

Is there any HPLC passivation procedure available for sensitive applications like e.g. analysis of some proteins, pyrophosphates etc. or for applications using ion exchange columns?

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While the inertness of surfaces in the flow path is very good and the level of elution of metal ions from Agilent HPLC systems is very low and therefore perfect for most applications, a small group of highly sensitive applications require a higher level of inertness.

The procedure described here allows preparing the system for highly demanding applications.

In principle, materials used in the flow path of any HPLC system can interact with solvents and samples in two ways: on the one hand ions or molecules can be eluted from materials inside the instrument. On the other hand, the sample can bind to the surface of these materials.

As Agilent uses high quality materials like stainless steel and resistant polymers, the extent of these effects is very small and cannot be observed for most applications. While some HPLC systems use polymers instead of stainless steel, which not only limits the applicable pressure for such systems, but target substances can be adsorbed selectively by polymer surfaces or cavities causing biased measurement results.

However there are some applications using samples (e.g. some proteins, pyrophosphates etc.) or columns (e.g. ion exchange columns) which may be sensitive to even very small amounts of metal ions. Metal complexes formed with samples or solvents may show up when using mass spectrometers for detection.

Solution/Action:

In such cases we recommend applying the subsequent passivation procedure.

1. Remove any and all columns from the system. Columns might get damaged by the passivation solution.

2. Pre-mix 200 ml of Acetonitrile with 800 ml of Water containing 1% formic acid (20% Acetonitrile + 80% Water with 1% formic acid) in a solvent bottle and put both bottle-heads A and B into the solvent bottle.

3. Use the existing stainless steel capillaries to connect the modules in the following order (see figure in at the bottom). This example utilizes the binary pump but if you are using quaternary pump repeat this procedure for other two channels C and D, and for Isocratic pump use the suggested solvents one after the other.

A. Connect the pump outlet to the thermostatted column compartment's heat exchanger inlet using the pump's outlet capillary. Make sure to connect it to the same heat exchanger used by the application, (i.e. left heat exchanger or right heat exchanger).

B. Connect the thermostatted column compartment heat exchanger outlet to the autosampler's injection valve port 1 (inlet) using the autosampler's outlet capillary. Again make sure to connect it to the same heat exchanger used by the application, (e.g. left heat exchanger or right heat exchanger).

C. Connect the autosampler's injection valve port 6 (outlet), to the detector inlet using the detector's inlet capillary.

Note: if any of the existing capillaries does not have sufficient length to fit this configuration, please add a zero dead volume union and an additional capillary to enable them to reach. This way all of the capillaries which are normally a part of the system will be cleaned by this procedure. Try to avoid using long capillaries.

4. The Autosampler needs to be in main-pass position such that the entire module is flushed.

Note: Do not perform any injections as a part of or during this procedure.

5. Set the Thermostatted Column Compartment's temperature to 80 °C for G1316A modules or to 100 °C for G1316B modules, ensuring that the temperature is set for the same heat exchanger used by the application, (e.g. left heat exchanger or right heat exchanger).

Note: Be sure to install the Thermostatted Column Compartment's front cover.

6. Using a Flow Rate of 0.4 ml/min and a composition of 50% B flush system for a minimum of 3 hours or overnight if possible.

Note: Do not allow the pump to run out of solvent and shut off by making sure that there is enough solvent to pump at the set flow rate for the desired time duration, (e.g. 0.4 ml/min. pumps 384 ml in 16 hours, 192 ml from each bottle).

7. After flushing the system as described in step 6, add 5 mmol/l phosphate (e.g. NaH_2PO_4) to the solvent mixture in both bottles.

8. Set the thermostatted column compartment's temperature to 40 °C, ensuring that the temperature is set for the same heat exchanger used by the application, (e.g. left heat exchanger or right heat exchanger).

9. Using a flow rate of 0.4 ml/min and a composition of 50% B flush system for an overnight, or if possible, over the weekend.

Note: Do not allow the pump to run out of solvent and shut off by making sure that there is enough solvent to pump at the set flow rate for the desired time duration, (e.g. 0.4 ml/min. pumps 384 ml in 16 hours, 192 ml from each bottle).

Additional Information:

Other passivation procedures have been published previously, e.g. SN 01100-086: Passivation Procedure for Agilent 1100/1200 Instruments SN 01100-016: Background Masses with HP 1100 Systems and MS Detection - Flushing Procedure. Please contact your Agilent representative for details.

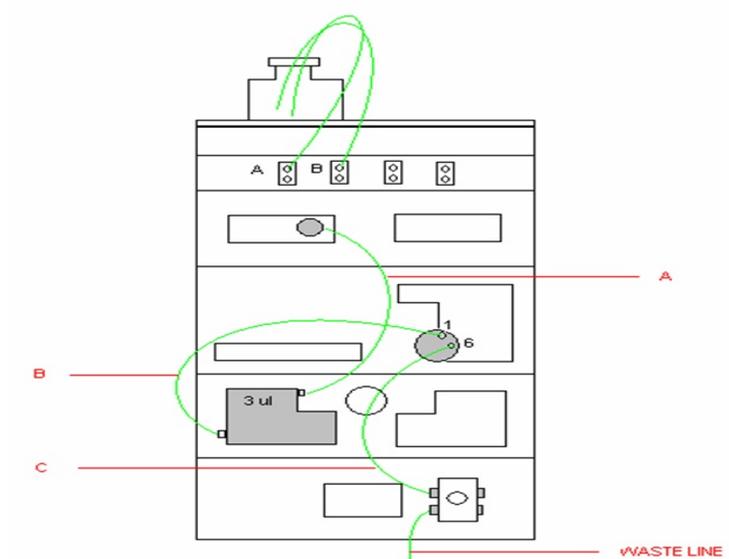
As these procedures involve the use of concentrated nitric acid 65%, they are more difficult to handle. Measurements of surface activity and ICP-MS detection of eluted metal ions have shown that results of the procedure described in this note have been superior compared to previous passivation procedures regarding most applications. First tests at many sites using sensitive applications have yielded good results which were accepted by our customers.

In case of applications which are especially sensitive on copper ions and applying this procedure does not meet customer requirements, we recommend applying the passivation procedure described in SN 01100-086. Please contact your Agilent representative for more details.

The surface activity/release of metal ions of instruments decreases over time, as the metal surface self-passivates. So

this procedure is especially useful for new systems. Systems once passivated maintain a low activity level unless treated with highly corrosive substances.

This passivation procedure is not part of the installation or other standard services.



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Printed in USA August 7, 2007