Verbesserung der LC-Performance durch Integration neuer HDR-DA-Detektoren und Hybrid LC-SFC-Systeme von Agilent Technologies

APPLICA 2012

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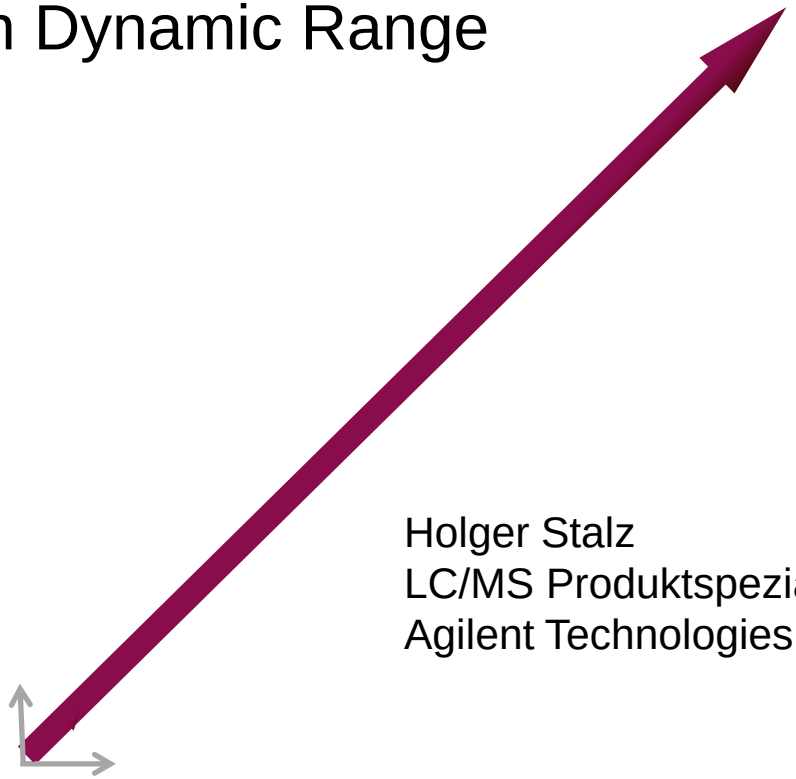
30x Wider Linear UV range - Quantification of widely different concentration levels in one single run

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

1260/1290 Infinity HDR-DAD



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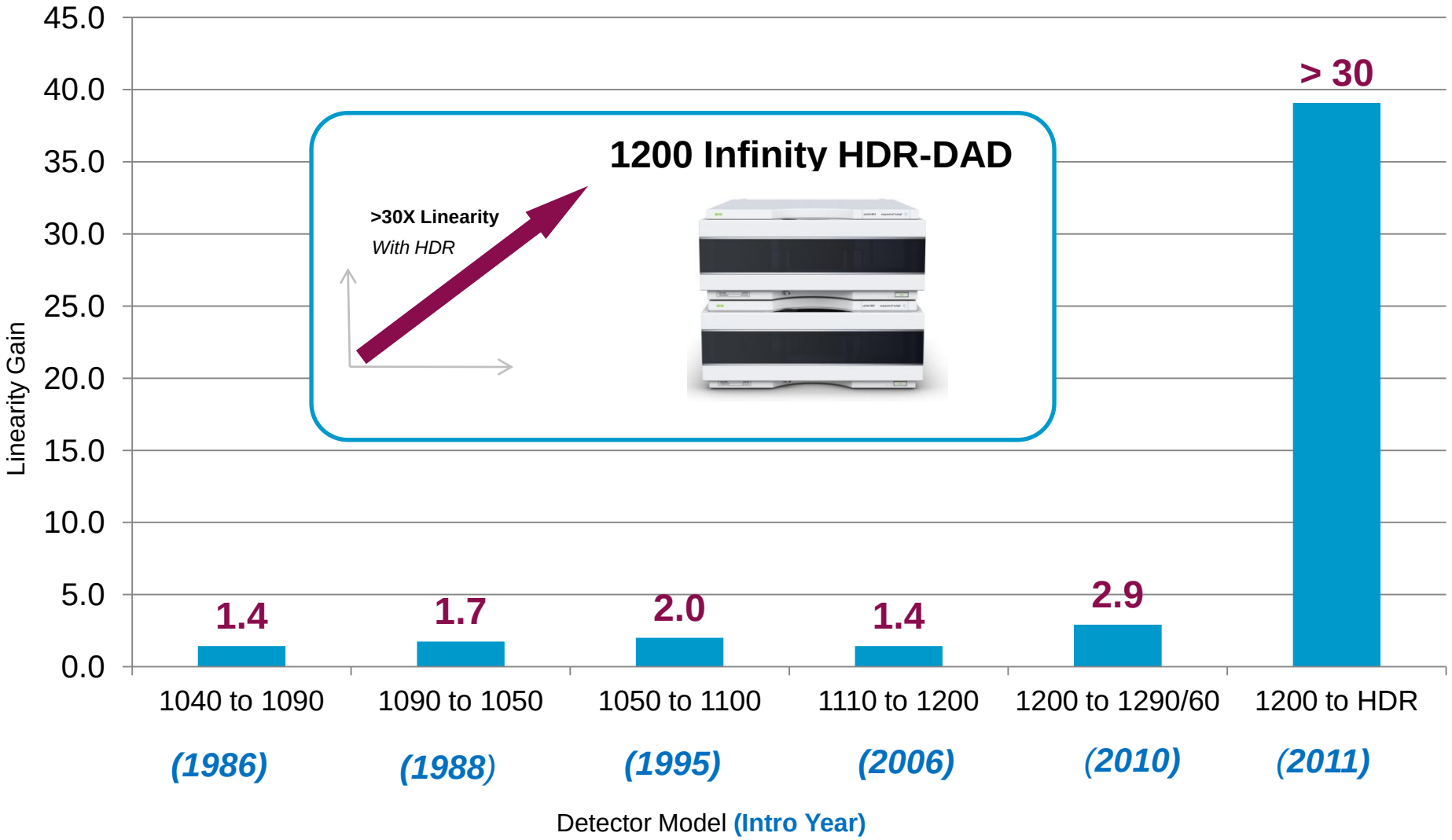
History of DAD Linearity Gain

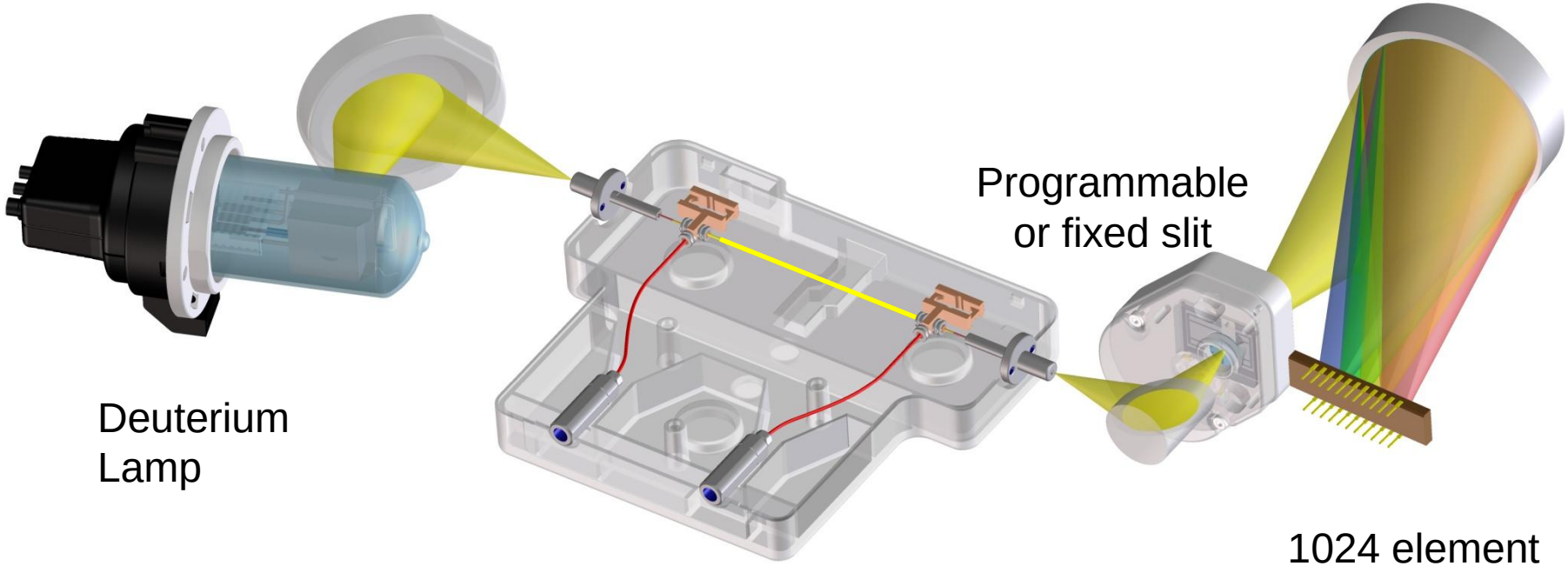
The last 30 years



Optofluidic Waveguides: Max-Light Flow Cells

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

1260 / 1290 Infinity DAD



Linearity, linear range and dynamic range

1200 Series Diode Array Detector, 10 mm flow cell

$$Linear\_range = \frac{Upper\_Limit}{(3 * Detector\_Noise)} \approx 10^5$$

10 mm

Detector Signal [AU]

Upper limit 2.0

*Detector noise 7*10^{-6}*

HDR- DAD

Slope = Response Factor (Sensitivity)

HDR- DAD

Linear range

Dynamic range

LOD (3x S/N)

Conc (upper limit) linear range

Conc (upper limit) dynamic range

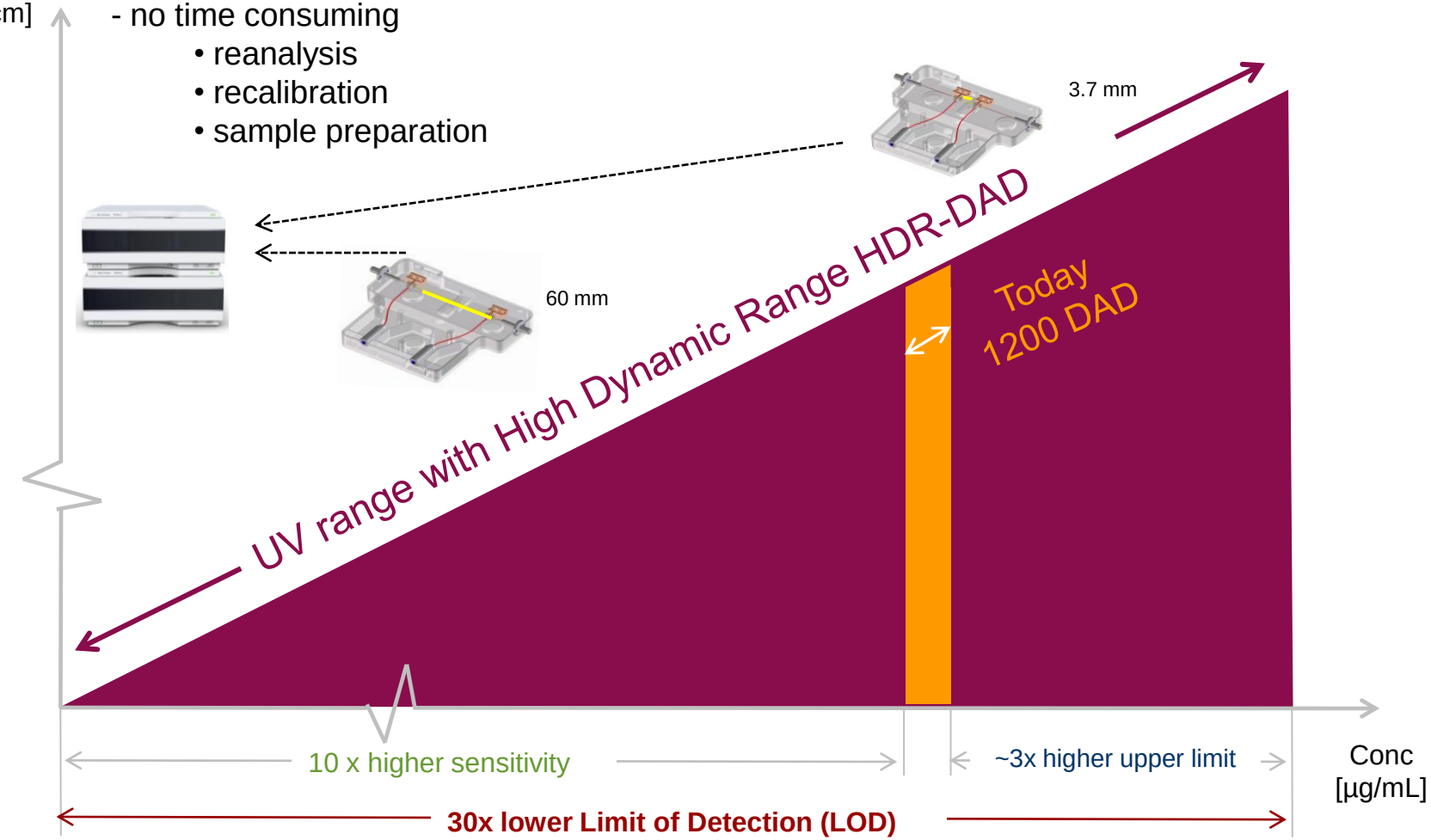
Conc [µg/mL]

30x Wider Linear Range with HDR-DAD

3.7 mm and 60 mm and Max-Light flow cell

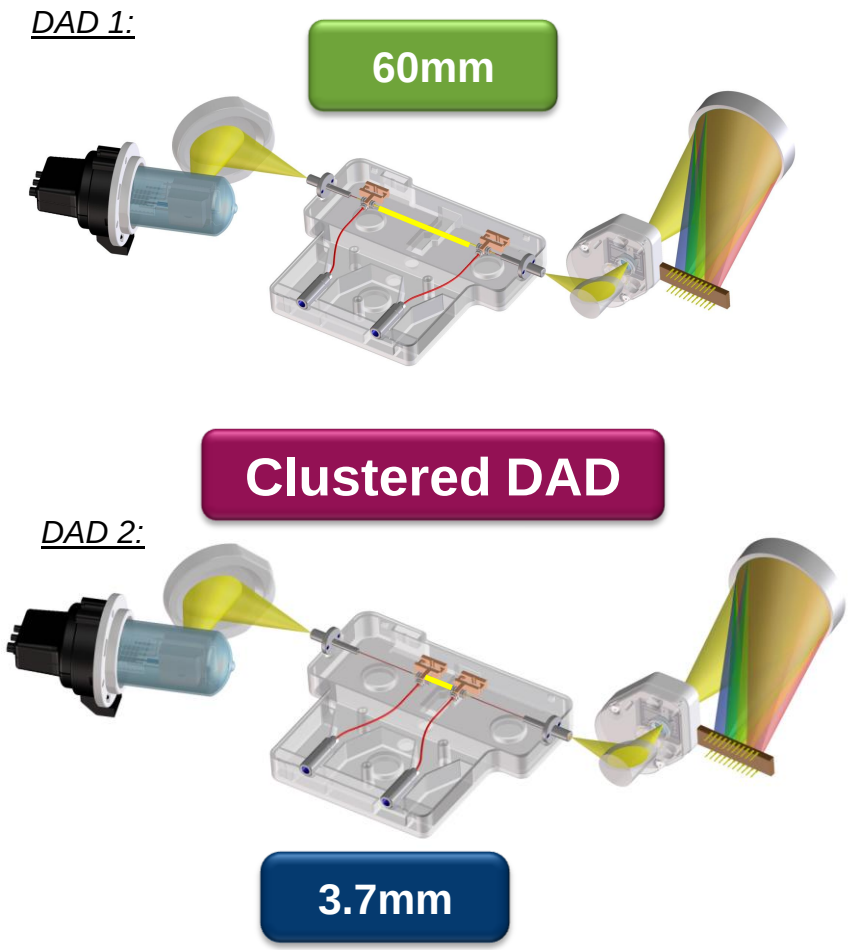
30x lower Limit of Detection (LOD) for impurity analysis

- For samples with widely different concentration levels
- no time consuming
  - reanalysis
  - recalibration
  - sample preparation



1290/1260 High Dynamic Range – HDR

Increasing Linear UV Range by >30x



Technology

- Cluster of two DADs
  - **DAD 1:** 60 mm Cell – for low concentrations
  - **DAD 2:** 3.7 mm Cell – for high concentrations
  - **Output:** One, combined signal, normalized to 10 mm path length (HDR range: 0.6 x 10^{-6} to 6.7 AU/cm) vs for 1200 Series: 7 x 10^{-6} to 2 AU/cm)

Control / Usability

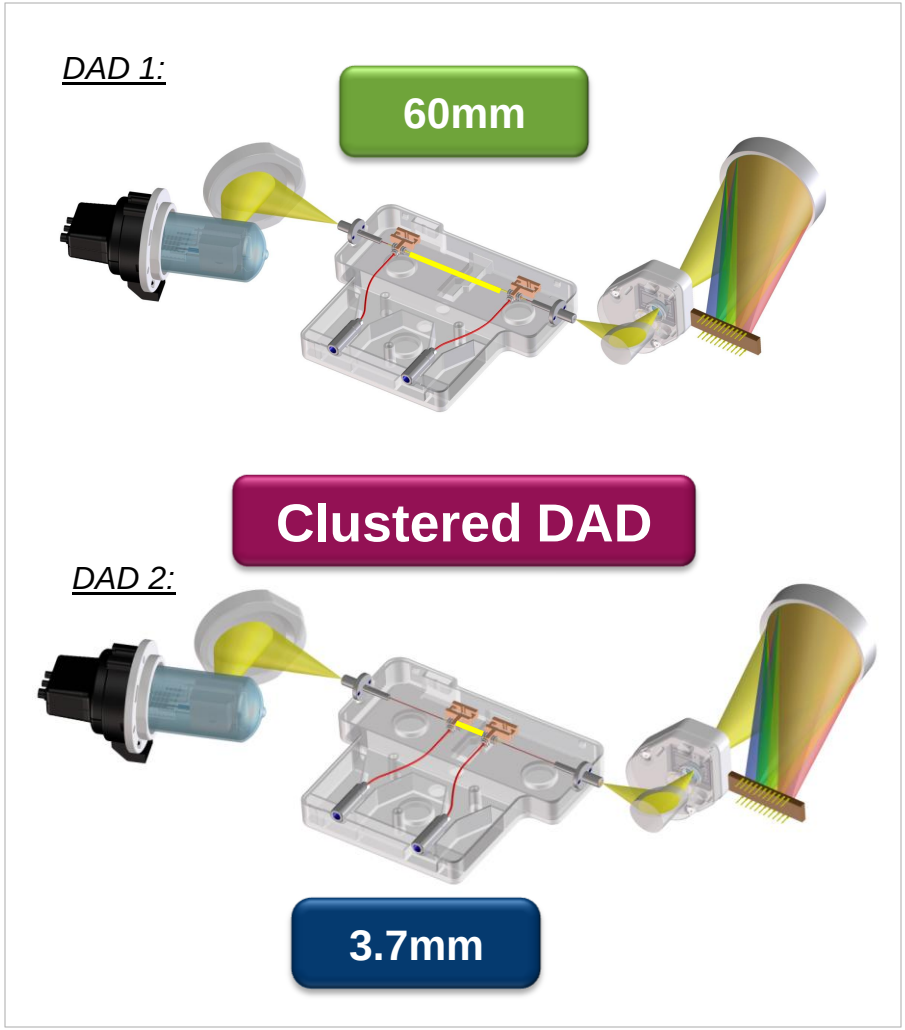
- Like standard 1290/1260 DAD: Control, data analysis, reporting

Investment Protection

- Existing 1290/1260 DAD can be upgraded (by 2nd DAD)
- Existing 1100/1200 Systems can be upgraded (by two 1290 or 1260 DAD)

1290/1260 High Dynamic Range – HDR

Increasing Linear UV Range by >30x



For sample mixtures with widely different concentration levels:

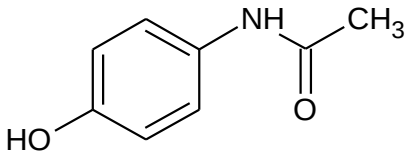
- All sample information in one run, e.g. for fixed dose combinations
  - shorter turnaround time
  - higher instrument utilization
  - reduced solvent and waste costs.
- Up to 30x lower LOD, 15x lower LOQ (compare to 1100/1200 Series)
  - for more reliable automated integration
  - higher area precision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

Experimental

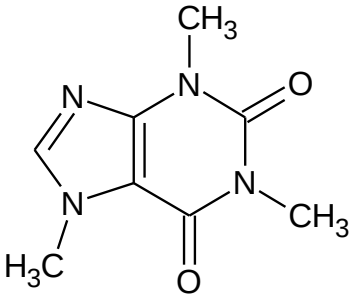
Sample:

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

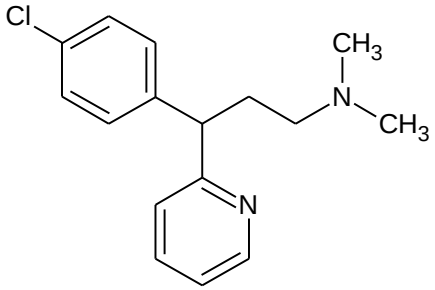
Paracetamol



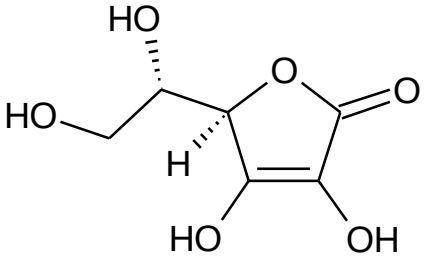
Caffeine



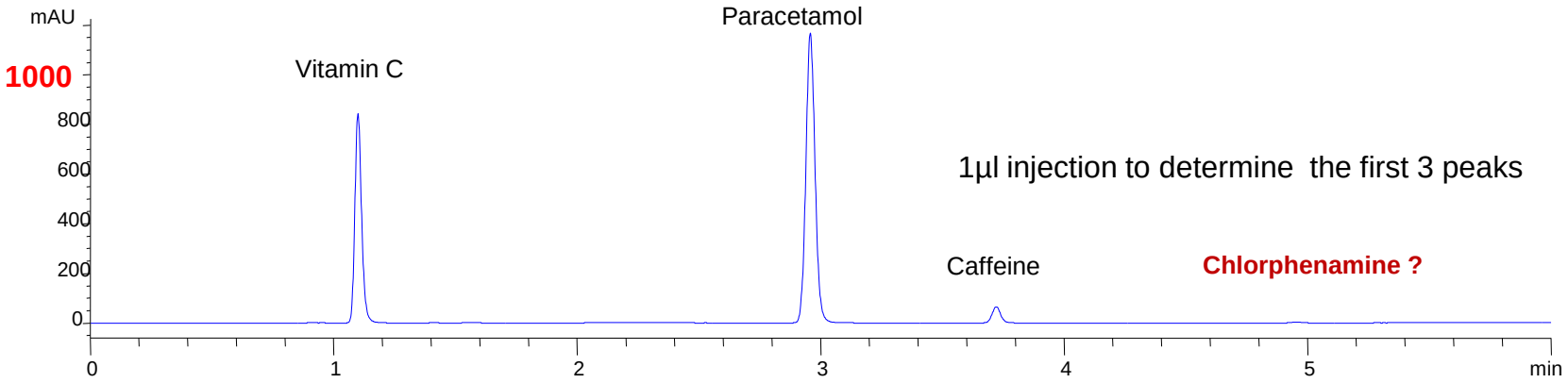
Chlorphenamine



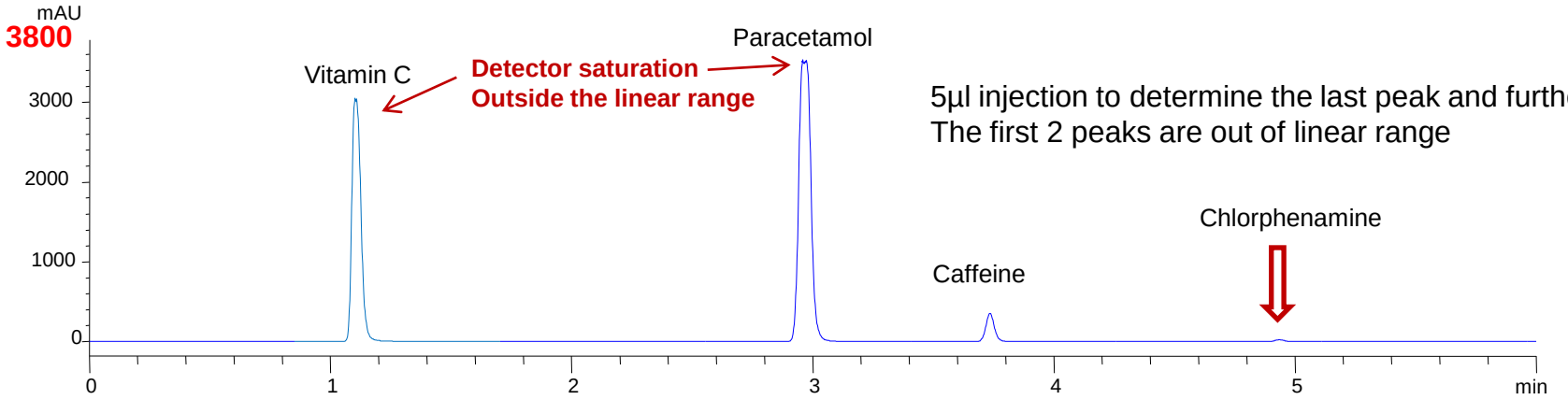
Vitamine C



2 Injections needed if a conventional DAD is used

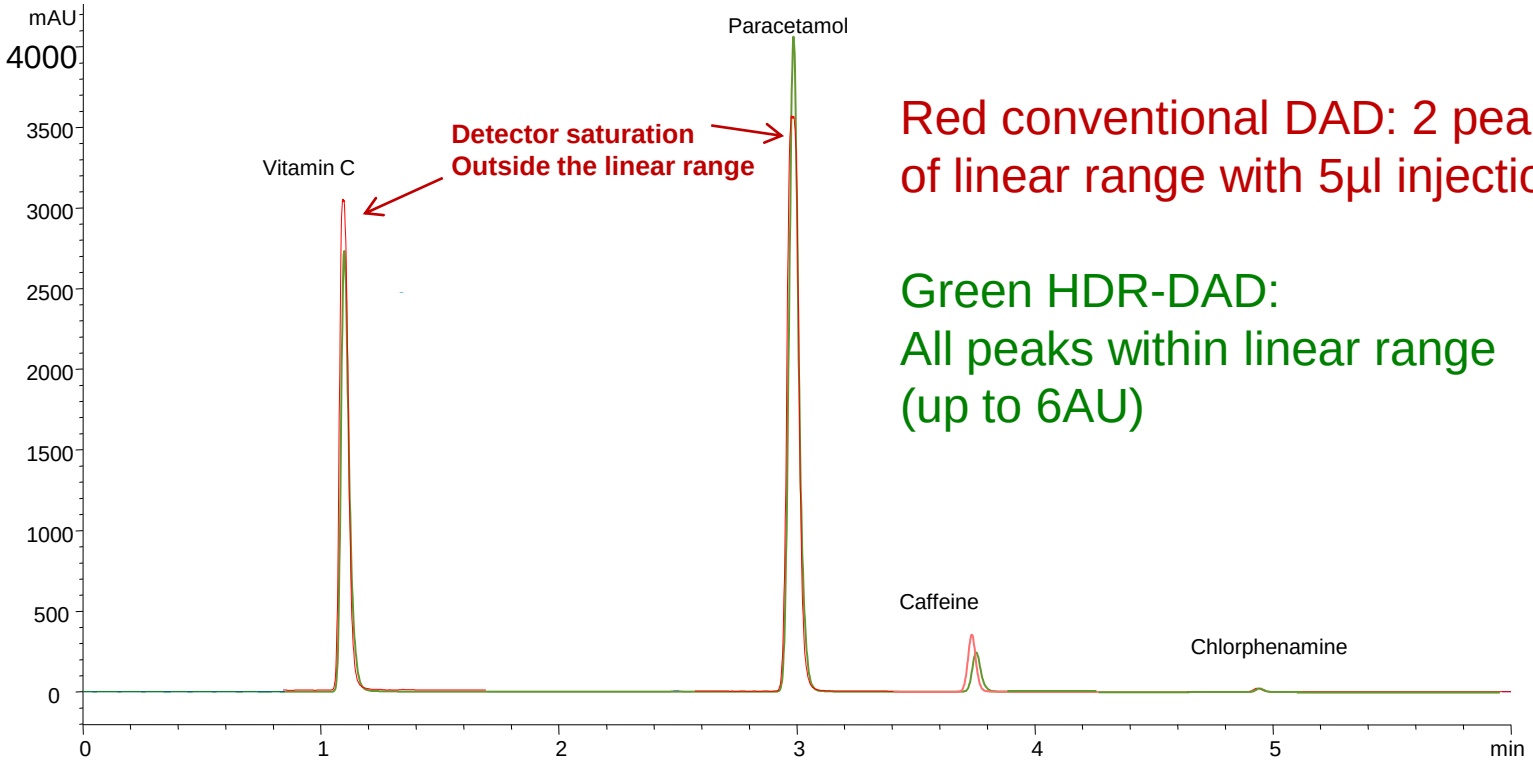


1µl injection to determine the first 3 peaks



5µl injection to determine the last peak and further impurities
The first 2 peaks are out of linear range

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



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

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

Summary

With the Agilent HDR DAD the determination of low and high doses drugs is possible in one run wheras 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

Infinitely better SFC performance

Agilent 1260 Infinity Analytical SFC Solution

What is Supercritical Fluid Chromatography?

Definitions:

1. Supercritical Fluid Chromatography is a chromatography technique based on the use of supercritical carbon dioxide as the mobile phase
2. Supercritical Fluid Chromatography (SFC) is a form of normal phase chromatography that is used for the analysis and purification of low to moderate molecular weight molecules. Principles are similar to those in HPLC however SFC typically utilizes CO_2 as the mobile phase; therefore the entire chromatographic flow path must be pressurized.

Benefits of SFC vs. LC

- Unique selectivity (orthogonality to HPLC)
- Premier choice for chiral compounds
- Replacement for Normal-Phase-HPLC
- Complimentary to RP-LC or HILIC
- Speed of analysis (faster than LC)
- Cheaper than LC
- Green
- Broad detector compatibility

Unique Features of the Agilent 1260 Infinity SFC

Performance

- ✓ HPLC-like UV-Sensitivity
- ✓ The only SFC with 600 bar power range
- ✓ Seamless MS and ELSD connection

Cost

- ✓ 10-15x lower operating costs with standard grade CO_2
- ✓ Lowest solvent consumption and waste generation
- ✓ From entry LC upgrade to complete high-end MS/SFC
- ✓ all in one hybrid SFC/UHPLC system

Time

- ✓ 3 x faster than LC
- ✓ no significant equilibration to change from UHPLC to SFC with the Hybrid SFC/UHPLC

Robustness

- ✓ Based on proven 1260 Infinity quality

Product Overview: 3 Ways to get SFC from Agilent ….

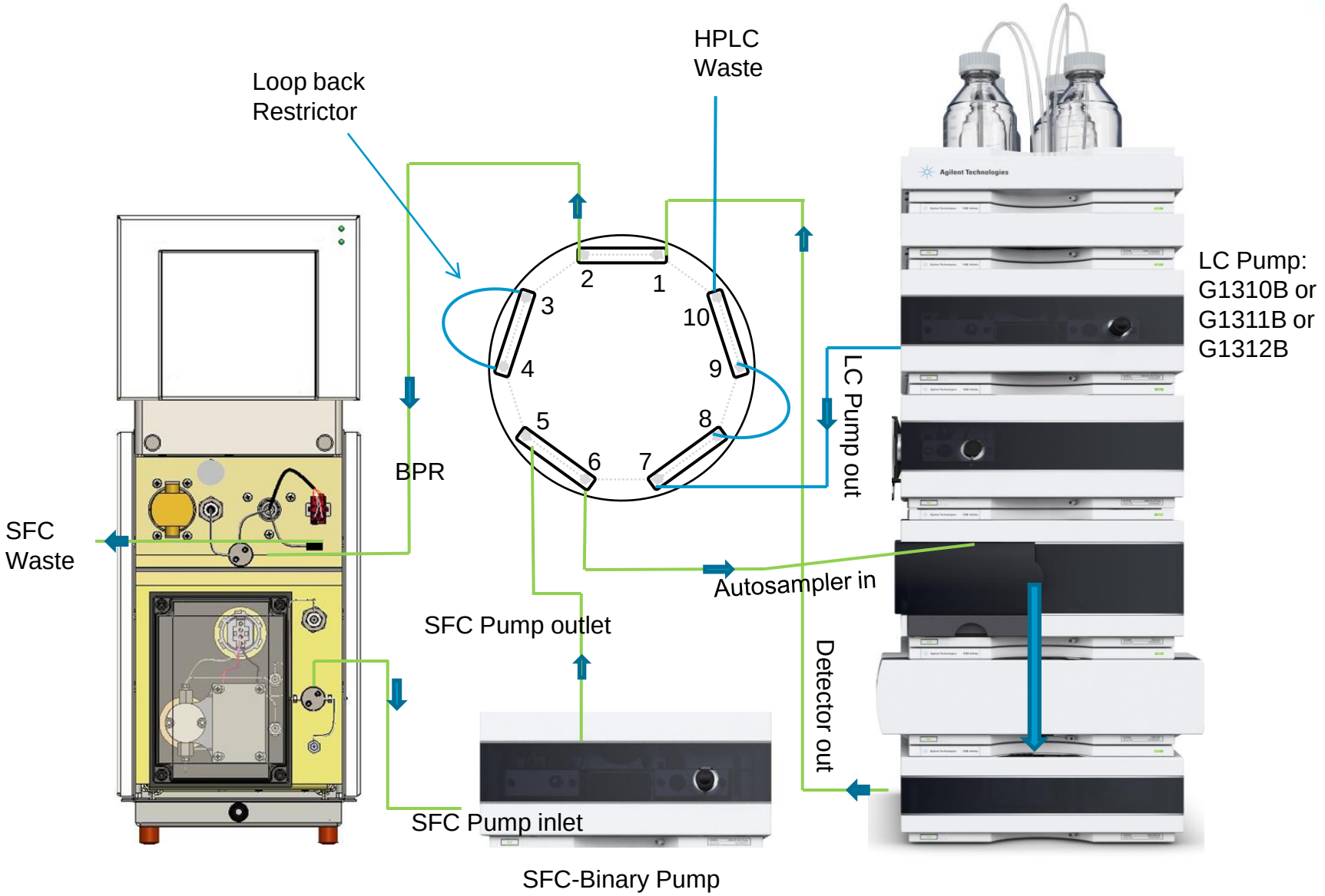
Hybrid SFC/UHPLC System	Analytical SFC	LC Upgrade to SFC
• 1260 Infinity analytical SFC • LC pump (Quat, Binary) • 2pos/10port valve • Hybrid SFC/UHPLC kit Change from SFC to UHPLC **Orthogonal Separation Power**	• 1260 Infinity analytical SFC **Best SFC performance**	• Fusion A5n (Aurora) • 1260 SFC binary pump • SFC accessory kit Convert 1100/1200 to SFC **Most affordable**

The Agilent 1260 Infinity SFC/UHPLC Hybrid System

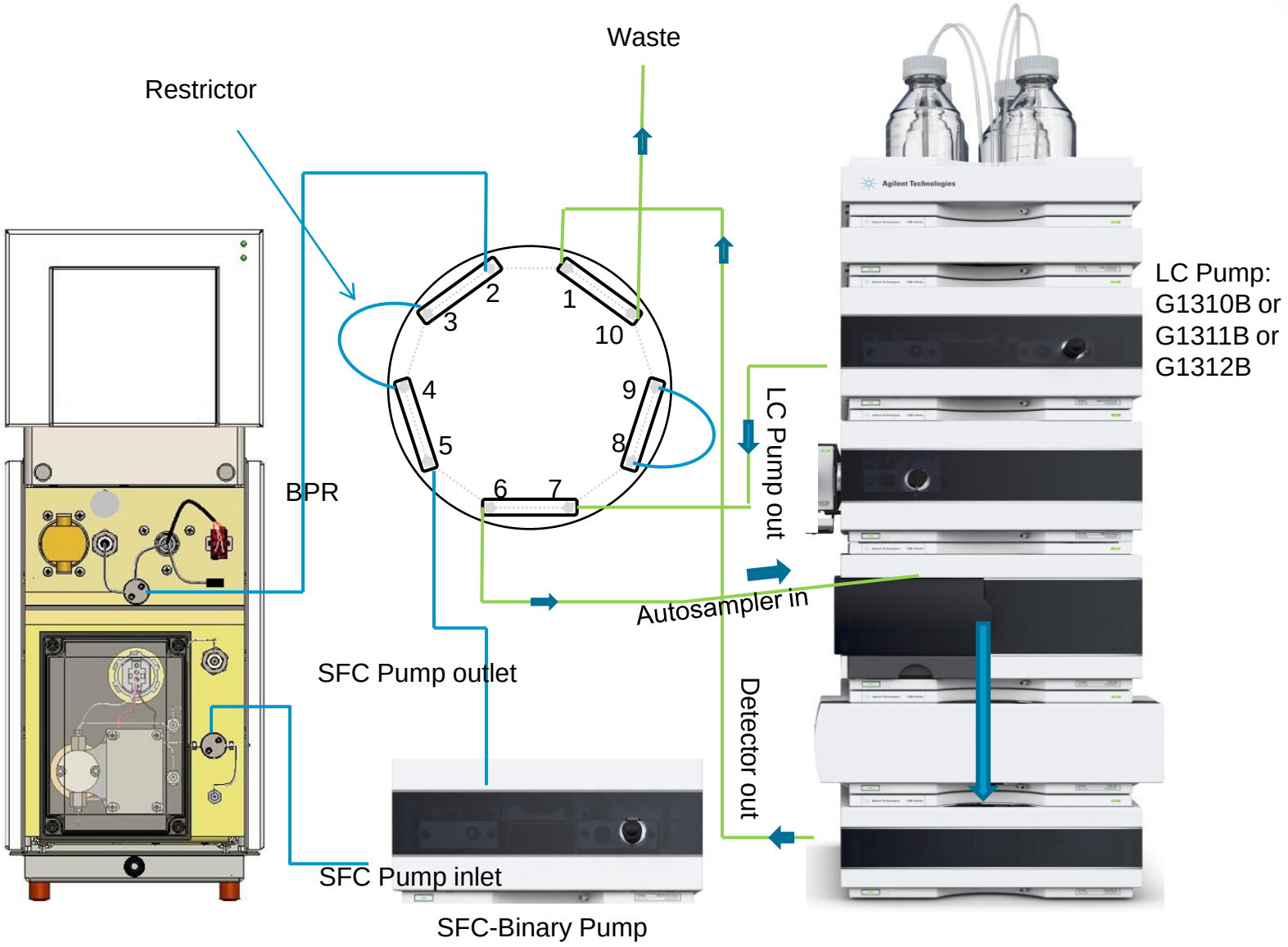
Two orthogonal techniques with a single system

Simply switch the valve

Hybrid System in SFC Mode



Hybrid System in UHPLC Mode



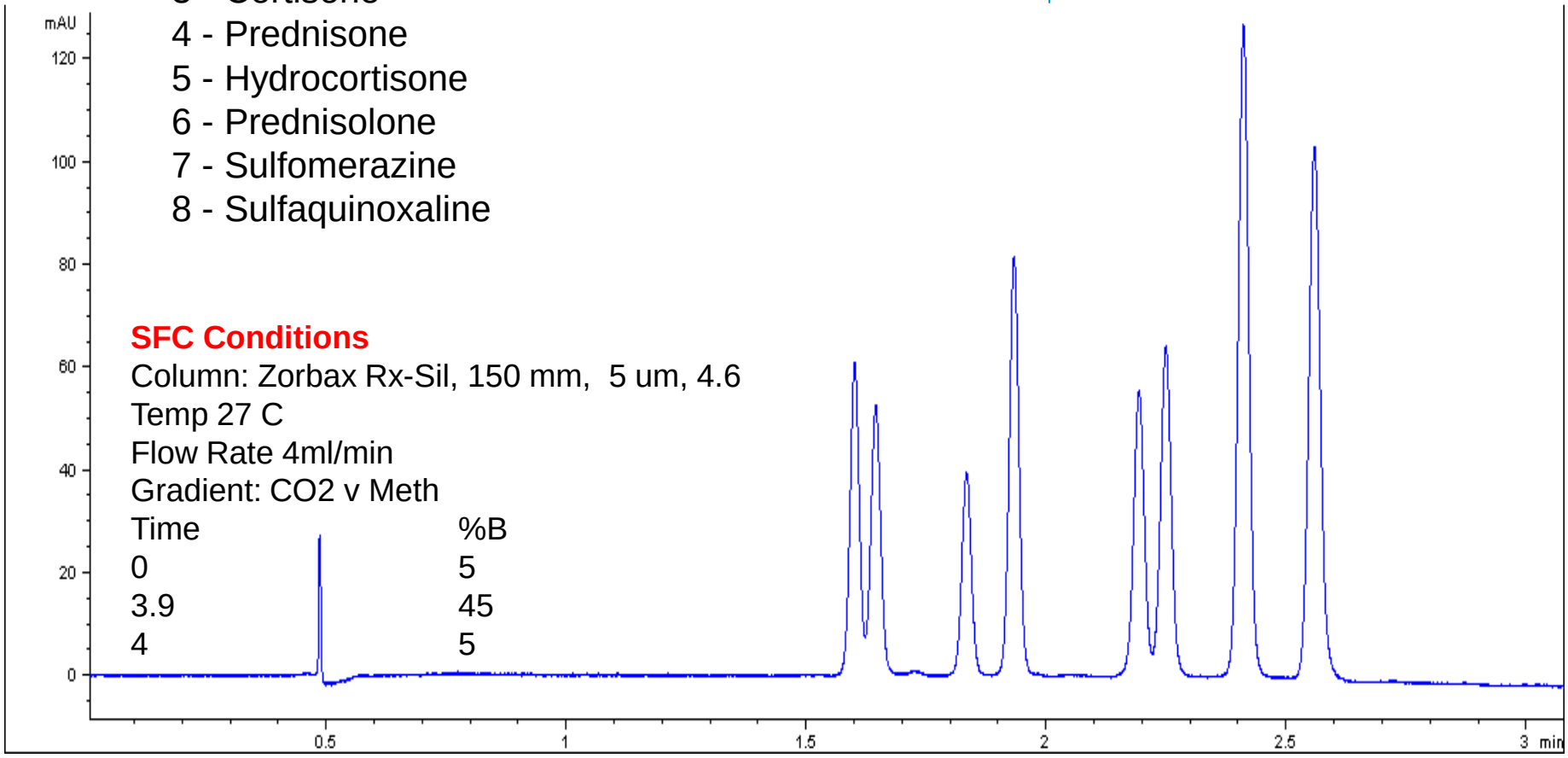
Why is orthogonal sample information important and what are the advantages of the Agilent Hybrid instrument?

- Orthogonal method screening in one single system
- Separate and detect impurities which co-elute when using a single technique
- Resolve peaks of complex samples
- Switch from SFC to UHPLC forth and back in a single sequence
- No equilibration time between LC and SFC
- Significant cost saving, only one system has to be purchased
- Direct results comparison between SFC and LC
- No instrument to instrument variation
- Save lab space
- The only vendor who can offer both techniques in one system

Performance: Orthogonal Separation: SFC Mode

1 - Caffeine
2 - Theophylline
3 - Cortisone
4 - Prednisone
5 - Hydrocortisone
6 - Prednisolone
7 - Sulfomerazine
8 - Sulfaquinoxaline

8 Components



SFC Conditions

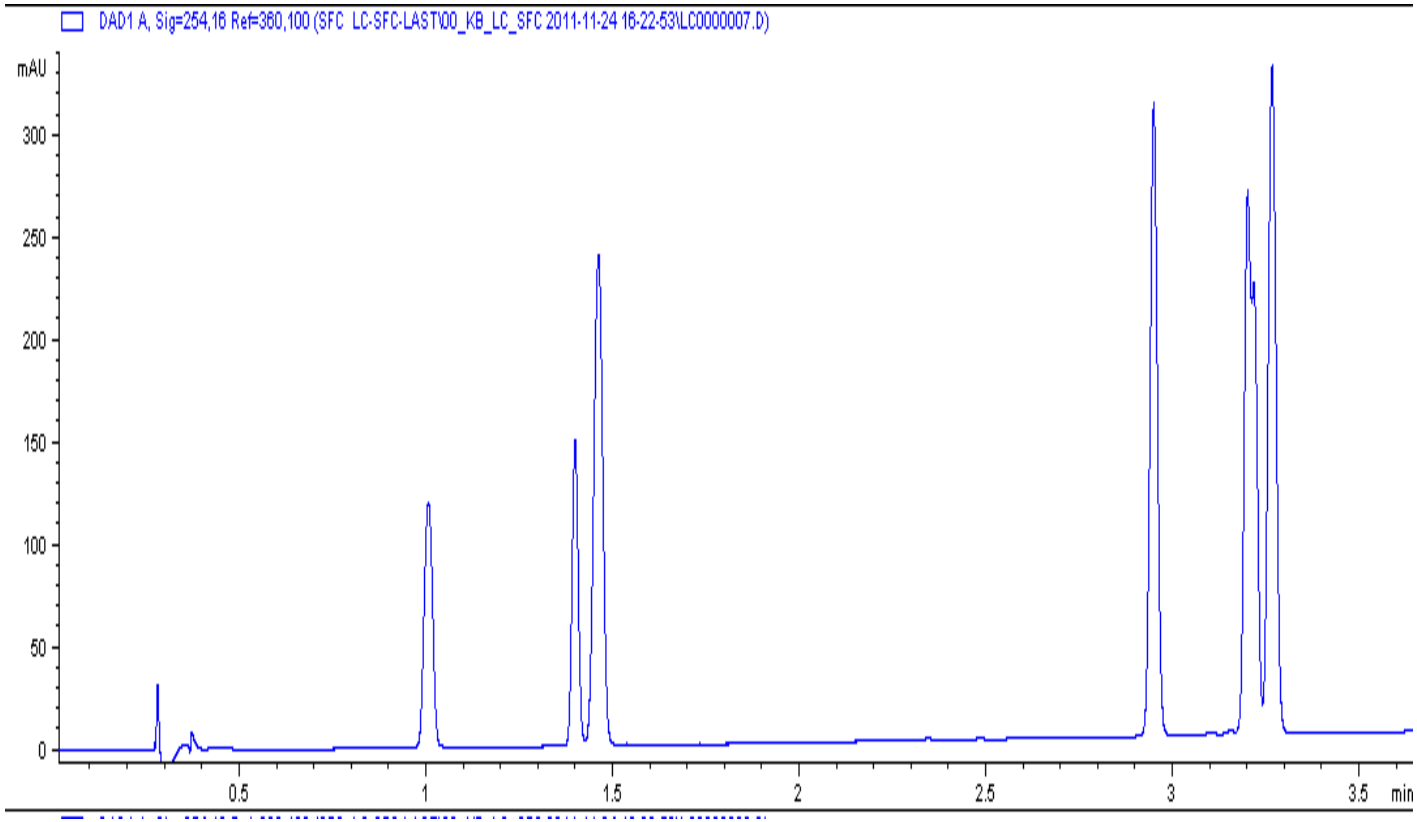
Column: Zorbax Rx-Sil, 150 mm, 5 um, 4.6
Temp 27 C
Flow Rate 4ml/min
Gradient: CO2 v Meth

Time	%B
0	5
3.9	45
4	5

Performance: Orthogonal Separation: UHPLC Mode

What to do if there is a resolution problem?

6 Components + 2 Co-Eluting Peak

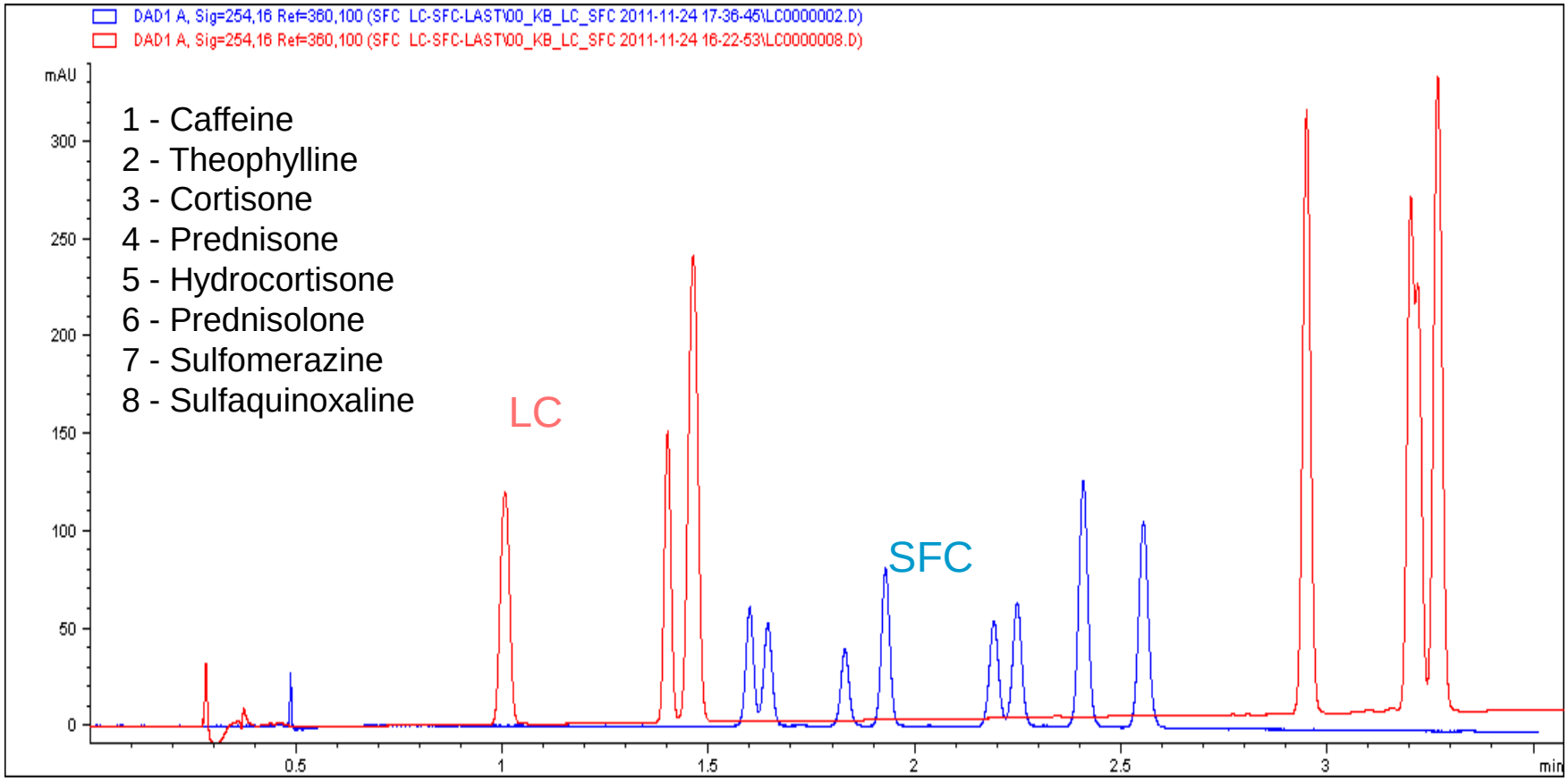


LC Conditions

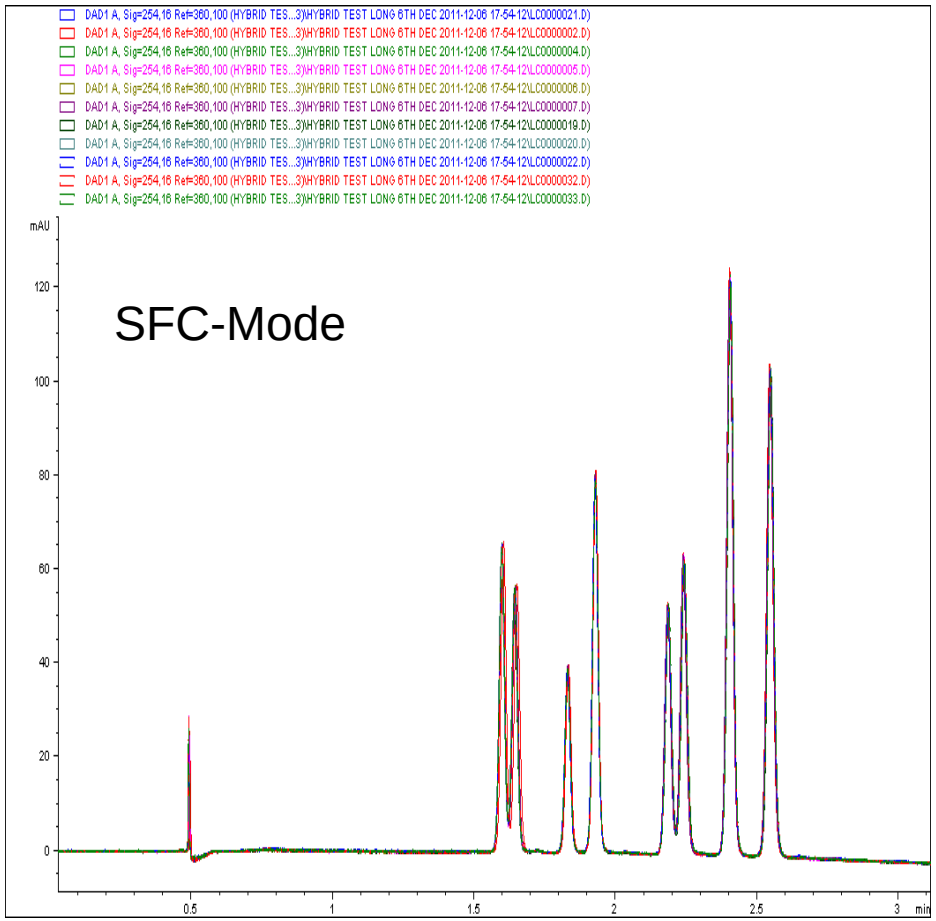
Column s–
ACE/Poroshell 3.0 x 150mm
Temp 45 C
Flow Rate 1ml/min
H20 v Meth

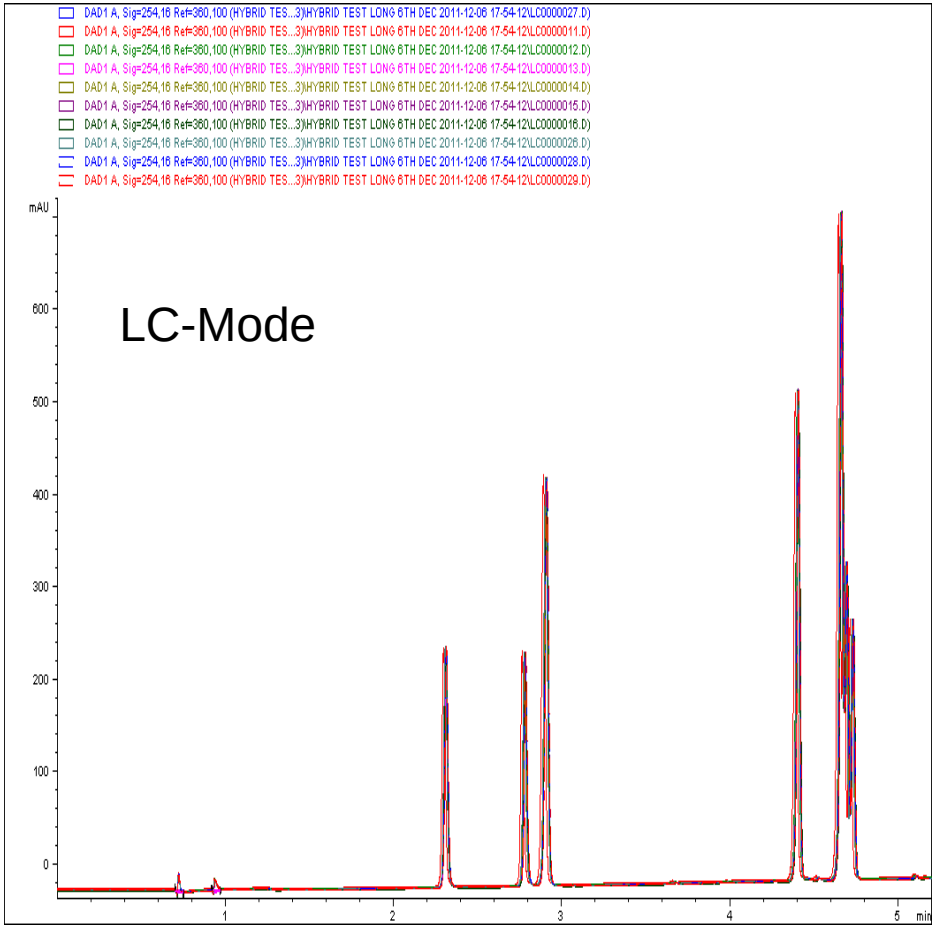
Time	%B
0	5
4.1	45
4.4	45
4.6	100
5.0	100
5.1	5

Overlay SFC versus UHPLC mode



Repeatability: SFC and LC in a single sequence





Part of a sequence with alternating SFC and LC batches

Ich danke Ihnen für Ihre Aufmerksamkeit!

Haben Sie Fragen?