

A hidden cause for peak tailing of small acidic compounds

There are many influences on a liquid chromatography separation which can lead to peak tailing. Some are quite obvious and therefore taken into account during method development: the composition of the mobile phase, pH of the buffer and the selectivity of the chosen stationary phase. But there are also less prominent factors. One of these influences is the corrosion of metal parts in the chromatography system includ-

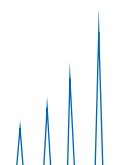
ing the column, which causes leaching of metal ions into the system. For neutral or basic substances, the presence of metal ions is usually insignificant. On the other hand, acidic analytes, which also contain, for example, phosphate or several carboxylate moieties, as well as substances that can undergo metal-catalysed reactions such as oxidation, can easily interact with metal ions.



New LC equipment shows low free metal content due to passivation of its metal surfaces, which impedes corrosion. However, this passivation is not permanent and corrosion will occur sooner or later depending on your method conditions.

As a result, you will see peak tailing, loss of recovery, ghost peaks and retention time shifts over time. This will get more pronounced as corrosion and contamination increases in your system.

This Expert Tip will go into detail on expected interactions between metal-ions and small acidic molecules, sources and promoting factors of metal corrosion as well as temporary and permanent solutions to mitigate peak tailing and restoring the quality of the chromatographic results.



Interaction of compounds with metal ions

Some substance classes such as (oligo)nucleotides, certain peptides and proteins, as well as small metal-coordinating compounds are well known to strongly interact with active metal surfaces. Effects are usually observable from the first use of the instrument and column. After initial improvement due to sample pre-conditioning effects (priming) within the first injections results will degrade again when corrosion of the system progresses^[1] These adsorption effects are described in more detail in the previous [YMC Expert Tip: Reducing adsorption on metal surfaces](#).

Other common effects include metal-catalysed reactions such as the oxidation of methionine side-chains in peptides and proteins, oxidation of benzoquinones and hydroquinones^[2] and nitrosation of amines^[3]. For the most critical analytes these interactions have already been well-known and

understood for decades, but in many cases, end-users aren't aware that their analytes of interest may be prone to similar interactions.

Usually, in these cases adverse effects on the chromatographic results are not visible at first and will only appear over time when system- and column corrosion increases. It is highly likely that the resulting peak tailing will be misinterpreted as early column ageing, because issues can be partially reset by using a fresh column. But due to the contamination of the system with metal ions, the same issues will appear sooner and sooner with each new column.

Sensitive analytes are usually small molecules containing carboxylates, hydroxyl groups and three-bonded nitrogen atoms with lone pairs of electrons that are able to form a 5- or 6-membered ring with metal ions.

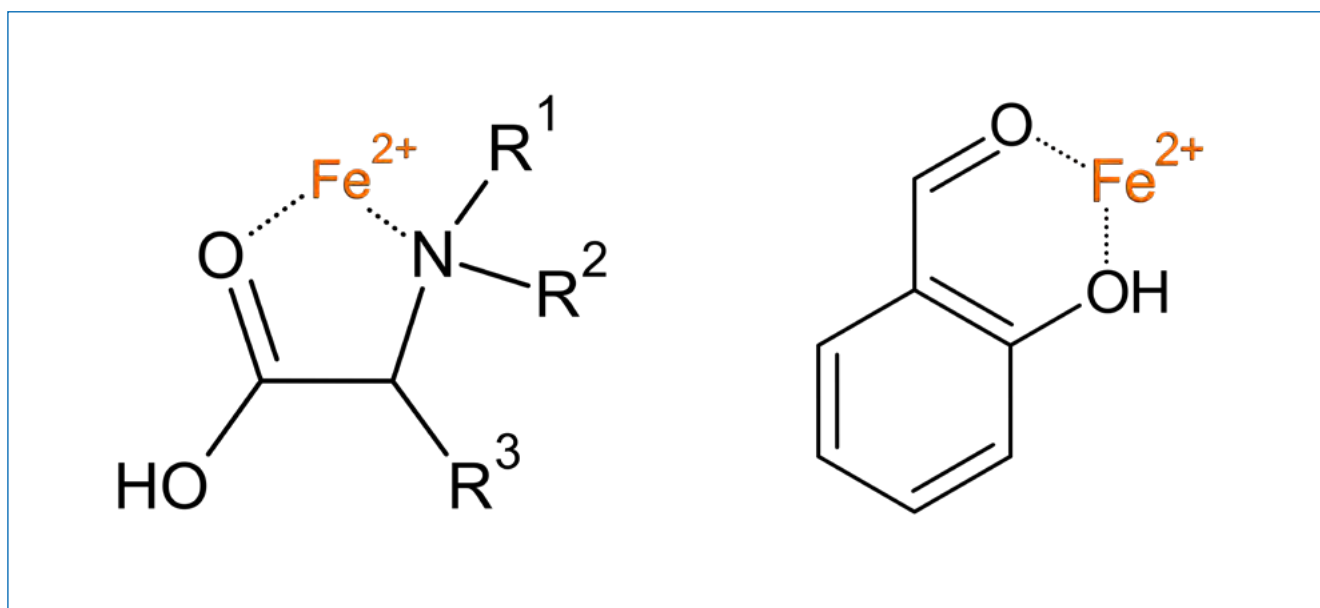


Figure 1: Examples for small compounds able to interact with Fe²⁺ ions.

Sources for metal ion contamination

LC columns may be exposed to different levels of metal-ion contamination during their life-time. The three main sources for this contamination are:

1. **Corroding steel or titanium parts from the LC instrument**
2. **Corroding steel or titanium parts from the LC column**
3. **Metal-ion content in sample**

Depending on the exact composition of the eluents and mobile phase in an application, the following components will fall prone to corrosion and subsequently leach metal-ions into the mobile phase:

- **Filter frits in eluent bottles**
- **In-line filter frits**
- **Solvent lines**
- **Injection needle**
- **Column body, inlet and outlet frit**

A number of conditions can promote corrosion and will lead to a quicker degeneration of the chromatographic results. The most common one is the use of 100% organic solvent (acetonitrile or methanol) as eluent or mobile phase. It has been shown that, especially in small diameter openings such as found in porous frits, just a few hours of exposure will corrode stainless steel^[4] or titanium^[5] and subsequently influences quality of the results.^[3, 6]

Other promoters for corrosion are the use of strong acids or oxidising agents in eluents and sample solvent as well as using mobile phase buffers above pH 5, which will lead to a creeping enrichment of metal ions in the LC-system and the stationary phase.

Usually, the column inlet frit is the most critical component when it comes to corrosion and its effects.^[7] This is due to its placement between the injector and stationary phase on the high-pressure side of the system as well as its high metal surface area compared to other parts of the system. This was shown several times in different experiments. For example, Myers *et al.* showed that by replacing the inlet frit of a new column with one of a used column metal catalysed sample oxidation increased immediately to levels previously shown on the used column.^[3] On the other hand, the use of an old column in reversed flow direction eliminated the on-column oxidation reaction on the inlet completely.^[8]

Procedures to restore method performance

Even though it is not trivial at first to identify metal-ion contamination as the root cause for peak tailing of small acidic compounds, it is quite easy to mitigate this problem again.

To reduce quick corrosion of steel or titanium eluent filter frits, the organic eluent can simply be diluted with 5% buffer.^[9] Metal-ions from stainless steel components are easily washed away from any surface and stationary phase by using a 0.1%–4% phosphoric acid solution at room temperature. Usually, if phosphoric acid is already added to the eluents, this contamination does not even happen with conventional hardware. Another option is to use chelating agents, such as EDTA, in the same way.^[10]

This is also the only way to keep titanium-ion contamination in check, because titanium is not easily dissolved by forming a pyrophosphate complex unlike iron.

One way to eliminate metal corrosion from the LC-system completely is by using a **bioinert** instrument and column. Here, it is very important to distinguish between truly bioinert technologies, that implement PEEK components (**YMC metal-free hardware**) or different organic-inorganic coatings on metal surfaces (**YMC-Accura hardware**) instead of bare metal parts and **bio-compatible** technologies. The latter ones usually use titanium linings in their flow path and therefore still fall prone to the above-mentioned effects.

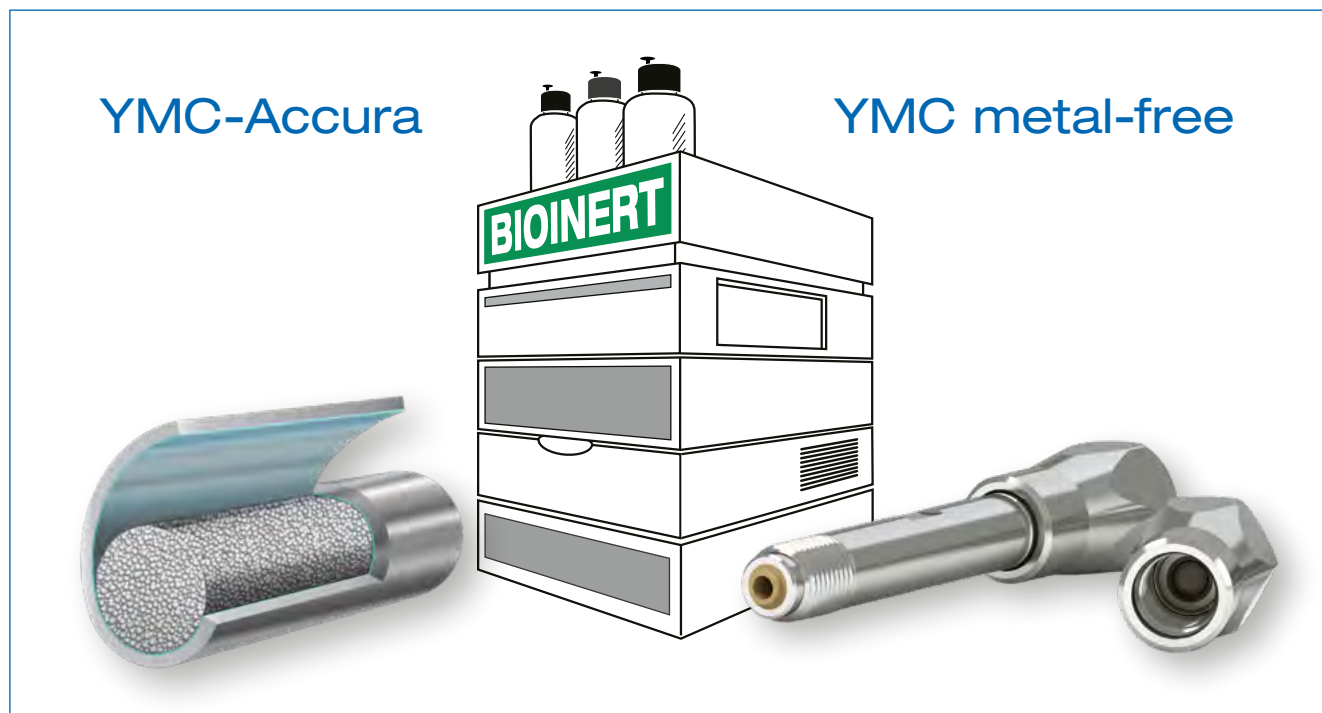


Figure 2: Bioinert hardware options from YMC.

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