InfinityLab Deactivator Additive User Guide

The InfinityLab deactivator additive is a unique mobile phase additive that increases overall sensitivity of the instrument, reduces instrument preparation time, and increases throughput of the system. Thoroughly tested with Agilent columns, this additive will significantly improve peak shape and recovery of analytes that interact with stainless steel.

How to use the additive

1. Conduct a 0.5 % phosphoric acid wash of the system before using the additive. See 0.5 % Phosphoric acid wash section below for instructions.
2. To make up buffer solutions with organic solvents, a more concentrated buffer solution is made up in water at a specified pH first. See Example section below.
3. Add 1 mL of InfinityLab deactivator additive to 1 L of aqueous solvent.
4. Add 1 mL of InfinityLab deactivator additive to 1 L of organic solvent containing at least 10 % water and a pH level greater than 7. See Precaution 1 below.

Precautions

1. Organic solvents greater than 90 % acetonitrile and pH less than 7 are prone to solvent polymerization that can cause issues with check valves. If the additive is introduced into these conditions, the risk of solvent polymerization increases.
2. The InfinityLab deactivator additive concentration has been optimized to 5 µm. At higher additive concentrations greater than 5 µm, the additive can cause ion suppression.

Examples

1. **Creating mobile phase stock solution:**
   A 100 mM ammonium acetate stock solution is first made in water, and adjusted to pH 9 with ammonium hydroxide. Mobile phase A is made by diluting the 100 mL of stock solution with 900 mL of water. Mobile phase B is made by diluting 100 mL of stock solution with 900 mL of acetonitrile (ACN). The final concentration in both mobile phase solvents is 10 mM ammonium acetate.
2. An optimized LC/MS condition for metabolite analysis by HILIC-LC/MS is shown in Tables 1 and 2.

Table 1. Optimized LC conditions for metabolite analysis.

Column	Agilent InfinityLab Poroshell 120 HILIC-Z, PEEK-lined, 2.1 × 150 mm
Mobile phase A	10 mM ammonium acetate, pH 9.0 in water + 5 µm deactivator additive
Mobile phase B	10 mM ammonium acetate, pH 9.0 in 90 % ACN + 5 µm deactivator additive
Gradient	Time (min) %B 0 90 2 90 12 60 15 60 16 90 24 90
Flow rate	0.25 mL/min
Column temperature	30 °C
Injection volume	1 µL
Total run time	25 min

Table 2. Optimized 6545 Q-TOF MS conditions for metabolite analysis.

Ionization mode	ESI negative
Gas temperature	200 °C
Gas flow	10 L/min
Nebulizer	40 psi
Sheath gas temperature	300 °C
Sheath gas flow	12 L/min
Capillary voltage	3,000 V
Nozzle voltage	0 V
Fragmentor voltage	125 V
Skimmer voltage	65 V
Oct RF Vpp	750 V
Acquisition parameters	Data were acquired at 2 GHz extended dynamic range MS mass range: 50–1,000 *m/z*

3. Figure 1 shows representative chromatograms of metabolite standards (1 µL injection of a 5 ng/µL sample) acquired using the above method.

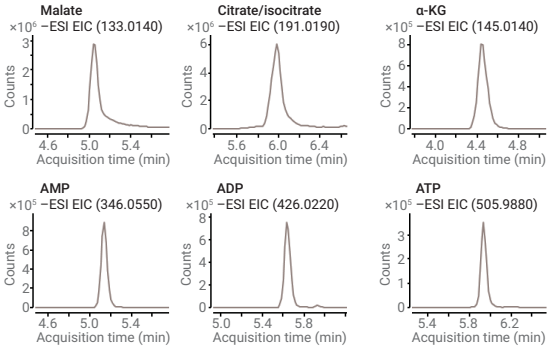

Figure 1. Representative chromatograms of metabolite standards.

4. For more information, please reference the Agilent HILIC Method Development and User Guide publication (5991-9271EN).

Storage recommendations

- The deactivator additive may be stored at room temperature.
- Opened bottles should be refrigerated to limit bacteria growth.

0.5 % Phosphoric acid wash instructions

Hands-on time: 30 minutes

Total preparation time: 3 hours + overnight (approximately 15 hours)

DO NOT run phosphate into the mass spectrometer.

1. Set the MS so that the source can be handled safely. This is normally the same setting that would be used for source cleaning. See Notes section below.
2. Change the flow rate to 0 mL/min and switch the solvent to pure water.
3. Set the system to *Purge On*, flowing directly to waste, or remove the inlet capillary from the HPLC column and place it in an appropriate waste container. See Notes section below.
4. Purge the LC pump with water at 5 mL/min for 5 minutes.
5. Set the system to *Purge Off* or stop the flow and re-attach the HILIC column.
6. Set the flow of water to 0.5 mL/min for 4.6 mm and 3.0 mm diameter columns, or 0.25 mL/min for 2.1 mm diameter columns. Run for 30 minutes through the system and column.
7. Change the flow rate to 0 mL/min and switch the solvent to 0.5 % phosphoric acid (in 90 % ACN/10 % water).
8. If using an MS or other detector with a nebulizer, detach and remove the spray needle and place it spray-end down into an appropriate waste container, then re-attach the inlet capillary. See Notes section below.
9. Set the system to *Purge On*, flowing directly to waste, or remove the inlet capillary from the HPLC column and place it in an appropriate waste container.
10. Purge the LC pump with 0.5 % phosphoric acid at 5 mL/min for 5 minutes.
11. Set the system to *Purge Off* or stop the flow and re-attach the HILIC column.
12. Set the flow of 0.5 % phosphoric acid at 0.1 mL/min and run overnight (12 hours minimum).
13. Change the flow rate to 0 mL/min and switch the solvent to pure water.
14. Set the system to *Purge On*, flowing directly to waste, or remove the inlet capillary from the HPLC column and place it in an appropriate waste container.
15. Purge with water at 5 mL/min for 5 minutes.
16. Repeat this purge with fresh water until the pH of the LC effluent from the capillary outlet has a pH >4.5.
17. Set the system to *Purge Off* or stop the flow and re-attach the HILIC column.
18. Set the flow of water to 0.5 mL/min for 4.6 mm and 3.0 mm diameter columns, or 0.25 mL/min for 2.1 mm diameter columns. Run for 1 hour through the system and column.
19. Change the flow rate to 0 mL/min and switch the solvent to the desired mobile phase.
20. Set the system to *Purge On*, flowing directly to waste, or remove the inlet capillary from the HPLC column and place it in an appropriate waste container.
21. Purge with mobile phase at 5 mL/min for 5 minutes.
22. Set the system to *Purge Off* or stop the flow and re-attach the HILIC column.
23. Set the flow of mobile phase to 0.5 mL/min for 4.6 mm and 3.0 mm diameter columns, or 0.25 mL/min for 2.1 mm diameter columns. Run for 1 hour through the system and column.
24. Reconnect nebulizer to MS and proceed with analysis.

Notes

- Contact the MS manufacturer's technical support if there is any doubt as to how to appropriately handle the source.
- An appropriate waste container should be clean, empty, compatible with the solvent, and large enough to hold the waste solvent without spillage. A large glass beaker or solvent bottle is generally recommended.
- The ESI needle, capillaries, and any other components should not be immersed in solvent or waste. Containers should be emptied regularly.
- If the nebulizer needle cannot be removed easily, the same steps can be followed with the capillary end that connects to the MS system. However, some interactions may occur within the needle.
- Passivate as much in the flow path as possible: HPLC system, capillaries, column, and detector.
- Use appropriate safety measures when handling all solvents and HPLC components.
- **DO NOT run phosphate into the mass spectrometer.**

www.agilent.com/chem/infinitylab-additive

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