Fraction Tube Filling Variability

on Combi*Flash*® Rf-200 Systems

Abstract

Variability in test tube delivery volume is observed when running Combi*Flash* Rf-200 systems, particularly at high flow rates associated with large columns. This variability is normal, caused by the system assigning higher priority to data collecting, peak cutting, flow rate, and gradient accuracy functions. Monitoring tube fill volume is given a lower priority. Excessive filling of tubes can be reduced by lowering the tube volume setting in the Method Editor or Configuration windows. Use of larger collection tubes at high flow rates also ameliorates the issue.

Discussion

Background

The main processor on the Combi*Flash* Rf-200 focuses its priority on collecting data and performing peak detection on anything from a single wavelength through All-Wavelength Collection or external detectors. Due to this emphasis on monitoring the incoming data, tube changes due to peak cutting are very tightly controlled and precise.

While monitoring these important activities, the main processor also monitors the pumping status once each second. At high flow rates, this may cause noticeable fill volume variability due to pump refill strokes.

The pumping status is read from a separate processor which controls the pump flow rate and gradient formation. Because of this dedicated processor, the flow rate and gradient accuracy specifications equal or exceed that of any other contemporary automated flash chromatography system.

Modifying the Tube Volume Setting

If a user feels the system fills the tubes to an uncomfortably high level during a purification run, the tube volume setting may be changed in either the Method Editor or the Configuration windows.

If the issue is rarely observed, such as an occasional large column, high flow rate run, the tube volume should be changed using the Method Editor window.

If the system frequently fills the tubes too high, the default tube volume should be changed on the Configuration window. From the Instrument Configuration tab, click the Default Tube Volume button and enter the change for the appropriate racks. Changing the default tube volume will affect all subsequent purifications that use the tube sizes that were changed. This solution is useful if large columns (with higher flow rates) are commonly used.

From either window, change the tube volume setting from Max to 2 mL less than the capacity printed on the front of the tube rack. For example, an 18 mm tube rack lists the default maximum volume as 25 mL per tube. One would enter 23 mL for a reduced fill volume.

Increasing the Tube/Rack Capacity

Selecting larger capacity tubes will also reduce the variations in tube volumes.

Conclusion

Although tubes are filled to slightly differing volumes, this variation is due to the emphasis on data collection and peak cutting. The pump flow rate and gradient are controlled by a separate processor system to ensure accurate gradients and flow rates.

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