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Thermo Scientific Chromeleon

Operational Qualification/ Performance Qualification for HPLC Instruments

Annex to Operating Instructions version 9.5

Annex version D to DOC 4828.3250A, Rev. 9.5 • August 2023

ThermoFisher SCIENTIFIC

Contents

1 Using this Manual

1.1 About this Manual

This annex is an addendum to *HPLC OQ/PQ Operation Instructions*, version 9.5. It describes the changes on sequence templates of a version later than 9.5. The version number of the sequence template is defined in the name of the report template.

2 Known Restrictions

The qualification of the Vanquish fraction collector contains two tests:

- Delay volume determination
- Fraction collection and carryover test

Both tests are covered by the AutoQ templates and include automatic evaluations. The results of the tests are documented in the qualification checklist for Thermo Scientific Fraction Collectors according to section 7, page 19.

3 Standard Test Procedures

The qualification of the new supported module follows section 3, page 15 of the HPLC OQ/PQ Operating Instructions.

3.1 New Supported Module

3.1.1 Refractive Index Detectors

3.1.1.1 Thermo Scientific

Series	Variant
Vanquish	VC-D60-A

3.2 Overview of Checks and Limits

3.2.1 Refractive Index Detectors

For a detailed description of the test procedures, see section 6.3.9, page 163 of the HPLC OQ/PQ Operating Instructions.

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
VC-D60-A	Baseline noise	Temperature: 35°C	≤ 50 nRIU	≤ 50 nRIU
	Drift		≤ 500 nRIU/h	≤2500 nRIU/h
	Linearity	Signal range: up to approx. 500 µRIU	r ≥ 99.9%	r ≥ 99.9%

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

3.3 Requirements and Preparations

3.3.1 Parts Required

3.3.1.1 Standards Kits

General

The standards kit (part no. 3323.0010) contains the seven required caffeine and pyrene standards (not for sale part no. 3323.0002). Due to legal shipping restrictions, the pyrene standard is shipped in solid form. Before you can use the standard, dissolve the solid pyrene in 1 mL of HPLC-grade methanol (see section 3.1.3.1, page 19 of the HPLC OQ/PQ Operating Instructions). For single-wavelength detectors (including the VWD-3400RS, VF-D40-A and VC-D40-A), caffeine is used for the wavelength accuracy check, so you do not have to prepare (that is, dissolve) the pyrene standard.

RI Detectors

The kit with part no. 3325.0010 contains the five standards with various concentrations required for qualifying an RI detector (not for sale part no. 3325.0001).

Substance	Concentration
Glycerin in water	5 mg/mL
Glycerin in water	10 mg/mL
Glycerin in water	15 mg/mL
Glycerin in water	25 mg/mL
Glycerin in water	35 mg/mL

4 Special Test Procedures

4.1 Thermo Scientific Fraction Collector

4.1.1 Prerequisites

Before qualifying the fraction collector, make sure that the other modules in the system (for example, the pump and autosampler) have been qualified successfully. Qualification of the other system modules requires a supported UV detector.

4.1.2 Parts Required

The table lists all items required for qualifying the fraction collector.

Part	Part No.	Description
Eluent A: water	-	Approx. 500 mL, HPLC grade
Caffeine	3323.0010	300 µg/mL, can be obtained from HPLC OQ/PQ standards kit (not for sale part no.: 3323.0002)
Caffeine	3323.0010	2000 µg/mL, can be obtained from HPLC OQ/PQ standards kit (not for sale part no.: 3323.0002)
Caffeine	3323.0010	10 µg/mL, can be obtained from HPLC OQ/PQ standards kit (not for sale part no.: 3323.0002)
Caffeine (Carryover reference)	-	0.2 µg/mL in water (add 6 µL 10 µg/mL caffeine standard in 294 µL water)
Bottles	-	1 L, rinsed
Viper connection capillary	6040.2330	0.13 mm x 850 mm
Viper connection capillary	4832.5000A	0.18 mm x 15 m (optional, can be obtained from Performance Qualification Kit – part no.: 4832.5000A)
Viper delay capillary	6040.2340	0.10 mm x 350 mm

4.1.3 Performing the Tests

4.1.3.1 Delay Volume Determination

Configuring the system

In the Chromeleon **ePanel** → **More Options**… → **Delay Volume**, select **Delay Capillary IDxL**: 100 µm x 350 mm.

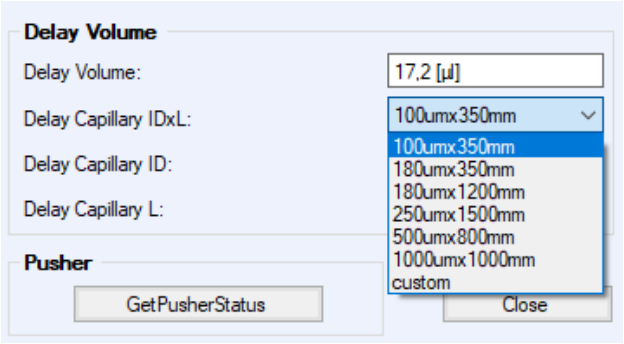


Figure 1: Set the delay capillary

TIP Do not set the delay capillary after performing the delay volume determination. Otherwise, the measured delay volume is overwritten by the theoretical value.

Preparing the system

1. Fill a bottle with HPLC grade water and label the bottle HPLC Water.
2. Connect the bottle labeled HPLC Water to the pump solvent line A (1).
3. Ensure that the 0.13 mm x 850 mm capillary is connected between the autosampler and absorbance detector.
4. Ensure that the 0.10 mm x 350 mm Delay Capillary is connected between the absorbance detector and fraction collector.
5. Prepare one empty vial and one vial filled with water (1.5mL, Vanquish 54 Vial Rack) with pre-split cap. Position the vials in the autosampler according to following table.

Test	Replicates	Substance	Rack Position Autosampler	Rack Position Fraction Collector
Equilibration water	1	Air	R:A9	As default
Delay volume determination	1 + 6	Air	R:A9	As default

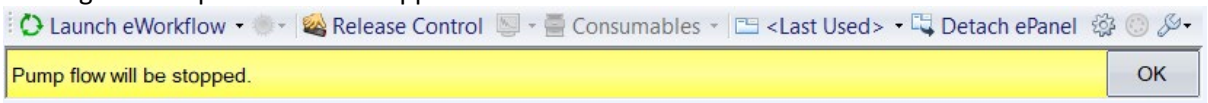
6. Ensure that all supporting LC components are powered on and properly communicating, ensure also that the fraction collector is connected and ready for operation.
7. Purge the HPLC system with water at 3 mL/min about 5 minutes.
8. Start the pump at a flow rate of 1mL/min and equilibrate the system approximately for 5 minutes.

TIP Flush the whole system, including HPLC and Fraction Collector, with water to remove any air in the fluidic path. If you have problems to flush out the potential air, addition of organic solvents may help. In addition, please refer to Operating Manuals for Vanquish HPLC systems and modules for proper maintenance of HPLC system or HPLC modules.

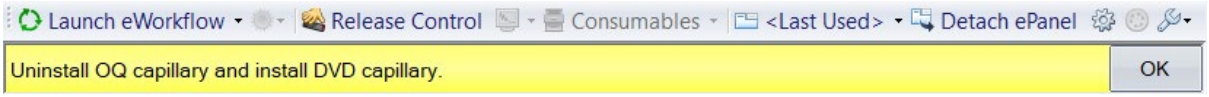
Performing the test

1. Create the test sequence [select (Operational Qualification -> Special Test if available): Vanquish Fraction Collector Delay Volume Determination] according to section 5.3 of the HPLC-OQ/PQ Operating Instructions.
2. Start the queue.
3. During sequence running, multiple of operation steps are required for the operator. Pay attention to the pop-up messages and follow the instructions strictly.

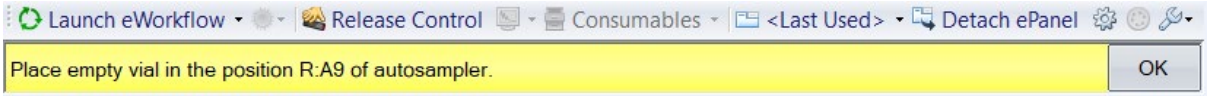
Message 1: Pump flow will be stopped



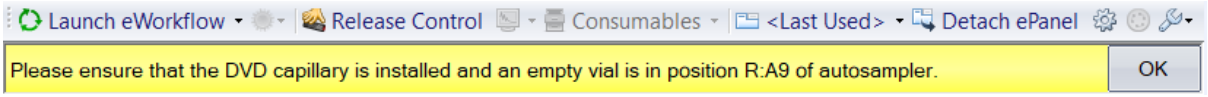
Message 2: Uninstall OQ Capillary and install DVD Capillary
DVD Capillary (Autosampler to Detector: 0.13mm x 850mm)



Message 3: Place empty vial at the position R: A9 of autosampler



Message 4: Please ensure that the DVD capillary is installed and an empty vial is in position R: A9 of autosampler

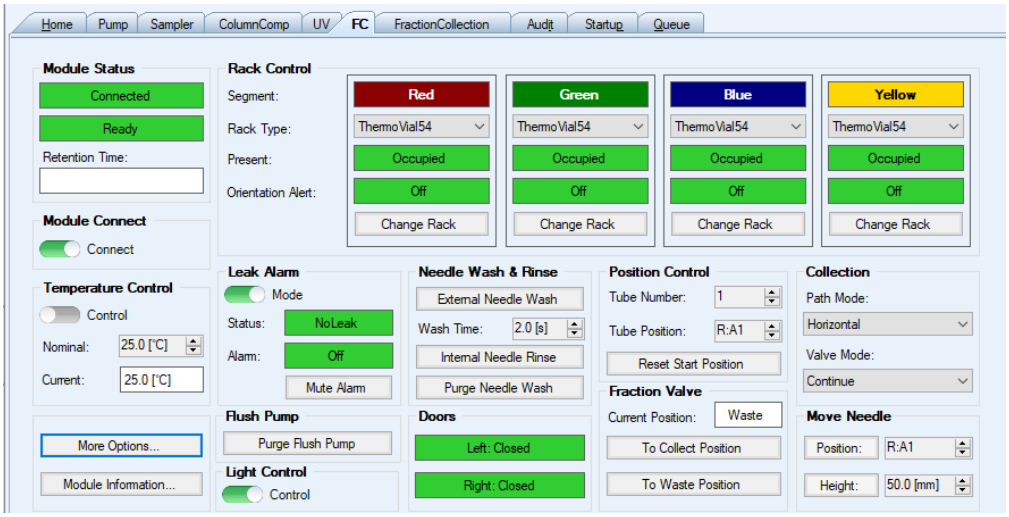


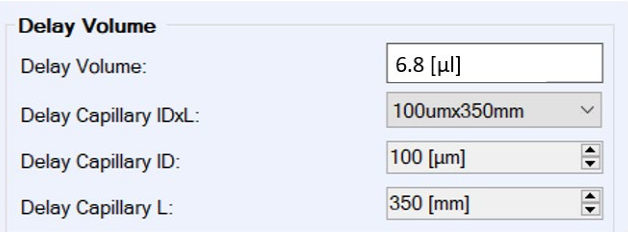
4. After the “OQ_VFC_DELAY_VOLUME” sequence finished, VFC_DELAY_VOLUME Report will be offered.
5. Open VFC_PQ_OQ _Report by double click. Then check the Delay Volume Determination value in the VFC_DELAY_VOLUME report, and make sure the test result is **“OK”**.

• Data for Delay Volume Determination Test

Injection	Observed Value µL n.a.
VFCDVD_injection 1	7.2
VFCDVD_injection 2	6.2
VFCDVD_injection 3	6.5
VFCDVD_injection 4	8.3
VFCDVD_injection 5	6
VFCDVD_injection 6	6.7
Average:	**6.82**
RSD:	**12.29 %**
RSD Limit:	**<= 1.0 %**
SD:	**0.84**
SD Limit:	**<= 1.5 µL**
Result:	**ok**

6. The delay volume value is applied to the system which is shown in **ePanel** → **FC** → **More Options**… → **Delay Volume**.



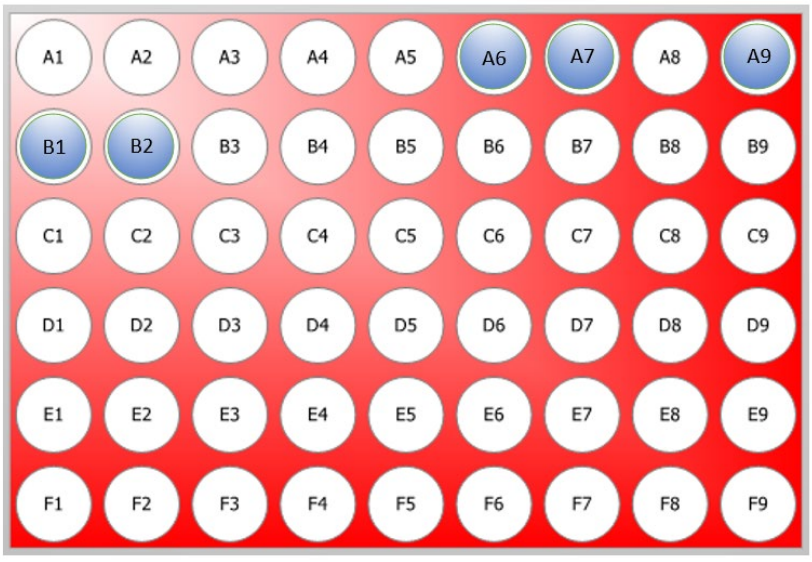


4.1.3.2 *FC Collection and Carryover Test*

Preparing the system

1. Uninstall the 0.13 mm x 850 mm Capillary between the absorbance detector and autosampler.
2. Install the 0.18 mm x 15 m OQ Capillary between the absorbance detector and autosampler.
3. Place a vial with 300 µg/mL Caffeine at R: A6 of autosampler for fraction collection test.
4. Place a vial with 2000 µg/mL Caffeine at R: A7 of autosampler. Place a vial with 0.2 µg/mL caffeine reference at R: B1 for carryover test.
5. Place a vial with water at R: B2 of autosampler.

Vial positions in the **R tray** of the **autosampler**:



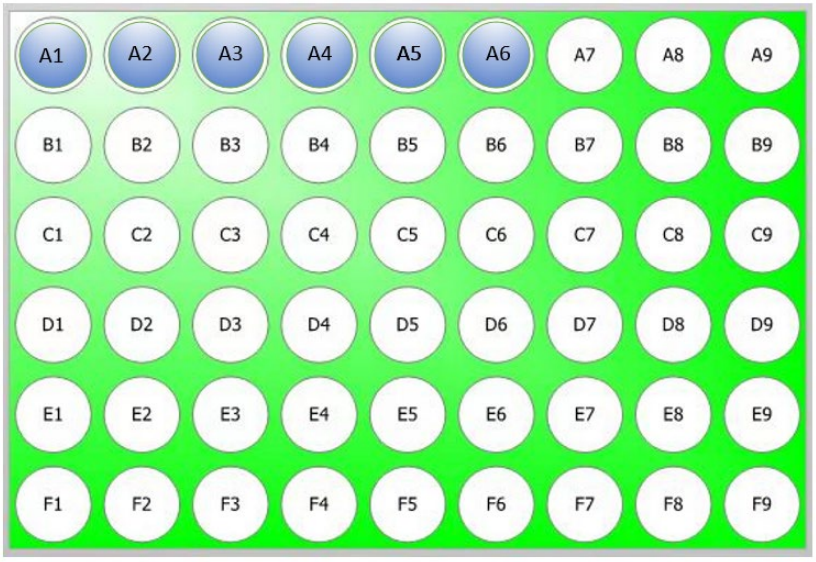
A6: 300 µg/mL caffeine
A7: 2000 µg/mL caffeine
A9: empty vial
B1: 0.2 µg/mL caffeine
B2: water

Figure 2: Vial positions in the R tray of the autosampler

6. Place 6 empty vials at position G: A1 to G: A6 in the fraction collector. Vials in G: A1 to G: A3 are used for fraction collection test. Vials in G: A4 and G: A5 are prepared for carryover test.

Note: Vial in G:A6 is used to avoid 'vial missing' error.

Vial positions in the **G tray** of the **fraction collector**:



A1-A6: empty vials

Figure 3: Vial positions in the G tray of the fraction collector

Performing the test

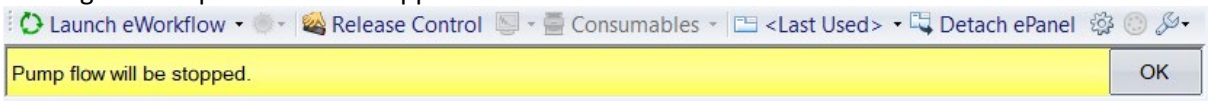
1. Create the test sequence [select (Operational Qualification -> Special Test if available): Vanquish Fraction Collector Carryover and Collection according to section 5.3 of the HPLC-OQ/PQ Operating Instructions.
2. Start the queue.

Overview of the test sequence:

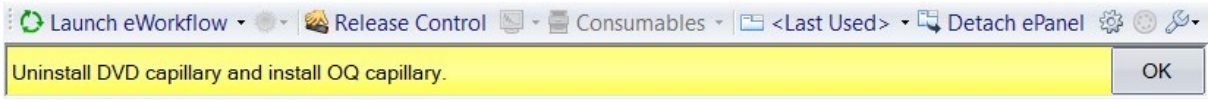
Test	Replicates	Substance	Vial Position Autosampler	Vial Position Fraction Collector
Equilibration	1	Water	R: B2	Collection off
Collection of Caffeine standard	3	300 µg/mL Caffeine in Water	R: A6	G: A1 – G: A3
Collection of Caffeine standard	1	2000 µg/mL Caffeine in Water	R: A7	G: A4 – G: A5
Solvent injection	1	Water	R: B2	Collection off
Carryover reinjection	1	Collected Carryover	G: A5	Collection off
Carryover reference	1	0.2 µg/mL Caffeine in Water	R: B1	Collection off
Fraction reinjection	3	Collected fractions	G: A1 – G: A3	Collection off

3. During sequence running, multiple of operation steps are required for the operator. Pay attention to the pop-up messages and follow the instructions strictly.

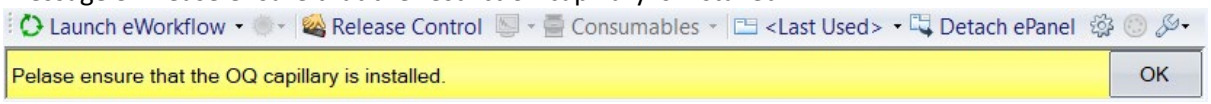
Message 1: Pump flow will be stopped



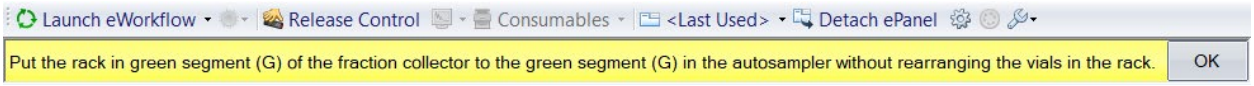
Message 2: Uninstall DVD Capillary and Install OQ Capillary
DVD Capillary (Autosampler to Detector: 0.13mm x 850mm)
Restriction Capillary (Autosampler to Detector: 0.18 mm x 15 m)



Message 3: Please ensure that the restriction capillary is installed

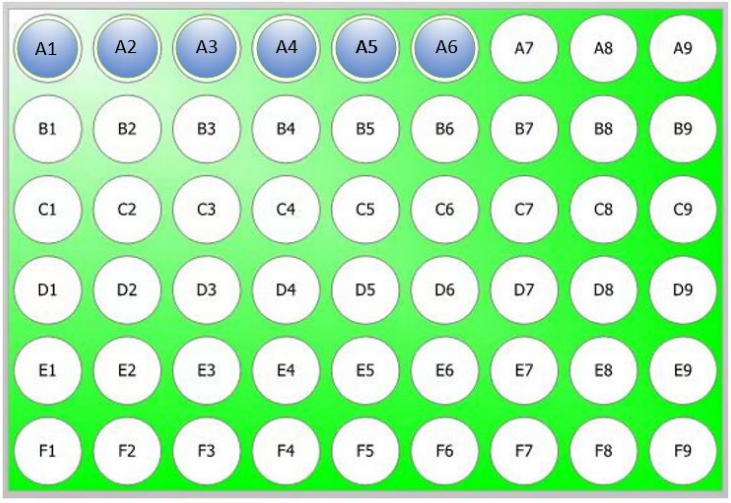


Message 4: Put the rack in green segment (G) of the fraction collector to the green segment (G) in the autosampler without rearranging the vials in the rack.



4. Move the sample rack from G tray of fraction collector to G tray of the autosampler for fraction reinjection. (Move rack only, the vials' position should not be changed.)

Vial positions in **G tray** of **autosampler:**



A1: 300 µg/mL caffeine fraction 1
A2: 300 µg/mL caffeine fraction 2
A3: 300 µg/mL caffeine fraction 3
A4: 2000 µg/mL caffeine fraction
A5: 2000 µg/mL caffeine carryover
A6: empty vial

Figure 4: Vial positions of the G tray in the autosampler

5. After the "OQ_VFC_CARRYOVER_ COLLECTION" sequence finished, VFC_COLLECTION Report and VFC_CARRYOVER Report will be offered respectively.
6. Open VFC_PQ_OQ _Report by double click. Then check the VFC_COLLECTION report and make sure the test result is **"OK".**

• Data for Collection Test

Injection Name	Ret. Time min UV_VIS_1 Caffeine	Area n.a. UV_VIS_1 Caffeine
Collection 1	0.3684	100.5108
Collection 2	0.3684	100.4827
Collection 3	0.3683	100.5369
Average:		**100.5101**

Injection Name	Ret. Time min UV_VIS_1 Caffeine	Area n.a. UV_VIS_1 Caffeine
Reinjection 1	0.3684	2.2572
Reinjection 2	0.3675	2.2728
Reinjection 3	0.3684	2.2974
Limit:		**>= 1.1168**
Result:		**ok**

7. Open VFC_PQ_OQ _Report by double click. Then check the VFC_CARRYOVER report and make sure the test result is **"OK".**

• Data for Carryover Test

Injection Name	Ret. Time min UV_VIS_1 Caffeine	Area n.a. UV_VIS_1 Caffeine
Carryover Reference	0.3717	0.1730

Injection Name	Area n.a. UV_VIS_1 Caffeine	Carryover
Carryover Reinjection	0.0049	0.003 %
Limit:		**<= 0.15 %**
Result:		**ok**

4.1.4 OQ/PQ Review and Completion

Once the qualification has been completed, the Service Representative must review all data and results and indicate that the Qualification tests **Passed** or **Failed**. The checklist should then be signed by the Service Representative / Operator and approved by the Customer.

4.1.5 Returning the System to Original Configuration

Return the customer system to the configuration and state it was in prior to the qualification, including replacing the solvents.

5 Additional Supported Instruments

5.1 Additional Supported Instruments

The procedures described in the operating instructions apply to the following additional modules.

Instrument	Supported Module
Thermo Scientific Vanquish Fraction Collector	• VF-F20-A

5.2 Overview of the Automated Checks

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
VF-F20-A	Delay volume determination	Delay capillary: 0.10 mm x 350 mm VAS to UV capillary: 0.13mmx850mm	RSD ≤ 1.0 % or SD ≤ 1.5 µL	RSD ≤ 1.0 % or SD ≤ 1.5 µL
VF-F20-A	Fraction Peak	Delay capillary: 0.10 mm x 350 mm VAS to UV capillary: 0.18mmx15m	Caffeine peak is identified	Caffeine peak is identified
VF-F20-A	FC Carryover	Delay capillary: 0.10 mm x 350 mm VAS to UV capillary: 0.18mmx15m	≤0.15%	≤0.15%

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

6 Test Design

6.1 Fraction Collector

6.1.1 Delay Volume Determination Test

Procedure and evaluation

Air is injected into the system to determine the fraction collection delay volume. Replicated injections are used to identify the determination method precision. The delay volume is calculated and applied automatically after successful execution of this test.

6.1.2 Fraction Collection Test and Carryover Test

Procedure and evaluation

Water is run through the system at 1 mL/min, a Caffeine standard (300 µg/mL) is injected three times and another Caffeine standard (2000 µg/mL) is injected one time. Fractions from the injections are collected in Time-Based mode. The fractions are re-injected and the Caffeine peak at 272 nm is verified using an absorbance detector.

For qualifying a Fraction Collector, a sequence will be offered to perform collection test and carryover test. The results of the tests can be used to evaluate fraction collection and carryover performance of Fraction collector.

1) Collection Test:

300 µg/mL caffeine standard is used in this test. 10 µL of 300 µg/mL caffeine sample is injected and caffeine peak is collected as target fraction. The collected fraction is re-injected to evaluate fraction collection effort. If the caffeine is presented and its peak area meet the requirement, the test will be passed.

2) Carryover Test:

0.2 µg/mL and 2000 µg/mL caffeine standard are used in this test. Inject 2000 µg/mL caffeine sample, the overloaded caffeine peak is collected as the 1st fraction, and the baseline in the same time frame with 1st fraction one minute later is collected as the 2nd fraction. Caffeine amount in the 2nd fraction is used to indicate the carryover performance by reinjection analysis. The caffeine peak area of the 2nd fraction reinjection is compared with the reference standard (0.2 µg/mL) injection. If the carryover caffeine peak area is less than reference caffeine peak area, the test will be passed.

Test criteria is carryover value ≤ 0.15% (µg/µg), which meets specification requirement, and 0.15% is a mass fraction not concentration fraction. Reference caffeine is a solution which carryover value is 0.15%. Then it only needs to compare the practical carryover with reference. If the carryover is less than reference, it means carryover is less than 0.15%.

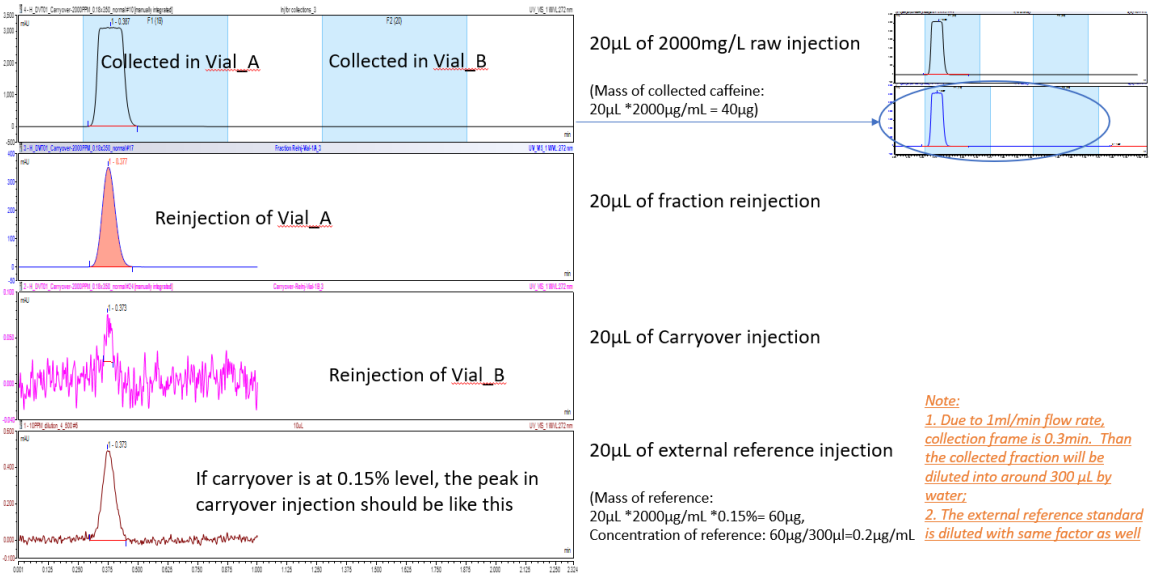
How to prepare reference caffeine:

$$C(\text{Reference}) = \frac{\text{Mass of injected caffeine} * 0.15\%}{\text{Volume of collection}} = \frac{2000\ \mu g/mL * 20uL * 0.15\%}{0.3\ \text{min} * 1\ \text{ml/min} * 1000} = 0.2\ \mu g/mL$$

Therefore the carryover value is：

$$\text{Carryover value} = \frac{\text{Area of reinjection}}{\text{Area of reference}} * 0.15\%$$

Carryover Test with 2000µg/mL Standard and 0.2µg/mL Reference (+0.1min)



7 Attachments

7.1 Qualification Checklist for Thermo Fraction Collectors

See next page.

I.QUALIFICATION CHECKLIST FOR THERMO FRACTION COLLECTORS

Date: _____ Customer: _____
Site: _____ Lab: _____ System: _____
Make: _____ Model: _____ Serial No.: _____

Power-Up Diagnostics (i.e. self-test) ❒ **Passed** ❒ **Failed**

Notes: _____

Software Communication Successful ❒ **Yes** ❒ **No**

Notes: _____

Delay Volume Determination Test

Test	Delay Volume Identified	Delay Volume Test Result
Delay Volume Determination	❒ Yes ❒ No	❒ Yes ❒ No

Delay Volume Determination Test ❒ Passed ❒ Failed

FC Carryover Test

Injection	Caffeine Peak Present in Reference Injection	Caffeine Peak Present in Fraction Injection
Carryover Reference and Reinjection	❒ Yes ❒ No	❒ Yes ❒ No

FC Carryover Test ❒ Passed ❒ Failed

Fraction Collection Test

Injection	Caffeine Peak Present in Initial Injection	Caffeine Peak Present in Fraction Injection
Caffeine Collection and Reinjection #1	❒ Yes ❒ No	❒ Yes ❒ No
Caffeine Collection and Reinjection #2	❒ Yes ❒ No	❒ Yes ❒ No
Caffeine Collection and Reinjection #3	❒ Yes ❒ No	❒ Yes ❒ No

Fraction Collection Test ❒ Passed ❒ Failed

Comments:

Performed By: _____ Date: _____
Date not required if using Digital Signature

Accepted By: _____ Date: _____
Date not required if using Digital Signature

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