



Determination of Low-Metal Release Using the Agilent 1260 Infinity II Bio-inert LC with ICP-MS

Technical Overview

Authors

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Abstract

Proteins are chelating agents, and metals such as iron are often found in protein-metal complexes. The Agilent 1260 Infinity II Bio-inert LC is based on the stainless-steel Agilent 1260 Infinity II LC, but contains completely metal-free components in the sample flow path to ascertain the integrity of biomolecules. The bio-inertness of the 1260 Infinity II Bio-inert LC was investigated by determining the metal content of different system-passing eluents using inductively-coupled-plasma mass spectrometry (ICP-MS). In comparison to the 1260 Infinity II LC, the 1260 Infinity II Bio-inert LC releases fewer metals when using acidic, basic, and salt-containing buffers.



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Introduction

Biochromatographers are faced with two challenges when using a traditional stainless-steel HPLC system:

- First, biological samples such as proteins might interact with the sample flow path, thereby leading to a lower protein yield.
- Second, the liquid chromatography system must be able to withstand harsh cleaning procedures (such as cleaning in place with HCl), which are applied to remove potential contaminations in bio-analysis.

The bio-inertness of the system is essential, for example, for the accuracy of protein recovery, as some proteins are chelating agents. For example, the metal ions Fe^{2+} and Fe^{3+} are often found in protein-metal complexes such as in iron-containing oxygen-transporting hemoglobin in the red blood cells of all vertebrates.

The Agilent 1260 Infinity II Bio-inert LC is based on the stainless steel Agilent 1260 Infinity II LC, but contains metal-free components in the sample flow path to ascertain the integrity of biomolecules. The absence of iron and steel in solvent delivery minimizes unwanted surface interactions, and increases column lifetime. All capillaries and fittings throughout the multisampler, multicolumn thermostat, and detectors are completely metal-free so that the biomolecules come in contact only with ceramic, sapphire, zircon oxide, FEP, and PEEK. In front of the sample path, the Agilent 1260 Infinity II Bio-inert Quaternary Pump is assembled using only bio-inert metals (titanium, gold, and platinum-iridium).

Due to the iron-/steel-free design, the system has a higher salt tolerance and a wider pH range (pH 1–13, short term 14) than a standard stainless steel system. Based on the proven technology of the Agilent 1260 Infinity II liquid chromatography platform, the 1260 Infinity II Bio-inert LC has the same performance specifications as the standard 1260 Infinity II LC, resulting in compatibility with standard methods¹.

To verify low-metal release from the 1260 Infinity II Bio-inert LC, different eluents were run through the system. The metal contents of the eluents were measured using inductively coupled-plasma mass-spectrometry (ICP-MS) after the system passage. In parallel, the same procedure was conducted on a 1260 Infinity II LC stainless steel system.

Experimental

The Agilent 1260 Infinity II LC consisted of the following modules:

- Agilent 1260 Infinity II Quaternary Pump (G7111B)
- Agilent 1260 Infinity II Multisampler (G7167A)
- Agilent 1260 Infinity II Multicolumn Thermostat (G7116A)
- Agilent 1260 Infinity II Diode Array Detector WR (G7115A) with standard flow cell (option #018)

The Agilent 1260 Infinity II Bio-inert LC consisted of the following modules:

- Agilent 1260 Infinity II Bio-inert Quaternary Pump (G5654A)
- Agilent 1260 Infinity II Bio-inert Multisampler (G5667A)
- Agilent 1260 Infinity II Multicolumn Thermostat (G7116A) with bio-inert heat exchanger (option #019)
- Agilent 1260 Infinity II Diode Array Detector WR (G7115A) with Bio-inert Flow Cell (option #028)

Agilent 7900x ICP-MS system

Software

Agilent MassHunter 4.3 ICP-MS

Materials

The following eluents were used:

- Double distilled water (ddH_2O)
- Acetonitrile/ ddH_2O (50:50)
- 0.1 % Trifluoroacetic (TFA) in ddH_2O
- 0.1 % Formic acid (FA) in ddH_2O
- 100 mM Sodium hydroxide (NaOH)
- Phosphate-buffered saline (150 mM sodium phosphate, 150 mM sodium chloride (NaCl))

The same bottle of each eluent was used for both systems. Blanks of all eluents were analyzed before the system was flushed with the respective eluent. All solvents used were of LC grade. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 μm membrane point-of-use cartridge (Millipak). Acetonitrile (LiChrosolv) was purchased from Merck KGaA, Darmstadt, Germany. TFA (Reagent Plus 99 %), FA (98–100 % puriss. p.a.), phosphate buffered saline tablets, and NaOH (Sodium hydroxide granulated >98 %) were purchased from Sigma-Aldrich, St. Louis, USA.

The HPLC system was flushed with each eluent for 10 minutes at a flow rate of 1 mL/min. After flushing, the mobile phase was collected in three time segments, starting from 10–20 minutes, 20–30 minutes, and 30–40 minutes, resulting in three samples per eluent per system, plus the blank sample.

Using ICP-MS (He mode), the following metals were determined in the LC system eluents:

- Titanium (Ti)
- Chromium (Cr)
- Manganese (Mn)
- Iron (Fe)
- Cobalt (Co)
- Nickel (Ni)
- Copper (Cu)
- Zinc (Zn)
- Zirconium (Zr)
- Molybdenum (Mo)

Results and Discussion

Table 1 shows the amount of metals in the eluents before (blanks) and after passing through the two different LC systems. Results are divided into three categories:

- Metals below the detection limit of the ICP-MS are marked green
- Metals with concentrations below 10 µg/L are colored in yellow
- Concentrations above 10 µg/L are marked in orange

In summary, of the six different solvents used, ACN/ddH₂O (50:50) and ddH₂O were the two solvents, which had the smallest impact concerning metal leaching out of both LC systems. No metals were detected after flushing both systems with ACN/ddH₂O (50:50). However, small amounts of manganese and nickel were found after flushing the stainless steel system with ddH₂O in contrast to the 1260 Infinity II Bio-inert LC, which is free of these metals.

Regarding acidic solvents, 0.1 % TFA had a greater impact on the metals eluting from the 1260 Infinity II LC than the Bio-inert LC. Due to the high corrosiveness of TFA, manganese and iron were detected. Additionally, small amounts of nickel and chromium were found in the acidic solvents flushed through the stainless steel system. In contrast, the acidic solvents passing the 1260 Infinity II Bio-inert were free of manganese, iron, nickel, and chromium.

Some manganese leaching can be noticed from the 1260 Infinity II LC while using phosphate buffer (PBS), which is used in many biological separation applications. Conversely, the 1260 Infinity II Bio-inert LC does not show manganese or any other metal leaching using this important buffer.

No leaching of metals, except for titanium, was observed using the 1260 Infinity II Bio-inert LC. Titanium is considered a bio-inert material with high corrosion resistance and excellent biocompatibility². The 1260 Infinity II Bio-inert LC shows excellent bio-inertness, particularly when using NaOH, 0.1 % TFA, and 0.1 % FA.

Table 1. The metal content of different eluents run through two different LC systems (Agilent 1260 Infinity II LC and Agilent 1260 Infinity II Bio-inert LC) and blanks (pure solvent before passing the LC systems).

	Blanks	Agilent 1260 Infinity II LC System	Agilent 1260 Infinity II Bio-Inert LC System
ddH ₂ O	Ti Cr Mn Fe Co Ni Cu Zn Zr Mo	Ti Cr Mn Fe Co Ni Cu Zn Zr Mo	Ti Cr Mn Fe Co Ni Cu Zn Zr Mo
ACN/ddH ₂ O	Ti Cr Mn Fe Co Ni Cu Zn Zr Mo	Ti Cr Mn Fe Co Ni Cu Zn Zr Mo	Ti Cr Mn Fe Co Ni Cu Zn Zr Mo
0.1 % FA	Ti Cr Mn Fe Co Ni Cu Zn Zr Mo	Ti Cr Mn Fe Co Ni Cu Zn Zr Mo	Ti Cr Mn Fe Co Ni Cu Zn Zr Mo
0.1 % TFA	Ti Cr Mn Fe Co Ni Cu Zn Zr Mo	Ti Cr Mn Fe Co Ni Cu Zn Zr Mo	Ti Cr Mn Fe Co Ni Cu Zn Zr Mo
100 mM NaOH	Ti Cr Mn Fe Co Ni Cu Zn Zr Mo	Ti Cr Mn Fe Co Ni Cu Zn Zr Mo	Ti Cr Mn Fe Co Ni Cu Zn Zr Mo
Buffer (150 mM NaPO ₄ , 150 mM NaCl)	Ti Cr Mn Fe Co Ni Cu Zn Zr Mo	Ti Cr Mn Fe Co Ni Cu Zn Zr Mo	Ti Cr Mn Fe Co Ni Cu Zn Zr Mo

< Detection limit
 <10 µg/L
 >10 µg/L

Conclusion

The Agilent 1260 Infinity II Bio-inert LC releases an almost negligible amount of metals when flushed with acidic, basic, and salt-containing buffers. It has a significantly lower release compared to the Agilent 1260 Infinity II LC, which contains stainless steel. We conclude that the 1260 Infinity II Bio-inert LC is highly recommended for bio-inert UHPLC applications.

References

1. Schneider, S.; Schweikert, U. Performance Characteristics of the Agilent 1260 Infinity II Bio-Inert LC, Agilent Technologies Technical Overview, publication number 5991-7980EN, **2017**.
2. Tschernitschek, H.; Borchers, L.; Geurtsen, W. Nonalloyed titanium as a bioinert metal - a review. *Quintessence International* **2005**, *36(7-8)*, 523-530.

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© Agilent Technologies, Inc., 2017
Published in the USA, August 1, 2017
5991-8314EN



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