

Neueste LC Entwicklungen

2D-LC, High Dynamic Range
Detektion, quaternäre Pumpe
mit 1200 bar

Sebastian Krahe
Produktspezialist LC
Agilent Technologies,
Deutschland/Österreich
2. Oktober 2012 Hamburg

Agilent 1290 Infinity 2D-LC Solution

A new flexible and user-friendly 2D-LC solution for the most complex samples



What is 2D-LC?

2D-LC: Injecting the effluent or a part of the effluent of one column to a second column, ideally with orthogonal separation behavior.

Purpose: increase total separation power.

Peak capacities **multiply** for orthogonal separation mechanisms!

Two different modes:

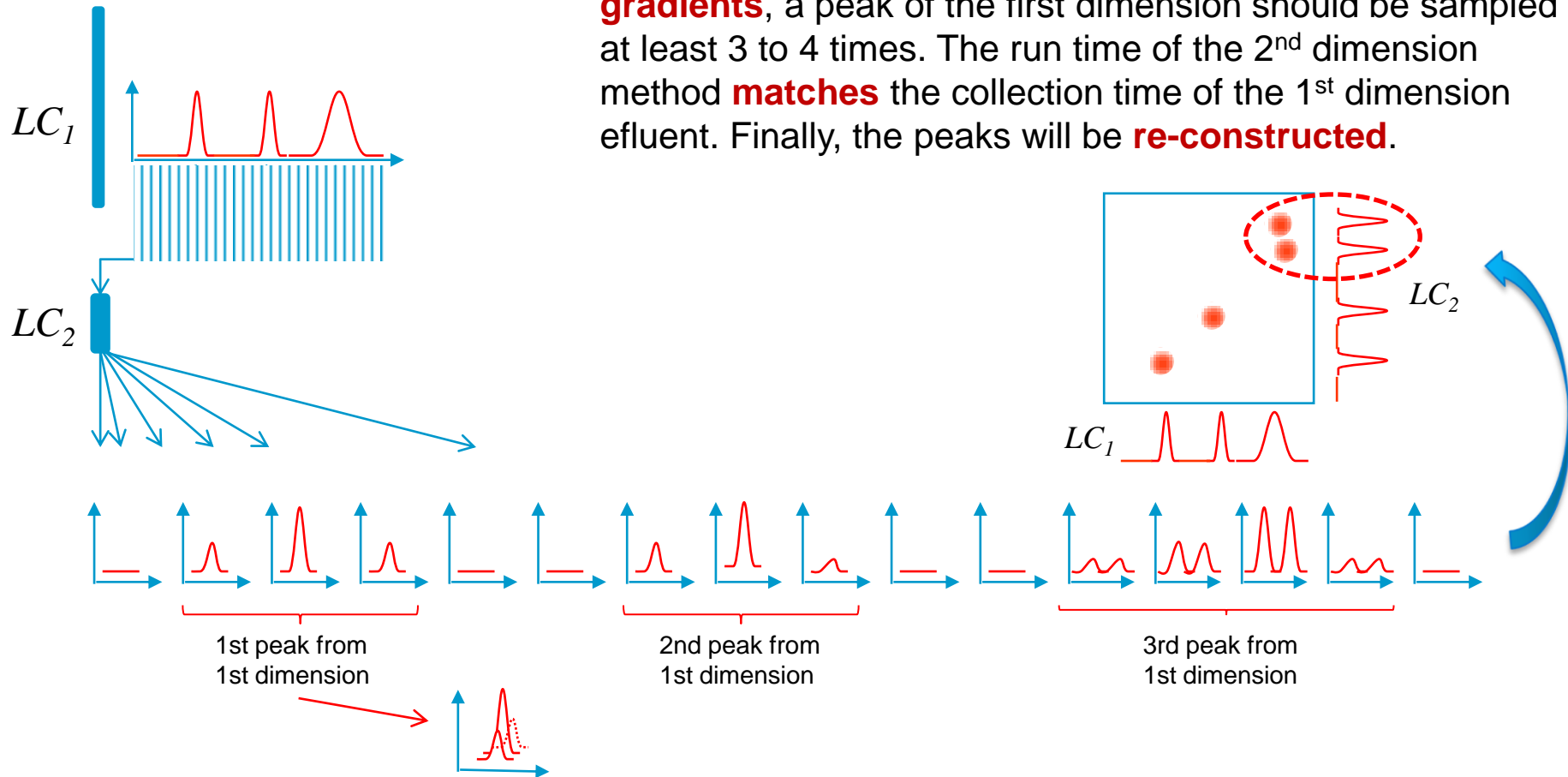
Comprehensive 2D-LC (“LCxLC”)

Heart-cutting 2D-LC (“LC-LC”)

2D-LC - Difference between comprehensive 2D-LC and heart-cutting 2D-LC

Comprehensive 2D-LC (LCxLC):

The **complete effluent** of the first column will be injected to the second column and will be analyzed with **very fast gradients**, a peak of the first dimension should be sampled at least 3 to 4 times. The run time of the 2nd dimension method **matches** the collection time of the 1st dimension effluent. Finally, the peaks will be **re-constructed**.



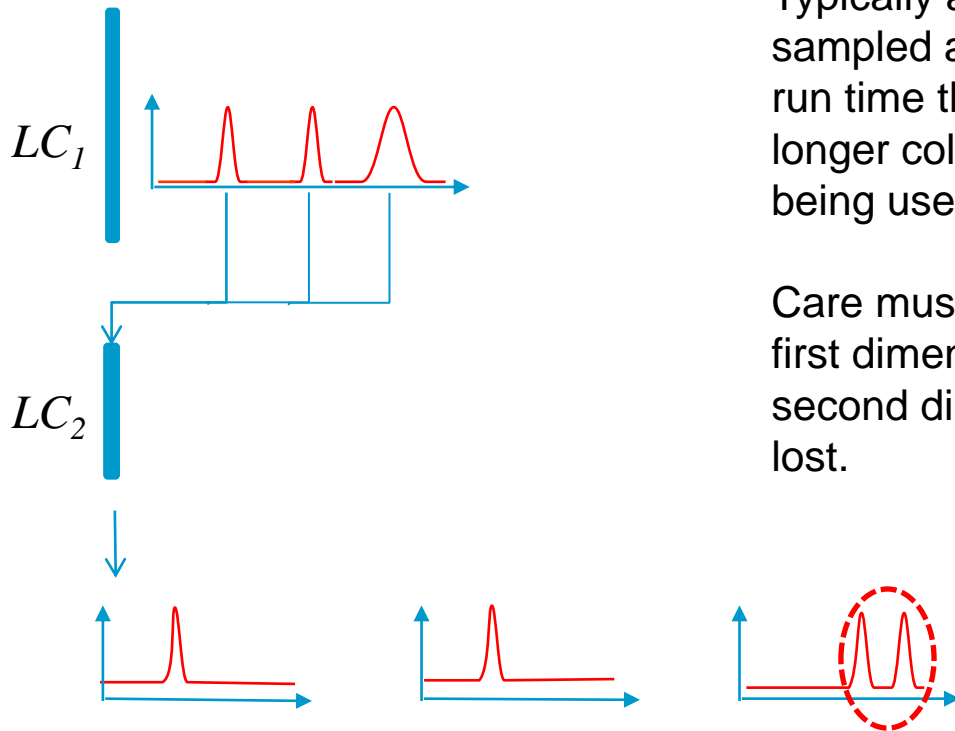
2D-LC - Difference between comprehensive 2D-LC and heart-cutting 2D-LC

Heart-cutting 2D-LC (LC-LC):

Only **parts** of the effluent of the first column – the peaks eluted from the 1st dimension column - will be injected to the second column.

Typically a peak from the first dimension will be sampled as a whole and a gradient with a **longer** run time than the collection time will be used. Also longer columns with higher separation efficiency are being used in as 2nd dimension column.

Care must be taken if peaks are eluting from the first dimension column when a gradient on the second dimension is still running – this peak will be lost.



Hardware – Module-flexibility

1. Dimension



2. Dimension



1290 Infinity Binary Pump

One or two 1290 Infinity TCC

1290 Infinity Binary Pump

1290 Infinity Autosampler or 1260 HiP Autosampler

Optional 1260/1290 Infinity Detector

Optional 1260/1290 Infinity Detector



1260/1290 Infinity detector

↓
For 1st dimension chromatogram and peak-triggering

↓
To monitor waste-line

1260 Infinity Capillary Pump

1260 Infinity Autosampler



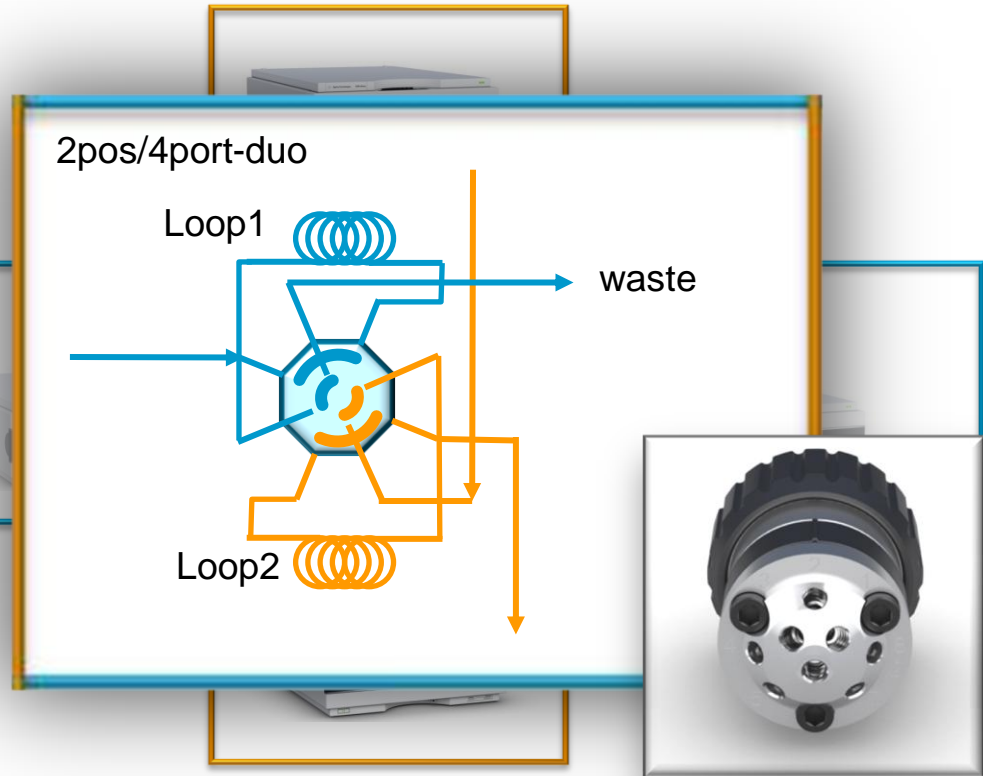
1260 Infinity Binary or Quaternary Pump

**Almost any Agilent pump or autosampler in the 1st dimension!
Almost any detectors are supported!
A 1290 Infinity Binary Pump for the 2nd dimension is required.**

Hardware – Valves, uniqueness and flexibility

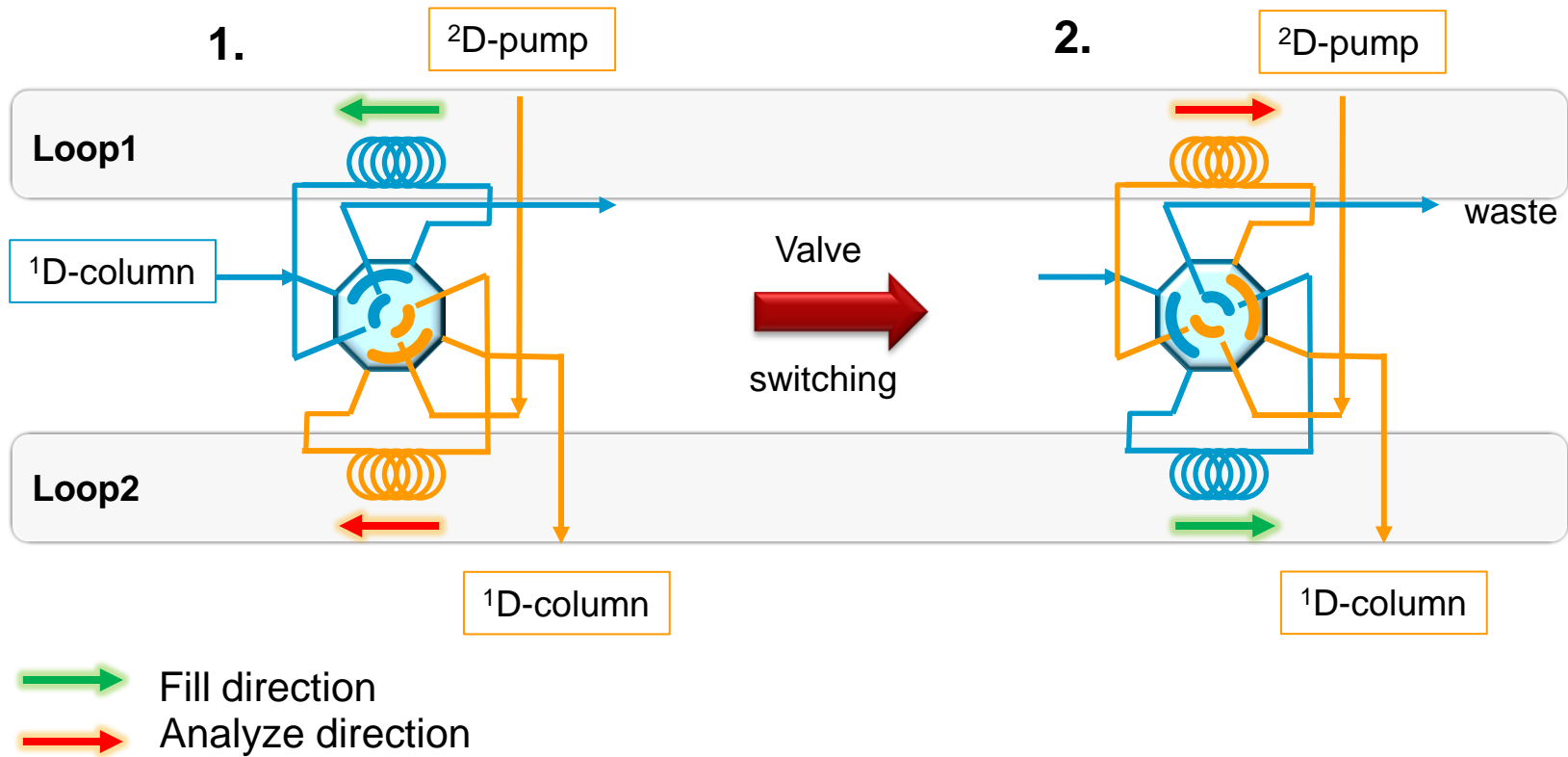
2. Dimension

1. Dimension



New Agilent-only special 2D-LC-QuickChange valve. Single valve with fully symmetric flow-paths and symmetric fill/flush-out behavior. Only valve that allows countercurrent flush-out of both loops.

Advantages of the new Agilent 2D-LC QuickChange valve head



All flow paths are equal (no additional bridging loops)
Symmetric countercurrent fill/analyze direction of loops (reducing band-spread)
All in one valve (no synchronization, costs)

2D-LC Acquisition software

- an add-on to Agilent's OpenLAB CDS ChemStation edition

Dashboard:

All modules in one dashboard can be related individually, e.g. „BinPump-1st-Dim“

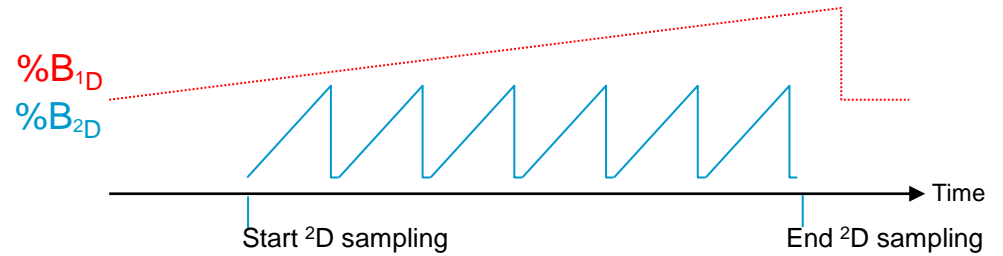
The screenshot displays the 'Mein LC (online): Method and Run Control' software interface. The main dashboard is divided into several sections:

- Left Panel:** A tree view showing the sequence structure, including folders like 'C:\CHEM32\1\SEQUENCE' and files such as 'APCDELAY.S', 'BATCH.S', 'DEF_IC.S', 'DENOISE.S', 'INSTPERF.S', 'LOADTEST.S', 'ROBUST.S', 'ROUTINE.S', 'SEQDOC.S', 'SEQSUM.S', and 'STATIST.S'.
- Top Panel:** 'Method and Run Control' status bar showing 'Ready', 'Last Run', and '0.0'.
- Instrument Control Panel:** A grid of control panels for different instruments:
 - Hip Sampler:** Shows a syringe icon and '0.0 µL'.
 - Binary Pump:** Shows two pumps (A1, B2) with flow rates of 80.0 and 20.0, and a pressure of 0.200 ml/min / 0.47 bar.
 - Column Comp.:** Shows 'Port 1 -> 2' and temperatures of 23.40 °C and 23.38 °C.
 - DAD:** Shows a detector icon and 'EMF' status.
- Bottom Panel:** A table for 'Single Sample' with columns for Location, Sample Name, Sample Info, Datafile, and Data Dir. Below it is a 'Sampling Diagram' window showing a grid of sampling points.
- Bottom Status Bar:** Shows 'wadi1140 0.00 / 0.00' and 'Instrument Idle'.

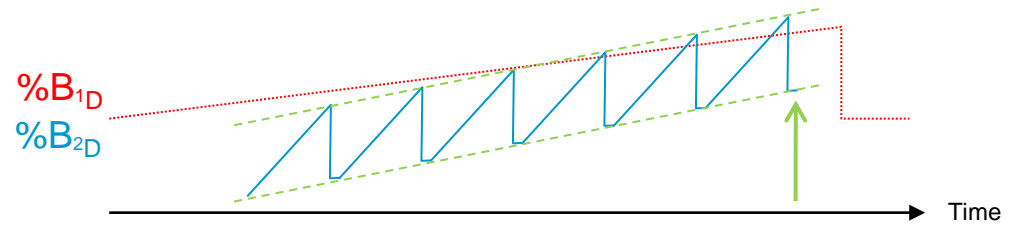
Comprehensive 2D-LC – gradient modes

- special ²D-gradient modes to improve resolution

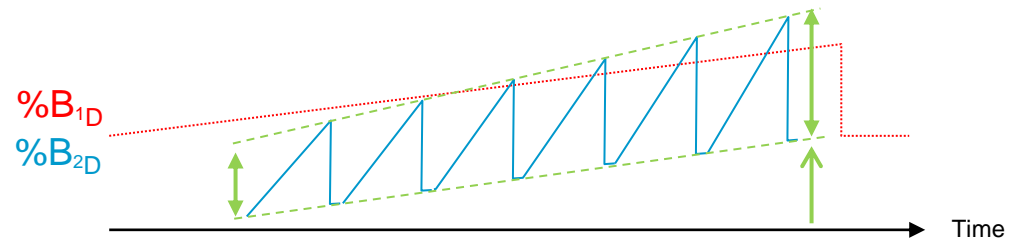
- standard repeating with start- and end-time



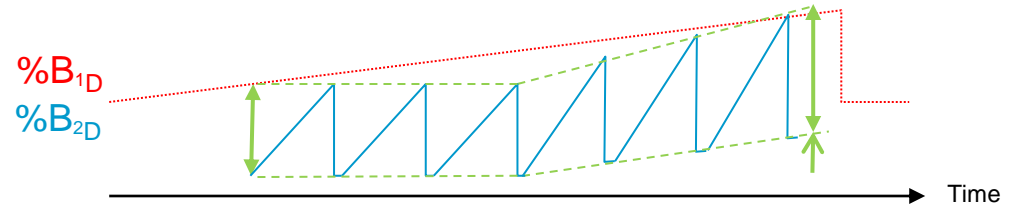
- constantly shifted %B_{2D}



- constantly shifted %B_{2D} and shifted Δ%B_{2D}

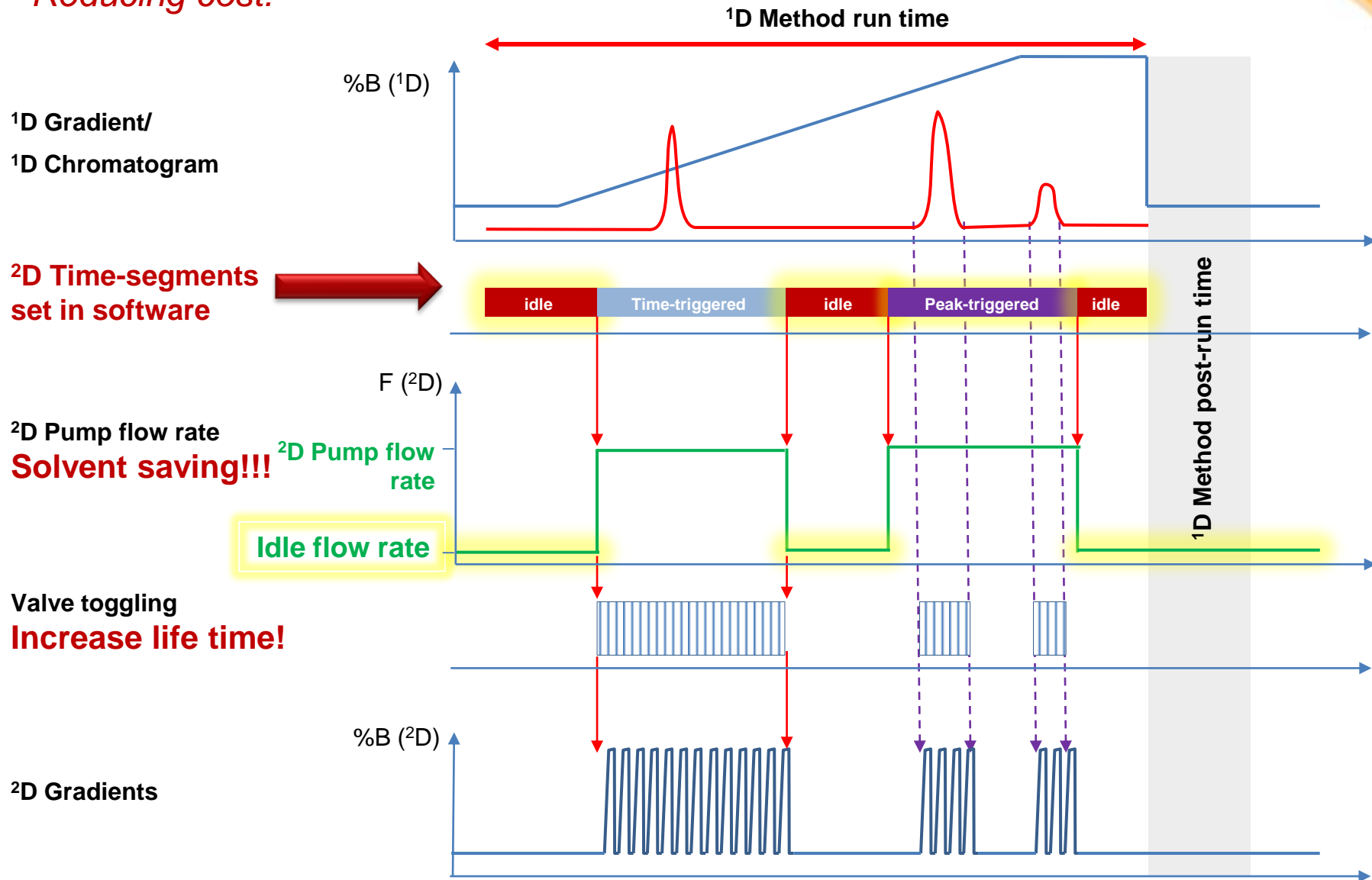


- **Any combination**



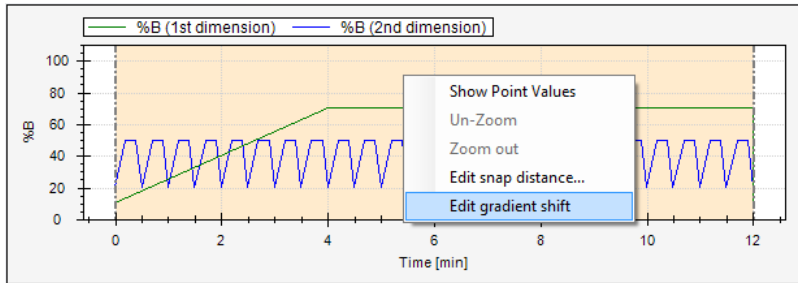
Time-segmenting in comprehensive 2D-LC

- Reducing cost!

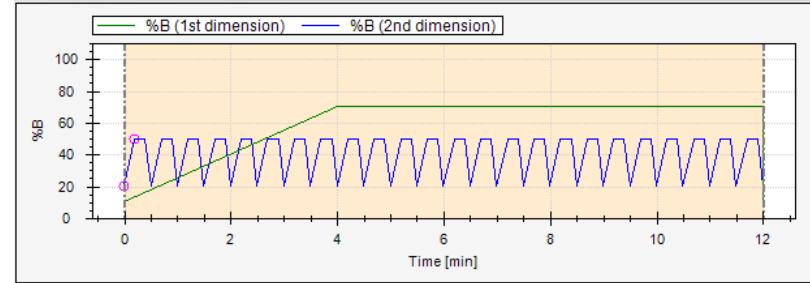


Example: graphical editing of a gradient shift – in less than a minute!

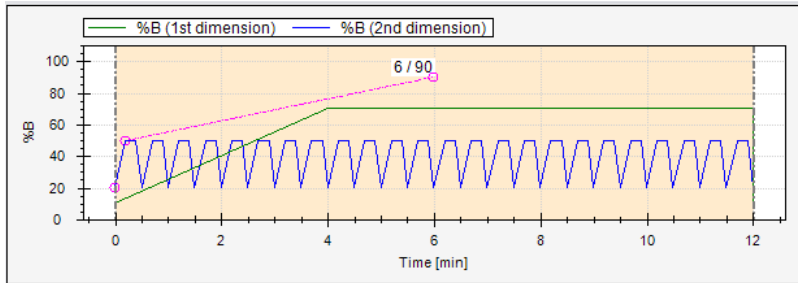
- replace editing of large timetables by a few mouse operations



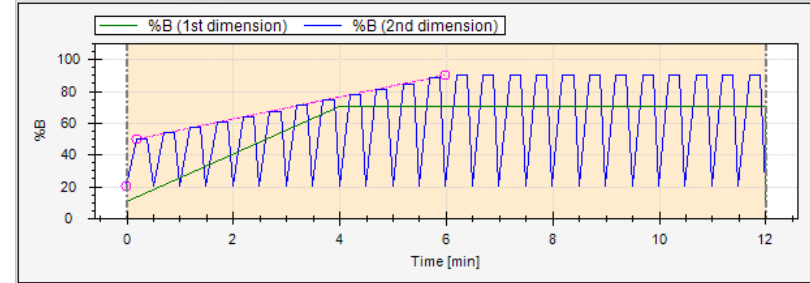
1 Use context menu to enter the editing mode



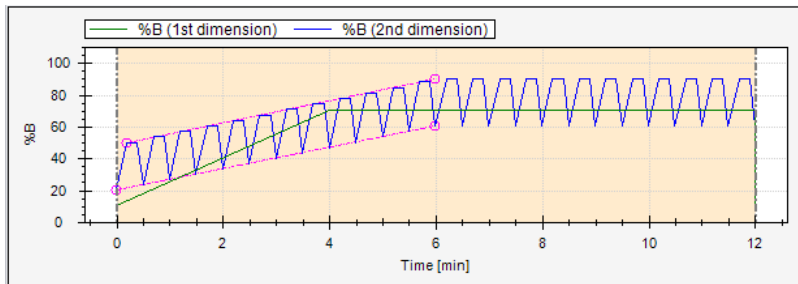
2 Timetable entries are marked with circles



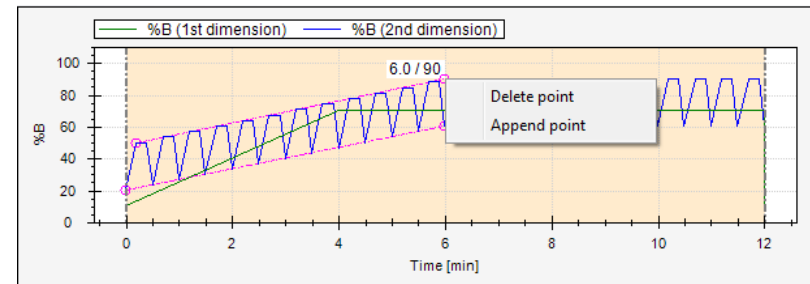
3 Draw a straight line by dragging the mouse to a new %B value at a specified runtime of the 1st dimension



4 When releasing the mouse, a new TT entry is made and the gradient rollout is automatically updated



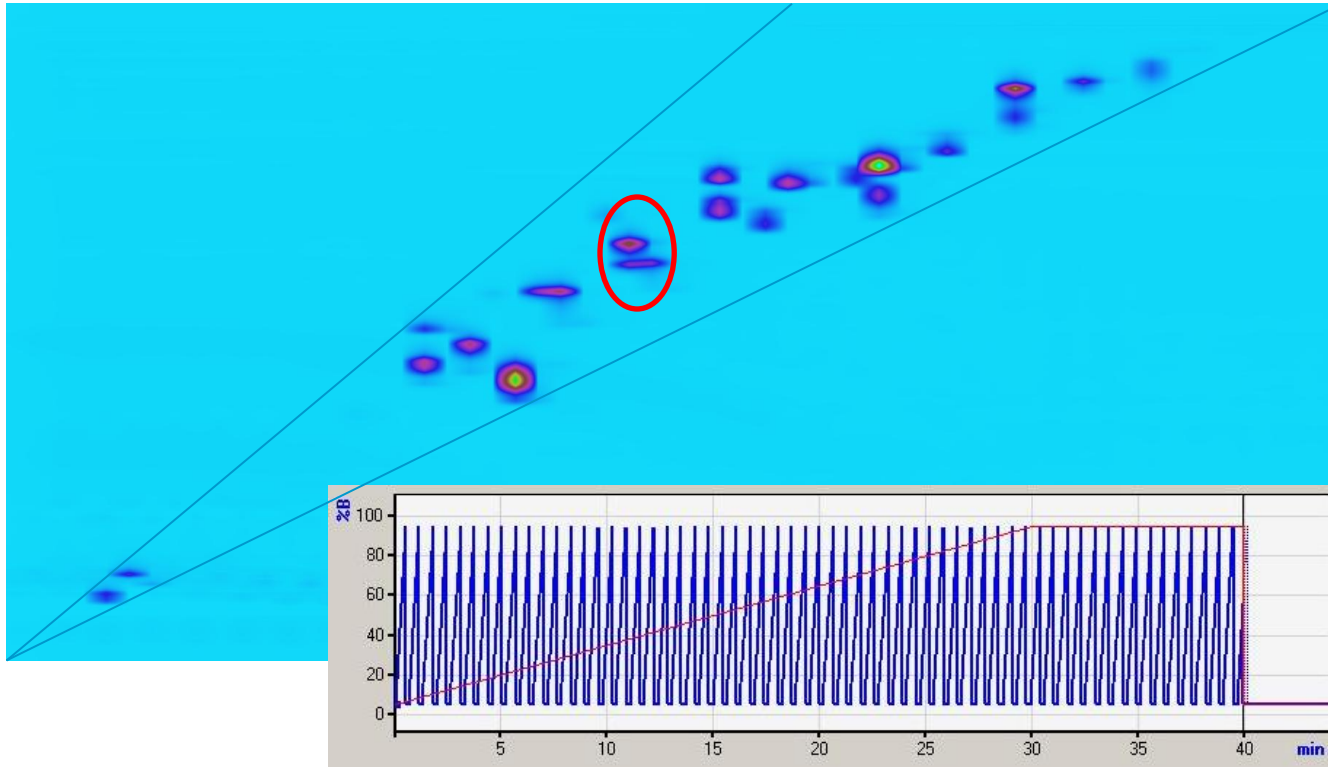
5 Repeat step 3 + 4 with other TT entries



6 Insert / Delete shift points (mouse cursor and context menu changes near to a shift line)

Application examples

-Advantage of shifted gradient features



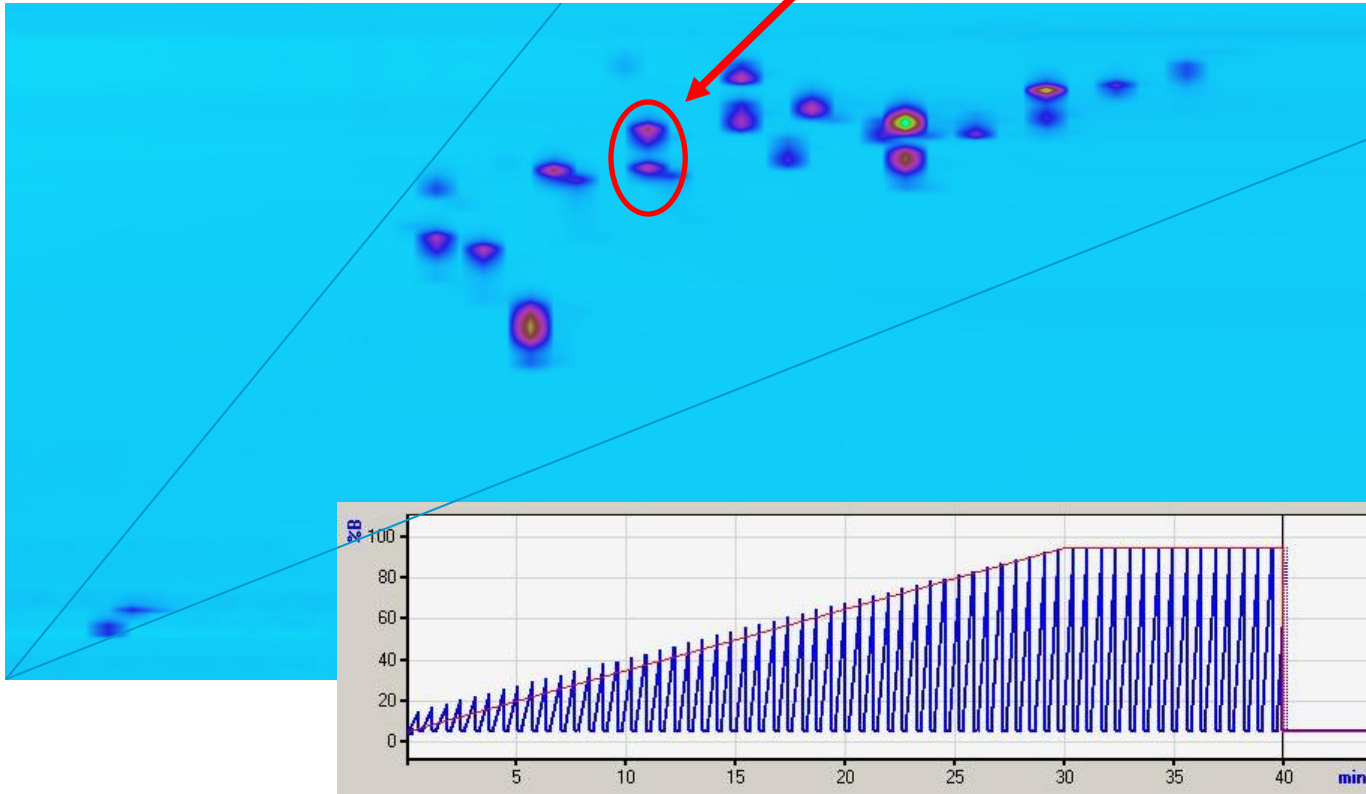
RPLC x RPLC
Easy method set-up but only little orthogonality

Current state-of-the-art 2D-LC – narrow spread of peaks

Application examples

-Advantage of shifted gradient features

Resolution optimized!



Use of shifted gradient feature

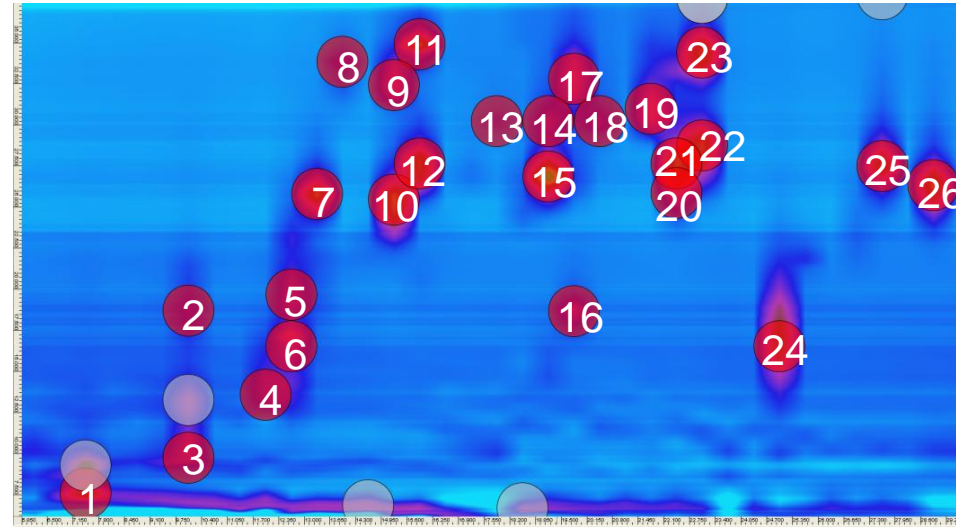
Imagine to program this gradient manually!
With the Agilent 2D-LC Acquisition software a matter of a minute!

Applications examples

- Polyphenols from food matrix

Compound		RT 1st dim (min)	RT 2nd dim (sec)	Peak Volume
Esculin	mean	9.75	18.58	177,383
	s.d.	nd	0.11	4,713
	RSD (%)	nd	0.57	2.7
Rutin	mean	13.65	33.68	72,375
	s.d.	nd	0.07	853
	RSD (%)	nd	0.22	1.2
Coumaric acid	mean	13.00	25.57	660,541
	s.d.	nd	0.13	13,037
	RSD (%)	nd	0.52	2.1
Reservatrol	mean	18.85	26.65	1,122,219
	s.d.	nd	0.10	16,089
	RSD (%)	nd	0.37	1.4
Salicylic acid	mean	19.50	18.53	211,092
	s.d.	nd	0.10	6,895
	RSD (%)	nd	0.51	3.3
Luteolin	mean	19.50	32.70	695,601
	s.d.	nd	0.11	17,592
	RSD (%)	nd	0.33	2.5
7-Hydroxy-Flavone	mean	22.75	34.26	1,388,226
	s.d.	nd	0.10	17,195
	RSD (%)	nd	0.30	1.2
Pinoslyvin	mean	24.70	18.85	1,588,654
	s.d.	nd	0.23	57,580
	RSD (%)	nd	1.24	3.6
Chrysin	mean	27.30	27.42	808,916
	s.d.	nd	0.11	14,768
	RSD (%)	nd	0.39	1.8
Flavone	mean	28.60	26.35	1,008,012
	s.d.	nd	0.13	20,911
	RSD (%)	nd	0.48	2.1

**Peak volume:
all compounds <3.6% RSD**



Summary

The Agilent 1290 Infinity 2D-LC solution brings the power of two-dimensional LC-separation to you at a never before experienced ease-of-use!

Highest separation power by 2D-LC combined with outstanding performance of the 1290 Infinity LC – the ideal tool for complex samples from biological origin, polymers, food-extracts, and many more.

Innovative new hardware and software features for ease-of-use, reduced operation costs and highest performance.

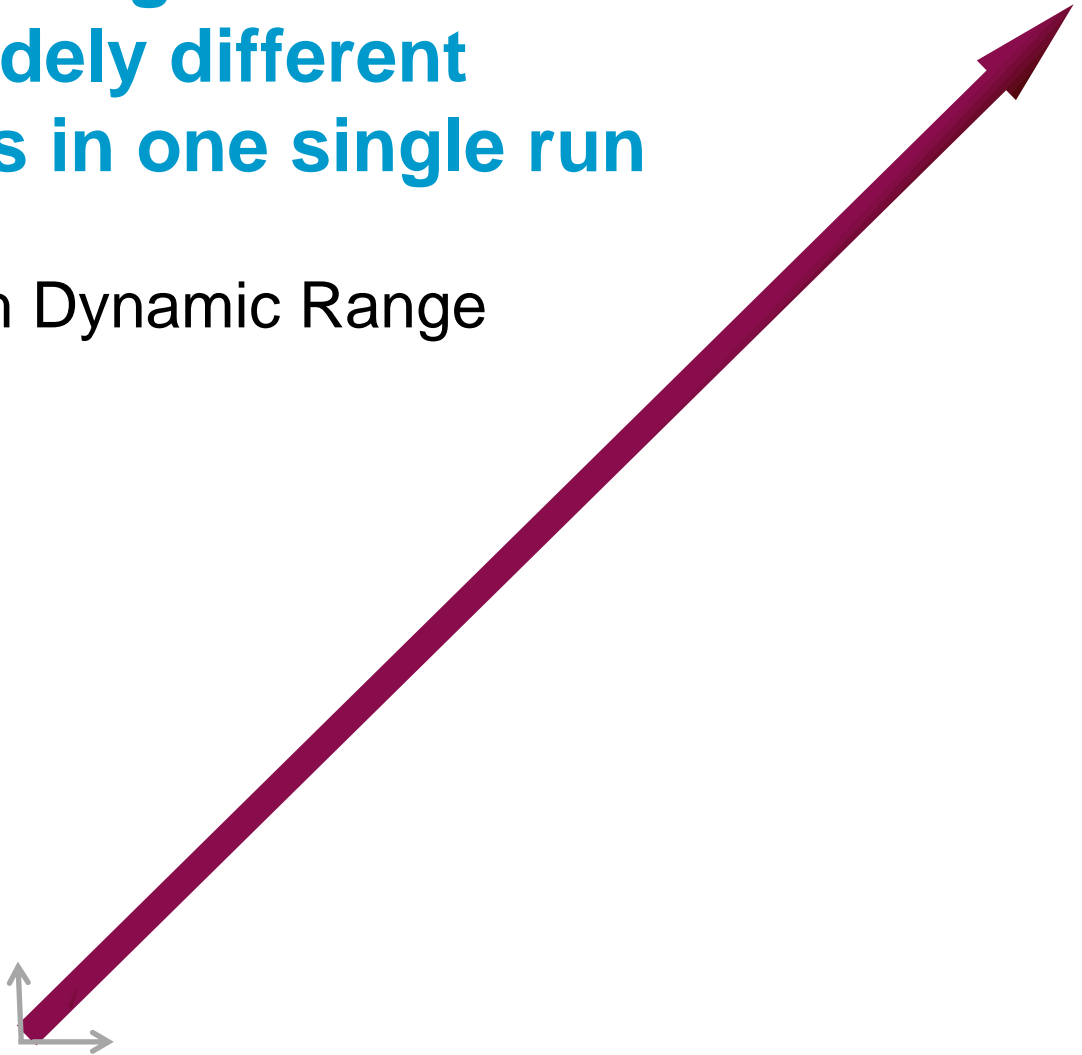
Upgradability or re-use of existing Agilent LC equipment.

30x wider linear UV range - Quantification of widely different concentration levels in one single run

Agilent 1200 Infinity High Dynamic Range
(HDR-DAD) Solution

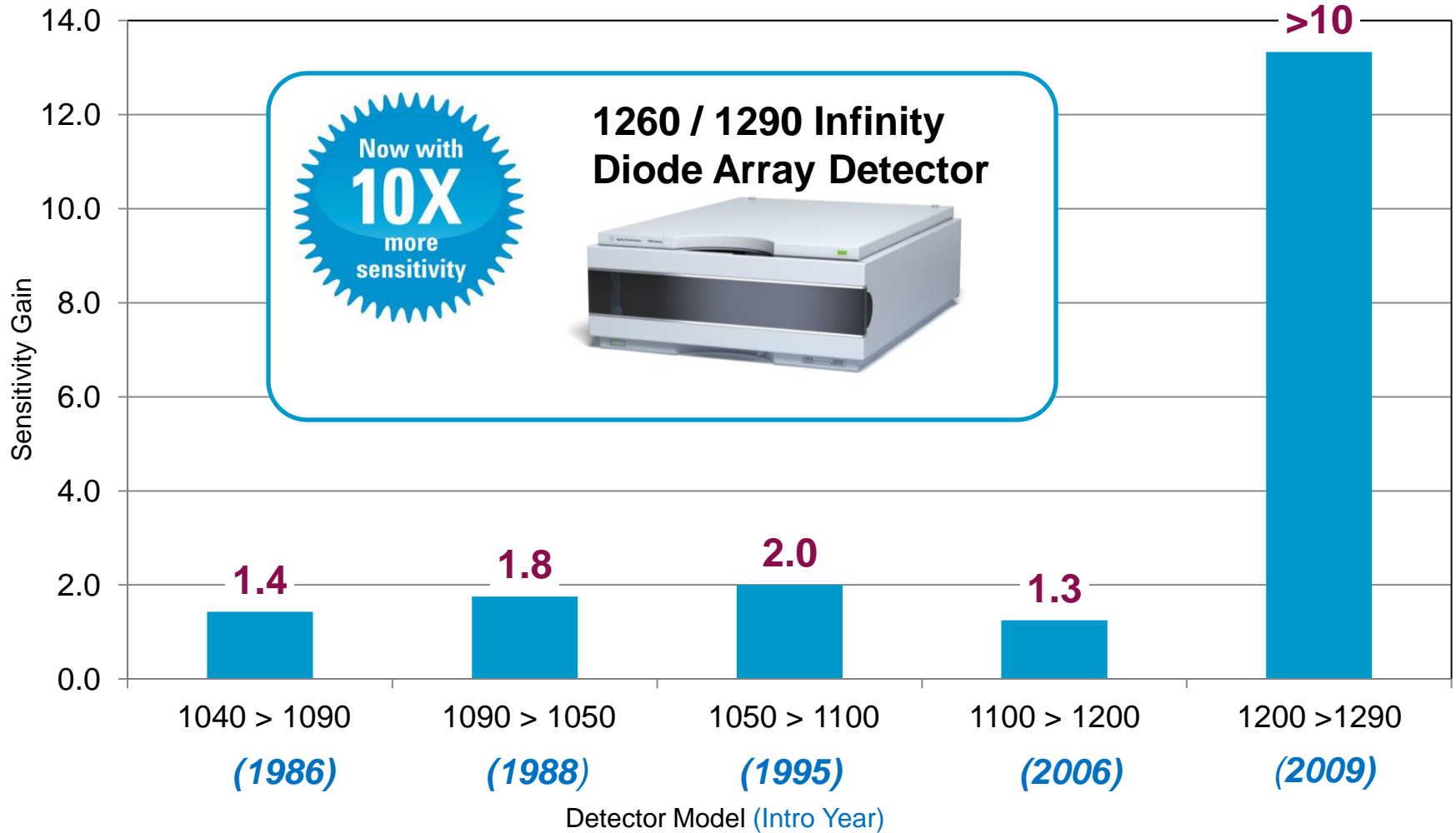


1260/1290 Infinity HDR-DAD



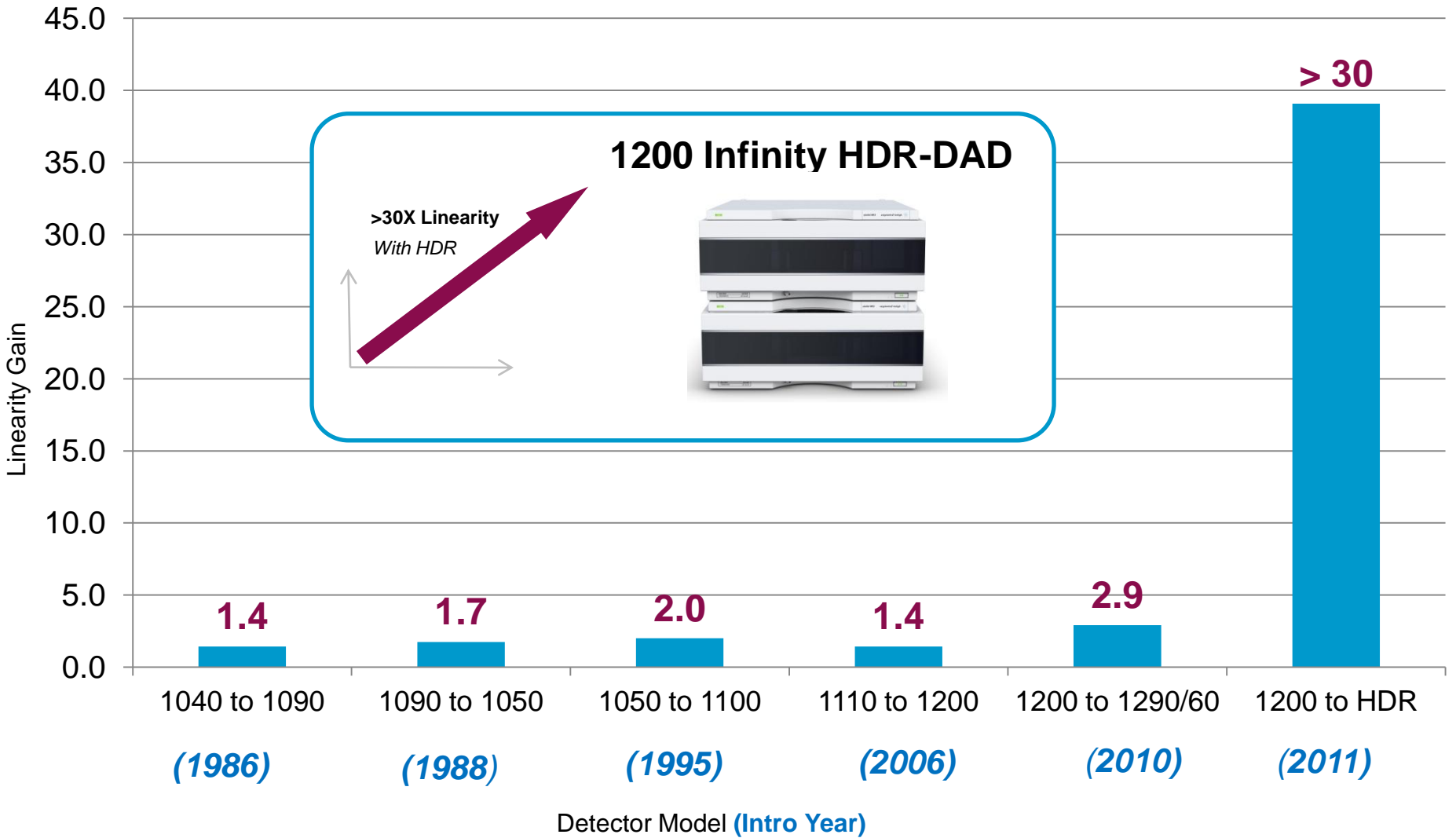
History of DAD Sensitivity Gain

The last 30 years



History of DAD Linearity Gain

The last 30 years



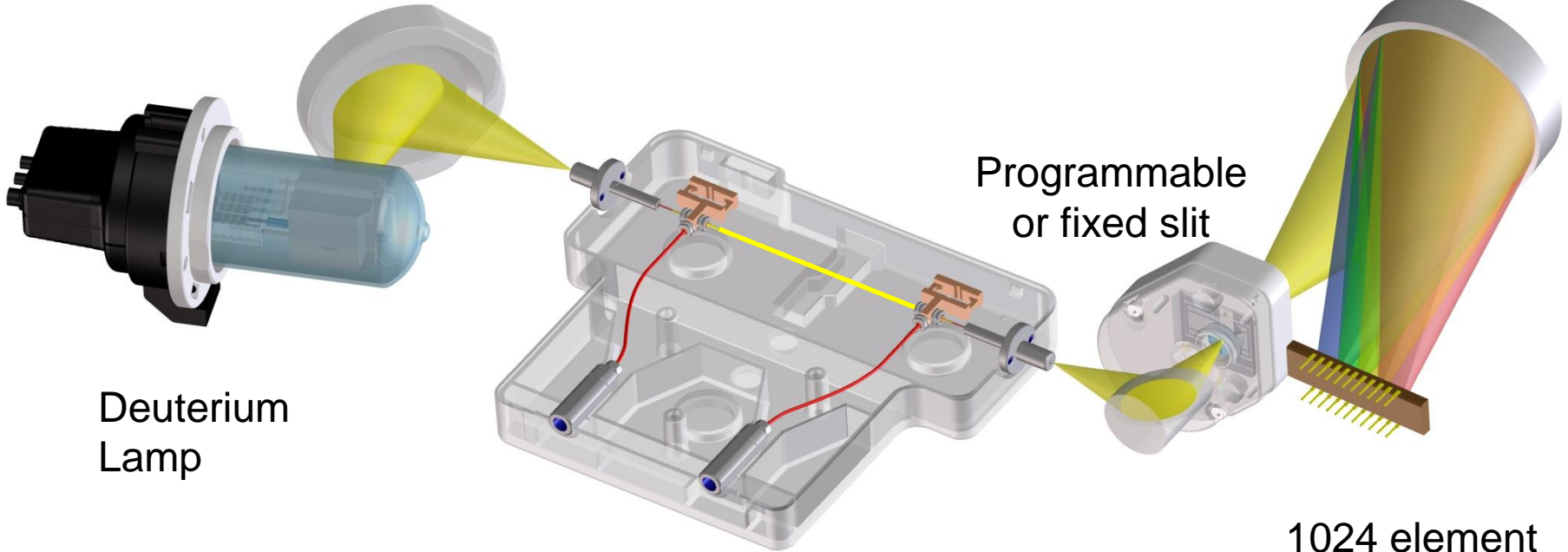
Optofluidic Waveguides: Max-Light Flow Cells

Total-internal reflection in a non-coated fused silica fiber

1260 / 1290 Infinity DAD

Mirror

Grating



Deuterium Lamp

Programmable or fixed slit

1024 element diode-array

Max-Light Cartridge Cell
10 mm or 60 mm path length

Linearity, linear range and dynamic range

1200 Series Diode Array Detector, 10 mm flow cell



10 mm

$$Linear_range = \frac{Upper_Limit}{Detector_Noise} \approx 10^5$$

Detector Signal [AU]

Upper limit
2.0

Slope = Response Factor
(Sensitivity)

Detector noise
 7×10^{-6}

HDR-DAD

HDR-DAD

Linear range

Dynamic range

LOD
(3x S/N)

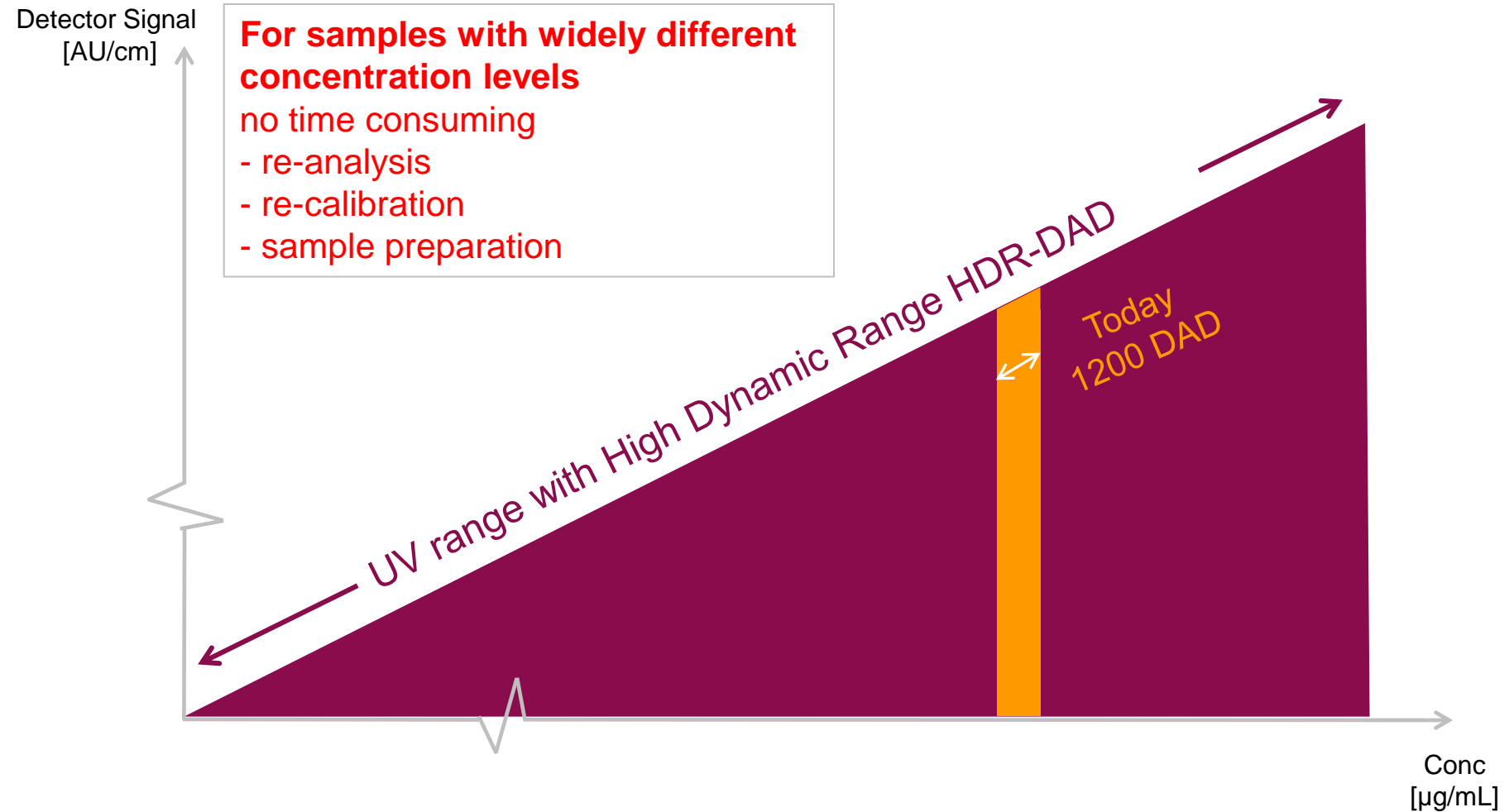
Conc (upper limit)
linear range

Conc (upper limit)
dynamic range

Conc [µg/mL]

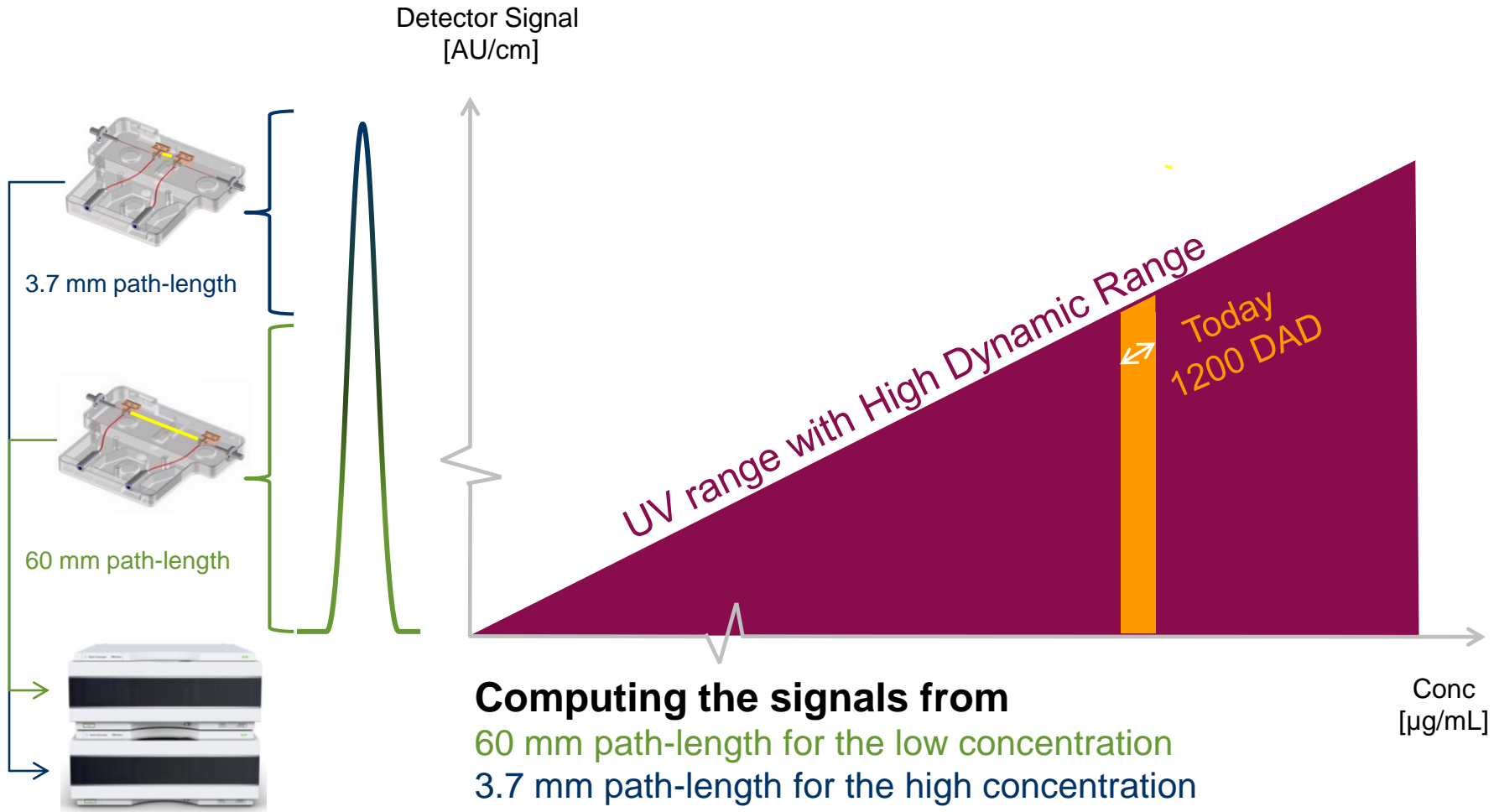
30x Wider Linear Range with HDR-DAD

All sample information in one run



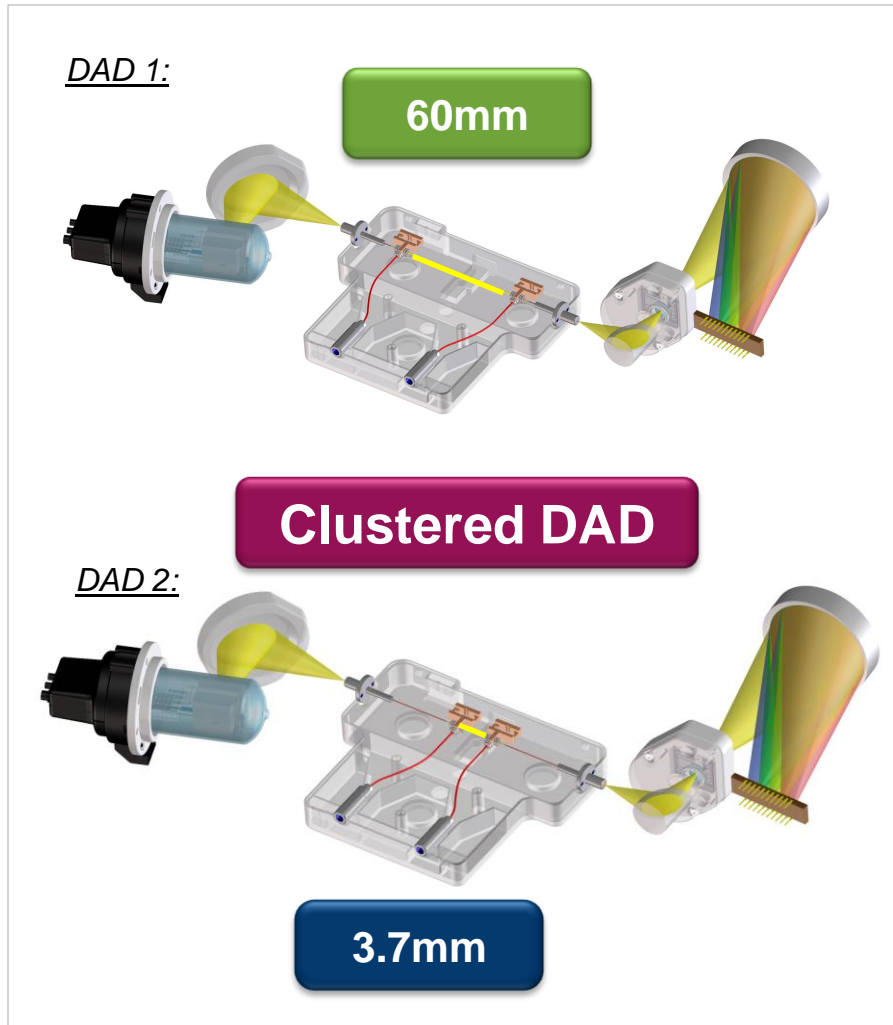
30x Wider Linear Range with HDR-DAD

3.7 mm and 60 mm and Max-Light flow cell



1200 Infinity High Dynamic Range (HDR-DAD)

Increasing linear UV Range by 30x: Benefits



For sample mixtures with widely different concentration levels:

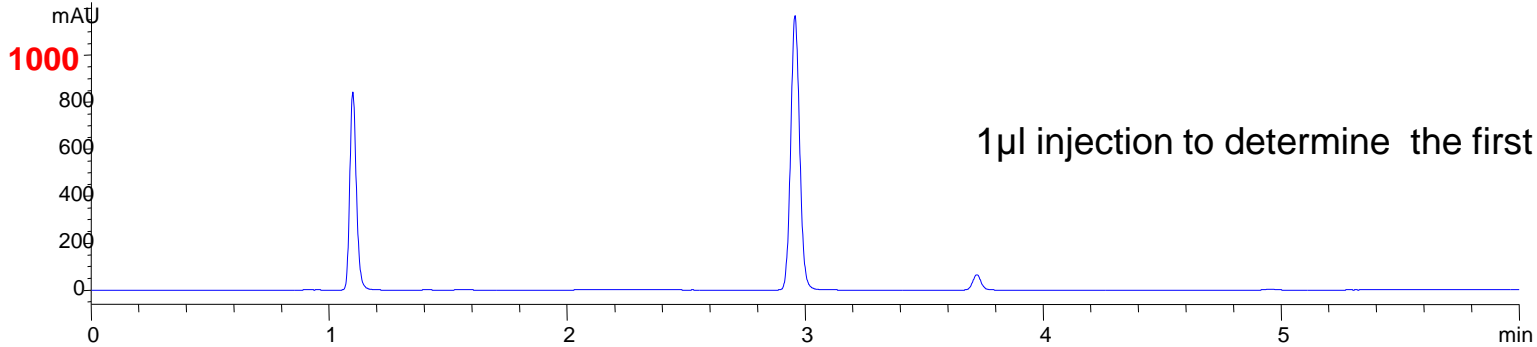
- All sample information in one run, e.g. for fixed dose combinations
 - shorter turnaround time
 - higher instrument utilization
 - reduced solvent and waste costs.
- Up to 30x lower LOD, 15x lower LOQ (compare to 1100/1200 Series)
 - for more reliable automated integration
 - higher area precision of low levels impurities
 - prepared for stronger regulations
 - genotoxins

Experiment

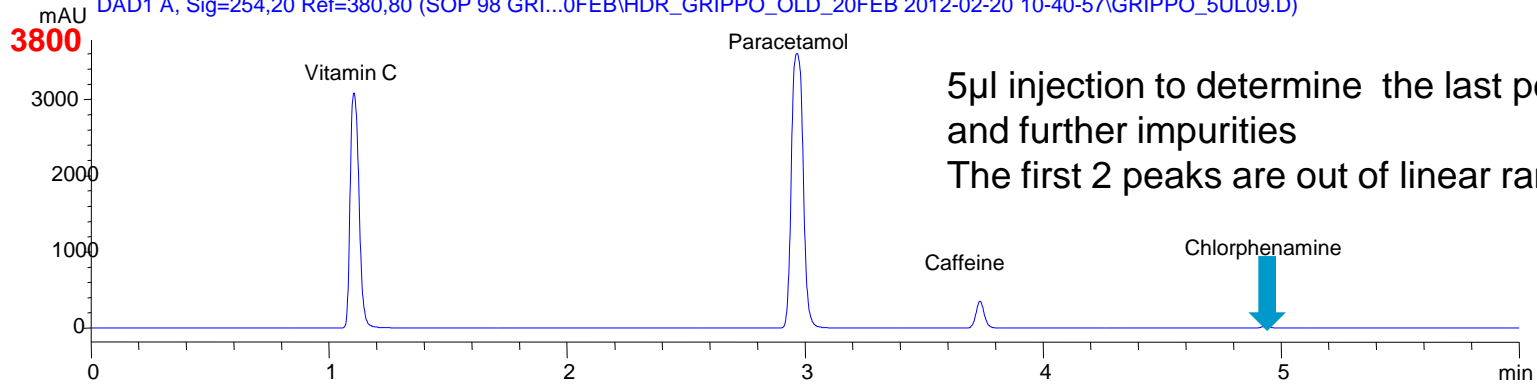
- *As sample a Fixed Dose Combination drug was used:*
 - *Paracetamol (200mg) and Chlorphenamine (2.5mg)(1:80), other compounds are Vitamin C (150mg) and Caffeine (25mg) + further small impurities*
- *Analysis on Agilent 1290 Infinity LC with conventional DAD using 2 injections with 1 μ L and 5 μ L injection volume to determine high and low dosed drugs*
- *Inject of 5 μ L injection volume on Agilent 1290 Infinity HDR DAD system to determine low-dose and high-dose drug within linear range (6 AU) with one single injection*

2 Injections needed if a conventional DAD is used

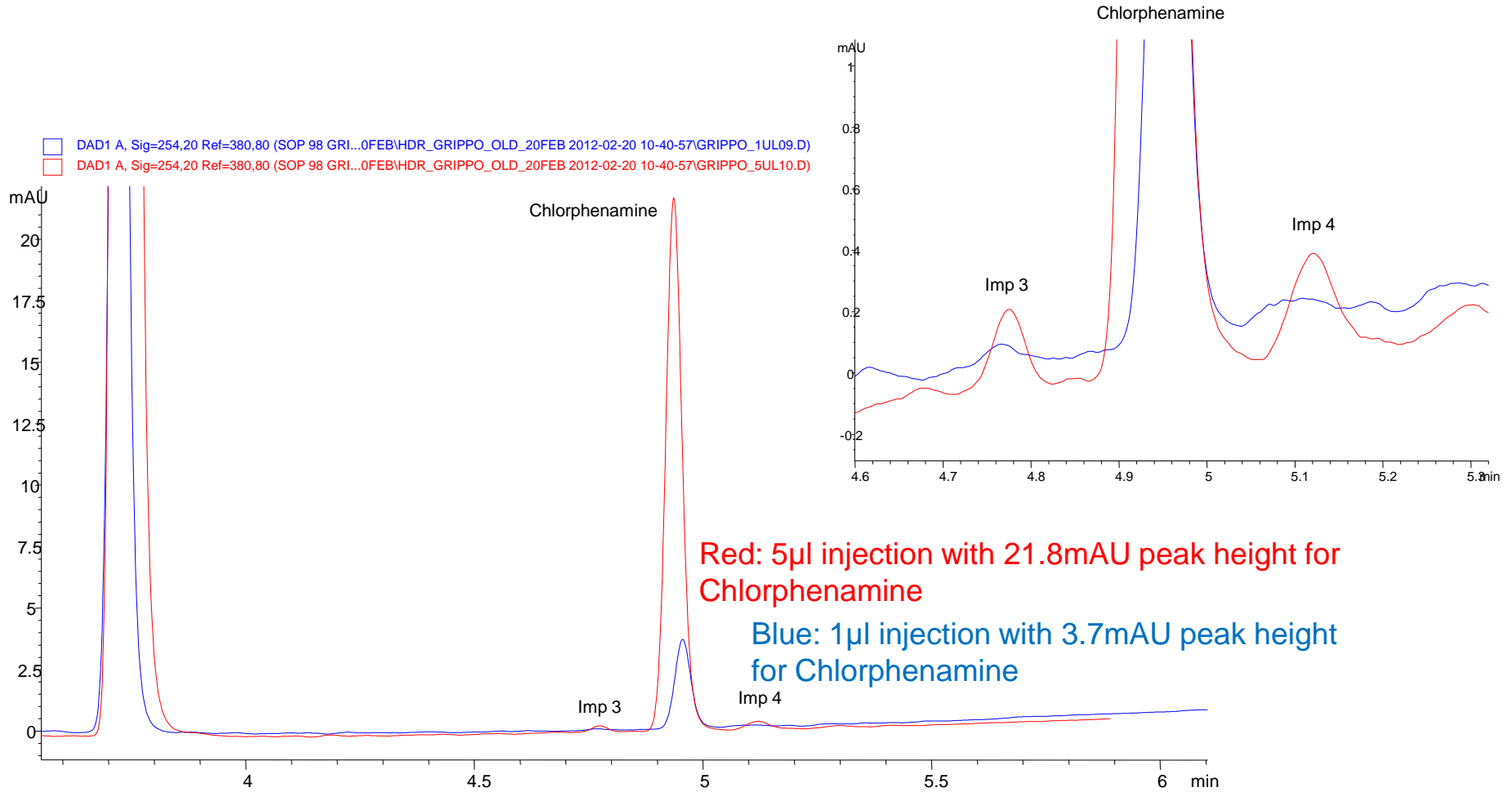
DAD1 A, Sig=254,20 Ref=380,80 (SOP 98 GRI...0FEB\HDR_GRIPPO_OLD_20FEB 2012-02-20 10-40-57\GRIPPO_1UL09.D)



DAD1 A, Sig=254,20 Ref=380,80 (SOP 98 GRI...0FEB\HDR_GRIPPO_OLD_20FEB 2012-02-20 10-40-57\GRIPPO_5UL09.D)



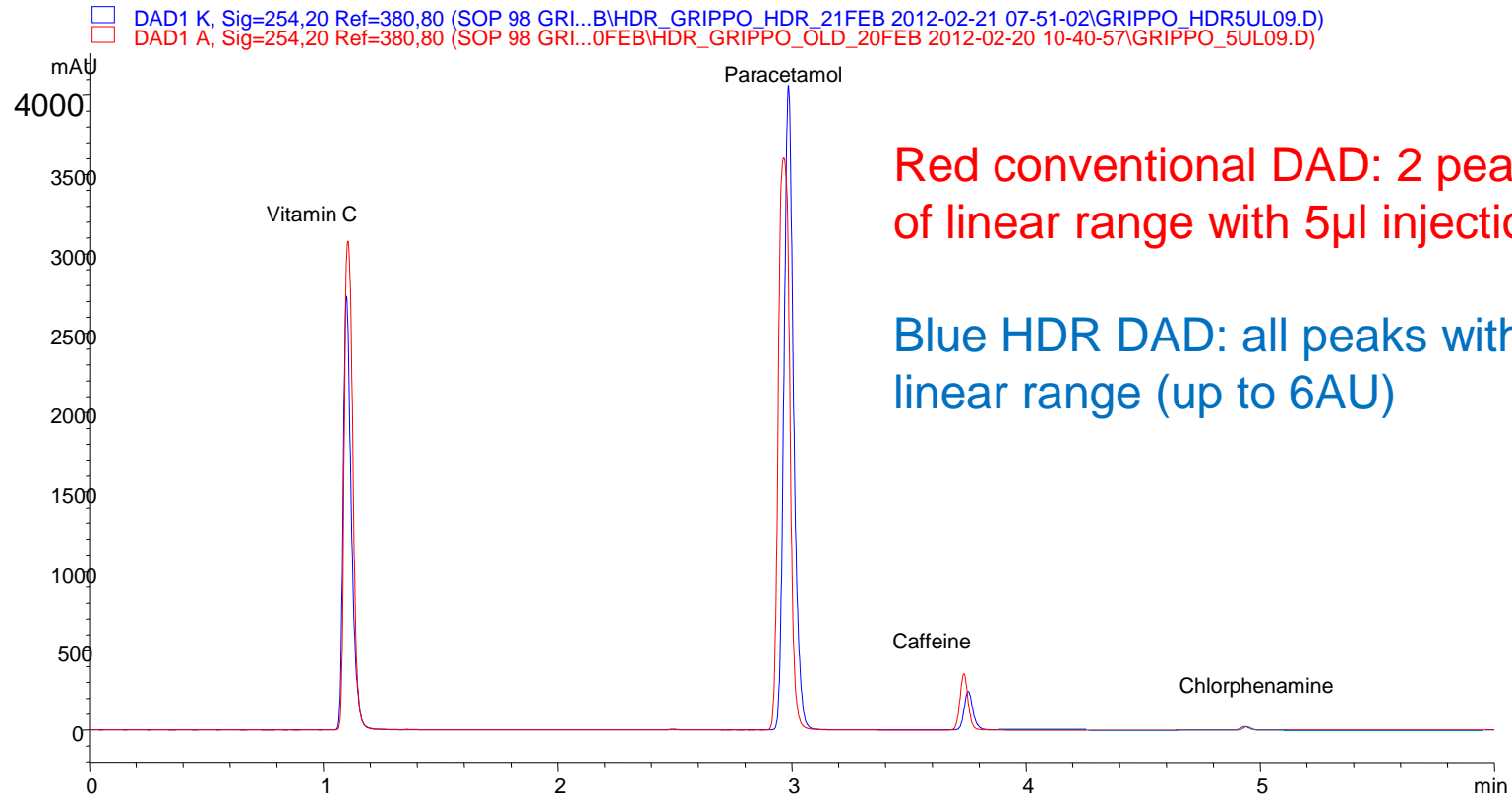
2 Injections needed if a conventional DAD is used



Red: 5µl injection with 21.8mAU peak height for Chlorphenamine

Blue: 1µl injection with 3.7mAU peak height for Chlorphenamine

Comparison of old DAD vs. HDR DAD, 5µl injection

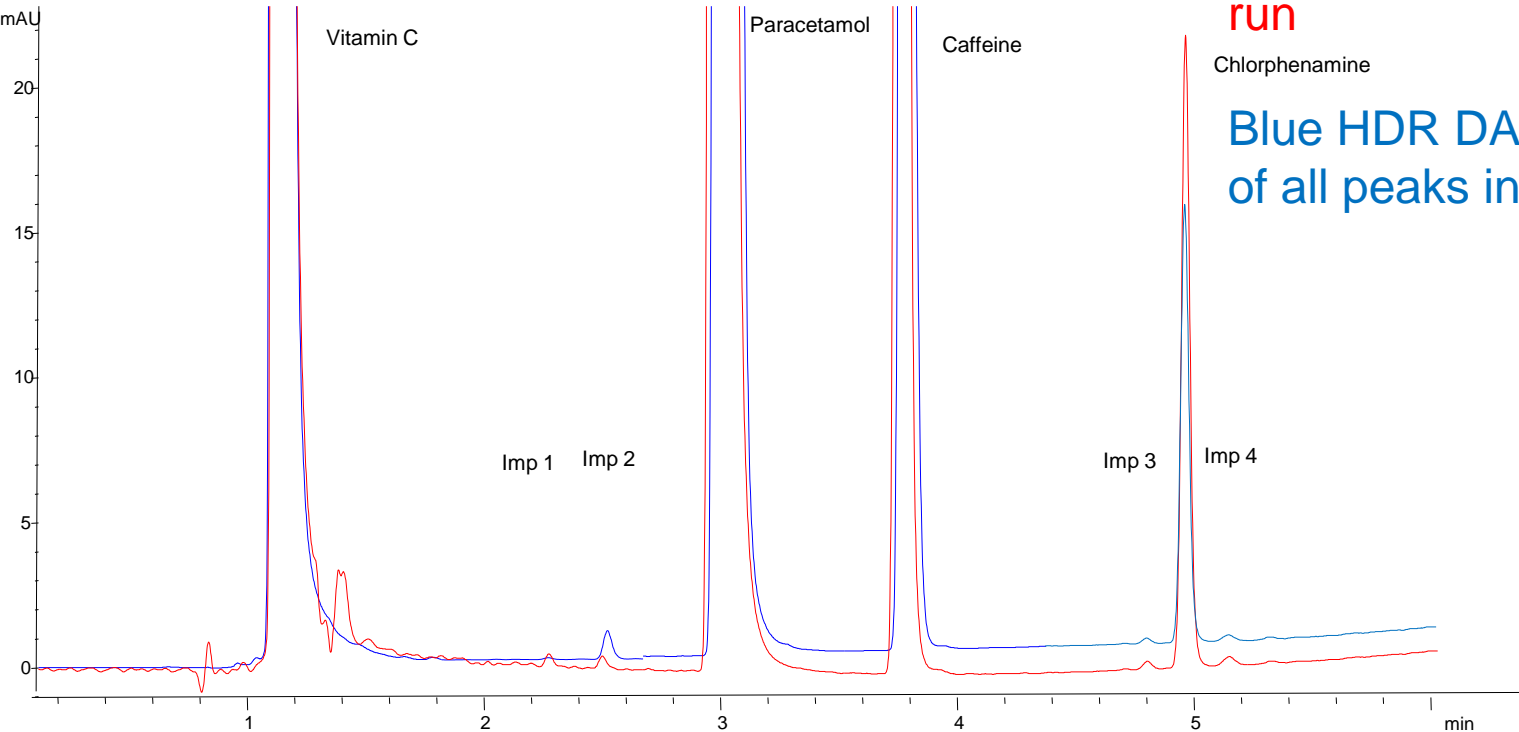


Red conventional DAD: 2 peaks out of linear range with 5µl injection

Blue HDR DAD: all peaks within linear range (up to 6AU)

Comparison of conventional DAD and HDR DAD, 5 μ l injection

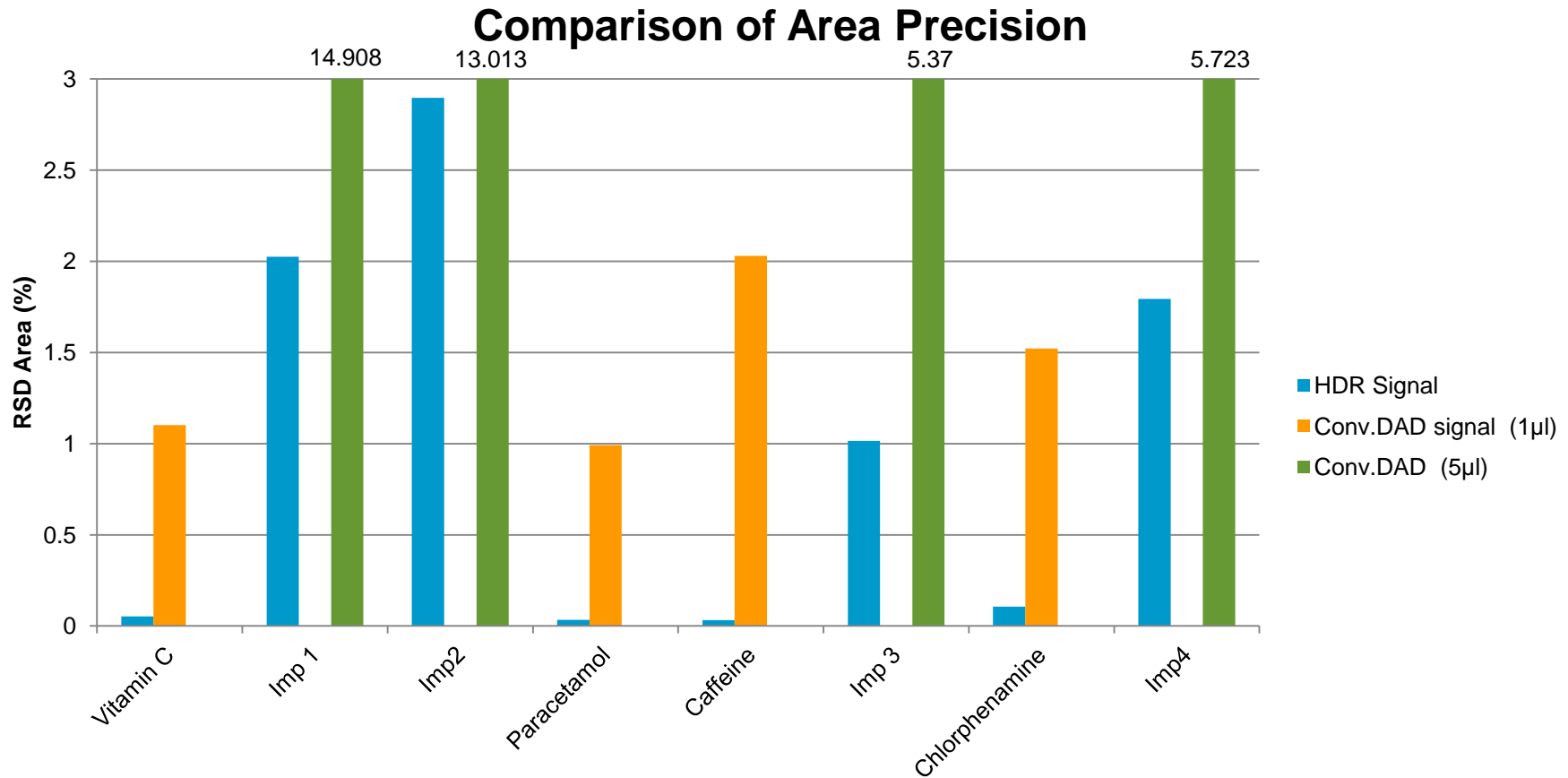
□ DAD1 K, Sig=254,20 Ref=380,80 (SOP 98 GRI...B\HDR_GRIPPO_HDR_21FEB 2012-02-21 07-51-02\GRIPPO_HDR5UL09.D)
□ DAD1 A, Sig=254,20 Ref=380,80 (SOP 98 GRI...0FEB\HDR_GRIPPO_OLD_20FEB 2012-02-20 10-40-57\GRIPPO_5UL09.D)



Red conventional DAD:
determination of impurities and
chlorphenamine in a second
run

Blue HDR DAD: determination
of all peaks in one run

Comparison of area precision



HDR DAD

5µl injection volume for determination of all compounds (blue)

Conventional DAD: 1µl injection volume for determination of Vit C, paracetamol and Caffeine (orange)

5µl injection volume for determination of Chlorphenamine and further impurities (green)



Summary

With the Agilent HDR DAD the determination of low and high doses drugs is possible in one run whereas the complete determination of all drugs with a conventional DAD needs 2 injections with different injection volumes

Further improved precision and lower detection limits are achieved compared to a conventional DAD

The new Agilent 1290 Infinity Quaternary LC

How binary pump-like performance
can enhance your lab efficiency



The 1290 Infinity Quaternary LC System

Leveraging the Performance of the 1290 Infinity Binary LC System



**1290 Infinity DAD with 60mm cell
(& HDR optional)**

**1290 Infinity TCC with broadest
valve selectivity**

**1290 Infinity Autosampler
(with Flexible Cube for lowest
carry-over)**

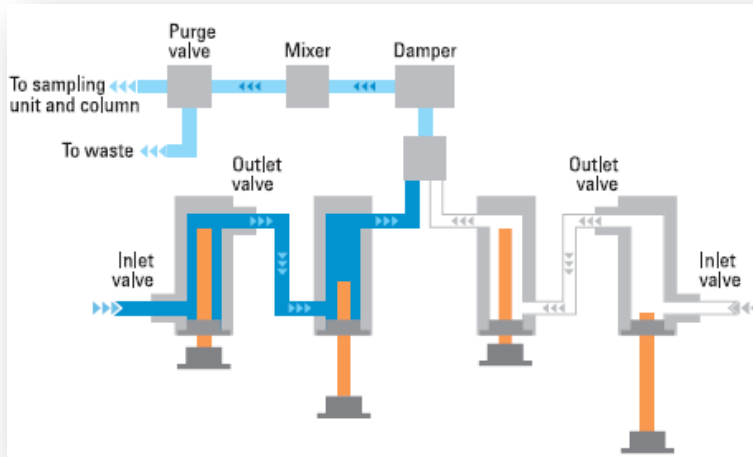
1290 Infinity Quaternary Pump



High Pressure vs. Low Pressure Mixing Principle

Binary pump

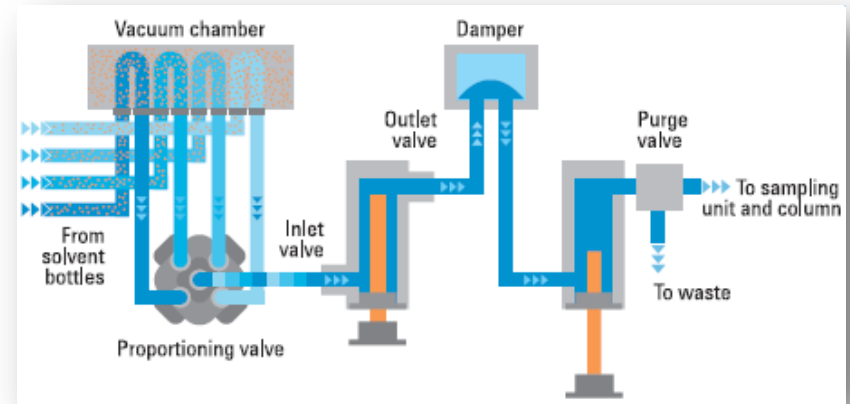
→ High Pressure Mixing



- Combination and mixing of mobile phases **after** the pump head
- Possible influence of excess volume on gradient (→ flow accuracy!)

Quaternary pump

→ Low Pressure Mixing



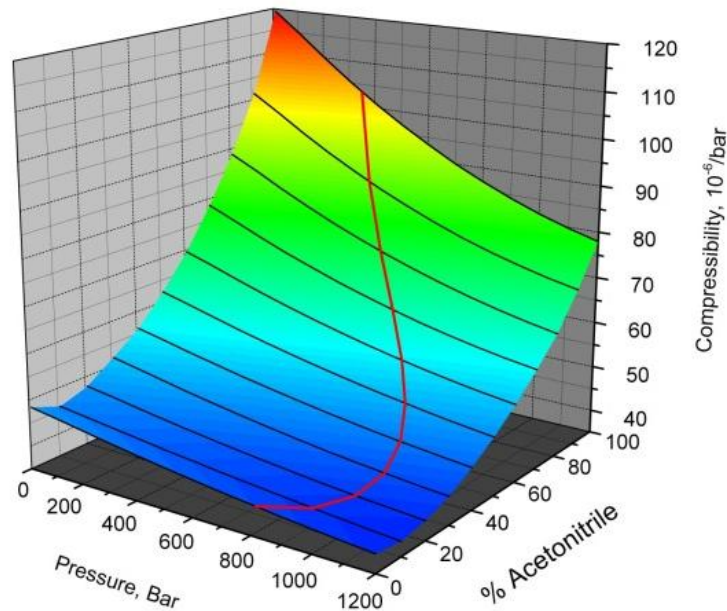
- Combination and mixing by low-pressure proportioning valve **before** the pump head
- No influence of excess volume on gradient, but on composition accuracy!

The Story of the 1290 Infinity Quaternary Pump is a Story about **Accuracy!!**

3 Crucial Points to achieve ultimate quaternary pump performance (low pressure mixing!):

1. For best performance you must know your eluents

Compressibility of Water-Acetonitrile Mixtures



The Story of the 1290 Infinity Quaternary Pump is a Story about **Accuracy!!**

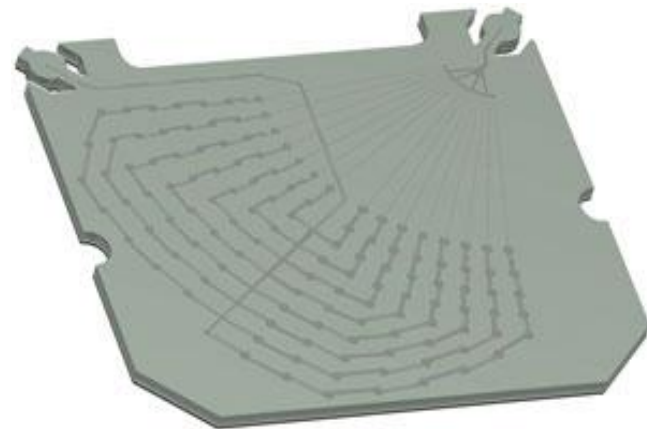
3 Crucial Points to achieve ultimate quaternary pump performance (low pressure mixing!):

1. For best performance you must know your eluents
2. Pump intelligence - Active Damping - with high pump resolution (~300 pL minimum step)

The Story of the 1290 Infinity Quaternary Pump is a Story about **Accuracy!!**

3 Crucial Points to achieve ultimate quaternary pump performance (low pressure mixing!):

1. For best performance you must know your eluents
2. Pump intelligence - Active Damping - with high pump resolution (~300 pL minimum step)
3. New InletWeaver using proven Jet Weaver technology



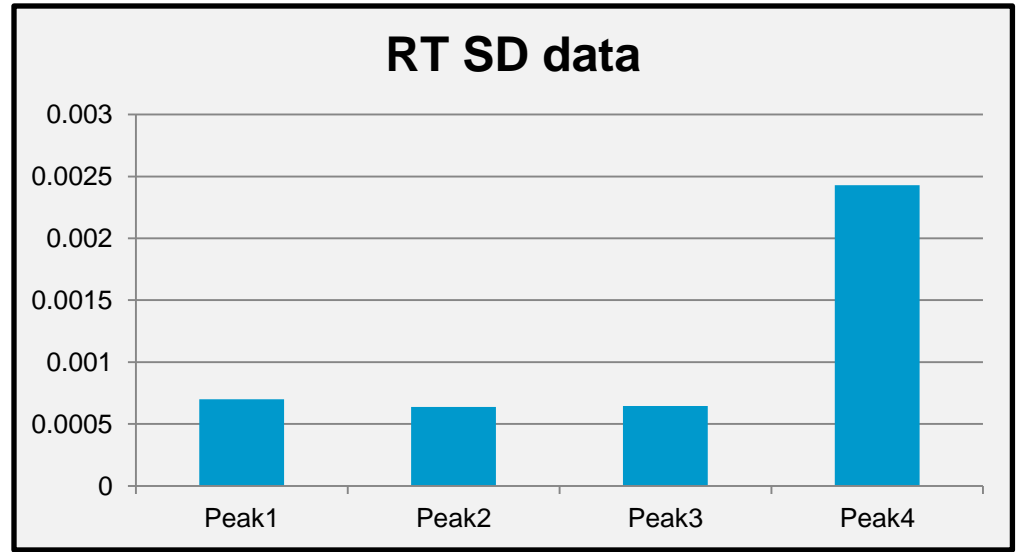
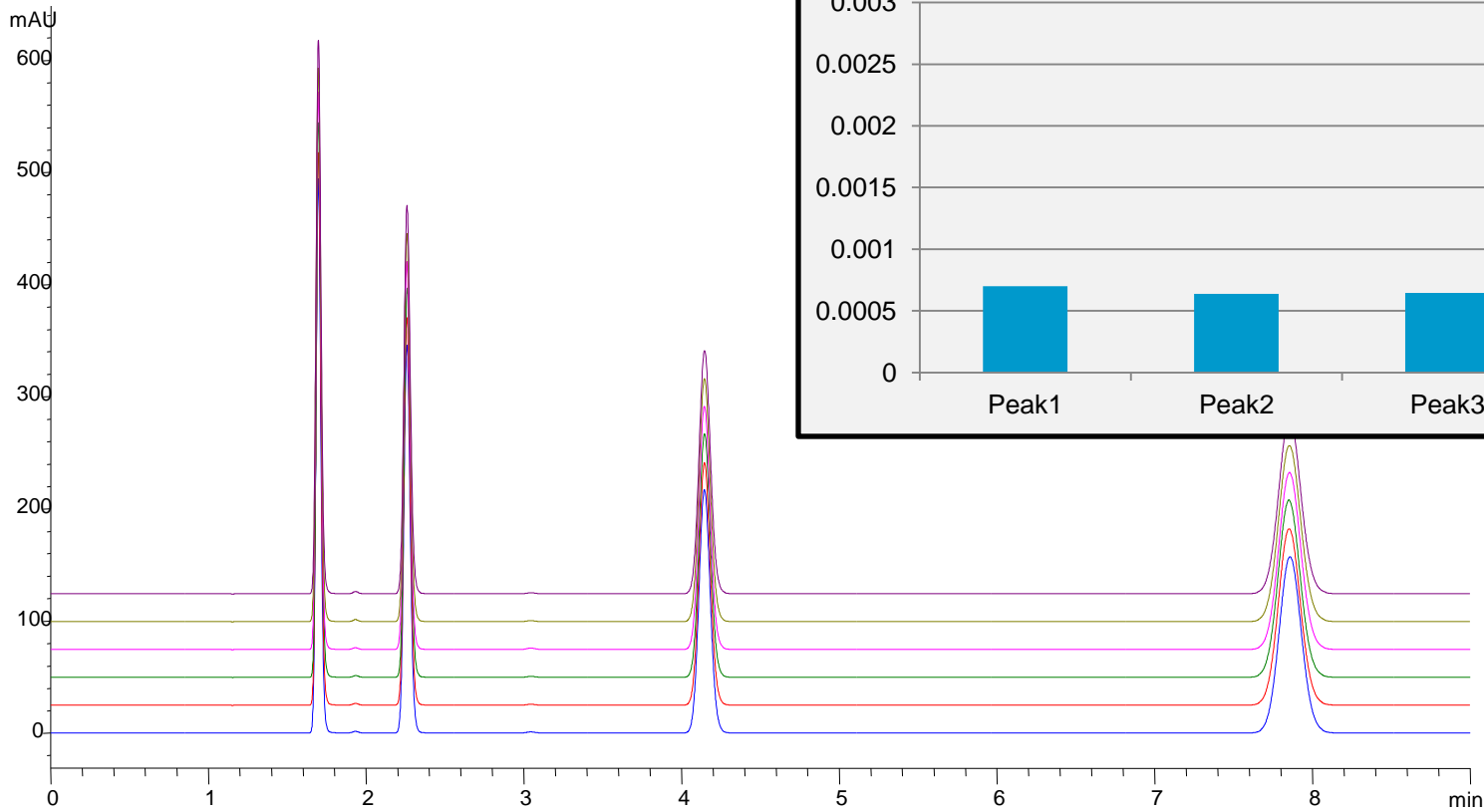
The Story of the 1290 Infinity Quaternary Pump is a Story about **Accuracy!!**

3 Crucial Points to achieve ultimate quaternary pump performance (low pressure mixing!):

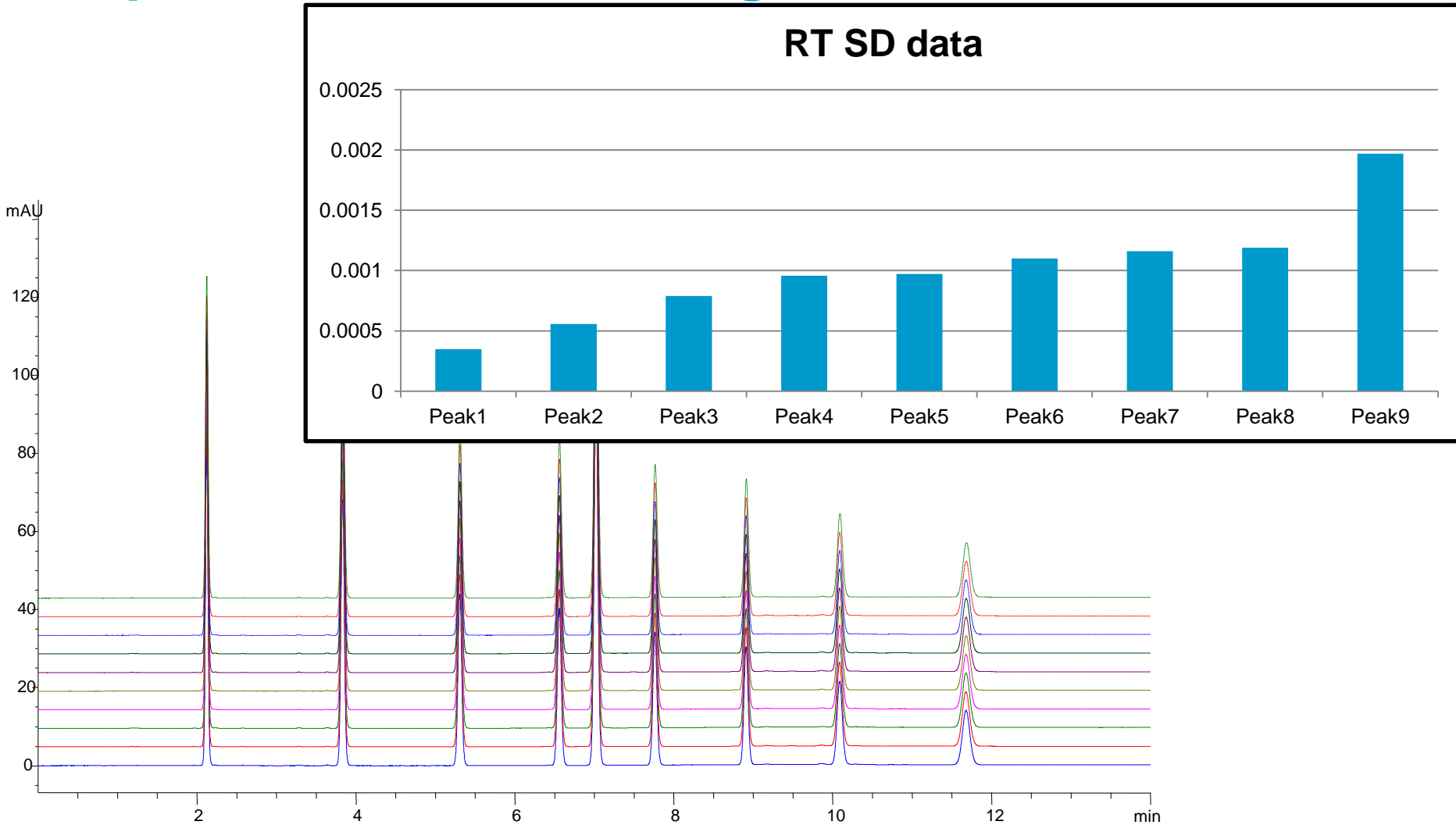
1. For best performance you must know your eluents
2. Pump intelligence - Active Damping - with high pump resolution (~300 pL minimum step)
3. New InletWeaver using proven Jet Weaver technology

The 1290 Infinity Quaternary Pump offers the highest accuracy and precision over the **full application range!**

1290 Infinity Quaternary Pump - Isocratic Analysis

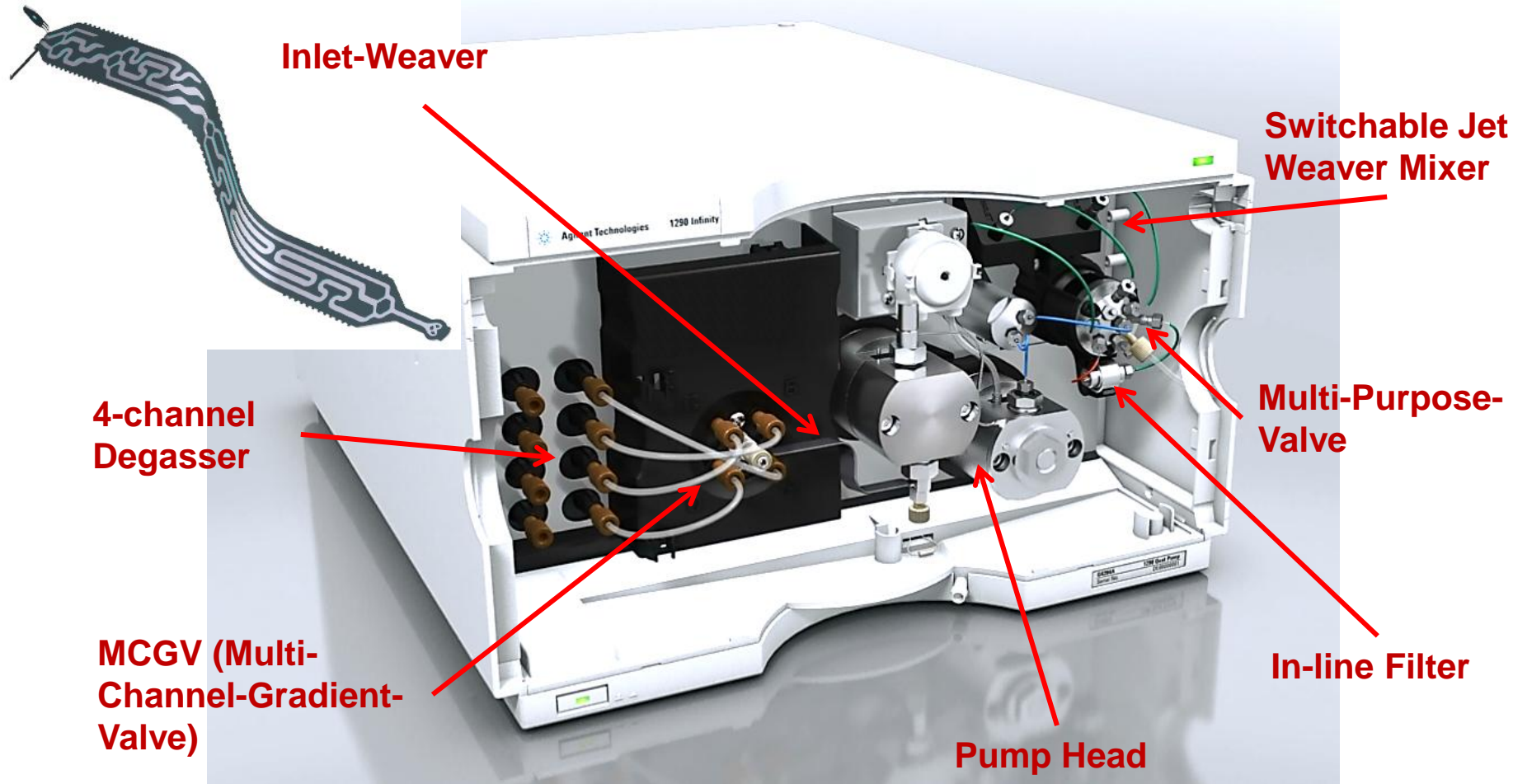


1290 Infinity Quaternary Pump - Analysis of RR sample with conventional gradient method



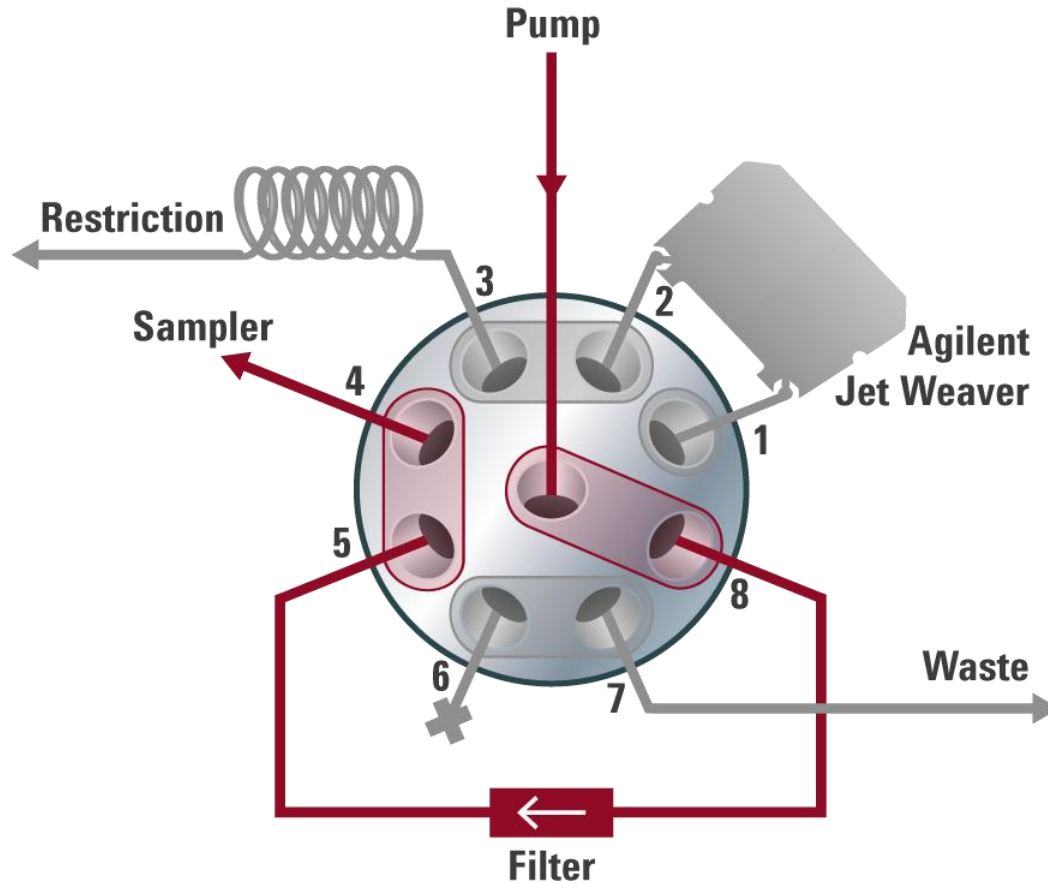
1290 Infinity Quaternary Pump

Various tools enabling the high performance



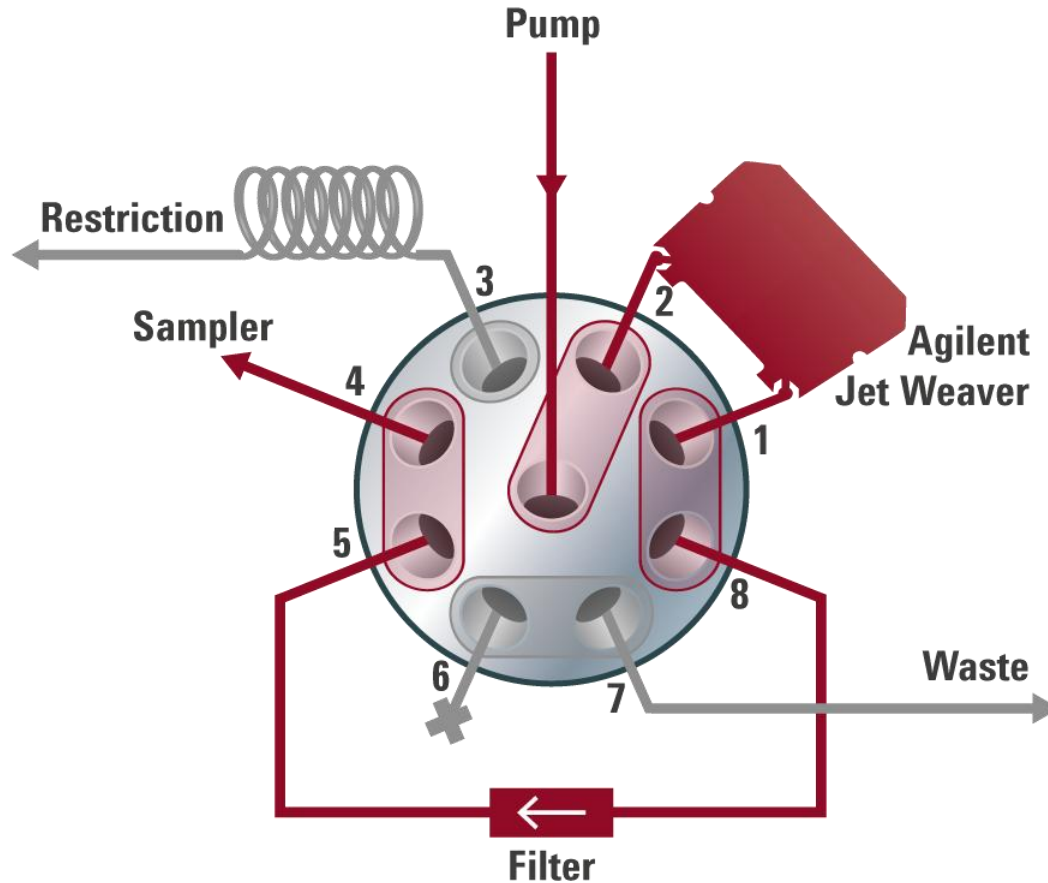
Multipurpose valve functions for highest comfort

Standard Application for standard Delay Volume



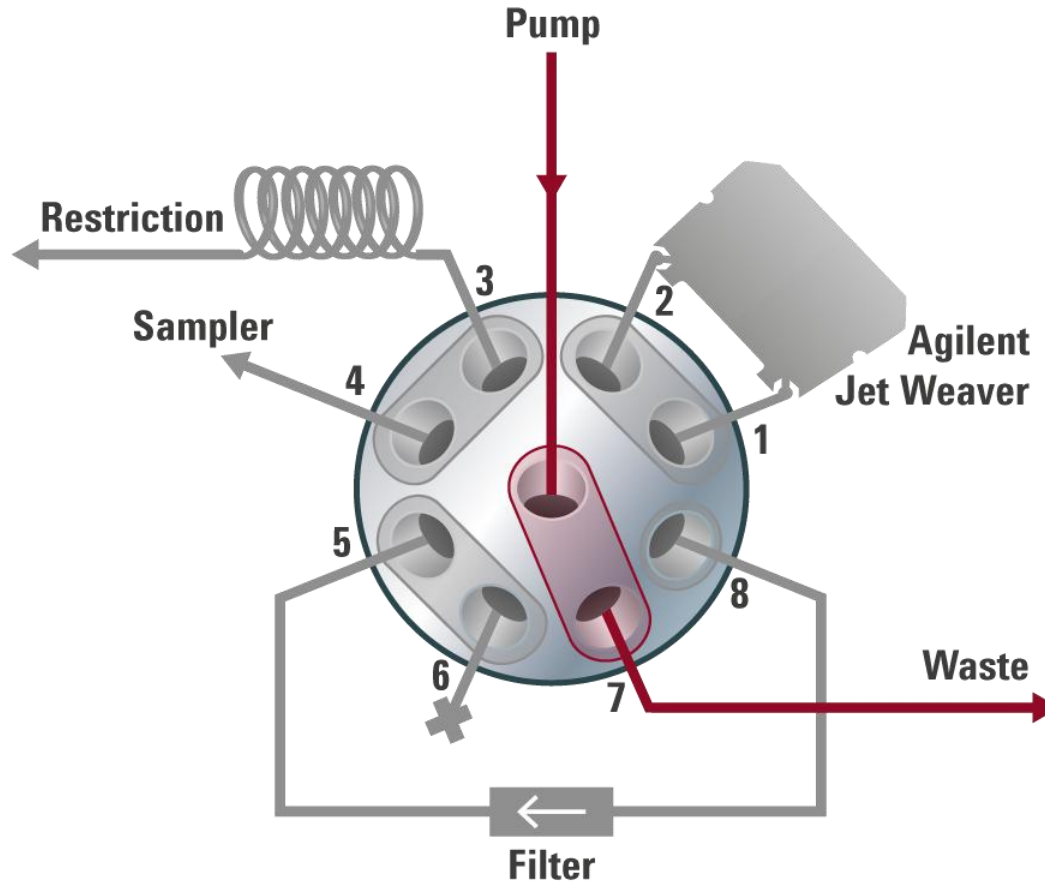
Multipurpose valve functions for highest comfort

Extra Mixing Volume for lowest baseline ripple (TFA applications)



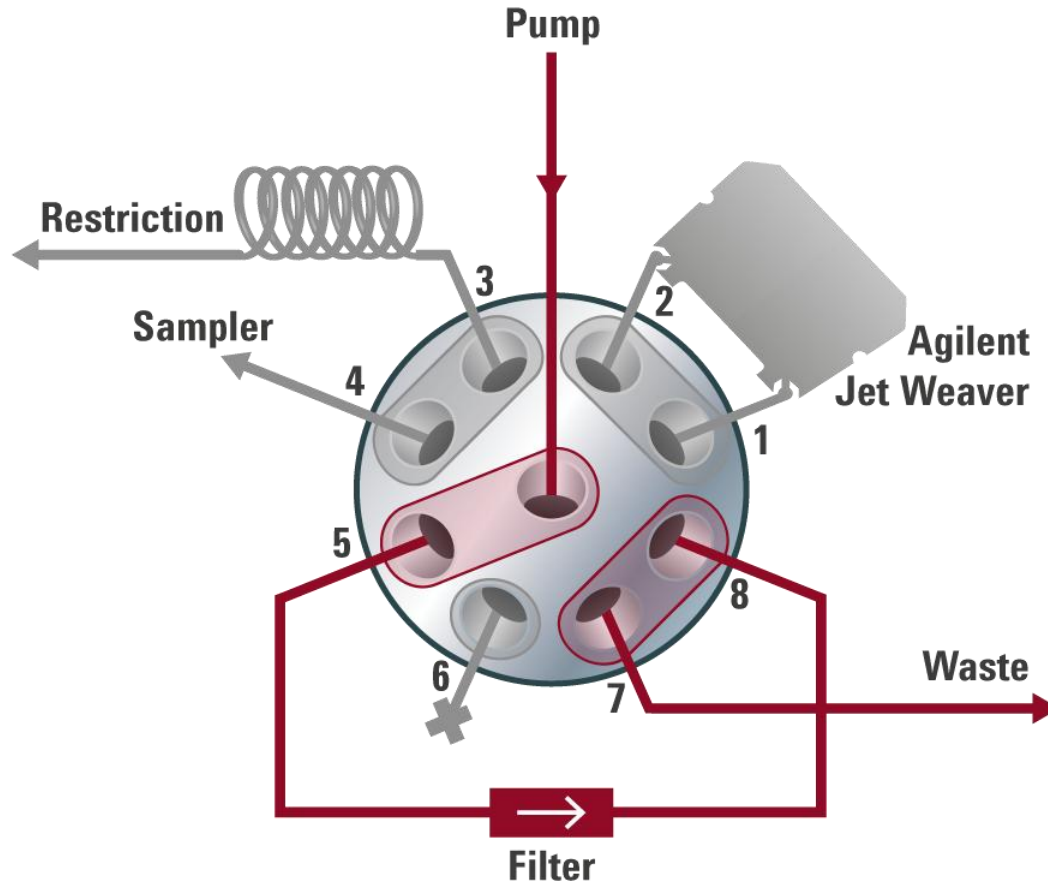
Multipurpose valve functions for highest comfort

Automatic Purging



Multipurpose valve functions for highest comfort

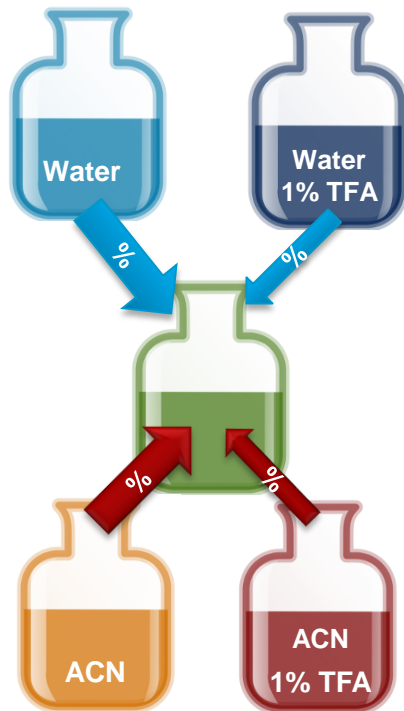
Automatic filter backflush



1290 Infinity Quaternary Pump - BlendAssist

Simple tool for online-dilution of modifiers and gradient set-up

You need different concentrations of modifiers in your analysis, would like to have just one stock-solution and do online dilution to profite from the quaternary mixing capability of your pump? Here is a simple tool – **BlendAssist!**



Desired method conditions - example:

1. 5 to 95% gradient of ACN with 0.1% TFA in Water and 0.08% TFA in ACN
2. 20 – 80% gradient of ACN with 0.5% TFA in Water and 0.4% TFA in ACN

Without BlendAssist you need to either pre-mix the required solvents or by using stock-solutions of TFA in Water and ACN to program complex gradients (%A, B, C, D).

With BlendAssist: just program your binary organic/aqueous gradient and define the dilution factor!

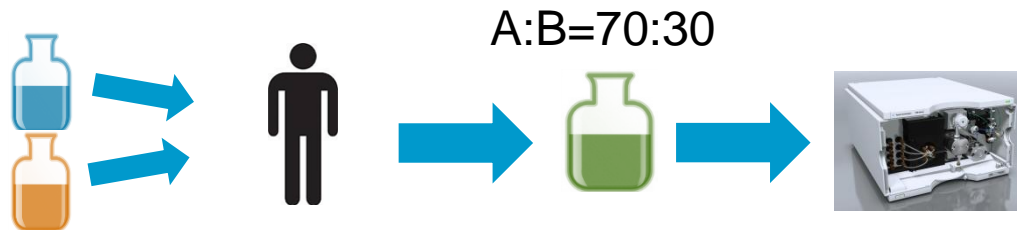
Example: Isocratic analysis of Glucocorticoids

– *dynamically vs. premixed solvents*

Isocratic composition by **dynamic** mixing:



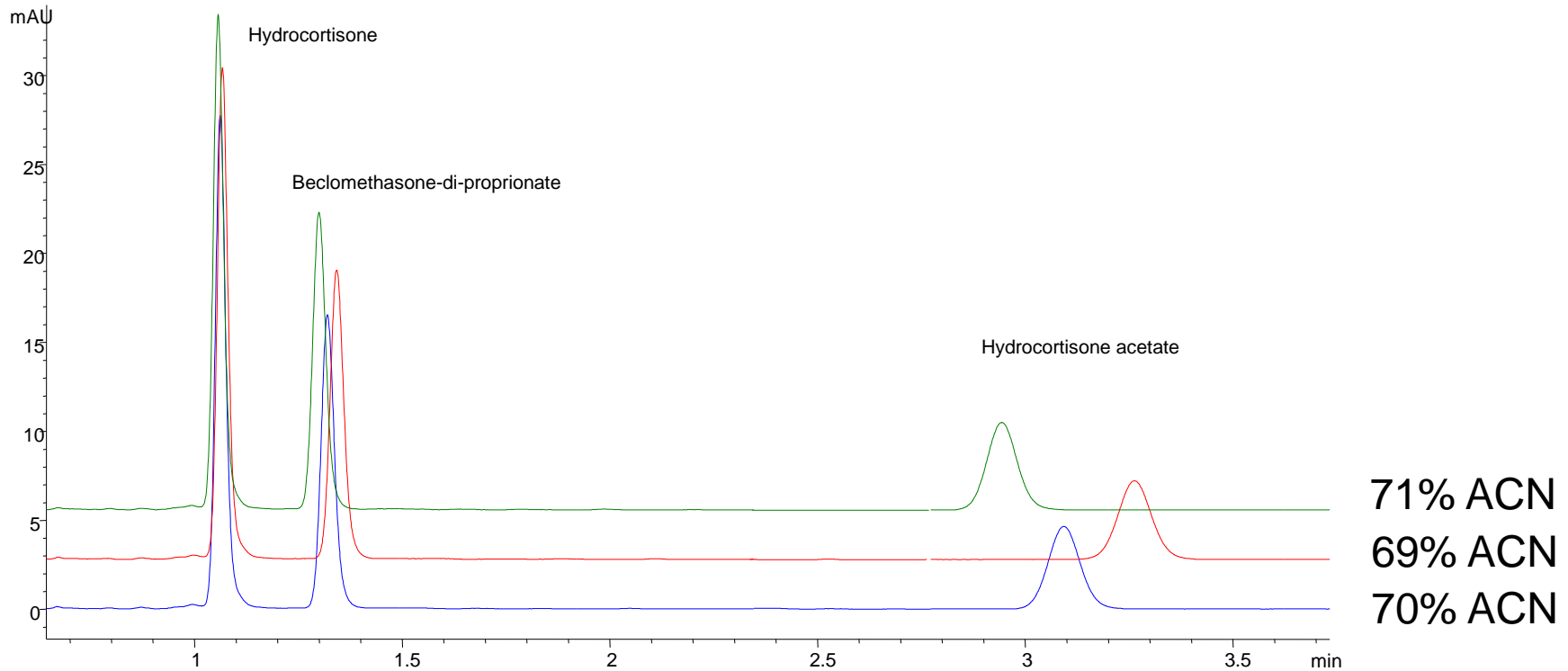
Isocratic composition by **premixed solvent**:



Which method is better?

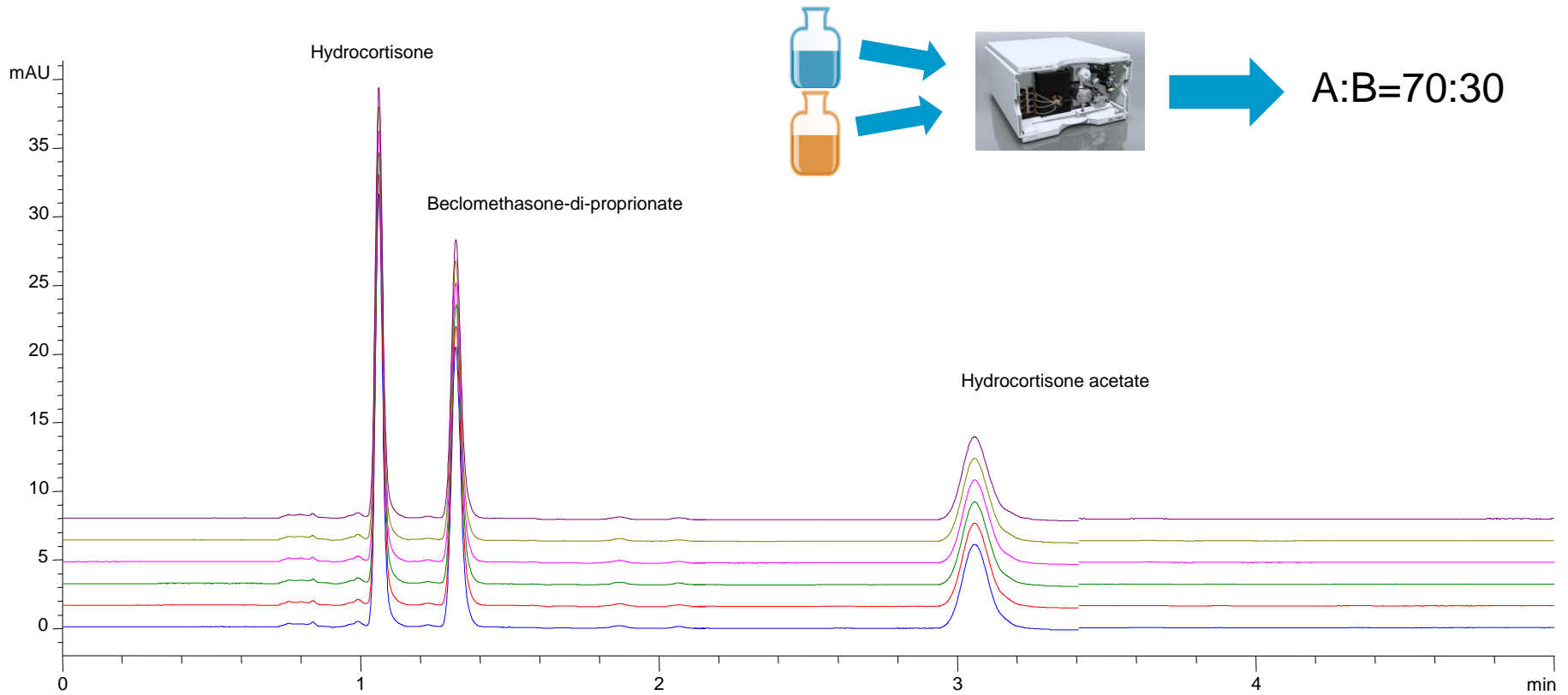
RT Variation based on solvent mixtures

- Influence of 1% deviation of solvent composition



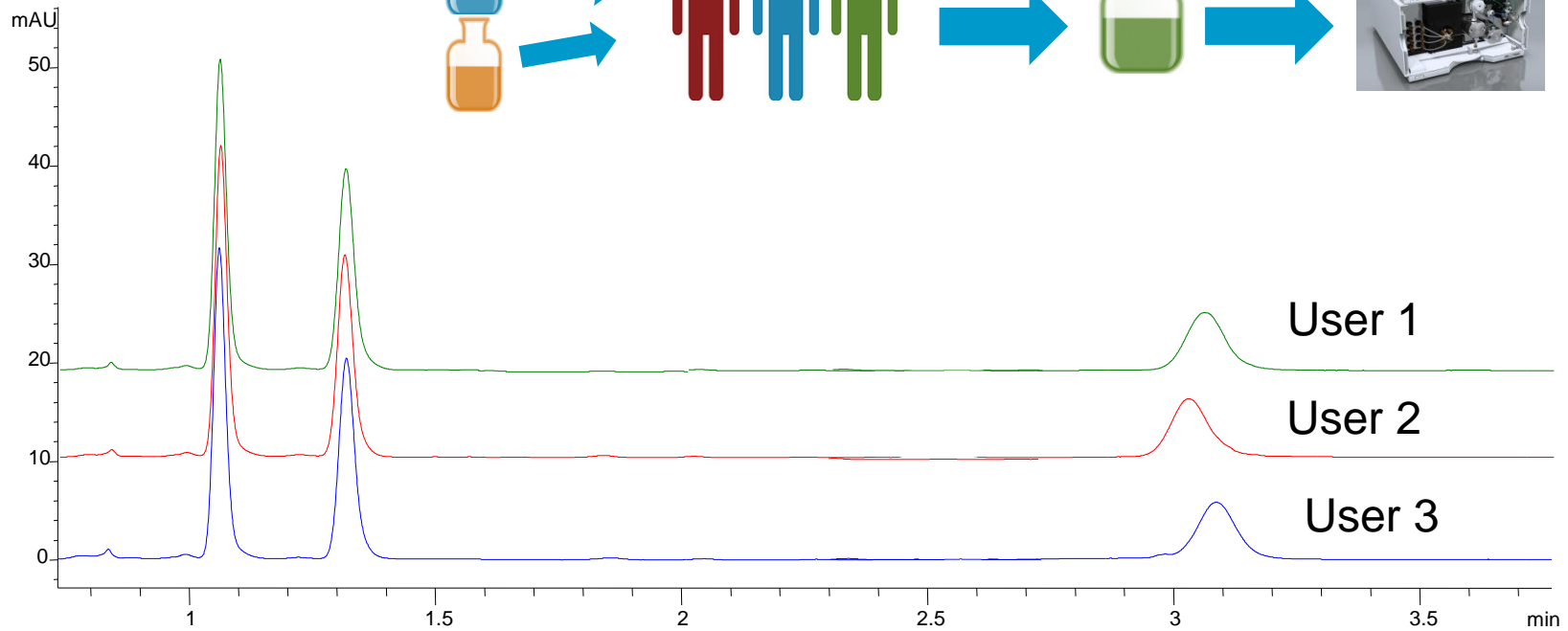
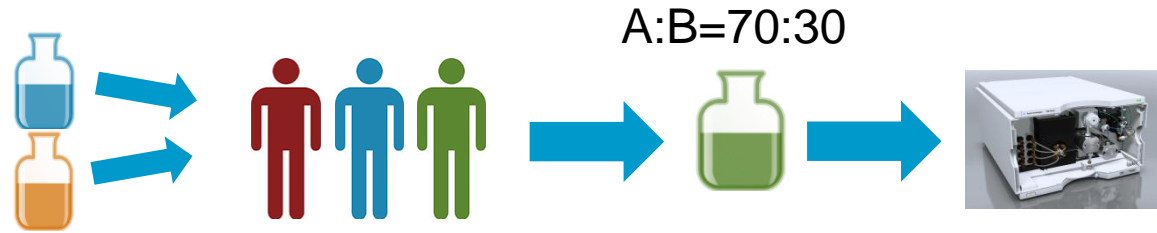
Analysis of Glucocorticoids

- Using a dynamically mixed mobile phase



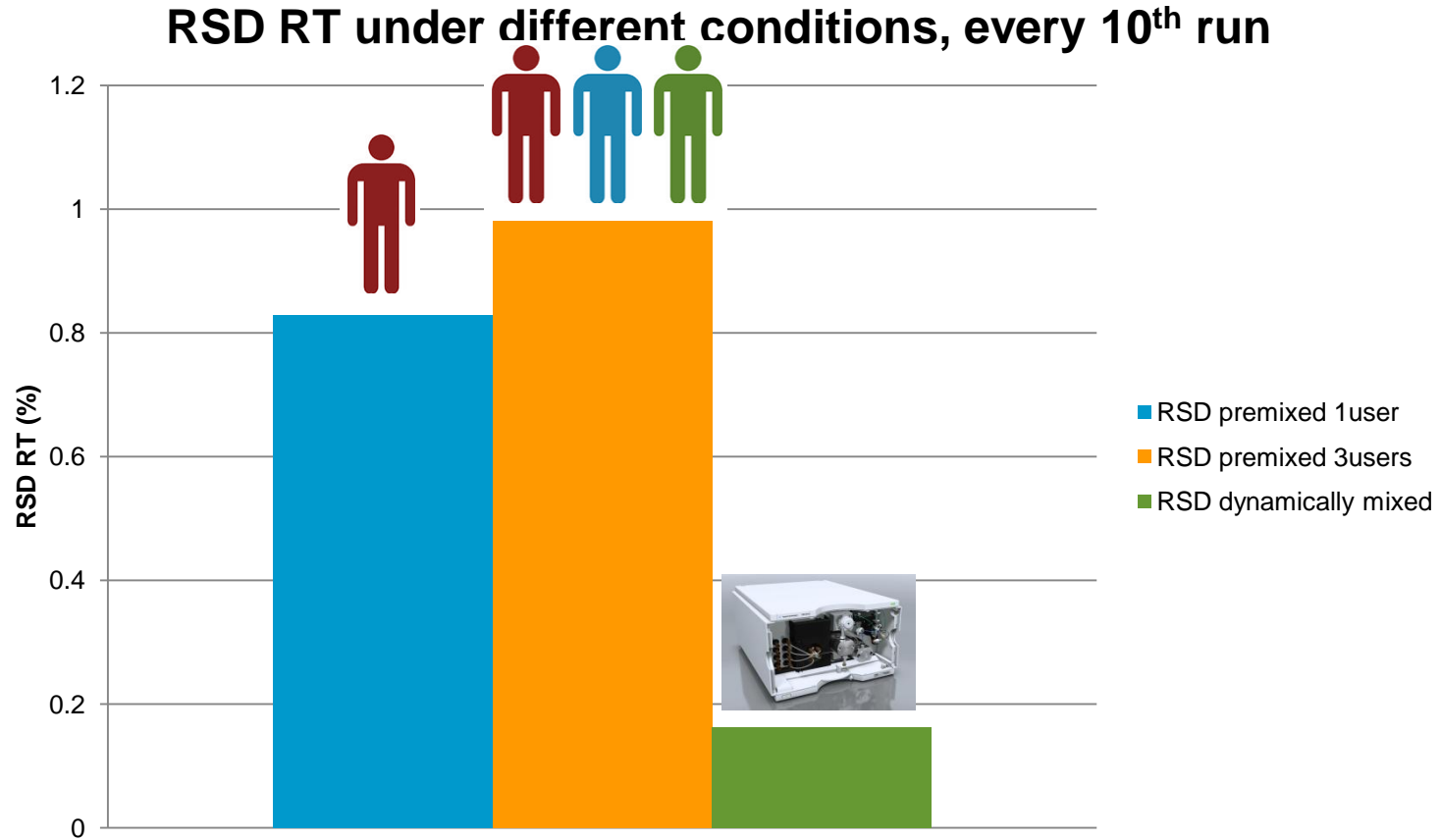
Analysis of Glucocorticoids

- Comparing dynamically mixed mobile phase vs. user influence



Analysis of Glucocorticoids

- Comparing dynamically mixed mobile phase vs. user influence



1290 Infinity Quaternary Pump

Application Areas

- Method Development
- Automated Workflows & Walkup Systems
- Quality Control & Quality Assurance (QA/QC)
- Drug Discovery
- Drug Development
- Food Safety & Environmental Analysis
- Metabolomics
- Forensics & Doping Control
- Front-End for Mass Spectrometer

1290 Infinity Quaternary Pump

Conclusion

- Full 1290 Infinity LC Power range
- Highest composition precision and accuracy, solvent independent and across pressure and flow rate range

→ Full application range with binary-like pump performance!

- Blend Assist for Buffer/Additive Blending
- ISET-ready!

→ Full flexibility!

**VIELEN DANK FÜR IHRE
AUFMERKSAMKEIT!!**

