



Neue Agilent LC Produkte für unterschiedliche Einsatzgebiete

Sebastian Krahe
Produktspezialist LC
Agilent Technologies

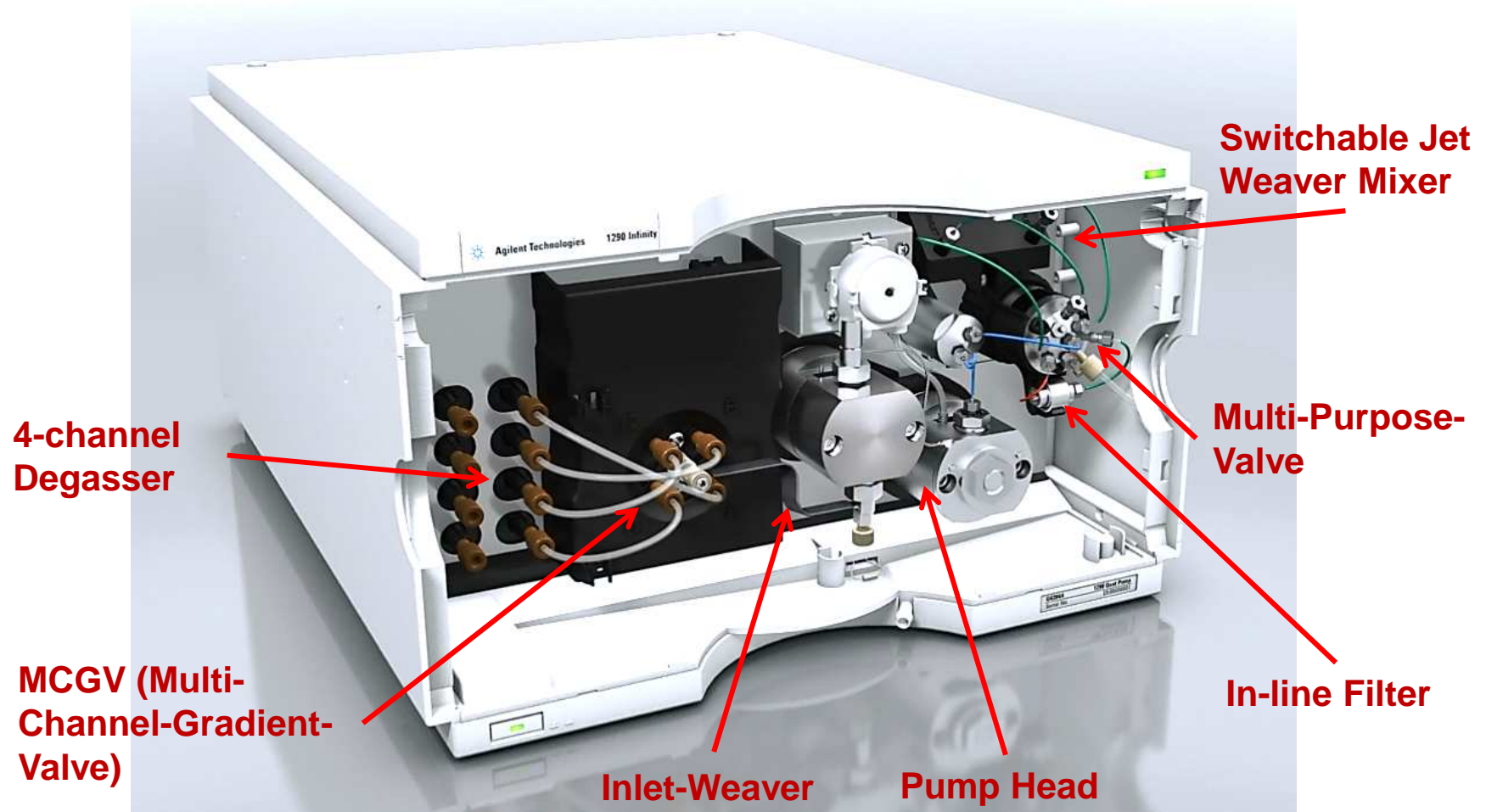
The new Agilent 1290 Infinity Quaternary LC

How binary pump-like performance
can enhance your lab efficiency



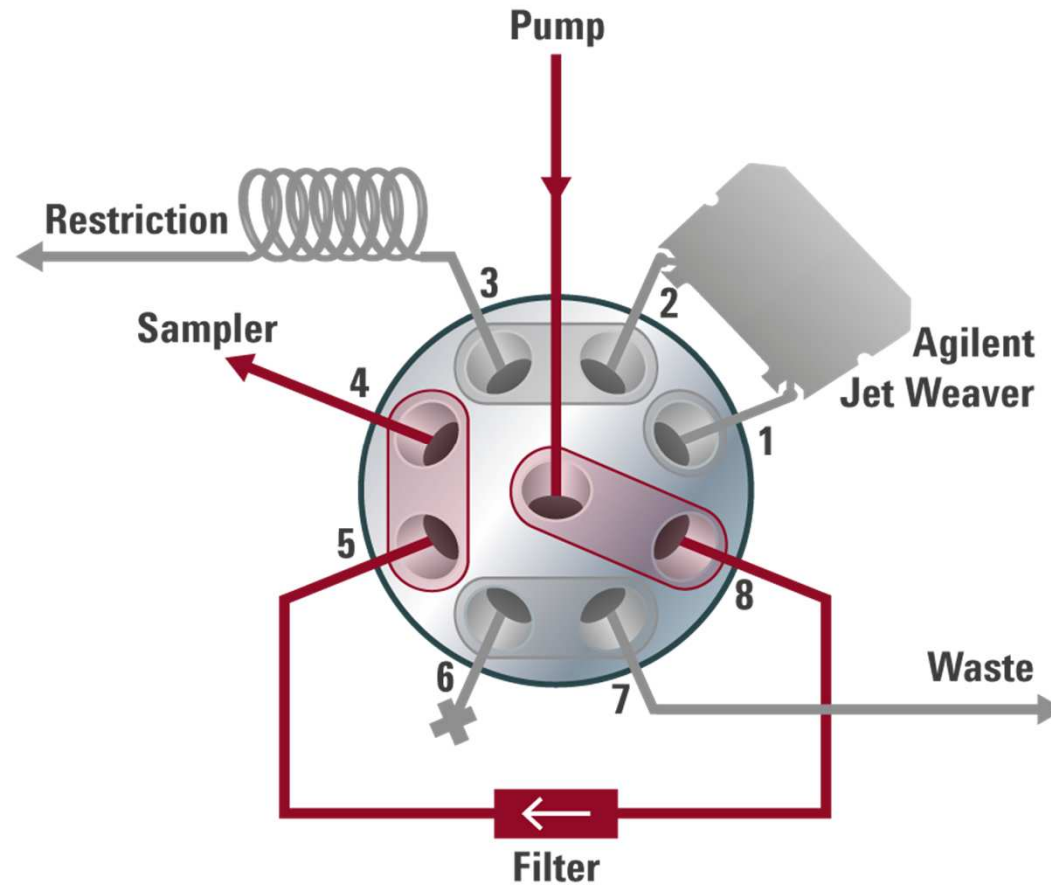
1290 Infinity Quaternary Pump

Various tools enabling the high performance



Multipurpose valve functions for highest comfort

Standard Application for standard Delay Volume



Agilent 1290 Infinity Quaternary Pump

Specifications & Benefits

Power Range

- For any kind of analysis

Composition Accuracy and Precision

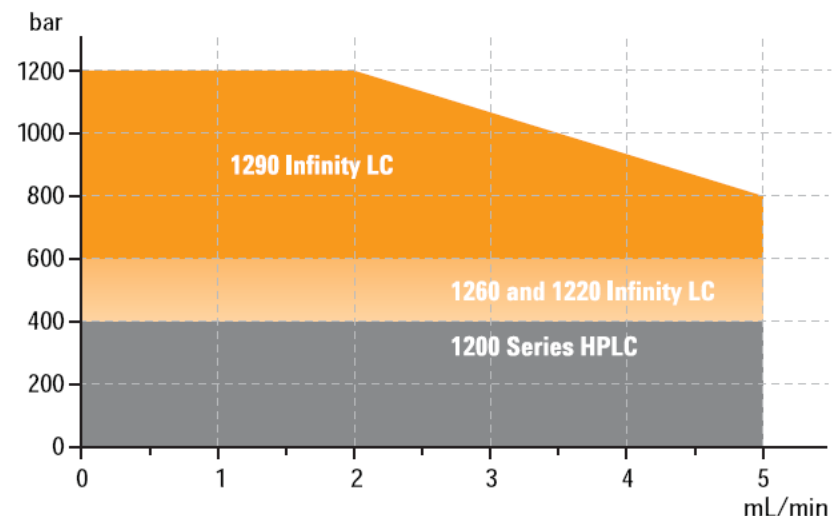
- < 0.15 % RSD or 0.02 min SD
- 0.4 % (1-99 % Composition B)

- High RT precision in gradient runs

Flow Accuracy and Precision

- < 0.07 % RSD or 0.01 min SD
- 1.0 % or 10 μ L

- High RT precision in isocratic runs



Composition Range

- 1-99 %
- Wide analytical range

Delay Volume

- < 350 μ L
- For fast quaternary gradients

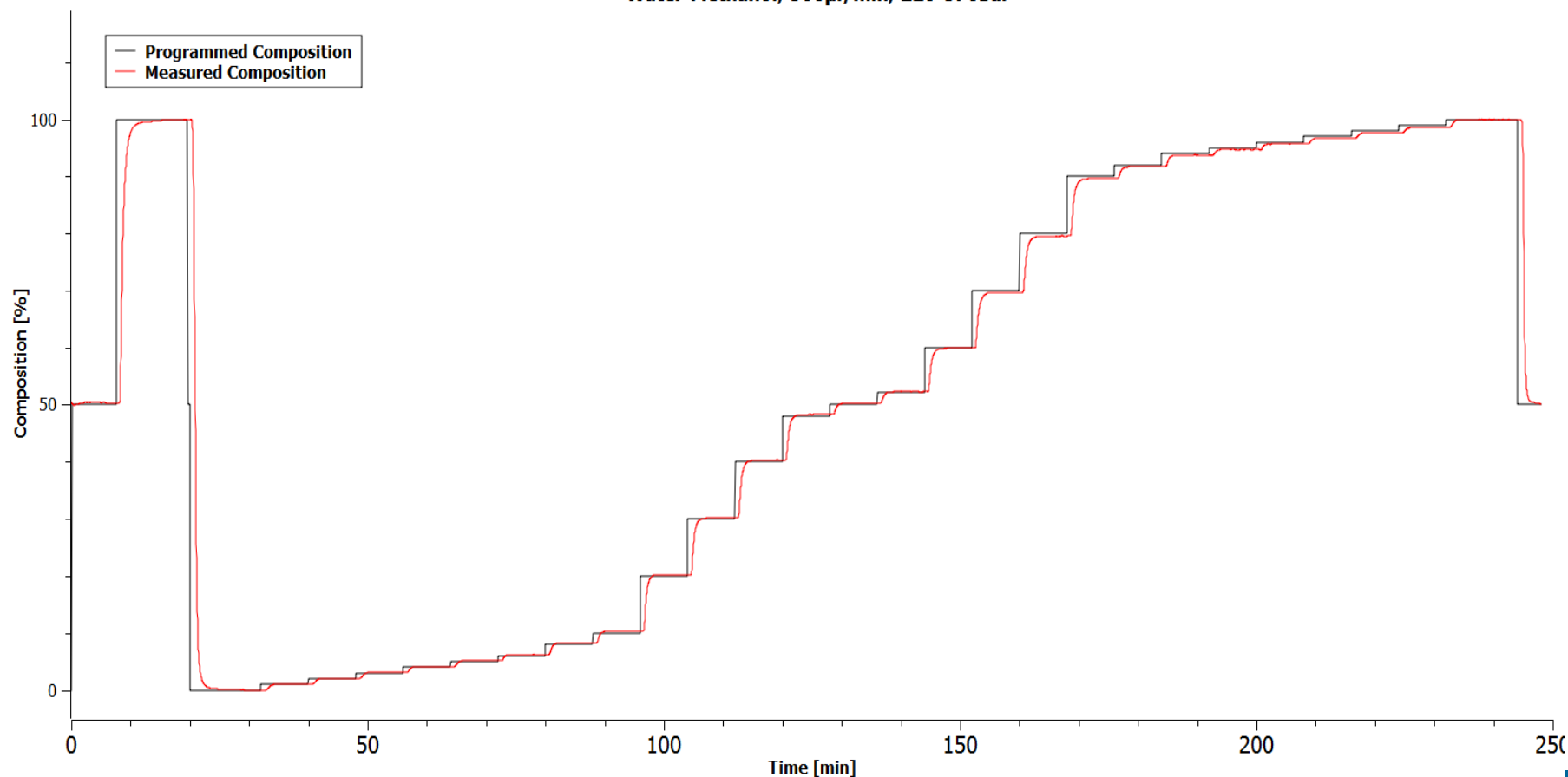
Composition Accuracy and Precision

Step Gradient Analysis with Tracer

- Different mixtures (H₂O, MeOH, ACN) at
- different pressures and
- different flow rates

→ Full application range!

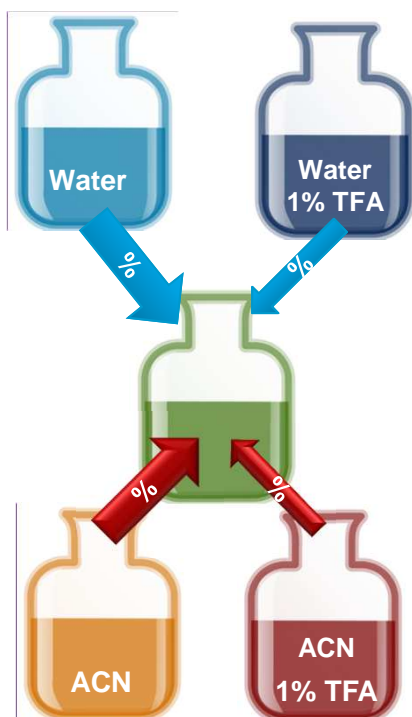
Water-Methanol, 500µl/min, 220-570bar



Ternary/Quaternary Gradients

Blend Assist

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:

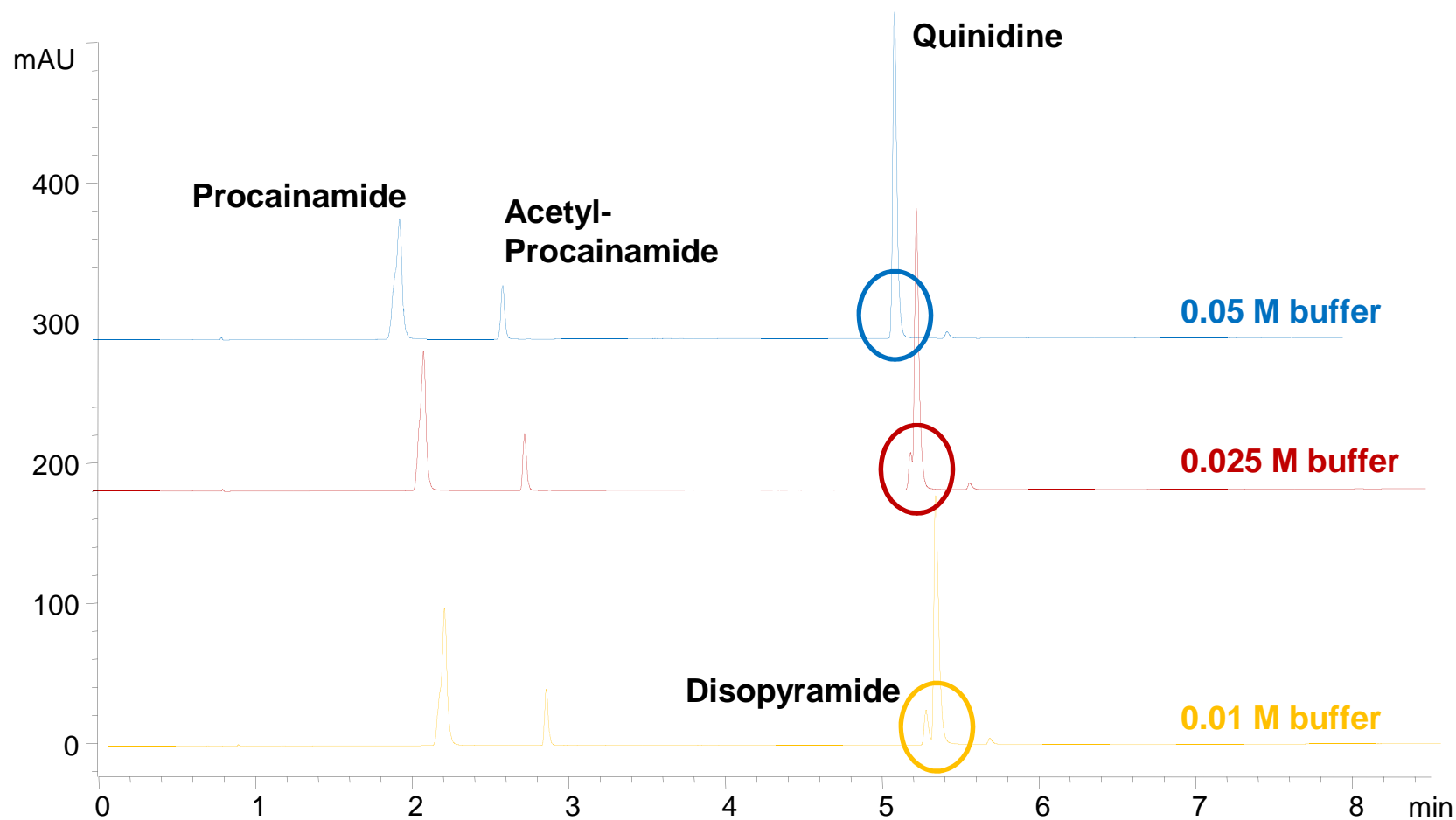
1. 5 to 95% gradient of ACN with 0.1% TFA in Water and 0.08% TFA in ACN
2. 20 – 80% gradient of ACN with 0.5% TFA in Water and 0.4% TFA in ACN

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!

Ternary/Quaternary Gradients

Blend Assist, different Phosphate buffer concentrations



Agilent 1290 Infinity 2D-LC Solution

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



2D-LC - What is it?

2DLC: 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”)

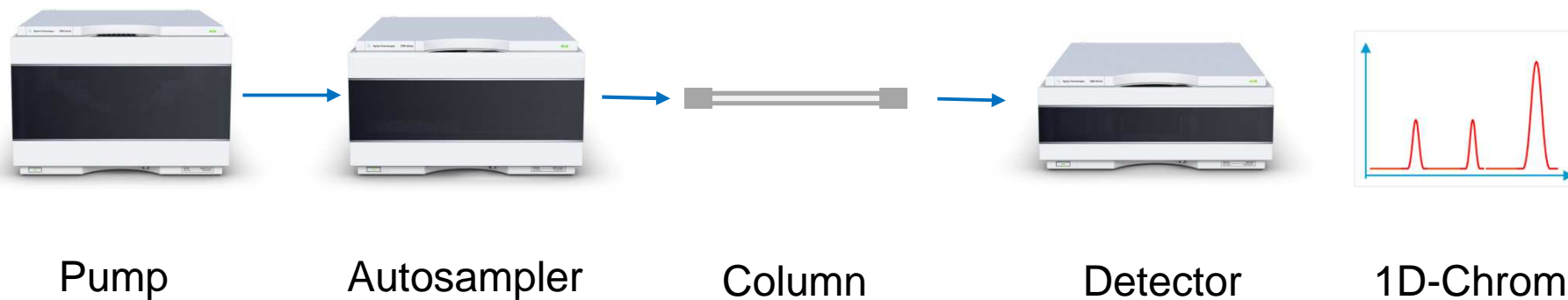
Complex/unknown samples:
bio-pharma, food, polymers....

Heart-cutting 2D-LC (“LC-LC”)

Known samples/improving
confidence: pharma, methdev...

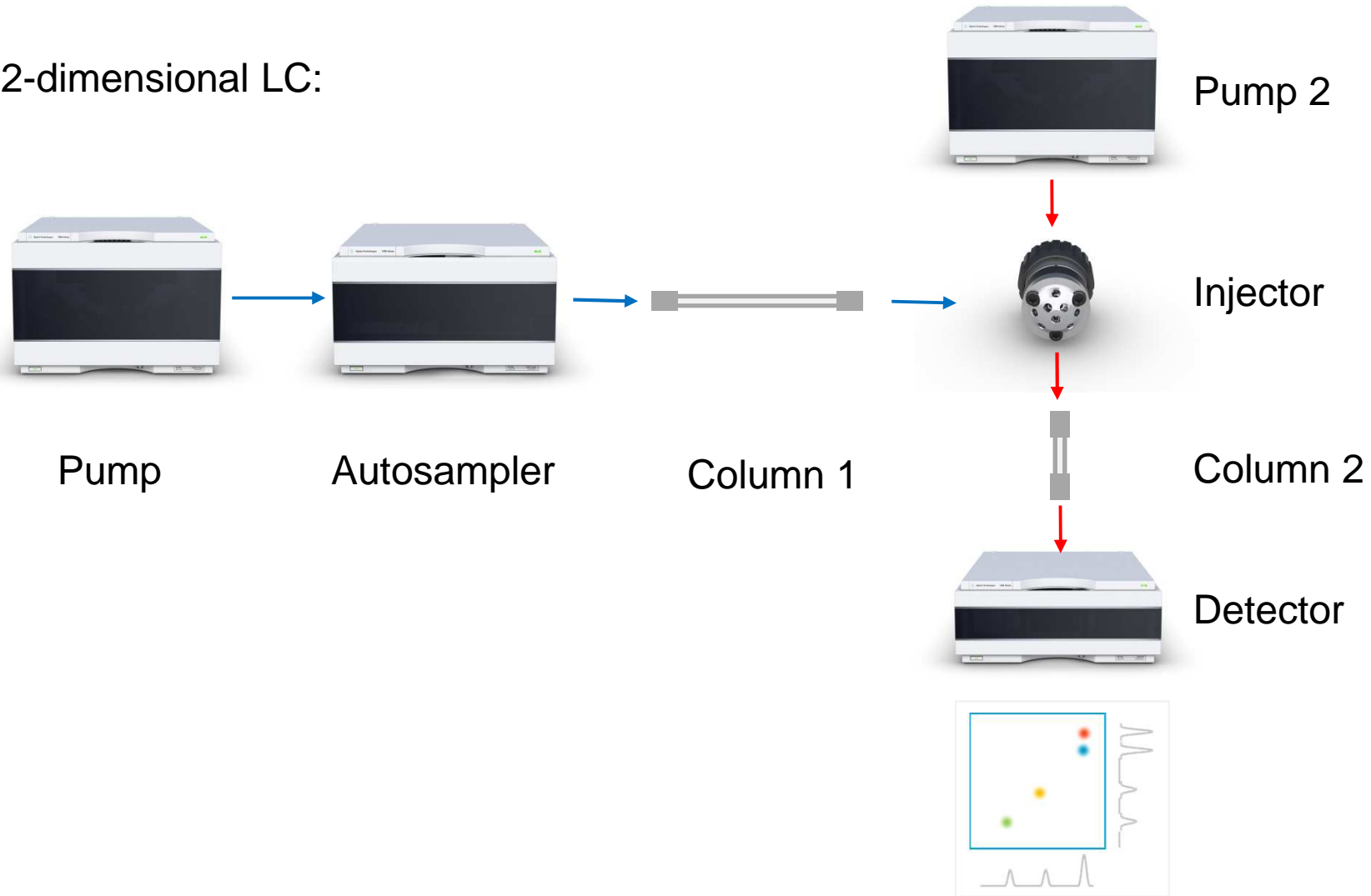
2D-LC - What is it?

Standard 1-dimensional LC:



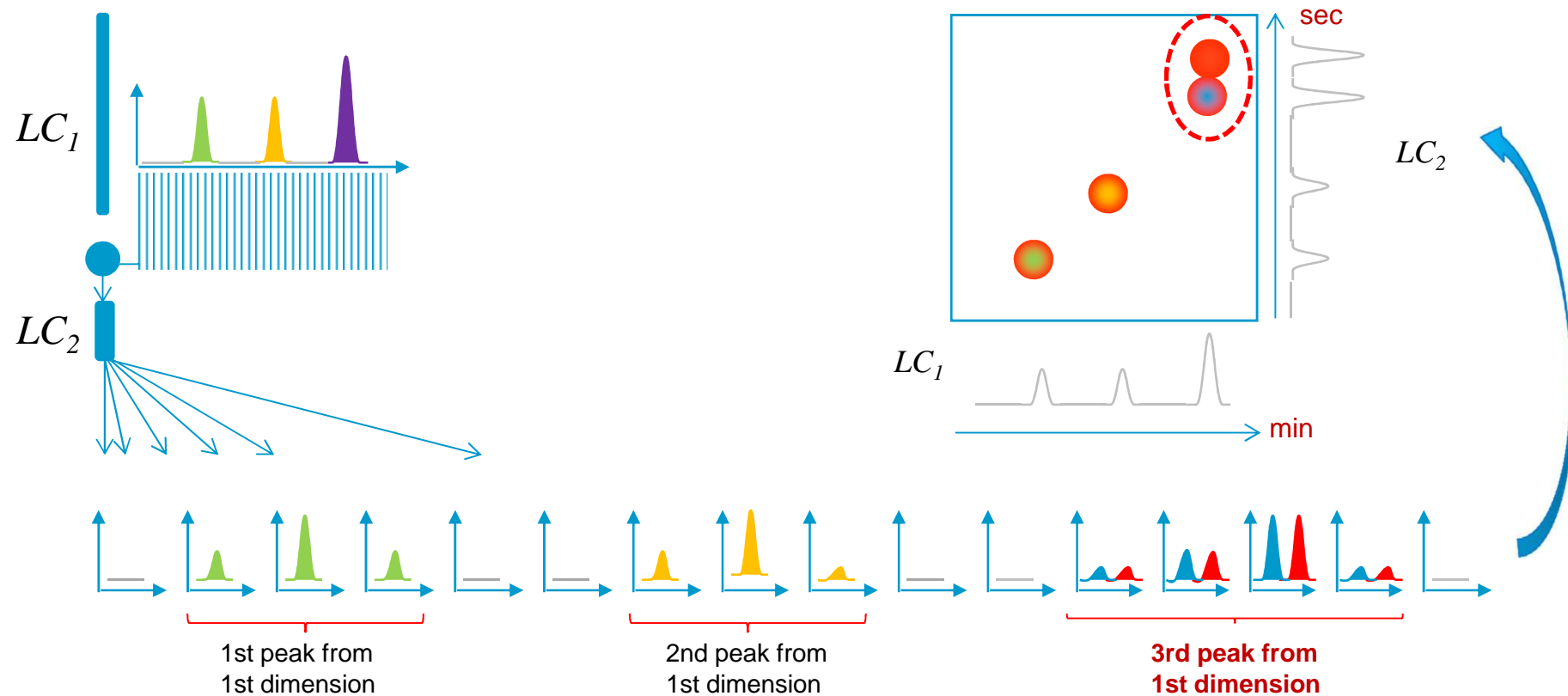
2D-LC - What is it?

2-dimensional LC:



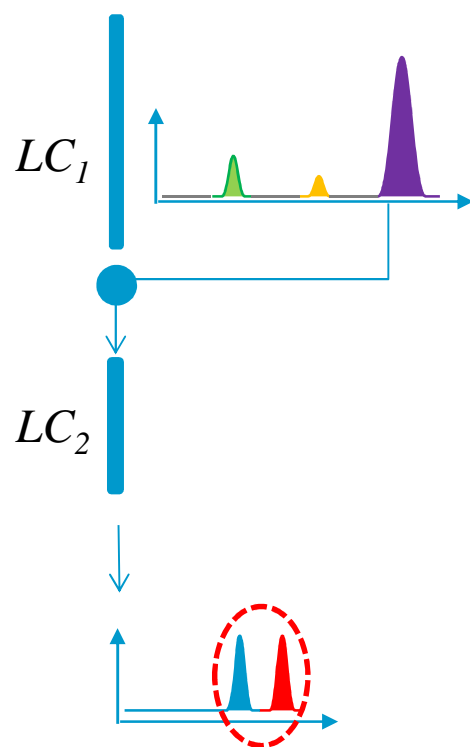
2D-LC - comprehensive vs. heart-cutting 2D-LC

Comprehensive 2D-LC (LCxLC):



2D-LC - comprehensive vs. heart-cutting 2D-LC

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



Only **parts** of the effluent of the first column will be injected to the second column.

The gradients in the 2nd dimension can be much longer as in comprehensive 2D-LC.

Loss of information.
But better quality data for peak of interest

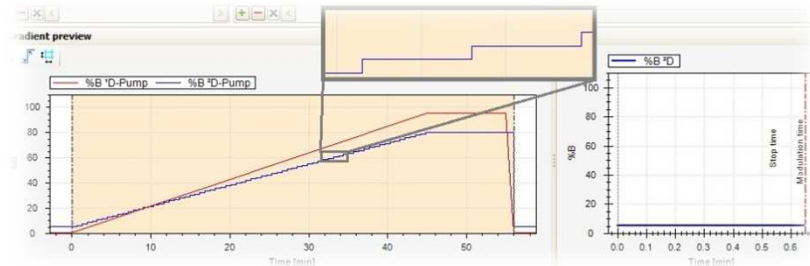
2D-LC Acquisition Software

- supported ²D-gradients modes

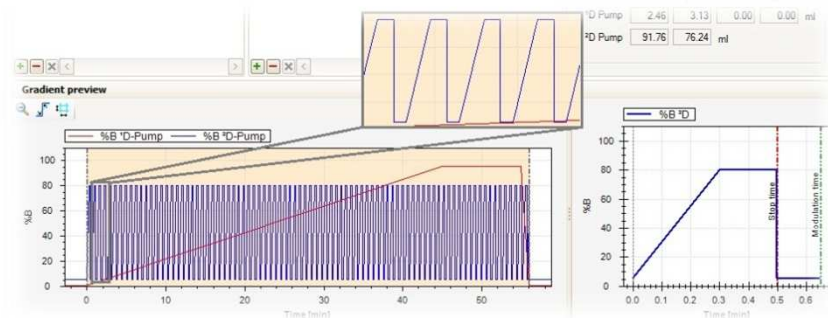
Isocratic



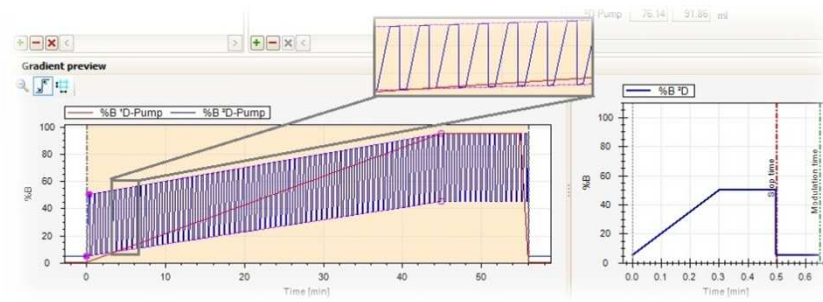
Advancing Isocratic



Std. Gradient



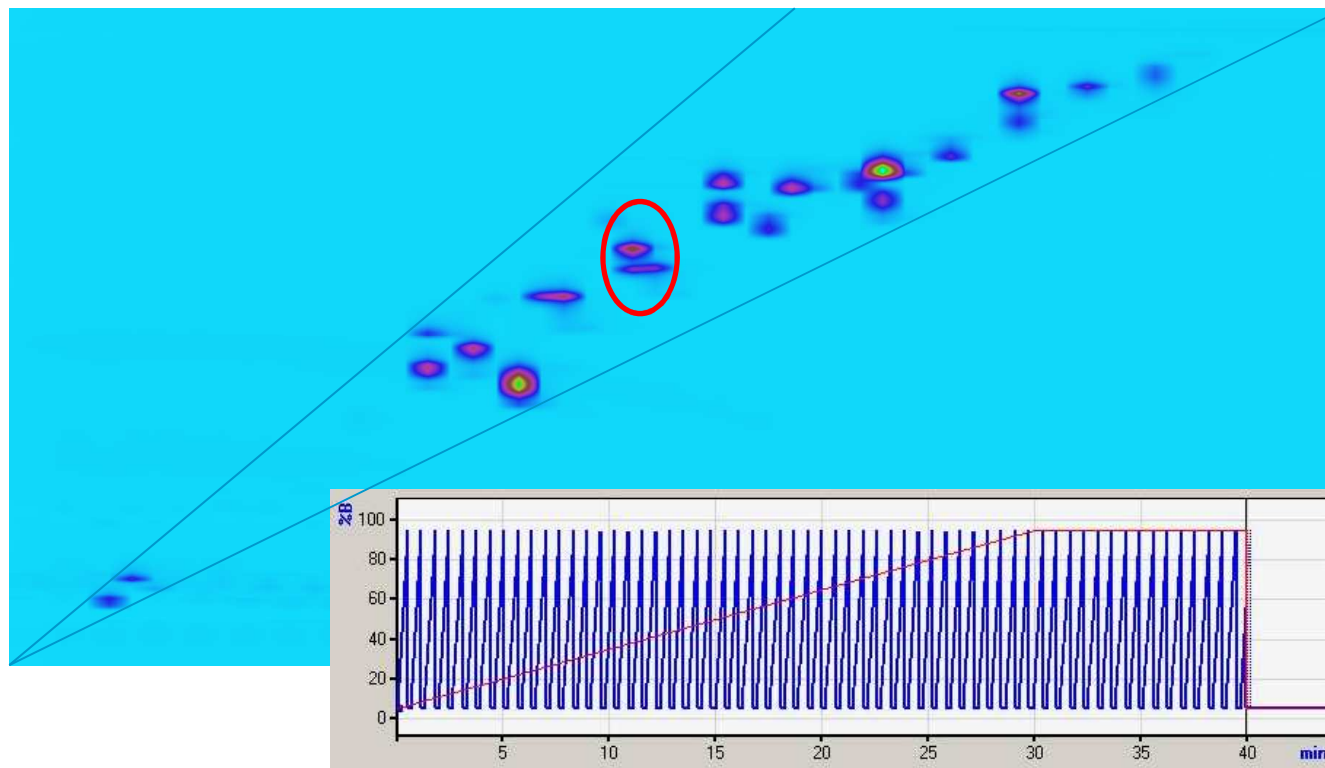
Shifted Gradient



...and most combinations!

Application examples

-Advantage of shifted gradient features



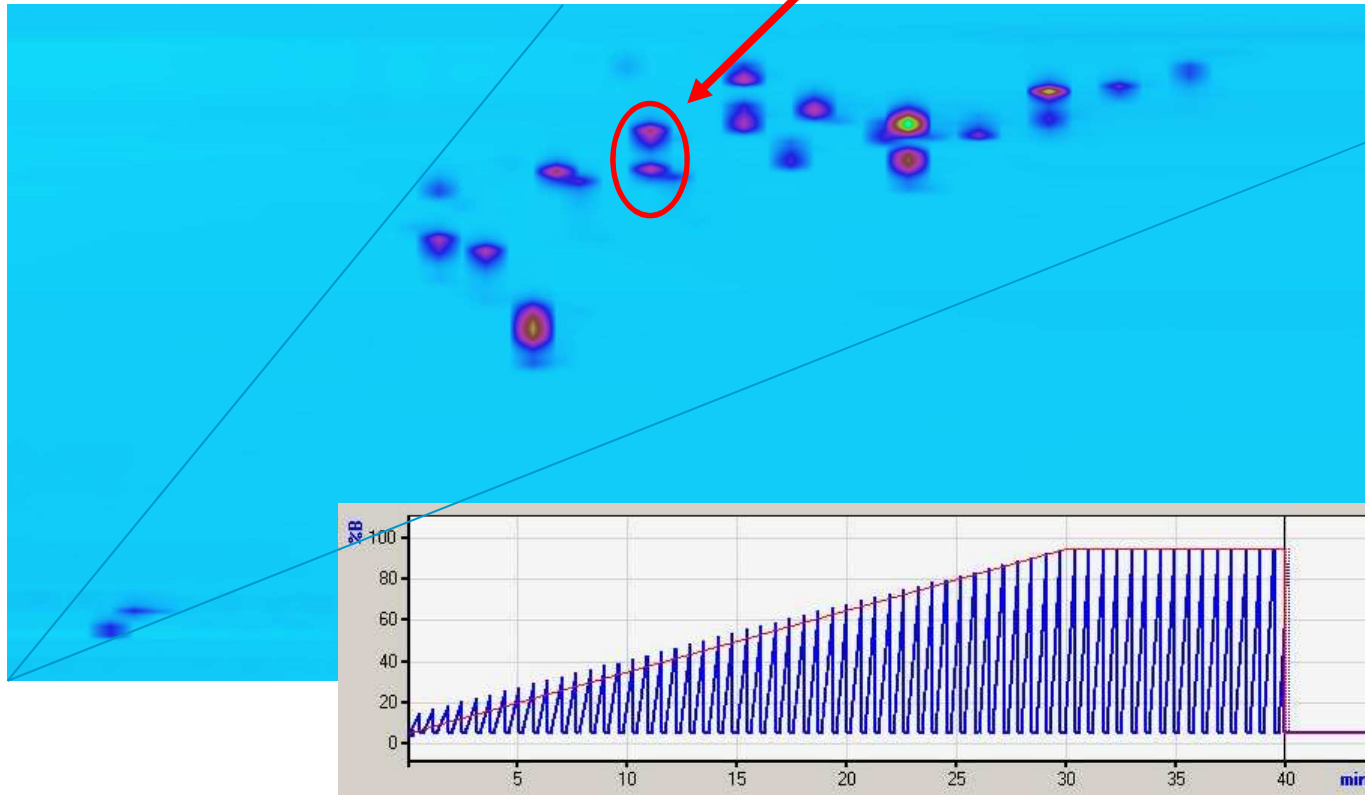
RPLC x RPLC
Easy method set-up but only little orthogonality

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!

THE ART OF EMULATION



Seamless Method Transfer 1260/1220 Infinity

By unchanged critical specification



	1100/1200 Series HPLC	1260 Infinity Quaternary LC	1200 Series RRLC – Std.	1260 Infinity Binary LC
Max Flow Rate	5 mL/min	5 mL/min	5mL/min	5 mL/min
Delay Volume	900-1200 µL	900-1200 µL	900-1200 µL*	900-1200 µL*
Capillary ID	0.17mm	0.17mm	0.17mm	0.17mm
Disp. Vol. w/o cell	15µl	15µl	15µl	15µl
Injection Principle	Variable Loop	Variable Loop	Variable Loop	Variable Loop
Inj. Volume – Std/Ext.	100 / 1500 µL	100 / 1500 µL	100 / 1500 µL	100 / 1500 µL
Area RSD	<0.25 %	<0.25 %	<0.25 %	<0.25 %
Oven Design	A	A	A	A
Column Length	300 mm	300 mm	300 mm	300 mm

Optimized for 3 – 4.6mm ID Columns

* Smaller delay volumes possible

Seamless Method Transfer

What if critical specification change ...?

* Optimized for 2.1 - 4.6mm ID
** Optimized for 2.1mm ID

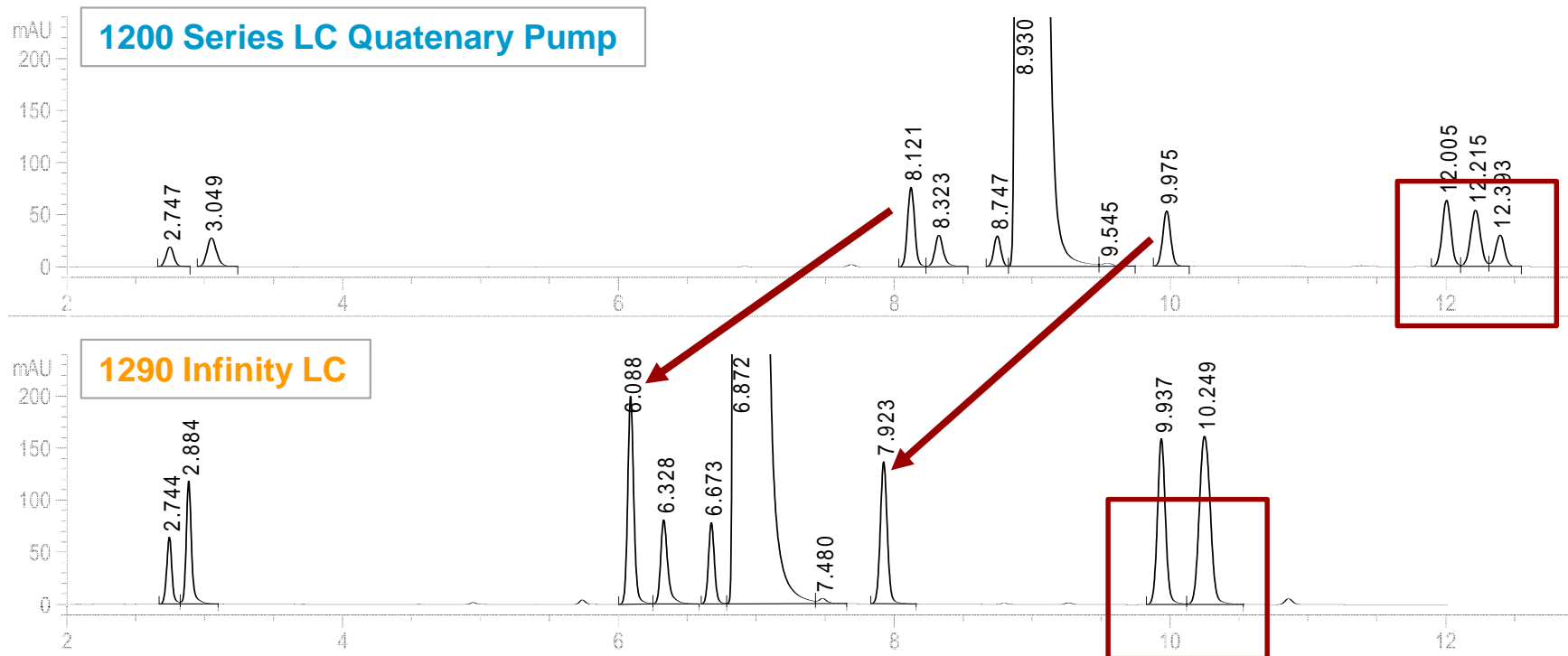


	1100/1200 Series HPLC	1260 Infinity Quaternary LC	1200 Series RRLC – Std.	1260 Infinity Binary LC	1260 Infinity Binary LC	1290 Infinity Binary LC
Max Flow Rate	5 mL/min	5 mL/min	5mL/min	5 mL/min	5 mL/min	5 mL/min
Delay Volume	900-1200 µL	900-1200 µL	900-1200 µL*	900-1200 µL*	340 µL**	10 – 110 µL*
Capillary ID	0.17mm	0.17mm	0.17mm	0.17mm	0.12mm**	0.12mm*
Disp. Vol. w/o cell	15µl	15µl	15µl	15µl	7.5µL**	7.5µL*
Injection Principle	Variable Loop	Variable Loop	Variable Loop	Variable Loop	Variable Loop	Variable Loop
Inj.Vol. – Std/Ext.	100 / 1500 µL	100 / 1500 µL	100 / 1500 µL	100 / 1500 µL	100 / 1500 µL	100 µL
Area RSD	<0.25 %	<0.25 %	<0.25 %	<0.25 %	<0.25 %	<0.25 %
Oven Design	A	A	A	A	A	A
Column Length	300 mm	300 mm	300 mm	300 mm	300 mm	300 mm

* Smaller delay volumes possible
The Measure of Confidence

Gradient Analyses with Different LC Systems

- Impact of delay volume and mixing behavior



Sample: 0.5% impurities in formulation (metoclopramide)
 Xbridge C18, 150x3 mm, 3.5 μ m dp,
 0.45 ml/min, Eluent: A = 0.25 % AmAc, B = ACN,
 Gradient: 0-15 min; 5-57 % B

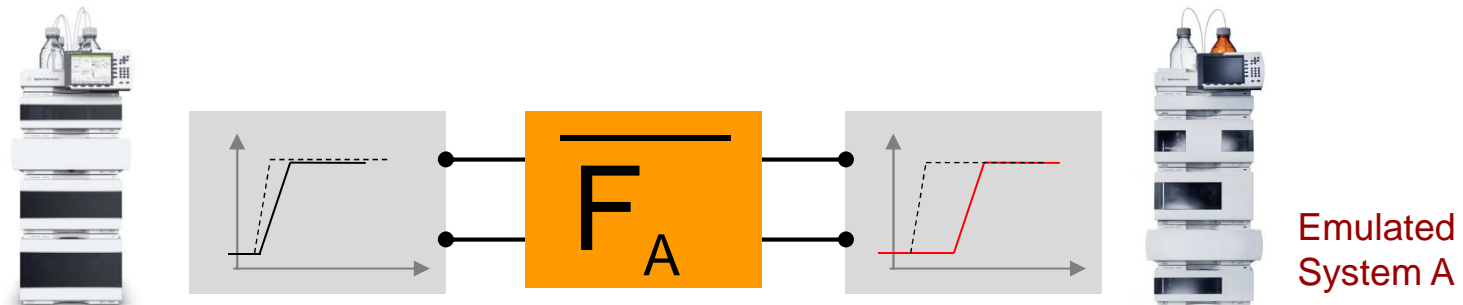
The result:

- Difference in RT and Resolution
- One peak is missing!

Method Transferability: 1290 Infinity LC

- System Emulation Technology

Concept



Select System to be emulated by a simple mouse click:



Agilent
1100 Series



Agilent 1200
Series LC



Agilent 1260
Infinity LC



Agilent 1220
Infinity LC



Alliance
(August 2012)

Intelligent System Emulation Technology ISET Implementation



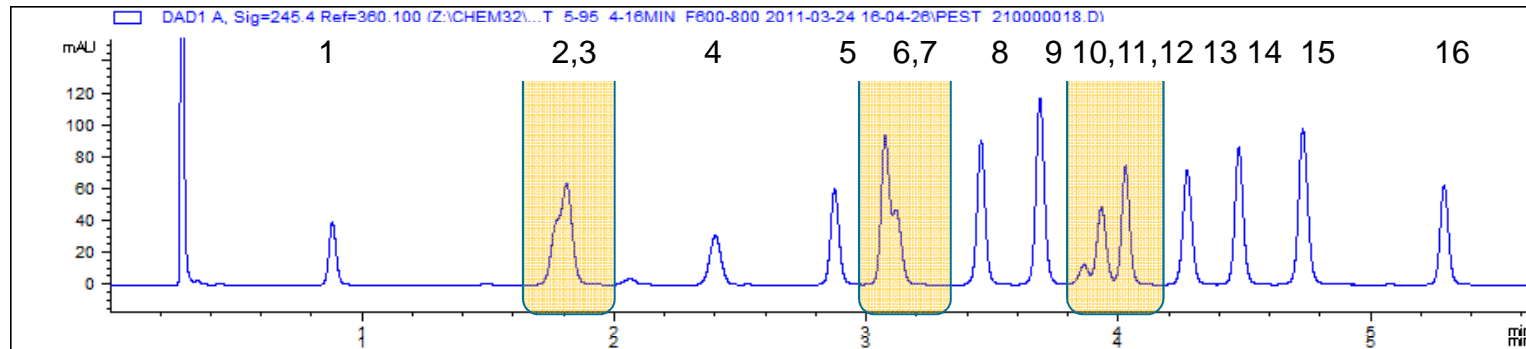
Select pump

Select sampler

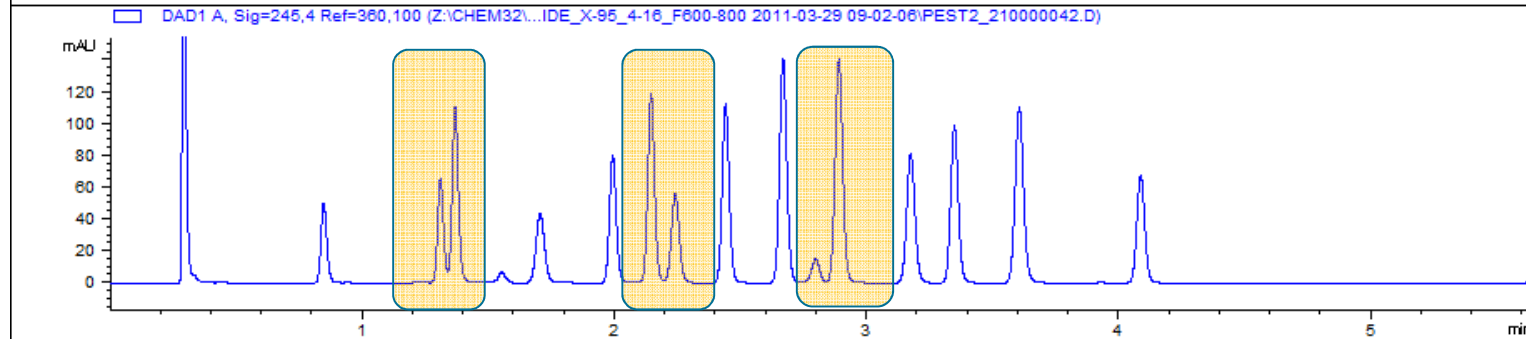
Method transfer, pesticide example

2.1x100 mm Zorbax Eclipse Plus, 1.8 μm column, flow: 0,8 mL/min

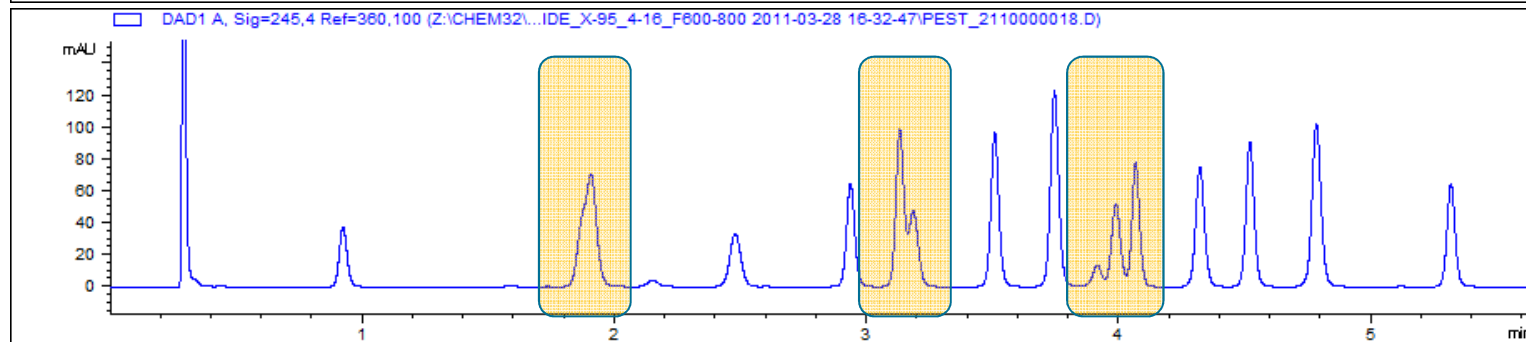
(A)
1100
Quaternary
400 bar



(B)
1290 Infinity
1200 bar



(C)
1290 Infinity
1200 bar
with ISET



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

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

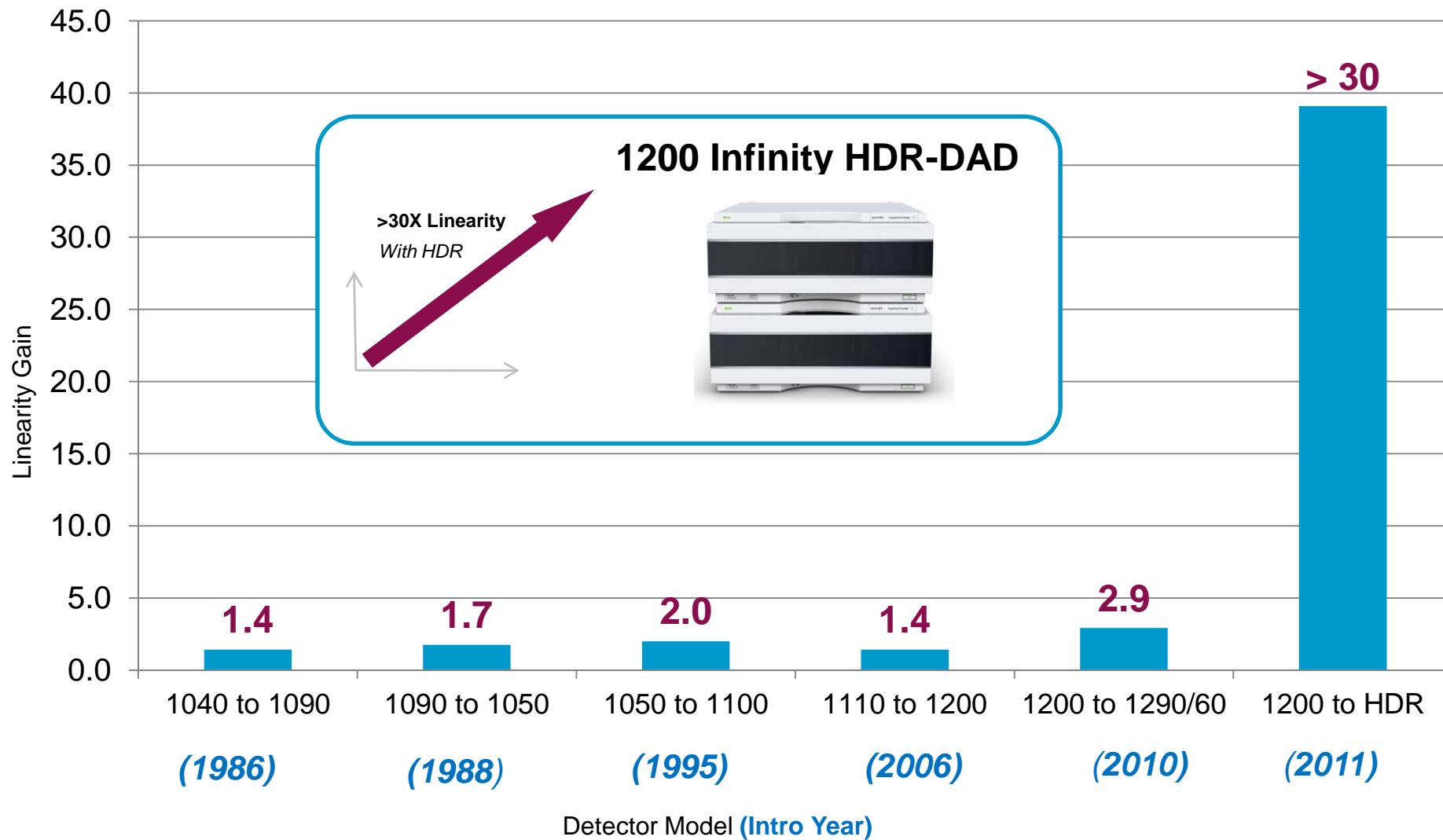


1260/1290 Infinity HDR-DAD



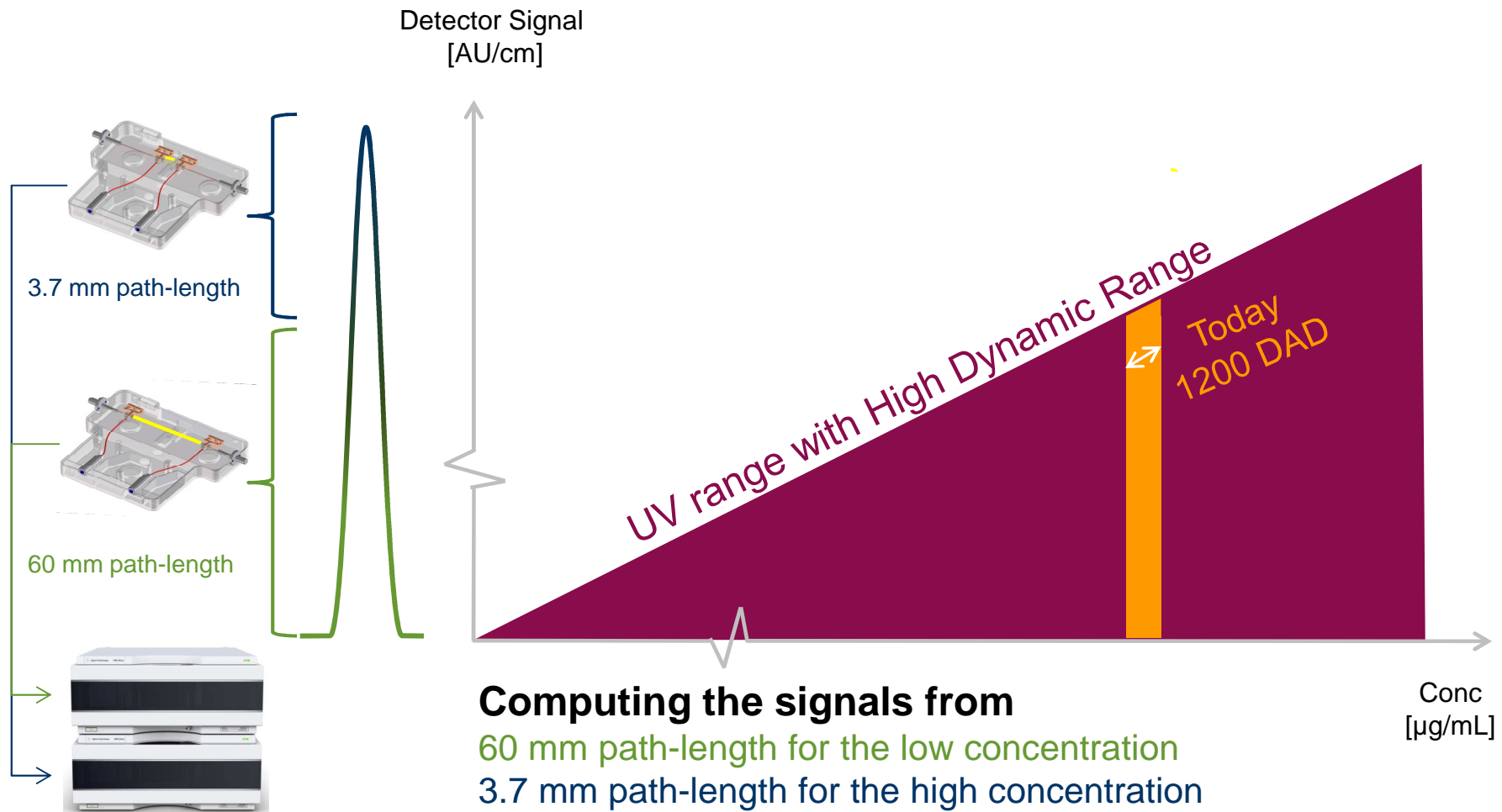
History of DAD Linearity Gain

The last 30 years

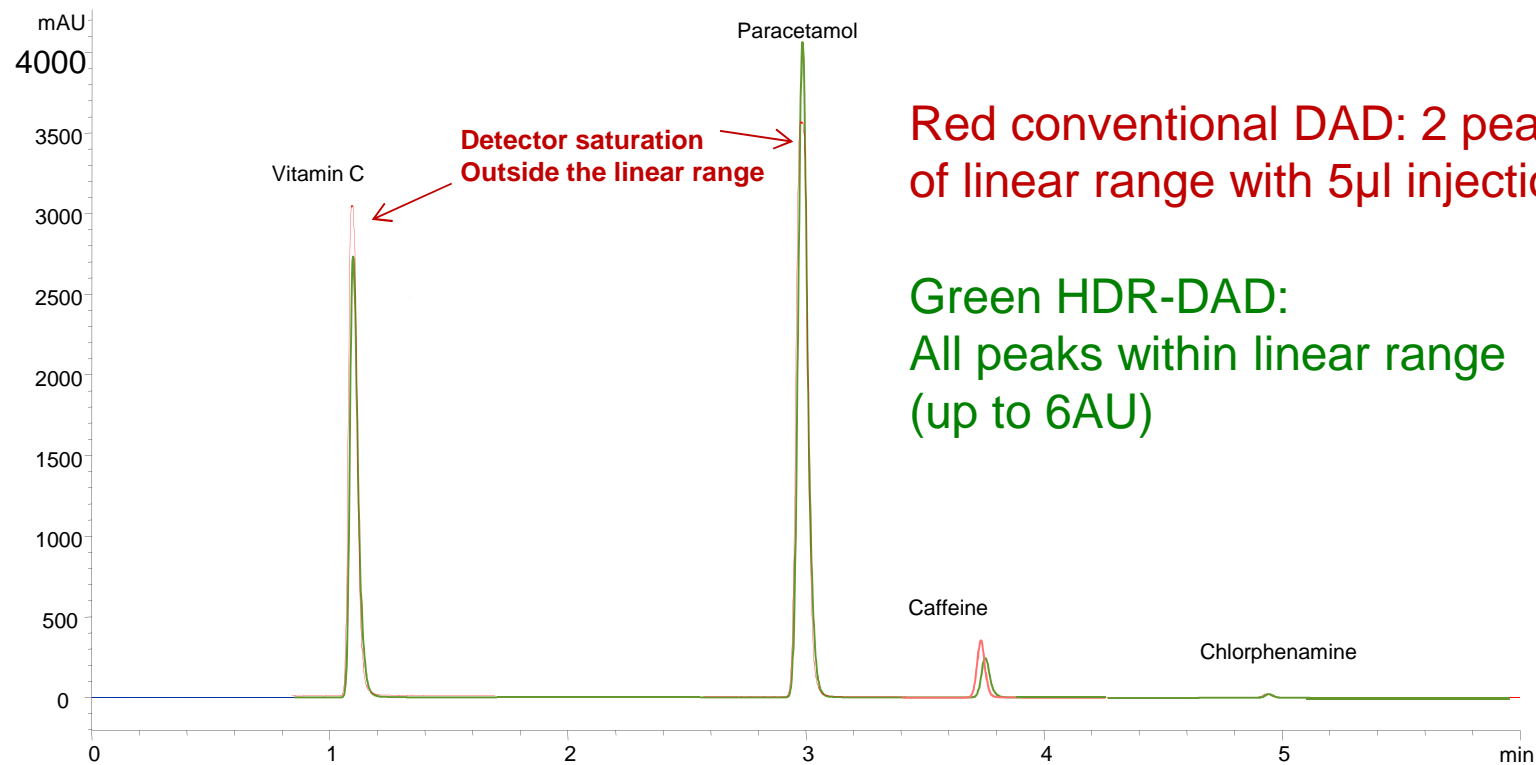


30x Wider Linear Range with HDR-DAD

3.7 mm and 60 mm and Max-Light flow cell



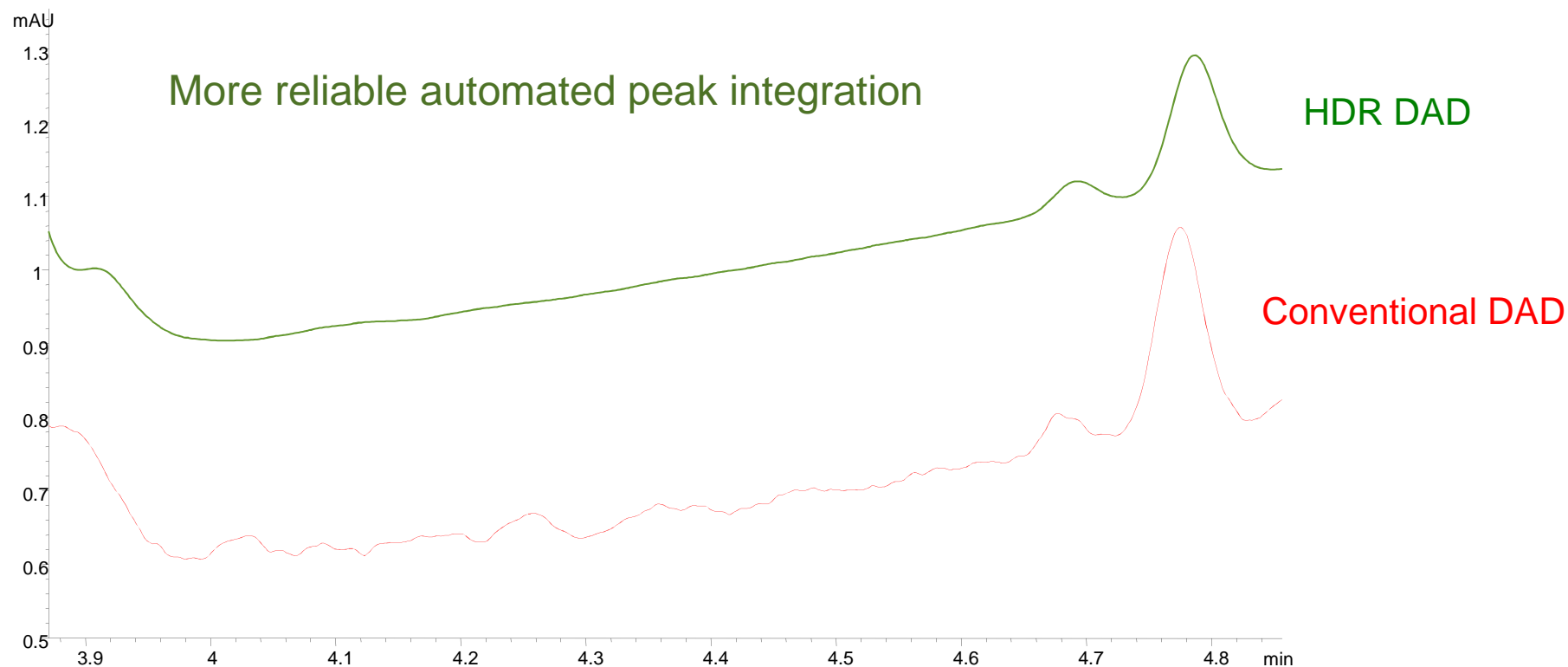
Comparison of conventional DAD vs. HDR DAD, 5 μ l injection



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

Green HDR-DAD:
All peaks within linear range (up to 6AU)

Comparison of LOD



Compound	LOD with S/N=3 for Conventional DAD	LOD with S/N=3 for HDR- DAD solution
Chlorphenamine	~1ng	~0.1ng

*What needs to be considered with
narrow-bore columns:*

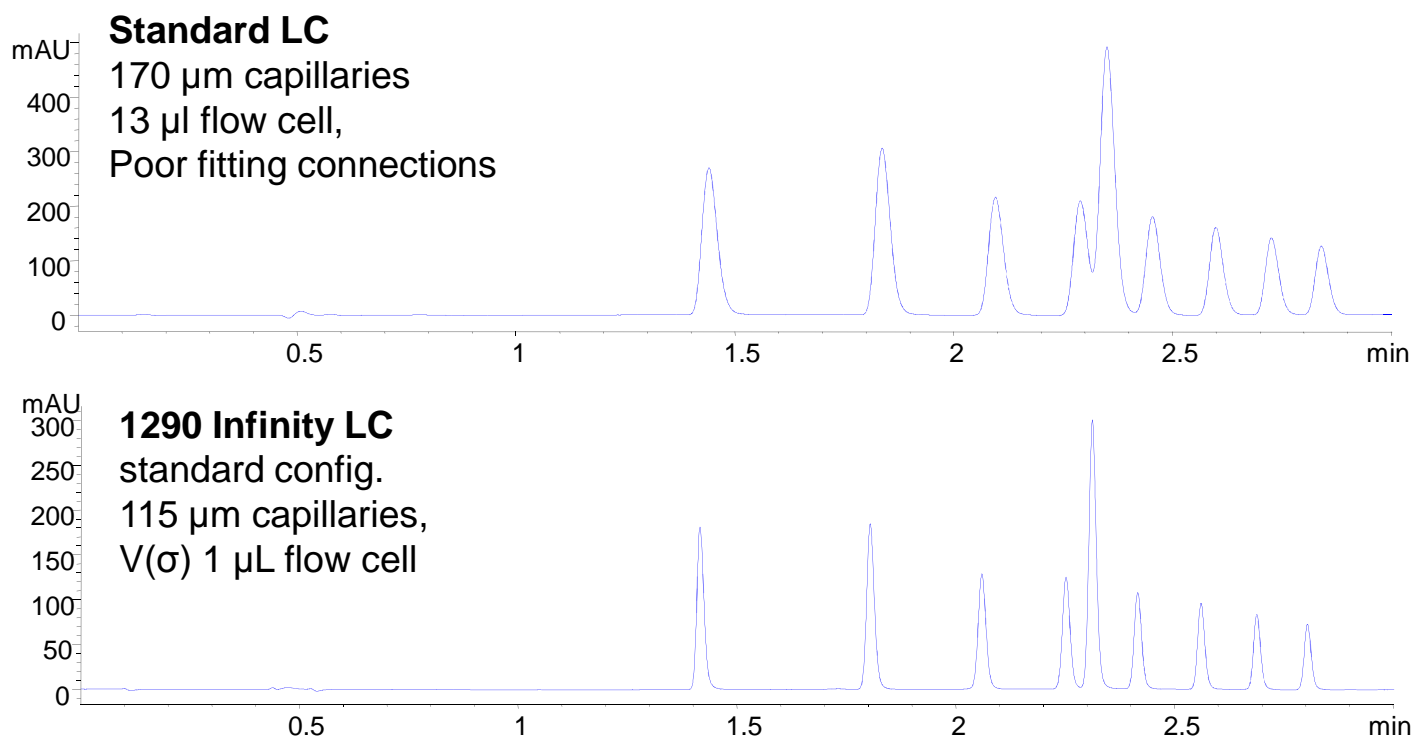
Ultra Low Dispersion Kit Rev.2 for 1290 Infinity LC



Extra column volume, extra column dispersion

Smaller & more efficient columns (ID,L, particle size) => Smaller peak volume
 Smaller peak volume requires smaller extra column volume

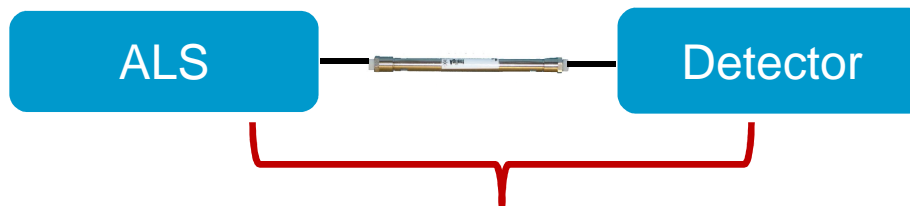
Example: 2.1 x 50 mm 1.8 μm particles



Extra column volume, extra column dispersion

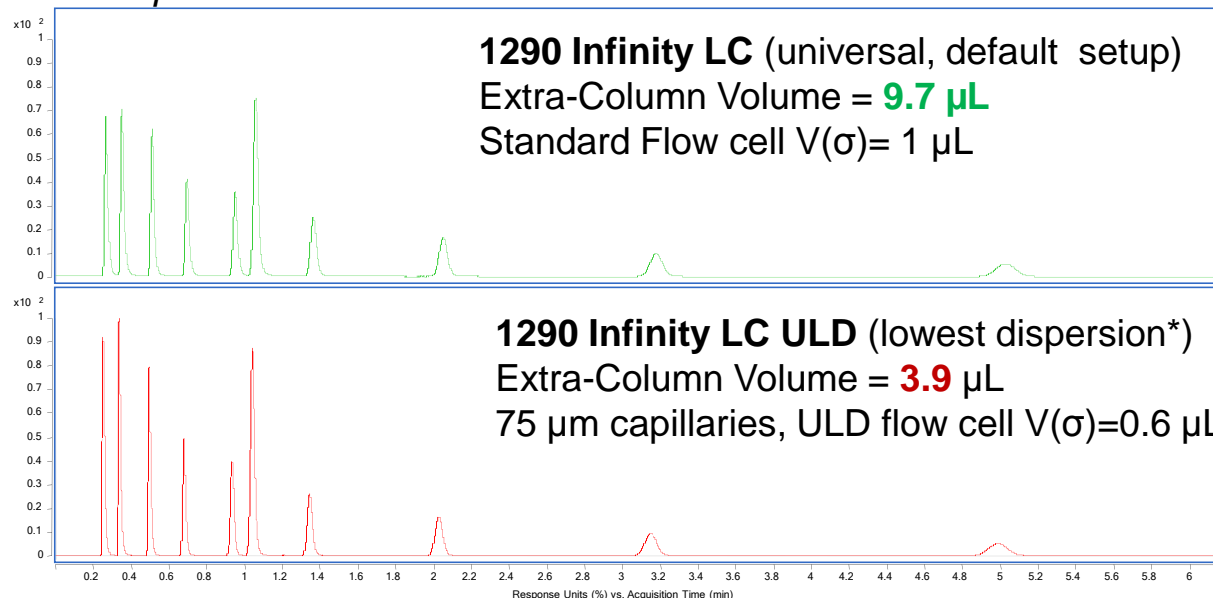
- Facts

- Effects are **huge** for isocratic runs with **early eluting peaks**



Extra column dispersion for isocratic separation

Example on 2.1 x 50 mm STM column



Preliminary results:

$$R_{s_{5,6}} = 2.38$$

$$N_4 = 5529$$

$$N_8 = 9697$$

$$N_9 = 9947$$

$$R_{s_{5,6}} = 2.77 \rightarrow \text{16\% increase}$$

$$N_4 = 8864 \rightarrow \text{60\% increase}$$

$$N_8 = 11251 \rightarrow \text{16\% increase}$$

$$N_9 = 10898 \rightarrow \text{10\% increase}$$

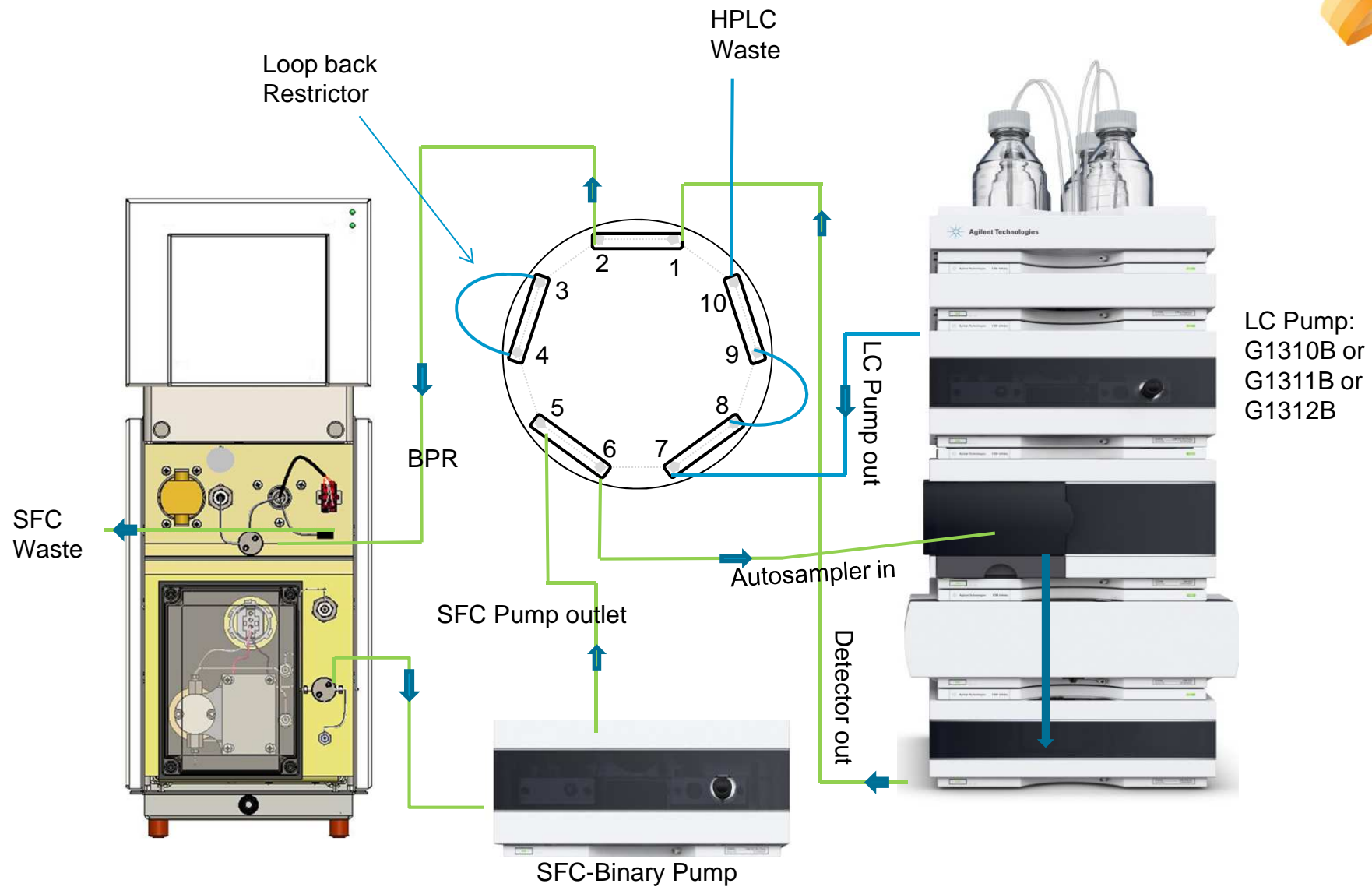
The Agilent 1260 Infinity SFC/UHPLC Hybrid System



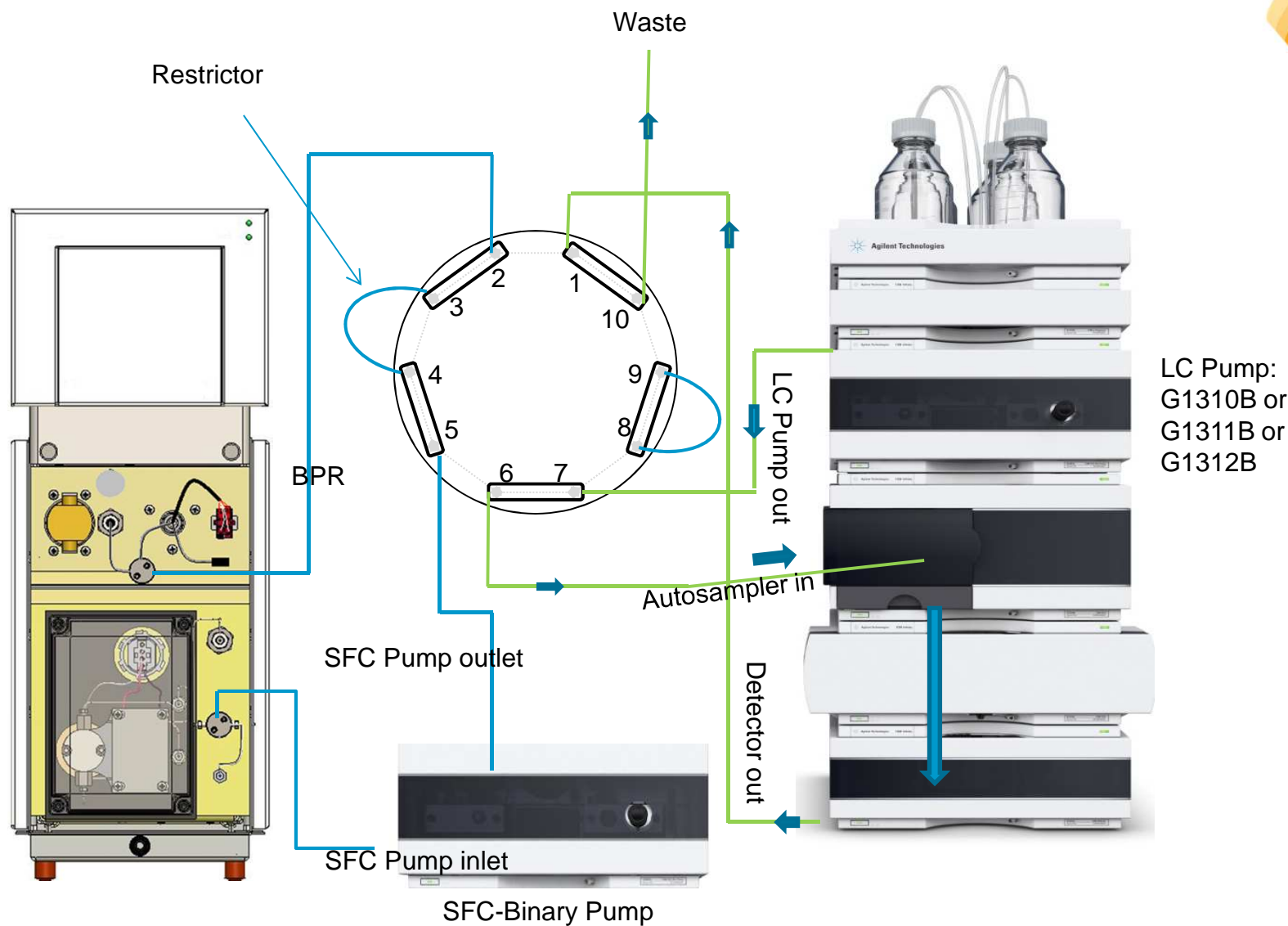
Two orthogonal techniques with a single system

Simply switch the valve

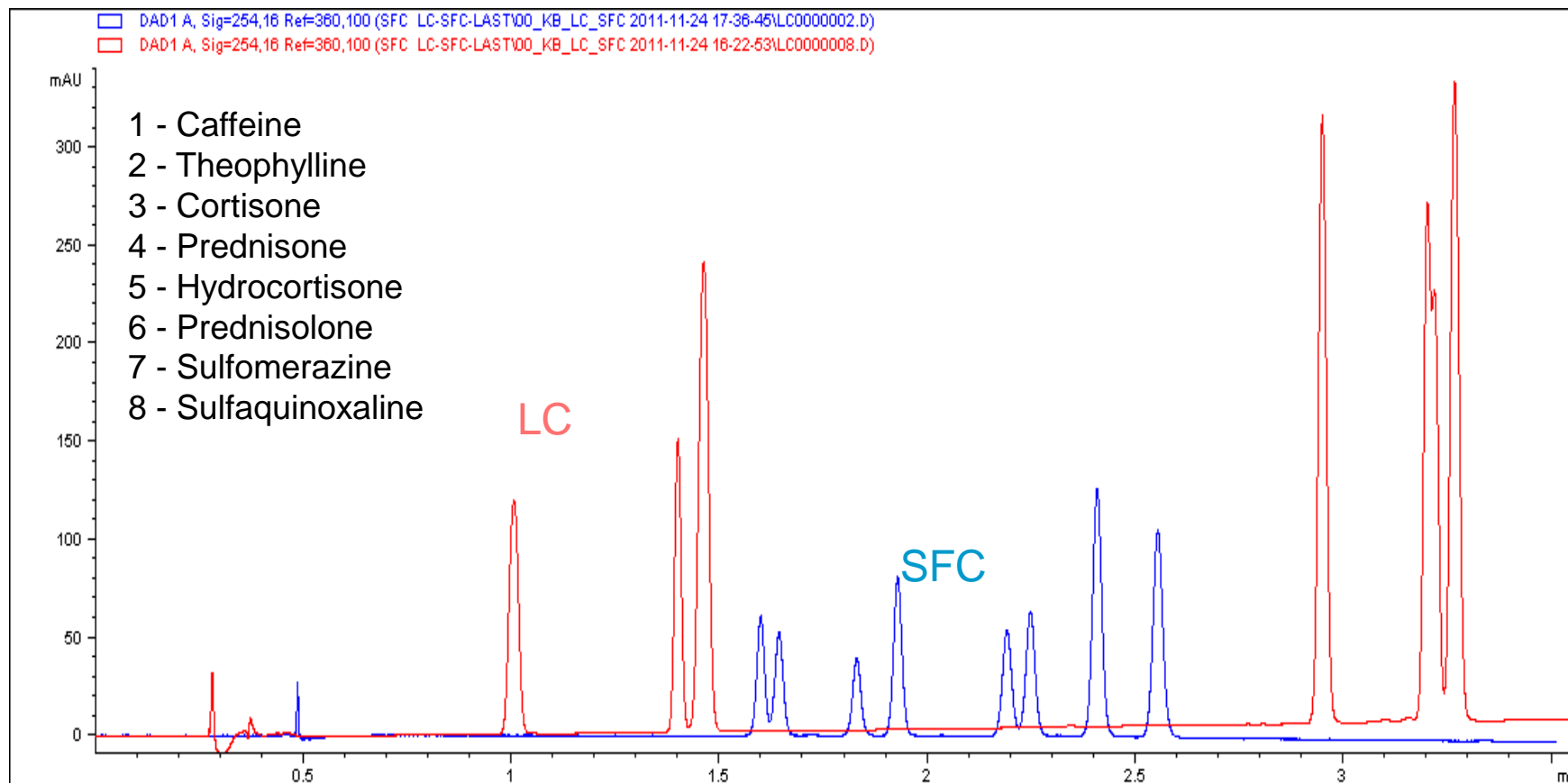
Hybrid System in SFC Mode



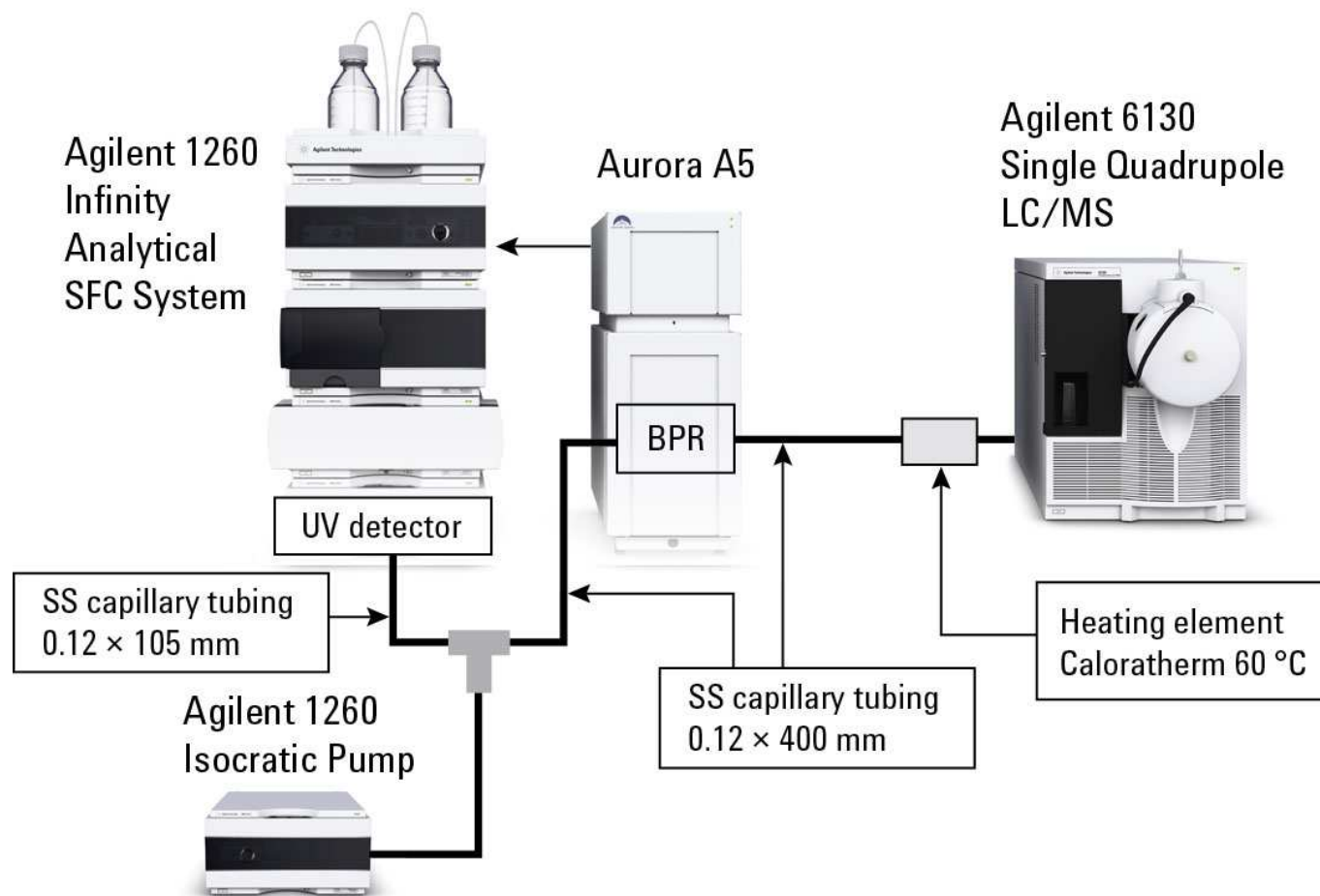
Hybrid System in UHPLC Mode



Overlay SFC versus UHPLC mode



G6100 series MS connection to SFC



**with make-up
flow pump**

Advantages
Superior area
reproducibility
and robustness,
acids can be
added to
enhance
selectivity

Limitations:
only up to 3
ml/min

MS-setup with make-up flow pump G1310B

Are any other non-UV detectors suited for connection with SFC?



Evaporative Light-Scattering perfectly fits with SFC



For compounds which do not contain chromophores

Evaporative Light Scattering Detection (ELSD)

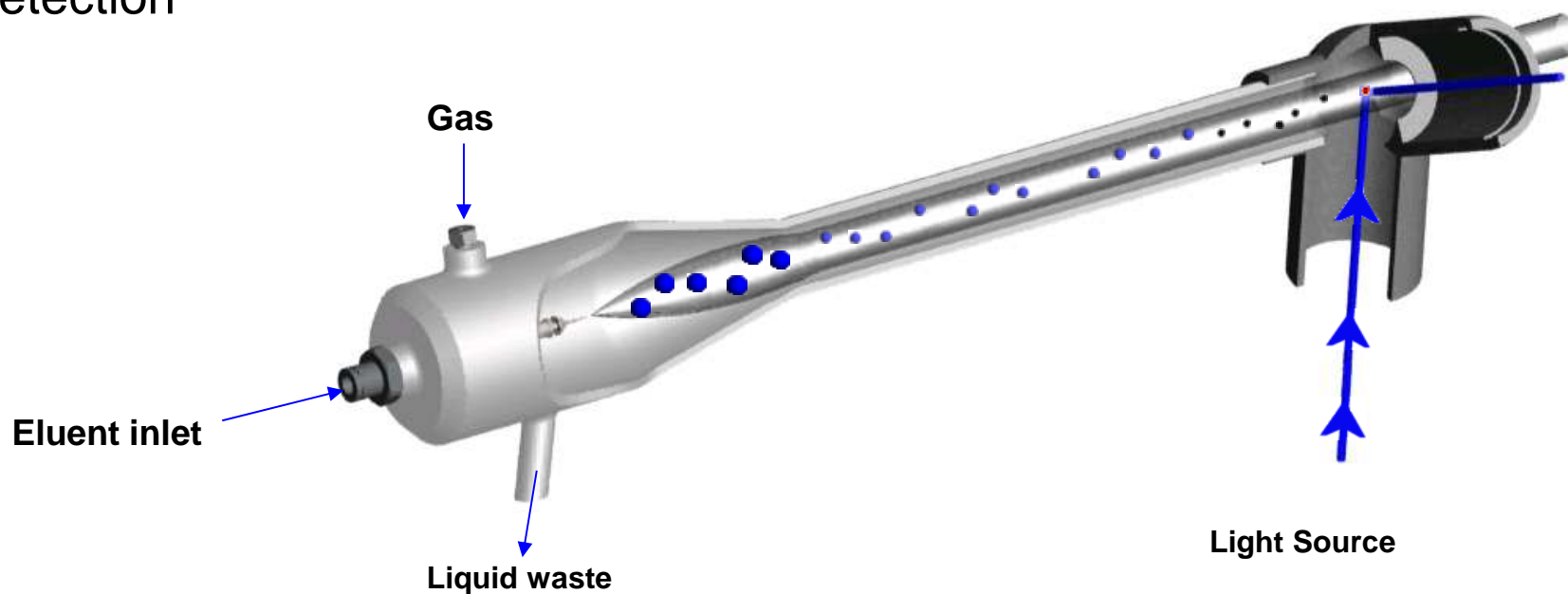
Agilent 1260 Infinity ELSD &
1290 Infinity ELSD
G4260B and G4261B



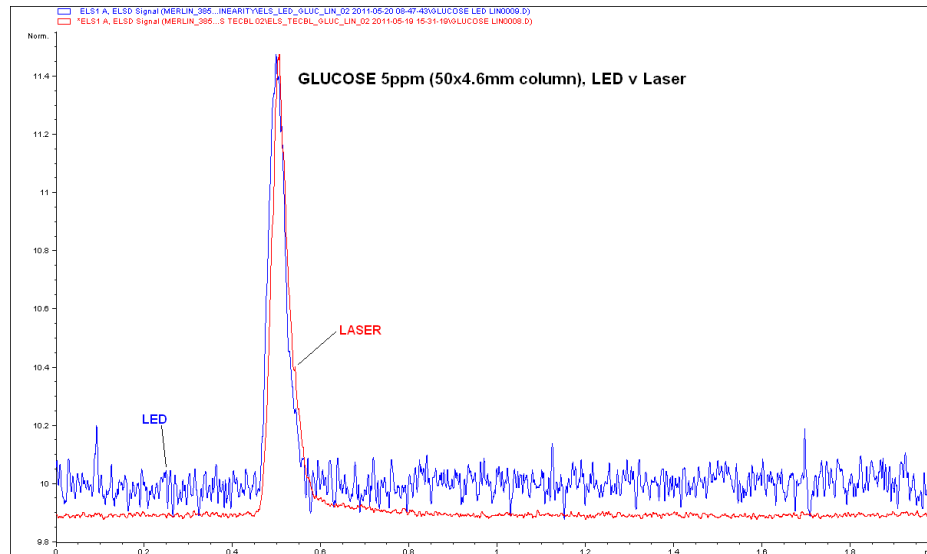
Introduction: Principle of Operation

The ELSD principle of operation employs three distinct stages :

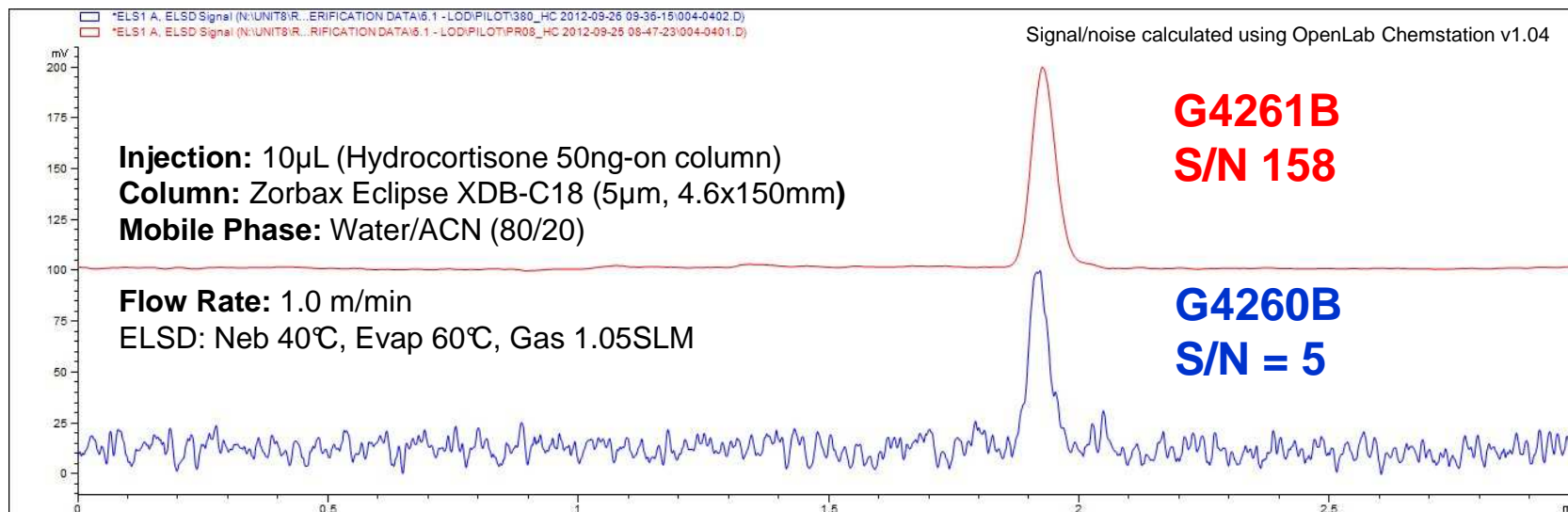
- Nebulisation
- Evaporation
- Detection



Increased sensitivity, reduced baseline noise



The increased laser intensity coupled with a high gain photomultiplier and digital signal processing increases signal and reduces noise.





Agilent ELSD Portfolio November 2012



	G4260B		G4261B	
	1260 Infinity ELSD		1290 Infinity ELSD (Cooling deleted)	1290 Infinity ELSD (Standard, cooled)
Light Source	Blue LED (480nm)		Blue Laser (405nm)	
Temperature range	Nebulizer: 25-90°C Evaporator: ambient – 120°C		Nebulizer: 25-90°C Evaporator: ambient – 120°C	Nebulizer: 25-90°C Evaporator: 10°C – 80°C
Features	<ul style="list-style-type: none"> Hydrocortisone limit of detection 30ng RS232 Communication only 		<ul style="list-style-type: none"> Hydrocortisone limit of detection 4ng 5-9 x improvement in sensitivity over current model Unique sub-ambient operation (greater response for volatile compounds) RS232 and LAN Communication 	
	<ul style="list-style-type: none"> Voltage selection (110/240V) no longer required Variable gas flow for uniformity of response <ul style="list-style-type: none"> Fast data collection (up to 80Hz) Controlled via ChemStation / EZChrom Front Panel control of all parameters (Temperature, gas flow, data rate..etc) 			

The Agilent 1260 Infinity bio-inert quaternary LC

The New Standard in Bioanalysis

**BIO
inert**



100% Bio-inert

- ✓ Precious sample does not touch metal surfaces
- ✓ pH range 1-13 (shortterm 14)
- ✓ 2 M salt, 8 M urea
- ✓ No stainless steel immobile phase flow path
- ✓ New capillary technology

UHPLC capability

- ✓ 600 bar

Superior Ease of Use and Robustness

- ✓ Buffer Advisor Software
- ✓ Highly corrosion resistant
- ✓ Active seal wash
- ✓ Quaternary buffer mixing
- ✓ Agilent proven quality
- ✓ Agilent warranty and service quality (enhanced PM possible)
- ✓ Superior Bio-HPLC columns for biotherapeutic characterization

The choice for both, bioanalytical and biopurification up to 10 ml/min

Proof of concept: surface activity

ATP Analysis – 1260 Infinity standard LC system

Chromatographic conditions

Flow rate: 0.5 mL/min

Isocratic run with buffer A, B, C, D or E

Stop time: 5 minutes

Injection volume: 0.2 μ L

Temperature TCC: 40 $^{\circ}$ C

Diode array detector: 254 nm

No column, PEEK restriction

Solvents

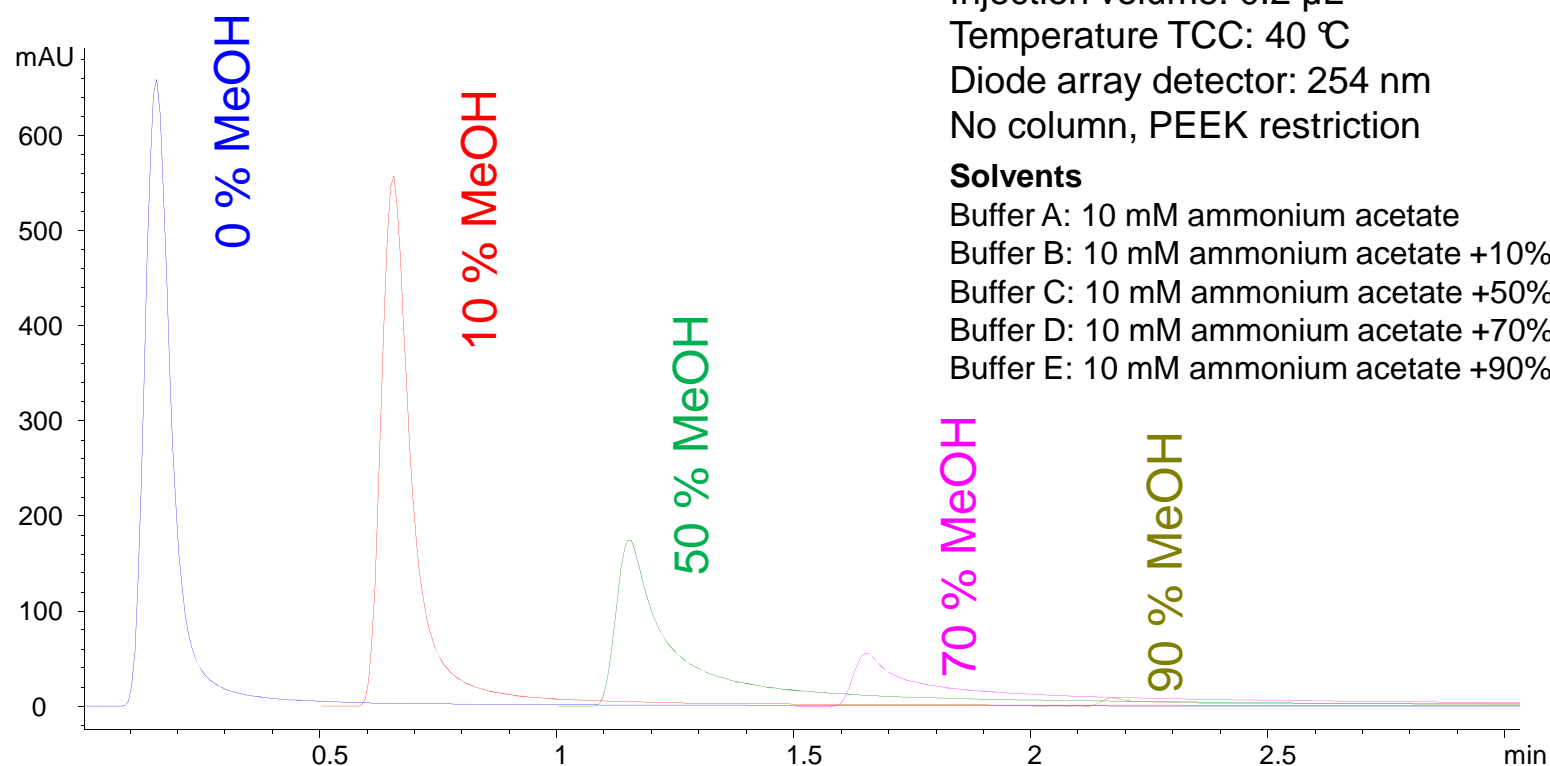
Buffer A: 10 mM ammonium acetate

Buffer B: 10 mM ammonium acetate +10% methanol

Buffer C: 10 mM ammonium acetate +50% methanol

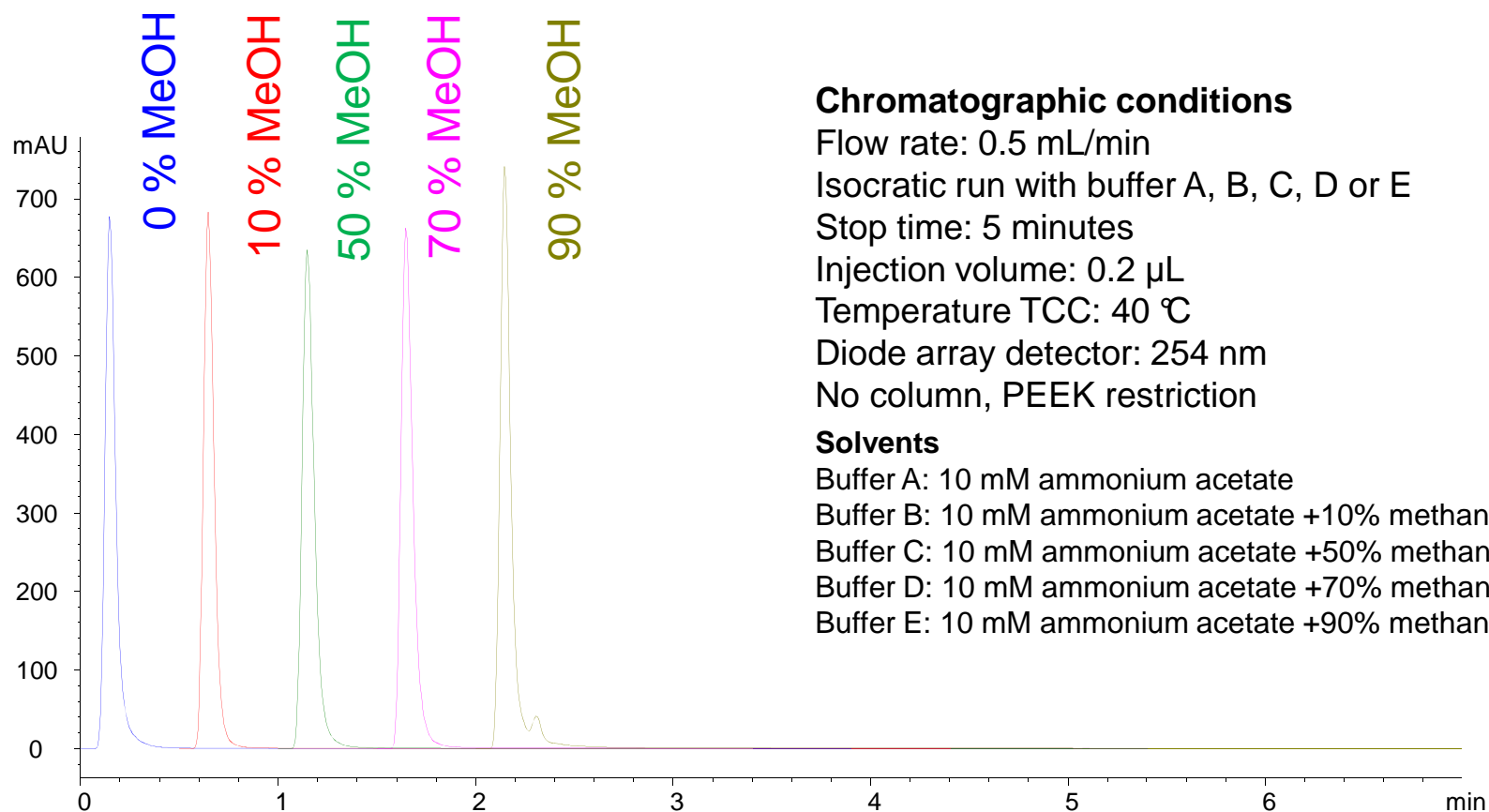
Buffer D: 10 mM ammonium acetate +70% methanol

Buffer E: 10 mM ammonium acetate +90% methanol



Proof of concept: low surface activity

ATP Analysis – 1260 Infinity Bio-inert LC



Chromatographic conditions

Flow rate: 0.5 mL/min

Isocratic run with buffer A, B, C, D or E

Stop time: 5 minutes

Injection volume: 0.2 μ L

Temperature TCC: 40 $^{\circ}$ C

Diode array detector: 254 nm

No column, PEEK restriction

Solvents

Buffer A: 10 mM ammonium acetate

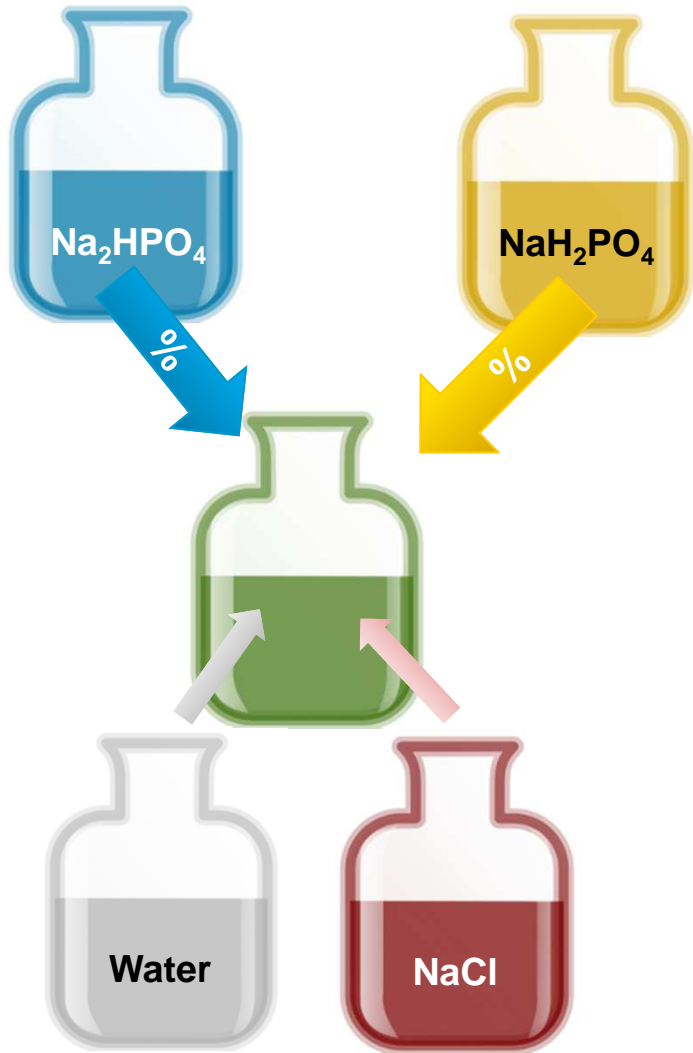
Buffer B: 10 mM ammonium acetate +10% methanol

Buffer C: 10 mM ammonium acetate +50% methanol

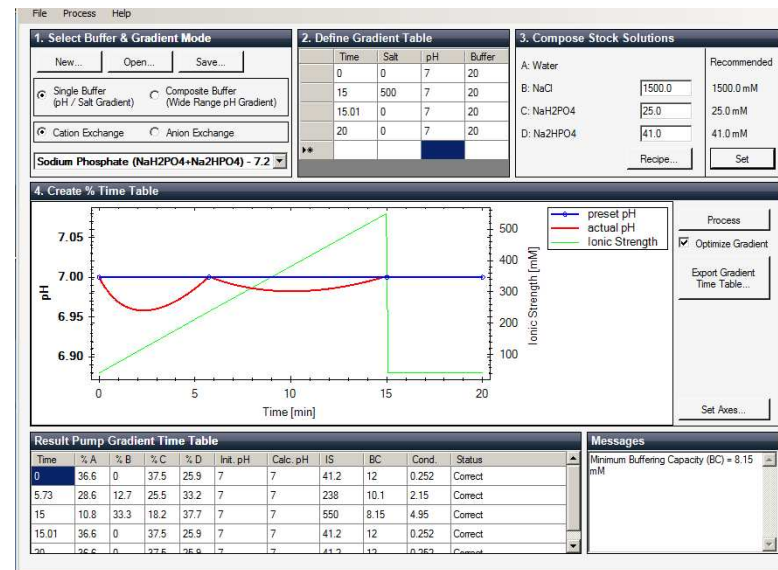
Buffer D: 10 mM ammonium acetate +70% methanol

Buffer E: 10 mM ammonium acetate +90% methanol

...a Quat LC and Buffer Advisor enables Automation....



Mix 2 components directly to get desired pH



Dilute online or add salt for salt gradients

Using the 1260 Infinity LC for Protein SEC

Agilent 1260 Infinity Multi Detector Suite



Agilent 1260 Infinity Multi Detector Suite for Protein SEC

...helps to differentiate aggregates of monoclonal antibodies

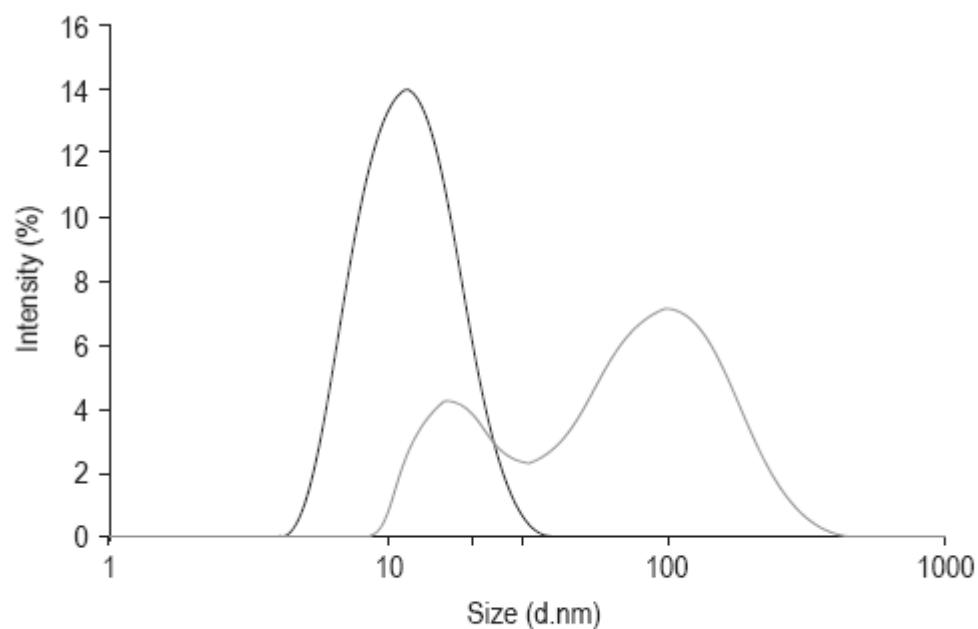


Figure 1. Size distribution (by intensity) from two antibody samples, Ab1-a (black) and its treated form Ab1-b (grey). Ab1-b is aggregated, while Ab1-a shows no evidence of larger components.





Danke für Ihre Aufmerksamkeit!