

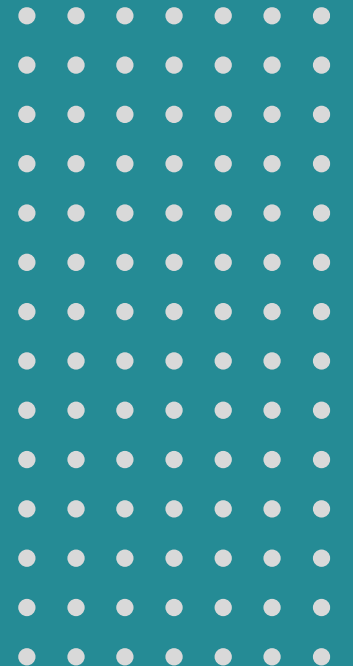
The Allotrope 2023 Spring Connect Event

LC-UV Methods and Results Interoperability using ADF

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25th April 2023





Acknowledgements



+ The extended project team

High Performance Liquid Chromatography (HPLC) instruments:



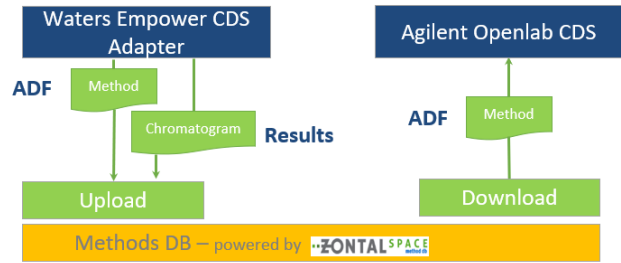
Pistoia Methods Database Project

Pistoia chartered a project to transform analytical methods into standardized, machine-readable instructions that can be stored centrally and shared across different vendors / models of HPLC-UV to execute the methods

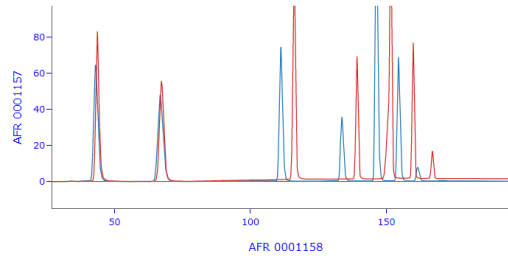


Methods Database Timeline

Building ADF adapters



OpenLab CDS
Empower CDS.



Scope extension:
Result

Chromatograms and
Peaks

Next Phase

More companies to
implement

2018

2021

Agilent 1260
Agilent 1290

2022

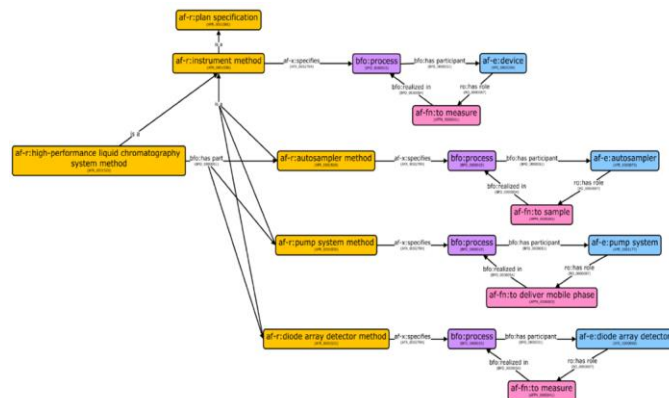
2022 Testing

2023

Extension Instrumental coverage
Waters hardware

HPLC-UV Data
model

Building
architectural
solution and
testing



Digital, automatic
methods transfer between
CDSs and between
collaborators



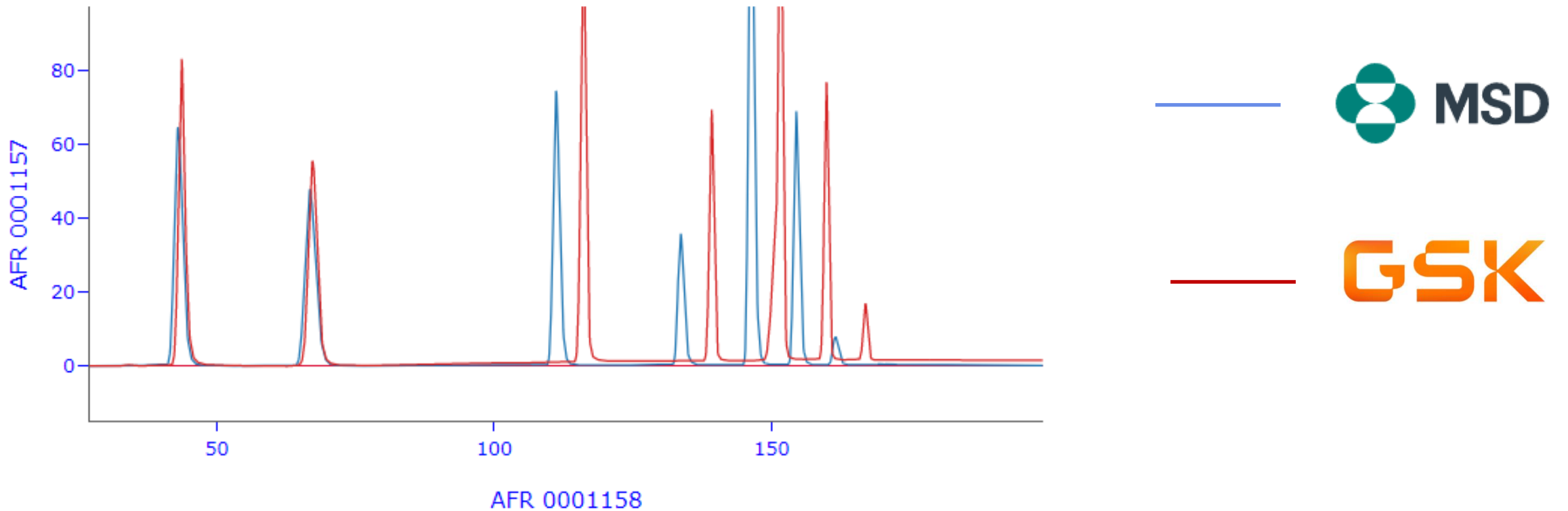
Methods DB Phase 1 Proof of Concept – Completed

Transfer methods using OpenLab CDS through ZONTAL Cloud

Objectives	Import and export methods using OpenLab CDS Transfer methods across Agilent 1260 and 1290 instruments Overlay OpenLab CDS data on ZONTAL Cloud
Results	<ol style="list-style-type: none">1. Export and import gradient methods using OpenLab CDS2. Export and import isocratic methods using OpenLab CDS3. Transfer methods between Agilent 1260 and 12904. Import third party method on OpenLab CDS5. Overlay Merck and GSK data

Methods Portability across Companies: Comparison

Method's transfer on Agilent 1290



Methods DB Phase 2 Proof of Concept – Completed

Transfer methods between Empower CDS and OpenLab CDS through ZONTAL Cloud and Orbis Gateway

Objectives

Import and export methods using Empower, OpenLab CDS and vice versa
Transfer methods across Agilent 1260 and 1290 instruments
Submit sample set from ZONTAL cloud to Empower CDS
Overlay Empower and OpenLab data

Results

1. Export and import gradient methods using Empower CDS
2. Export and import isocratic methods using Empower CDS
3. Transfer methods between Agilent 1260 and 1290
4. Transfer methods between Empower and OpenLab CDS
5. Import third party method on Empower CDS
6. Overlay OpenLab and Empower data
7. Sequence submission

Methods DB Phase 2 Proof of Concept

Video Demo – Sequence Submission from ZONTAL to Empower CDS™

Method DB Phase 1 Report AD 10FEB2022 - Last Modified: 20 April

was introduced into the system and hence impacted the retention time reproducibility.

Column details	BEH C18, 2.1 x 50 mm, 1.7 µm particle size		
Column temperature	40°C		
Mobile phase A	10% MeOH in 90% Water		
Mobile phase B	Acetonitrile		
Flow rate	0.5 mL per minute (HPLC)		
Gradient profile - A	Times (mins)	% Mobile Phase A	% Mobile Phase B
	0.00	90	10
	0.75	90	10
	3.00	5	95
	3.10	5	95
	3.30	90	10
	5.00	90	10
Detectors	DAD		
Detector wavelength	254 nm, 4nm bandwidth and 254 nm, 4nm bandwidth, Ref = 360, 100		
Peak Width	Not specified		
Injection volume	5 microlitres		
Blank/Diluent	1:1 water/ methanol		
Autosampler needle wash solvent	50/50 (v/v) water/ acetonitrile		
Seal Wash	10:90 IPA: Water		
Data collection time/Run time	5 minutes		
Number of samples	Blank + 1 sample		
Sample concentration	Standard 2 - Purchased from Waters		
Sequence Template	Blank (x3), Test mix (x6)		
Method File Names	AcqMethod_RY80B-2800CCDS2_Agilent_1290_Pistoia_Test_Method_5 AcqMethod_RY801-B200-UPLC7_TestMethod2 AcqMethod_RY818_C_208_ImportedMethod8_DF		

(a) – Test Method 5

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Methods DB Phase 2 Proof of Concept

Video Demo – Empower and OpenLab Data Overlay on ZONTAL Space

The screenshot displays the Empower software interface. The top menu bar includes File, Home, Processing, Audit/E-Sign, and Injection Tree. The main workspace is divided into several panels:

- Data Processing:** A tree view on the left showing a folder structure for 'ad418058_N81553_10_1290_Original_Gradient_17JAN2022'. It contains sub-folders for 'Blank Water' and 'Standard Mix' with various data files (e.g., 'Standard Mix - 2022-01-17 12-12-13+00-00-02-r001.dx').
- Chromatograms:** A central plot area showing a chromatogram for 'Standard Mix | DAD1A, Sig=220.0,4.0 Ref=360.0,100.0'. The y-axis is labeled 'Response' and the x-axis is 'Retention time'. The plot shows several distinct peaks. A yellow circle highlights the 'Chromatograms' tab in the top right of this panel.
- Sample Information:** A table at the bottom right providing details for the selected sample.

Sample Information	
Description	
Type	Sample
Level	
Sample amount	0
Multiplier	1; 1; 1; 1; 1
Dil. factor	1; 1; 1; 1; 1

At the bottom of the interface, the status bar shows 'Current user: SYSTEM' and 'Connected'. The Windows taskbar at the very bottom indicates the time is 17:12 on 28/04/2022.

Phase 2 – Overlay of Empower and OpenLab CDS Data

Overlay of data collected on Agilent 1290 instrument using Empower and OpenLab CDS

The slight difference in peak height and retention time was expected as different lot of standards and mobile phases were used.

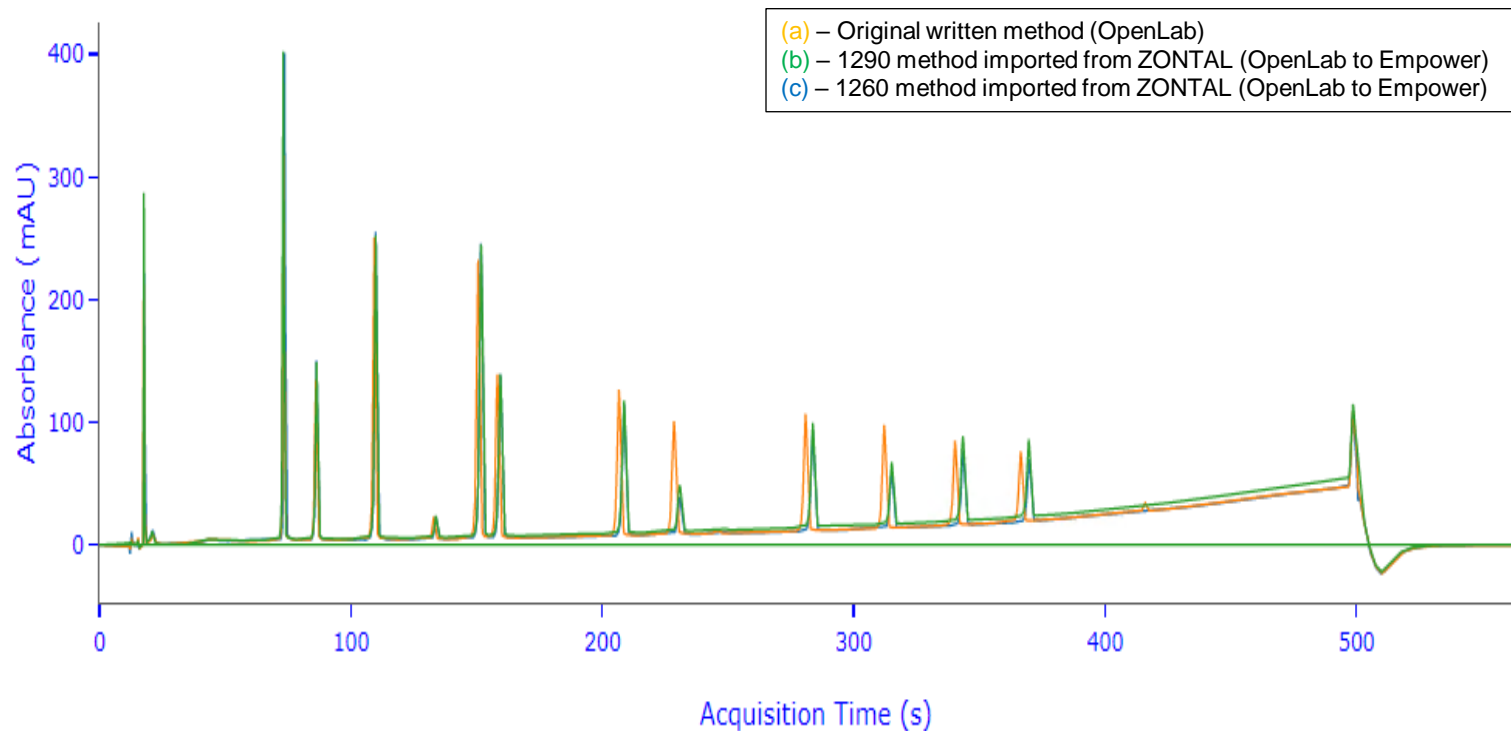


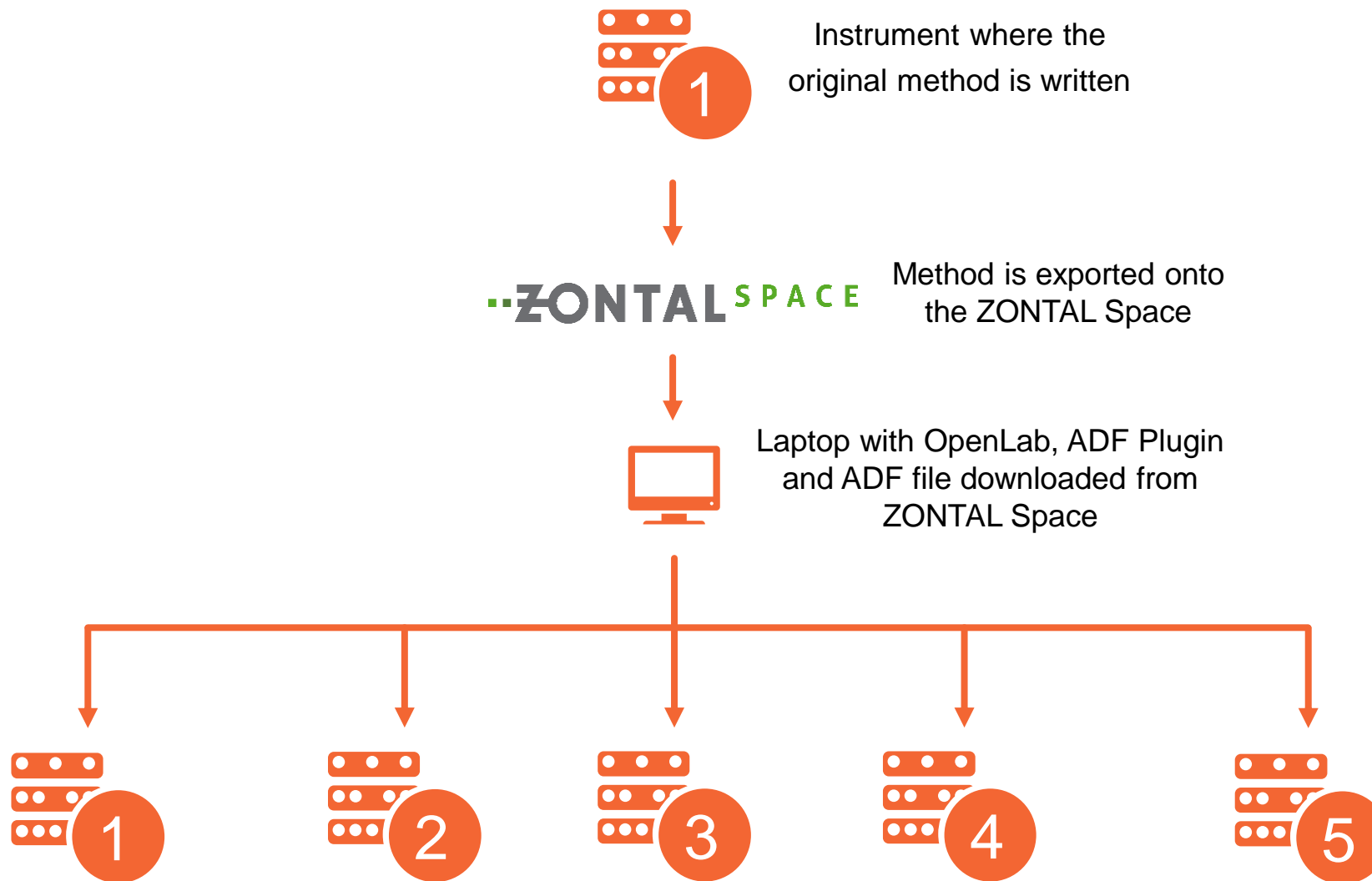
Figure 2: HPLC chromatogram overlay of non-GMP 8-minute generic method (1290 Instrument): (a) original written method on 1290 using OpenLab CDS, (b) 1290 method imported from ZONTAL on Empower CDS and (c) 1260 method imported from ZONTAL on Empower CDS

Methods DB Phase 3 Proof of Concept – Completed

Transfer methods using OpenLab CDS through the ZONTAL Cloud on a diverse set of instruments

Objectives	Import and export methods using OpenLab CDS Transfer methods across different Agilent Instruments (with different modules) Transfer methods from binary to quaternary pump
Results	<ol style="list-style-type: none">1. Install OpenLab and ADF plugin on laptop to facilitate instrument control2. Update the ADF plugin to support method transfer from binary to quaternary pump3. Export and import gradient methods using OpenLab CDS4. Transfer methods between Agilent 1290, 1260 and 1200 modules
Next steps	<ul style="list-style-type: none">– Repeat the experiments on two diverse instruments in the US– Share findings internally and externally.

15 Minute Gradient Method Setup



ADF method files imported onto instruments with different modules stacking

HPLC/UPLC Instrument Configuration

HPLC/UPLC Module Stacking

Module	Instrument 1: WU_STV11	Instrument 2: WU_S29	Instrument 3: STV_02	Instrument 4: S56 (Biopharma)	Instrument 5: LC2
Pump	G7120A: 1290 Infinity II Binary Pump	G1312A: 1200 Binary Pump	G7120A: 1290 Infinity II Binary Pump	G7111B: 1260 Infinity II Quaternary Pump	G1312B: 1260 Infinity Binary Pump
Injector	G7167B: 1290 Infinity II Multisampler	G1329A: 1100 Autosampler	G7167B: 1290 Infinity II Multisampler	G7167A: 1260 Infinity II Multisampler	G7167A: 1260 Infinity II Multisampler
Column Compartment	G7116B: 1290 Multicolumn Thermostats	G1316A: 1200 Thermostatted Column Compartment	G7116B: 1290 Multicolumn Thermostats	G7116B: 1290 Multicolumn Thermostats	G1316C: 1200 Thermostatted Column Compartment
Detector	G7117B: 1290 Infinity II Diode Array Detector	G1314B: 1200 Infinity Variable Wavelength Detector	G7117A: 1290 Infinity II Diode Array Detector	G7115A: 1260 Infinity II Diode Array Detector Wide Range (WR)	G4212B: 1260 Infinity Diode Array Detector

15 Minute Gradient Method

Results and discussion

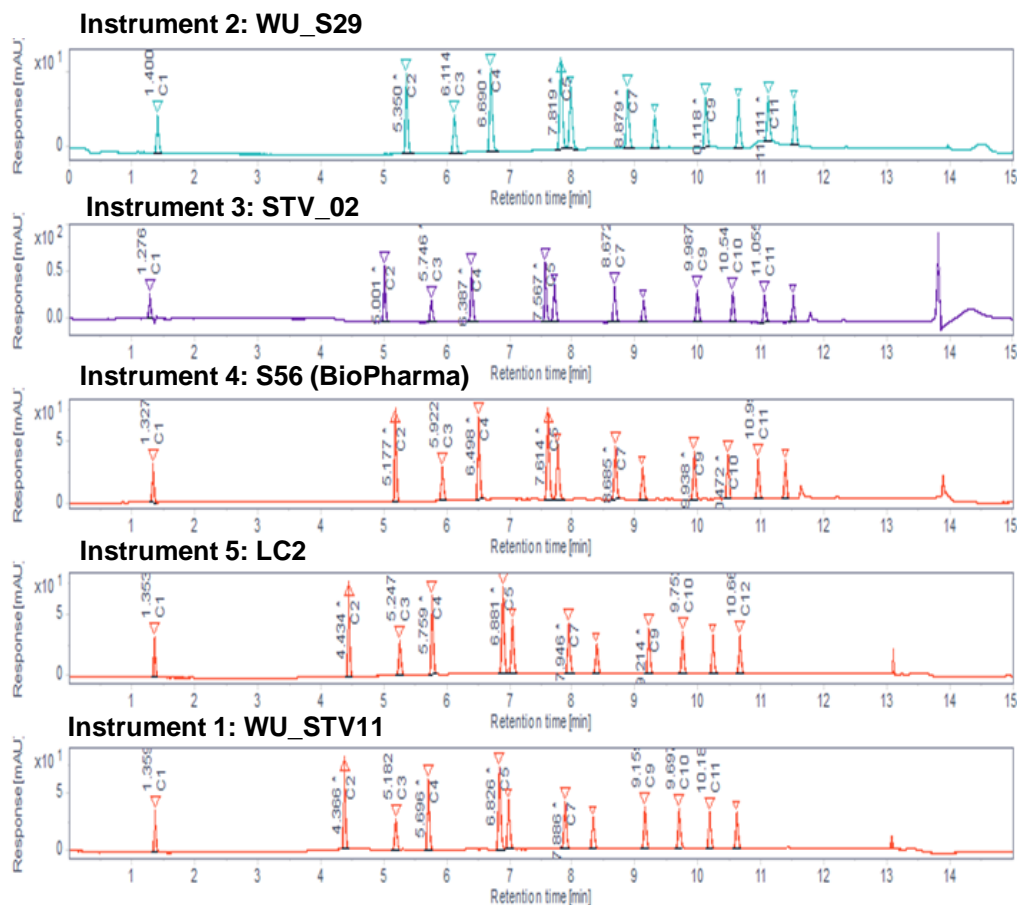


Figure 3: HPLC chromatogram of 15 minute CSH method collected on instruments 1 to 5.

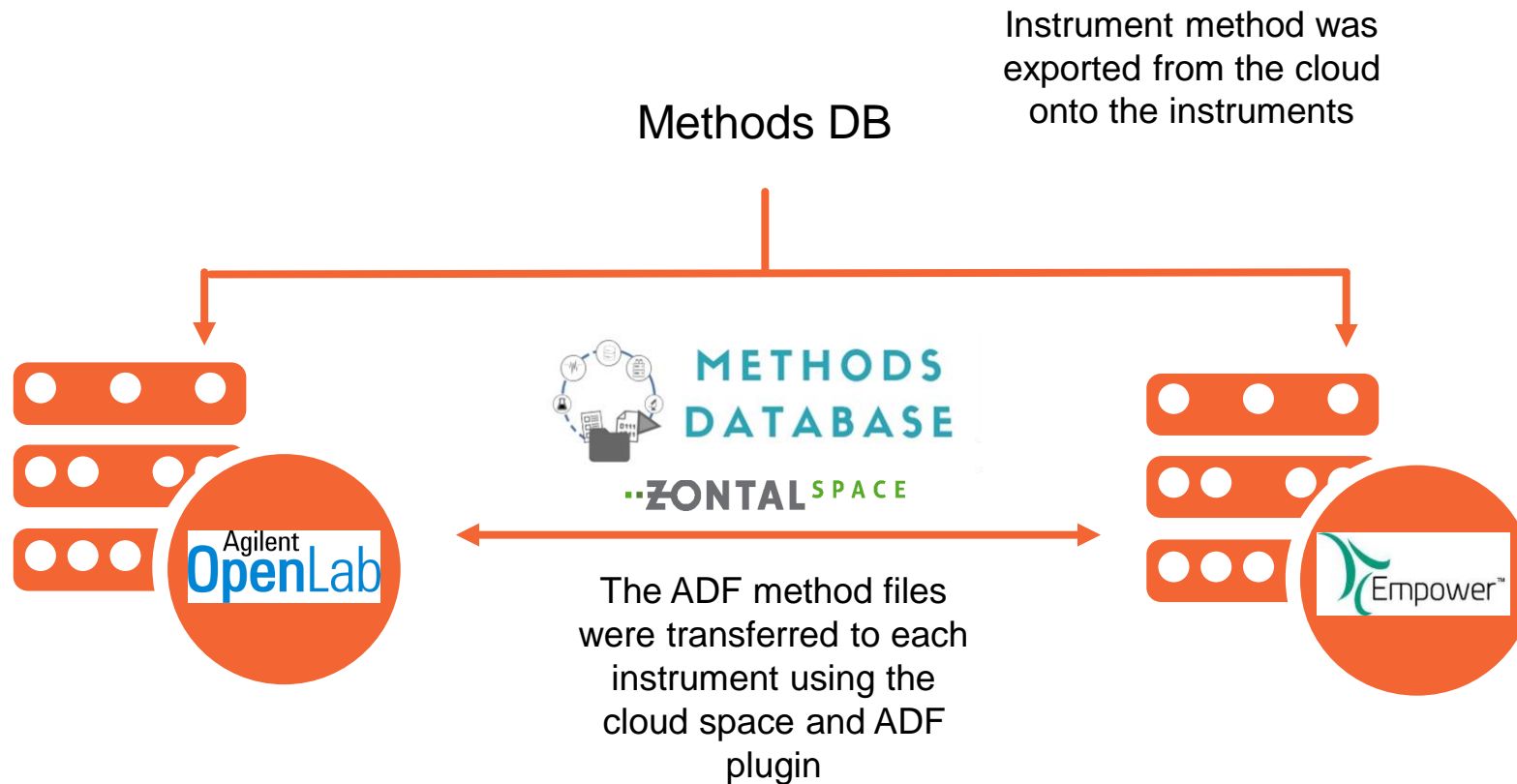
- As shown in Figure 3, the data collected on instrument 1 is comparable to instruments 2-5.
- The data obtain confirms our theory that the 1200/1260 has a lag in applying the gradient in comparison to the 1290 pump
- As expected, the same elution order was observed throughout all the 5 instruments.
- A slight change in difference in retention time was observed on various instruments but this was expected as each instrument will have a different dwell volume.
- The data proves that the ADF plugin can be used to transfer method across a wide range of Agilent HPLC/UPLC instruments.

Methods DB Phase 4 Proof of Concept – Completed

Transfer methods between different manufacturers (hardware and CDS)

Objectives	Transfer methods between Waters and Agilent Hardware Transfer method between Empower CDS and OpenLab CDS
Results	<ol style="list-style-type: none">1. Transferred a gradient method between Waters Acquity and Agilent 1290 instrument (including autosampler temperature)2. Transferred a gradient method between Empower CDS and OpenLab CDS3. Overlay data acquired using both CDS
Next steps	– Draft article to share results with analytical community

Phase 4 Step Up



ADF files were shared between the two different manufacturers using the Zontal Space

Instrument Configuration

UPLC Module Stacking

Module	Agilent Hardware WU_STV11	Waters Hardware WU_STV0188
Pump	G7120A: 1290 Infinity II Binary Pump	Acquity UPLC H Class Quaternary Pump
Injector	G7167B: 1290 Infinity II Multisampler	Acquity UPLC H Class Sample Manager FTN
Column Compartment	G7116B: 1290 Multicolumn Thermostats	Acquity UPLC H Class Column Manager
Detector	G7117B: 1290 Infinity II Diode Array Detector	Acquity UPLC Photodiode Array Detector

Method Comparison

Diode Array Detector: Photodiode Array Detector

Signals

Acc	Wavelength	Bandwidth	Wavelength	Bandwidth
Signal A	<input checked="" type="checkbox"/> 254.0	4.0	<input checked="" type="checkbox"/> 360.0	100.0
Signal B	<input checked="" type="checkbox"/> 254.0	4.0	<input type="checkbox"/> 360.0	100.0
Signal C	<input type="checkbox"/> 254.0	4.0	<input type="checkbox"/> 360.0	100.0
Signal D	<input type="checkbox"/> 230.0	4.0	<input type="checkbox"/> 360.0	100.0
Signal E	<input type="checkbox"/> 260.0	4.0	<input type="checkbox"/> 360.0	100.0
Signal F	<input type="checkbox"/> 273.0	4.0	<input type="checkbox"/> 360.0	100.0
Signal G	<input type="checkbox"/> 280.0	4.0	<input type="checkbox"/> 360.0	100.0
Signal H	<input type="checkbox"/> 290.0	4.0	<input type="checkbox"/> 360.0	100.0

Peakwidth
> 0.025 min (0.5 s response time) (10 Hz)

Stop time: As Pump/Injector Off
 min

Post time: Off On
 min

Advanced

Spectrum

Store: All

Range from: 190.0 nm to 400.0 nm

Step: 2.0 nm

Analog Output

Zero Offset: 5 %

Attenuation: 1000 mAU

Margin for negative Absorbance: 100 mAU

Autobalance: Prerun Postrun

Lamps on required for acquisition: UV Lamp

PDA Detector

General | 2D Channels | Analog Out | Events

Lamp On

Enable 3D Data

λ Range: 191 nm to 400 nm

Resolution: 2.4 nm

Sampling Rate: 10 points/sec

Filter Time Constant: Fast 0.1000 sec

Exposure Time: Auto msec

Interpolate 2nd order filter Region

Use UV blocking filter (below 210nm)

Negative Absorbance Margin: -0.40 AU

Comment:

PDA Detector

General | 2D Channels | Analog Out | Events

Data mode: λ

Channel 1 Absorbance-Compensat 254 3.6 nm resolution

Channel 2 Absorbance 254 3.6 nm resolution

Channel 3

Compensation reference λ Start: 310 End: 410 nm

Method Comparison

Binary Pump: Quaternary Pump

Flow: 0.500 mL/min

Solvents

A: 1 100.0 % Methanol in Water
2 100.0 % Water V.03

B: 1 100.0 % Acetonitrile V.03
2 100.0 % Acetonitrile V.03

Pressure Limits: Min: 0.00 bar, Max: 1,200.00 bar

Stoptime: As Injector/No Limit (selected), 5.00 min

Posttime: Off (selected), 1.00 min

Advanced

Timetable (5/100 events)

Time [min]	A [%]	B [%]	Flow [mL/min]	Max. Pressure Limit [bar]
0.00	90.00	10.00	0.500	1200.00
0.75	90.00	10.00	---	---
3.00	5.00	95.00	---	---
3.10	5.00	95.00	---	---
3.30	90.00	10.00	---	---
5.00	90.00	10.00	---	---

Quaternary Solvent Manager

Auto-Blend Plus™

General Misc Data

Solvents: A, B, C, D

Pressure Limits: Low: 0 psi

Gradient

Time	Flow (mL/min)	%A	%B	%C	%D	Curve
1 Initial	0.500	90.0	10.0	0.0	0.0	Initial
2 0.75	0.500	90.0	10.0	0.0	0.0	6
3 3.00	0.500	5.0	95.0	0.0	0.0	6
4 3.10	0.500	5.0	95.0	0.0	0.0	6
5 3.30	0.500	90.0	10.0	0.0	0.0	6
6 5.00	0.500	90.0	10.0	0.0	0.0	6

OK Cancel

Method Comparison

Column Compartment: Column Manager

The image displays a software interface for configuring a column compartment. It is divided into several sections:

- Temperature:** This section is split into 'Left' and 'Right' columns. Each column has radio buttons for 'Not Controlled', 'As Detector Cell', 'Unchanged', and 'Combined'. The 'Left' column has a temperature input field set to 40.0 °C, which is highlighted with a green box. The 'Right' column has a temperature input field set to 20.0 °C.
- Valve Position/Column:** This section has radio buttons for 'Use Current Column / Position' and 'Use Selected Column / Position'. The 'Use Selected Column / Position' option is selected, and a dropdown menu below it shows 'Position 1', which is also highlighted with a green box. Below this are two circular diagrams representing valve positions.
- Advanced:** This section includes an 'Enable Analysis' checkbox (unchecked) and another set of 'Left' and 'Right' radio buttons for 'With any temperature' and 'When temperature is within'. The 'When temperature is within' options are selected. Below these are input fields for temperature range (± 0.8 °C) and time (0.0 min).
- Valve Position/Column After Run:** This section has radio buttons for 'Do not switch', 'Switch to position / column at beginning of run', 'Increase valve position / column', 'Decrease valve position / column', and 'Use valve position / column'. The 'Do not switch' option is selected, and a dropdown menu below it shows 'Position 1'.
- Column Manager:** This is a separate dialog box with two tabs: 'General' and 'Data'.
 - Temperature:** A 'Column' dropdown menu is set to '40.0', highlighted with a green box. To its right, an 'Alarm Band' checkbox is checked, with a value of ± 1.0 °C.
 - Active Preheater:** A dropdown menu is set to 'Disabled'.
 - Column Selection:** A 'Valve Position' dropdown menu is set to 'Column 1', highlighted with a green box. To its right, an 'Equilibration Time' input field is set to 0.1 min.
 - Comment:** A text input field with a search icon.

Method Comparison

Multi sampler: Sample Manager

The screenshot shows the 'Advanced' settings panel in Empower. The 'Injection' section has 'Injection volume' set to 5.00 µL. The 'Needle Wash' section has a dropdown set to 'Standard Wash'. The 'Stoptime' section has 'As Pump/No Limit' selected. The 'Posttime' section has 'Off' selected. The 'High Throughput' section has 'Sample Flush-Out Factor' set to 5.0. The 'Thermostat' section has 'Off' selected.

The screenshot shows the 'Sample Manager FTN' interface. The 'General' tab is active. The 'Solvents' section has 'Wash Solvent Name' set to 'Water' and 'Purge Solvent Name' set to 'Water'. The 'Pre-Inject Wash' is set to 0 sec and 'Post-Inject Wash' is set to 6 sec. The 'Temperature Control' section has 'Column' and 'Sample' both set to 'Off' °C, with 'Alarm Band' set to ± 5.0 °C. The 'Loop Offline' checkbox is unchecked, and 'Load Ahead' is also unchecked. The 'Active Preheater' is set to 'Disabled'. There is a 'Comment' field and an 'Advanced...' button.

The injection volume in Empower is specified in the sequence table.

The ADF method file can also contain the auto sampler temperature which can be transferred across both manufacturers.

Phase 4 – Data from different vendors

Data Comparison on Zontal Space – Agilent and Waters Hardware

The difference in retention time between the two manufacturers is due to the different dwell volumes of the instruments used to execute the method.

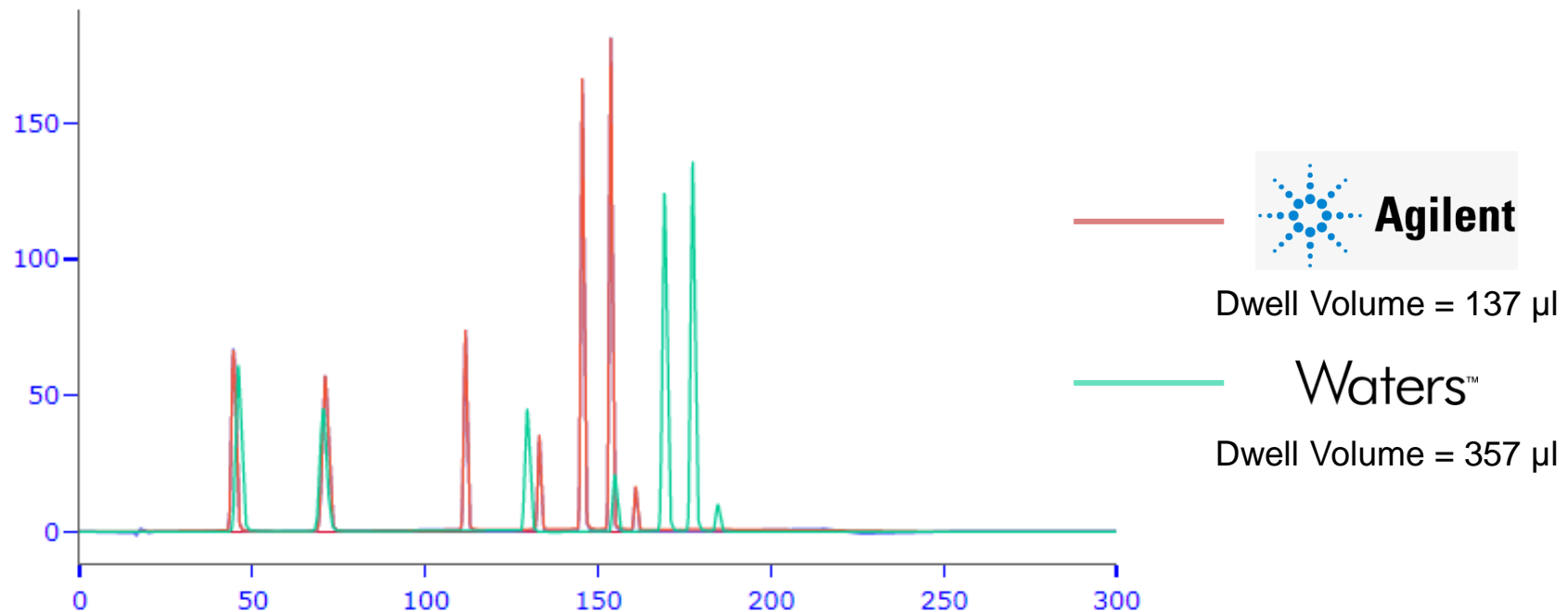
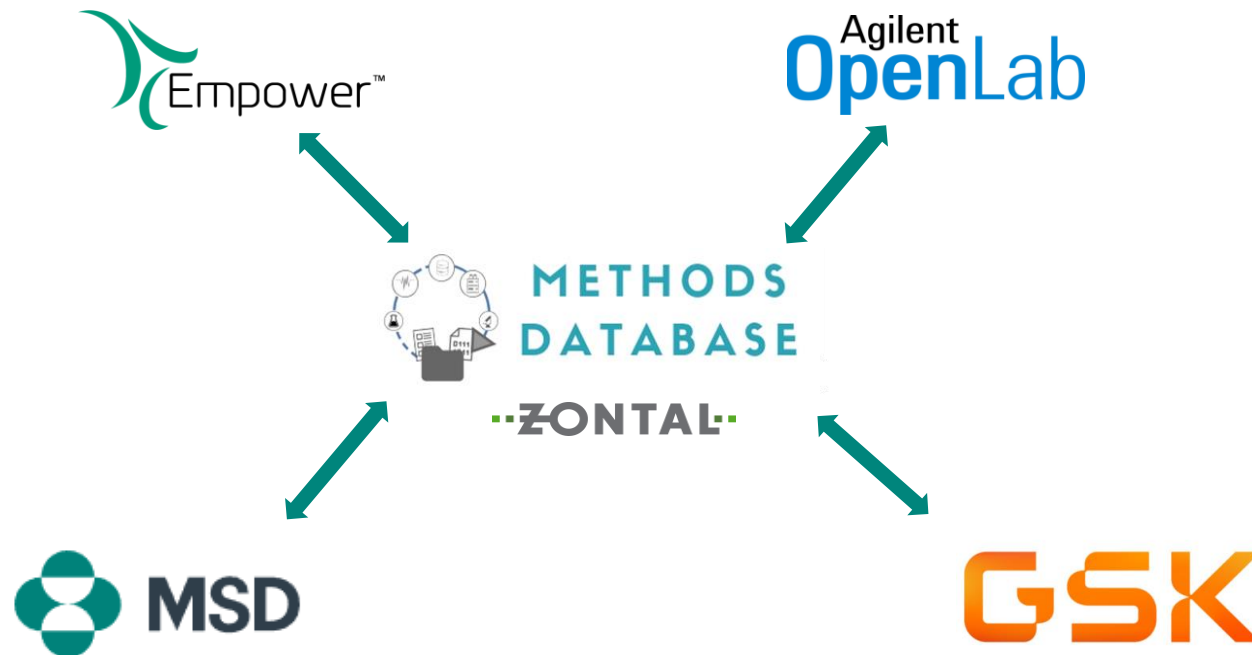


Figure 1: HPLC chromatogram comparison of the data collected Merck's gradient method on Agilent 1290 and Waters Acquity instruments at GSK.

Conclusion

☐ Method portability shown across

- ✓ Different instrument vendors
- ✓ Different chromatography data systems (CDS)
- ✓ Different pharmaceutical companies



How to get involved?

- Start implementation of the Methods Db
 - Lab/Instruments/Technical requirements
 - Interoperability across more hardware or software?
 - Own use case?
- Support the project/CoE

	NOW 6 MONTHS	NEXT 6+ MONTHS
Next Phase of POC	<ul style="list-style-type: none"> • Define next phase to test a different dimension, perhaps pharma to CRO • Documented plan for an implementable product • Publish specifications for more software companies to test • ROI calculator for Pharma companies • Calculation of pharma investment • Plan for sources of funding 	<ul style="list-style-type: none"> • Efforts to launch implementable product • Additional software companies test in order to make product software agnostic • Involve new instrument company • Progress with columns + data analytics
Socialization	<ul style="list-style-type: none"> • Material plan to use broad-based marketing to socialize progress so far and upcoming plan • Publish webinar series from the day • Publish pharma test cases story in addition to June 2022 press release • Strengthen pitch with ROI and investment data • Bring together more pharma companies to join initiative • Tap into support of new Pistoia member, Charles River 	<ul style="list-style-type: none"> • Bring together more pharma companies to join initiative • Proliferation through organizations to warrant future development • Gather contacts to target from Pistoia commercial partners • Identify mutual customers across businesses represented to expand to



**METHODS
DATABASE**



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