SARTORIUS

Guideline

# Column Integrity Test for CIMmultus and CIM Ray Monolithic Columns

## Introduction

The column integrity test must be performed before first use and is recommended when column damage is suspected. Column integrity is evaluated with a column integrity test, which measures the detector response (UV, conductivity) of a non-binding tracer. The tracer is injected into the flow path of the system and eluted by an isocratic elution with a suitable mobile phase. The duration from tracer injection to elution is the retention time of the tracer and should conform to a defined criterion. To account for dead volume of the system, it is necessary to first perform a tracer injection without connecting the column. A column integrity test is therefore performed on the LC system with the column connected to the system and without. The peak shape and the retention time of the tracer give an indication of column integrity.

For more information, contact
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# Before you begin

Before you begin with the testing procedure, please check that the operating conditions are in accordance with Instructions for Use.

# Testing conditions

| | | |
|---|---|---|
| **Tracer & Mobile phase*** | IEX, OH, H-Bond ADC, SO4: | 5 % acetone in water (v/v) |
| | C4 HLD, Oligo dT18, PrimaS, PrimaS HR, PrimaT, Swiper: | 0.1 mg/mL uracil in water |
| | SDVB: | 0.1 mg/mL uracil in 15 % ethanol, 0.47 M NaCl |
| **Method** | Isocratic | |
| **Flow rate** | 1 column volume (CV)/min<br>A different flow rate can also be used if there are system limitations. | |
| **Detection** | UV 280 nm for acetone<br>UV 260 nm for uracil<br>UV 200 nm or conductivity for NaCl | |

*Tracer type and concentration can be adjusted. Tracer should not interact with the stationary phase. If NaCl is used as a tracer it is recommended to use a salt-containing mobile phase as well (e.g. 100 mM NaCl) to minimize tracer retention on the column. The volume of injection can be adjusted as needed to provide adequate signal response. Mobile phases should be filtered using 0.22 µm filter.

| **Column size** | Specimen CIMmic | 1 mL | 4 mL | 8 mL | 40 mL | 80 mL | 400 mL | 800 mL | 4000 mL | *8000 mL |
|---|---|---|---|---|---|---|---|---|---|---|
| **Tracer volume** | 5 µL | 25 µL | 100 µL | 200 µL | 1 mL | 2 mL | 10 mL | 20 mL | 100 mL | 200 mL |

*Installation and testing of 4000 mL and 8000 mL units can be performed by a qualified Sartorius BIA Separations engineer.

# Procedure

**1. Determine the suitability of the LC system (the column is not connected at this stage):**

1.1. Measure the exact flow rate φ (mL/min) on the system.

Collect $dH_2O$ for 2-3 minutes into a pre-weighed container or volumetric flask. Determine the flow rate. The measured flow rate should not differ from the system flow rate by more than 5 %.

1.2. Measure the pressure drop on the system.

Double the flow rate of the testing procedure (i.e. 2 CV/min) or use the highest flow rate achievable on the pump. The pressure drop on the system should not exceed the maximum pressure specified in the Instructions for Use.

1.3. Check the system for any leaks.

Check all connections and valves on the system for leakage.

**2. System tracer injection**

2.1. Measure the retention time of tracer ($t_0$) on the system (the column is not connected at this stage).

Inject the tracer solution and run the test procedure using the conditions outlined above. The peak should be uniform and smooth without any fronting or other deformation. Record the time $t_0$ at the apex of the absorbance signal peak. See the dashed absorbance signal on reference chromatogram for guidance -Figure 1.

**3. Column Integrity Test**

3.1. Column preparation

Connect the column to the system per Instructions for Use. Wash the column with at least 15 CV*[1] of purified water*[2] or until stable baseline is observed.

*[1] For CIM Ray wash the column according to Instructions for Use.

*[2] In the case of SDVB column wash with appropriate buffer.

WARNING: Reversing the flow on the column will irreversibly damage the monolith.

3.2. Perform Column Integrity Test

Inject the tracer solution and run the test procedure using conditions outlined above. The peak should be uniform and smooth without any fronting or other deformation. Record the time $t_r$ at the apex of the absorbance signal peak. See the blue absorbance signal on reference chromatogram for guidance-Figure 1.

3.3. Wash the column with at least 10 CV of $dH_2O$*[3]

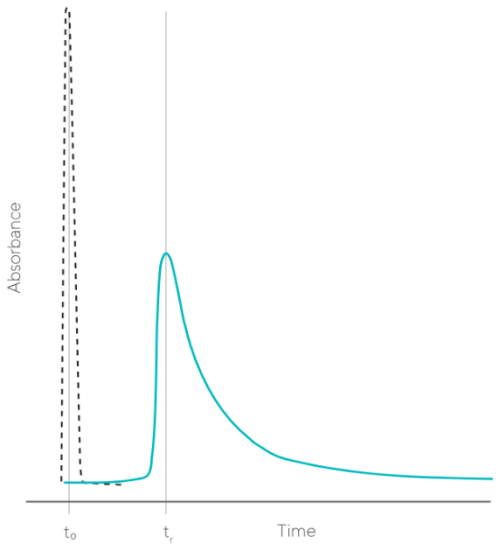
This step concludes the column integrity test of the column. For result interpretation see next section.

*[3] In the case of SDVB column wash with 10 CV of 20 % ethanol.

3.4. Proceed with the chromatographic run or store the column according to the conditions specified in the Instructions for Use.

# Determining tracer retention time

Calculate the difference in retention time of tracer with the equation below. The obtained peak should be smooth and without any fronting or other deformations.
NOTE: In case of CIM Ray columns, the peaks are wider due to the higher dead volume of the column setup.




Tracer retention time, $\Delta t = \frac{(t_r - t_0) \times \varphi_{used}}{\varphi_{1CV}}$

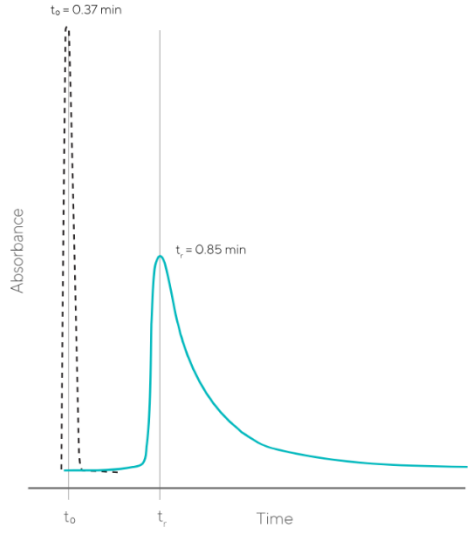
$\varphi_{used}$...used flow rate, in mL/min

$\varphi_{1CV}$...flow rate equivalent to 1 column volume (CV)/min, in mL/min

$t_0$...tracer retention time on the system, in min

$t_r$...retention time of tracer when the column is connected to the system, in min

**Figure 1**: *Calculating tracer retention time.*




$\varphi_{used}$ = 2 mL/min

$\varphi_{1CV}$ = 1 mL

$t_0$ = 0.37 min

$t_r$ = 0.85 min

$$\Delta t = \frac{(t_r - t_0) \times \varphi_{used}}{\varphi_{1CV}} = \frac{(0.85\ min - 0.37\ min) \times 2\ mL/min}{1\ mL/min} = 0.96\ min$$

**Figure 2**: *Calculating tracer retention time, CIMmultus 1 mL column was used in example above.*

# Examples of unsuitable traces

The following examples show column integrity test traces that could indicate column damage. Peak broadening, fronting, tailing, or split peaks should not be present on the system nor column integrity test chromatograms. System artefacts can sometimes occur, for example, due to pulsation of the pumps. Address the issue and consider repeating the procedure.

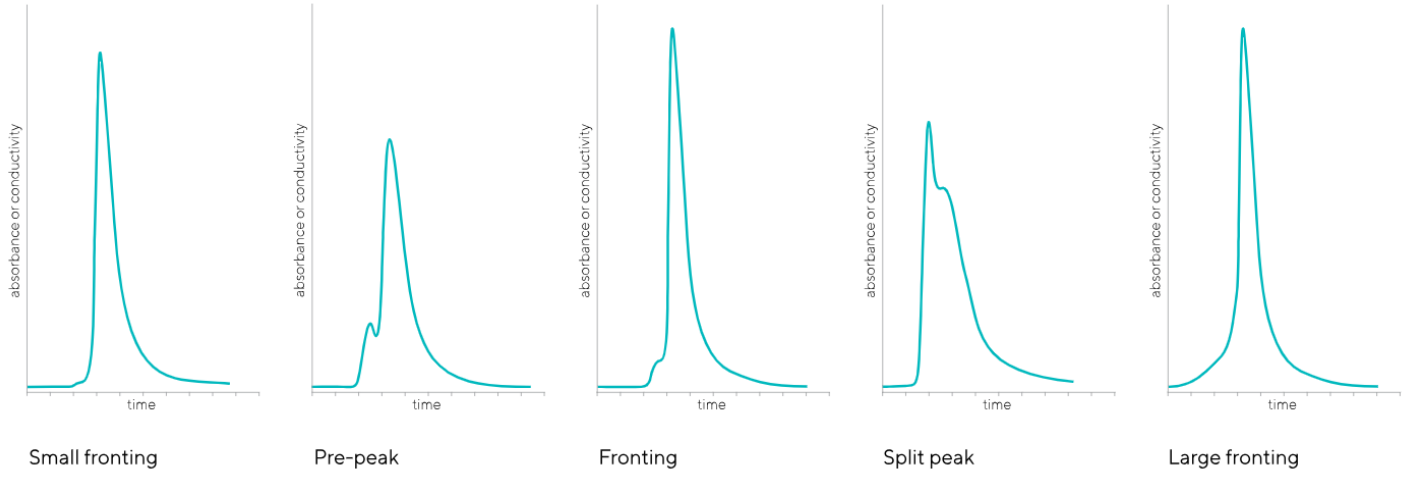



**Figure 3**: *Examples of failed integrity test peak shapes.*

# Acceptance criteria

Deviations can be expected when using a different tracer/mobile phase system than recommended due to interactions between the solid and liquid phases.

Criteria for custom made products are not defined; however, the same procedure can be applied for testing. The peak should not elute in the void volume of the column, and its shape should conform to the requirements of peak shape outlined above.

| **Column size** | Specimen CIMmic | 1 mL | 4 mL | 8 mL | 40 mL | 80 mL | 400 mL | 800 mL | 4000 mL | *8000 mL |
|---|---|---|---|---|---|---|---|---|---|---|
| **Acceptance criteria [min]** | ≥ 0.75 | ≥ 0.75 | ≥ 0.75 | ≥ 0.75 | ≥ 0.75 | ≥ 0.75 | ≥ 0.75 | ≥ 0.75 | ≥ 0.75 | ≥ 0.75 |

# FAQ

**How often should the integrity of the column be verified?**
The column integrity test should be performed before first use and is the recommended test when column damage is suspected. Column damage does not happen often. Typical scenarios leading to it could be: column falling on the ground, reverse flow, pressure spikes during use or monolith drying up.

**Can I change the tracer for the column integrity test?**
It is possible to perform column integrity test with a different tracer/mobile phase systems, such as a 1 M NaCl tracer. If NaCl is used as a tracer it is recommended to use a salt-containing mobile phase as well (e.g. 100 mM NaCl) to minimize tracer retention on the column. In this instance, conductivity signal is measured instead of absorbance. Bridging study between standard tracer and salt tracer is recommended.

**The column retention time is outside of acceptance criteria, is the column damaged?**
Values of retention time may differ due to interaction tracer-medium when using a different tracer. It is best to do an intial test with recommended tracers followed by an alternative tracer to perform a bridging study and confirm validity of the criteria.

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