



CHROMATOGRAPHIC SPECIALTIES INC.

Addressing Bacterial and Fungal Growth in HPLC Mobile Phases and Columns

To prevent or limit bacterial and fungal growth when using 100% aqueous mobile phases, always remove your column and store it in the fridge when the system is shut down overnight or longer.

For shut down, use a 1/16 union or a length of 1/16" tubing the length of the column with fittings on either end in place of the column, then pump 15% methanol 85% water through the entire system (should only take 2-3 minutes, run at elevated flow to generate some backpressure) before shutting down for the night. That much organic will prevent growth of microbes. In the morning, pump mobile phase for 2-3 minutes again at elevated flow to get say 25-50 psi back pressure, then reinstall the column. This keeps biofilms from becoming established.

Note that prevention is much easier than cure! We recommend daily maintenance rather than relying on the cleaning steps below that should be followed should a biofilm become established.

Getting rid of an established biofilm is much worse.

First – if you're going to replace any frits, seals or check valves **WAIT UNTIL AFTER FLUSHING WITH MAGIC MIX**. If this step works, you'll have chunks of dead biofilm flowing through your system. Then flush your system with 100% water. You will be using high concentrations of organic solvents, so no point in risking any precipitates.

You need to remove your column and run the line from autosampler to column to waste instead. You don't want your detector hooked up until the pump and autosampler stop releasing chunks. Next make up 40:40 or "Magic Mix". This is a solution of 40% isopropanol, 40% acetonitrile and 20% water. You'll want 200mL or so of Magic Mix so you can saturate and flush your entire system.

Start by pumping or sucking (drawing with a disposable syringe) Magic Mix through all your standard flow path up to the end of the autosampler to column line. After running 20mL through your system, let everything sit for 20-30 minutes. In this time, the Magic Mix will dehydrate the fungi / bacteria and diffuse into their cells.

After this, you have a choice. You can disconnect the solvent inlet line from your HPLC pump and draw Magic Mix through the solvent lines and degasser lines until all the biofilm is flushed out (you stop seeing black or white floaty bits coming out of your tubing). Then reattach the solvent line to the pump and go on to the next step.

If you have only a small amount of microbial growth, you can use your pump to flush Magic Mix through and clear the debris out of solvent intake lines and your degasser. The drawback is that the filters in your pump will be clogged with debris before you finish flushing your intake lines. You are going to replace them at the end of the process anyhow, but this would mean replacing filters part way through your flush, then replacing the new filters at the end of the next step.

If you flushed the inlet lines and degasser separately from the pump, now run the pump to flush it and the autosampler. When you stop seeing floaties coming out of the autosampler to column line, it's time to replace your frits, check valve, and likely the pump seals and autosampler injection valve seal.

Now you can attach your detector back in with a union or jumper tube. Pump Magic Mix through until your detector flow cell is full, then let everything sit for 20-30 minutes. After that, flush through with Magic Mix until you stop seeing floaties come out of the detector.

You can now flush with water, reinstall your column and get back to work.