

Robustness and Metal Release Testing of the New Agilent 1260 Infinity Bio-inert LC with Agilent 7700 ICP-MS

Nicole Fellner, Jochen Strassner, Katja Kornetzky
Agilent Technologies, R&D and Marketing GmbH, Hewlett Packard-Str. 8,
76337 Waldbronn, Germany

Hyphenated separations: LC-MS,
CE-MS, and LC-NMR
P1-G-183-M0



Introduction

In Bio-Chromatography it is crucial that the chromatography system has lowest interaction with protein samples demanding it to be mostly metal free. Furthermore, it must convey a high robustness in order to withstand harsh cleaning procedures.

We therefore stressed the Agilent 1260 Infinity Bio-inert Quaternary LC System under extreme conditions and subsequently determined metal content and evaluated pump performance.

Conclusions

Three stress experiments prove that the Agilent 1260 Infinity Bio-inert LC System displays even under harsh conditions no metal leaching or impairment of parts, showing it is ideal for Bio-Chromatography.

Furthermore, its low surface activity and low metal content make it an attractive front-end for ICP-MS.

Results and Discussion

The 1260 Infinity Bio-inert Quaternary LC is a fully inert UHPLC system. It consists of a titanium based pump and a metal-free sample flow path comprising of ceramic and inert polymers. Metal clad PEEK capillaries enable chromatography up to 600 bar.

Figure 1 depicts three experiments that were defined in order to show high corrosion resistance, Bio-inertness and robustness of the 1200 Infinity Bio-inert LC system.

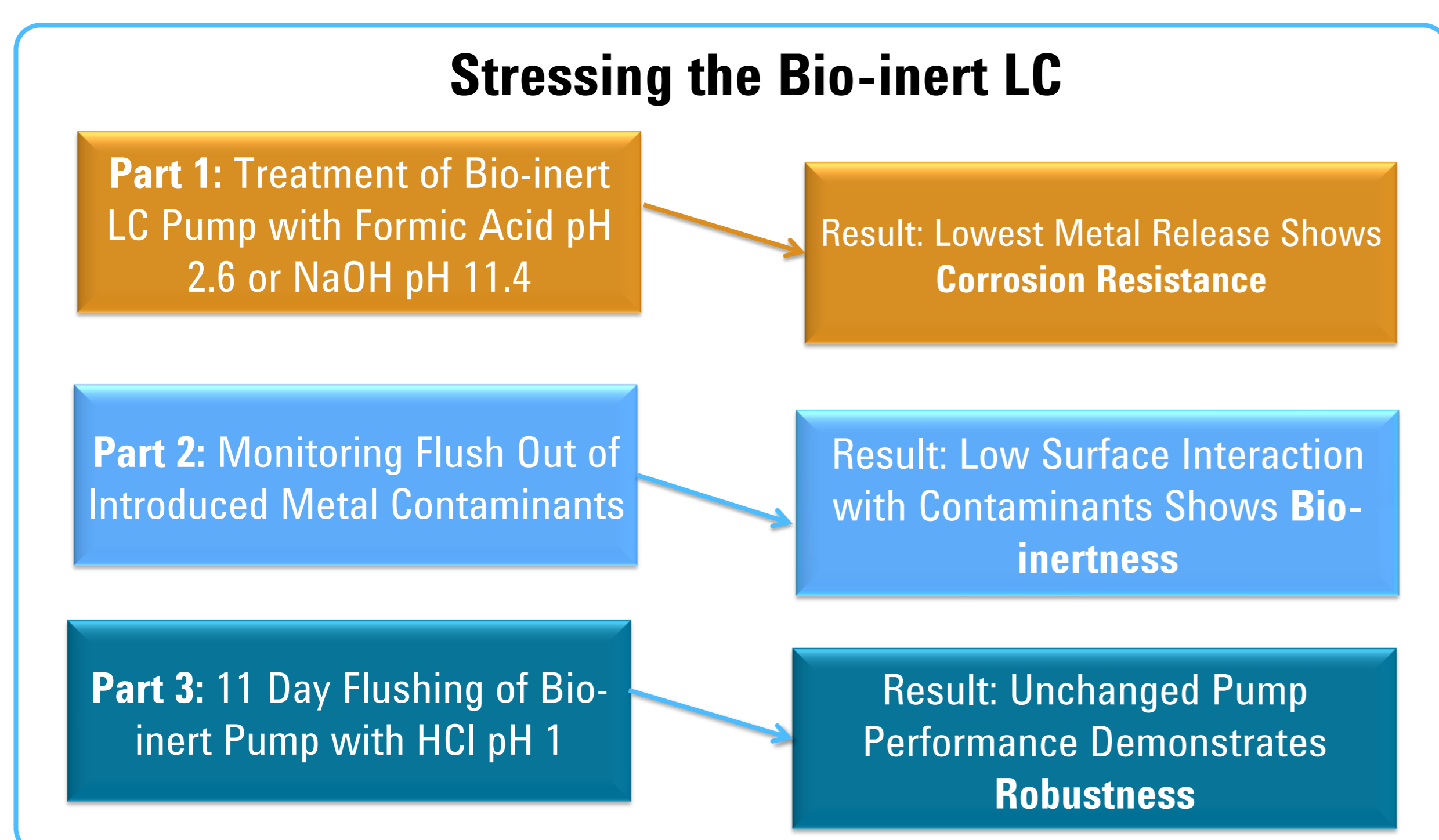


Figure 1. Three experiments were conducted showing corrosion resistance, Bio-inertness and robustness of the Bio-inert LC

Part 1: Corrosion Resistance Test

To check for corrosion resistance we flushed the pump of the Bio-inert LC with NaOH pH 11.4 and Formic Acid pH 2.6 for 30 minutes. Then we took three fractions of 10 ml each over 30 min and determined the metal content with the Agilent 7700 ICP-MS¹. These data were then compared to data obtained after treatment of a stainless steel Agilent 1100 series LC system (Figure 2). The Bio-inert LC's low metal release (<1 µg/L) proves the corrosion resistance of the Bio-inert LC.

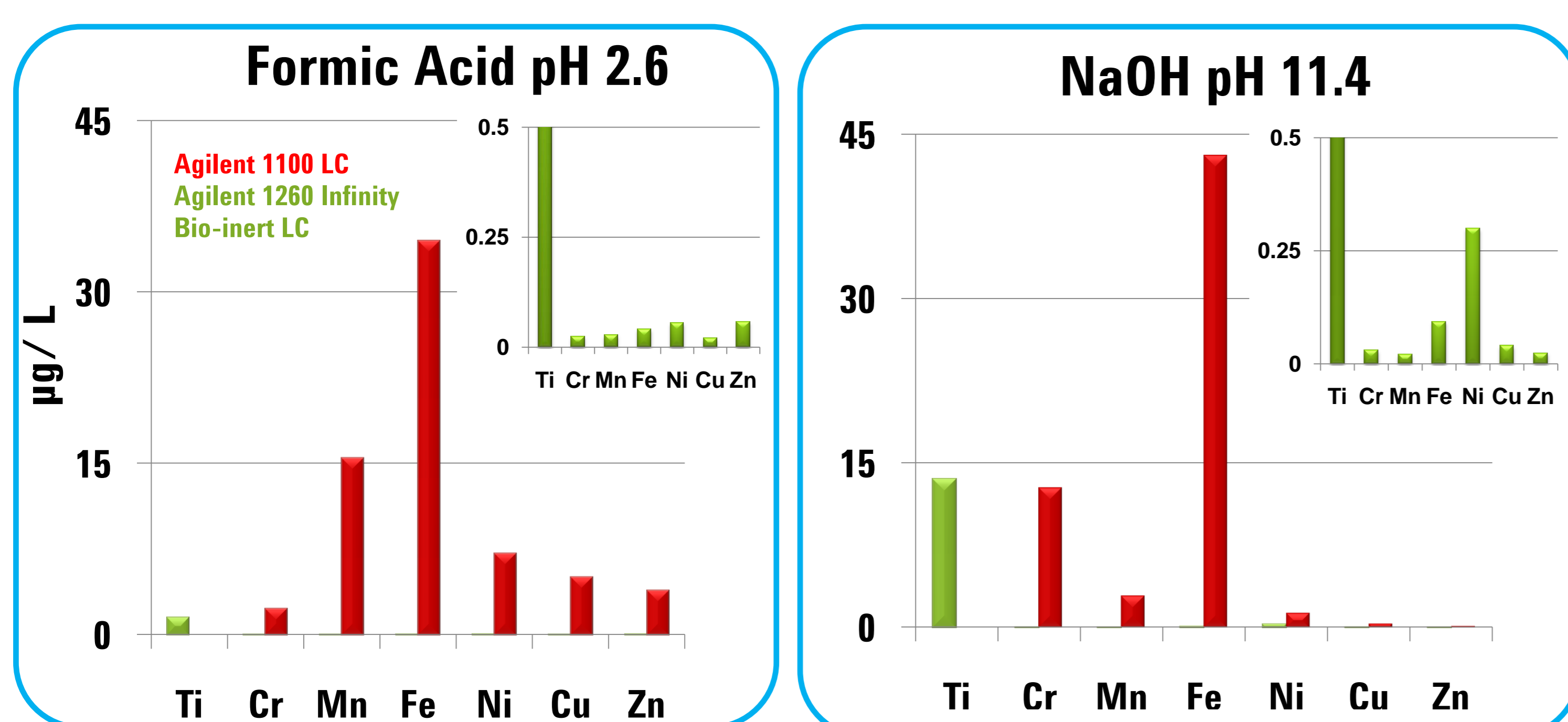


Figure 2. Metal release of the Bio-inert LC pump (range of the green bar) is extremely low. This is confirmed when compared to an Agilent 1100 series stainless steel LC (red bars)

Part 2: Bio-inertness Test

In this experiment we introduced metal contaminants (Ti, Fe, Ni, Cu, Zn) to the Bio-inert LC pump by flushing the system with formic acid which had previously washed out metal from a stainless steel system. By determining metal content with ICP-MS after one, two and four hours we monitored how quickly those were flushed out. Already after one hour metal content was reduced to one fifth, demonstrating lowest surface activity and hence system inertness (Figure 3).

Wash Out of Metal Contaminants in 0.1% Formic Acid pH 2.6 of a Bio-inert LC Pump

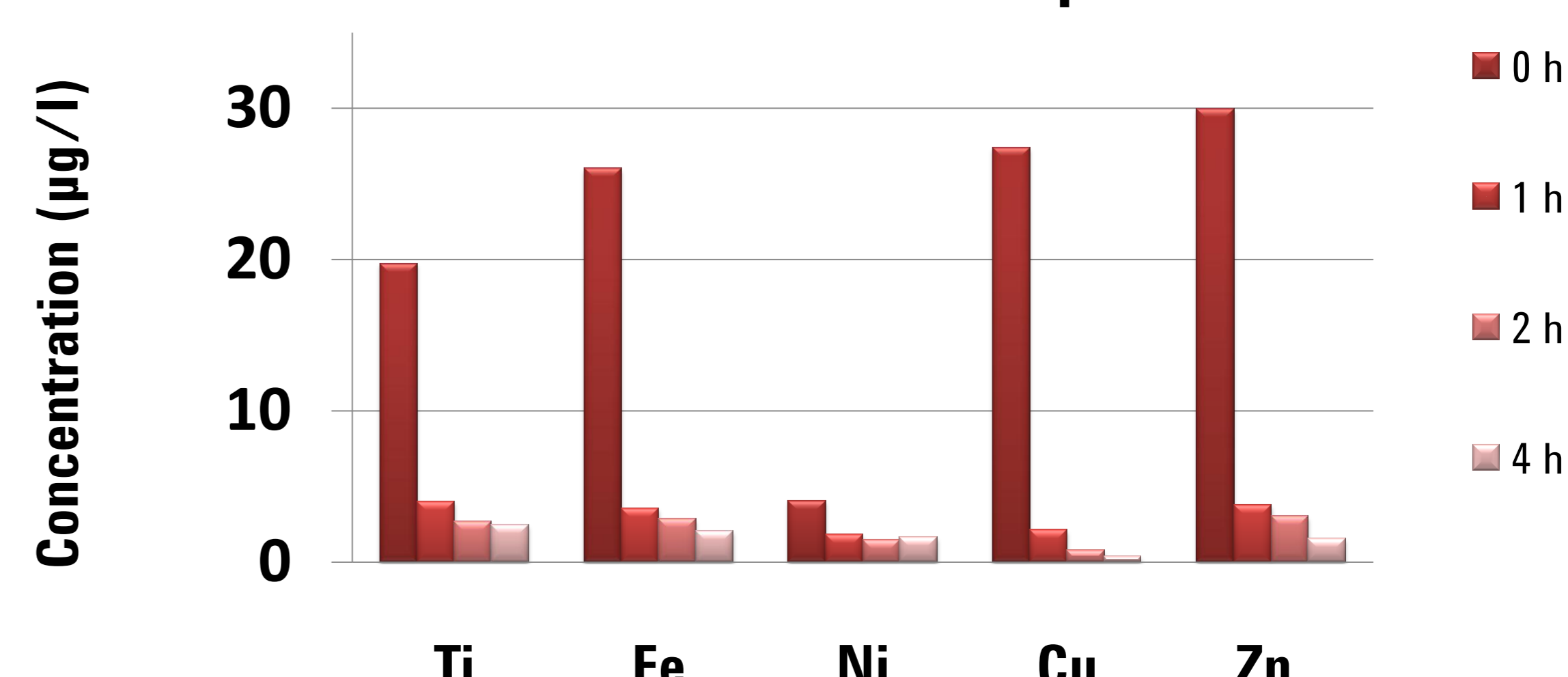


Figure 3. Fast wash out of metal contaminants from the Bio-inert LC pump

Part 3: Robustness Test

To simulate Cleaning in Place Procedures, the Bio-inert pump was flushed for 11 days with 0.1 M HCl (pH 1). For measurement the pump outlet was blocked and the flow rate was subsequently lowered from 3 µL to 1 µL/min. Even at lowest flow of 1 µL/min the pressure still rises which demonstrates absolute tightness of the pump seals (Figure 4). Also a composition accuracy test revealed that performance is the same as a non-HCl treated Bio-inert pump (data not shown). Results make evident that even after a strenuous treatment the pump still exhibits its reliable performance.

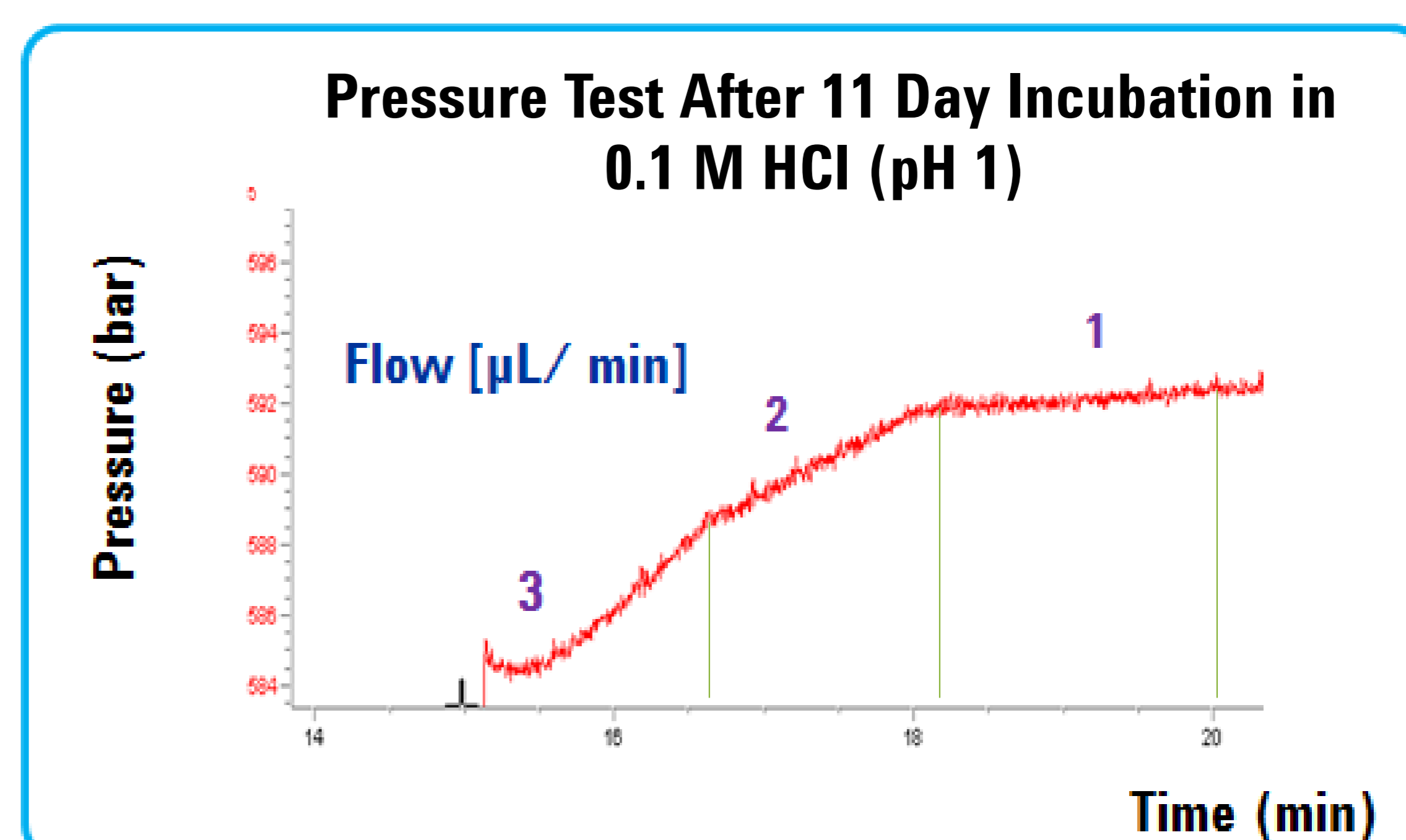


Figure 4. Bio-inert pump pressure profile showing unchanged performance of the Bio-inert LC pump after treatment with HCl pH 1 for 11 days

References:

¹ Fellner, N., Strassner, J.: Metal Release Analysis of the New 1260 Infinity Bio-inert HPLC System by 7700 ICP-MS