UHPLC Expert eSeminar Curriculum

Agilent Liquid Phase Separation News from Pittcon 2010!

Christian Gotenfels
Product Manager UHPLC

1290 Infinity LC
- 10 x Higher Sensitivity
- Higher sample capacity
- Total cost of ownership
- Third party Software Control

Michael Frank
Product Manager HPLC

- Ultra-High Pressure Valve Solutions

Martin Vollmer
Product Manager SFC

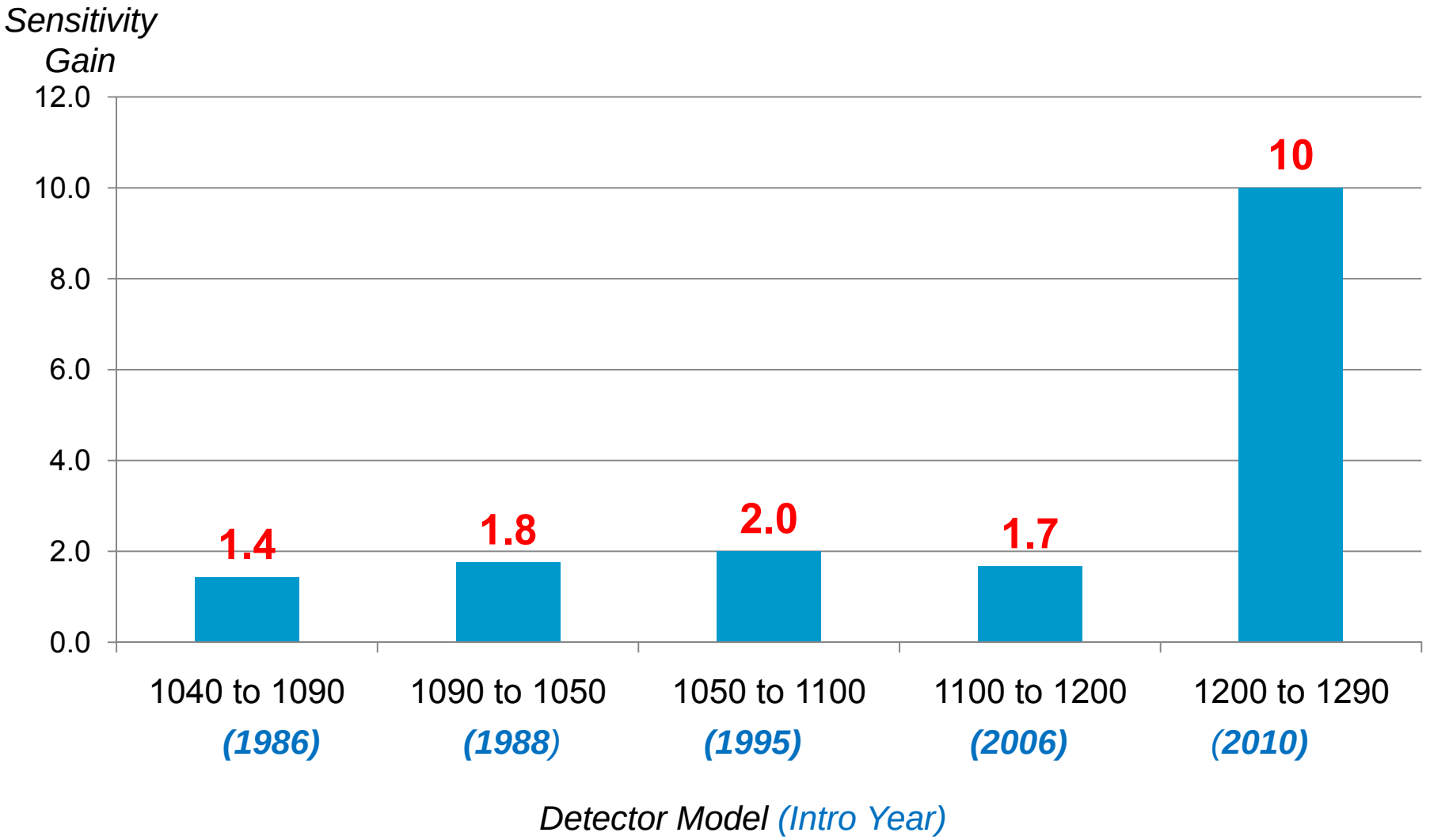
- Supercritical Fluid Chromatography Superior Sensitivity with green chemistry

The world's most sensitive UV detector!

1290 Infinity Diode Array Detector

History of Sensitivity Gain (DAD)

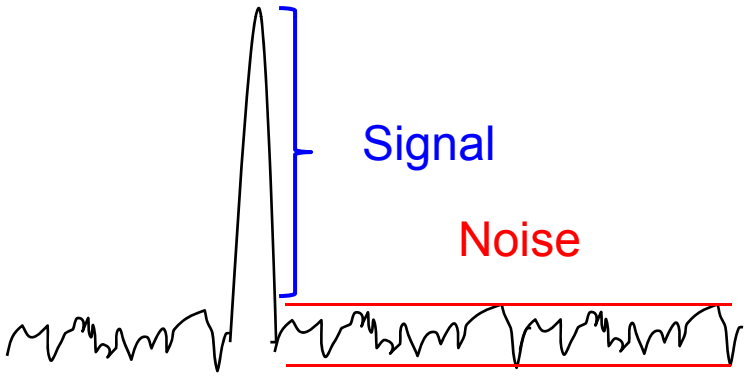
- The last 30 years



What is Sensitivity?

Limit of detection (LOD):

The lowest concentration, or smallest mass flow, which can be distinguished from the noise by a certain predefined probability (Signal/Noise).

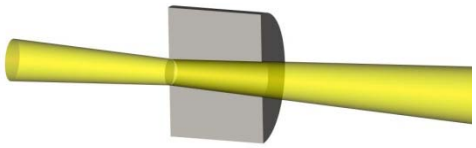


Limit of detection (LOD):

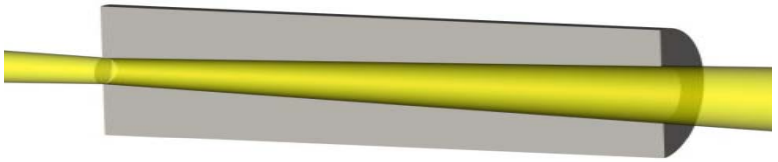
$$h_{Signal} = 3(2) \times h_{Noise}$$

Effects of path length increase

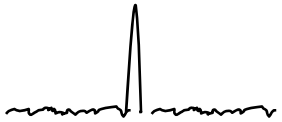
Conventional flow cells:



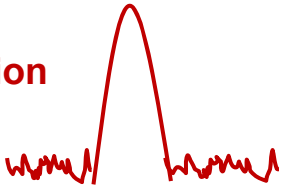
10 mm pathlength
13 µL geom. volume



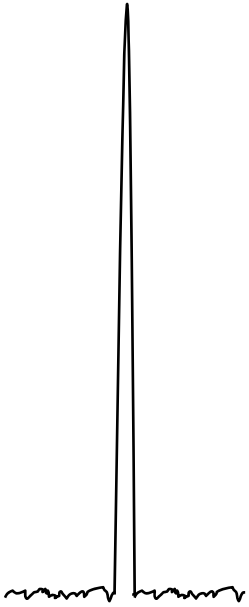
60 mm pathlength
78 µL geom. volume



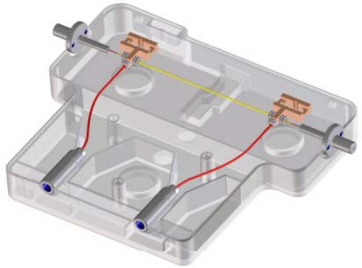
Peak Dispersion



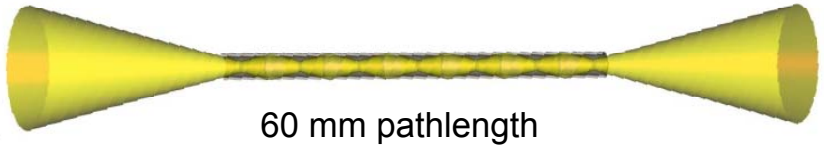
- Loss of resolution
- Loss of signal height
- Inrease of Noise



Max-Light <u>High Sensitivity</u> cell:

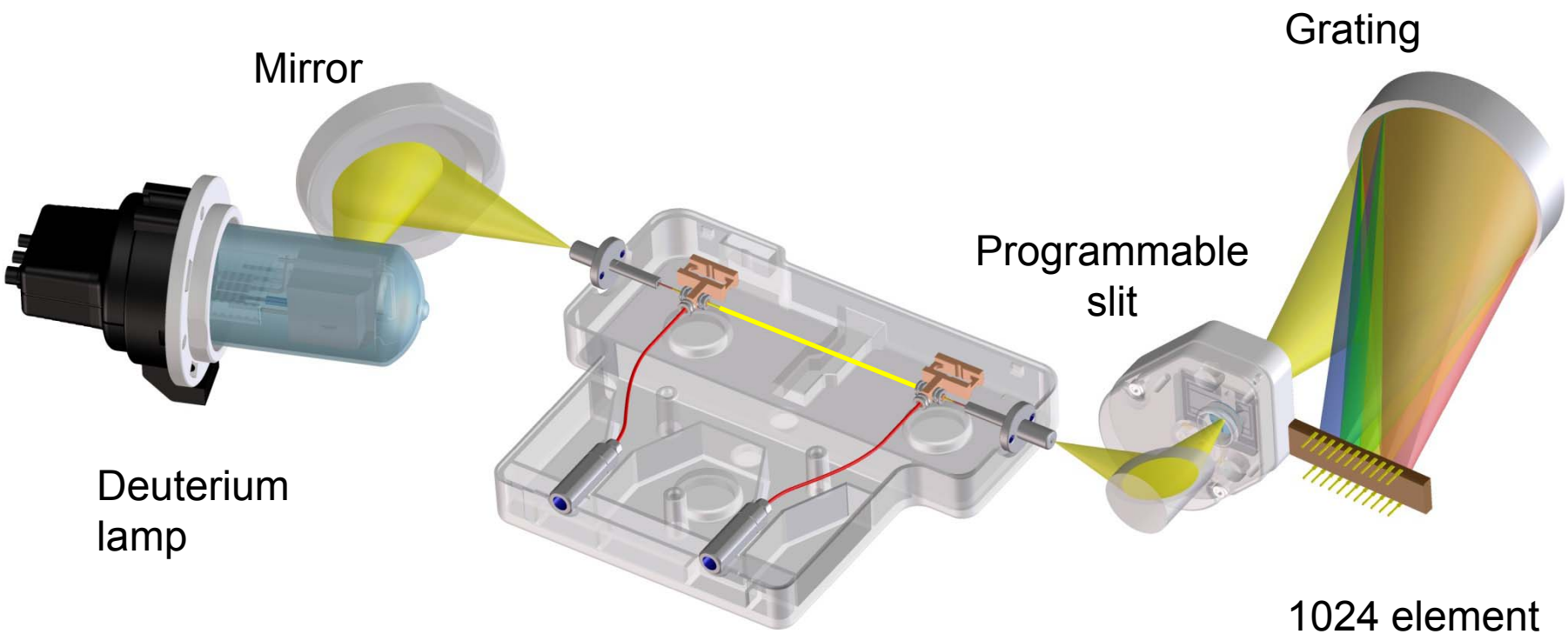


Optofluidic waveguides
(total internal reflection)



60 mm pathlength
4 µL σ_V dispersion volume

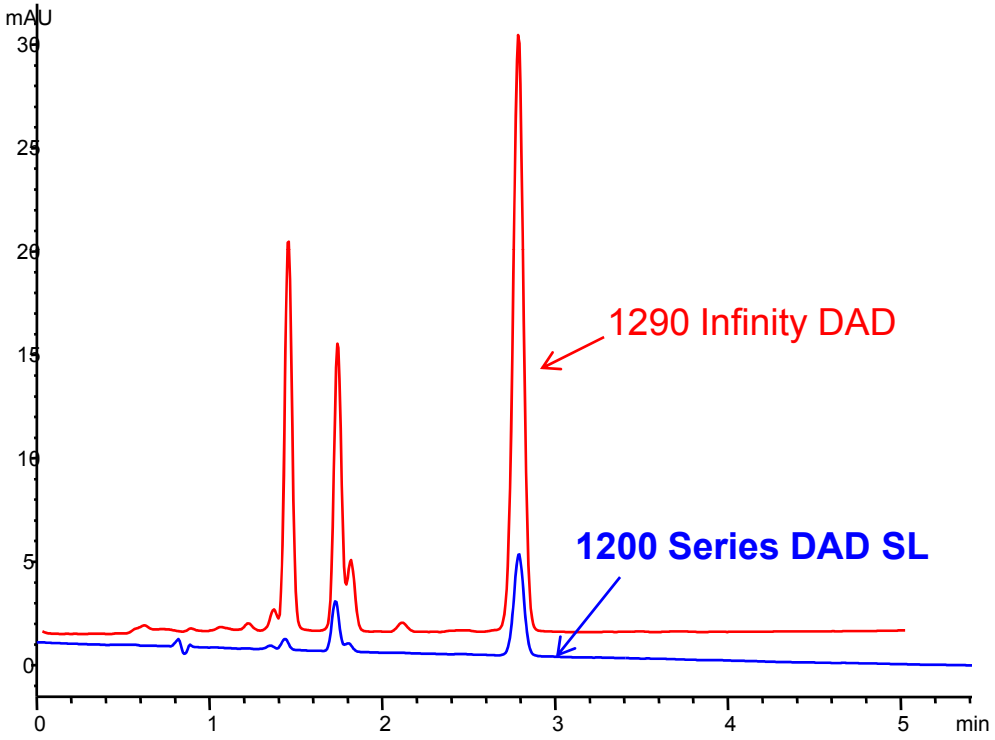
Optofluidic waveguides – Agilent Max-Light cartridge cell utilize total-internal reflection in a non-coated fused silica fiber



Check DAD video on the website

11.4 x Higher Sensitivity

-1290 Infinity DAD compare to 1200 Series DAD SL

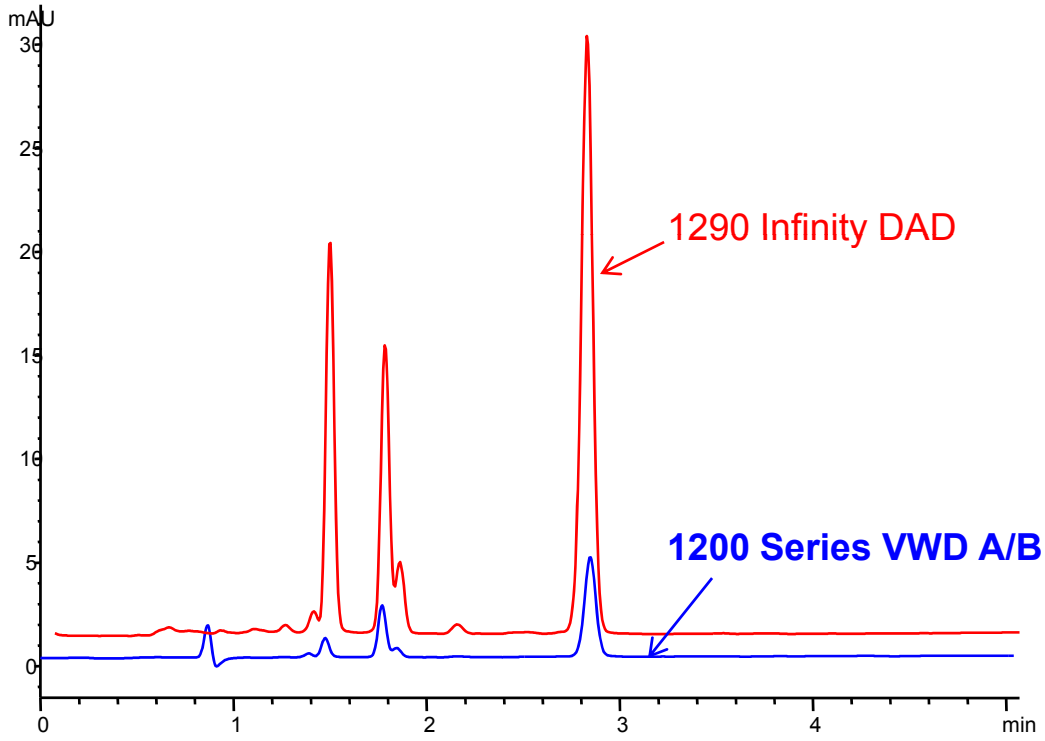


Columns: 150 x 4.6mm Zorbax SB C18, 5µm
Sample: Anthracene: 835 pg/µL
Mobile phase: A: Water, B: Acetonitrile
Elution: isocratic 80 % B
Injection volume: 5 µL
Flow: 1.5 mL/min
DAD: 251/4nm, Ref= 450/80nm,
2.5Hz, slit width 4nm

	1290 DAD 60 mm	1200 DAD SL 10mm
Height [mAU]	28.87579	4.93845
Noise [mAU]	0.009806	0.01908
Signal/ noise	2944	259
Sensitivity increase	**+11.4**	

11.6 x Higher Sensitivity

- 1290 Infinity DAD compare to 1200 Series VWD A/B



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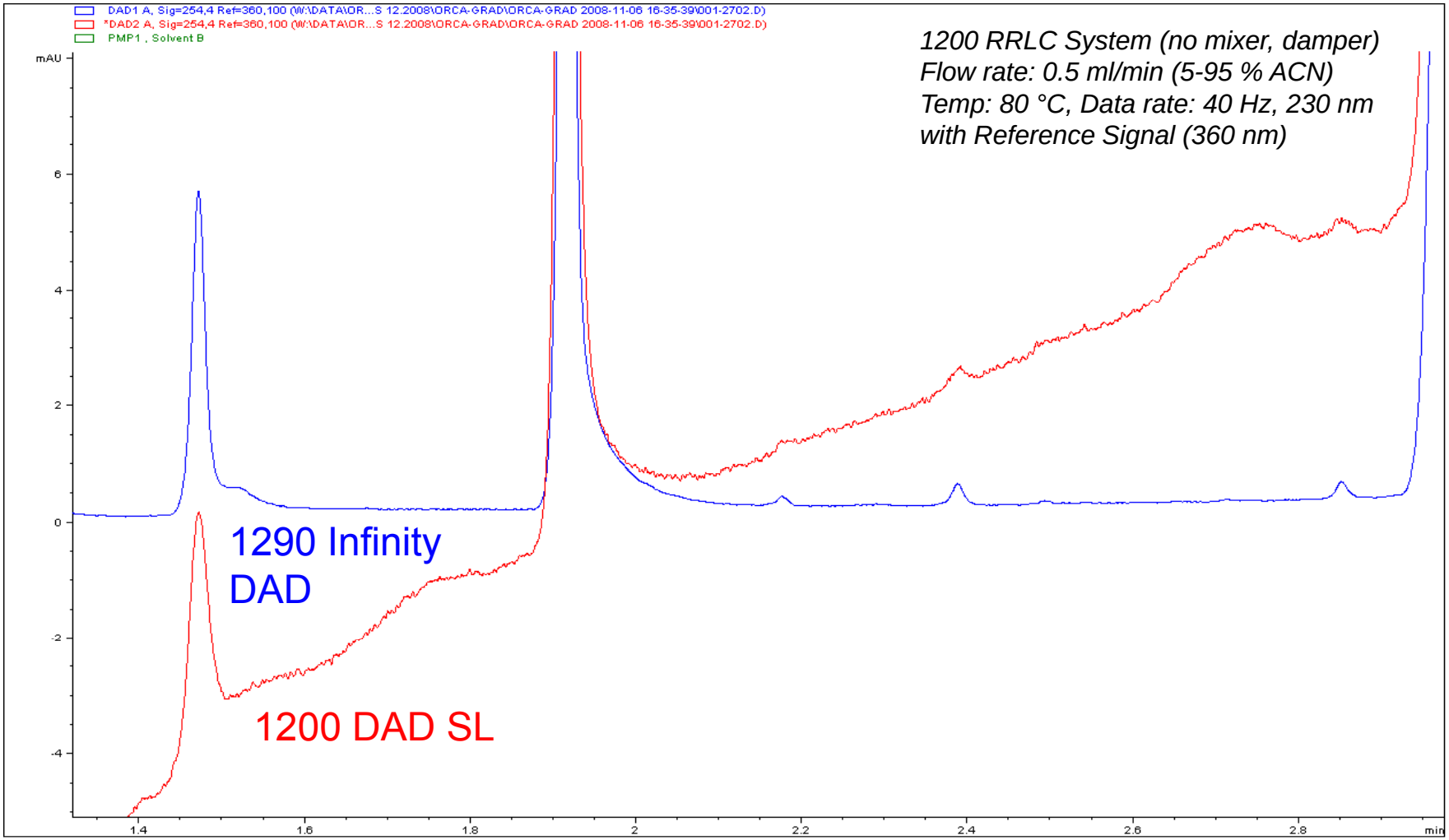
Mobile phase: A: Water, B: Acetonitrile
Elution: isocratic 80 % B
Injection volume: 5 µL
Flow: 1.5 mL/min
DAD: 251/4nm, Ref= 450/80nm, 2.5Hz, slit width 4nm
VWD: 251 nm, 2.5 Hz

	1290 DAD 60 mm	1200 VWD 10mm
Height [mAU]	28.87579	4.79998
Noise [mAU]	0.009806	0.01894
Signal/ noise	2944	253
Factor	+11.6	

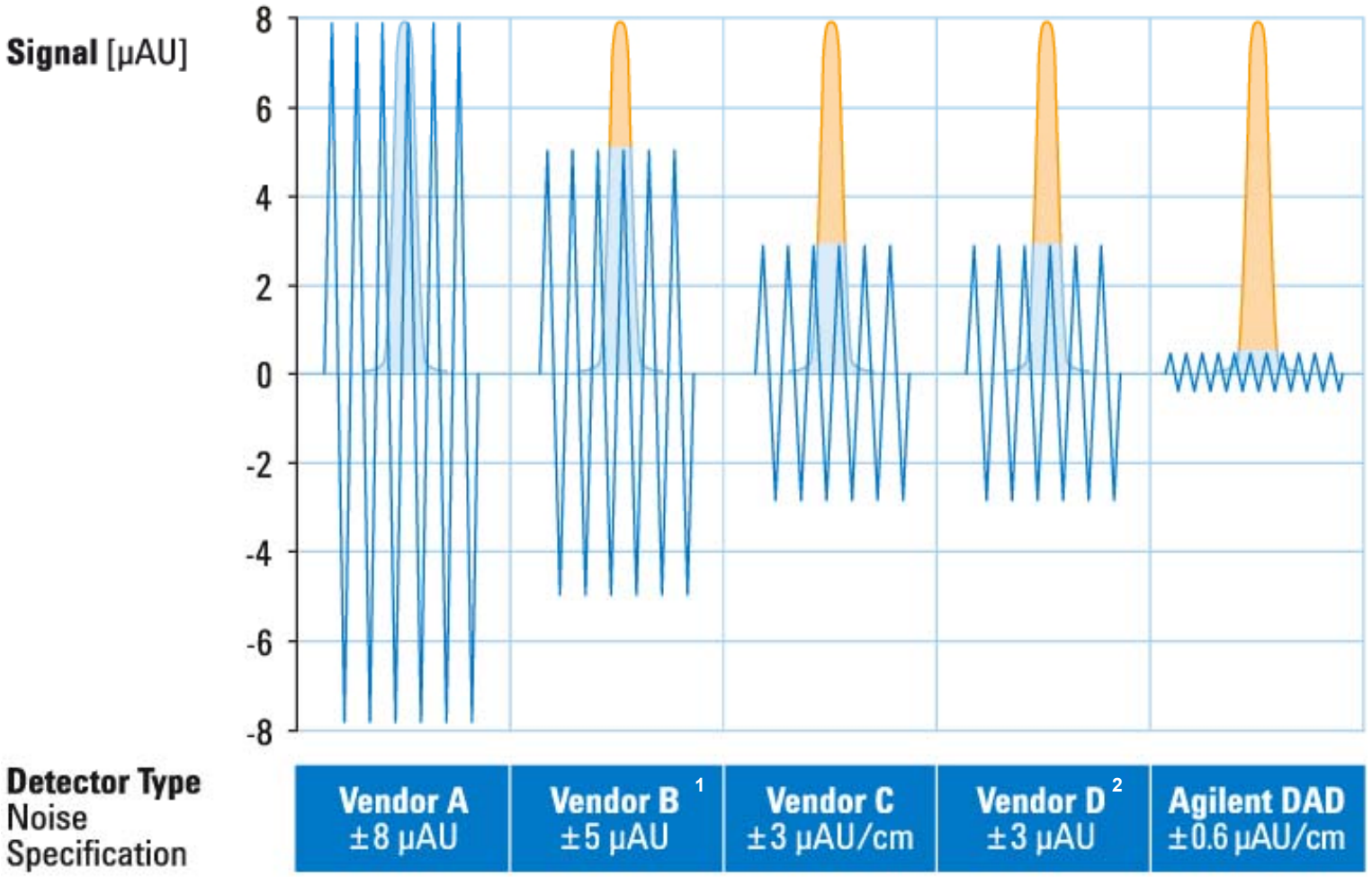
1290 Infinity Diode Array Detector

- Baseline robustness due to significant reduced RI effects



Comparison with other vendors

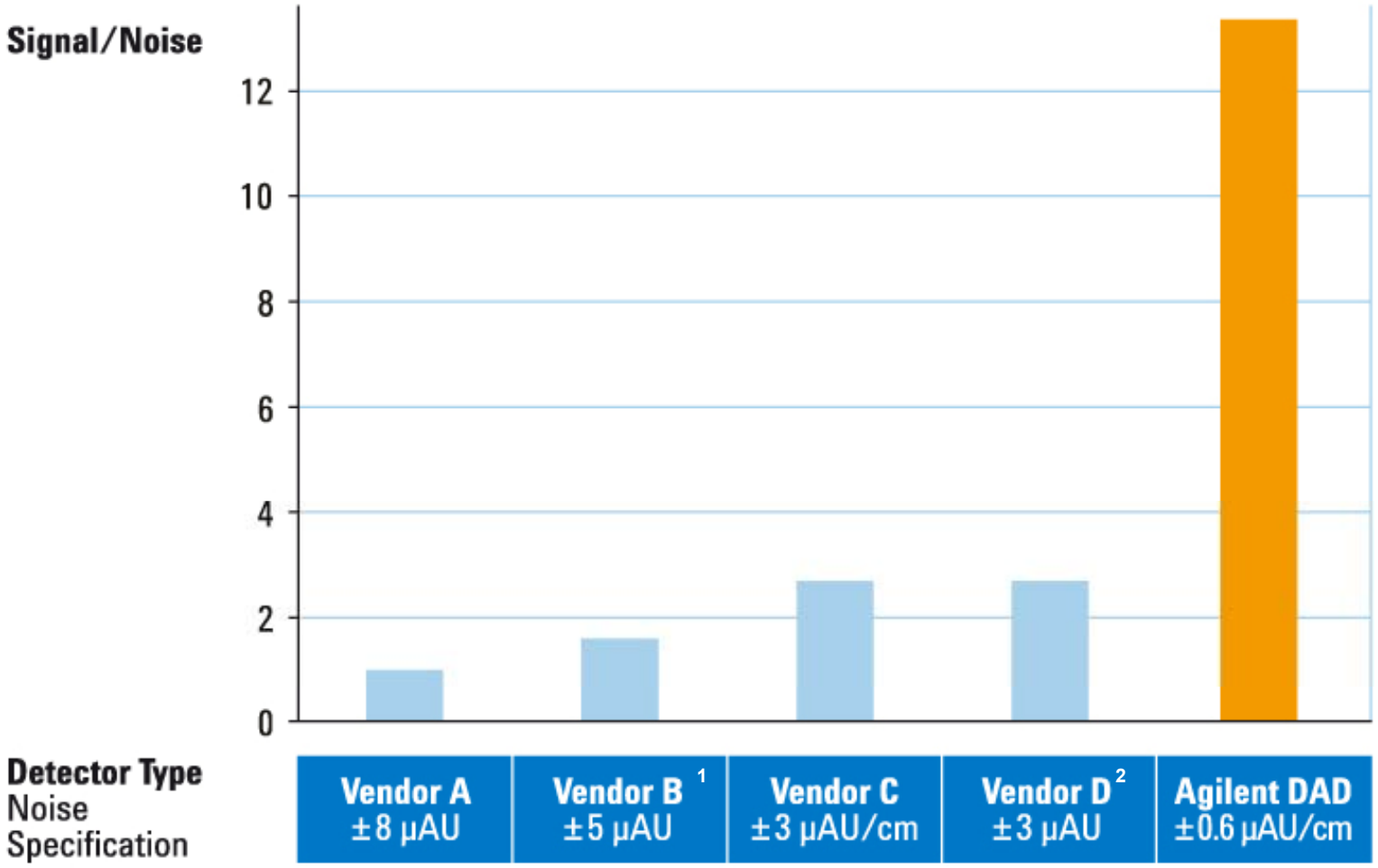
- Noise Specifications



[1] ***Dry flow cell***
[2] ***8 nm slit***

Comparison with other vendors

- *Signal/Noise*



[1] ***Dry flow cell***
[2] ***8 nm slit***

10 x Higher Sensitivity

- Customer benefits

- Higher data quality for more confidence
- Detection of lower levels of impurities
  (genotoxins)
- Simplified sample preparation methods
  (10 x less sample is required for extraction and clean up)
- Prepared to meet more stringent regulative requirements

1290 Infinity DAD Features & Benefits

- Ultra sensitivity through revolutionary Agilent Max-Light cartridge cell with 60 mm optical path length (typically noise: < ± 0.6 µAU/cm)
- Universal Agilent Max-Light cartridge standard cell with 10 mm optical path length provide high sensitivity (Noise: < ± 3 µAU) and lowest peak dispersion for 2.1, 3 and 4.6 mm ID columns
- More reliable and robust peak integration process due through less baseline drift
- Multiple wavelength and full spectral detection at high sampling rate of 160 Hz, keeping pace with the analysis speed of ultra fast LC
- Programmable slit from 1 to 8 nm provides optimum incident light conditions for rapid optimization of sensitivity, linearity and spectral resolution
- RFID technology for flow cells and lamp provide new levels of data traceability
- Electronic temperature control (ETC) provides maximum baseline stability and practical sensitivity under fluctuating ambient temperature and humidity conditions

THE UHPLC DEBATE IS OVER.

Agilent 1290 Infinity LC Injector HTS/HTC

Make your high throughput LC/MS analysis more robust

1290 Infinity Sample Injection Choices

THE UHPLC DEBATE IS OVER.

1290 Infinity Autosampler (G4226A)

- Up to 2 well plates cooled
- 40 ppm or 0.004% typical carryover
- Injection volume 40 uL and up to 100 uL possible*

1290 Infinity LC Injector HTC (G4278A) NEW

- 50 cm wide
- Up to 12 well plates cooled
- ~40 ppm or 0.004% typical carryover
- Injection volume up to 100 µL with DLW, and up to 5 mL** with Active or Fast wash

1290 Infinity LC Injector HTS (G4277A) NEW

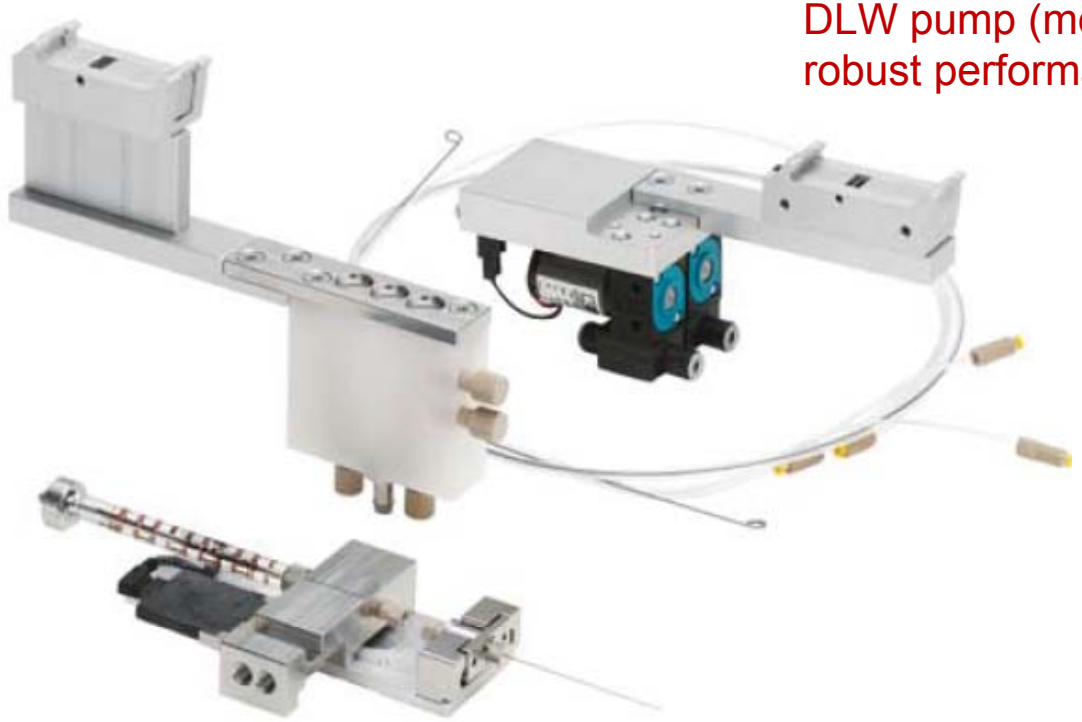
- 80 cm wide
- Up to 24 well plates cooled
- ~40 ppm or 0.004% typical carryover
- Injection volume up to 100 µL with DLW, and up to 5 mL** with Active or Fast wash

* May 2010
** Separate HW required

Fast Dynamic Load & Wash (DLW)

DLW wash station

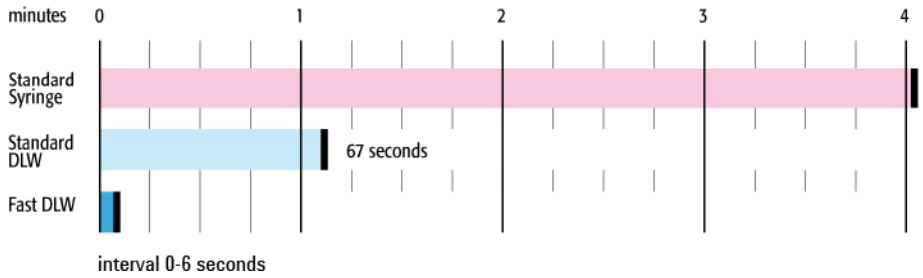
DLW pump (more robust performance)

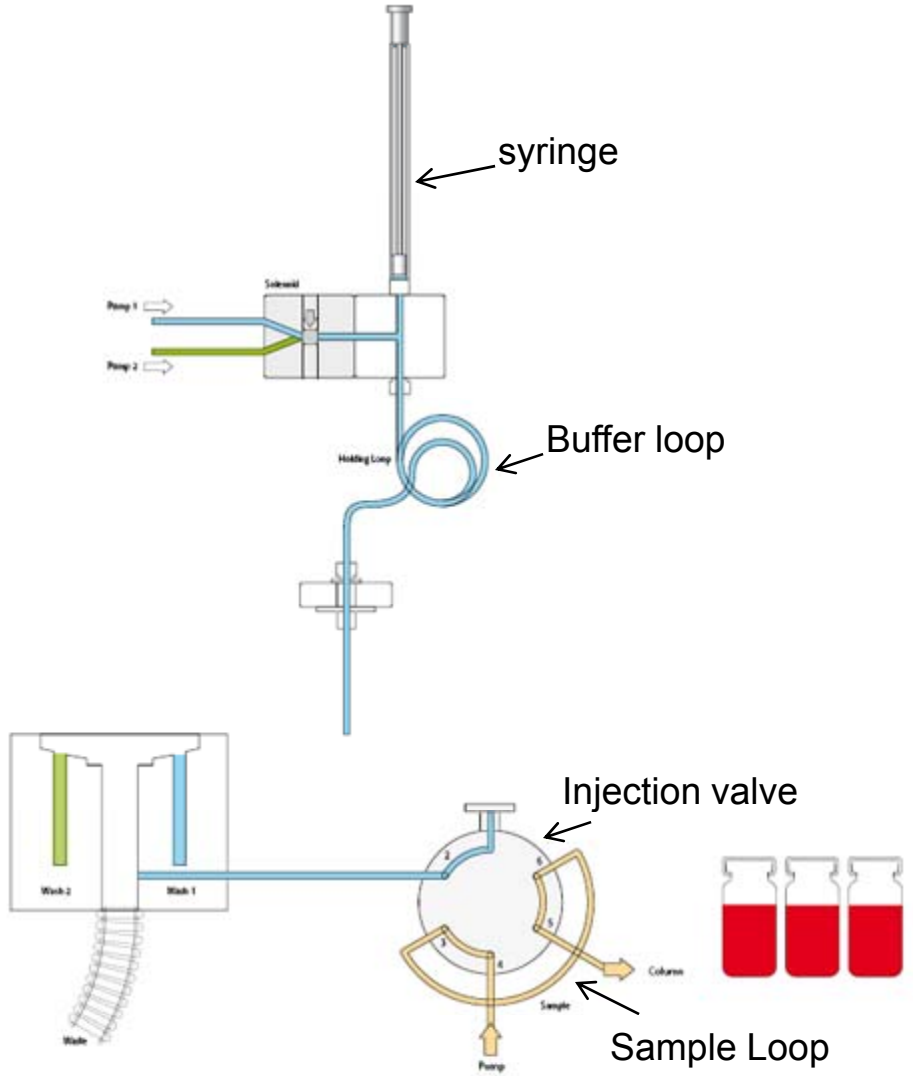


Syringe

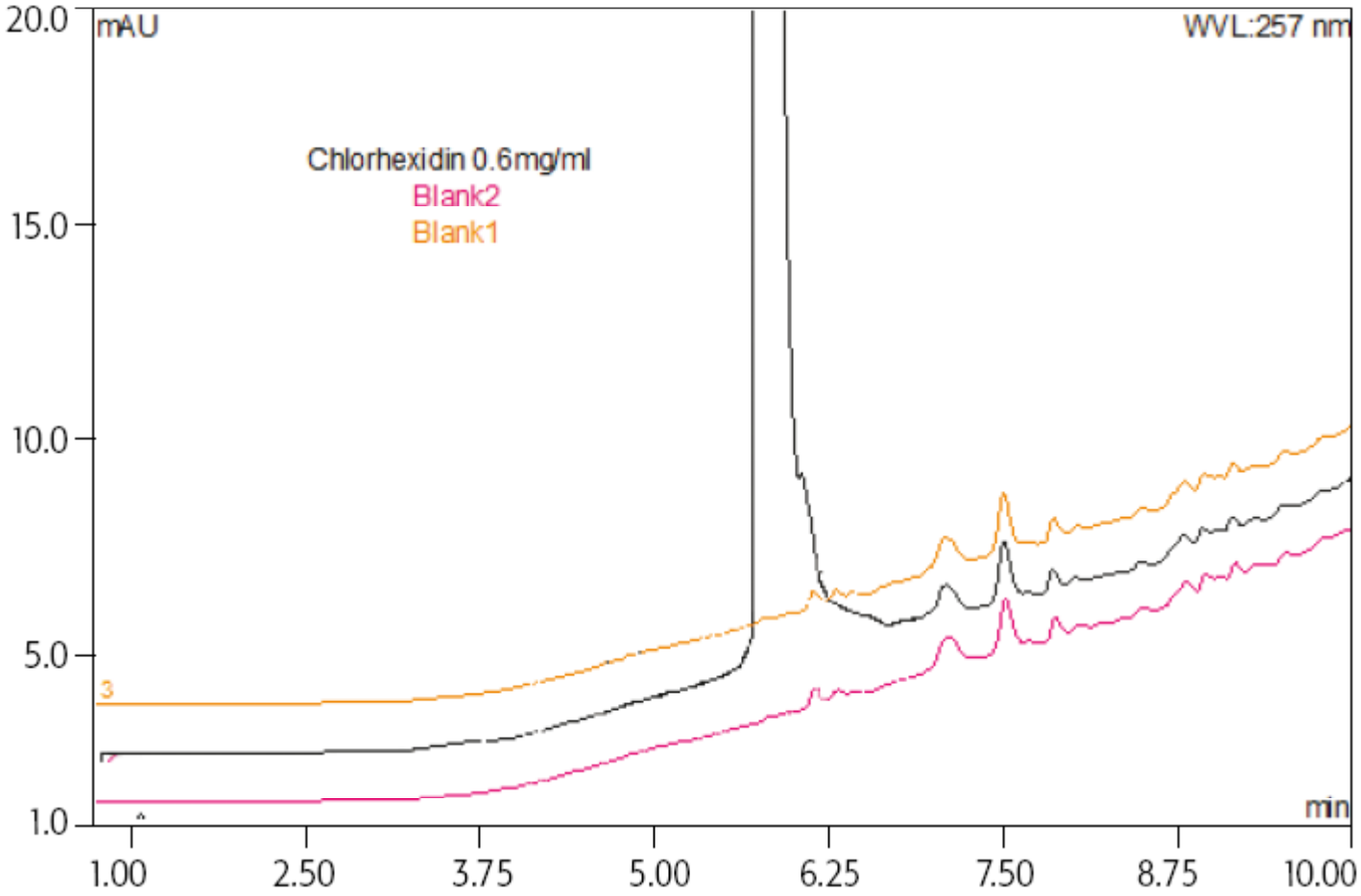
Fast Dynamic Load & Wash Cycle Demonstration

Cycle Start



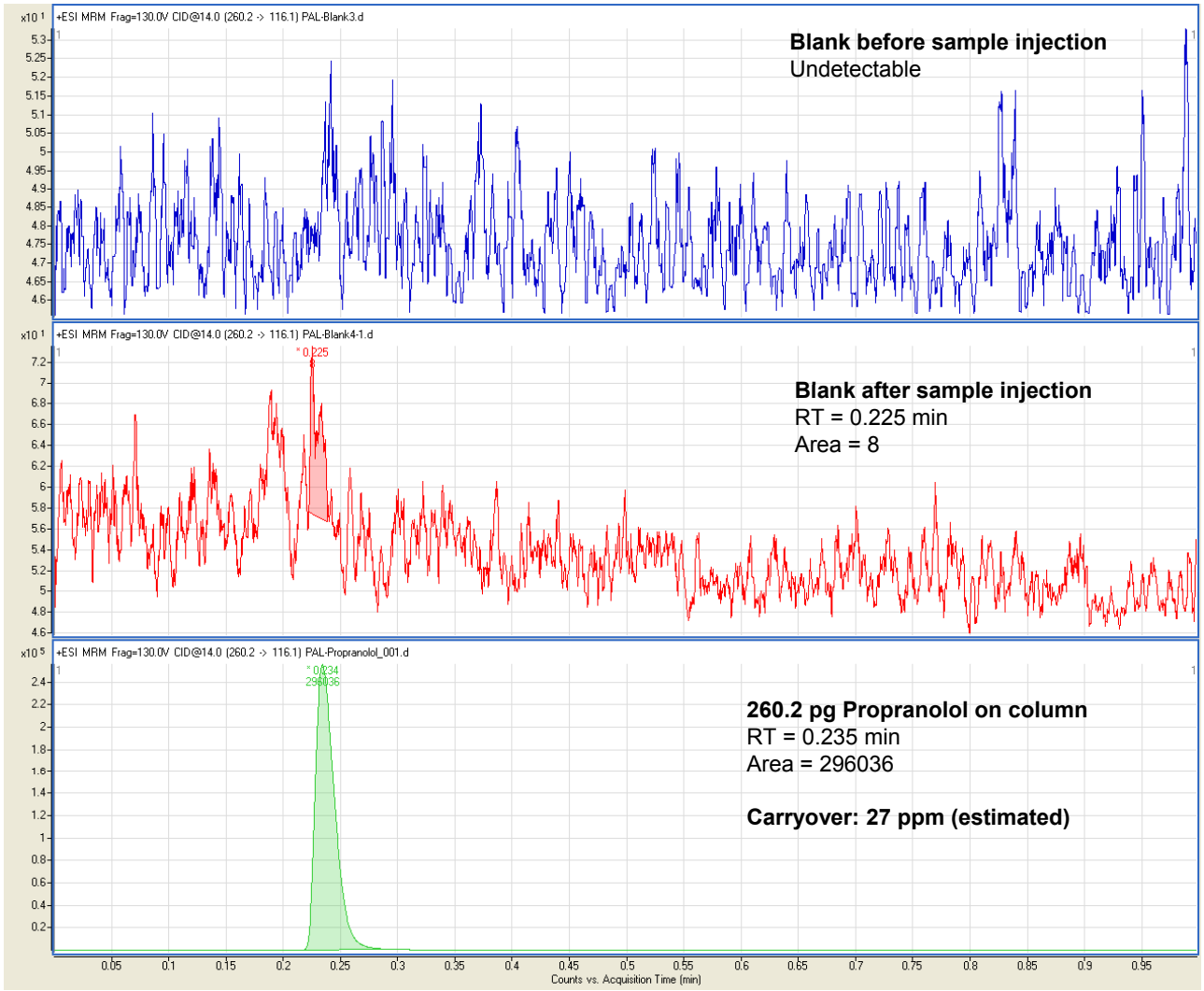


Carryover Test UV



Injection of 1200 ng Chlorhexidine on column; followed by 2 blank injections using the new DLW option. Less than 0.003% (30ppm) of carryover could be detected.

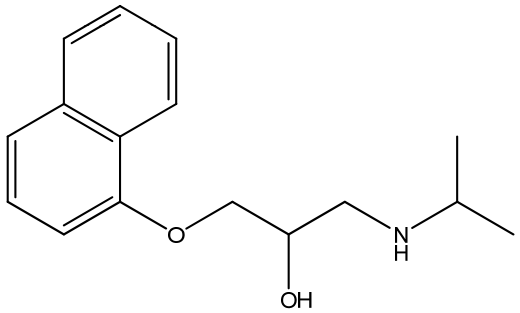
Carryover, 1.0 µM (+/-) Propranolol
(260.2 pg on column) (+MRM: 260.2 → 116.1)



Counts vs. Acquisition Time (min)

Dwell time = 20 ms
Interchannel delay= 3.5 ms
Cycle time = 50 ms

Flow rate = 0.8 mL/min



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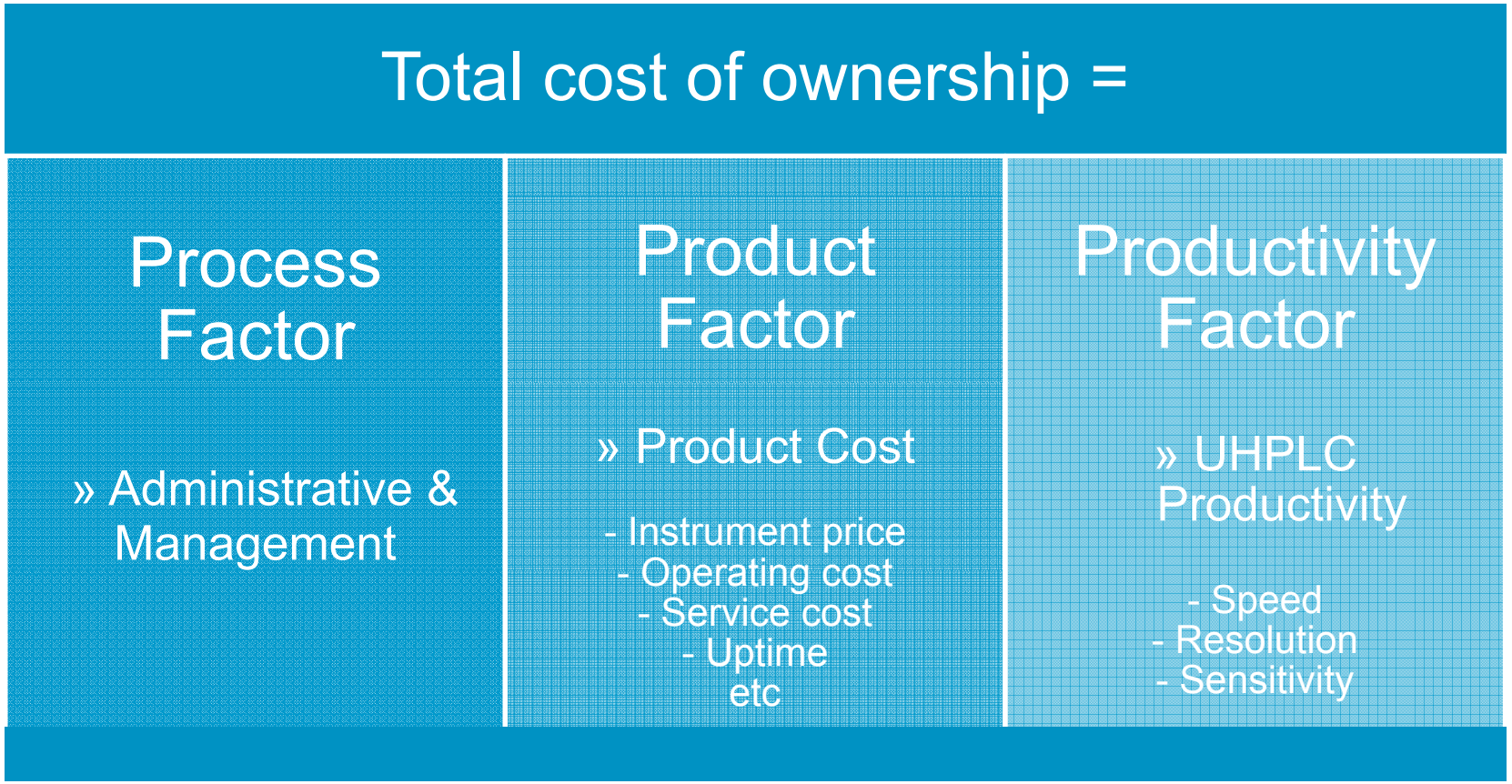
Total cost of ownership

"All costs, including direct and indirect costs, associated with owning capital assets required to support your business operations".



UHPLC Productivity with HPLC service costs

- Lowest total cost of ownership

"UHPLC systems are seen as less reliable and more complex to maintain, resulting in higher service costs compare to HPLC systems".

This has changed with 1290 Infinity LC, because of

1290 Infinity Design for supportability:

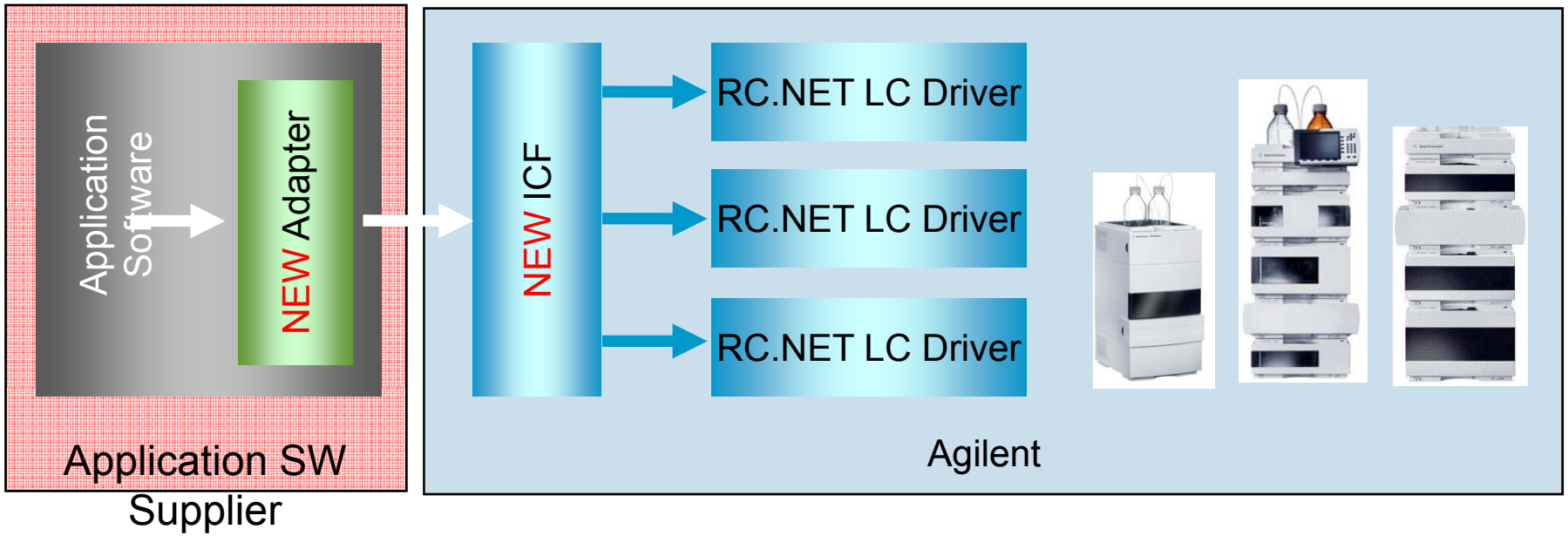
- Instrument monitoring
- Enhanced maintenance procedures
- Superior Diagnostic (onsite/remote)
- Increased lifetime of parts
- Easy and fast exchange of parts

Agilent 1290 Infinity LC

Third party Software Control

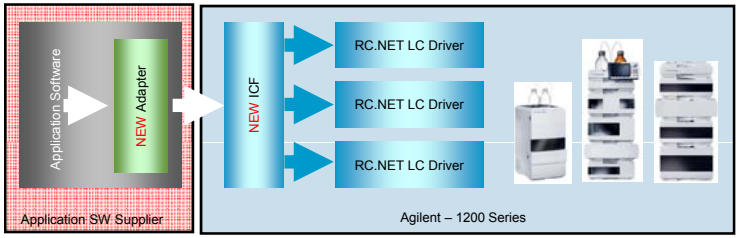
Instrument Control Framework

The Ultimate 3rd Party Connectivity Solution



Instrument Control Framework

The Ultimate 3rd Party Connectivity Solution



Advantages for Customers

Full Control

- All actual and future modules and systems supported – 1120, 1200, 1290, ...
- All features supported – complete Agilent RC.net driver functionality as Agilent SW

Fast, Easy, Trouble-free Control

- Immediate and easy access new releases – via Plug and Play of new/updated Agilent RC.net Driver

Future Proof Control

- All future modules, systems and updates supported

Dionex – Agilent Joined Statement

Dionex & Agilent Technologies
Joint Statement
on
Dionex Chromeleon Control of the Agilent 1290 Infinity LC

10th November, 2009

Dear Agilent 1290 Infinity LC and Dionex Chromeleon customer,

We have received a number of enquiries from joint customers regarding Dionex Chromeleon control of the Agilent 1290 Infinity LC.

Agilent and Dionex have a long standing agreement on cooperation in the development of software control of each other's instruments. The agreement provides Dionex access to the full set of Agilent 1200LC Series instrument control codes and/or drivers with assurance of continued updating of information should the codes/drivers change in any way and technical support from Agilent for instrument driver development and troubleshooting.

For the Agilent 1290 Infinity LC, the two companies are actively collaborating on developing Chromeleon control and early prototypes already exist. According to the current project plan, instrument control for the Agilent 1290 Infinity LC is planned to be available middle of 2010.

Regards

Dr. Stefan Schütte
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Liquid Phase Separations Business/LPAD-LSG
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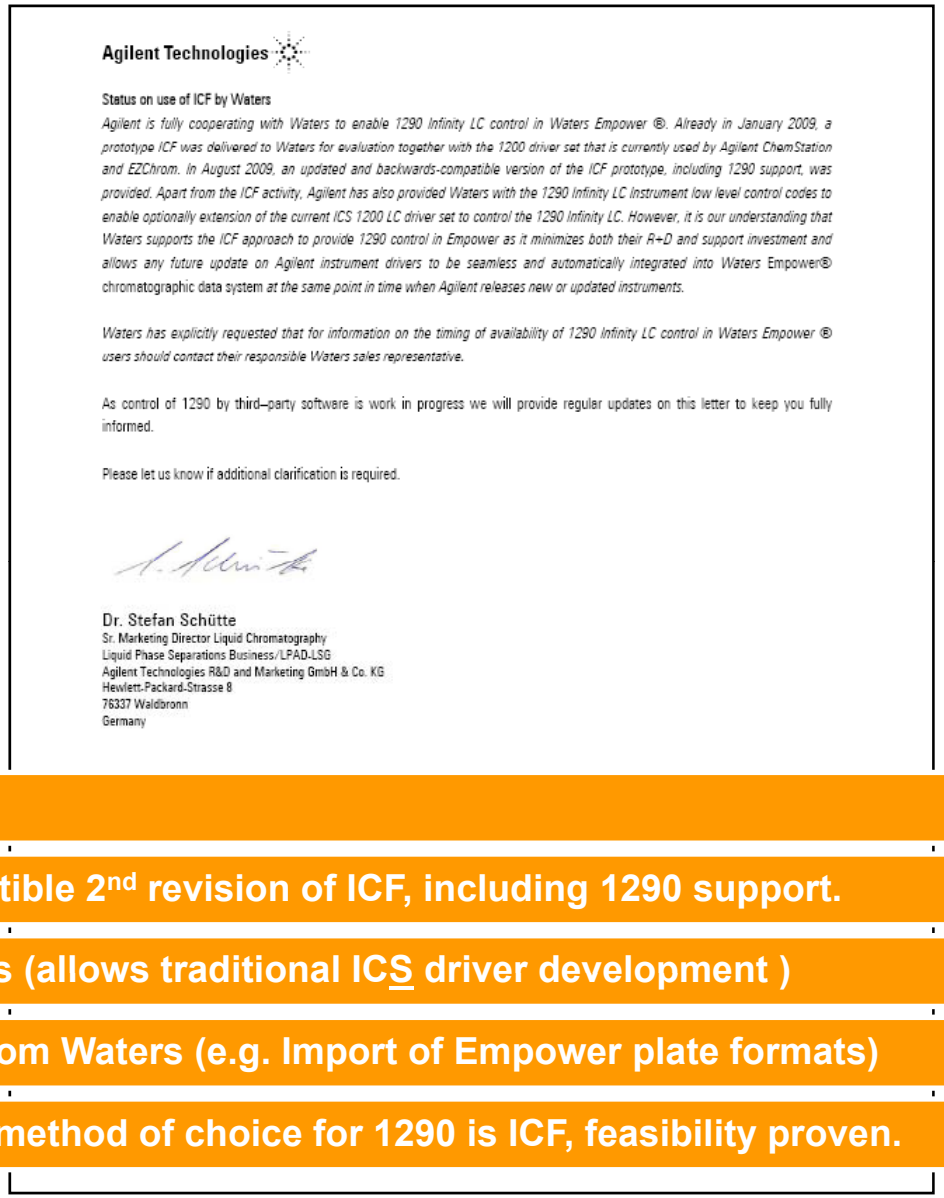
Life Sciences Business Unit
Dionex Softron GmbH
Domierstrasse 4
82110 Germering
Germany

Agilent Customer Letter on ICF



Agilent Technologies

Status on use of ICF by Waters

Agilent is fully cooperating with Waters to enable 1290 Infinity LC control in Waters Empower ®. Already in January 2009, a prototype ICF was delivered to Waters for evaluation together with the 1200 driver set that is currently used by Agilent ChemStation and EZChrom. In August 2009, an updated and backwards-compatible version of the ICF prototype, including 1290 support, was provided. Apart from the ICF activity, Agilent has also provided Waters with the 1290 Infinity LC instrument low level control codes to enable optionally extension of the current ICS 1200 LC driver set to control the 1290 Infinity LC. However, it is our understanding that Waters supports the ICF approach to provide 1290 control in Empower as it minimizes both their R+D and support investment and allows any future update on Agilent instrument drivers to be seamless and automatically integrated into Waters Empower® chromatographic data system *at the same point in time when Agilent releases new or updated instruments.*

Waters has explicitly requested that for information on the timing of availability of 1290 Infinity LC control in Waters Empower ® users should contact their responsible Waters sales representative.

As control of 1290 by third–party software is work in progress we will provide regular updates on this letter to keep you fully informed.

Please let us know if additional clarification is required.

Dr. Stefan Schütte
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76337 Waldbronn
Germany

Jan 2009: Waters received ICF from Agilent

Aug 8, 2009: Waters received backwards compatible 2nd revision of ICF, including 1290 support.

Aug 8, 2009: Waters received 1290 control codes (allows traditional ICS driver development)

2009: Agilent receives enhancement requests from Waters (e.g. Import of Empower plate formats)

Agilent‘s understanding is that implementation method of choice for 1290 is ICF, feasibility proven.

Thanks for your attention!