

Troubleshooting Guide: Sievers* M9 SEC DOC Detector

TEST REPORT

Purpose

The following troubleshooting guide aims to cover general issues encountered when using the SEC-TOC system. There are potential issues specific to an individual part or module of the system that are not explicitly covered. For example: this guide will not cover diminishing flow rate resulting from a specific isocratic pump issue. To deal with issues not covered in this guide, users are encouraged to contact the manufacturer of the component in question.

The following guide has been written specifically for the SEC-TOC system recommended by Veolia that incorporates Agilent Liquid Chromatography modules (degasser, isocratic pump, MWD/DAD/FLD detectors), the TOYOPEARL HW-50S SEC column by Tosoh, and the Sievers M9 TOC Analyzer by Veolia. This system is compatible with the appropriate version of Agilent Chem-Station (contact Agilent Analytical Technologies for further details regarding the appropriate version) for data collection, and the Agilent Universal Interface box is recommended for the live transfer of data from the M9 Analyzer to Chem-Station.

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1. Elevated or Depressed TOC Baseline

There are several reasons for the TOC baseline (of which live readings can be viewed on the TOC interface) to rise above or fall below reasonable and expected levels (e.g. microbial growth, clog due to particulate or sample build up within the system, etc.). These issues are most commonly experienced following long periods of inactivity. To recognize when a baseline is elevated and when it has returned to normal, it is very helpful to have some knowledge of what the expected baseline should be (i.e. an estimate of the TOC levels in the ultrapure water used for mobile phase, and how much will be added from minor impurities introduced from the mobile phase salts). If an additional standalone TOC (e.g. Sievers M9 or M5310 not connected to SEC system) is accessible, analysts can determine the exact mobile phase TOC (however, this may not be possible). Regardless of the timing or cause of an elevated baseline, users are first encouraged to thoroughly flush the entire system following the protocol outlined in the best practices guide.

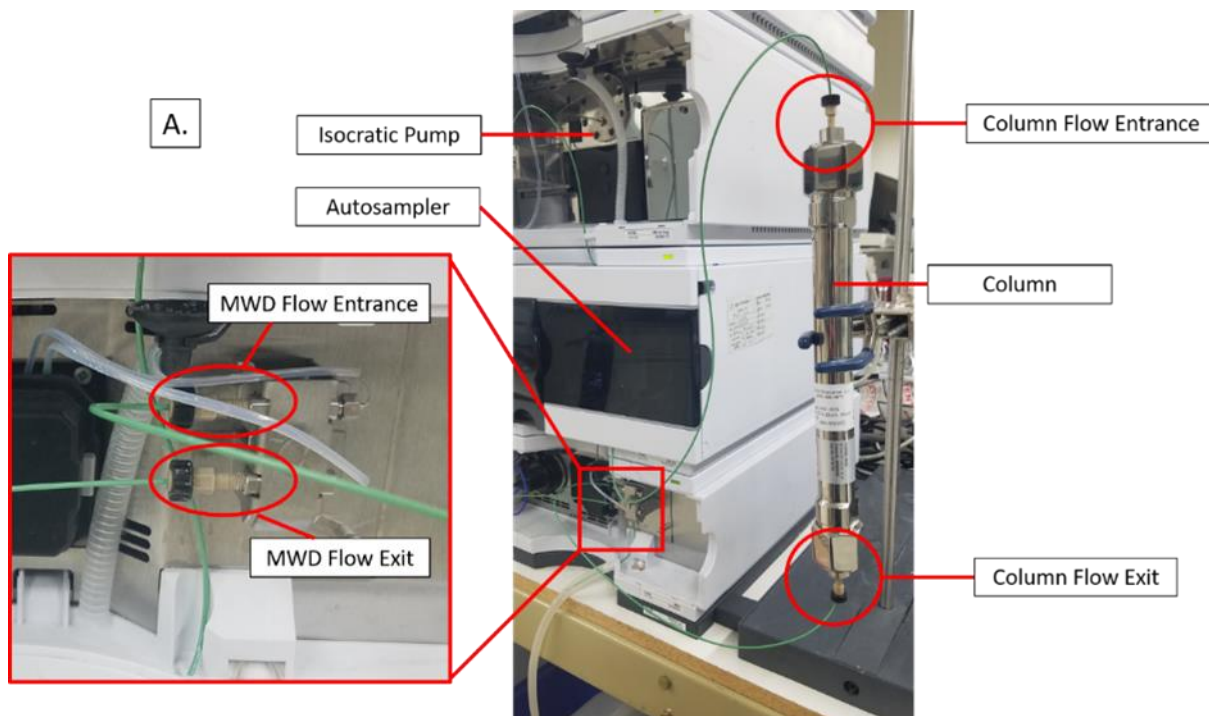
- Flush for extended lengths of time with ultrapure water (minimum 2-5 hours).
- Bypass the SEC column and then flush with hydrogen peroxide (3% H₂O₂) solution (1-2 hours minimum).
- Flush with ultrapure water before and after (both 1 hour minimum) reconnecting the SEC column.

For the following reasons, it is essential to ensure all peroxide has been thoroughly removed from the system before reconnecting the column and powering on the M9.

- Peroxide is damaging to the column.
- Peroxide can react with the oxidizer in the M9 potentially creating gas bubbles in the system. While not necessarily damaging, this gas would then need to be removed before reliable measurements can be recorded. This is not an issue when the M9 is powered off as oxidizer is only used when the M9 is in operation.

In general, if flushing procedures have already been performed (and repeated) it is wise to diagnose where in the system the issue is specifically arising from.

Rearrange system tubing to isolate each module, and then measure the baseline under normal instrument flow conditions (1 mL/min) with mobile phase or ultrapure water. For example, to test if the elevated baseline is arising from the column, bypass the column (close off column entrance by looping the tubing from the column exit around to the entrance), then resume flow under the same conditions (see **Figure 1** with description). If the baseline drops once the column has been removed, the elevated baseline is likely originating from the column.



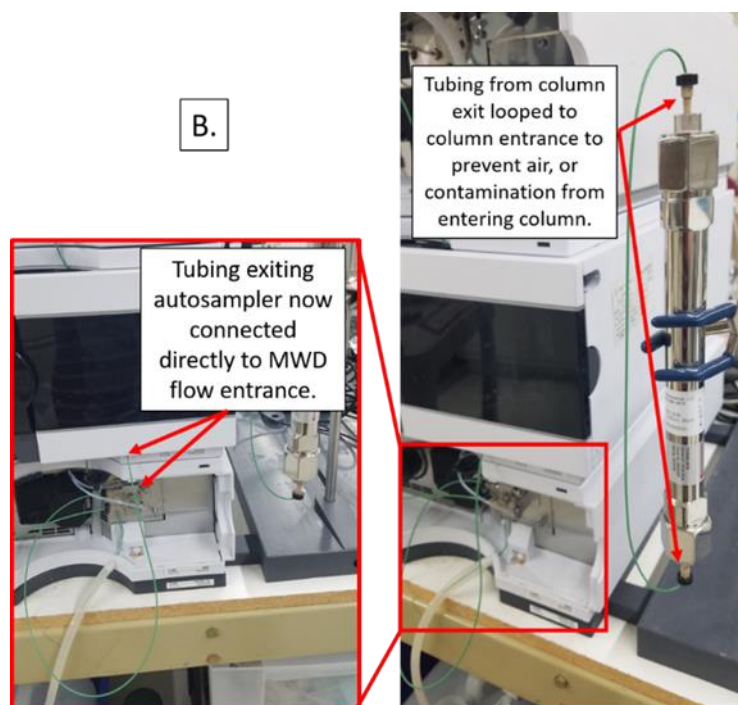


Figure 1. In system pictured in Figure 1.A (top), the flow path is as follows: isocratic pump, autosampler, SEC column (entrance to exit), MWD (entrance to exit), M9 Analyzer (not pictured). Therefore, to bypass the column, tubing should first be disconnected from the MWD entrance. The tubing connected to the column flow entrance should then be disconnected and re-connected to the MWD flow entrance. Figure 1.B (left) shows the new flow path: isocratic pump (not pictured), autosampler, MWD, M9 (not pictured). NOTE: Once the column has been disconnected, solvent will begin to flow out of the column. To prevent air, dust, or other contamination from being drawn into the column, the tubing exiting the column can be looped around to the entrance of the column (closing off both openings), as shown in Figure 1.B (right). If tubing cannot be looped from the column exit to entrance, parafilm can be used to seal both openings.

Once the origin of the elevated baseline is determined, the exit tubing of that component should be disconnected from the rest of the system as to not allow a clog or microbial growth to travel into another portion of the system (example shown in **Figure 2**).

a. Column

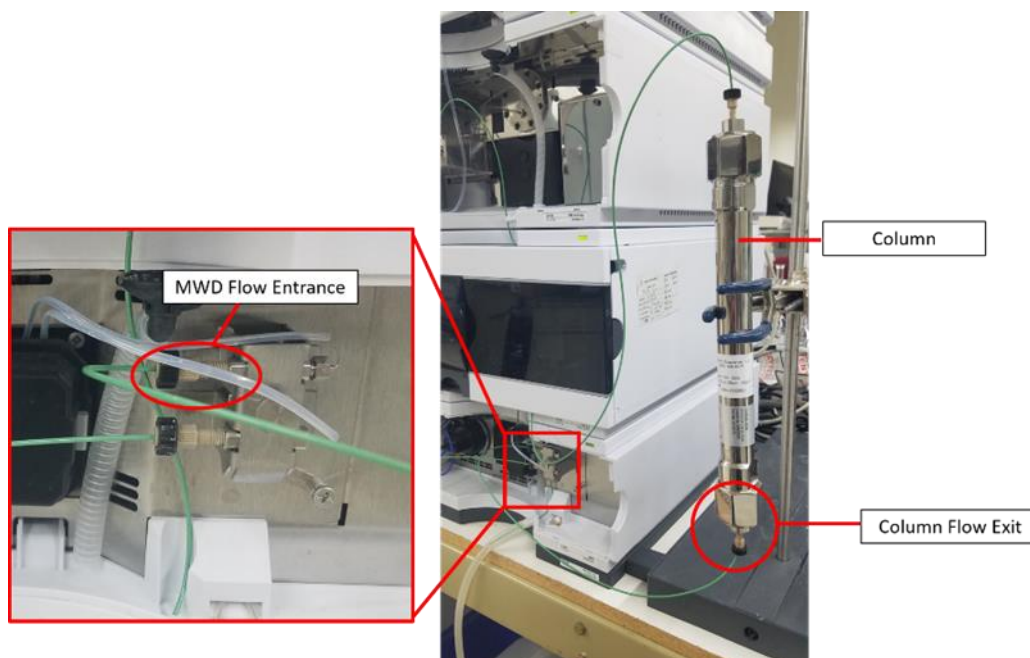


Figure 2. In the system pictured, flow travels through tubing connected from the column exit to the MWD entrance. To allow flow to travel to waste, disconnect the tubing from the MWD entrance and move tubing to a waste catch. NOTE: Cover opening to MWD entrance (i.e. using parafilm) to prevent possible contamination.

The following applies specifically to the TOSOH TOYOPEARL column recommended in the system description. Connect the column directly to the isocratic pump and disconnect any parts or modules later in the flow path (i.e. solution should flow out of the column to waste, see **Figure 2**).

Flush with ultrapure water for minimum 1 hour.

Disconnect tubing from entrance and exit of column, invert the column, reconnect tubing to column entrance and exit, and repeat typical flushing procedures with ultrapure water (see **Figure 3**).

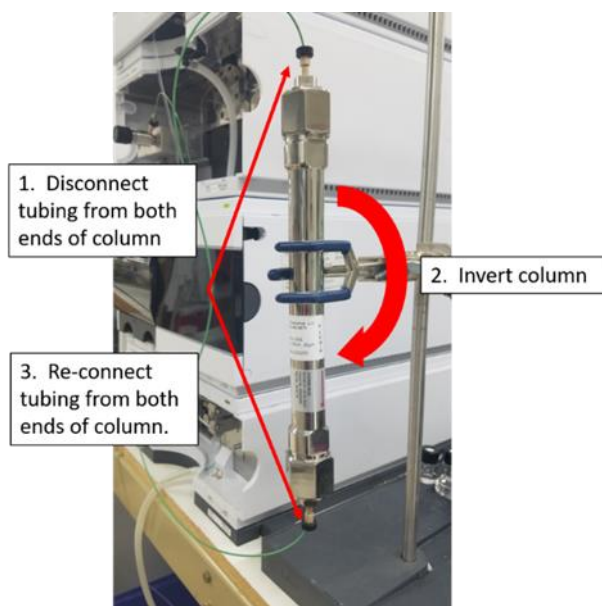


Figure 3. Instructions for Inverting SEC Column

Additional flushing to remove strongly bound materials should be conducted as follows (the following information can be referenced in the TOYOPEARL instruction manual):

- First flush with an alkaline solution of 0.1-0.5M sodium hydroxide.
- Then flush with acidic solution of 0.1-0.5M hydrochloric or sulfuric acid. **WARNING: NEVER FLOW NITRIC ACID THROUGH A TOYOPEARL RESIN COLUMN.** Nitric acid can react violently with TOYOPEARL resins.
- **NOTE:** TOSOH operating manuals states the TOYOPEARL resin used in this column is stable in the pH range of 2-13 for long-term use and stable in the range of 1-14 for short periods. These conditions should be met when flushing with alkaline or acidic solutions.
- 10-20% solutions of methanol, ethanol, or isopropanol can be used to flush out hydrophobically bound material. Organic solvent flush should also be conducted only after alkaline flush.
- The above flushes should be conducted for a minimum of 1 hour. Between alkaline, acidic, and organic solvent flushes, system should be flushed with ultrapure water before the next flush.

After flushing the column with the above solutions, thoroughly flush the column again with ultrapure water (minimum 2 hours) before reconnecting it to the rest of the system.

b. Sievers M9 SEC DOC Detector

Disconnect the M9 from the entire system.

Using a squirt bottle, connect the nozzle to either the entrance or exit tubing of the M9 and force (this will require applying significant pressure to be applied to the squirt bottle) ultrapure water or the recommended hydrogen peroxide solution through the instrument. It is possible for clogs to form in a gradient and is therefore helpful to repeat this step from both the forward and reverse flow directions (see **Figure 4**).

If hydrogen peroxide is used, a flush with ultrapure water (of ~40-50 mL with a squirt bottle) should follow.

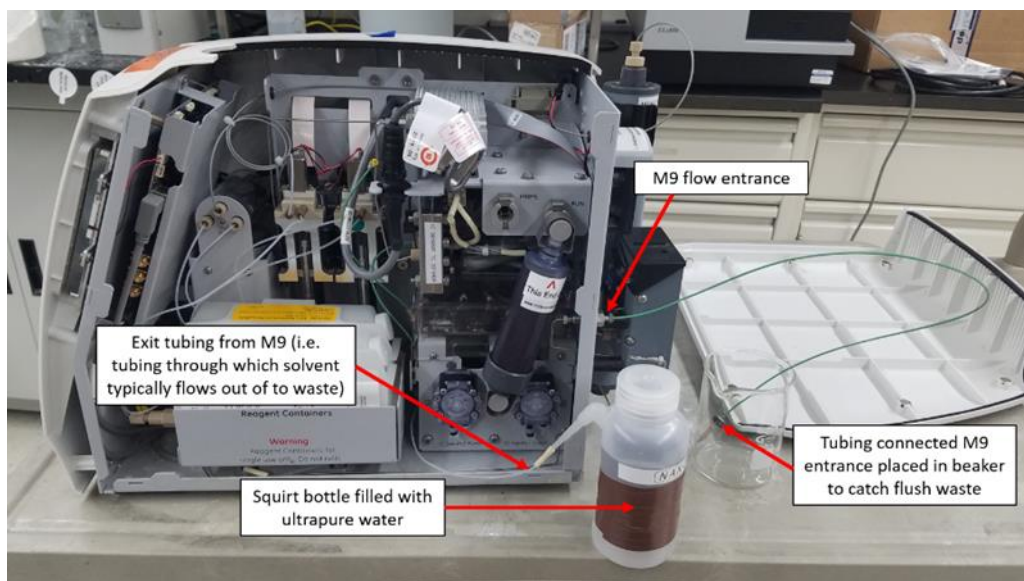


Figure 4. Pictured above is an M9 Analyzer that has been disconnected from the SEC system. The nozzle of the squirt bottle filled with ultrapure water (hydrogen peroxide can also be used) has been inserted into the exit tubing of the M9 (i.e. in this image the M9 will be flushed in reverse). Tubing connected to the M9 entrance is typically connected to the exit of the MWD of the HPLC stack. Here it has been placed in a beaker to catch the flush waste. Pressure can now be applied to the squirt bottle to force solution (either ultrapure water or hydrogen peroxide) through the M9 flow path.

Using this method, flush several instrument volumes of solution (~25-50mL). DO NOT ATTEMPT THE ABOVE STEP USING A SYRINGE AS IT IS POSSIBLE TO DAMAGE OR RUPTURE THE CO₂ PERMEABLE MEMBRANE. More details can be found in the M9 Instrument Manual.

c. Isocratic Pump/Degasser Module

Open the purge valve of the isocratic pump, turn on the pump and increase the flow rate to the maximum (or the maximum flow rate that is safe to operate the pump). For the recommended system, the flow rate can be increased to 5 mL/min (**Figure 5**).

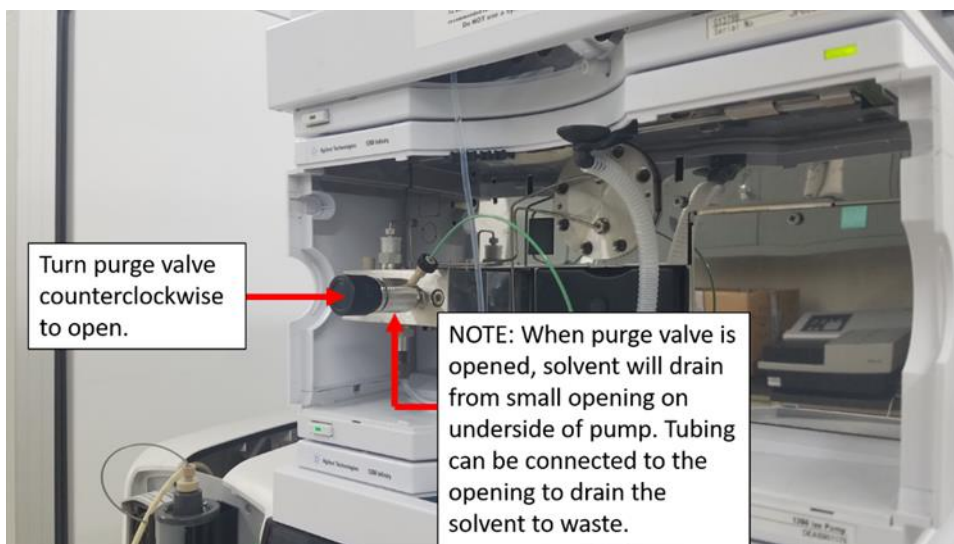


Figure 5. Pictured above is the isocratic pump module of the HPLC stack with an arrow highlighting the purge valve.

Using a Luer lock syringe (ideal volume of ~15mL or larger) outfitted with a piece of PEEK tubing, with the flow stopped, screw the tubing into the inflow or outflow of the isocratic pump/ degasser and push several syringe volumes of ultrapure water, hydrogen peroxide solution or isopropyl alcohol (IPA), through the LC modules (see **Figure 6**).

If isopropyl alcohol or peroxide are used, a flush with ultrapure water should follow of 30-50mL to ensure thorough removal of peroxide or IPA.

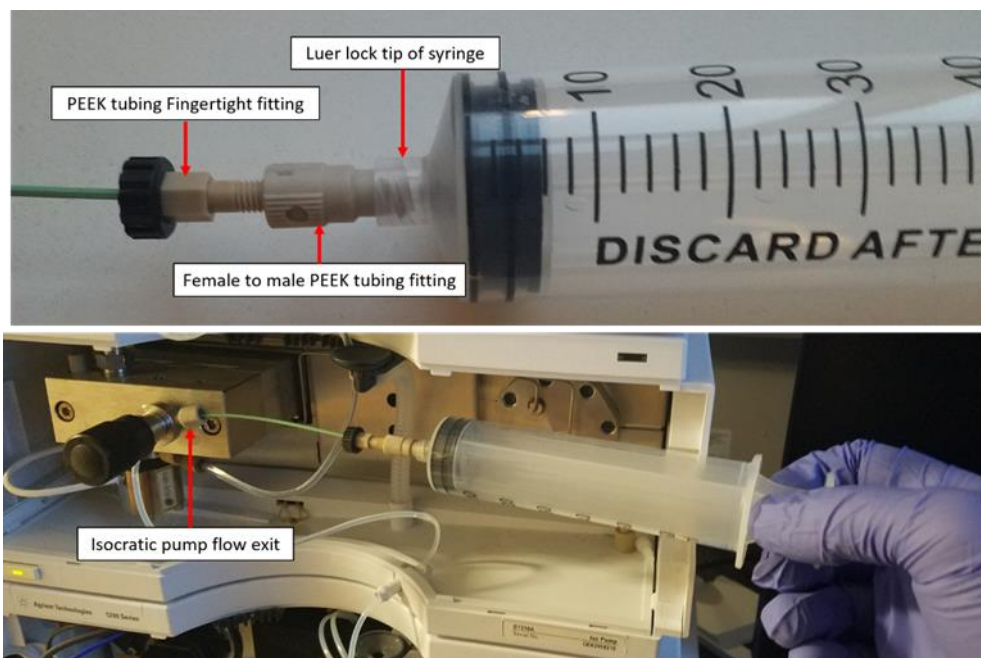


Figure 6. Pictured above is a plastic syringe with a Luer Lock tip into which peek tubing is connected (top) and shown connected to the flow exit of the isocratic pump with PEEK tubing. This configuration can be used to push solution (ultrapure water, hydrogen peroxide, or isopropanol) through the isocratic pump.

d. Individual tubing

Many of the individual pieces of tubing both between liquid chromatography modules/column, and within the M9 can be removed, thoroughly flushed (using Luer Lock syringe seen in Figure 6) or replaced in severe cases.

2. Improper M9 Acid/Oxidizer Flow Rates

Depending on the composition of mobile phase/sample, the acid/ oxidizer of the M9 may need to be adjusted. Appropriate selection of flow rates will be most impacted by the pH and buffer capacity of the mobile phase as well as TOC concentration present in samples.

Acid rate:

A low acid flow rate will lead to insufficient removal of inorganic carbon, and thus possibly an inflated TOC signal. When the M9 is operating in SEC configuration, there is no calculation and subtraction of IC during signal calculation. The instrument instead relies on efficient removal of IC before the TOC signal is measured. Thus, sufficient acid flow rate is crucial to accurate TOC measurements. While it is not possible to set the acid flow rate too high, a rate that far exceeds that which is necessary for IC removal will use up the acid at a very rapid rate increasing the rate at which it will need to be replaced. A flow rate of 5 μ L is typically enough (see "Best Practices Guide" for further discussion).

Oxidizer rate:

Low oxidizer rates will lead to a depressed TOC signal. Users may notice the TOC signal begin to rise on the front end of a peak to a certain point. Once the TOC concentration exceeds this point, the signal will then appear wavy and variable, until the TOC concentration again falls below that same point. An example is shown below in **Figure 7**.

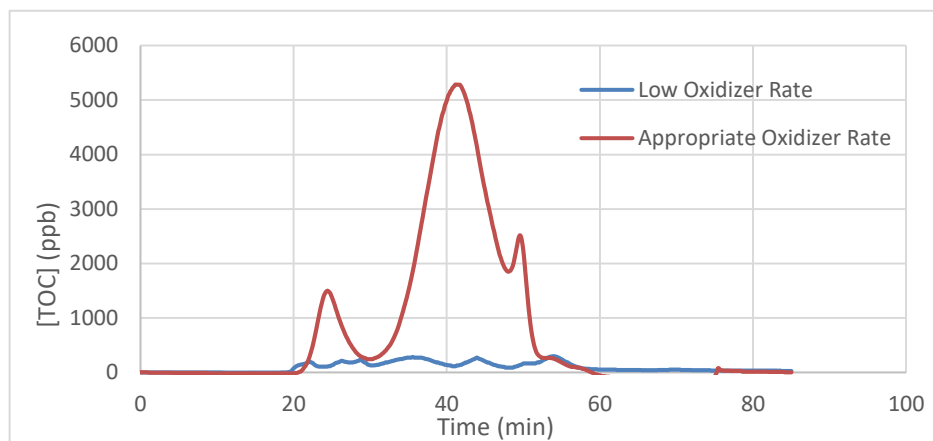


Figure 7. The figure above displays two chromatograms of the same sample from different runs. The red line represents the analysis conducted with an appropriate oxidizer rate. The blue line represents the analysis in which the oxidizer rate was too low leading to a depressed signal.

If the oxidizer rate is too great, it is possible for gas bubbles to form within the M9 tubing. Evidence of an exceedingly high oxidizer rate is a TOC signal rising during the start of a chromatogram peak to a certain level. The TOC signal may then appear to drop almost entirely (where it should be continuing to rise) and only resume when the chromatogram peak again drops to the concentration at which the signal dropped. An example of a chromatogram displaying this issue is shown in **Figure 8**. It is also possible for the signal to appear highly variable in the same region in which it drops (not pictured).

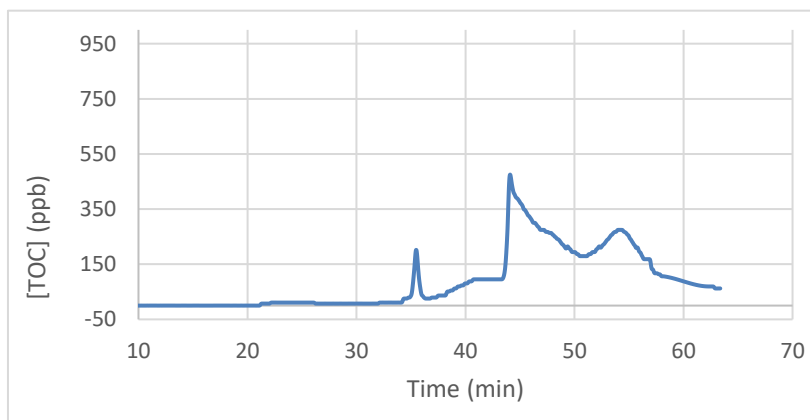


Figure 8. The figure above displays a chromatogram for which the oxidizer rate setting on the M9 was excessively high. Circled in the dashed red circle is the portion of the chromatogram in which the signal drops almost entirely as described above. This may be due to the formation of gas in the tubing of the M9 following sample oxidation.

3. Increased System Pressure

There are several causes for a buildup of pressure in the system. Clogs due to sample/ salt/ particulate build up, or microbial growth are the most common. Many of the components of the SEC-TOC system have upper pressure limits, and therefore these issues should be diagnosed and resolved before analysis continues. In the SEC-TOC system recommended by Veolia (see “Best Practices Guide”), pressure is only directly measured by the isocratic pump. To test an individual system component, begin by disconnecting the component in question (i.e. unscrew the tubing that enters the component). The initial pressure (before component was disconnected) can then be compared to that after the component is disconnected. For example, if an abnormally high pressure (more than 4 bar, compared to typical operating pressure) is suspected to originating from the column, unscrew the tubing connected to the top of the column and observe the pressure drop that occurs (see **Figure 9**).

It needs to be understood that all additional modules connected to the pump will increase pressure. Therefore, when testing a component for a clog, the pressure measured with and without a module attached needs to be compared to **typical operating conditions**. Therefore, the analyst needs to have knowledge regarding how the pressure of the system typically behaves. In the example above, if the pressure returns to the expected level (that is, the expected level with the column disconnected) after the tubing entering the column is disconnected, it is likely that the issue is originating from the column, if not, the same test should be performed on additional system components.

- If pressure originates from the column or the M9, follow the protocols outlined in the “Elevated or Depressed TOC Signal” section above for these components respectively.
- If pressure originates from the isocratic pump, additional detectors (i.e. FLD, DAD), or the degasser, please contact Agilent Analytical Technologies (or the manufacturer of the component in question) for further instruction.

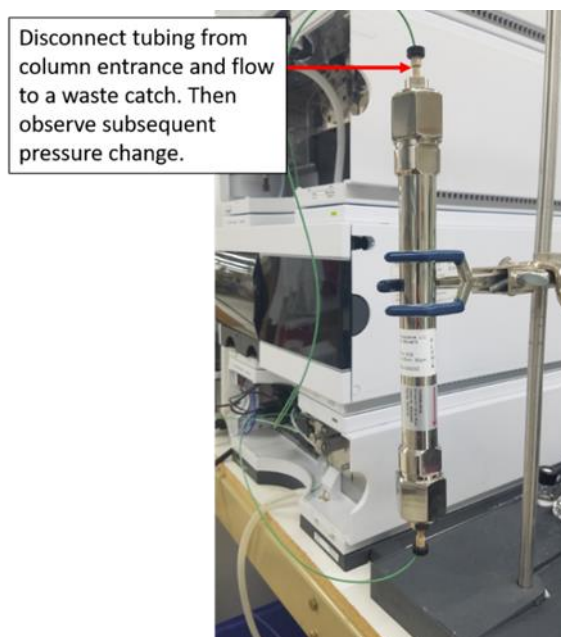


Figure 9. As described above, if a pressure increase is suspected to be originating from the column, disconnect the tubing from the column flow entrance.

4. Artifacts

It is possible for artifacts to appear in the TOC chromatogram from time to time (possible causes discussed below). The best way to tease out an artifact from the actual signal is to conduct duplicate (at minimum) runs of every sample. Artifacts are rarely repeatable and therefore easy to identify when comparing two runs of the same sample. If it is not

possible for duplicates to be analyzed, artifact peaks often show up as a very sharp peak (steeper and more abrupt than sample peaks) at unexpected times. An example is shown in the plots below (**Figure 10**). In the top plot, artifacts have been circled in red, which have been removed from the bottom plot. Programs such as MATLAB or R can be used to effectively remove artifacts and interpolate through the gap in data.

Possible causes of artifacts include (but are not limited to):

- Sample mass may become lodged in the column and elute at a later time (during a later run). This would appear as an unexpected TOC peak of varying shape and intensity.
- Small amounts of column packing material are thought to occasionally break down and elute as an unexpected TOC peak.
- Within the M9, syringes constantly inject the acid and oxidizer reagents into the sample stream (needed for the calculation of TOC by the M9). The periodic refill process of these syringes may result in sharp, periodic TOC peaks. This particular type of artifact is shown in **Figure 10**. Optimization of the acid/oxidizer selected should minimize the intensity of this type of artifact.

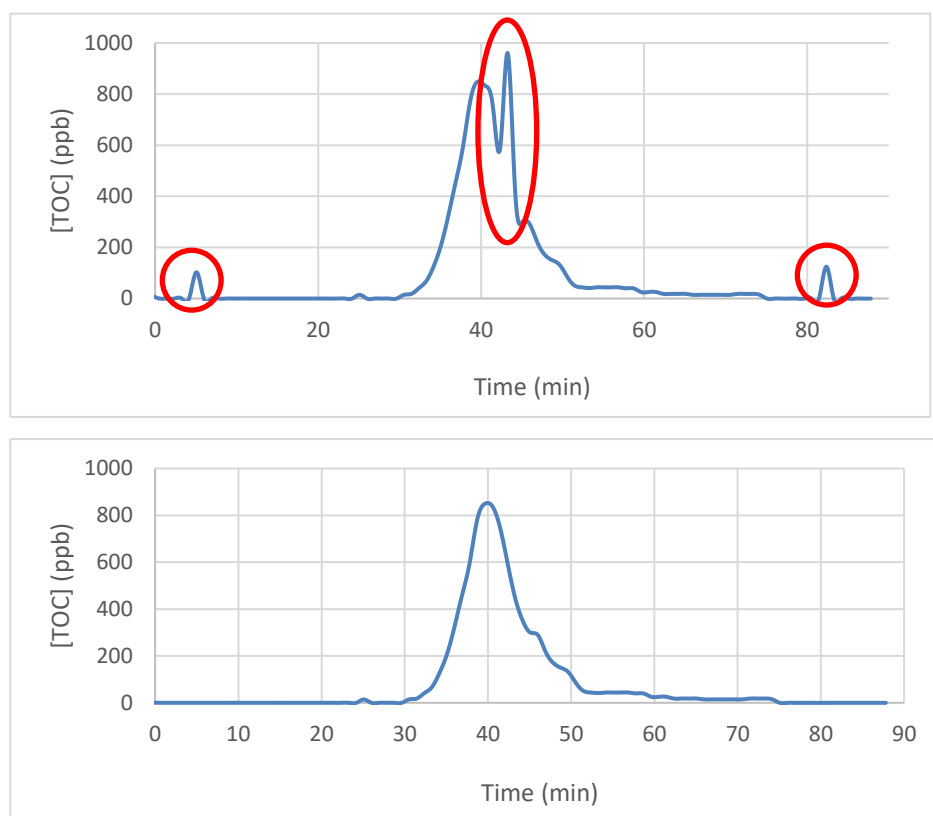


Figure 10. Chromatograms of the same analysis, before (top) and after (bottom) artifact removal.

5. Conclusion

This document is intended to provide tips for trouble shooting the specific SEC-TOC system described above (system description section). Common issues include an elevated or depressed baseline, decreased flow rates, increased pressure, and artifacts observed during analysis. While addressed individually and with respect to specific system components, some of the issues described may arise in combination and possibly because of another underlying issue. For example, if bacterial growth were to occur in the column, analysts might observe an increased system pressure (recorded by the isocratic pump), decreased flow rate exiting the column and an elevated TOC baseline measured by the M9. This list is not exhaustive about both possible issues or the potential causes. If a problem is not resolved using the tips in this guide, users are referred to the specific component manufacturers (Veolia, Agilent, or TOSOH).