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Biocompatibility in (U)HPLC Technology

Material choices and their impact on Bioanalytical Performance

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Table of contents

1. Overview

Biocompatibility is a crucial factor in UHPLC systems when dealing with bioanalysis. While stainless steel components are commonly used in UHPLC systems due to their durability and cost-effectiveness, they pose significant challenges for certain bioanalytical applications. Issues like corrosion under high salt concentrations and unwanted interactions with samples such as proteins, highlight the need for materials that do not affect the application they are part of, referred to as a biocompatible system.

Understanding Biocompatibility in UHPLC Systems

Biocompatibility has two main aspects: general inertness and analyte-specific inertness. General inertness refers to the chemical resistance of the system against the mobile phase, sample solution, and wash solution. Materials like titanium, known for their higher corrosion resistance compared to stainless steel, are preferred in harsh conditions involving high salt or buffer concentrations across a wide pH range.

Analyte-specific inertness, the primary focus of this research, pertains to preventing the UHPLC system from influencing the analyte. Key concerns include the adsorption of analytes to stainless steel and the leaching of metal ions. Adsorption and leaching can lead to various analytical issues, such as increased retention time, peak tailing, carry-over, reduced peak height, lower analyte recovery, and poor reproducibility.

> **This article provides insight into biocompatibility based on various analytical experiments to show the importance of using the right material for your application.**

Adsorption effects

To measure the effects of a biocompatible PEEK flow path compared to a stainless steel flow path, analytical tests are performed. These tests will indicate the adsorption effect of a biomolecule to the flow paths. To perform these tests, the nucleotide Adenosine-5′-triphosphate (ATP) will be used. As a reference sample, known for its inert behavior towards stainless steel and PEEK, uracil will be used.

To monitor the adsorption effects, several injections will be carried out using different flow paths. The first test to measure these adsorption effects, is to look at the analyte recovery in the chromatograms. When the ATP molecules flow through the stainless steel tubing, the tubing will adsorb some of the particles, leading in analyte recovery loss in the first peak. However, as more injections will be performed, the less active spots (spots that do not have ATP adsorbed yet) will be present. The expectation is, that with every injection, the analyte recovery will be higher until the tubing is completely saturated with ATP.

With biocompatible flow paths made from PEEK, the analyte recovery is expected to be reproducible during the entire run. To test this, 30 injections will take place using ATP using stainless steel and a biocompatible material, PEEK. A subsequent test will be performed using uracil, a component that does not interact with any material used during these experiments. The results are shown in the graphs below.

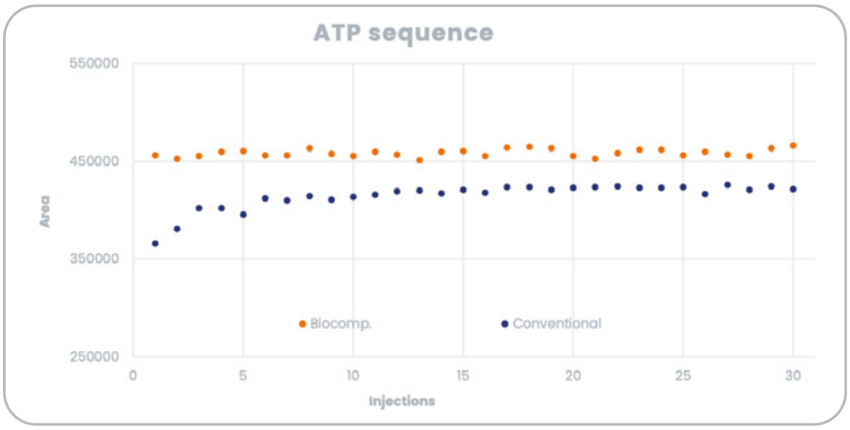


Figure 1. 30 consecutive injections of ATP using a PEEK- & Stainless Steel flow path.

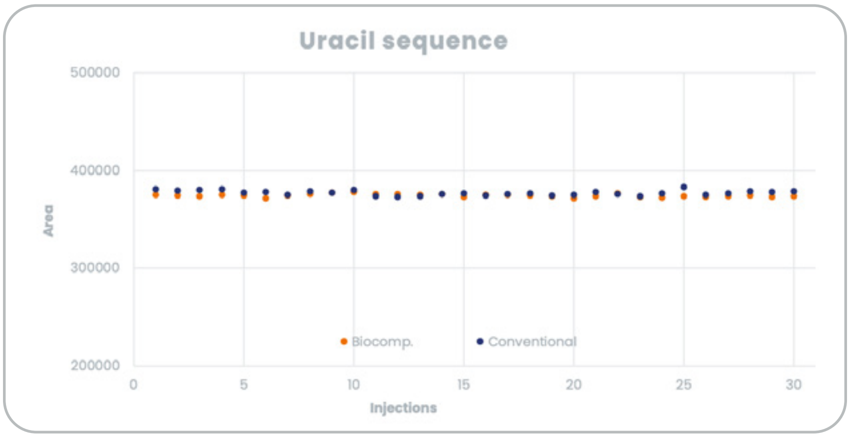


Figure 2. 30 consecutive injections of Uracil using a PEEK- & Stainless Steel flow path.

When looking at figure 1, a clear difference is observed between the two flow paths. The PEEK flow path shows a good replicable result. The stainless steel flow path on the other hand shows that a minimum of 10 injections are needed before an equilibrium is reached. Also a difference in maximum analyte recovery can be seen in this figure, even though the same sample was used. This phenomenon is not evident when an Uracil sample is used (figure 2). This implies that the loss in analyte recovery is caused by the interaction between ATP and stainless steel, and not the difference in flow path.

Figures 3 and 4 show the chromatogram overlays of the first injections for this experiment. These figures show a difference in analyte recovery between PEEK and stainless steel for ATP injections. The stainless steel flow path shows a 32% sensitivity loss compared to the biocompatible flow path. The uracil, however, shows no difference in peak shape between the two flow paths.

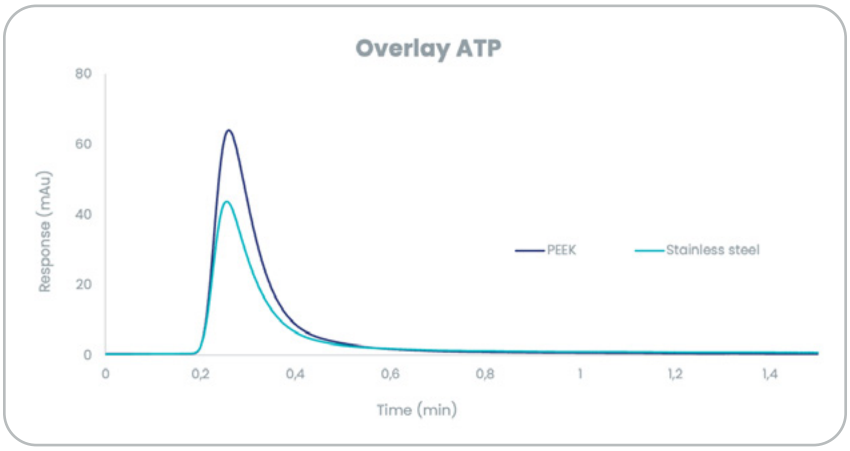


Figure 3. Peak overlay of the first injection of ATP

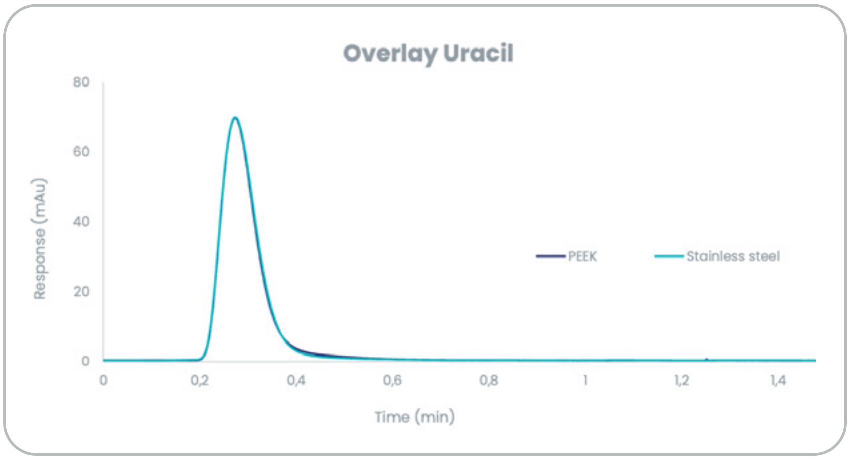


Figure 4. Peak overlay of the first injection of Uracil

To further investigate the influence of stainless steel on bioanalysis, an additional piece of stainless steel tubing is added to the flow path; doubling the contact area for the analyte. By increasing the contact area, the more active spots are present in the flow path, which would lead to even more analyte loss. The results of this experiment are shown in the figures below.

These results show that it not only takes longer to reach maximum analyte recovery, but also the maximum analyte recovery is lower. Uracil however still shows a stable analyte recovery, even while the length of the flow path has doubled.

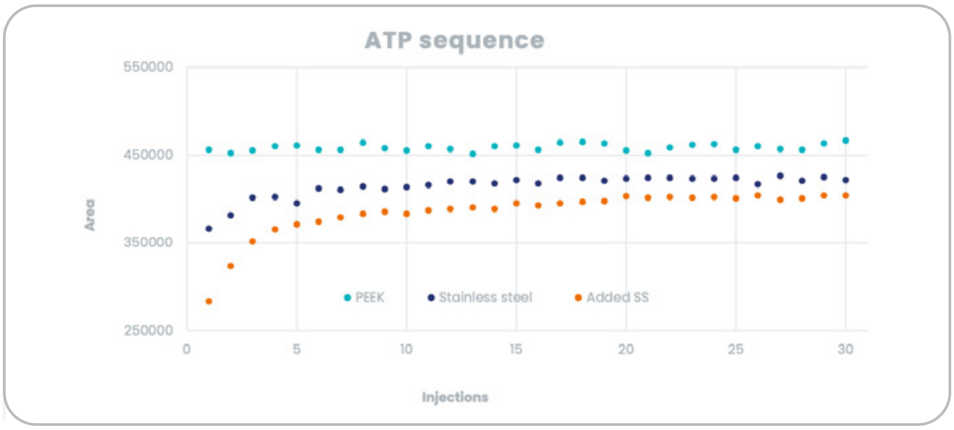


Figure 5. 30 consecutive injections of ATP using a PEEK-, Steel, and doubled (added) stainless steel length flow path

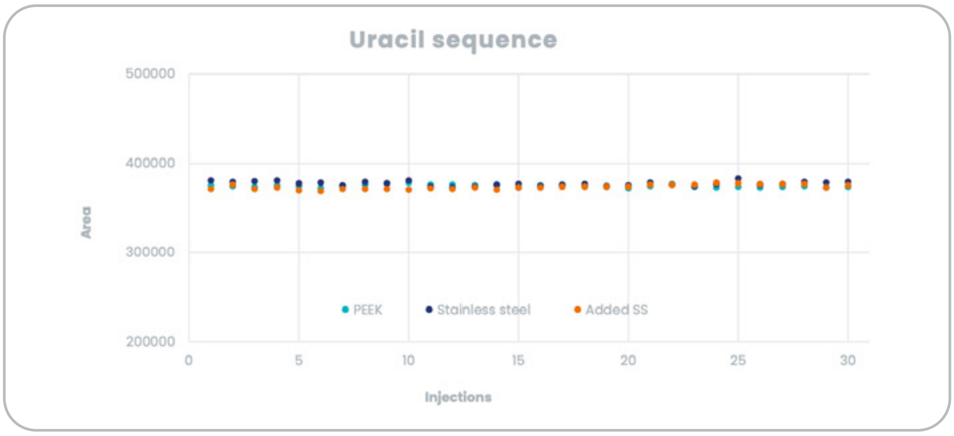


Figure 6. 30 consecutive injections of Uracil using a PEEK-, Steel, and doubled (added) stainless steel length flow path

When looking at the peak shape of the first injections of this experiment, a 52% loss in sensitivity can be measured between a biocompatible (PEEK) flow path and the stainless steel flow path with the additional tubing. This flow path does however not differ in peak shape when using uracil, implying that the loss in analyte recovery is due to the adsorption of ATP to the stainless steel tubing in the flow path.

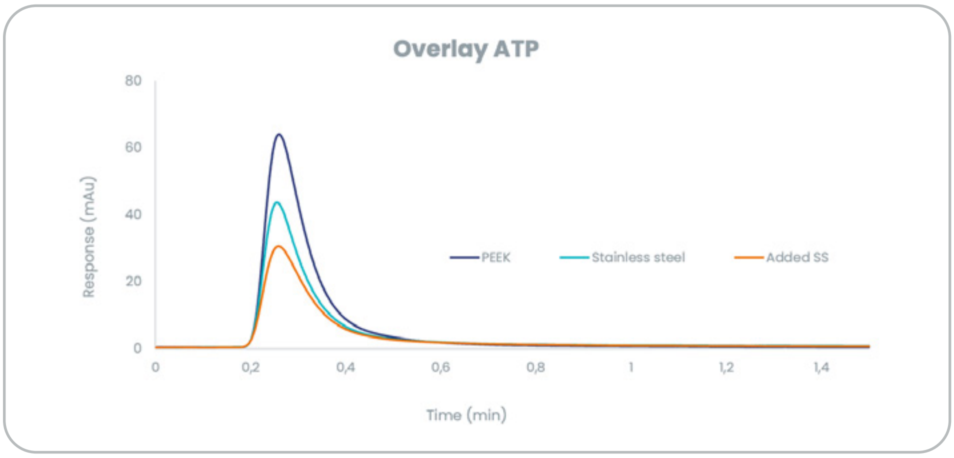


Figure 7. Peak overlay of the first injection of ATP, with added stainless steel

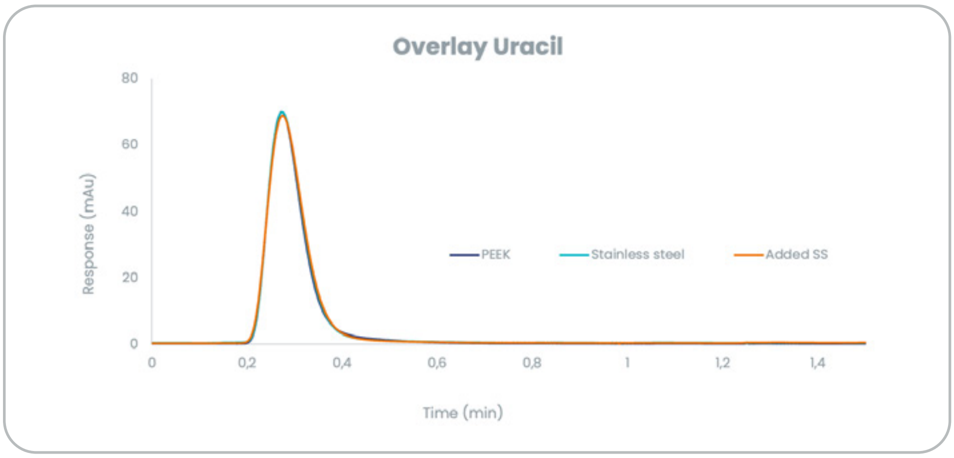


Figure 8. Peak overlay of the first injection of Uracil, with added stainless steel

Carry over

When using a stainless steel flow path for bioanalysis, the adsorption effects of the analyte can lead to carry over in subsequent analyses. The carry over is measured on both the biocompatible PEEK flow path as the stainless steel flow path, using ATP as the analyte. These carry over experiments were conducted by injecting a highly concentrated ATP sample (20.000 ppm), followed by a blank sample. The results of these experiments are shown in the graphs below.

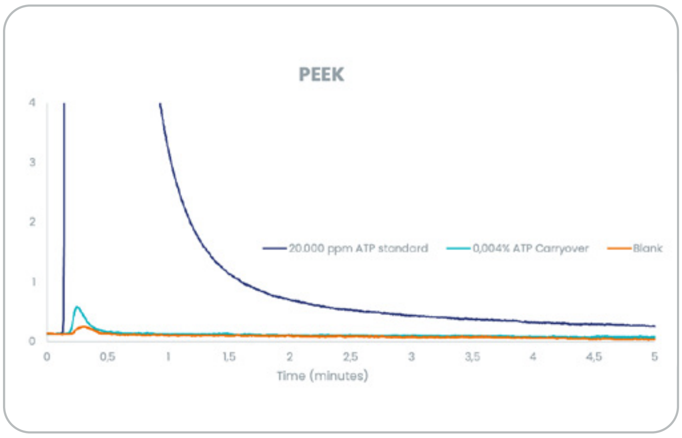


Figure 9. Carry over results PEEK flowpath

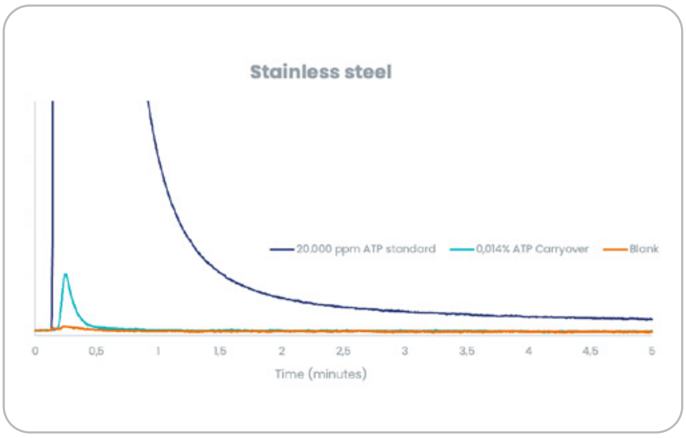


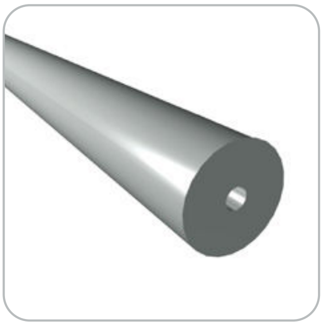
Figure 10. Carry over results SS flowpath

These graphs show that the usage of the stainless steel flow path with bioanalysis results in 3.5 times more carry over compared to a biocompatible PEEK flow path.

2. Alternative materials

While PEEK has served as one of the most chosen biocompatible options for many LC applications, it has some physical limitations; a maximum pressure rating of 480 bar, PEEK is well-suited for HPLC but falls short for UHPLC. This means that there is a need for other alternatives, not only biocompatible but also able to withstand these ultra-high pressures.

Currently, various alternatives to typical stainless steel flow paths are available on the market for bioanalysis. This chapter looks into these materials and their advantages and disadvantages.



Metal alternatives

Because of the fact that metal components generally are more resistant to high-pressures, metal components are often looked at for the replacement of stainless steel flow paths. This section looks at often used metal-based alternatives for stainless steel.

Titanium

Titanium is a biocompatible material used in (U)HPLC, but has some physical limitations. Because it becomes very brittle when used into capillary tubing, titanium is mainly used for larger components, such as pump heads. Besides this limitation, titanium-ions can leach in to the mobile phase, when a high concentration of organic solvents (such as methanol and acetonitrile) are used. These leached ions can attach to the stationary phase which can interact with the sample.

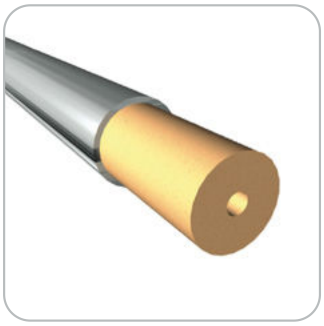
MP35N

Another biocompatible metal that is often used is the cobalt based Co–35Ni–20Cr–10Mo alloy, also known as MP35N. MP35N was originally developed for aerospace fasteners but is also used in biomedical engineering due to the high strength and biocompatibility.

MP35N has an increased corrosion resistance, because of the formation of an oxide layer. The oxide layer formed on MP35N shows a high resistance to salt water, chloride-containing solutions, and inorganic acids. This makes this metal more corrosion resistant (general inert) compared to stainless steel. MP35N does however has some downsides. MP35N is a hard and strong material. This makes it more challenging to manufacture in to capillary tubing, leading to a more expensive material.

The other downside is that MP35N is made out of an iron alloy. This means the flow path may be biocompatible, but not iron-free. The iron ions can leach into the mobile phase and analyte adsorption can still occur. These effects are however minimal and are mostly induced by iron in the material. MP35N has a maximum iron percentage of 1%, compared to the 69% iron in stainless steel.

This means that these effects are minimal for MP35N. MP35N has therefore gained acceptance as an additional biocompatible metal-based alternative to stainless steel in liquid chromatography.

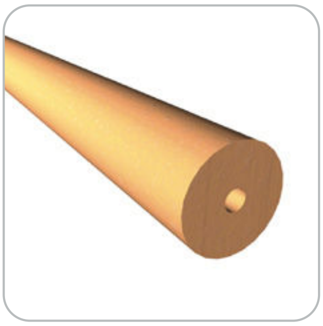


PEEK

Polyetheretherketone, also known as PEEK, is a high-strength biocompatible polymer. PEEK was developed around the late 1990s, to replace metal implants in orthopedics and trauma. Several studies have shown that PEEK is biocompatible and bioinert and is chemically inert due to its hydrophobic surface. The benefit of this hydrophobic surface is that there is no protein adsorption, which is one of the shortcomings of a standard, non-biocompatible LC system. Another advantage of PEEK is that there is no metal leaching into the mobile phase, because PEEK is a polymer. This is not only a challenge in biocompatibility, but is also a useful feature in ICP-MS where these unwanted metal ions can be detected. PEEK also has the benefit of having a lower roughness, compared to stainless steel and other metals. A lower roughness on the inside of the capillary, as well as fewer defects and grooves, decreases band dispersion. This means that PEEK is a perfect candidate to tackle most of the problems of a biocompatible system.

The biggest disadvantage of PEEK is its maximum pressure rate. A PEEK capillary can withstand up to 480bar. This is acceptable for HPLC applications, but not for UHPLC. To combat this, a stainless steel lining can be applied on the outside of the tubing to make it more pressure resistant; creating PEEK lined stainless steel (PLS). By using PLS, the maximum rated pressure can improve to 1300 bar. PLS is however a relatively new technology and is challenging to manufacture, resulting in less cost-effectiveness. Besides this, PEEK poses two main challenges. Because PEEK is a polymer, it cannot withstand some extreme solvent conditions such as concentrated HNO_3, and may cause swelling when Methylene chloride, DMSO, or THF is used. Besides this, the usage of PEEK in a flow path can induce hydrophobic interactions that can occur with some samples.

Overall PEEK is a suitable option, especially when combined with stainless steel cladding (PLS) to increase the maximum pressure. There are some limitations, but PEEK can be a suitable option for most biocompatible applications.



Fused Silica

The concept of fused silica was first introduced as GC column material, but has made an appearance in LC applications as a biocompatible material. The main drawback of fused silica is that it is very brittle and only provide a maximum pressure up to 690 bar. Often times, PEEK will be used as a cladding material, which makes PEEK-fused silica, better known as PEEKsil. This makes the material pressure resistant up to 2068 bar.

The main benefit of using fused silica is the chemical inertness to most organic solvents (unlike PEEK), and is completely metal free.

Fused silica is however a very brittle material. The fused silica can be reinforced with PEEK, to not only make it stronger but still have a bit of flexibility in the tubing. Bending this type of tube too much may cause some cracks, which means the tubing must be handled with care. Besides this, fused silica has a limited pH range of pH 0-10, making it less suitable for bioanalysis using basic buffers. •