

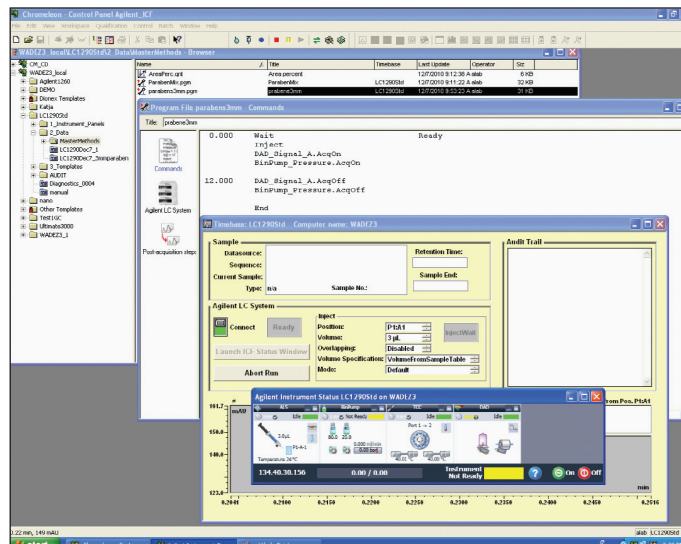
Operating the Agilent 1290 Infinity LC with Dionex Chromeleon software using Instrument Control Framework

Instrument set up and performance

Technical Overview

Author

Angelika Gratzfeld-Huesgen
Agilent Technologies, Inc.
Waldbronn, Germany



Abstract

The Agilent Instrument Control Framework software (ICF) enables third party LC data acquisition and processing software providers to simplify the development of instrument control software for Agilent LCs. Chromeleon 6.80 SR 10 in combination with Agilent ICF A.01.02 provides enhanced control functions for all Agilent 1100 and 1200 Series LC systems.

As an example, the Agilent 1290 Infinity LC system was connected to the new software combination in the Dionex Chromeleon 6.8 architecture. Nearly all Agilent 1290 Infinity LC instrument features are now accessible by combining ICF and Chromeleon software, for example, injector programming, column regeneration with a second pump or acquisition of up to 8 DAD signals.



Agilent Technologies

Introduction

Recently, Agilent Technologies introduced the Instrument Control Framework (ICF), a software component, making it faster and easier for third party software to enable and control Agilent liquid chromatography (LC) systems in chromatographic data systems or workstations^{1,2}. Based on new standard instrument drivers from Agilent, ICF eliminates much of the delay and effort of using low-level instrument control codes by software developers to write their own native drivers.

In the following we will demonstrate:

- What prerequisites have to be fulfilled to ensure seamless interaction with Agilent 1290 Infinity LC systems, Dionex Chromeleon software and ICF software
- Which modules and instrument features are supported
- How instrument methods for the Agilent 1290 Infinity LC system are set up in the Dionex Chromeleon software
- That the Agilent 1290 Infinity LC system also fulfills performance specifications under Dionex Chromeleon

Experimental

The instrument used was an Agilent 1290 Infinity LC system, equipped with the following modules:

- Agilent 1290 Infinity Series Binary Pump with vacuum degasser
- Agilent 1290 Infinity Series High Performance Autosampler
- Agilent 1290 Infinity Series Thermostatted Column Compartment

- Agilent 1290 Infinity Series Diode Array Detector SL for 160-Hz operation
- Agilent ZORBAX RRHT Eclipse Plus C-18 column packed with 1.8- μ m particles and Poroshell 120 column packed with 2.7 μ m particles

Results and Discussion

In the following, the prerequisites mentioned are the minimum needed to be able to run the ICF with Chromeleon.

Prerequisites

- To operate Chromeleon with the Agilent ICF, the minimum supported Chromeleon version is Chromeleon 6.80 SR10 and driver update DU10A⁴
- The Instrument Control Framework (ICF) and the Agilent LC Driver Package (version A.01.01 SP1 or higher) must first be installed on the PC.
- Agilent LC module must have "RC.Net drivers"

- Before installing the software, ensure all hardware is installed. Connect the individual Agilent modules via CAN. Connect the whole instrument to the PC via LAN, use the LAN card in the Agilent module that produces the largest amount of data (DAD > FLD > MWD > VWD > RID³)

Supported Agilent LC modules

See Appendix for details

Using the ICF, it is now possible to support Agilent instrument features which were not supported with previous Chromeleon version using native Dionex drivers. All features available in the new *On-Line* screen which is added to the known Chromeleon screens, are now supported (Figure 1). Using a right mouse-click in one of the module windows give access to all control, method and other features of that module. Also, RF ID tags can be seen if the cursor is moved over the label pictogram for the lamp and cell of the DAD module, for example.



Figure 1
Agilent Instrument Status screen under Chromeleon and ICF software.

Newly supported instrument features are shown in Table 1.

Software Installation and setting up methods

The installation of the Dionex Chromeleon software with the Agilent ICF is done by the Dionex service.

After configuration and integration of the Agilent 1290 Infinity LC system in the Chromeleon architecture, the starting screen for setting up an instrument method is shown in Figure 2.

Click *File* and *New* and the little window (close to the middle of Figure 2) appears. New methods (called Program File), sequences, report etc are created here. Click *Program File* to open the dialog for setting up an instrument method, (Figure 3).

In Figure 2 (lower part of Figure 2) the user interface for direct control of the Agilent 1290 is shown. Right mouse click in the *Binary Pump* and then *Method* opens the Method screen for the pump. Flow rate and organic percentage can be selected to equilibrate the system for example. This is for *On-Line* only, no instrument method is created here.

The following configurations and tasks are now fully supported by Chromeleon with ICF

- Second pump for automated column regeneration
- Built-in valves in the thermostatted column compartment (TCC)
- Injector programming, external needle wash
- All 8 signals for the DAD or MWD
- Creation of additional compressibility curves by the user
- Flexible Cube (G4227A)
- RF ID tags: lamp and detector cell tags and column tags can be accessed in the "Agilent instrument status" screen by moving the mouse over the label pictograms
- Plate numbers must be entered like P1B5
Vial numbers must be entered as: VIAL: 1

Features not yet supported in the Chromeleon version used for this application note

- EMF
- Manual injection
- Fraction collection system
- Purge kit (G1373A)
- DAD recovery card
- Clustered thermostatted column compartments for column selection
- Clustered pump with valves for solvent selection
- Overlapped injections
- "Post time" available in the pump set up screen of Agilent pumps

Table 1
Supported instrument features.

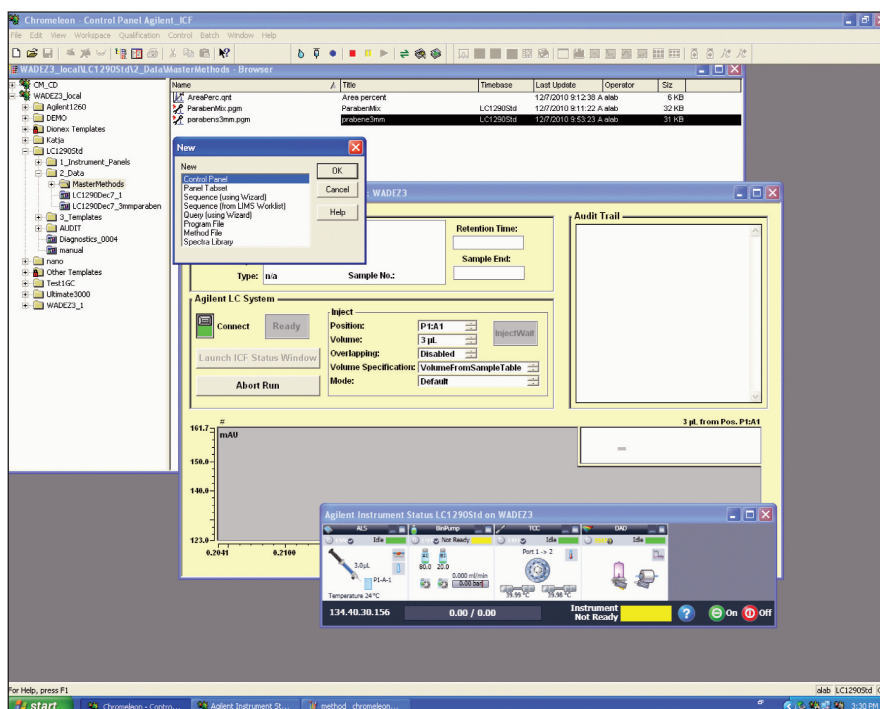


Figure 2
Start screen to create a new instrument method (Program File).

Entries to create an instrument method (Program file) shown in Figure 3 and 4.

Under *Commands*, entries have to be made according to the chromatographic needs, which were the following in our example for the analysis of Parabens entries.

After a *Wait* and an *Inject* command, the signal acquisition for the DAD and pressure signal was set to On at 0.00 min. Signal acquisition was set to Off after 12 min, (Figure 3).

This *Acquisition OFF* command at 12 min and the stop time entry in the pump set up screen must have the same value, or sequences and runs might be aborted.

Having set these commands, click *Agilent LC System* and the screen shown in Figure 4 appears.

Click *Launch Agilent ICF IME* to start the Agilent 1290 Infinity method setup screens for pump (Figure 4), autosampler, column compartment and DAD.

Having selected appropriate method values for all parameter the Method (Program) file can be stored in an appropriate directory.

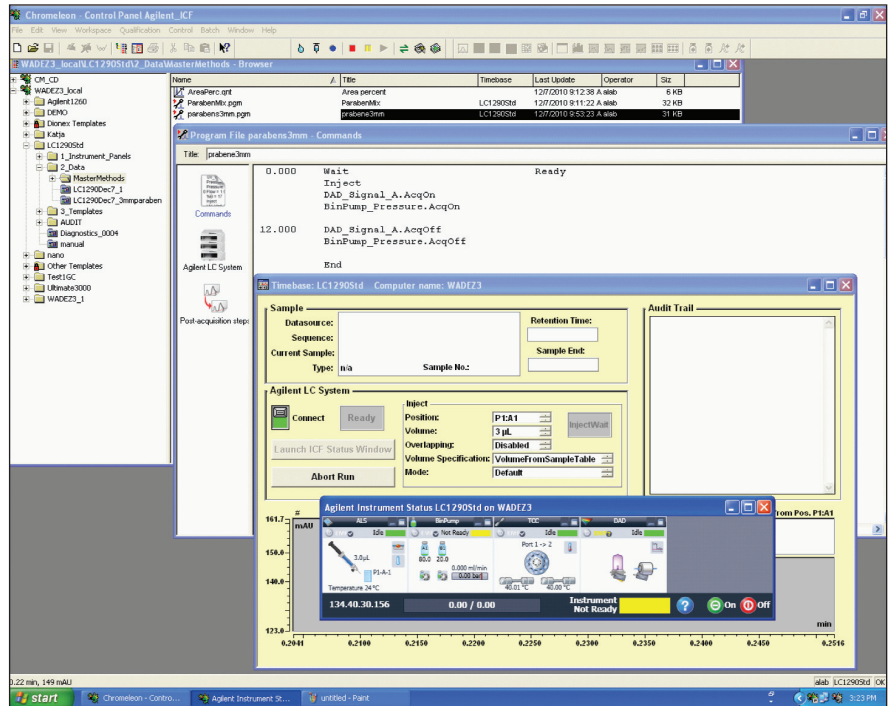


Figure 3
Start creating an instrument method (Program File).

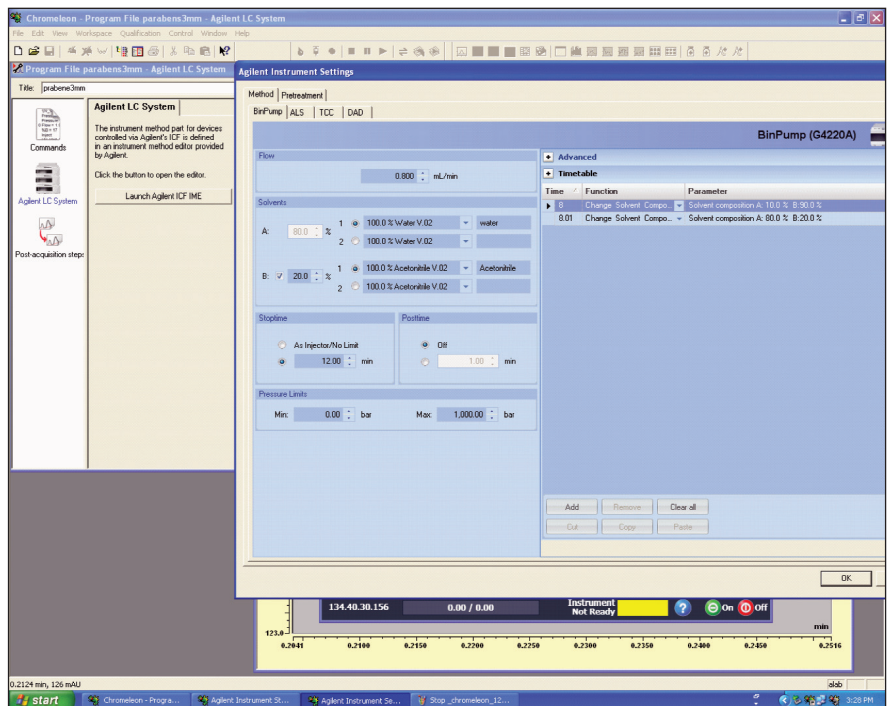


Figure 4
Creating an instrument method (Program File) using the AGILENT INSTRUMENT SETTING screen by clicking on LAUNCH AGILENT ICF IME.

Creating a sequence

A sequence is created using the Sequence Wizard. The wizard leads the user through a dialog for: name of sample, setting position of vials, number of injection per vial, instrument method to be used, and some more entries. The created sequence file is saved and the sequence is started by clicking *Batch* and *Start*. If all runs are done, the *Status* column shows *Finished* for all runs and processing of the data can be done.

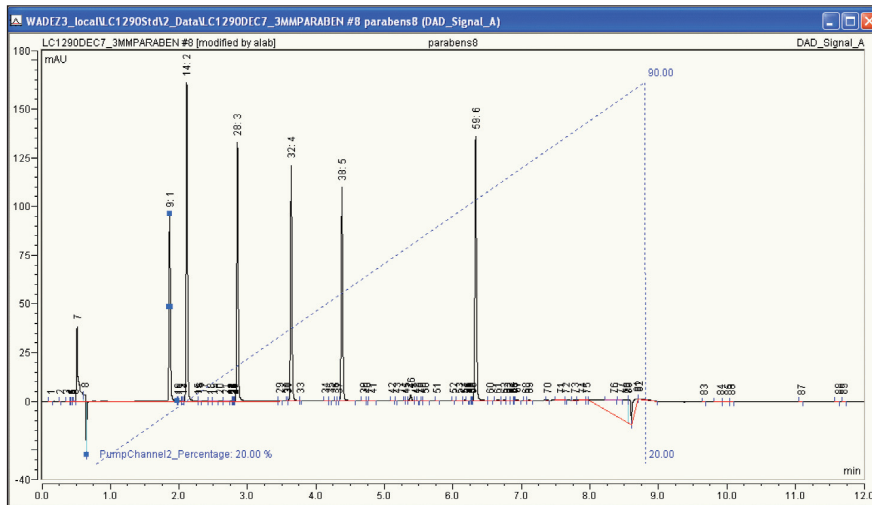
Performance of the Agilent LC systems using Dionex Chromeleon data processing tools

To demonstrate that the Agilent 1290 Infinity LC system fulfills the expected performance, the following tests were done:

- Precision of retention time and areas for parabens
- Carry over for Caffeine
- Detector linearity using Caffeine standards

Precision of retention time and areas

In Figure 5, the chromatogram of the Paraben sample is shown. Precision of retention times and areas for a 3 μL injection are combined in Table 2. Data were evaluated using Chromeleon Peak summary report. The precision for the retention times for 6 consecutive runs is $< 0.035\%$ RSD, for the area the precision is $< 0.27\%$ RSD. Both values are well within the specification limits for the Agilent 1290 Infinity LC system.



Chromatographic conditions

Compounds: Uracil, Phenol, methyl-, ethyl-, propyl-, butyl- and heptylparaben
 Column: Agilent ZORBAX Eclipse C18 RRHT, 4.6 mm \times 100 mm, 1.8 μm
 Mobile phases: Water/Acetonitrile
 Gradient: 20% to 90% in 8 min, at 8.01 min 10%
 Flow rate: 1.2 mL/min
 Stop time: 12 min
 Column temperature: 40 $^{\circ}\text{C}$
 Injection volume: 3 μL
 DAD: 254/10 nm Ref. 360/100 nm, 20 Hz

Figure 5
 Chromatogram of Paraben sample for evaluation of retention time and area precision.

Peak number/name	Peak name	RSD RT (%)	RSD area (%) (3 μL injection volume)
1/Phenol	Phenol	0.033	0.193
2/Methylparaben	Methyl-	0.026	0.266
3/Ethylparaben	Ethyl-	0.025	0.172
4/Propylparaben	Propyl-	0.034	0.157
5/Butylparaben	Butyl-	0.024	0.141
6/Heptylparaben	Heptylparaben	0.011	0.110

Table 2
 Precision of retention times and areas for 6 consecutive runs.

Carry over

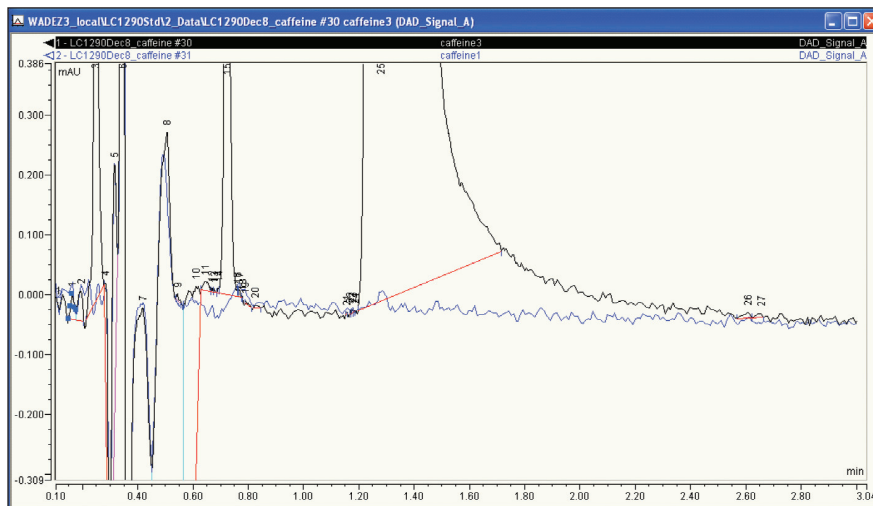
In Figure 6, the results of the carry over experiment is shown. 1500 ng of Caffeine were injected followed by an injection of 3 μ L of pure water. No carry over could be observed, see blue trace in Figure 6.

Detector linearity

Detector linearity was tested using caffeine standards from 0.5 up to 500 μ g/mL. The coefficient of correlation was 99.9989 over an absorbance range from 2.7 up to 2475 mAU. The data were evaluated using Dionex linearity report style.

Conclusion

The Agilent Instrument Control Framework (ICF) is a software component, making it faster and easier for third party software to enable and control Agilent liquid chromatography (LC) systems in chromatographic data systems or workstations. In our application example, the new ICF software was used to control the Agilent 1290 Infinity LC system in combination with Dionex Chromeleon software. The instrument was configured in Chromeleon and data were acquired and processed. The combination of ICF software and the Chromeleon 6.80 SR10 software and driver update DU10A allows accessing nearly all available Agilent instrument features such as injector programming, external needle wash, column regeneration with a second pump and more. The Agilent Instrument Status screen is used to set up *On-line* methods, to switch the system On or Off, to equilibrate columns, to view the status of single modules and to access special features using the *Control* function available for each Agilent LC module. As expected, the Agilent 1290 Infinity LC system shows the same excellent performance for data acquired and processed using Chromeleon and ICF software.



Chromatographic conditions

Column:	Agilent Poroshell 120 EC, 3.0 mm \times 50 mm, 2.7 μ m
Mobile phases:	A = Water B = Acetonitrile
Isocratic:	10% B
Flow rate:	0.8 mL/min
Stop time:	3 min
Injection volume:	3 μ L
Column temperature:	30 $^{\circ}$ C
DAD:	273/10 nm
Ref:	380/80 nm
Flow cell:	10 mm
Peak width:	< 0.0125 min (20 Hz)

Figure 6

Carry over experiment injecting 1500 ng Caffeine followed by the injection of 3 μ L water.

References

1. "The Agilent Technologies Instrument Control Framework," Technical Overview, Agilent Technologies publication number 5990-6504EN, November 2010
2. "The Agilent Technologies Instrument Control Framework" Short overview, Agilent Technologies publication 5990-5756EN, June 2010
3. "Chromeleon[®] and Agilent ICF – Quick Start Guide," Dionex publication, 2010-07
4. "Product Release Notes for CHROMELEON[®] 6.80, Driver Update DU10a," Dionex publication, November 2010

Appendix - Supported Modules

	Model number	Firmware
Detectors		
Variable Wavelength UV Detector (VWD)	G1314A	A.06.10
	G1314B	A.06.10
	G1314C	A.06.10
	G1314D	B.06.20
	G1314E ¹	B.06.20
Diode Array Detector (DAD)	G1315A	A.06.10
	G1315B	A.06.10
	G1315C ¹	B.06.13
	G1315D ¹	B.06.13
Fluorescence Detector (FLD)	G4212A ¹	B.06.23
	G1321A	A.06.10
Refractive Index Detector (RID)	G1362A	A.06.10
Multiple Wavelength Detector (MWD)	G1365A	A.06.10
	G1365B	A.06.10
	G1365C ¹	B.06.13
	G1365D ¹	B.06.13
Pumps		
Isocratic Pump	G1310A	A.06.10
Low-pressure gradient pump	G1311A	A.06.10
High-pressure gradient pump with (optional) solvent selection valves	G1312A	A.06.10
	G1312B	A.06.10
Binary Pump	G4220A ¹	B.06.23
Autosamplers		
Autosampler	G1313A	A.06.10
	G1329A	A.06.10
	G1329B	A.06.10
Wellplate LC Autosampler	G1367A	A.06.12
	G1367B	A.06.12
Thermostated Column Compartments		
Column Compartment	G1316A	A.06.10
	G1316B	A.06.10
	G1316C	A.06.14
External Valves		
2-position/10-port valve	G1157A	A.06.02
2-position/6-port valve	G1158A	A.06.02
2-position/6-port valve, high pressure	G1158B	A.06.02
6-position selection	G1159A	A.06.02
12-position/13-port valve	G1160A	A.06.02
Compact Systems		
Compact System	G4286A ¹	_2
	G4287A ¹	_2
	G4288A ¹	_2
	G4289A ¹	_2
	G4290A ¹	_2

¹ The modules marked with 1 are equipped with a LAN card that allows multi-controller access. This is required for simultaneous communication by Agilent Lab Advisor and Chromeleon.

² For firmware versions of the Compact LC components, please refer to: <http://www.chem.agilent.com/en-US/Products/Instruments/lc/Pages/specification-sicf.aspx>

www.agilent.com/chem/lc

© Agilent Technologies, Inc., 2011
Printed February 1, 2011
Publication Number 5990-7215EN



Agilent Technologies