

# Using the Waters Fraction Collector III under Empower Control

Waters

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**Note:** This document is an addendum to the *Waters Fraction Collector III Operator's Guide*. Refer to that document for information about hardware.

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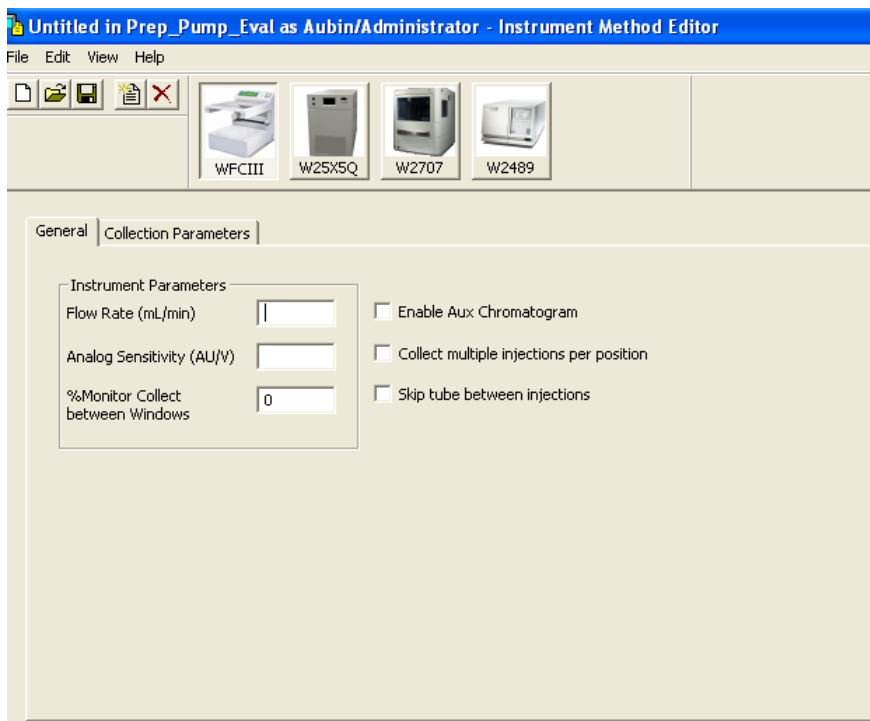
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## Setting up the WFC III method using the method editor

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This section lists the parameters for setting up the Waters Fraction Collector III (WFC III) method using the method editor under Empower control.

### General parameters tab



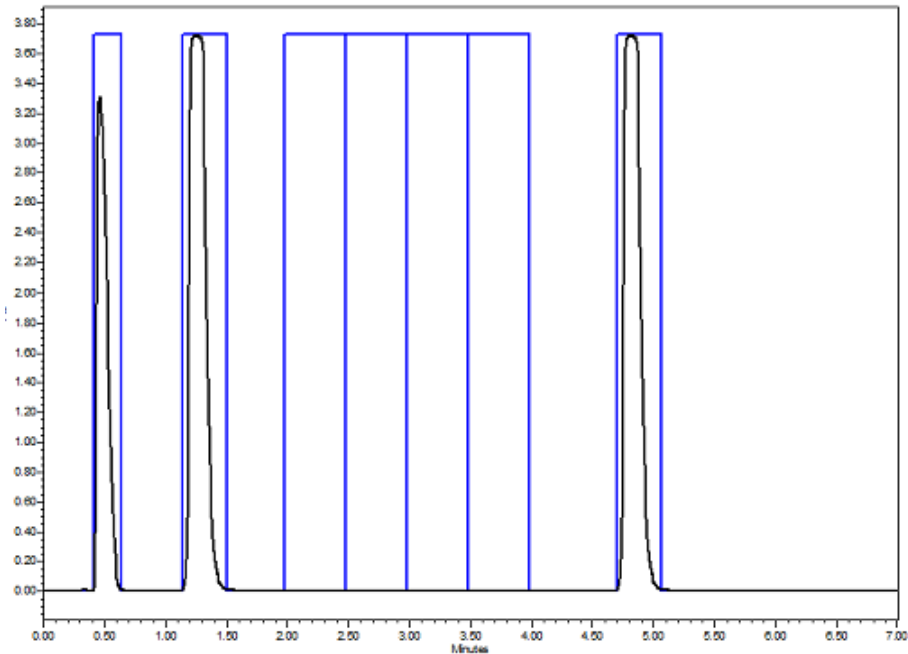
## Instrument Parameters panel

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- **Flow Rate** – This is the flow rate of the separation method. The software uses this value (along with others) to fill the tubes to the desired level and prevent overfilling.
- **Analog Sensitivity** – For information purposes only. This should be set to the same value as the detector's analog sensitivity (usually 2.0).
- **% Monitor Collect between Windows** – This is the percentage of tube fill between collection windows (0 = no volume collected between windows,

100 = the entire collection tube is filled between collection windows). Any value greater than zero will cause collection to occur between windows. You must enter a value greater than zero if you want to collect waste between the collection windows.

The example below shows two collection windows (0.01 to 2.0 minutes and 4.0 to 7.0 minutes) with % Monitor Collect between Windows selected, which results in mobile phase being collected between the windows.



### Check boxes

- Enable Aux Chromatogram – Collects the data channel that is visible to the WFC III. The data channel displays as WFC III Peak in the channel tab. Collecting this data can be helpful when setting threshold collection values. Once the threshold collection values have been set, this data has little use and should not be collected.
- Collect multiple fractions per position – For users injecting the same sample, this option collects peaks from replicate injections into the same vessels. Injections must be from a single sample line of Empower to

collect into the same set of tubes. The WFC III advances with every new line of run samples.

| Sample Set Method: 1 |              |           |            |          |                            |                    |                           |      |
|----------------------|--------------|-----------|------------|----------|----------------------------|--------------------|---------------------------|------|
| Plate/Vol            | Inj Vol (uL) | # of Injs | SampleName | Function | Method Set / Report Method | Run Time (Minutes) | Next Inj. Delay (Minutes) |      |
| 1                    | 1:F,1        | 1.0       | 10         | time     | Inject Samples             | WFC3_Test_Sept9 2  | 2.00                      | 0.00 |

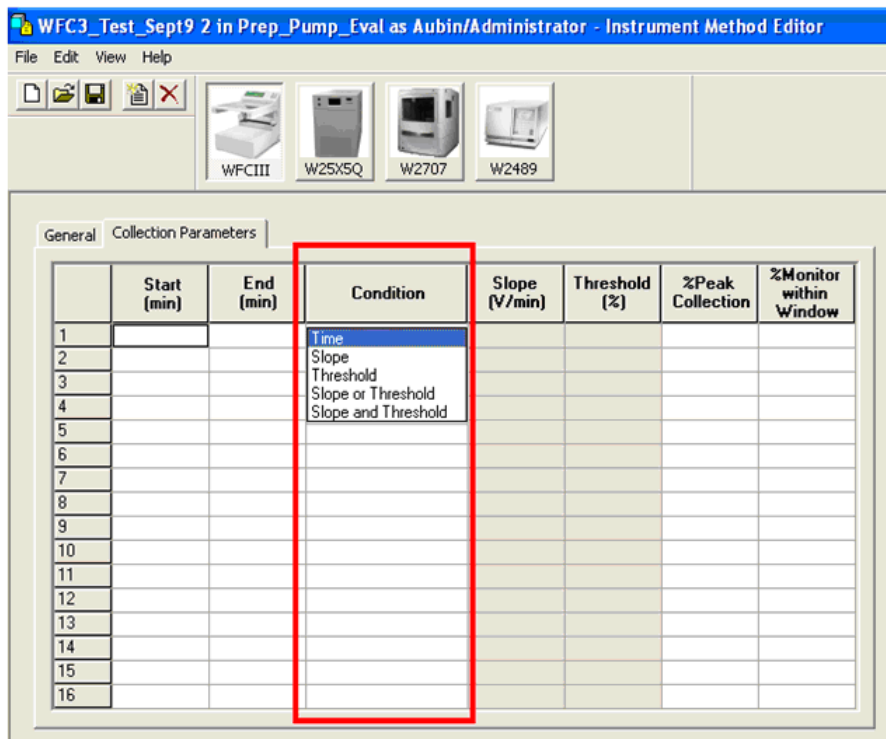
- Skip tube between injections – Leaves an empty tube between sets of collected fractions. This is usually left unchecked.

## Collection parameters tab

The Collection parameters tab defines the conditions for peak collection.

|    | Start (min) | End (min) | Condition | Slope (V/min) | Threshold (%) | %Peak Collection | %Monitor within Window |
|----|-------------|-----------|-----------|---------------|---------------|------------------|------------------------|
| 1  |             |           |           |               |               |                  |                        |
| 2  |             |           |           |               |               |                  |                        |
| 3  |             |           |           |               |               |                  |                        |
| 4  |             |           |           |               |               |                  |                        |
| 5  |             |           |           |               |               |                  |                        |
| 6  |             |           |           |               |               |                  |                        |
| 7  |             |           |           |               |               |                  |                        |
| 8  |             |           |           |               |               |                  |                        |
| 9  |             |           |           |               |               |                  |                        |
| 10 |             |           |           |               |               |                  |                        |
| 11 |             |           |           |               |               |                  |                        |
| 12 |             |           |           |               |               |                  |                        |
| 13 |             |           |           |               |               |                  |                        |
| 14 |             |           |           |               |               |                  |                        |
| 15 |             |           |           |               |               |                  |                        |
| 16 |             |           |           |               |               |                  |                        |

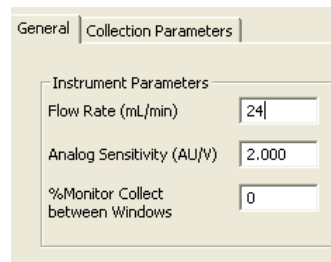
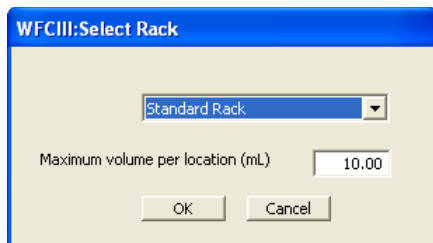
- Condition – The type of collection (Time, Slope, Threshold, Slope or Threshold, Slope and Threshold).



- Start (min) – Collection window start time
- End (min) – Collection window end time

## Rules:

- You cannot only instruct the fraction collector to collect a specific time fraction.
- You must calculate using flow rate, collection tube volume, and percent peak collection.



In the above example, 10 mL collection tubes are defined, and the method has a flow rate of 24 mL/min. Therefore,  $10 \text{ mL}/24 \text{ mL/min} = 0.417 \text{ min/tube}$  (i.e. 24 seconds to fill the tube).

Based on the above information:

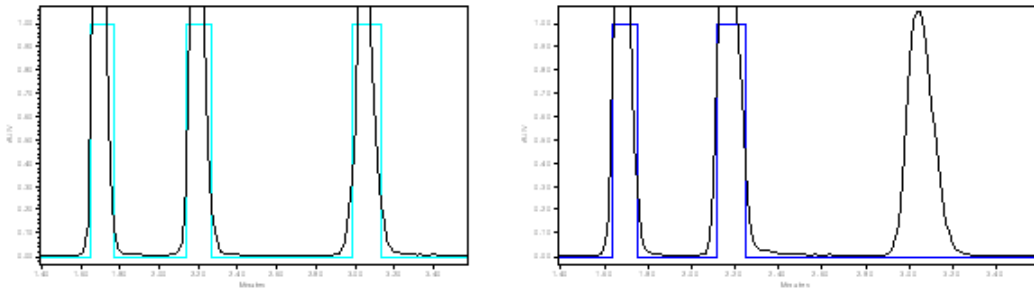
- 100% peak collection results in 24 second time fractions
- 75% peak collection results in 18 second time fractions
- 50% peak collection results in 12 second time fractions
- 25% peak collection results in 6 second time fractions
- Slope (V/min) – The collection start and end (within the time window). When you choose slope value as the condition, data above the preset slope value is collected. Collection begins when the slope of the signal exceeds the preset value and continues until the signal slope falls below the preset value. You can use slope value discrimination when there is a large drift in the baseline and/or the peaks are sharp.

Slope collection uses the signal from the Aux Chromatogram to trigger the collection based on the rise or fall of the peak.

Allowable values are 0 to 9.999. Lower values collect broader peaks. Zero (0 V/min) collects everything, but is not recommended.



In the example below, the slope is set to 2.5% in the chromatogram on the right, and to 5% in the one on the left.

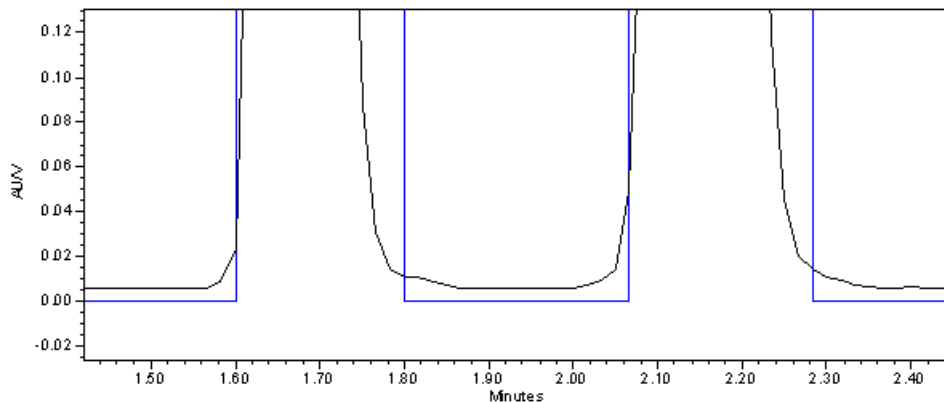


- **Threshold (%)** – Defines the collection start and end (within the time window) when you choose threshold as the condition. Only a peak larger than the preset percentage of the signal level is collected. Collection begins when the level rises above the preset value and continues until the level falls below the preset value.

Threshold collection uses the percent signal from the Aux Chromatogram to trigger the collection.

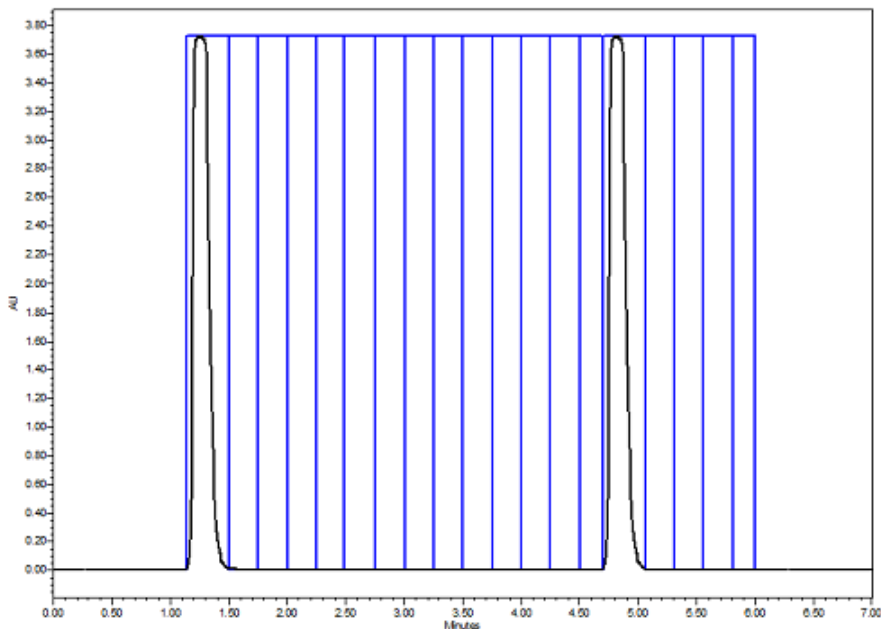
A 1% value is equal to about 0.012 AU on the WFCIII Peak data channel.

Experiments show 2% is about as low a threshold value as is practical.



- **% Peak Collection** – Defines the tube fill. A value of 100 completely fills the tube.
- **% Monitor within Window** – Percent of tube fill per collection vessel when the sample does not meet selected peak criteria. Use this function

to collect the mobile phase that elutes between peaks. 100% fills the tube volume.



The above example has one collection window (1.0 minutes to 7.0 minutes) and condition set to threshold and % Monitor within Window set to 50%. The two peaks are collected with the threshold along with the waste mobile phase between the peaks.

## And/Or collections

### “Or” collection

In an “Or” collection, either parameter can be met for the collection to start.

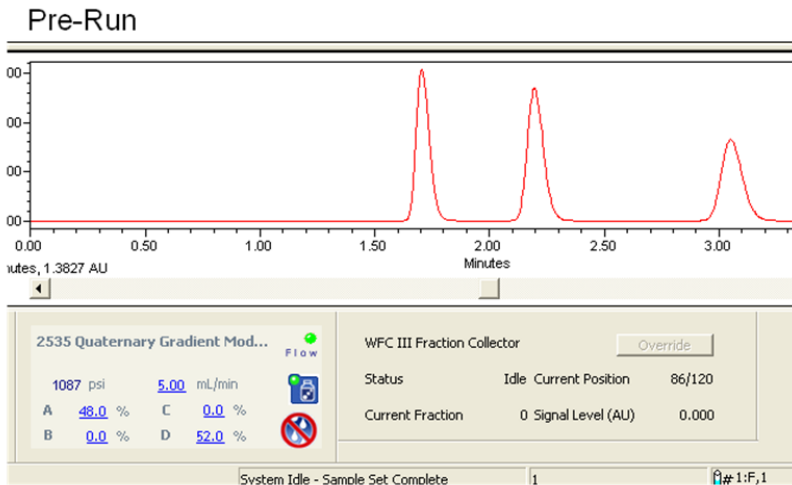
| General |             | Collection Parameters |                    |               |               |                  |                        |
|---------|-------------|-----------------------|--------------------|---------------|---------------|------------------|------------------------|
|         | Start (min) | End (min)             | Condition          | Slope (V/min) | Threshold (%) | %Peak Collection | %Monitor within Window |
| 1       | 0.10        | 4.00                  | Slope or Threshold | 1.000         | 5             | 100              | 0                      |
| 2       |             |                       |                    |               |               |                  |                        |
| 3       |             |                       |                    |               |               |                  |                        |

## “And” collection

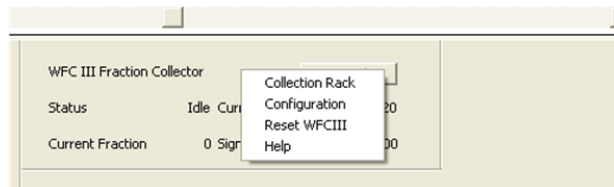
In an “And” collection, both parameters must be met for collection to start.

|   | Start (min) | End (min) | Condition           | Slope (V/min) | Threshold (%) | %Peak Collection | %Monitor within Window |
|---|-------------|-----------|---------------------|---------------|---------------|------------------|------------------------|
| 1 | 0.10        | 4.00      | Slope and Threshold | 1.000         | 5             | 100              | 0                      |
| 2 |             |           |                     |               |               |                  |                        |

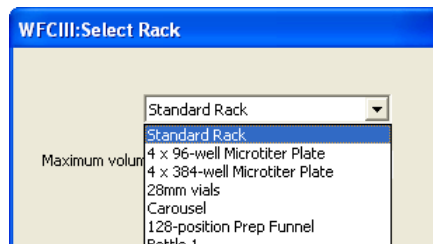
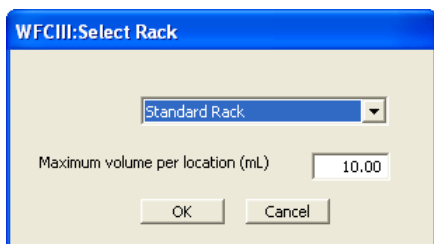
## Run Samples control panel



Right Click Brings up Menu



- Collection Rack – Defines the collection rack and collection tube volume.



- Configuration – Defines delay volume, buzzer, valve position, and drain position.



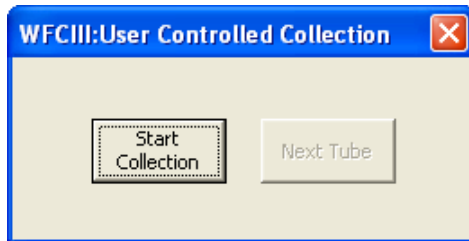
- Delay Volume – The volume from the outlet of the detector to the tip of the fraction collector.
- Buzzer – Defines if a sound is generated at the end of a collection (i.e. end of a run) or every time the arm moves. You can also define not to generate any sounds.
- Valve Position – The collection valve can be closed or open during movement. When collecting closely eluting peaks, it is sometimes beneficial to leave the valve open. When the valve is open, no mobile phase flows to waste during movement. The drawback of leaving the valve open is that some solvent will miss tubes entirely during movement. Most users keep the valve closed.
- Reset WFC III – Resets the WFC III. When pressed, the arm moves back to tube position 1. Use this choice to reset the collection bed.
- Help – Accesses the help files. Help is also available during the run time.

## Emergency collection (override)

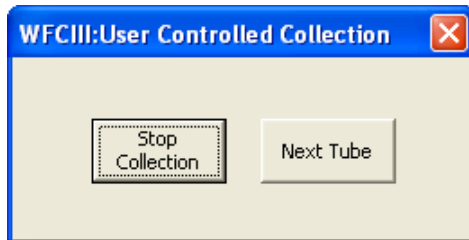
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|                            |   |                   |        |
|----------------------------|---|-------------------|--------|
| WFC III Fraction Collector | <input type="button" value="Override"/> |                   |        |
| Status                     | Collecting                              | Current Position  | 92/120 |
| Current Fraction           | 1                                       | Signal Level (AU) | 2.478  |

Click Override to start emergency collection. The following dialog box appears.



Click Start Collection to start a collection. The following dialog box appears.



The collector advances to the next tube when full or when you click Next Tube.

To stop the override collection, click Stop Collection.

Once you click Override, the WFC III window mode operation ends and the collection is under user control until the end of the current injection.

Window mode operation is restored on subsequent injections.

## What happens when the bed becomes full during a collection?

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When the bed becomes full during a collection, the WFC III generates an instrument failure and sample and data collection stop. The sample list is aborted and the pump continues to run, which is typical Empower instrument failure behavior. Error messages are generated in the message center.

|    |                           |            |                     |                |  |
|----|---------------------------|------------|---------------------|----------------|--|
| 53 | 9/16/2009 10:39:02 AM EDT | WFC3_Sept9 | Aubin/Administrator | Prep_Pump_Eval | Instrument Failure WFCIII@1                                |
| 54 | 9/16/2009 10:39:10 AM EDT | WFC3_Sept9 | Aubin/Administrator | Prep_Pump_Eval | WFCIII@1: Stopped the collection as Bed Full has happened. |

Click Reset WFC III in the control panel to reset the instrument (i.e. clear the error message), and reset the bed back to position 1.

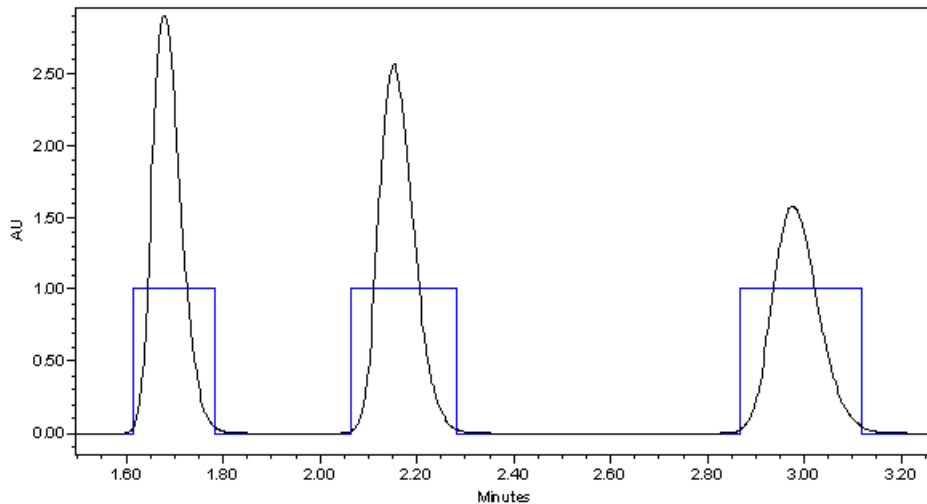
## Looking at data

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The WFC III generates one or two data channels.

WFC III Collection State is the indication of peak collection. You can overlay this data with the detector channel you are reviewing or in a report to show which peaks were collected.

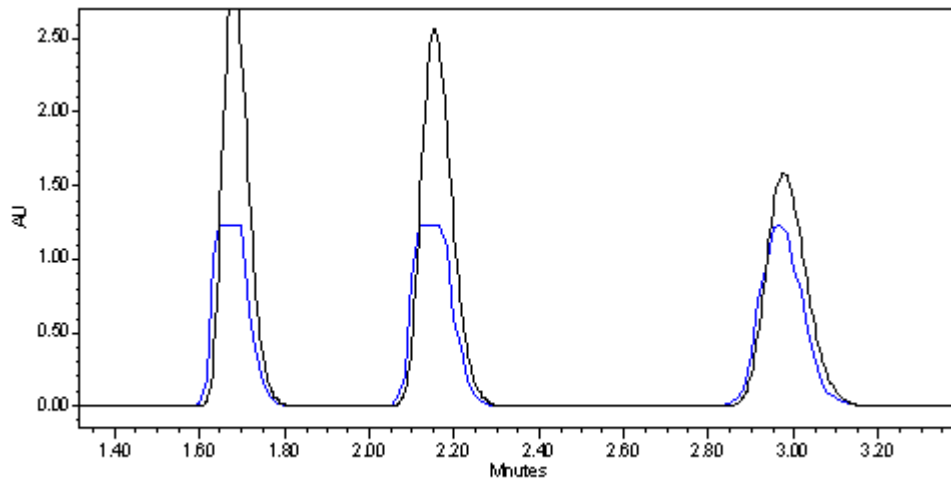
|   | SampleName | Vial  | Injection | Sample Type | Date Acquired             | Channel                   | Channel Description       |
|---|------------|-------|-----------|-------------|---------------------------|---------------------------|---------------------------|
| 1 | 0          | 1:F,1 | 10        | Unknown     | 9/16/2009 11:44:44 AM EDT | vWFC III Collection State | vWFC III Collection State |
| 2 | 0          | 1:F,1 | 10        | Unknown     | 9/16/2009 11:44:44 AM EDT | vWFC III Peak             | vWFC III Peak             |
| 3 | 0          | 1:F,1 | 10        | Unknown     | 9/16/2009 11:44:44 AM EDT | vW2489 ChA                | vW2489 ChA 254nm          |



The black line in the above example is the UV output from the 2489 UV/Visible detector and the blue line is the collection state. When the collection state = 0, mobile phase has gone to waste. When the collection state = 1, mobile phase collects in the collection vessels.

The WFC III peak channel is the analog channel that is visible to the WFC III. This data can be used to help set threshold values. Users have the option to collect, or not collect, this data channel.

**WFC III peak channel in blue, 2489 UV/Visible detector channel in black**



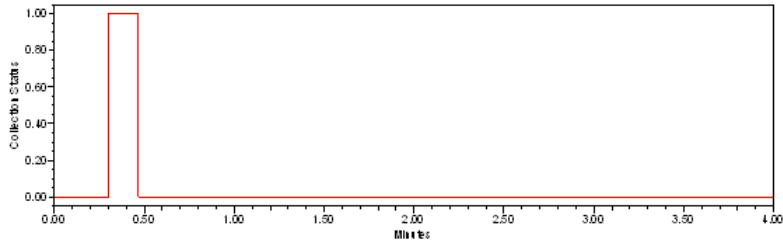
Note the lower data rate and maximum AUFS (1.25) of the WFC III peak channel; this channel should not be used for integration.



The WFC III comprehensive status report is the only means of determining what fraction is in what tube.



[fraction report](#)



— Sample Name: 0; Vial: 1:F,1; Injection: 1; Channel: WFC III Collection State; Date Acquired: 9/16/2009 10:09:39 AM EDT

**WFC III Comprehensive Status**

Sample: 0 Vial: 1:F,1 Inj: 1

Post Run Data Table

|   | Vessel Number | Peak Number | Collection Start Time (MM:SS) | Collection Duration (MM:SS) |
|---|---------------|-------------|-------------------------------|-----------------------------|
| 1 | 102           | 1           | 00:19                         | 00:09                       |

**WFC III Comprehensive Status**

Sample: 0 Vial: 1:F,1 Inj: 1

Some users find it beneficial to create a fraction report consisting of an overlay chromatogram (to overlay the collection state and the UV data channel) and the comprehensive status report to determine what peak is in what tube.

