

Using Agilent Fraction Collectors in Empower with ICF Integration

**Technical Note** 

## Introduction

Waters Corporation's adoption of the Agilent Instrument Control Framework (ICF) for their Empower Data System is called Agilent ICF Support Layer (ICF SL). The Waters ICF SL is part of the Waters Instrument Control Package (ICS) environment.

## Prerequisites

- 1 Make sure to use an Agilent-only instrument LC system for fraction collection, as built-in intelligence can only be applied among Agilent modules via CAN.
- 2 Make sure to define a Stoptime for any Agilent Fraction Collector (FC)/Fraction Collector Cluster (FCC), as for internal calculations such as time-based fractionation, the FC/FCC Stoptime is needed. Ensure that the defined runtime is in sync with the chosen sample run time.
- **3** Install the plate definition files for samplers and fraction collectors.

## Agilent Fractionation Features

The behavior of the hardware and software control of Agilent fraction collectors in Agilent CDS varies slightly from the behavior in Empower. Therefore, the information in this Technical Note supersedes what is described in the Online Help and manuals.

## **Fraction Preview**

Fraction preview is only available in Agilent's CDS. If you are using Empower with older ICF SL versions, you can select the feature but it is unusable.

## **Fraction Start Location**

• Set the absolute fraction start location in the method on the General tab.

🔒 Untitled in Defaults as System/Administrator - Instrument Method Editor
File Edit View Help
□☞■ 
Instrument Method   Pretreatment Method   Auxiliary Channels General   Instrument Configuration
Fraction Collector Options     Image: Start at Location:   1
Figure 1 Frection Stort Location (numeric)

Figure 1 Fraction Start Location (numeric)

_		
	Instrument Method   Pretreatment Method   Auxiliary Cha	annels General Instrument Configuration
	Fraction Collector Options	
	Start at Location: P1-A-1	

Figure 2 Fraction Start Location (alphanumeric)

- Use one of the following formats: alphanumeric (e.g. P1-A-1) for wellplates, numeric for vial and tube trays.
- The fraction start location can be left empty. In this case, fractioning starts at the first location and continues using the next location based on the selected fraction order.
- Enter an absolute location and each run of the sequence will start with this location.
- The fraction locations are defined in the method. This means that you can generate different methods with *different start locations* to define a new start location per run, if required.
- The fraction start location does not offer **Pooling**. **Pooling** starts fraction collection at the same position as in the previous run and keeps track of the fraction fill state to prevent overfills.

#### NOTE

Using an absolute start location, each run of a sequence starts at the same location, thereby mimicking pooling. However, this setup does not warn the users or stop the sequence if locations are overfilled. When reaching the maximum fill volume, the fraction collector advances to the next valid position and may contaminate fractions of the previous run. Therefore, the user must know the expected fractions upfront and needs to intervene if correction is necessary.

- The fraction start location does not offer *logic positions*, such as **Next Location**, **Next Row**, etc. as options.
- Hover the mouse over the plus on the fraction tile to open the fraction location preview.



Figure 3 Fraction location preview

## Configuration

#### G1364E/F, G7166A, G7158B, G7159B, G5664A

1 Right-click the instrument tile in the dashboard and select Modify.

	Fraction Coll	?	_ □ Idle EMF⊘ 	Status Dashboar			1
		+	Contr	ol			
		<u>el</u> è	Meth	od			-
			ldenti	ify Devic	e		_
		Ģ	Reset	Fraction	n Colle	ector	
			Switc	h off Tra	y Illun	nination	
			Reset	Fraction	n Volu	mes	
ly	i () (		Modi	fy		•	Wellplate Assignment
							Collection Settings
		5	System	n Idle	_		Detector Delay Volumes
_			-				Linked Pump
							Needle and Tubing
							Needle Position
_							Vessel Dimensions

2 Select the configuration settings you want to change.Changes are applied immediately without performing a new auto-configuration.

### G1364A/B/C/D, G5664A

Instrument tile

Figure 4

1.1

1 Select the Module Options tab in the LC status dashboard.

-			
Instrument Status	Module Options	Diagnostics Log	

Figure 5 Module Options tab

- 2 Change the parameters and click Apply.
- **3** To apply the changes, perform an auto-configuration. For details see *Using the Fraction Collector in Empower 3 Environment Technical Note*.

## Reporting

• To display fractions, select the corresponding check boxes in the **Chromatogram** tab in the **Plot Properties**.

Plot Properties	×
Plot Properties Plot Chromatogram Fonts Colors Scaling Chromatogram Tick Marks  Chromatogram Tick Marks Start Time minutes  Chromatogram Fraction Regions  Chromatogram Fraction Regions  Chromatogram Fraction Regions  Chromatogram Fraction Shading  Chromatogram Fraction Lines	×
	Help

Figure 6 Plot Properties window

- Overfilled locations are not graphically displayed.
- *Manual fraction collection* is available. The manually triggered fractions are not specifically labeled as such.



Figure 7 Manual fraction collection

### **Agilent Fractionation Features**

Filt	er By: Default		•	Edit Vie <u>w</u>	Update M.	ax Rows: 1000			
┛	Sample Sets Injections Channels Methods Result Sets Results Peaks Fractions Sign Offs Curves View Filters Custom Fields Audit Trails								
Ē	Fraction Name	Fraction Location	Inj ID (Fraction)	Fraction Index	Location Index	Fraction Start Time (min)	Fraction End Time (min)	Fraction Volume (mL)	
1	FRACTION 1	P1-A-1	1175		1	0.066	0.174	0.07	
2	FRACTION 2	P1-A-2	1175	2	2	0.492	0.600	0.07	
3	FRACTION 3	P1-A-3	1175	3	3	0.918	1.026	0.07	
4	FRACTION 4	P1-A-4	1175	4	4	1.353	1.452	0.07	
5	FRACTION 5	P1-A-5	1175	5	5	1.779	1.878	0.07	
6	FRACTION 6	P1-A-6	1175	6	6	2.196	2.304	0.07	
7	FRACTION 7	P1-A-7	1175	7	7	2.622	2.731	0.07	
8	FRACTION 1	P1-A-1	1185	1	1	0.068	0.169	0.07	
9	FRACTION 2	P1-A-2	1185	2	2	0.502	0.594	0.06	
10	FRACTION 3	P1-A-3	1185	3	3	0.920	1.020	0.07	

• The Data Analysis of the projects includes a dedicated **Fractions** table.

Figure 8 Fractions table

## Fraction Cluster

**1** Define the clusters in the Pre-Configuration tool.

Agilent 1100/1200/1260/1290 LC Agilent 1120/1220 LC Systems				Fraction Collector II (FCC) Quat. Pump (67111B:FN8221172)					Filter By: Default	t
gilent 68xx/78xx/9000/88xx/76	97/G1888 GC/HS			VWD (G7114A:XY10873890) Prep. Pump (G7161B:GE76377123)			vner	Create Date	FullAudit	
jilent ELSD					Prep. Sa	mpler II (G7169B:XK57168227)		stem	10.12.2019 15:33:00	
onfigure Fraction Collector II										+×
Communication				Topology						
Device name Frac	tion Collector			Selected Topology V(F)n-V(R)	n •	Optimize Topology				
Type ID FCC		•								
	Connection settin	gs				G7159B C			G7166	6A R
Module List					$\Gamma$	AFC1			AF	FC3
Module Identifier	Is combined device	Name Is us	ed		/	XK57168227			BA57	779581
G7159B:XK57168227	<b>V</b>	AFC1	2			G7159B E			G7166	6A R
G7159B:YB86096914 G7166A:BA57779581		AFC2	7					B1		<b>N</b>
G7166A:OA45890453		AFC4	2			AFC2	000	B2	Ał	-C4
G9322A:FN29971415		Valve2	1			YB86096914			OA45	890453
						J				
					_	_			_	
Add	Configure	Remo	/e							
				Digital Triggers	ffline Configure	tion				

2 To set the recovery locations, go to Modify >Recovery Settings.

				1.01	
🕙 Modify Recovery Set	ttings		-		×
Start Location Mode:	Use Start Location 💌	Start Location :	2-P1-A-1		
When Recovery out of Loca	ations				
	Switch to Waste	<b>•</b>			
			Ok	Canc	el

Figure 10 Recovery settings

# Features Requiring LC Driver 3.0 or Higher

New modules and new features require LC Driver updates. The following fraction collection features have been integrated into the LC Driver and will be available via the corresponding ICF SL Version.

#### Table 1 Driver features

LC Driver	Feature
3.0	Increased capabilities for analytical-scale Fraction Collector II Clusters Up to three collectors can be combined with dedicated rotary valves to collect fractions. For Recovery Collection, up to three more modules can be attached using a G9322A Agilent 1260 Infinity II Clustering Valve. For more information, see Agilent LC&CE Driver 3.0 Release Note (https://www.agilent.com/cs/library/technicaloverviews/public/LC-and-CE-Driver-Release-Note-3-0.pdf).
3.1	New module support: G7158B Open-Bed Sampler <i>and</i> Fraction Collector Support of autoscale cluster combination of modules to allow for seamless switching between analytical scouting runs and preparative-scale purification within the same sequence. For more information, see Agilent LC&CE Drivers 3.1 Release Note (https://www.agilent.com/cs/library/technicaloverviews/public/LC-and-CE-Driver-Release-Note-3-1.pdf).
3.2	Enhanced support of G7158B 1290 Infinity II Preparative Open-Bed Autosampler and Fraction Collector in Fraction Collector Cluster The LC and CE Drivers now support clustering of the G7158B with up to two more Fraction Collector modules and up to three more Recover Collector modules.
3.3	G4734B Preparative 6-column selector valve, 600 bar (5067-6722) (Works as generic valve until LC Driver 3.3)
3.4	Flow gradients during fractioning



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