



Proof of Performance

# Determination of low-metal release from the Agilent 1260 Infinity Bio-inert Quaternary LC system using ICP-MS

Technical Overview

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## Abstract

Proteins are chelating agents and especially iron is often found in protein-metal complexes. The Agilent 1260 Infinity Bio-inert Quaternary LC system is based on the stainless-steel Agilent 1260 Infinity Quaternary LC system, but comprises new metal-free components in the sample flow-path to ascertain the integrity of bio-molecules. The bio-inertness of the Agilent 1260 Infinity Bio-inert Quaternary LC system was investigated by determining the metal content of different, system passing eluents using inductively-coupled-plasma mass-spectrometry (ICP-MS). The results were compared with the Agilent 1260 Infinity LC system and with a bio-inert system from another vendor. The Agilent 1260 Infinity Bio-inert Quaternary LC system releases less metals using acidic, basic, and salt containing buffers compared to the Agilent 1260 Infinity LC system and to the bio-inert LC system from the other vendor.



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## Introduction

Bio-chromatographers 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 and, therefore, lead 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.

As some proteins are chelating agents, bio-inertness of the system is essential for the accuracy of, for example, protein recovery. Especially the metal ions  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$  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 Bio-inert Quaternary LC system is based on the stainless-steel Agilent 1260 Infinity Quaternary LC system, but it comprises new metal-free components in the sample flow-path to ascertain the integrity of bio-molecules. The absence of iron and steel in solvent delivery minimizes unwanted surface interactions and increases column lifetime. All capillaries and fittings throughout the autosampler, column compartment, and detectors are completely metal-free so that the bio-molecules come in contact only with ceramics or PEEK. In front of the sample path, the Agilent 1260 Infinity Bio-inert Quaternary Pump is assembled using only bio-inert metals (titanium, gold, and platinum-iridium).

Due to the iron and steel-free design, the system has a higher salt tolerance and a wider pH range (1–13, short term 14) than a standard system. Based on the proven technology of the Agilent 1200 Infinity Series liquid chromatography platform, the 1260 Infinity Bio-inert Quaternary LC has the same performance specifications as the standard 1260 Infinity LC system – resulting in compatibility with standard methods<sup>1</sup>.

To verify low-metal release from the 1260 Infinity Bio-inert Quaternary LC, different eluents were run through the system and the metal content of the eluents was measured by ICP-MS after the system passage. In parallel, the same procedure was conducted on a 1260 Infinity LC stainless steel system and a bio-inert LC system from another vendor.

## Experimental

### Systems

1. Agilent 1260 Infinity LC system consisting of:
  - Agilent 1260 Infinity Binary Pump (G1312B)
  - Agilent 1260 Infinity High performance Autosampler (G1392B)
  - Agilent 1260 Infinity DAD (G4212B)
  - Agilent 1290 Infinity Thermostatted Column Compartment (G1316A)
2. Agilent 1260 Infinity Bio-inert Quaternary LC system consisting of:
  - Agilent 1260 Infinity Bio-inert Quaternary Pump (G5611A)
  - Agilent 1260 Infinity Bio-inert High performance Autosampler (G5667A)
  - Agilent 1260 Infinity DAD (G4212B)
  - Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
3. Bio-inert LC system from other vendor
4. Agilent 7700x ICP MS system

### Software

Agilent MassHunter ICP-MS, B 1.02

### Eluents

- Double-distilled water ( $\text{ddH}_2\text{O}$ )
- Acetonitrile/ $\text{ddH}_2\text{O} \rightarrow 50:50$
- 0.1% Trifluoroacetic acid (TFA) in  $\text{ddH}_2\text{O}$
- 0.1% Formic acid (FA) in  $\text{ddH}_2\text{O}$
- 100 mM Sodium hydroxide (NaOH)
- Phosphate buffer (150 mM sodium phosphate, 150 mM sodium chloride (NaCl))

The same bottle of each eluent was used for all systems, and blanks of all eluents were analyzed before the system was flushed with the respective eluent. All solvents used were 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) and NaCl were purchased from Merck KGaA, Darmstadt, Germany. TFA (Reagent Plus 99%), FA (98% to 100% puriss. p.a.), NaOH (Standard Solution, 1M), and sodium mono- and diphosphate (99%) 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. Then the mobile phase was collected from 10–20 minutes, 20–30 minutes, and from 30–40 minutes, resulting in three samples per eluent, per system, plus blank.

With ICP-MS, the following metals were determined in the different eluents:

- Titanium
- Chromium
- Manganese
- Iron
- Cobalt
- Nickel
- Copper
- Zinc
- Zirconium
- Molybdenum

## Results and Discussion

Table 1 shows the amount of metals in the eluents before (→ blanks) and after flowing through the three different LC systems used. The blank amounts are already subtracted in the results.

Summing up the results of the six different solvents used, ddH<sub>2</sub>O and 50% ACN were the two solvents with the smallest impact concerning metal leaching out of the LC system. Small amounts of iron and manganese were detected in the eluents, which had been running through an Agilent 1260 Infinity LC system. Chromium, copper, and nickel were found in the bio-inert system from the other vendor.

	Blanks	Agilent 1260 Infinity LC	Bio-inert system from other vendor	Agilent 1260 Infinity Bio-inert LC
H <sub>2</sub> O <sub>dd</sub>	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	Ti Cr Mn Fe Co Ni Cu Zn Zr Mo
ACN/ H <sub>2</sub> O <sub>dd</sub>	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	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	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	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	Ti Cr Mn Fe Co Ni Cu Zn Zr Mo
Buffer (150 mM NaPO <sub>4</sub> , 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	Ti Cr Mn Fe Co Ni Cu Zn Zr Mo

**Table 1**  
Metal content of different eluents, run through three different LC systems (Agilent 1260 Infinity LC, Agilent 1260 Infinity Bio-inert LC, and a bio-inert system from another vendor). The blank amounts are already subtracted in the results.

Regarding acidic buffers, 0.1% TFA had a greater impact on the metals eluting from the systems. More and higher amounts of metals could be detected in these eluents. Iron is, in marginal amounts (0.3%), part of the titanium alloy used in the Agilent 1260 Infinity Bio-inert Quaternary LC system. Due to the highly corrosive abilities of TFA, small amounts of iron were found in the eluents passing the 1260 Infinity Bio-inert Quaternary LC system and also in the bio-inert system from the other vendor. However, the amount of iron, found in the Agilent 1260 Infinity LC system, was 10 times higher. Titanium was found in both bio-inert systems in higher amounts after acidic, basic, and salt-containing eluents, due to the high content of titanium, used in the pump modules. Though, titanium is considered a bio-inert material with high corrosion resistance and excellent biocompatibility.<sup>2</sup>

When chemicals, for example, salts, are part of the eluents, the blanks revealed already certain amounts of metals, particularly iron. Especially the use of sodium phosphate and sodium chloride made ICP-MS measurements difficult due to the high metal content of the salts.

## Conclusion

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

## References

1. Performance characteristics of the 1260 Infinity Bio-inert Quaternary LC system, Technical Overview, Publication number, 5990-9194EN, 2011.
2. H. Tschernitschek, L. Borchers, W. Geurtsen, W. (2005). Nonalloyed titanium as a bioinert metal—a review. Quintessence International 36 (7–8), 523-530.

[www.agilent.com/chem/bio-inert](http://www.agilent.com/chem/bio-inert)

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