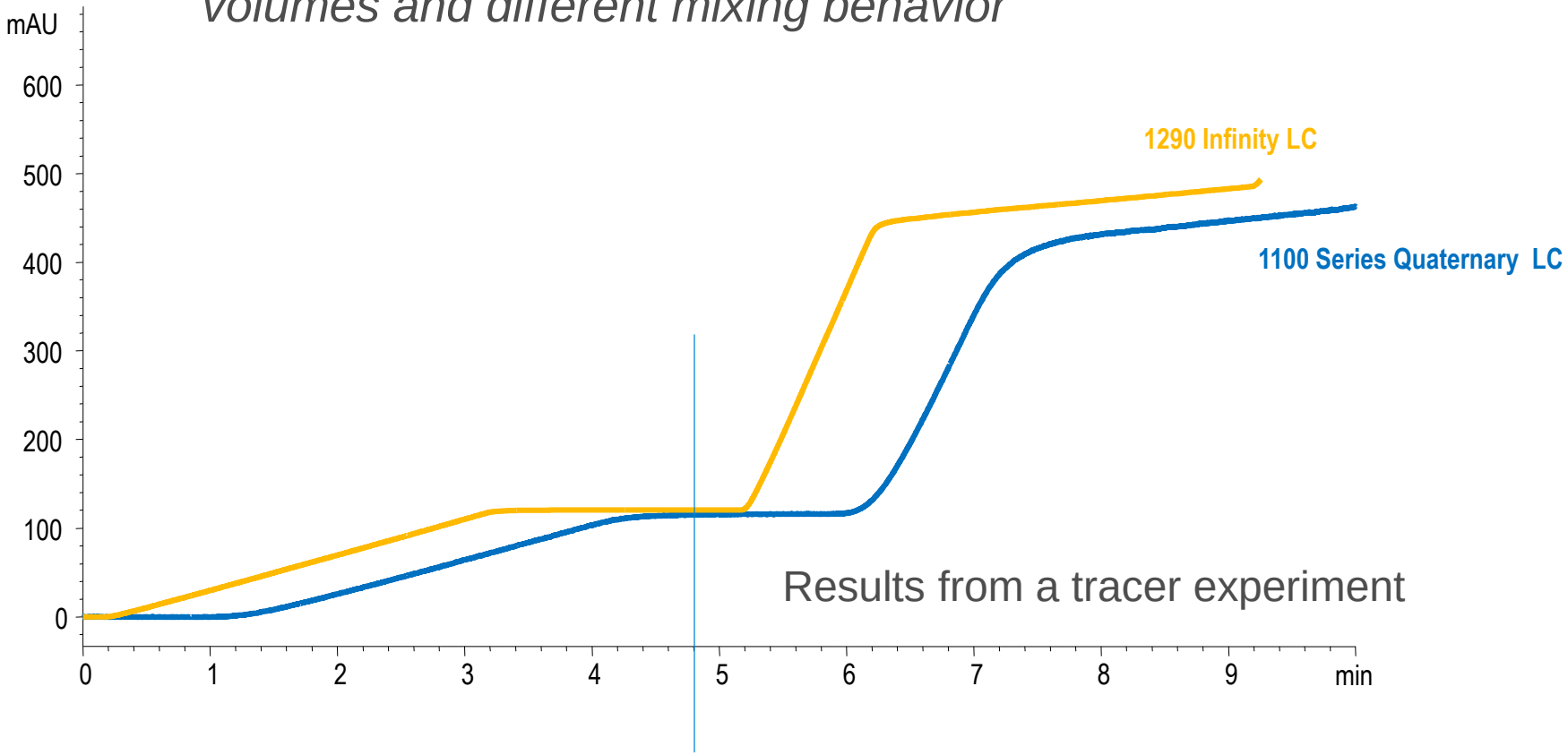
Method Transfer Between Different LC Instruments

- Dwell volumes and mixing behaviors are characteristic properties of the system fluidics. Chromatograms acquired on different LC instruments are not identical! CMAs (resolution, peak width, peak tailing, etc.) might change and can lead to different results!



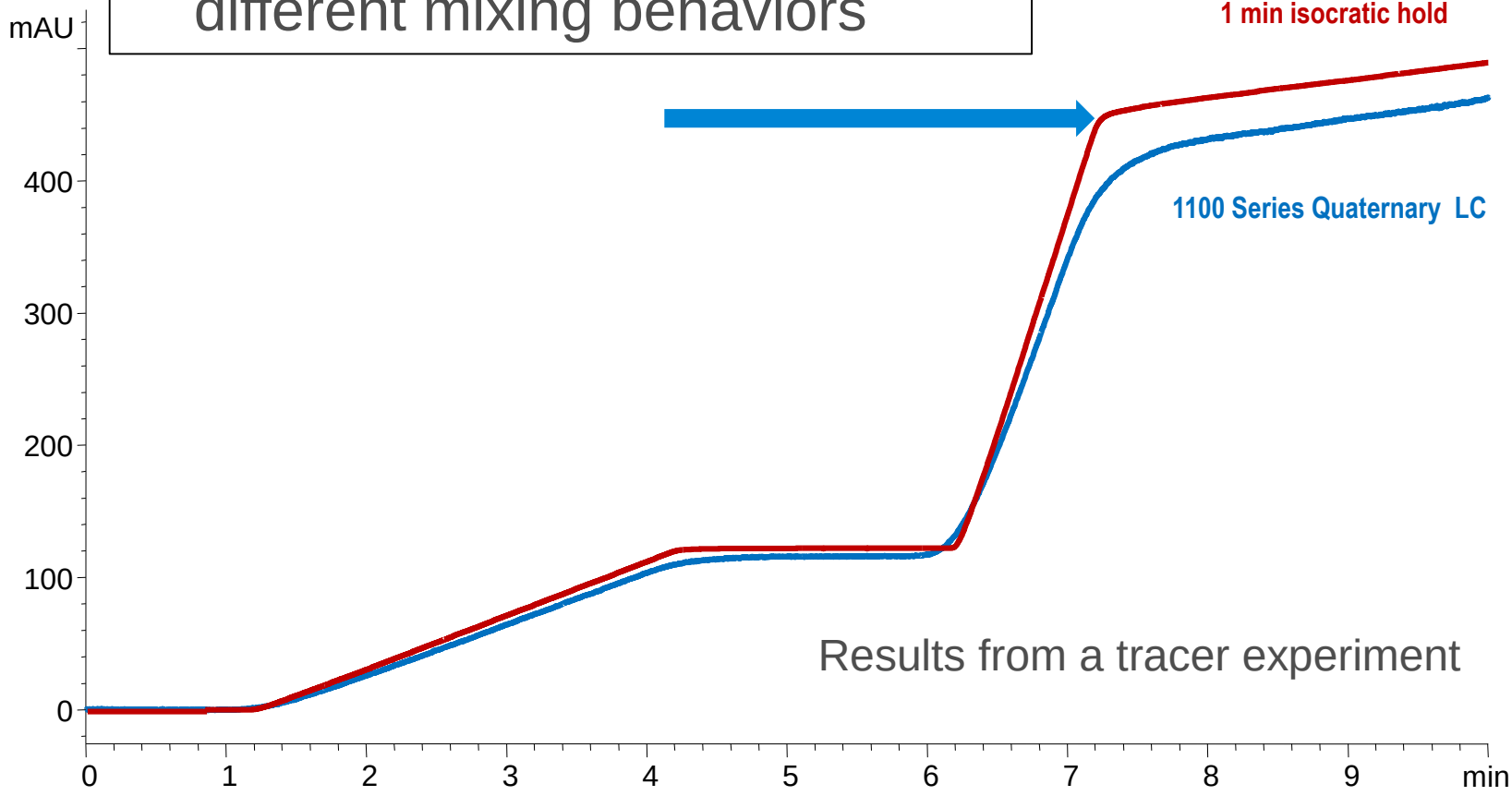
Monitoring The Gradient Generation on Different Instruments

Gradient differences due to different dwell volumes and different mixing behavior



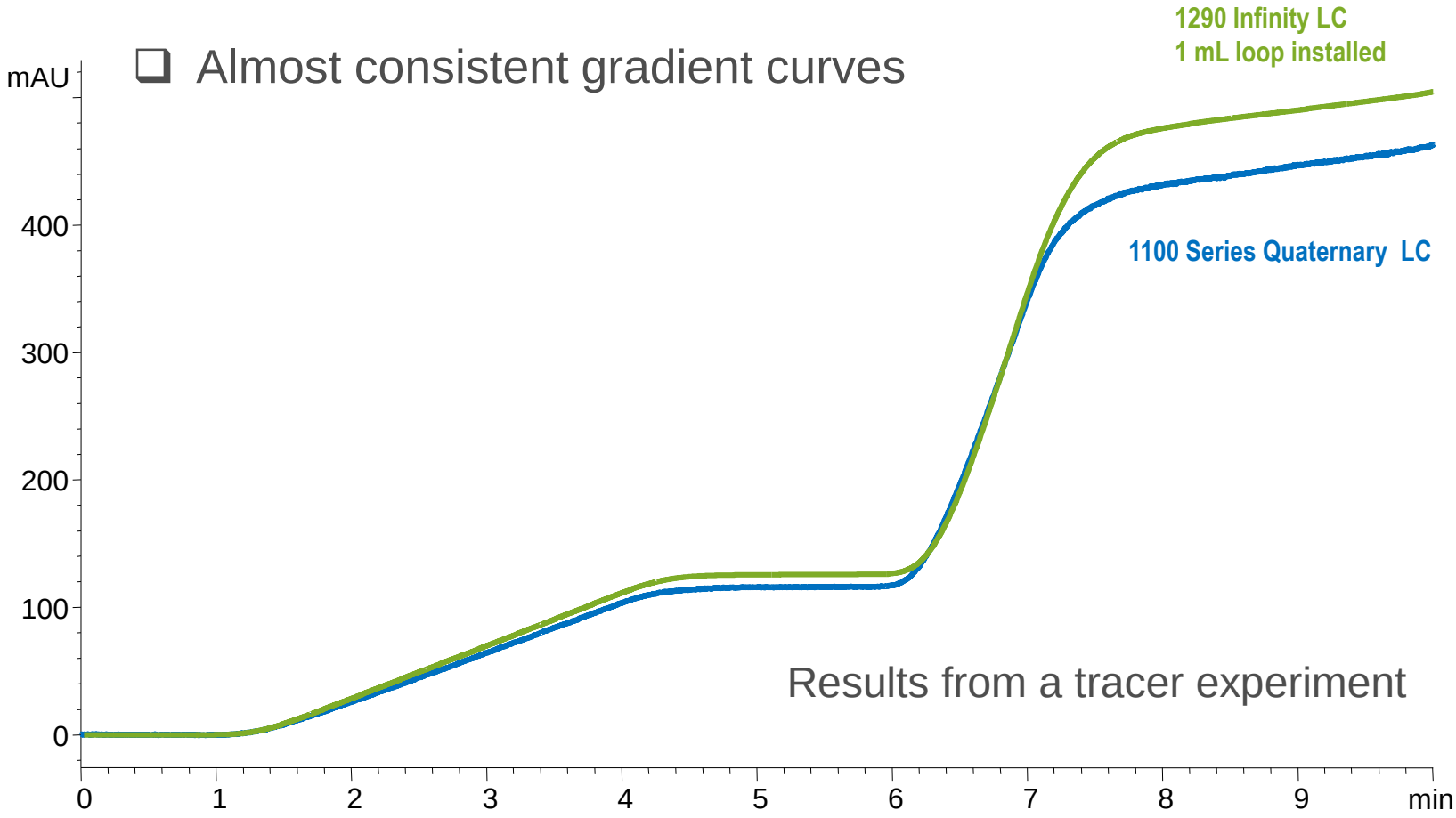
Applying an Isocratic Holding Step To Compensate The Different Dwell Volumes is a compromize

- Still gradient differences due to different mixing behaviors

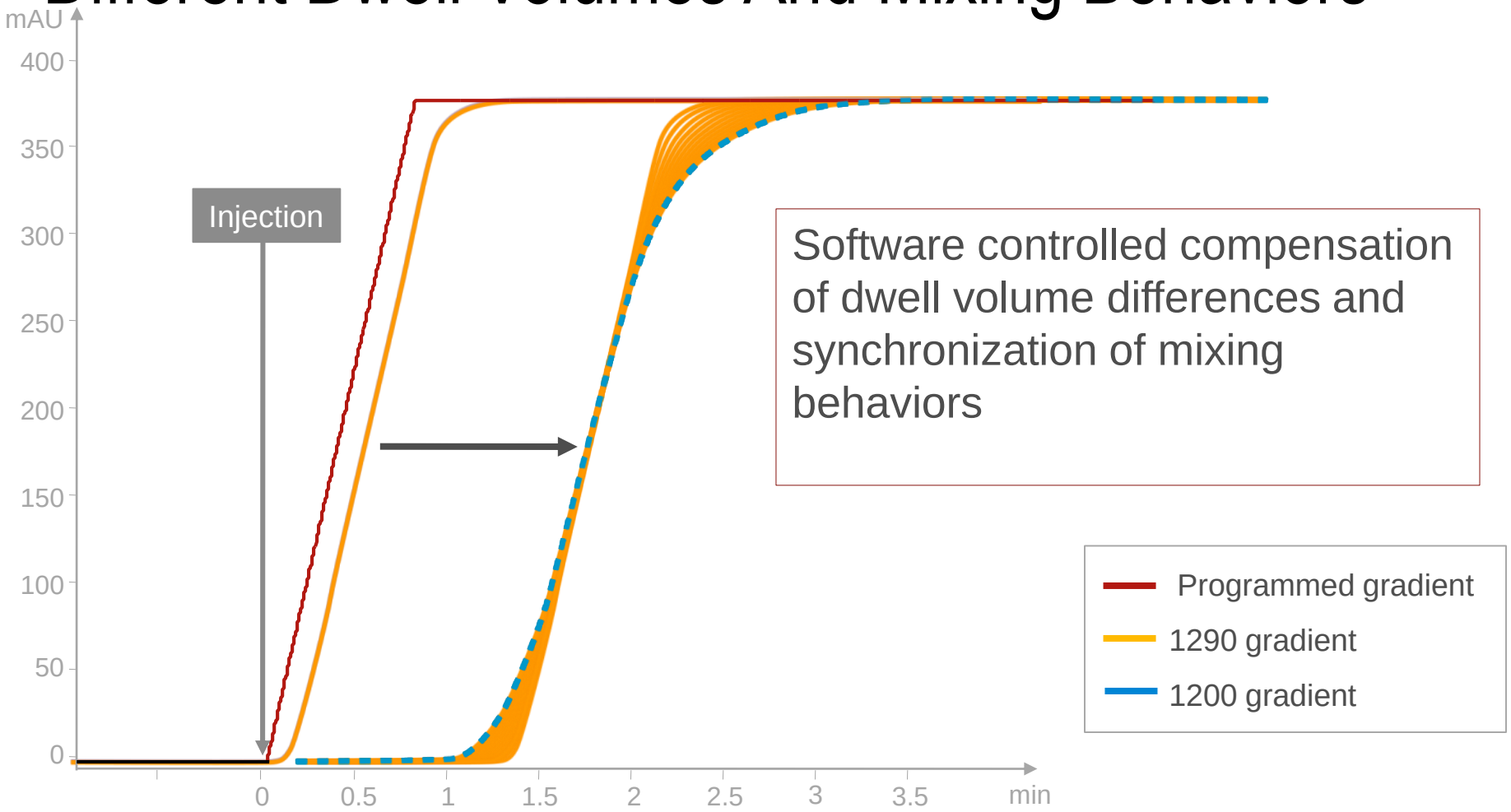


Is Adding A Physical Void Volume To Compensate The Different Dwell Volumes the right solution?

- Almost consistent gradient curves

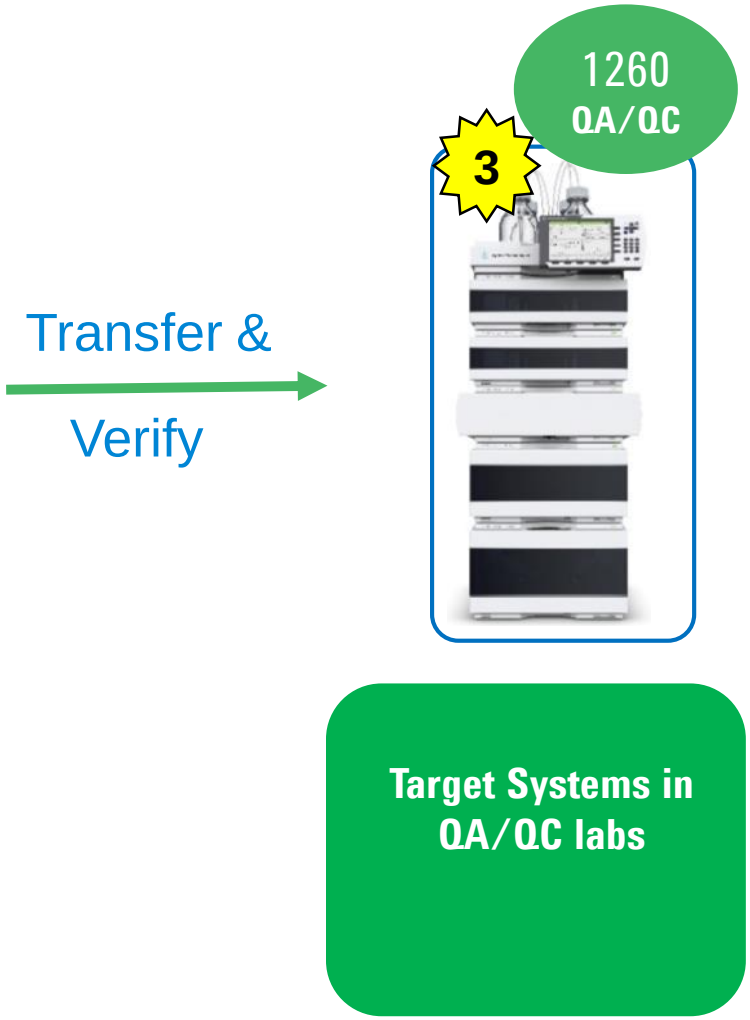


The Intelligent Agilent Solution To Compensate Different Dwell Volumes And Mixing Behaviors



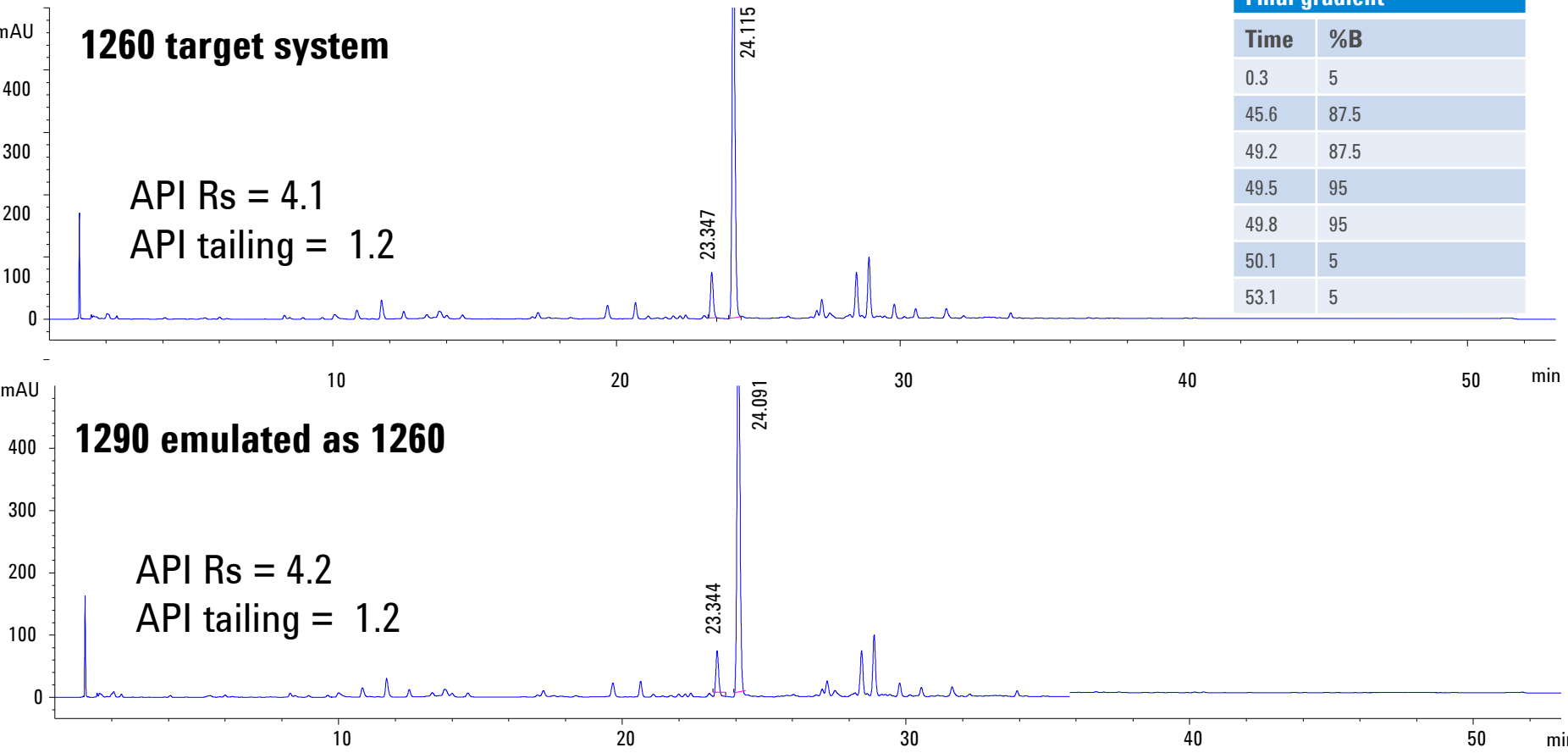
- Intelligent System Emulation Technology (ISET)

Step #3: Transfer to target system



Verification Of Results With The Target System

1260 Infinity data compared to 1290 Infinity data in emulation mode



Final gradient	
Time	**%B**
0.3	5
45.6	87.5
49.2	87.5
49.5	95
49.8	95
50.1	5
53.1	5

- ❑ Critical Method Attributes are within the defined limits




Demo Session

Dr. Wenlin Zhang

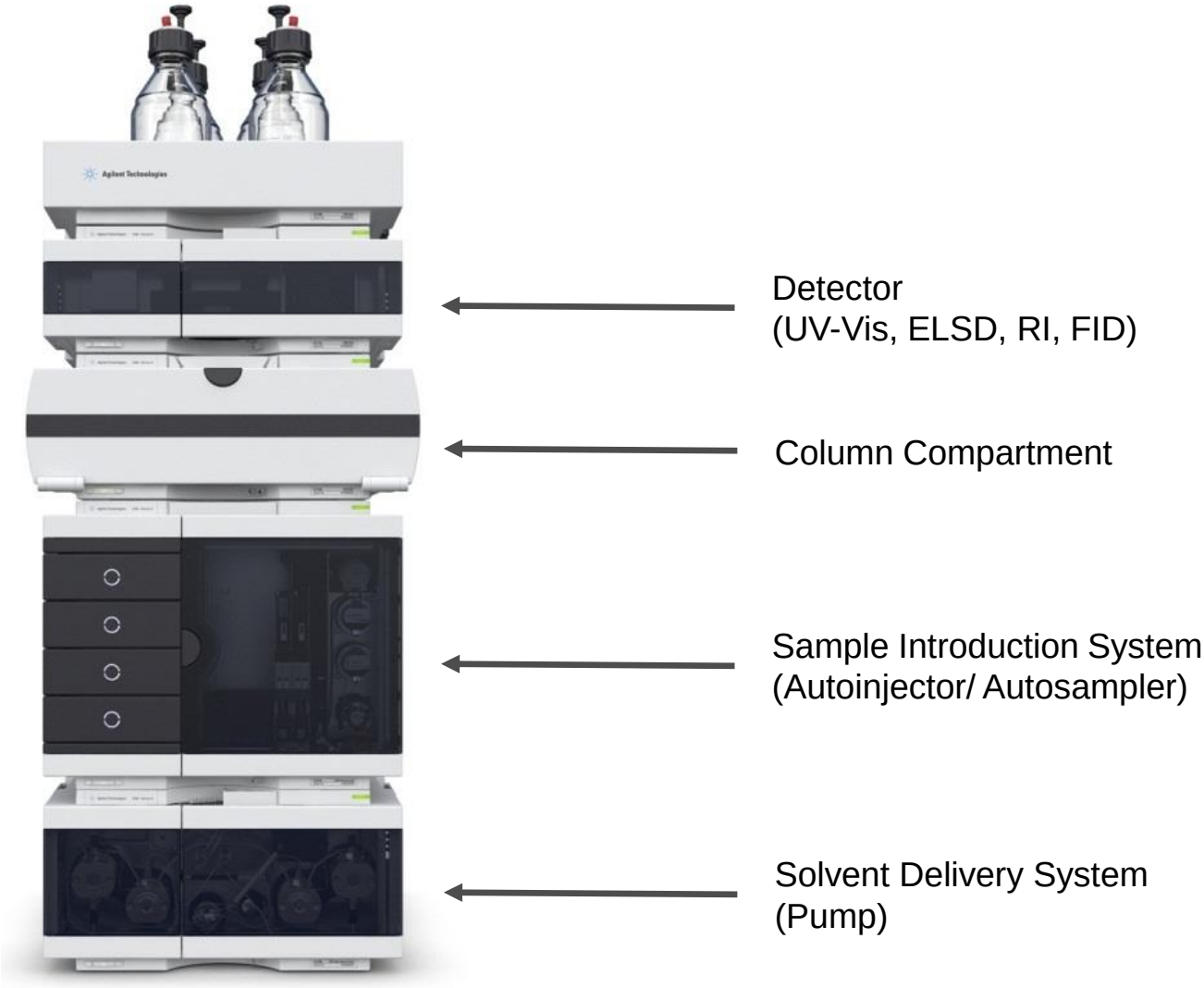
Application Scientist




❑1290 Infinity II Show-and-Tell

Agilent 1290 Infinity II LC System

In A Nutshell



1290 Infinity II High Speed Pump

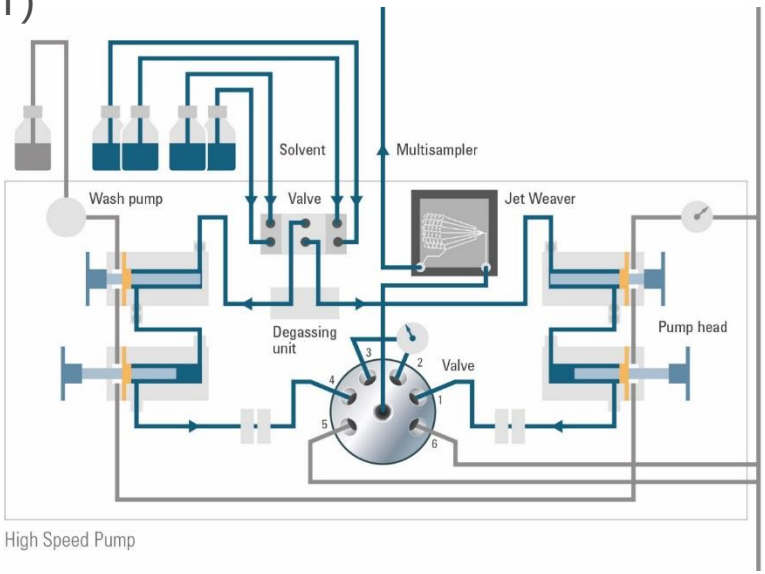
Binary Pump

G7120A 1290 Infinity II High Speed Pump 1300 bar

- \- More mechanical components
- \- 2-channel HPM (high-pressure mixing)
  (out of 4 mobile phases with internal solvent selection valve)
- \+ Lower delay volume (< 45 µL)
- \+ Seal Wash included
- \+ Prepare Pump
  - \+ Purge, Conditioning, Prime
- \+ Intelligent System Emulation Technology (ISET)

Applications

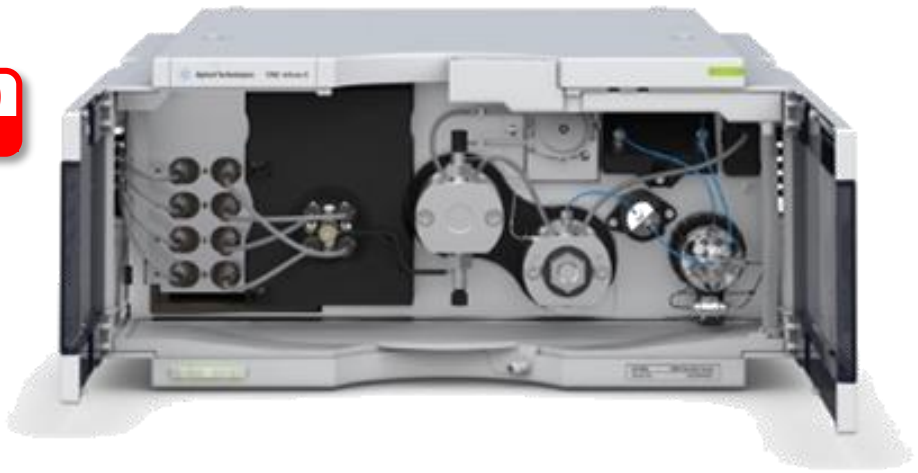
- Ideal for very fast/steep gradient changes
- Excellent front end for MS



High Speed Pump

1290 Infinity II Flexible Pump

Quaternary Pump



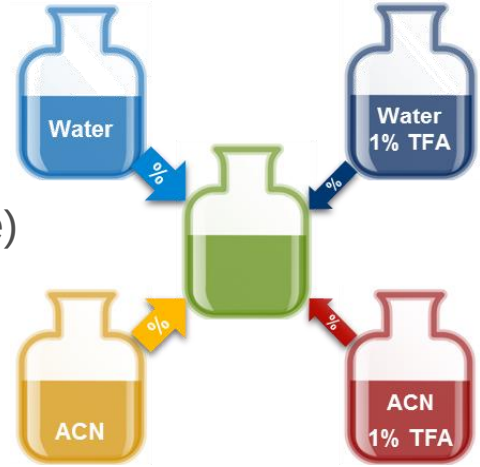
G7104A 1290 Infinity II Flexible Pump

1300 bar

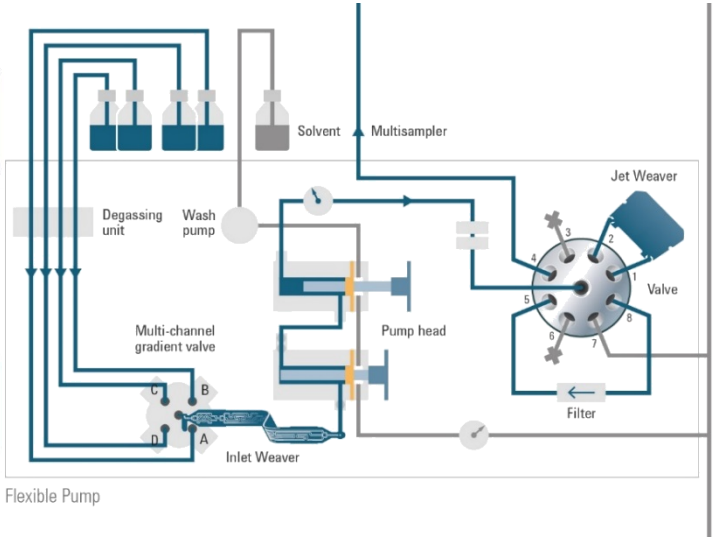
- \+ 4-channel LPM (low-pressure mixing)
- \- Higher delay volume, 350 µL or 500 µL
- \+ Seal Wash included
- \+ Blend Assist
- \+ Prepare Pump
  - \+ Purge, Conditioning, Prime
- \+ Intelligent System Emulation Technology (ISET)

Applications

- More than 2 solvent elution
- All other applications (Versatile)



Blend Assist



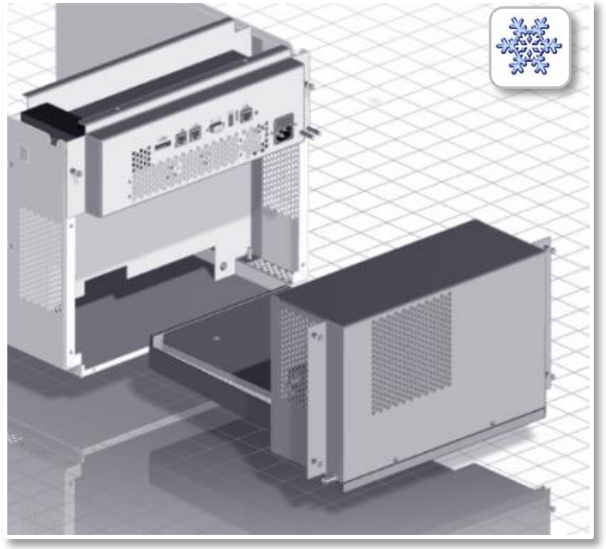
Flexible Pump

1290 Infinity II Autosampler

Vialsampler

G7129B 1290 Infinity II Vialsampler

- Vials only (2 x 66 vials), no well plates
+ Lower price
+ Lower carry-over, needle flush port
+ Relatively fast 18s (vials to injector)
+ Sampler cooling condenser (optional)
+ Walk up tray (option)
+ Optional integrated column compartment (ICC)
  + Up to 2 columns
  + Heating up to 80 °C
  - Cooling to -5 of ambient tempeature

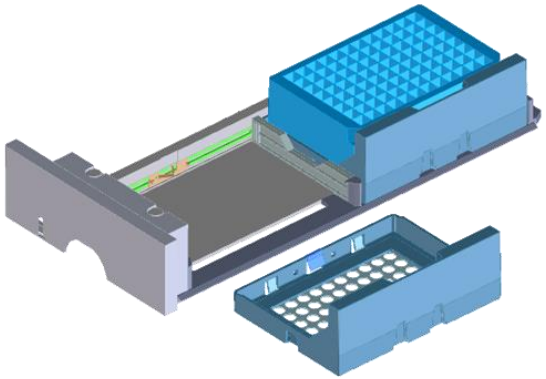


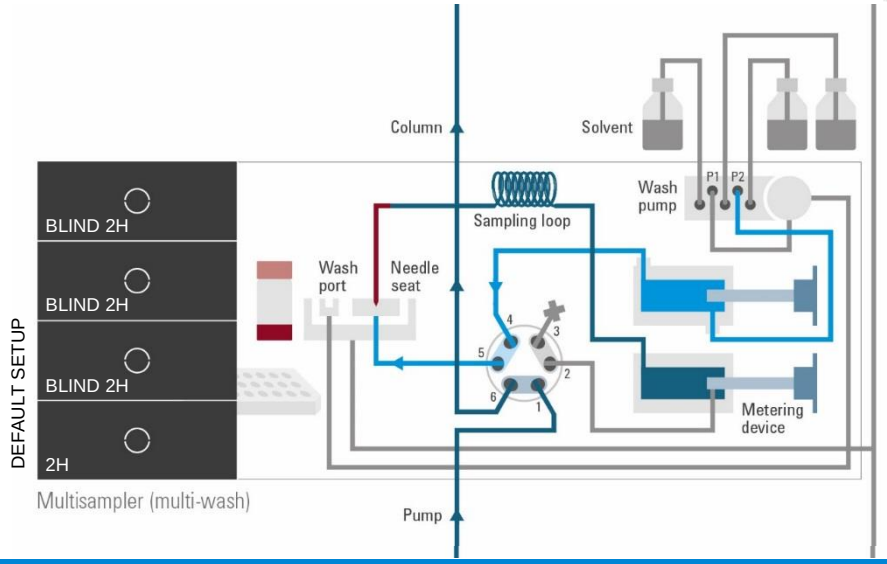
Agilent 1290 Infinity II Autosampler

Multisampler

G7167B 1290 Infinity II Multisampler  1300 bar

- \+ Vials and well-plates
- \+ Extremely fast (needle to vial)
- \+ High capacity up to 16 WPS (6144 samples)
- \+ Lowest carryover
- \+ Dual needle **or** Multi wash (3 solvents)
- \+ Highest Capacity per benchspace (8 x 54 vials)





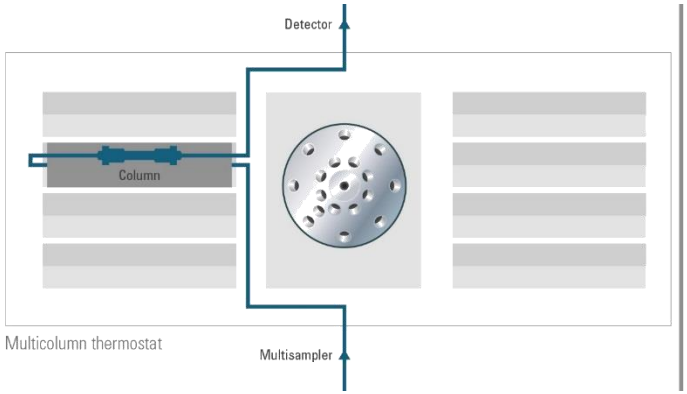
1290 Infinity II Column Compartment

Multi Column Thermostat (MCT)

G7116B 1290 Infinity II MCT

- \+ 4 x 300mm up to 8 x 100mm columns
- \+ Column heating and cooling
- \+ Up to 8 quick-connect heat exchangers
- \+ Quick-change valve (1300 bar)
- \+ Door flap can be opened to 90°, 180° or removed
- \+ Optional column tagging for up to 8 columns

RFID Column Tags



Quick-Change Valve

1290 Infinity II Detectors

UV Detectors

G7114B 1290 Infinity II VWD

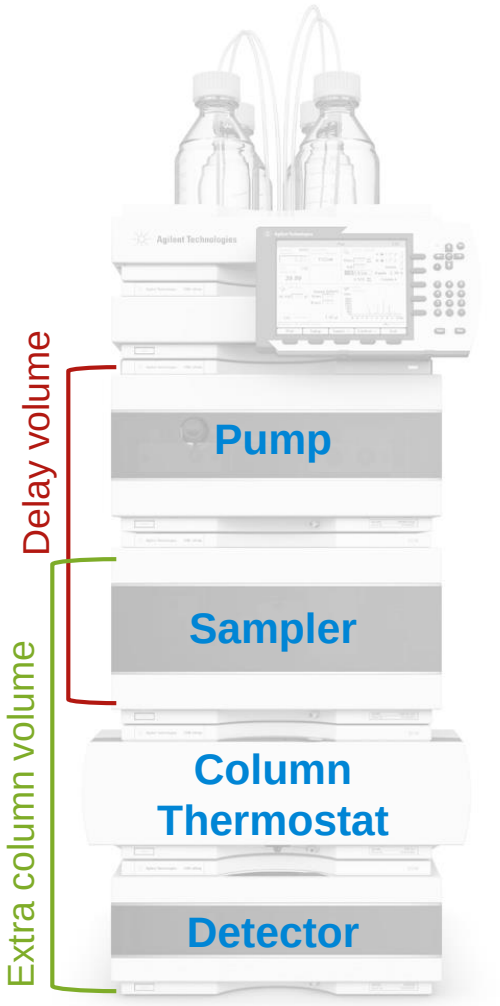
- \+ Dual λ mode (2.5 Hz)
- \+ Data rates 240 Hz for ultra-fast separation
- \+ 3 Prep flow cells
- \- Wavelength range 190 – 600 nm
- \- No spectra

G7114B 1290 Infinity II DAD *NEW DESIGN*

- \+ Programmable slit 1,2,4 8 nm
- \+ G7117A **FS** (Fixed Slit) version available

- Instrument to Instrument (I2I) Method Transfer using ISET

Parameters Affecting I2I Method Transfer



Delay volume Gradient mixing behavior Pressure x flow rate	Retention Time Resolution
Delay volume Extra column volume Injection volume	Retention Time Resolution Sensitivity
Temperature profile Extra column volume	Retention Time Resolution
Data rate Extra column volume Path-length	Resolution Retention Time Sensitivity

I-to-I Method Transferability

What Most Vendors Does?

- From a *lower to higher* dwell volume LC system

  (UHPLC → HPLC)

  A) Adding physical volume (plumbing solution)

  B) Modifying your gradient table with isocratic hold

- From a *higher to lower* dwell volume LC system

  (HPLC → UHPLC)

  C) Delay the injection using injector program

  Injection delay time = Dwell Volume ÷ Flow Rate

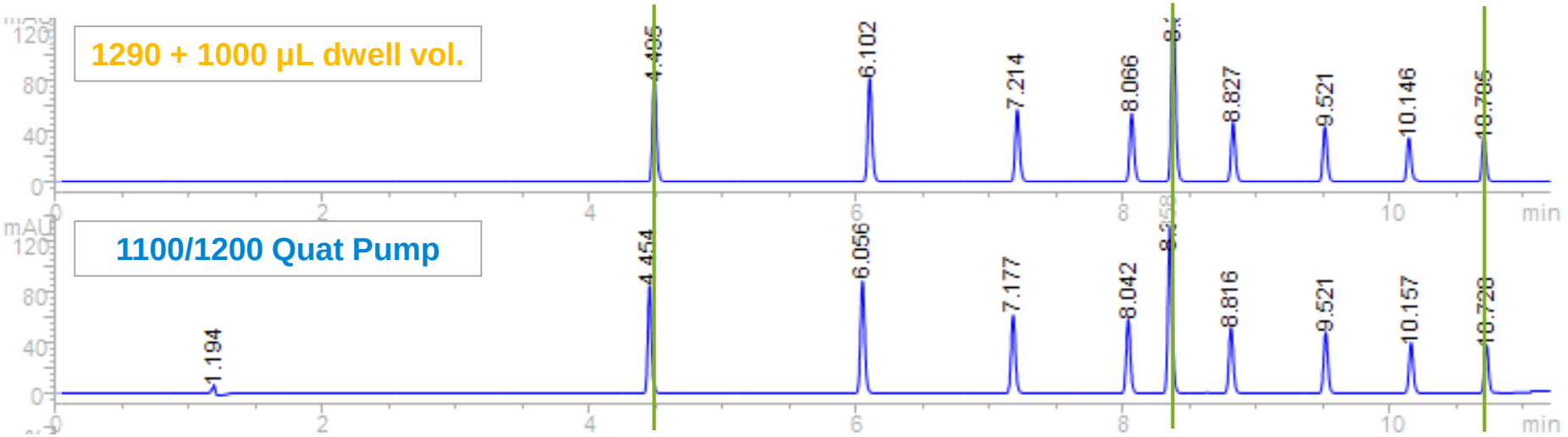
Method Transfer from Lower to Higher Dwell Volume

Adding Physical Volume (Plumbing Solution)

Examples:

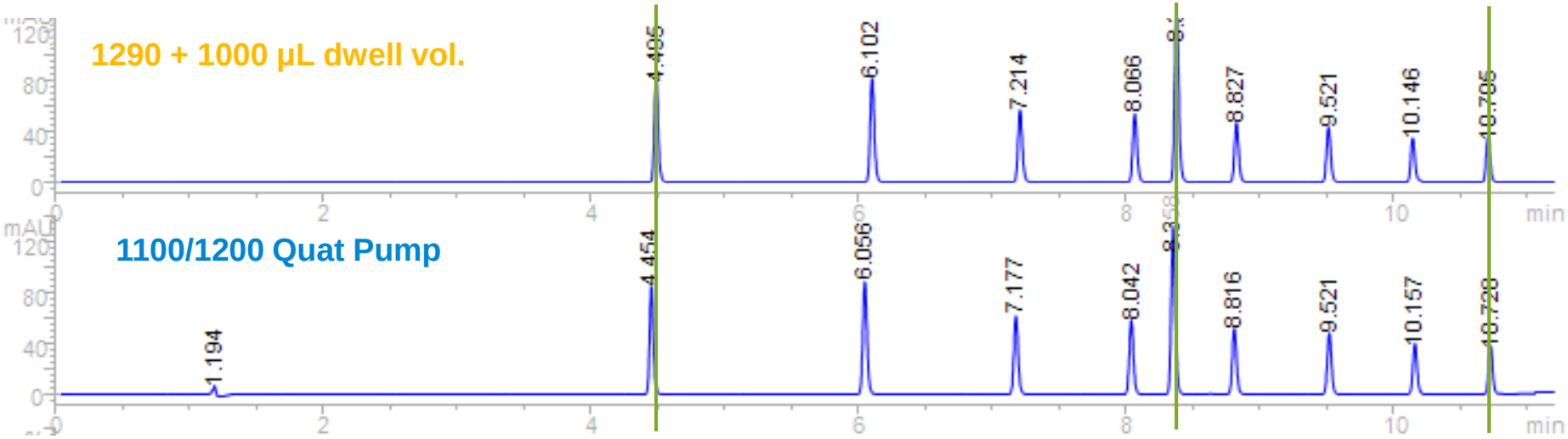
Binary LC Pump → Quaternary LC Pump

UHPLC → HPLC



Method Transfer from Lower to Higher Dwell Volume

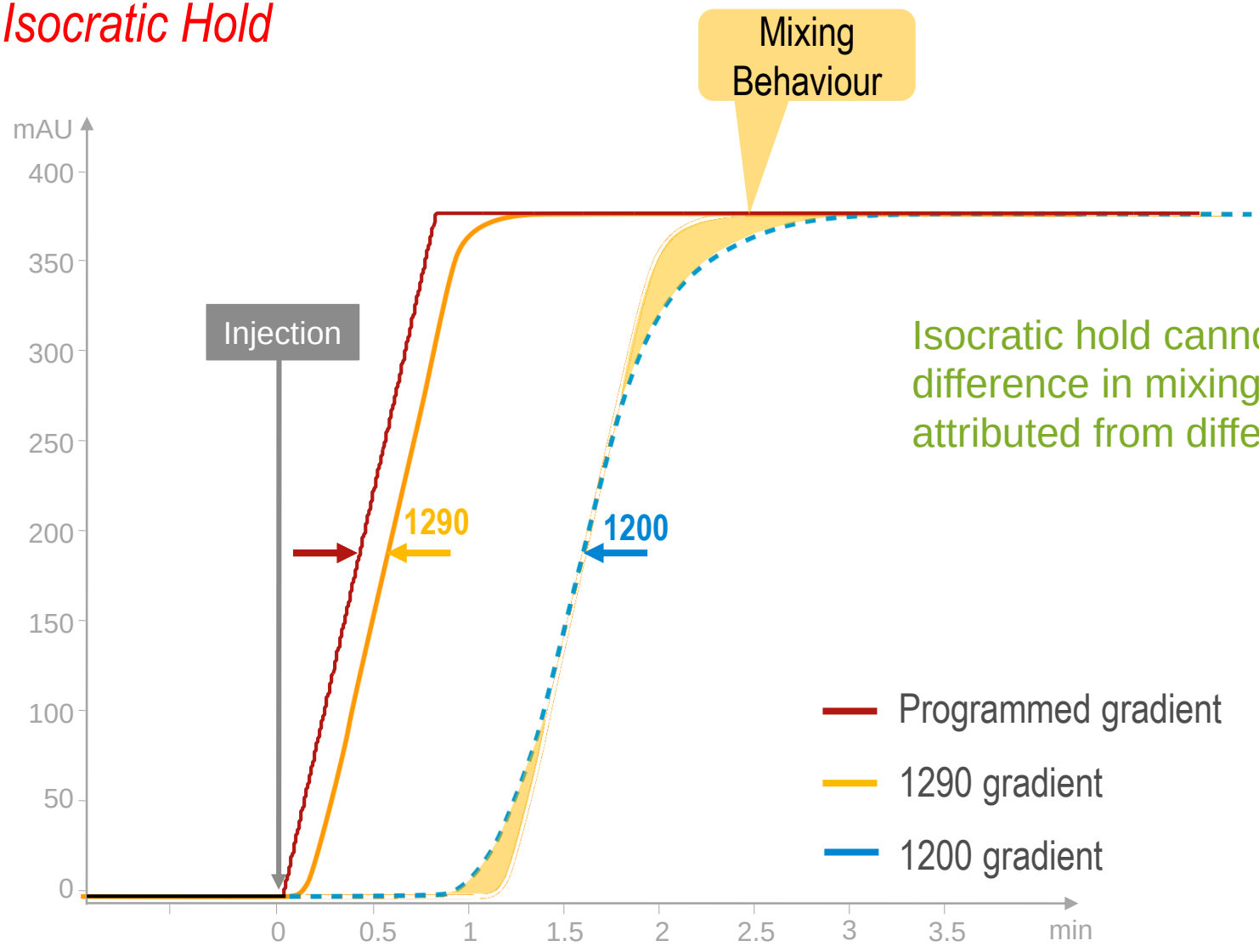
Adding Physical Volume (Plumbing Solution)



Advantages	Disadvantages
• Works well for not so fast analysis • No Method change	• Requires hardware change, no flexibility • Requires manual determination of the dwell volume (Pumps with damper the dwell volume is not constant and depends on pressure) • Hardware change difficult in validated environment

Method Transfer from Lower to Higher Dwell Volume

Isocratic Hold



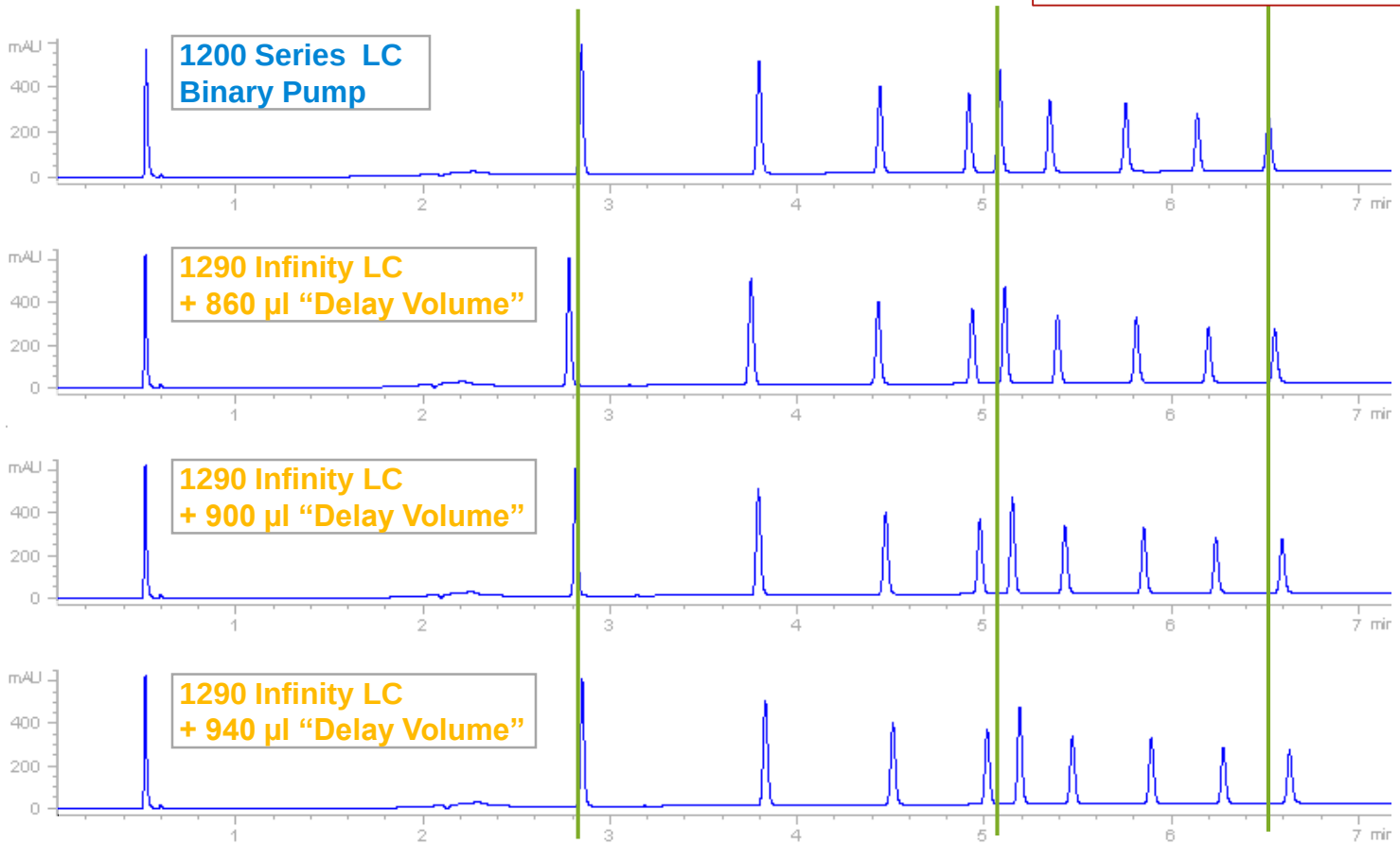
Isocratic hold cannot 100% resolve difference in mixing behavior attributed from different pumps

Method Transfer from Lower to Higher Dwell Volume

Isocratic Hold

Example: Infinity 1290 to 1200 series

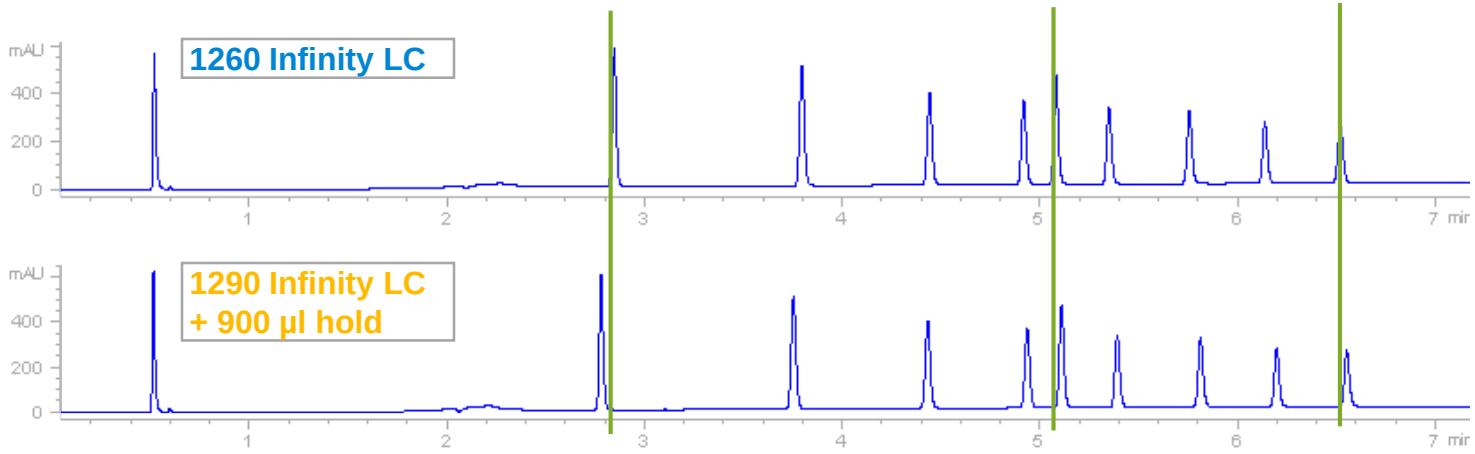
Does not work well with late eluting peaks



Method Transfer from Lower to Higher Dwell Volume

Isocratic Hold

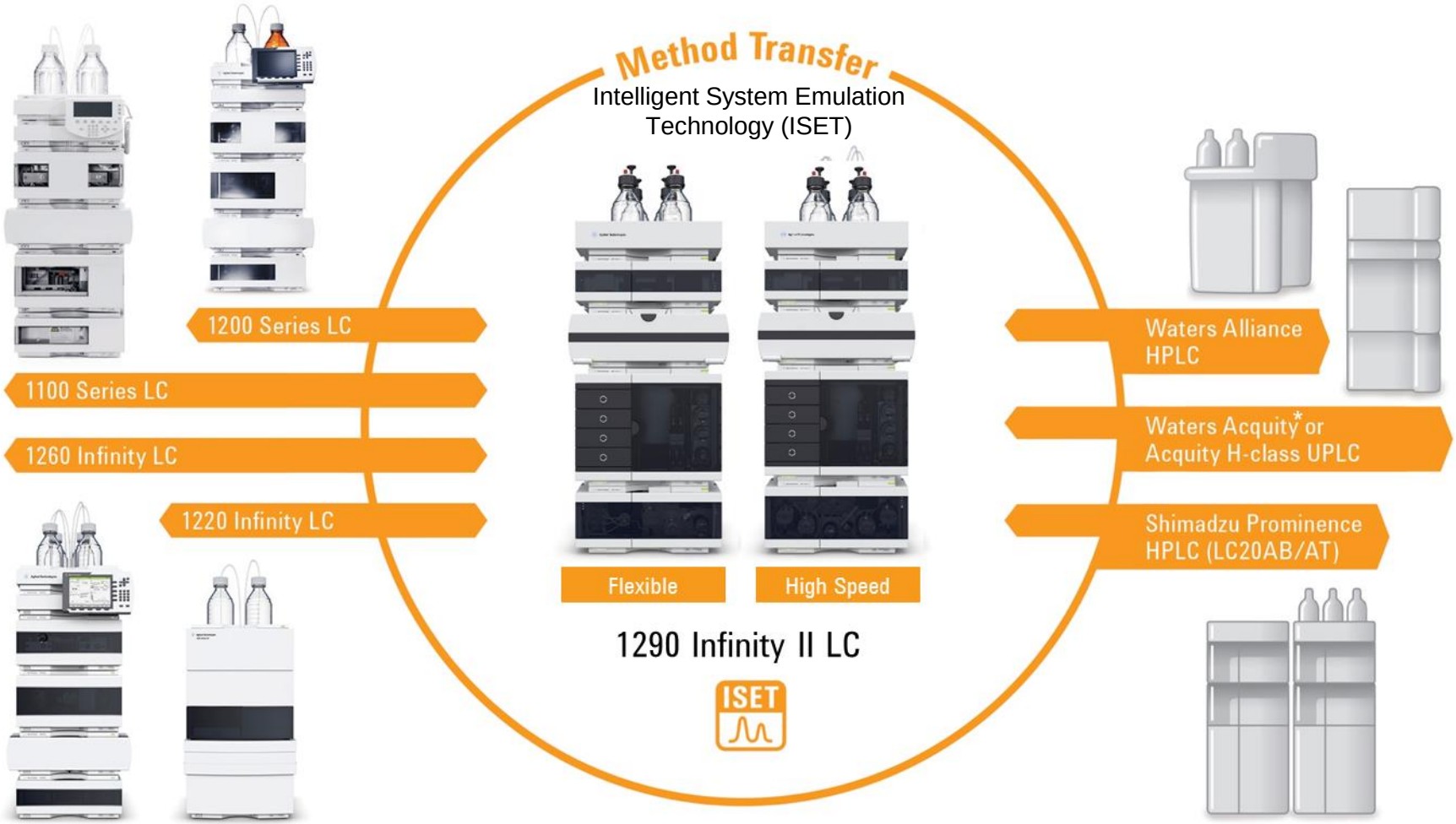
Example: Infinity 1290 to 1200 series



Advantages	Disadvantages
• Flexible • *Isocratic Hold* is part of the method	• Does not work in all cases • Requires manual determination of the dwell volume/ isocratic hold (Pumps with damper the dwell volume is not constant and depends on pressure) • Requires modification of the methods (should be avoided in validated environment)

I-to-I Method Transferability

What Agilent Does?



* Waters Acquity Binary UPLC can only be emulated by the 1290 Infinity II High Speed Pump

Intelligent System Emulation Technology (ISET)

ISET 4.0

Software compatibility

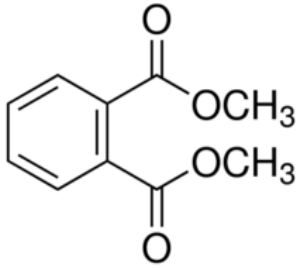
- Optional add-on to OpenLAB CDS ChemStation
- Use MSW to fully optimize instrument setup
- To run ISET 4.0, it is required to have the following installed:
  - OpenLAB ChemStation C.01.07
  - OpenLAB EZChrom EE A.04.07
  - Instrument Control Framework (ICF) A.02.03
  - Firmware version > B.06.07

Live Demonstration

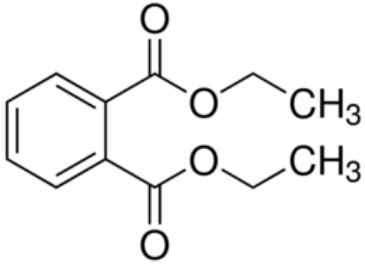
Method Transfer

Objective: Seamless Method Transfer from Agilent 1260 to 1290 Infinity II

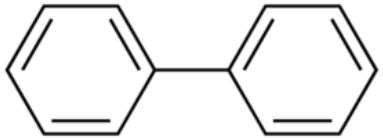
Compounds of Interest:



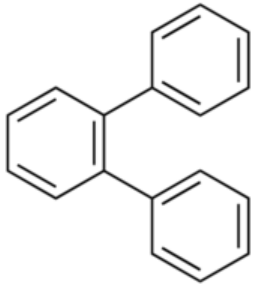
Dimethyl Phthalate



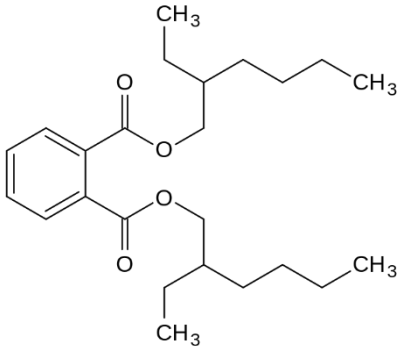
Diethyl Phthalate



Biphenyl



O-Terphenyl



Bis(2-ethylhexyl) Phthalate

Agilent 1260 LC System

LC Conditions

Instrument: Agilent 1260 Infinity LC system

Mobile Phase A: Ultrapure water, Mobile Phase B: HPLC grade Methanol

Injection volume: 1 µL

Flow Rate: 1.0 mL/min

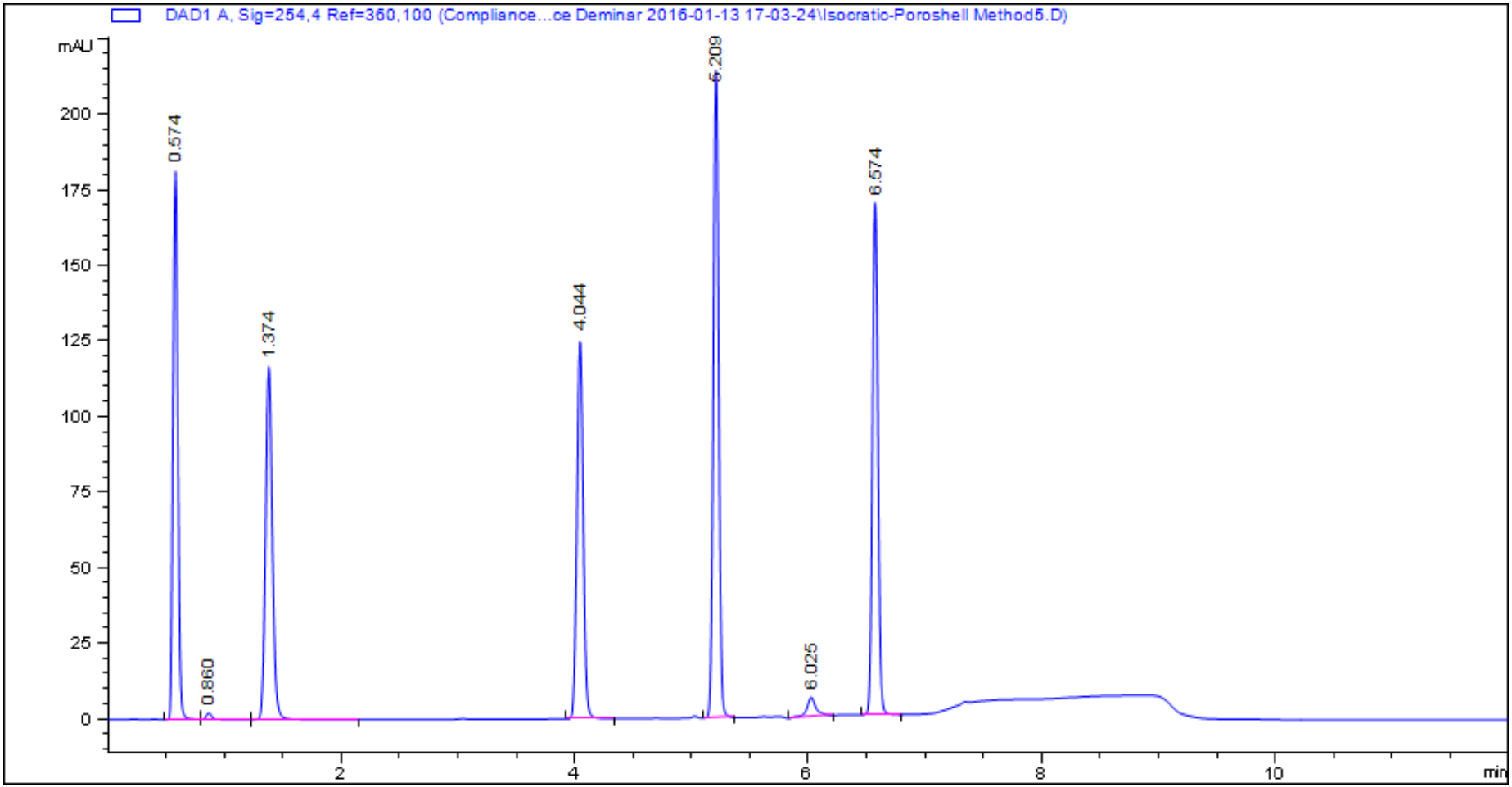
Elution Condition: 1 min – 50% B, 6 min – 100 % B, 8 min – 100% B, 8.1min - 70% Methanol (Gradient)

Column: Agilent Poroshell 120 (3.0 x 50 mm, 2.7 µm)

Wavelength: 254 nm, Reference: 360 nm

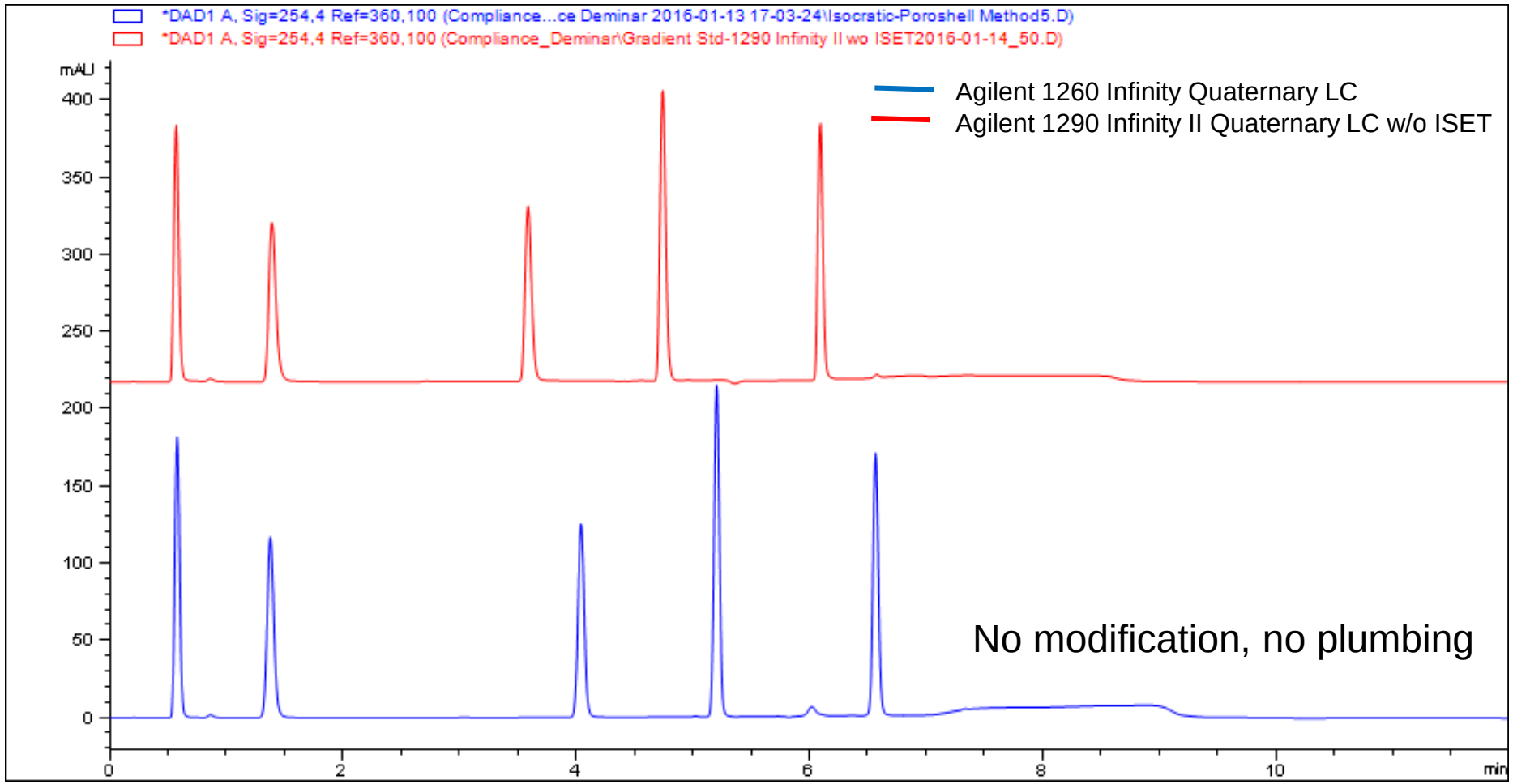
Chromatogram

Agilent 1260 Infinity LC System

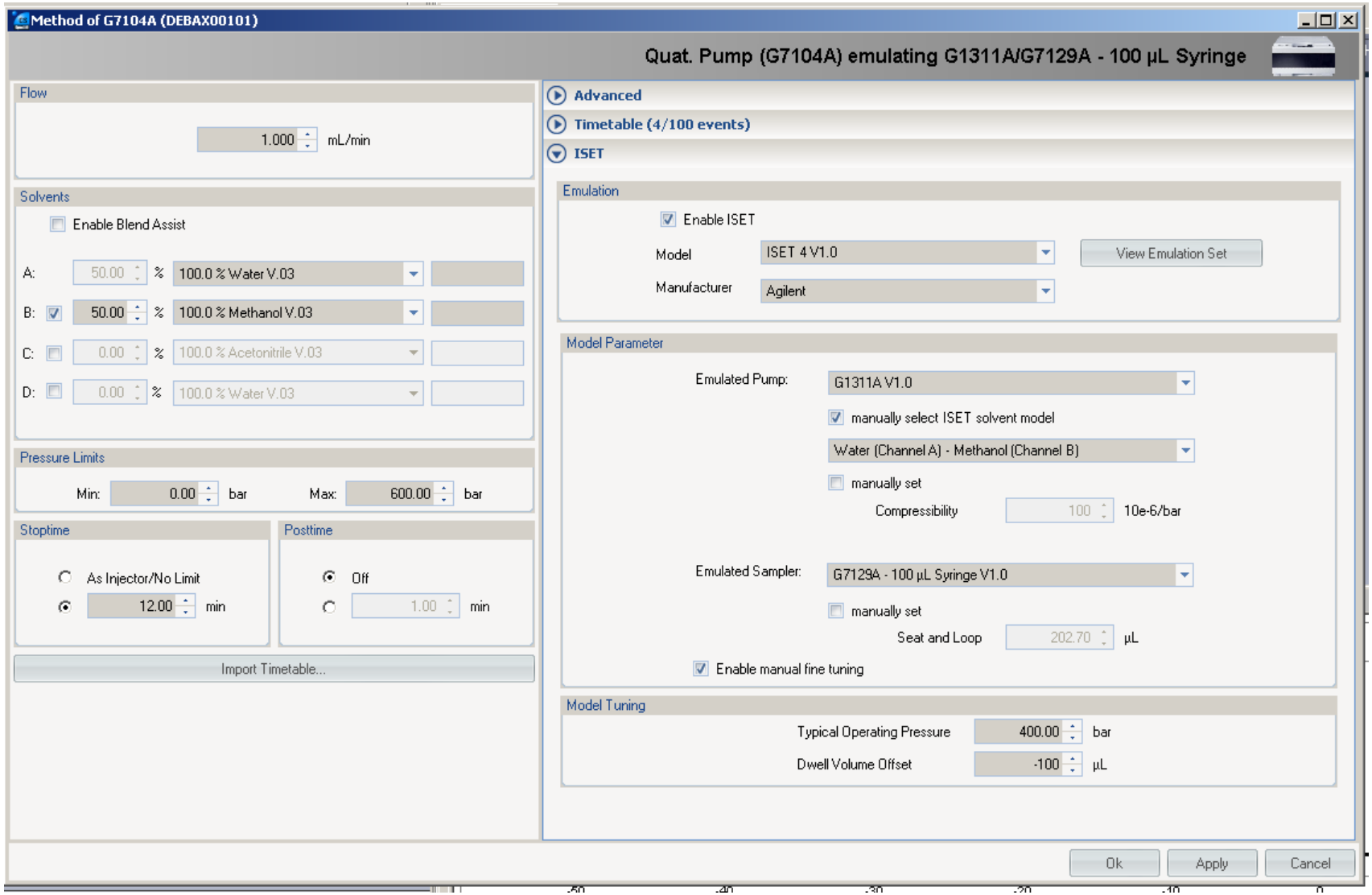


Method Transfer

From 1260 to 1290 Infinity II

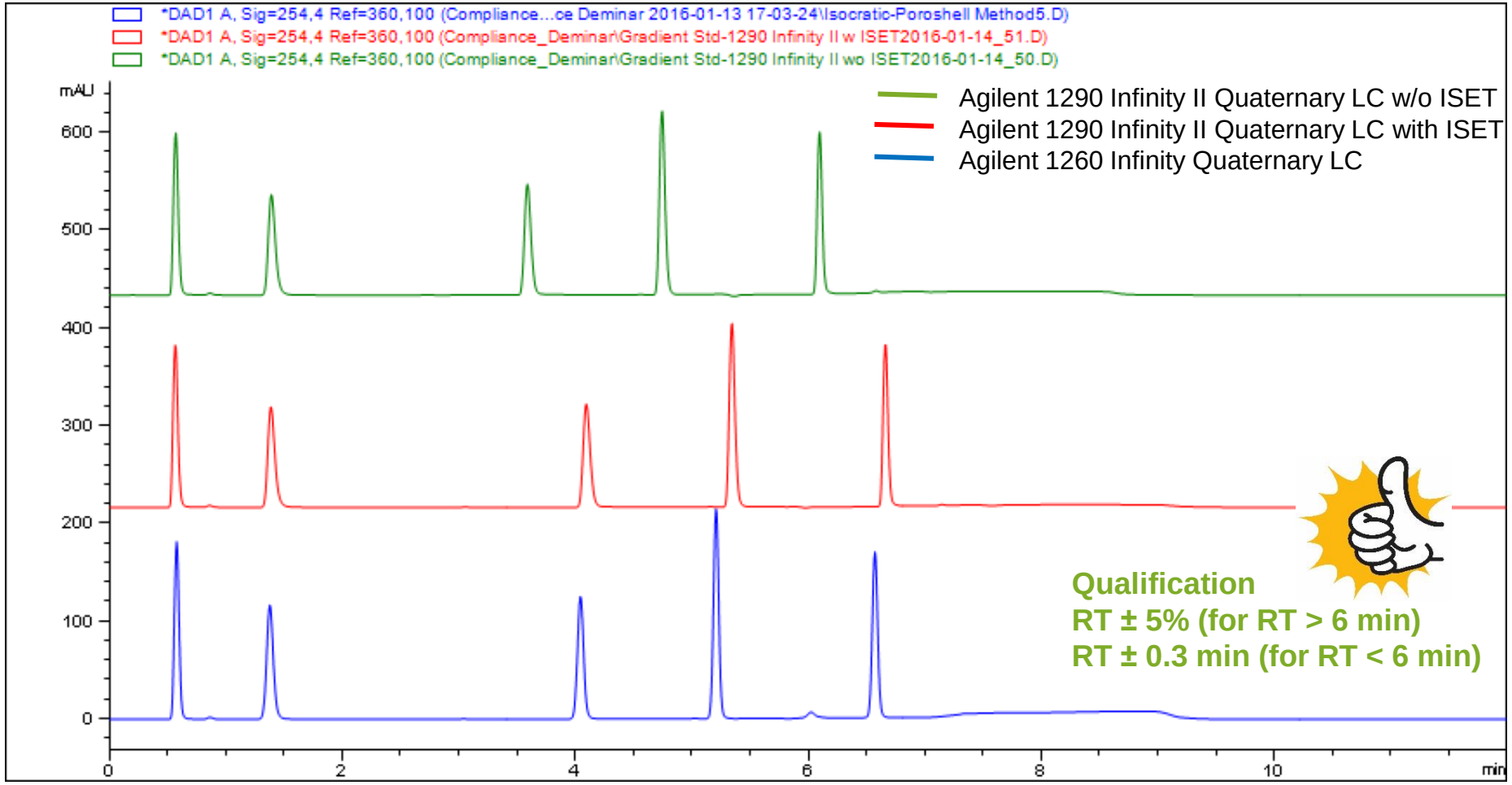


Select System for Emulation



Method Transfer with ISET

Agilent 1260 to 1290 Infinity II



- Automated Scouting of Stationary and Mobile Phases Using Agilent 1290 Infinity II Method Development Solution

Agilent 1290 Infinity II LC Setup

For Method Development

- Agilent 1290 Infinity II Flexible Pump (G7104A)
- Agilent 1290 Infinity valve drives (2 x G1170A) equipped with Agilent Quick-Change 12-position/ 13-port solvent selection valves (2 x G4235A) - for access up to 26 solvents (24 + 2 from pump)
- Agilent 1290 Infinity Multisampler (G7167B)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B) including valve drive (option #058) equipped with Agilent Quick-Change column selection valve (G4239C) and capillary kit (option #005) – for access up to 8 columns
- Agilent 1290 Infinity II Diode Array Detector (G7117B)

Agilent ChemStation Method Scouting Wizard (MSW)

MSW A.02.05

Software compatibility

- Optional add-on to OpenLAB CDS ChemStation
- Use MSW to fully optimize instrument setup
- To run MSW A.02.05 with all its features, it is required to have the following installed:
  - OpenLAB A.02.02, ChemStation C.01.07
    - This MSW version is not backwards compatible and cannot be installed on a lower ChemStation revision
  - LC driver package A.02.11, SP1 (minimal)
    - A.02.11, SP1 supports new 1290 Infinity II modules such as MCT, flex pump
- Currently neither MassHunter nor EZChrom support MSW

Demonstration

Method Development Solution

Objective: Determine best method for separation of HPLC standard mixture

Columns: 1) Zorbax SB-C18, 2) Eclipse Plus C18, 3) Eclipse Plus C8, 4) Extend C18, 5) SB CN, 6) Bonus RP, 7) Eclipse Plus Phenyl Hexyl

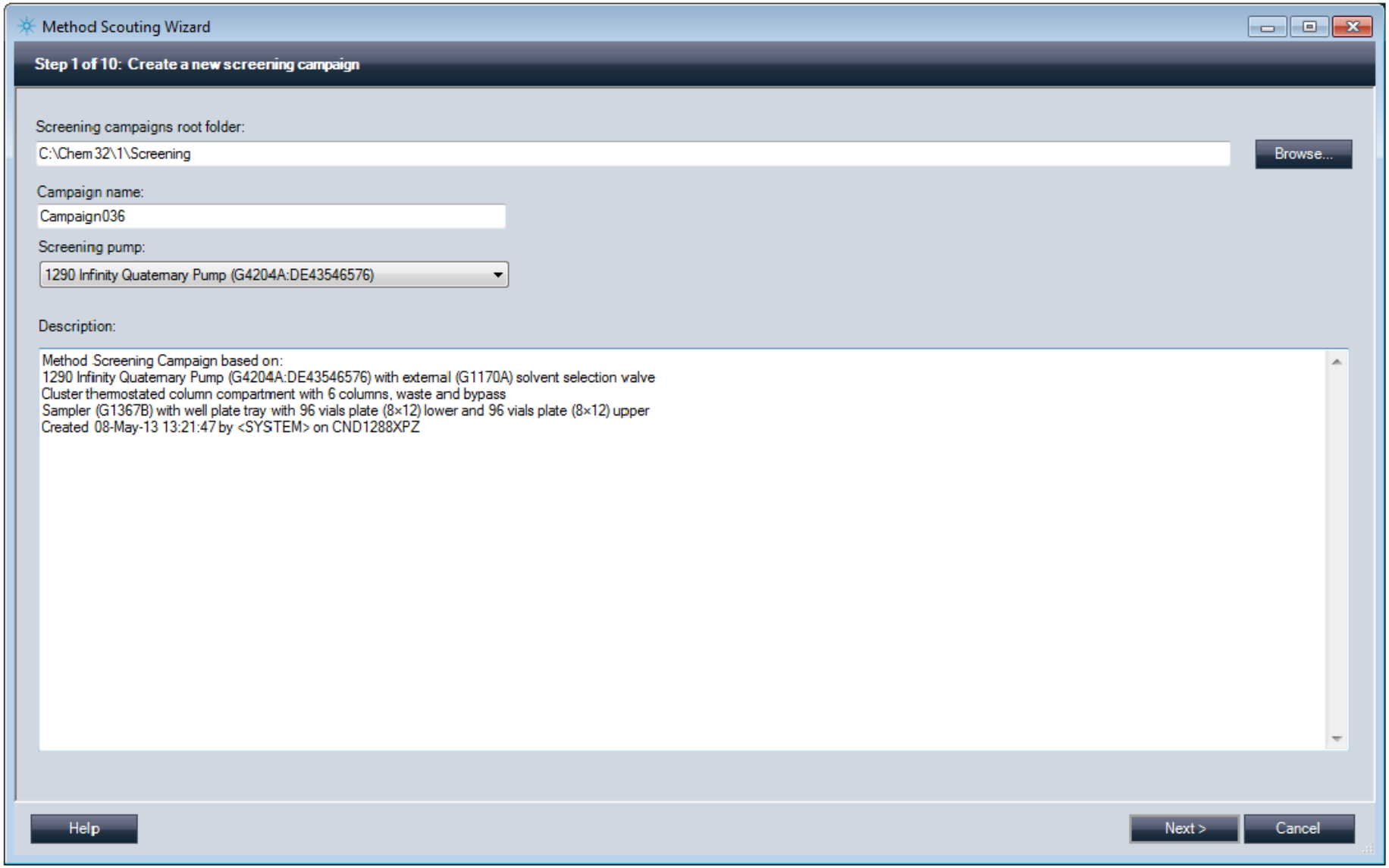
Solvents:

Mobile Phase A	Mobile Phase B
10 mm Ammonium, pH 8	Methanol
Water	Methanol + 0.1% TFA
Water + 0.1% TFA	Acetonitrile
	Acetonitrile + 0.1% TFA

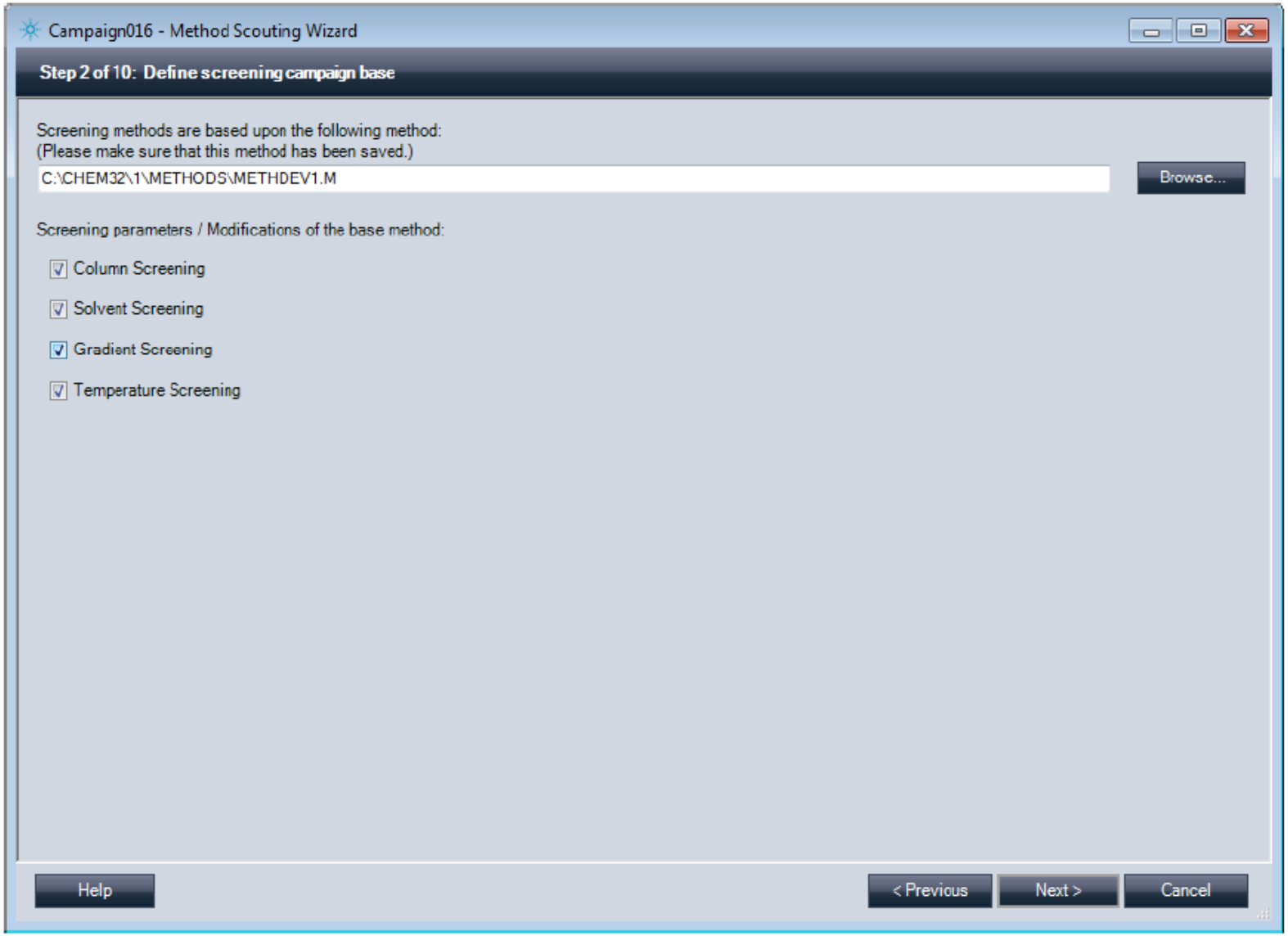
Temperature: 30, 40, 50, 60 ^{0}C

Sample: HPLC Standard Mixture

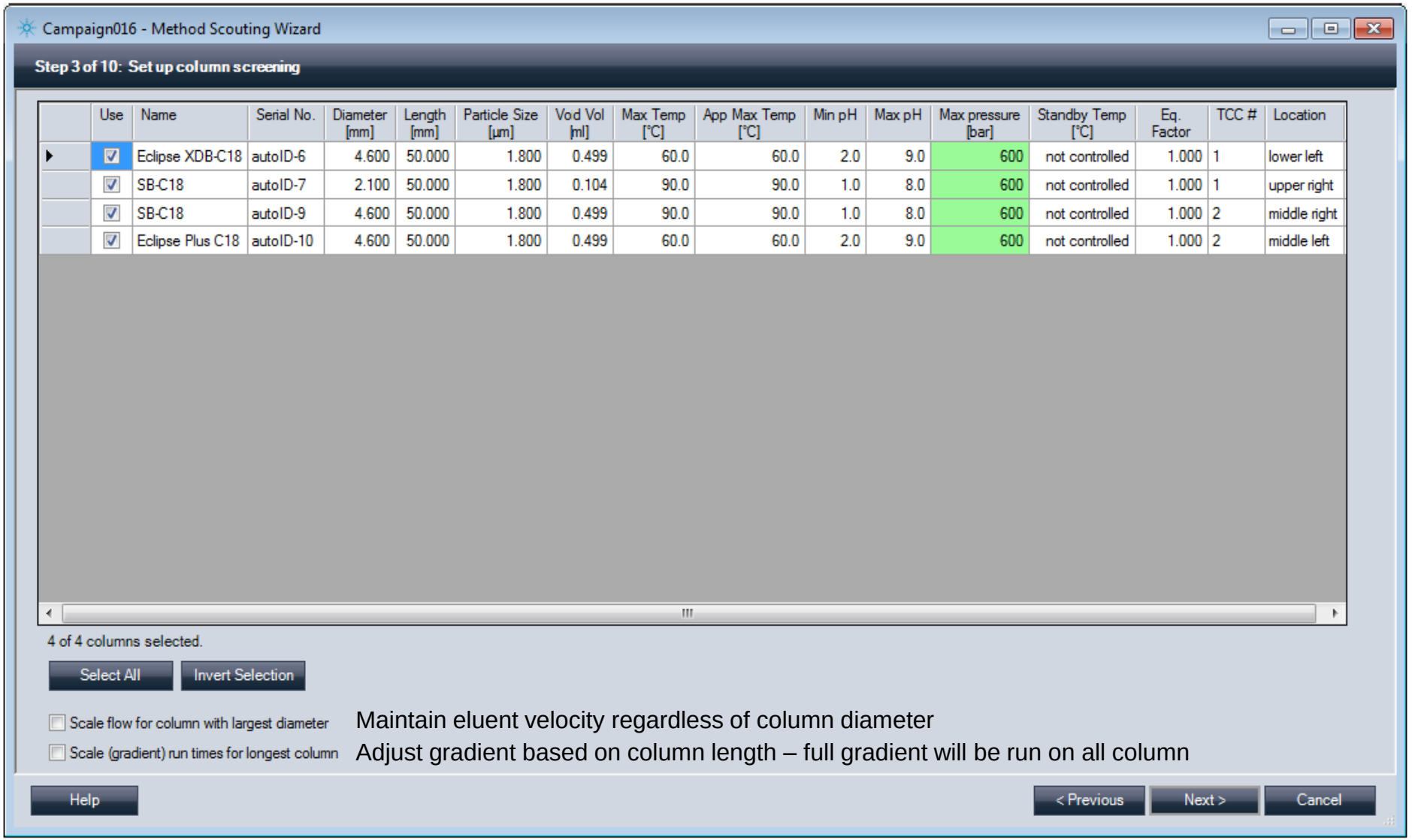
Starting New Screening Campaign



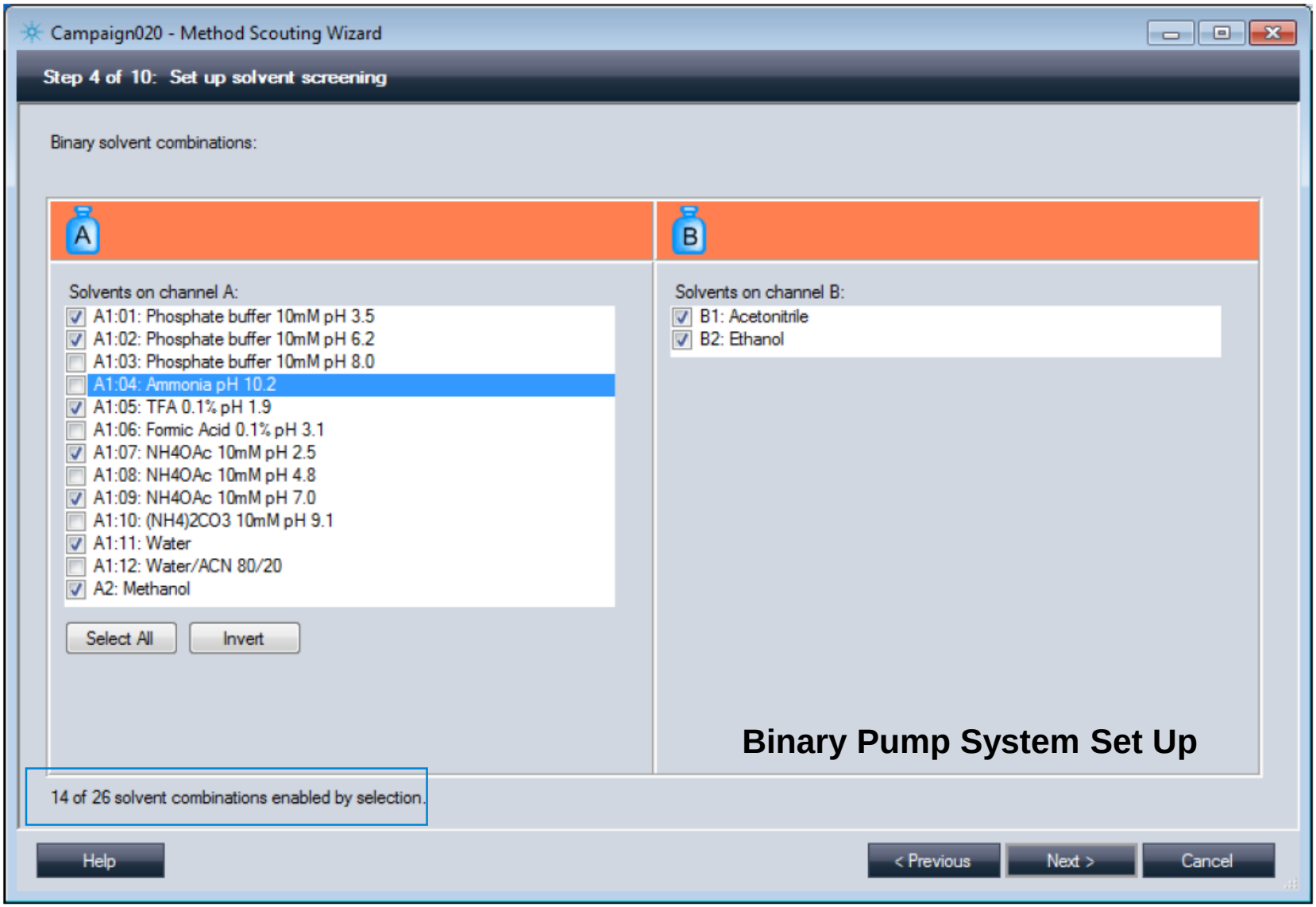
Define Master Method for New Campaign



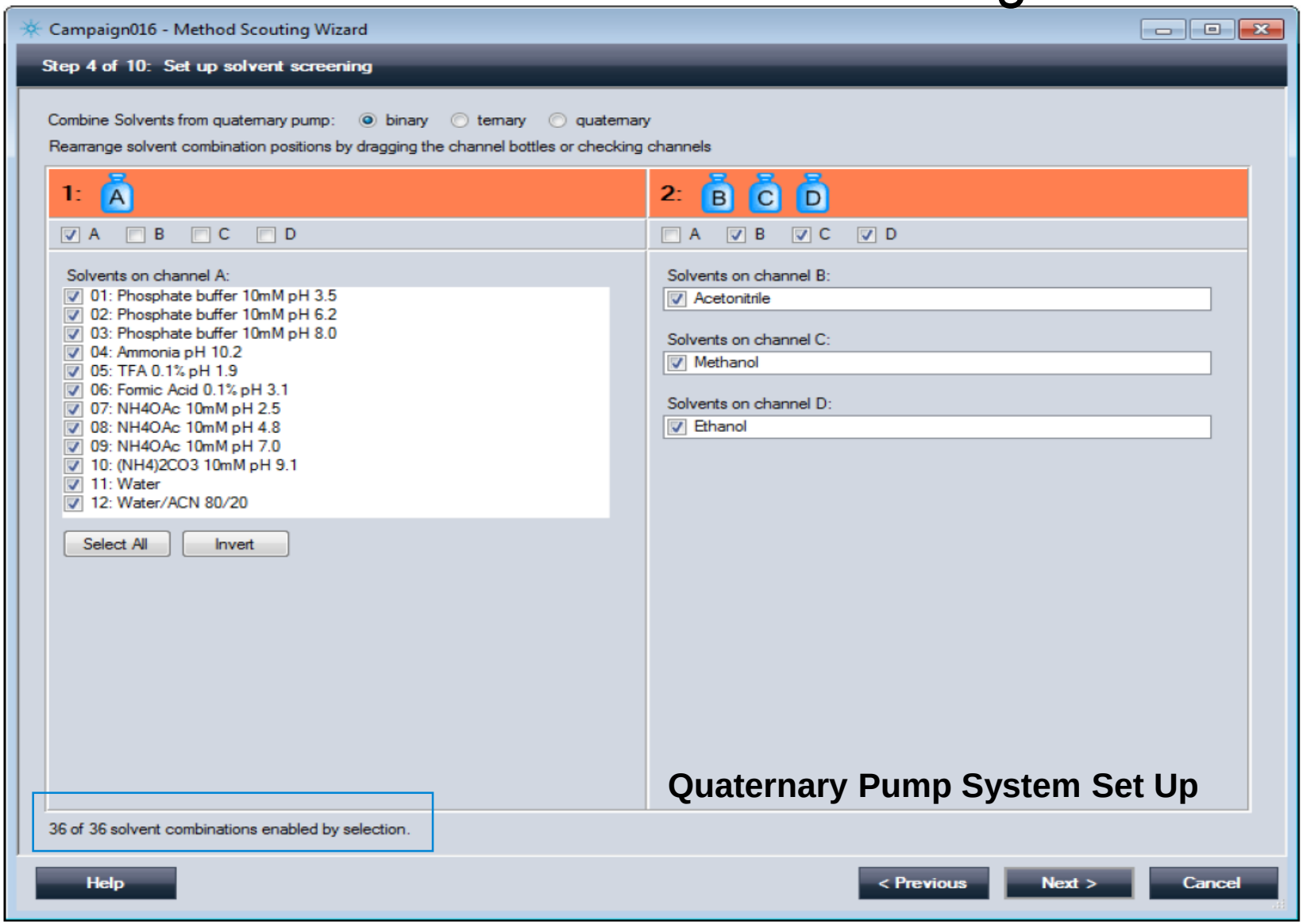
Selection of Columns – Column Screening



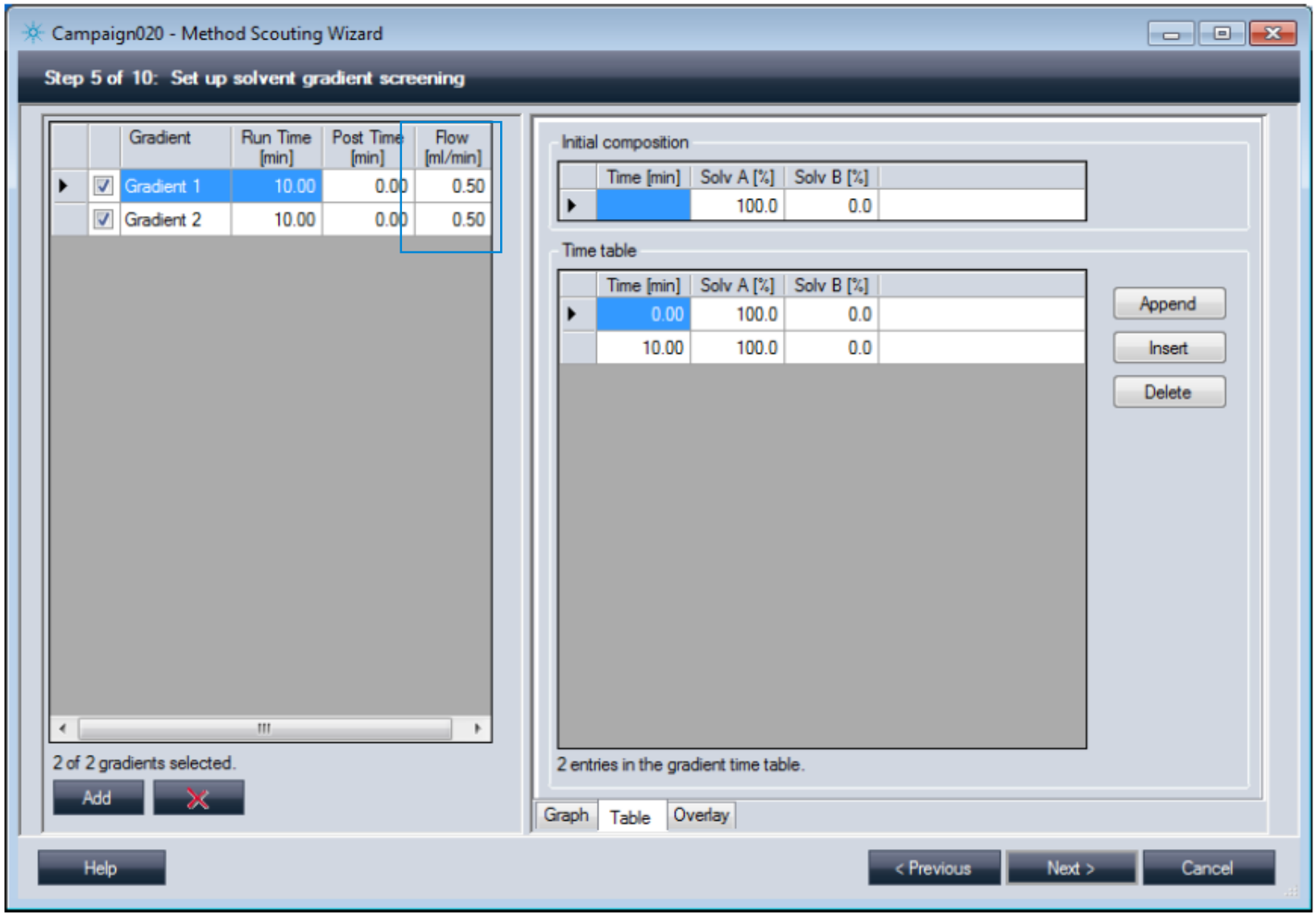
Selection of Solvents – Solvent Screening



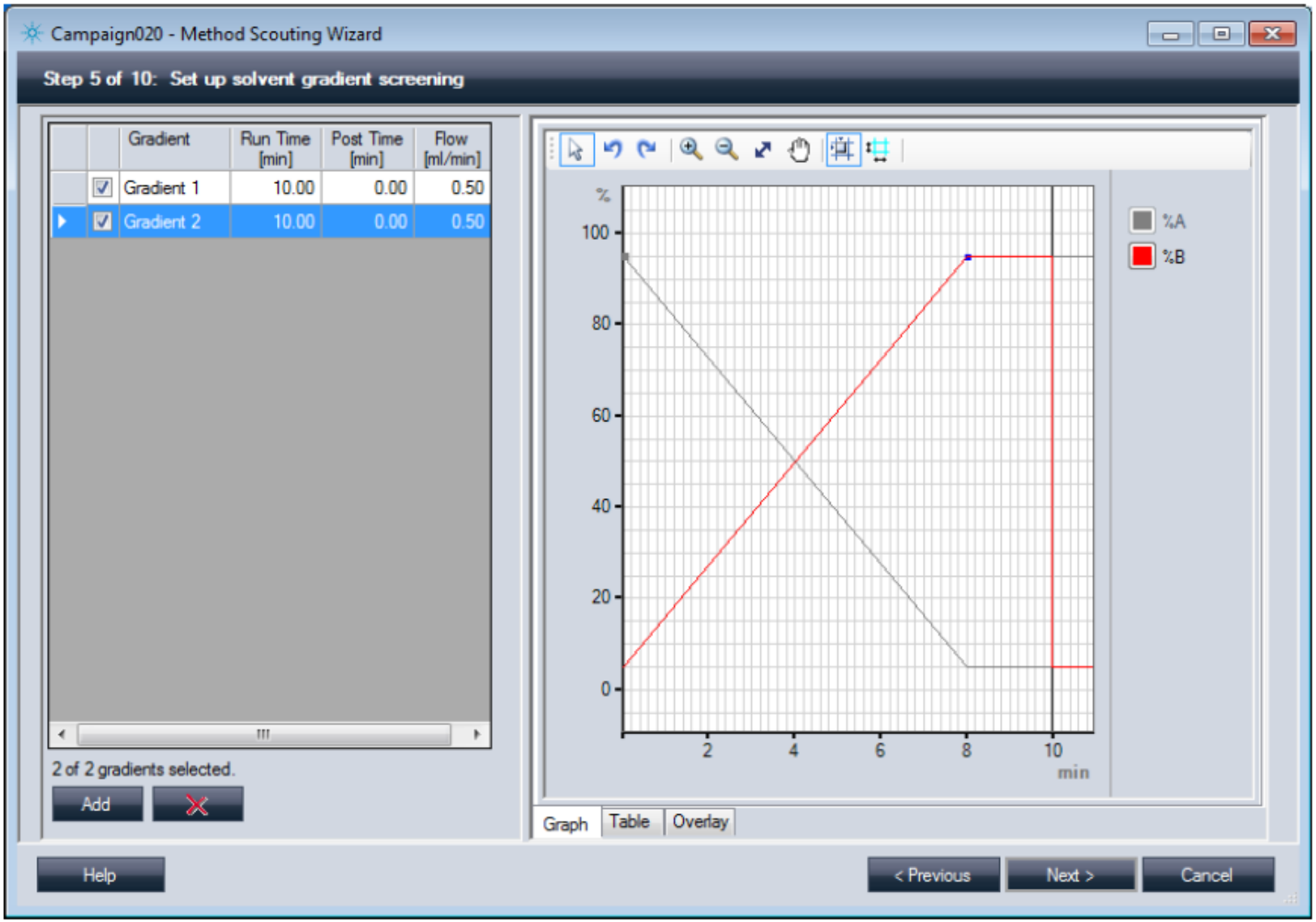
Selection of Solvents – Solvent Screening



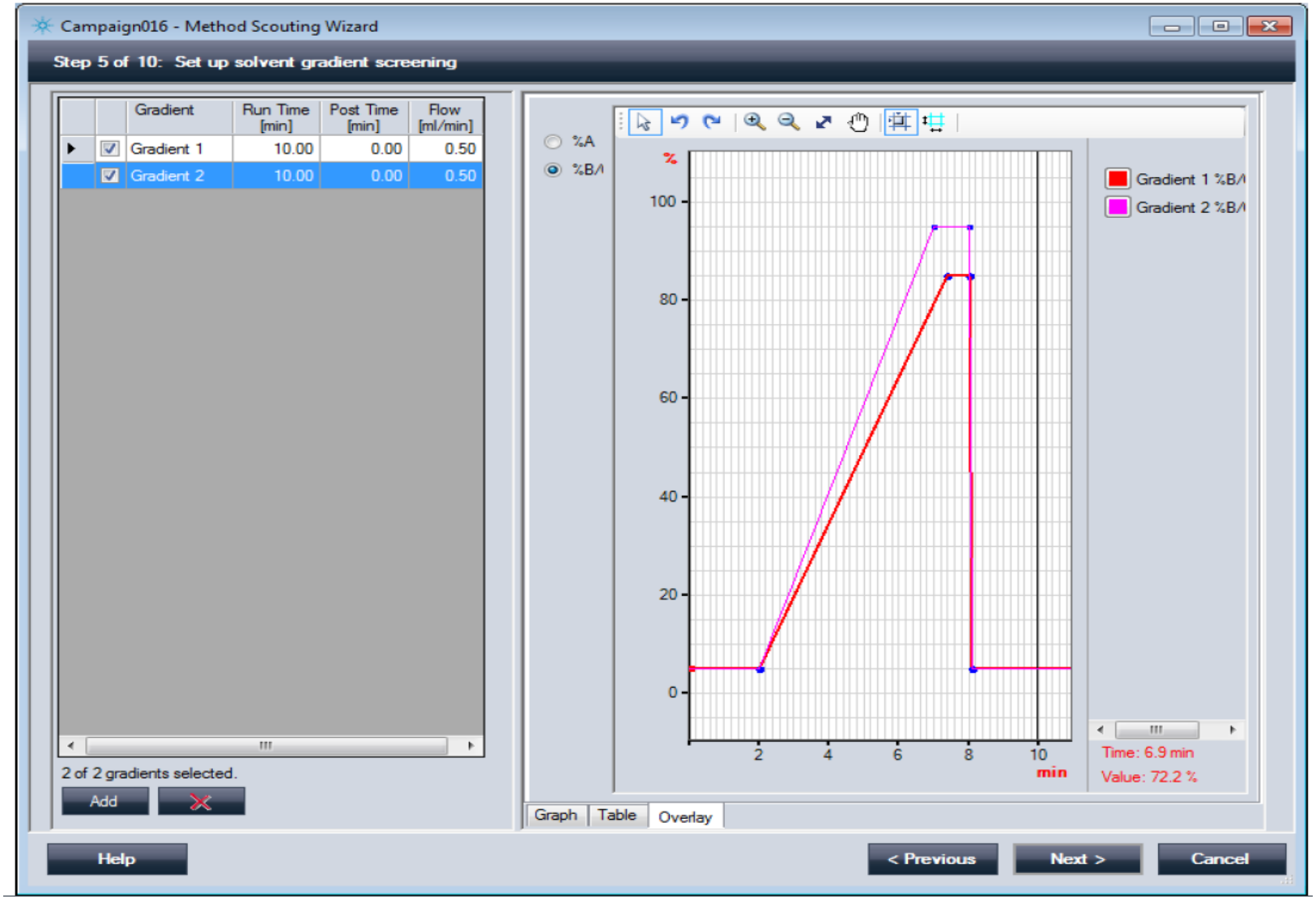
Selection of Elution Gradient – Table



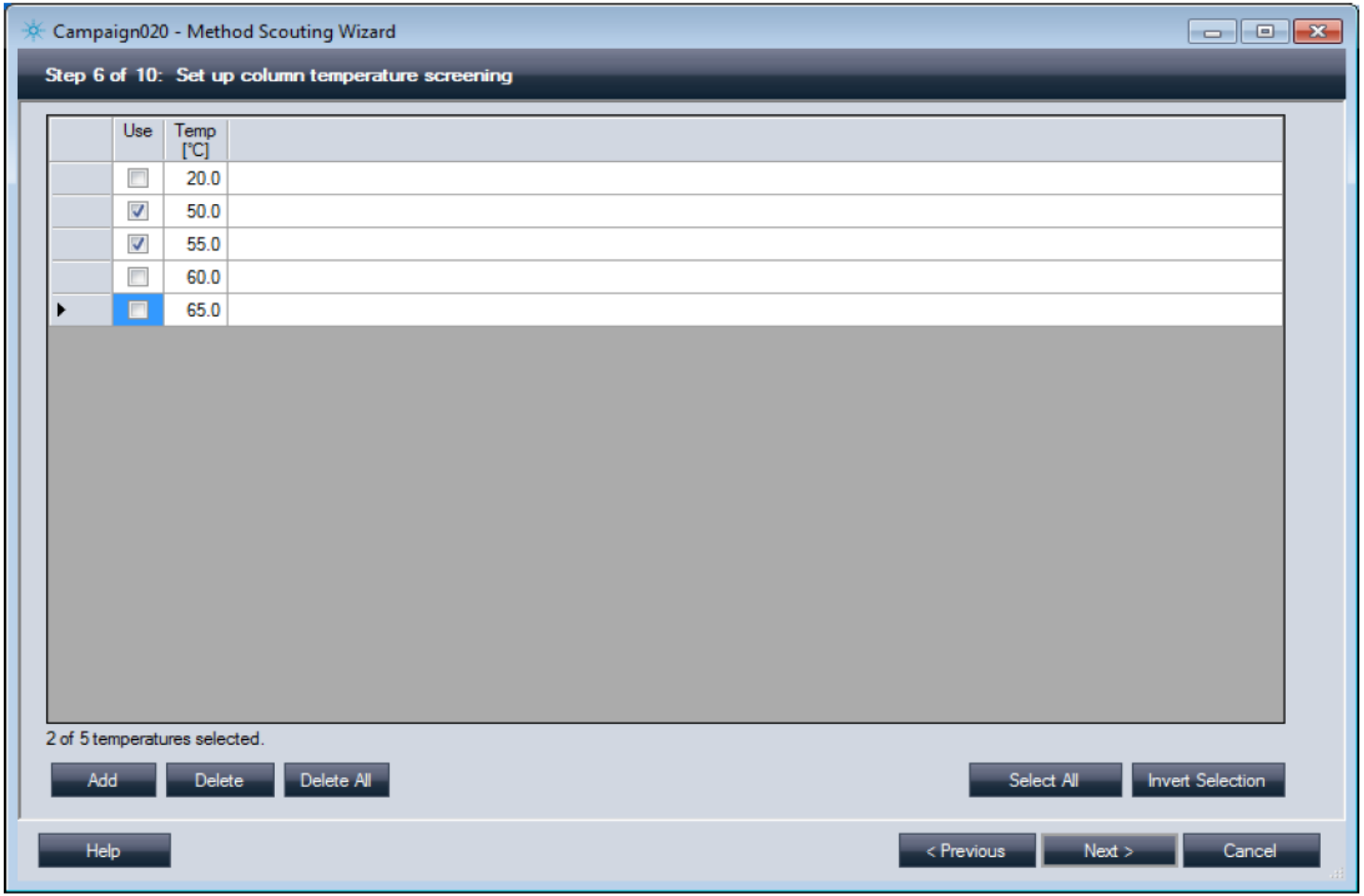
Selection of Elution Gradient – Graph



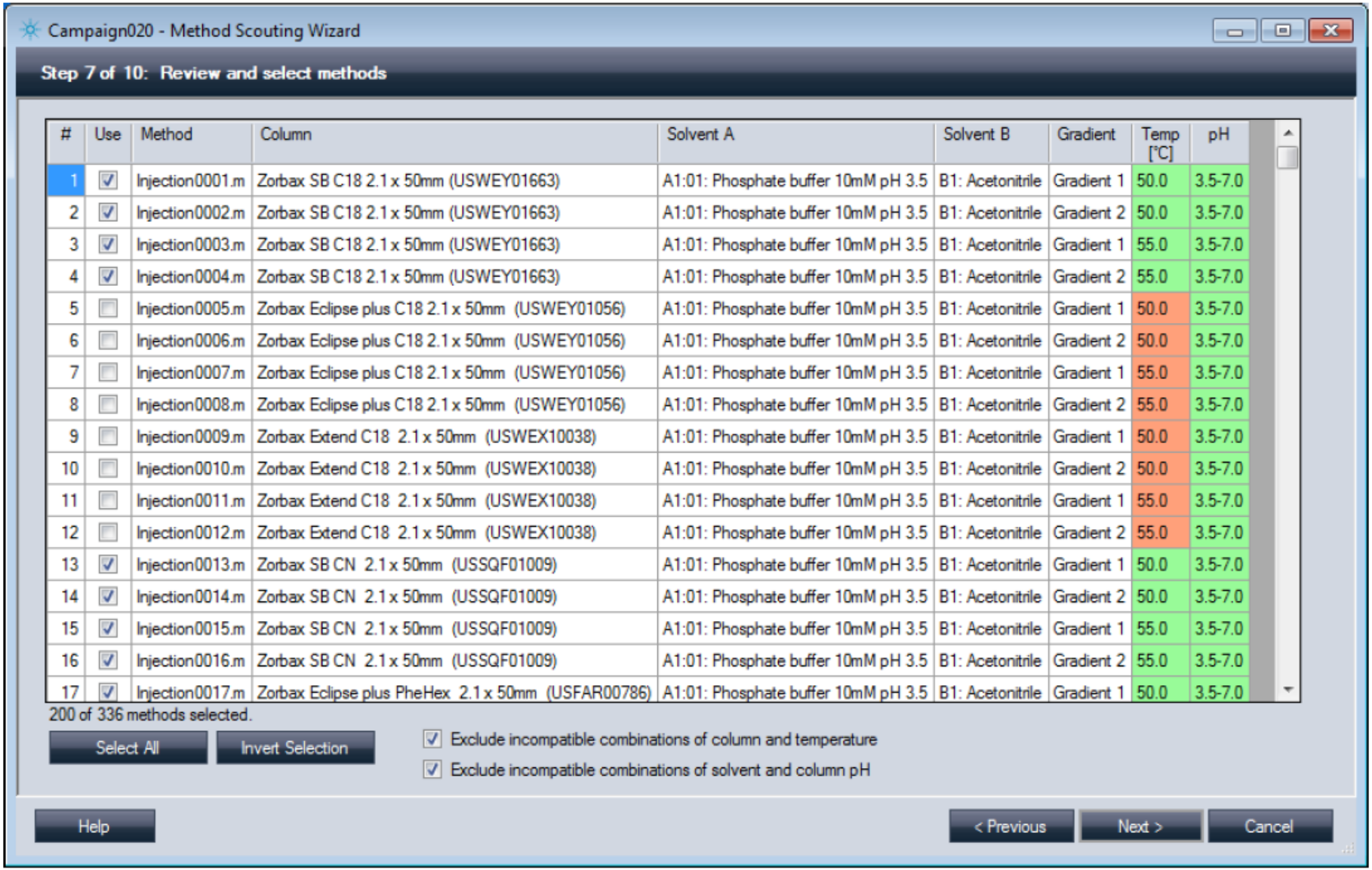
Selection of Elution Gradient – Overlay



Selection of Temperature – Temperature Screening

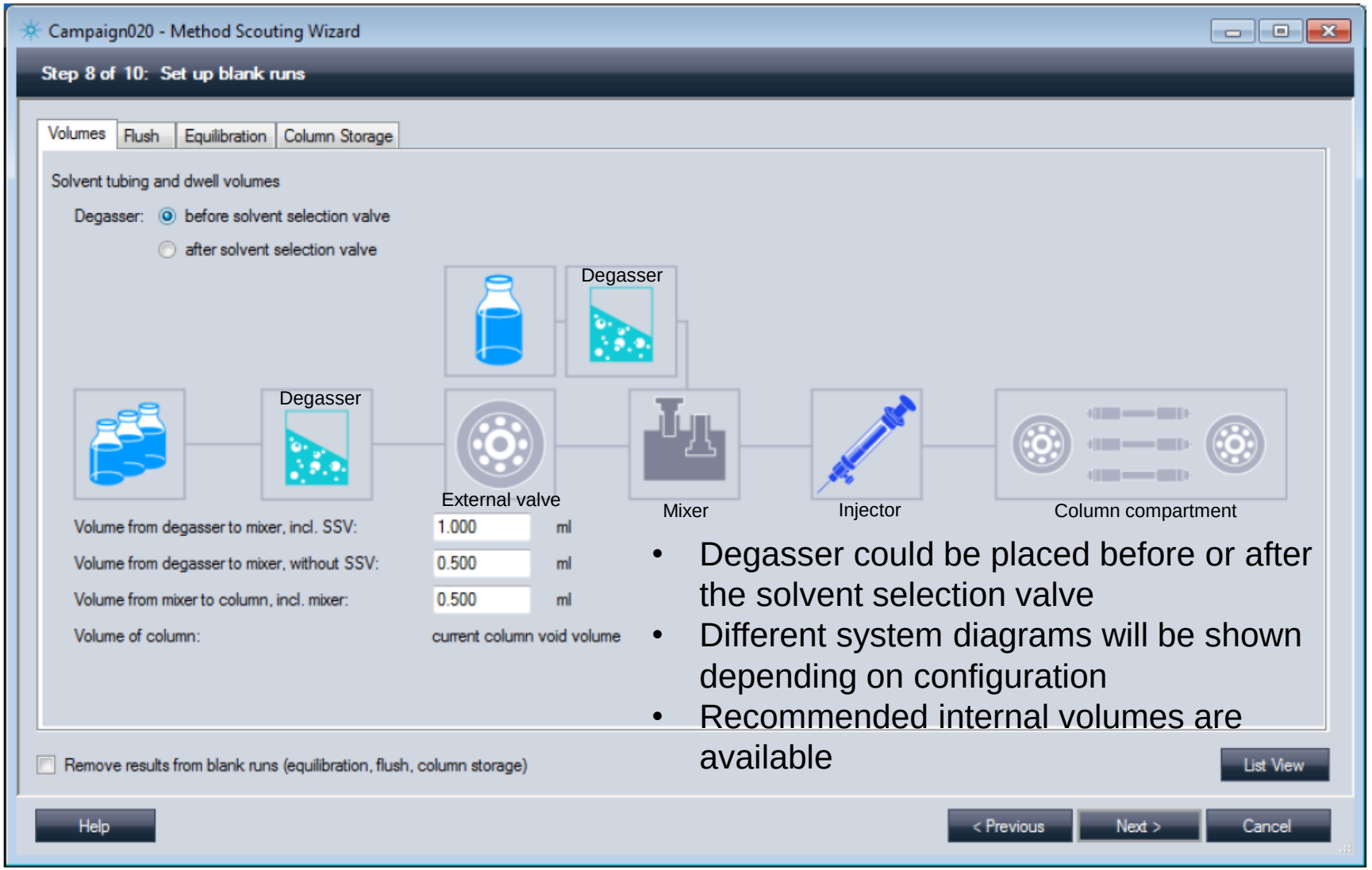


Review and Selection of Methods



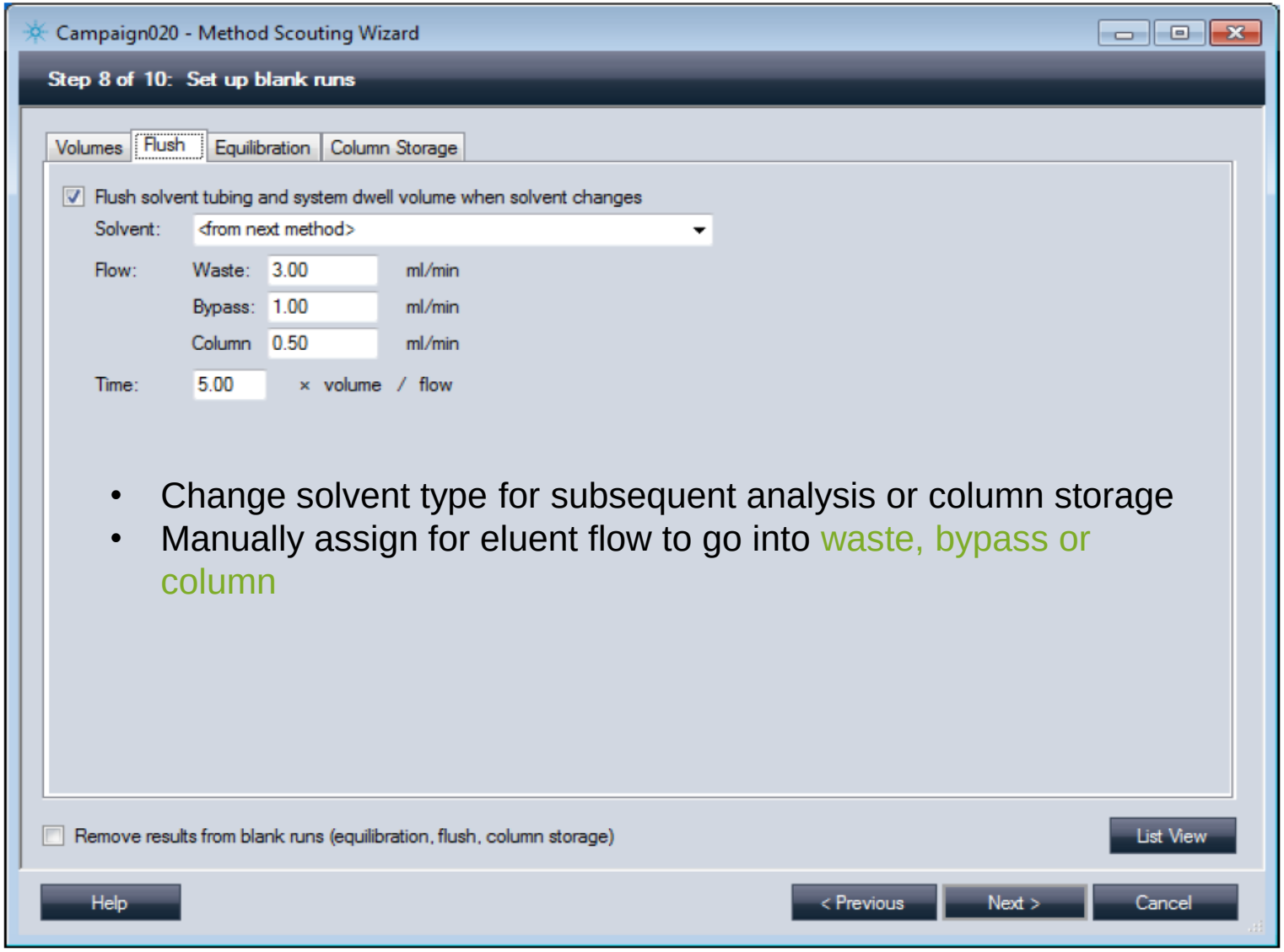
#	Use	Method	Column	Solvent A	Solvent B	Gradient	Temp [°C]	pH
1	☑	Injection0001.m	Zorbax SB C18 2.1 x 50mm (USWEY01663)	A1:01: Phosphate buffer 10mM pH 3.5	B1: Acetonitrile	Gradient 1	50.0	3.5-7.0
2	☑	Injection0002.m	Zorbax SB C18 2.1 x 50mm (USWEY01663)	A1:01: Phosphate buffer 10mM pH 3.5	B1: Acetonitrile	Gradient 2	50.0	3.5-7.0
3	☑	Injection0003.m	Zorbax SB C18 2.1 x 50mm (USWEY01663)	A1:01: Phosphate buffer 10mM pH 3.5	B1: Acetonitrile	Gradient 1	55.0	3.5-7.0
4	☑	Injection0004.m	Zorbax SB C18 2.1 x 50mm (USWEY01663)	A1:01: Phosphate buffer 10mM pH 3.5	B1: Acetonitrile	Gradient 2	55.0	3.5-7.0
5	☐	Injection0005.m	Zorbax Eclipse plus C18 2.1 x 50mm (USWEY01056)	A1:01: Phosphate buffer 10mM pH 3.5	B1: Acetonitrile	Gradient 1	50.0	3.5-7.0
6	☐	Injection0006.m	Zorbax Eclipse plus C18 2.1 x 50mm (USWEY01056)	A1:01: Phosphate buffer 10mM pH 3.5	B1: Acetonitrile	Gradient 2	50.0	3.5-7.0
7	☐	Injection0007.m	Zorbax Eclipse plus C18 2.1 x 50mm (USWEY01056)	A1:01: Phosphate buffer 10mM pH 3.5	B1: Acetonitrile	Gradient 1	55.0	3.5-7.0
8	☐	Injection0008.m	Zorbax Eclipse plus C18 2.1 x 50mm (USWEY01056)	A1:01: Phosphate buffer 10mM pH 3.5	B1: Acetonitrile	Gradient 2	55.0	3.5-7.0
9	☐	Injection0009.m	Zorbax Extend C18 2.1 x 50mm (USWEX10038)	A1:01: Phosphate buffer 10mM pH 3.5	B1: Acetonitrile	Gradient 1	50.0	3.5-7.0
10	☐	Injection0010.m	Zorbax Extend C18 2.1 x 50mm (USWEX10038)	A1:01: Phosphate buffer 10mM pH 3.5	B1: Acetonitrile	Gradient 2	50.0	3.5-7.0
11	☐	Injection0011.m	Zorbax Extend C18 2.1 x 50mm (USWEX10038)	A1:01: Phosphate buffer 10mM pH 3.5	B1: Acetonitrile	Gradient 1	55.0	3.5-7.0
12	☐	Injection0012.m	Zorbax Extend C18 2.1 x 50mm (USWEX10038)	A1:01: Phosphate buffer 10mM pH 3.5	B1: Acetonitrile	Gradient 2	55.0	3.5-7.0
13	☑	Injection0013.m	Zorbax SB CN 2.1 x 50mm (USSQF01009)	A1:01: Phosphate buffer 10mM pH 3.5	B1: Acetonitrile	Gradient 1	50.0	3.5-7.0
14	☑	Injection0014.m	Zorbax SB CN 2.1 x 50mm (USSQF01009)	A1:01: Phosphate buffer 10mM pH 3.5	B1: Acetonitrile	Gradient 2	50.0	3.5-7.0
15	☑	Injection0015.m	Zorbax SB CN 2.1 x 50mm (USSQF01009)	A1:01: Phosphate buffer 10mM pH 3.5	B1: Acetonitrile	Gradient 1	55.0	3.5-7.0
16	☑	Injection0016.m	Zorbax SB CN 2.1 x 50mm (USSQF01009)	A1:01: Phosphate buffer 10mM pH 3.5	B1: Acetonitrile	Gradient 2	55.0	3.5-7.0
17	☑	Injection0017.m	Zorbax Eclipse plus PheHex 2.1 x 50mm (USFAR00786)	A1:01: Phosphate buffer 10mM pH 3.5	B1: Acetonitrile	Gradient 1	50.0	3.5-7.0

Defining System Volumes



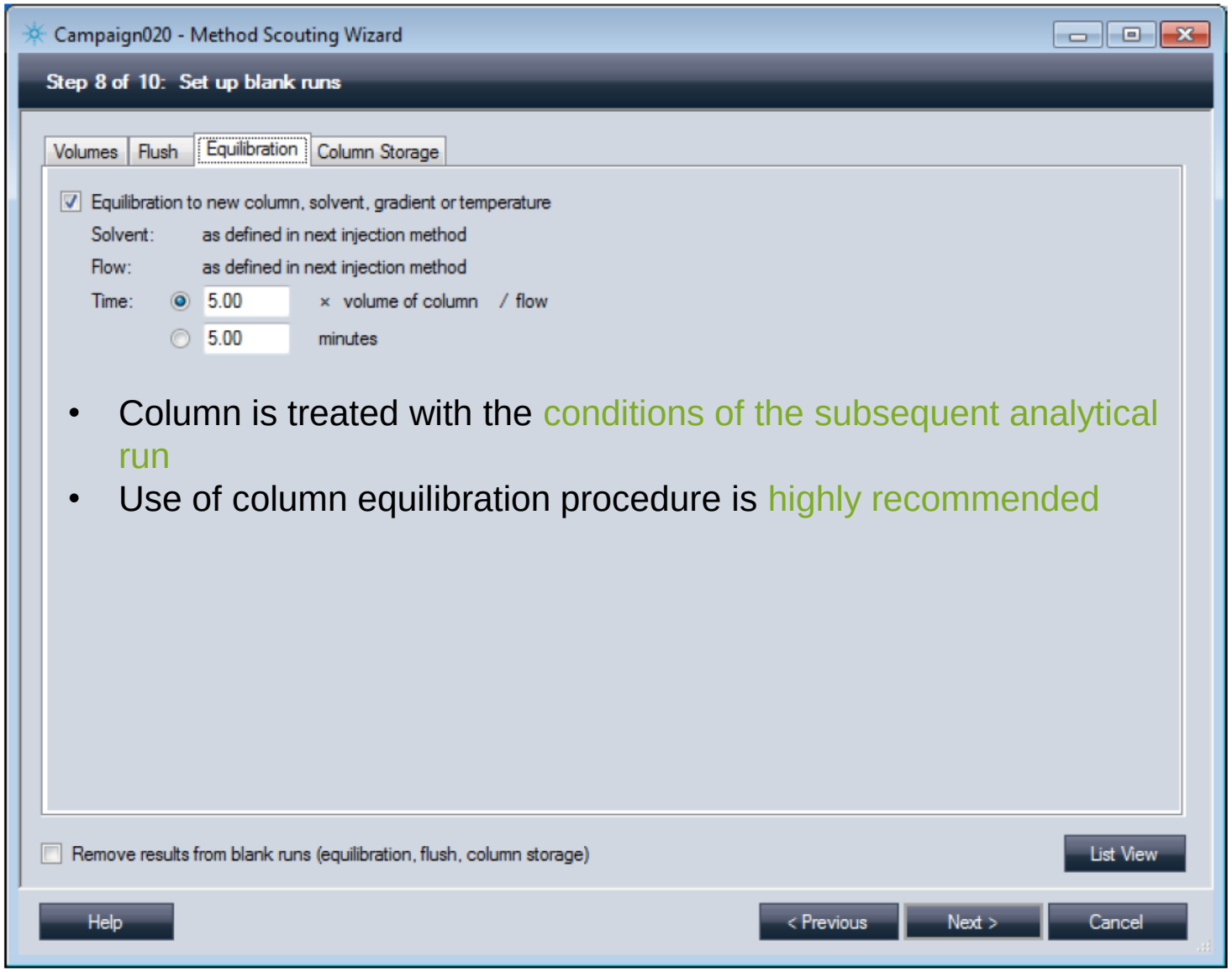
- Degasser could be placed before or after the solvent selection valve
- Different system diagrams will be shown depending on configuration
- Recommended internal volumes are available

Column Flushing



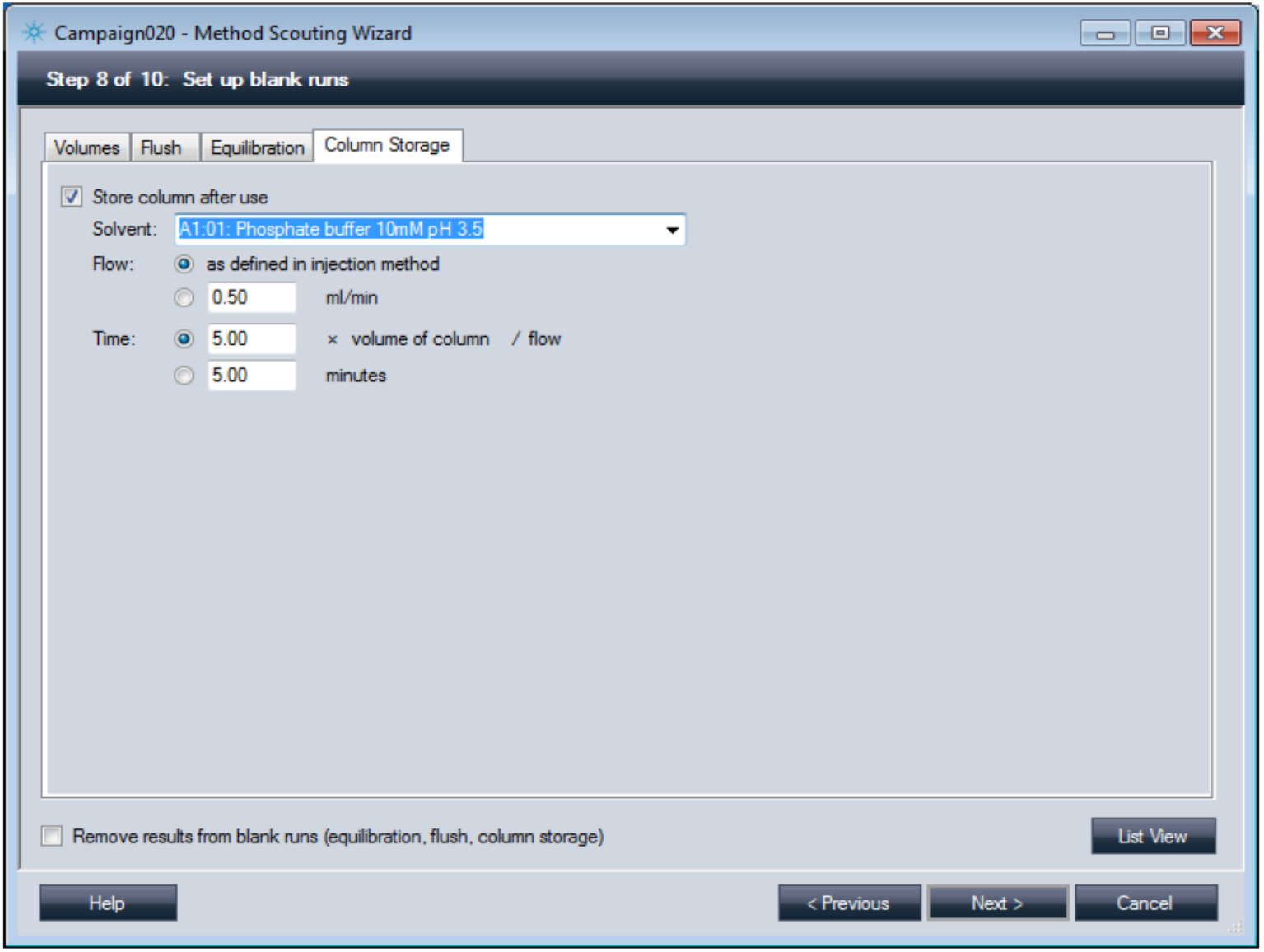
- Change solvent type for subsequent analysis or column storage
- Manually assign for eluent flow to go into waste, bypass or column

Column Equilibration

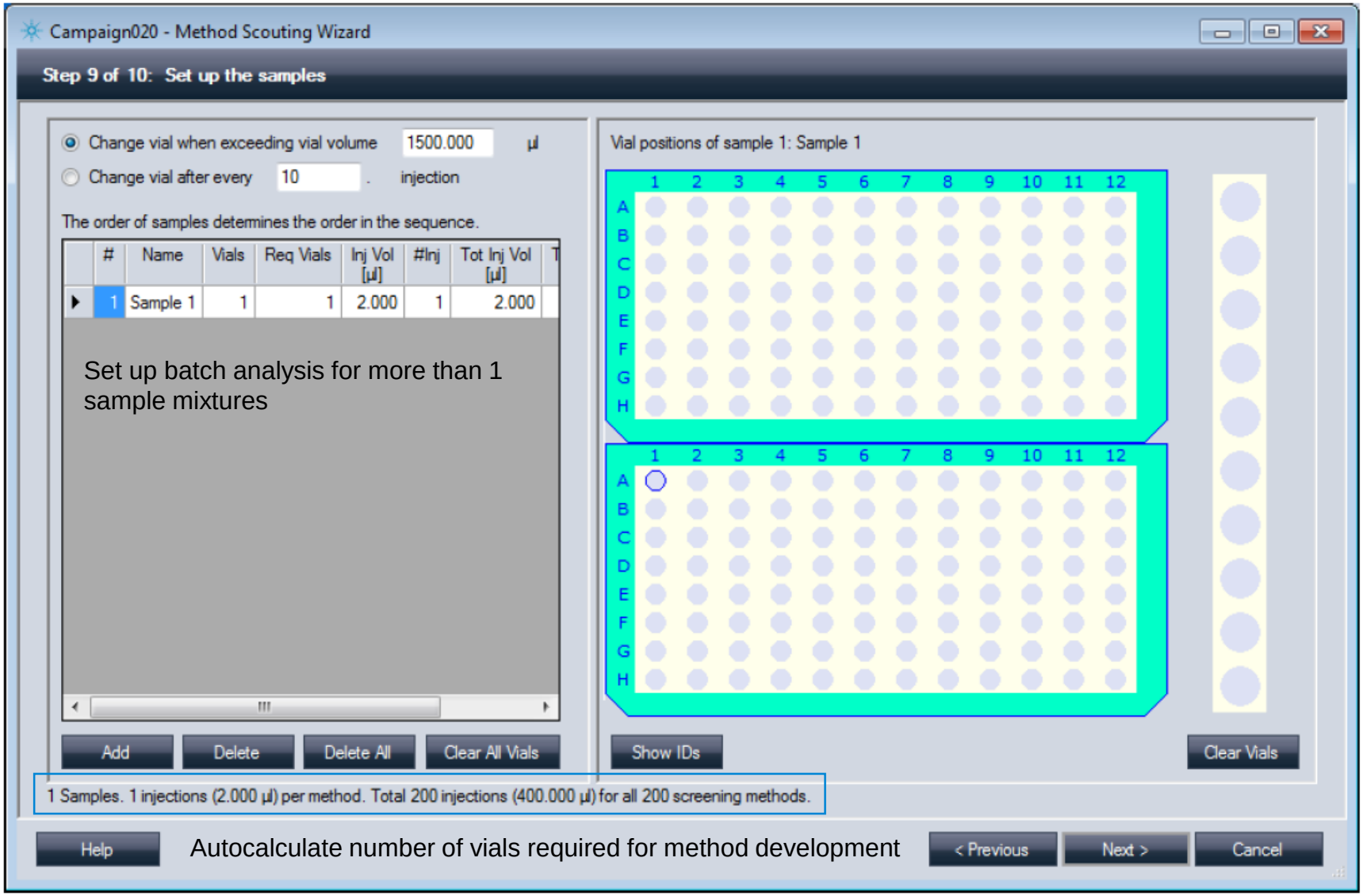


- Column is treated with the conditions of the subsequent analytical run
- Use of column equilibration procedure is highly recommended

Column Storage



Set Up Sample Queue



Method Development Summary

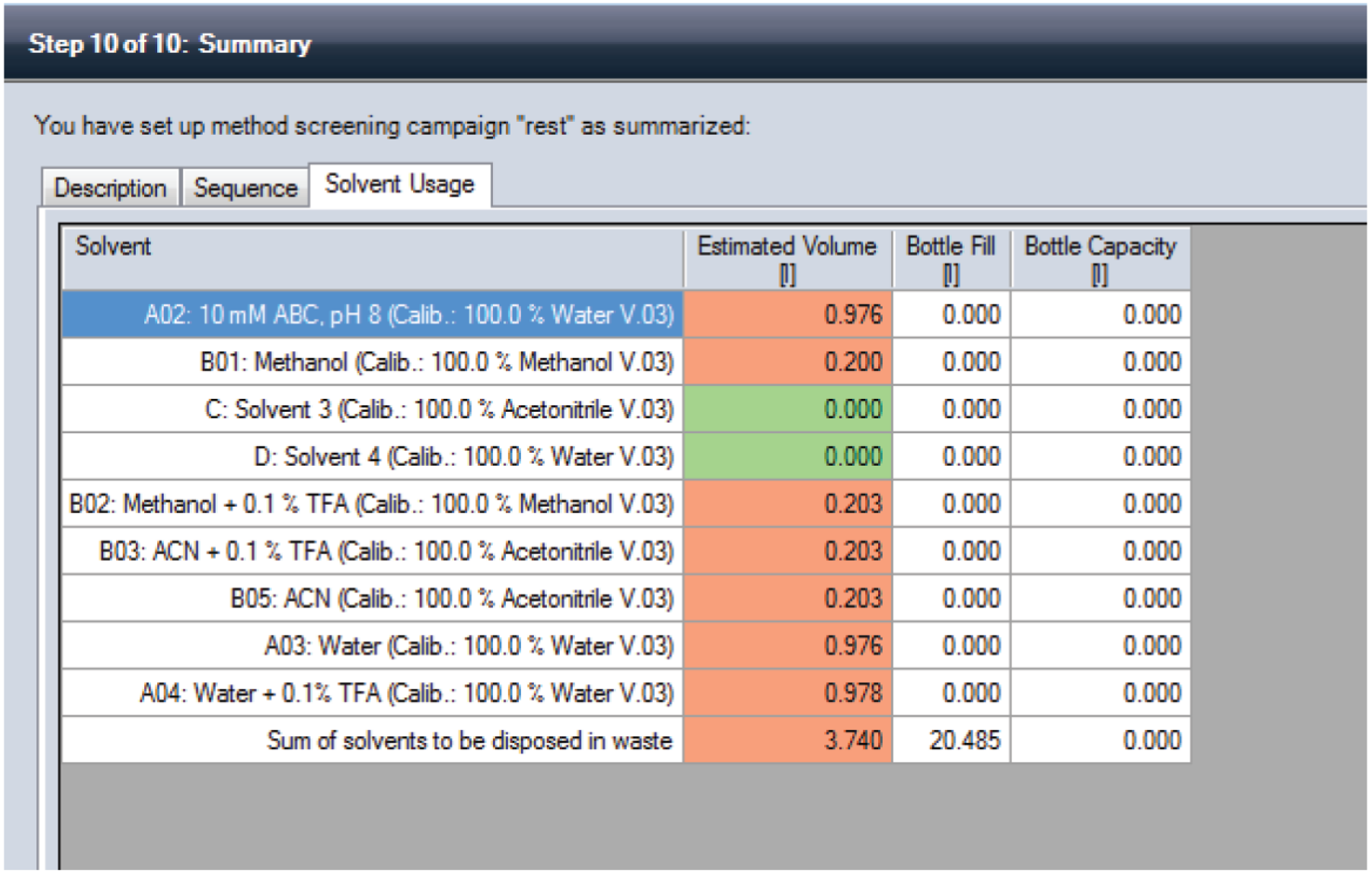
Step 10 of 10: Summary

You have set up method screening campaign "rest" as summarized:

Description | Sequence | Solvent Usage

#	Sample	Inj	Method	Type	Flow [ml/min]	Run Time [min]	Post Time [min]	Vial	Column	Solvent(s)	Gradient	Temp [°C]
1			FlushBypass0001.m	Flush	3.000	7.50	0.00		Bypass	50.0 % A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03), 50.0 % B01: Methanol (Calib.: 100.0 % Methanol V.03)		
2			FlushBypass0002.m	Flush	3.000	0.50	0.00		Bypass	95.0 % A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03), 5.0 % B01: Methanol (Calib.: 100.0 % Methanol V.03), 0.0 % C: Solvent 3 (Calib.: 100.0 % Acetonitrile V.03), 0.0 % D: Solvent 4 (Calib.: 100.0 % Water V.03)		
3			Equilibration0001.m	Equilbration	1.000	0.52	0.00		ZORBAX SB-C18	95.0 % A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03), 5.0 % B01: Methanol (Calib.: 100.0 % Methanol V.03), 0.0 % C: Solvent 3 (Calib.: 100.0 % Acetonitrile V.03), 0.0 % D: Solvent 4 (Calib.: 100.0 % Water V.03)		30.0
4	Sample 1	1	Injection0001.m	Injection	1.000	2.50	2.00	D1B-D1	ZORBAX SB-C18	95.0 % A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03), 5.0 % B01: Methanol (Calib.: 100.0 % Methanol V.03), 0.0 % C: Solvent 3 (Calib.: 100.0 % Acetonitrile V.03), 0.0 % D: Solvent 4 (Calib.: 100.0 % Water V.03)	Gradient 1	30.0
5			Equilibration0002.m	Equilbration	1.000	2.02	0.00		ZORBAX SB-C18	95.0 % A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03), 5.0 % B01: Methanol (Calib.: 100.0 % Methanol V.03), 0.0 % C: Solvent 3 (Calib.: 100.0 % Acetonitrile V.03), 0.0 % D: Solvent 4 (Calib.: 100.0 % Water V.03)		40.0
6	Sample 1	1	Injection0002.m	Injection	1.000	2.50	2.00	D1B-D1	ZORBAX SB-C18	95.0 % A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03), 5.0 % B01: Methanol (Calib.: 100.0 % Methanol V.03), 0.0 % C: Solvent 3 (Calib.: 100.0 % Acetonitrile V.03), 0.0 % D: Solvent 4 (Calib.: 100.0 % Water V.03)	Gradient 1	40.0
7			Equilibration0003.m	Equilbration	1.000	2.02	0.00		ZORBAX SB-C18	95.0 % A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03), 5.0 % B01: Methanol (Calib.: 100.0 % Methanol V.03), 0.0 % C: Solvent 3 (Calib.: 100.0 % Acetonitrile V.03), 0.0 % D: Solvent 4 (Calib.: 100.0 % Water V.03)		50.0
8	Sample 1	1	Injection0003.m	Injection	1.000	2.50	2.00	D1B-D1	ZORBAX SB-C18	95.0 % A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03), 5.0 % B01: Methanol (Calib.: 100.0 % Methanol V.03), 0.0 % C: Solvent 3 (Calib.: 100.0 % Acetonitrile V.03), 0.0 % D: Solvent 4 (Calib.: 100.0 % Water V.03)	Gradient 1	50.0
9			Equilibration0004.m	Equilbration	1.000	2.02	0.00		ZORBAX SB-C18	95.0 % A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03), 5.0 % B01: Methanol (Calib.: 100.0 % Methanol V.03), 0.0 % C: Solvent 3 (Calib.: 100.0 % Acetonitrile V.03), 0.0 % D: Solvent 4 (Calib.: 100.0 % Water V.03)		60.0
10	Sample 1	1	Injection0004.m	Injection	1.000	2.50	2.00	D1B-D1	ZORBAX SB-C18	95.0 % A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03), 5.0 % B01: Methanol (Calib.: 100.0 % Methanol V.03), 0.0 % C: Solvent 3 (Calib.: 100.0 % Acetonitrile V.03), 0.0 % D: Solvent 4 (Calib.: 100.0 % Water V.03)	Gradient 1	60.0
11			Storage0001.m	Column storage	1.000	1.81	0.00		ZORBAX SB-C18	95.0 % A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03), 5.0 % B01: Methanol (Calib.: 100.0 % Methanol V.03), 0.0 % C: Solvent 3 (Calib.: 100.0 % Acetonitrile V.03), 0.0 % D: Solvent 4 (Calib.: 100.0 % Water V.03)		
12			Equilibration0005.m	Equilbration	1.000	2.02	0.00		Eclipse Plus C18	95.0 % A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03), 5.0 % B01: Methanol (Calib.: 100.0 % Methanol V.03), 0.0 % C: Solvent 3 (Calib.: 100.0 % Acetonitrile V.03), 0.0 % D: Solvent 4 (Calib.: 100.0 % Water V.03)		30.0
13	Sample 1	1	Injection0005.m	Injection	1.000	2.50	2.00	D1B-D1	Eclipse Plus C18	95.0 % A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03), 5.0 % B01: Methanol (Calib.: 100.0 % Methanol V.03), 0.0 % C: Solvent 3 (Calib.: 100.0 % Acetonitrile V.03), 0.0 % D: Solvent 4 (Calib.: 100.0 % Water V.03)	Gradient 1	30.0
14			Equilibration0006.m	Equilbration	1.000	2.02	0.00		Eclipse Plus C18	95.0 % A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03), 5.0 % B01: Methanol (Calib.: 100.0 % Methanol V.03), 0.0 % C: Solvent 3 (Calib.: 100.0 % Acetonitrile V.03), 0.0 % D: Solvent 4 (Calib.: 100.0 % Water V.03)		40.0
15	Sample 1	1	Injection0006.m	Injection	1.000	2.50	2.00	D1B-D1	Eclipse Plus C18	95.0 % A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03), 5.0 % B01: Methanol (Calib.: 100.0 % Methanol V.03), 0.0 % C: Solvent 3 (Calib.: 100.0 % Acetonitrile V.03), 0.0 % D: Solvent 4 (Calib.: 100.0 % Water V.03)	Gradient 1	40.0
16			Equilibration0007.m	Equilbration	1.000	2.02	0.00		Eclipse Plus C18	95.0 % A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03), 5.0 % B01: Methanol (Calib.: 100.0 % Methanol V.03), 0.0 % C: Solvent 3 (Calib.: 100.0 % Acetonitrile V.03), 0.0 % D: Solvent 4 (Calib.: 100.0 % Water V.03)		50.0
17	Sample 1	1	Injection0007.m	Injection	1.000	2.50	2.00	D1B-D1	Eclipse Plus C18	95.0 % A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03), 5.0 % B01: Methanol (Calib.: 100.0 % Methanol V.03), 0.0 % C: Solvent 3 (Calib.: 100.0 % Acetonitrile V.03), 0.0 % D: Solvent 4 (Calib.: 100.0 % Water V.03)	Gradient 1	50.0
18			Equilibration0008.m	Equilbration	1.000	2.02	0.00		Eclipse Plus C18	95.0 % A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03), 5.0 % B01: Methanol (Calib.: 100.0 % Methanol V.03), 0.0 % C: Solvent 3 (Calib.: 100.0 % Acetonitrile V.03), 0.0 % D: Solvent 4 (Calib.: 100.0 % Water V.03)		60.0
19	Sample 1	1	Injection0008.m	Injection	1.000	2.50	2.00	D1B-D1	Eclipse Plus C18	95.0 % A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03), 5.0 % B01: Methanol (Calib.: 100.0 % Methanol V.03), 0.0 % C: Solvent 3 (Calib.: 100.0 % Acetonitrile V.03), 0.0 % D: Solvent 4 (Calib.: 100.0 % Water V.03)	Gradient 1	60.0
20			Storage0002.m	Column storage	1.000	1.81	0.00		Eclipse Plus C18	95.0 % A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03), 5.0 % B01: Methanol (Calib.: 100.0 % Methanol V.03), 0.0 % C: Solvent 3 (Calib.: 100.0 % Acetonitrile V.03), 0.0 % D: Solvent 4 (Calib.: 100.0 % Water V.03)		
21			Equilibration0009.m	Equilbration	1.000	2.02	0.00		Eclipse Plus C8	95.0 % A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03), 5.0 % B01: Methanol (Calib.: 100.0 % Methanol V.03), 0.0 % C: Solvent 3 (Calib.: 100.0 % Acetonitrile V.03), 0.0 % D: Solvent 4 (Calib.: 100.0 % Water V.03)		30.0
22	Sample 1	1	Injection0009.m	Injection	1.000	2.50	2.00	D1B-D1	Eclipse Plus C8	95.0 % A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03), 5.0 % B01: Methanol (Calib.: 100.0 % Methanol V.03), 0.0 % C: Solvent 3 (Calib.: 100.0 % Acetonitrile V.03), 0.0 % D: Solvent 4 (Calib.: 100.0 % Water V.03)	Gradient 1	30.0
23			Equilibration0010.m	Equilbration	1.000	2.02	0.00		Eclipse Plus C8	95.0 % A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03), 5.0 % B01: Methanol (Calib.: 100.0 % Methanol V.03), 0.0 % C: Solvent 3 (Calib.: 100.0 % Acetonitrile V.03), 0.0 % D: Solvent 4 (Calib.: 100.0 % Water V.03)		40.0
24	Sample 1	1	Injection0010.m	Injection	1.000	2.50	2.00	D1B-D1	Eclipse Plus C8	95.0 % A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03), 5.0 % B01: Methanol (Calib.: 100.0 % Methanol V.03), 0.0 % C: Solvent 3 (Calib.: 100.0 % Acetonitrile V.03), 0.0 % D: Solvent 4 (Calib.: 100.0 % Water V.03)	Gradient 1	40.0
25			Equilibration0011.m	Equilbration	1.000	2.02	0.00		Eclipse Plus C8	95.0 % A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03), 5.0 % B01: Methanol (Calib.: 100.0 % Methanol V.03), 0.0 % C: Solvent 3 (Calib.: 100.0 % Acetonitrile V.03), 0.0 % D: Solvent 4 (Calib.: 100.0 % Water V.03)		50.0
26	Sample 1	1	Injection0011.m	Injection	1.000	2.50	2.00	D1B-D1	Eclipse Plus C8	95.0 % A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03), 5.0 % B01: Methanol (Calib.: 100.0 % Methanol V.03), 0.0 % C: Solvent 3 (Calib.: 100.0 % Acetonitrile V.03), 0.0 % D: Solvent 4 (Calib.: 100.0 % Water V.03)	Gradient 1	50.0
27			Equilibration0012.m	Equilbration	1.000	2.02	0.00		Eclipse Plus C8			60.0
28	Sample 1	1	Injection0012.m	Injection	1.000	2.50	2.00	D1B-D1	Eclipse Plus C8	95.0 % A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03), 5.0 % B01: Methanol (Calib.: 100.0 % Methanol V.03), 0.0 % C: Solvent 3 (Calib.: 100.0 % Acetonitrile V.03), 0.0 % D: Solvent 4 (Calib.: 100.0 % Water V.03)	Gradient 1	60.0
29			Storage0003.m	Column storage	1.000	1.81	0.00		Eclipse Plus C8	95.0 % A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03), 5.0 % B01: Methanol (Calib.: 100.0 % Methanol V.03), 0.0 % C: Solvent 3 (Calib.: 100.0 % Acetonitrile V.03), 0.0 % D: Solvent 4 (Calib.: 100.0 % Water V.03)		
30			Equilibration0013.m	Equilbration	1.000	2.02	0.00		Extend C18	95.0 % A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03), 5.0 % B01: Methanol (Calib.: 100.0 % Methanol V.03), 0.0 % C: Solvent 3 (Calib.: 100.0 % Acetonitrile V.03), 0.0 % D: Solvent 4 (Calib.: 100.0 % Water V.03)		30.0
31	Sample 1	1	Injection0013.m	Injection	1.000	2.50	2.00	D1B-D1	Extend C18	95.0 % A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03), 5.0 % B01: Methanol (Calib.: 100.0 % Methanol V.03), 0.0 % C: Solvent 3 (Calib.: 100.0 % Acetonitrile V.03), 0.0 % D: Solvent 4 (Calib.: 100.0 % Water V.03)	Gradient 1	30.0
32			Equilibration0014.m	Equilbration	1.000	2.02	0.00		Extend C18	95.0 % A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03), 5.0 % B01: Methanol (Calib.: 100.0 % Methanol V.03), 0.0 % C: Solvent 3 (Calib.: 100.0 % Acetonitrile V.03), 0.0 % D: Solvent 4 (Calib.: 100.0 % Water V.03)		40.0
33	Sample 1	1	Injection0014.m	Injection	1.000	2.50	2.00	D1B-D1	Extend C18	95.0 % A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03), 5.0 % B01: Methanol (Calib.: 100.0 % Methanol V.03), 0.0 % C: Solvent 3 (Calib.: 100.0 % Acetonitrile V.03), 0.0 % D: Solvent 4 (Calib.: 100.0 % Water V.03)	Gradient 1	40.0
34			Equilibration0015.m	Equilbration	1.000	2.02	0.00		Extend C18	95.0 % A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03), 5.0 % B01: Methanol (Calib.: 100.0 % Methanol V.03), 0.0 % C: Solvent 3 (Calib.: 100.0 % Acetonitrile V.03), 0.0 % D: Solvent 4 (Calib.: 100.0 % Water V.03)		50.0

Method Development Summary



Step 10 of 10: Summary

You have set up method screening campaign "rest" as summarized:

Description | Sequence | Solvent Usage

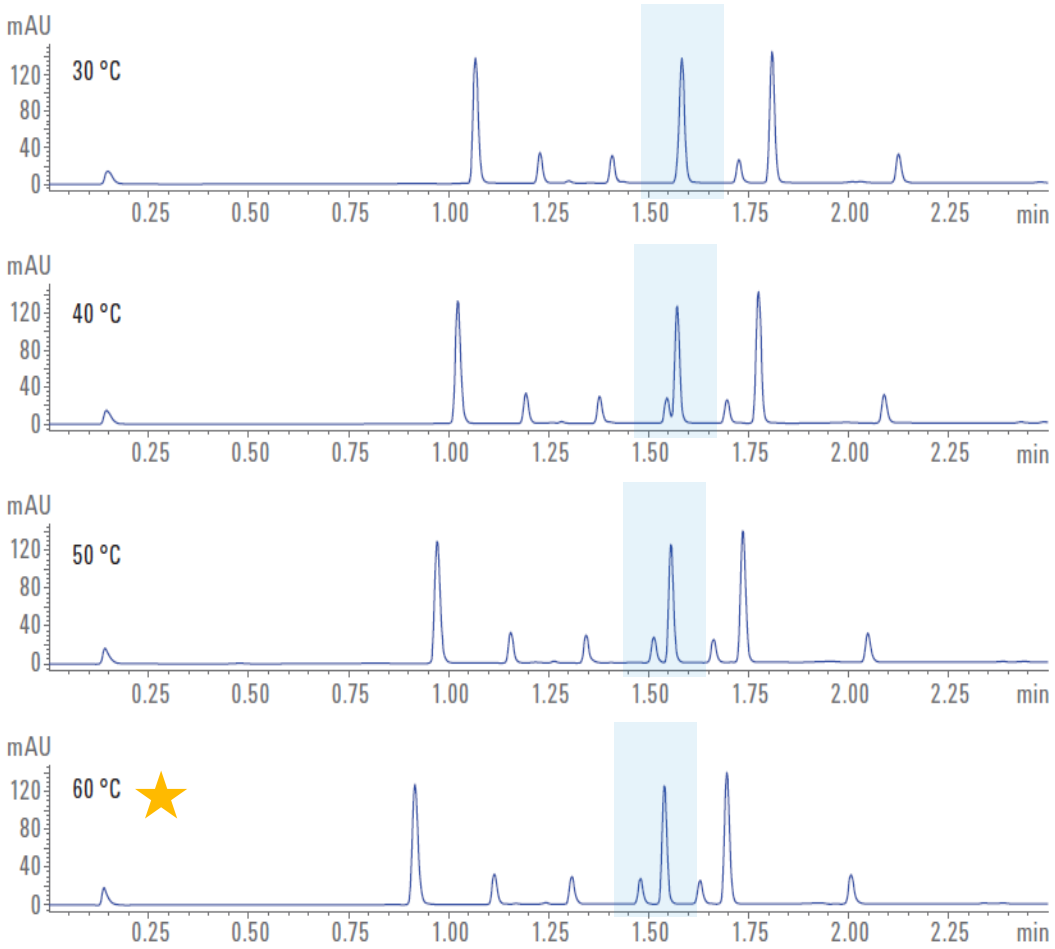
Solvent	Estimated Volume [l]	Bottle Fill [l]	Bottle Capacity [l]
A02: 10 mM ABC, pH 8 (Calib.: 100.0 % Water V.03)	0.976	0.000	0.000
B01: Methanol (Calib.: 100.0 % Methanol V.03)	0.200	0.000	0.000
C: Solvent 3 (Calib.: 100.0 % Acetonitrile V.03)	0.000	0.000	0.000
D: Solvent 4 (Calib.: 100.0 % Water V.03)	0.000	0.000	0.000
B02: Methanol + 0.1 % TFA (Calib.: 100.0 % Methanol V.03)	0.203	0.000	0.000
B03: ACN + 0.1 % TFA (Calib.: 100.0 % Acetonitrile V.03)	0.203	0.000	0.000
B05: ACN (Calib.: 100.0 % Acetonitrile V.03)	0.203	0.000	0.000
A03: Water (Calib.: 100.0 % Water V.03)	0.976	0.000	0.000
A04: Water + 0.1% TFA (Calib.: 100.0 % Water V.03)	0.978	0.000	0.000
Sum of solvents to be disposed in waste	3.740	20.485	0.000

Effect of Column on Selectivity

- Agilent ZORBAX SB-C18 and Agilent ZORBAX Eclipse Plus C8 columns showed the best separation
- Separation on Agilent ZORBAX SB-CN and Agilent ZORBAX Eclipse Plus Phenyl-Hexyl columns are not promising

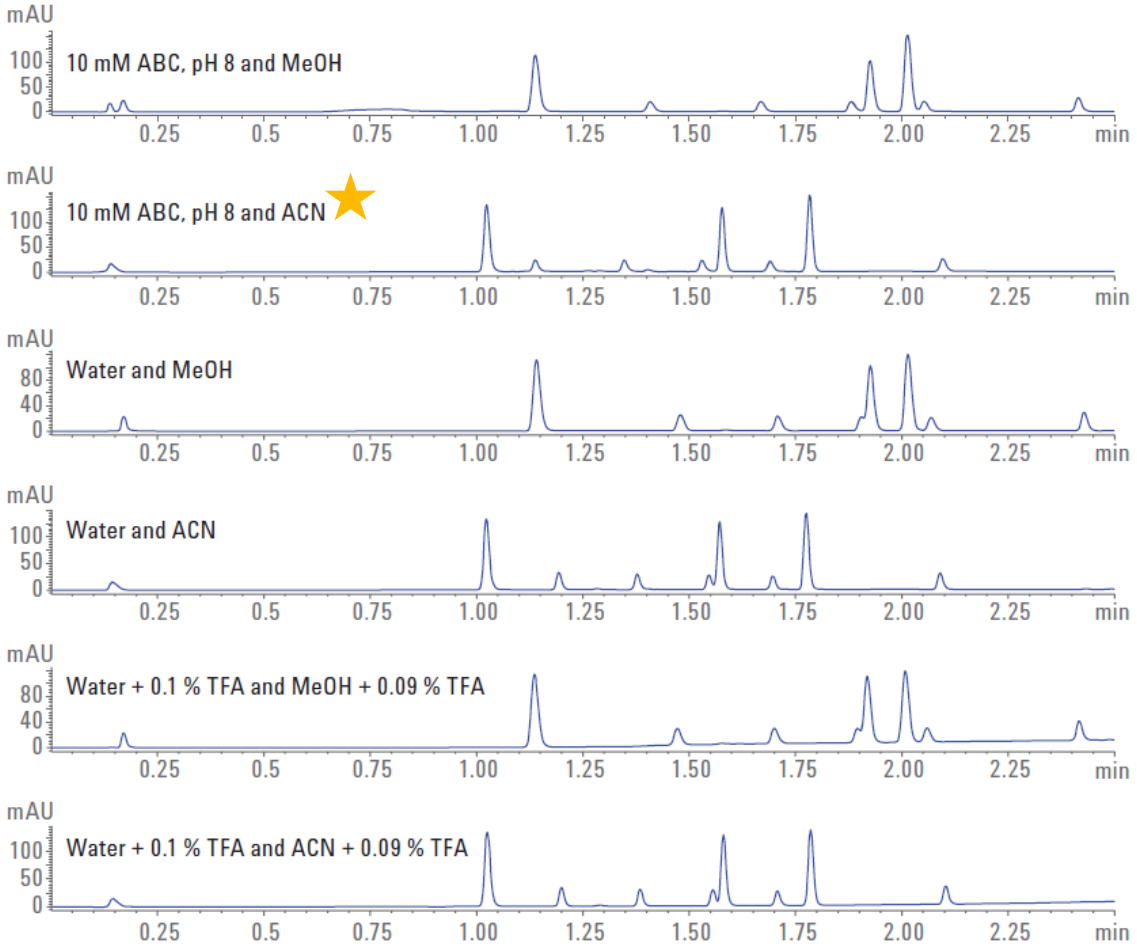
Effect of Temperature on Selectivity

- Temperature scouting on Agilent ZORBAX Eclipse Plus C18 with water and acetonitrile as mobile phase A and B
- 60 ^{0}C delivered best resolution



Effect of Solvent Selectivity

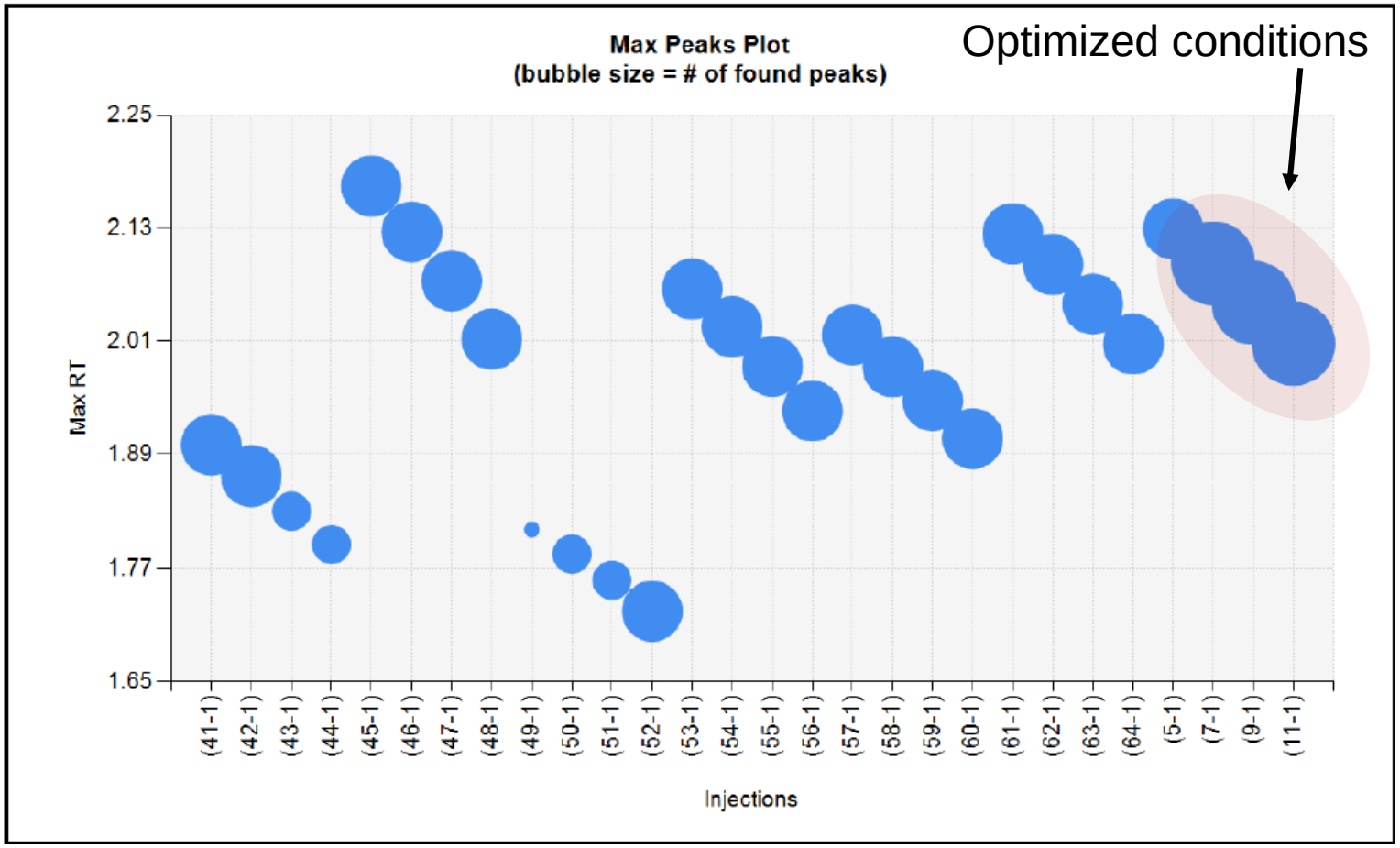
- Solvent scouting on Agilent ZORBAX Eclipse Plus C18 at 60 ºC
- 10 mM Ammonium Bicarbonate Buffer and Acetonitrile delivered the best resolution



Review Results

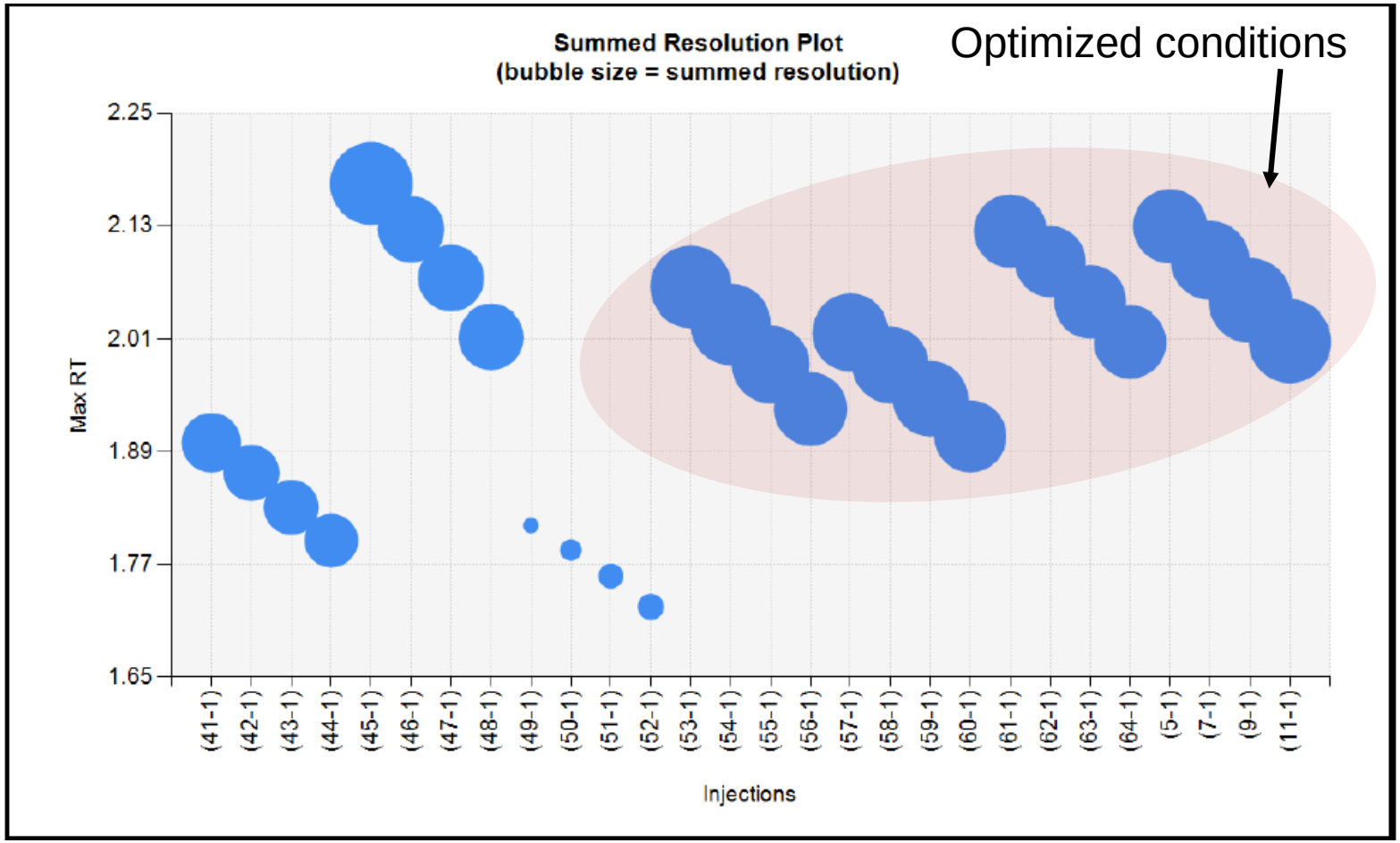
Intelligent Reporting – Max Peaks Plot

Comparison of different columns and temperature using water (A) and acetonitrile (B)



Review Results

Intelligent Reporting – Summed Resolution Plot



Application Note

5991-5934EN

Automated Scouting of Stationary and Mobile Phases Using the Agilent 1290 Infinity II Method Development Solution

Technical Overview

Authors

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Abstract

This Technical Overview demonstrates the use of the Agilent 1290 Infinity II Method Development Solution for automated scouting of stationary and mobile phases. The solution is equipped with an Agilent 1290 Infinity II Multicolumn Thermostat that enables automated switching between up to eight columns. The Agilent 1290 Infinity II Flexible Pump is clustered with two solvent-selection valves for the connection of up to 26 different solvents. The Agilent Method Scouting Wizard of the Agilent OpenLAB CDS ChemStation Edition Software facilitates easy setup of methods for different combinations of columns, mobile phases, and temperatures in a single sequence.

Agilent Technologies


THANK
YOU

Appendix

Agilent Application Notes

Method Transfer by ISET

- Fast screening of mobile and stationary phases with the Agilent 1290 Infinity LC and seamless method transfer to an Agilent 1200 Series LC using ISET

  Agilent Application Note 5991-0989EN

- Developing faster methods for generic drugs within USP <621>allowed limits

  Agilent Application Note 5991-0278EN

- Effective use of pharmacopeia guidelines to reduce cost of chromatographic analysis

  Agilent Application Note 5991-1053EN

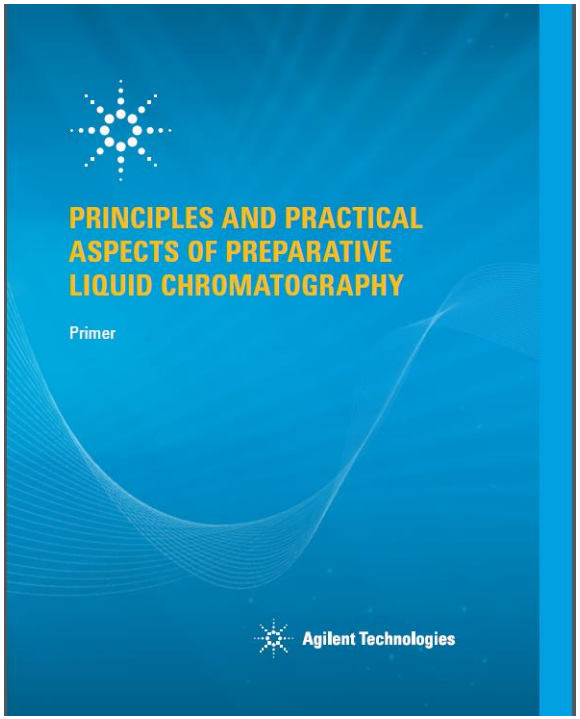
- Developing faster methods for generic drugs within EP 2.2.46E allowed limits

  Agilent Application Note 5991-0394EN

Agilent Application Notes
QbD based Method Development

- Quality-by-Design Approach to Stability Indicating Method Development for Linagliptin Drug Product
  - Agilent Application Note 5991-3834EN
- Automated QbD Based Method Development and Validation of Oxidative Degraded Atorvastatin
  - Agilent Application Note 5991-4944EN
- Development of an UHPLC Method for Azithromycin Tablets Using ChromSword Auto Software
  - Agilent Application Note 5991-5428EN
- QbD Based Method Development on an Agilent 1290 Infinity UHPLC system Combined with a Seamless Method Transfer to HPLC Using Intelligent System Emulation Technology
  - Agilent Application Note 5991-5701EN

Learn More About The Practical Aspects How To Measure Dwell Volumes And To Transfer & Optimize Methods



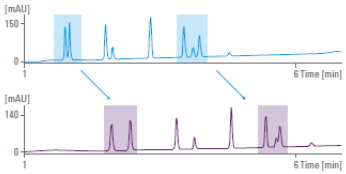


Figure 3.46 The effects of system dwell volume on separation efficiency. The upper chromatogram was obtained using a system with low dwell volume. In contrast, the lower chromatogram was obtained on a system with large dwell volume.

3.7.1.1 What constitutes optimized system setup?

A preparative LC system with appropriate dimensions can be used for both analytical scouting runs on 4.6 mm-id columns as well as for purification on a 50 mm id preparative column. In this case, the system dwell volume must be as small as possible to obtain good results when working in gradient mode.

As a general rule – system optimization is achieved when the ratio between dwell volume and column void volume is equal or less than one. Further, the ratio of the total system void volume to the applied flow rate should be equal to or less than 2:1 to obtain reasonable chromatographic performance.

Larger ratios extend the length of the chromatogram and will reduce chromatographic performance as shown in Figure 3.46. The volume of capillaries, mixer and injection loop have a strong impact on the dwell volume. Hence, it is important to keep loop sizes as small as possible or use two different loops and flow paths. Further, the correct capillary diameter for the applied flow rate must be used to reduce systems void volumes and as a consequence the peak dispersion.

3.7.1.2 What is the impact of capillary length and inside diameter?

The inside diameters of the capillaries have to be synchronized with the flow rate, see Table 3.2.

For most semi-preparative applications using a flow range between 10 and 100 mL/min, standard 1/16-inch capillaries of 0.02 or 0.03-inch id can be used.

46

5.2 Determining the system dwell volume

5.2.1 Determining the dwell volume of analytical systems

Two different methods for determination of the system dwell volume are available. For systems capable of delivering highly accurate analytical flow rates such as 1 mL/min, a method with a linear gradient profile delivers more accurate results than a step method. The method can also be used to measure the column void volume.

Use the following procedure to determine the dwell volume of analytical systems capable of delivering accurate flow rates.

1. Prepare solvent A: 100 % water
2. Prepare solvent B: 99 % acetonitrile with 1 % acetone as tracer
3. Prime the system with the solvents A and B.
4. Set the detection wavelength to 263 nm.
5. Replace the column by a low dead volume restriction (maintaining a backpressure of 50 bar).
6. Run a linear gradient from 0 to 10 minutes with 5 to 95 %B at a flow rate of 1 mL/min.

Note that when using preparative sample loops with volumes larger than 1 mL, the flow rate needs to be increased to finish the dwell volume determination in a reasonable time. A good compromise is to keep the flow rate equal to the loop size.

7. Determine the difference in time (t_{dwell}) between the programmed and actual elution time of the gradient at 50 % of the composition.
8. v_{dwell} can be calculated from $v_{dwell} = t_{dwell} \times f$

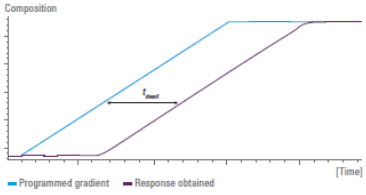


Figure 5.3 System dwell volume determination for analytical systems.

67

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This information is subject to change without notice.

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5991-2358EN

How To Measure Dwell Volumes

- Add a UV active tracer to solvent B (often acetone)
- Remove the column, fit a restriction capillary
- Run a step gradient from 10%B to 90%B
- Calculate the dwell volume by the delay between UV response and the step with respect to the flow rate