Septa performance

Septa performance components

Introduction

When developing or optimizing analytical workflows, research scientists, laboratory managers, and bench chemists tend to overlook the sample handling component in favor of column selection or optimal detector.

Far too often, the sample handling component, and more specifically the cap and septum, are ignored only to become sources of contamination or sensitivity suppression which are then difficult to track down.

Oliver Fiehn states in his '2016 Current Protocols in Molecular Biology' article, 'Metabolomics by Gas Chromatography–Mass Spectrometry: Combined Targeted and Untargeted Profiling', that failing septa can contribute to failed data acquisitions while Benke, Burla, Ekroos, Wenck, and Torta state in their initial sentence in the abstract of the 2020 Analtica Chimica Acta publication titled 'Impact of Ion Suppression by Sample Cap Liners in Lipidomics' that:

"Contamination from the polymeric material released by vial caps used for sample introduction in liquid chromatography can significantly affect the signal of the analyte of interest."

Because septa have contact potential with the analytical matrix, they can become a major contributor to unexpected analytical issues. From impure manufacturing techniques to physical characteristics, septa contribute more toward contamination/signal suppression than is routinely expected during the trouble-shooting phase of improving the analytical workflow.

The following are key contributors to consider when selecting the appropriate septum for your analytical workflow.

Performance components

Early users of chromatography consumables tried to limit their operational expenses by washing and reusing many components. Vials and septa were the leading candidates for reuse but in due time, these practices were called out as leading sources of analytical contamination and overall lack of performance. And while these practices have largely gone away, chromatography consumables can still be a major contributor to poor analytical performance. So, what are the contributors to poor analytical performance? For starters there needs to be chemical compatibility between the sample matrix and the sample handling system. If the septum material is not compatible with the matrix any number of issues will arise. Most chemical interactions between sample and septum are well known/documented and can easily be avoided. That said, there are many interactions that can have more subtle impacts of cap and septa that can negatively impact chromatography performance. Particular focus on the following septa components will likely lead you well down the path of excellent chromatographic results:

- **Septa curing process**
  - The value of the platinum catalyst process.
- **Septa leachables**
  - Minimizing the impact by minimizing leachables.
- **Evaporation**
  - Improper sealing negatively impacts sample integrity.
- **Appearance**
  - Septa that doesn't look good, doesn't perform well.
- **Strength PTFE/silicone bond**
  - Keeping the septa together enhances performance.
- **Resealing**
  - Samples vials are often pierced more than once, this shouldn't impact results.

Septa curing process

There are two major curing techniques for manufacturing silicone septa: peroxide based curing system and a platinum-based approach. The highest purity approach is the platinum cured system which eliminates the creation of by-products. Peroxide based curing introduces contaminants which then require an additional post-curing step in an attempt to remove the by-products. The highest quality and most pure septa available on the market today are manufactured utilizing the platinum-based approach. Where purity is of the utmost concern, principally food and medical methods, it is important to know which of the curing techniques are used and if you are unsure, you should always ask the septa provider which curing methodology is utilized.

Because of the increased costs associated with platinum cured silicone it is quite common for providers of septa to offer lower quality peroxide cured septa and higher quality peroxide septa thus leaving the burden of choice of quality over cost to the end-user. Trajan's approach is to only offer the highest quality, platinum quality septa to provide the highest quality septa.

Septa leachables

The impact of septa purity typically manifests itself through leachable contaminants that occur once sample matrix is placed inside a closed glass vial. Once the sample is within the sample handling vessel, only the glass of the vial and the septa are in contact with the sample.

A relatively straight forward extraction of the septa and subsequent analysis is a good indicator of what can possibly leach out of a septum. The extraction consists of soaking a septum in dichloromethane for 21 hours and then analyzing the extract via GC-FID. The extraction is aggressive in nature as it is unlikely that any sample matrix will have that prolonged of an interaction with the septa, but it does an excellent job of leaching out impurities from the septa. No silicone septa will ever show a completely flat baseline during analysis, as siloxanes are easily detected on a GC-FID, but comparing one sample extract to another as an indication of relative purity.

Below are two example chromatograms showing a Trajan manufactured septa compared to a chromatogram from another leading manufacturer. The septa are both PTFE/silicone septa but show a striking difference between leachables detected.

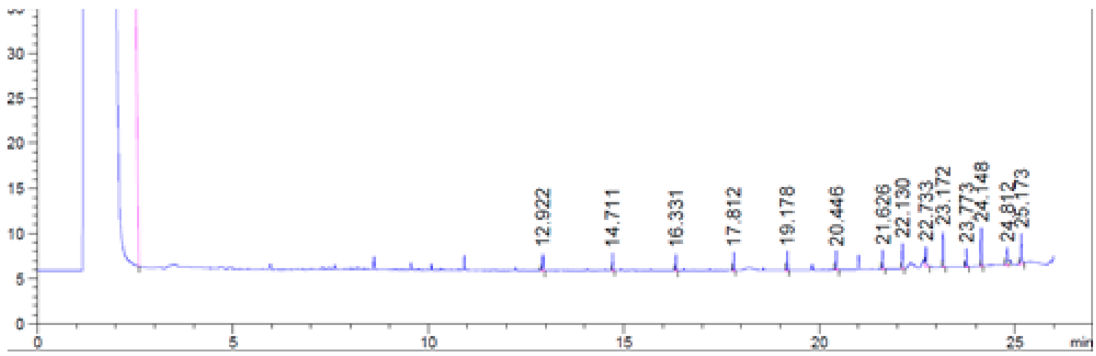

Figure 1. Trajan platinum cured PTFE/silicone septa

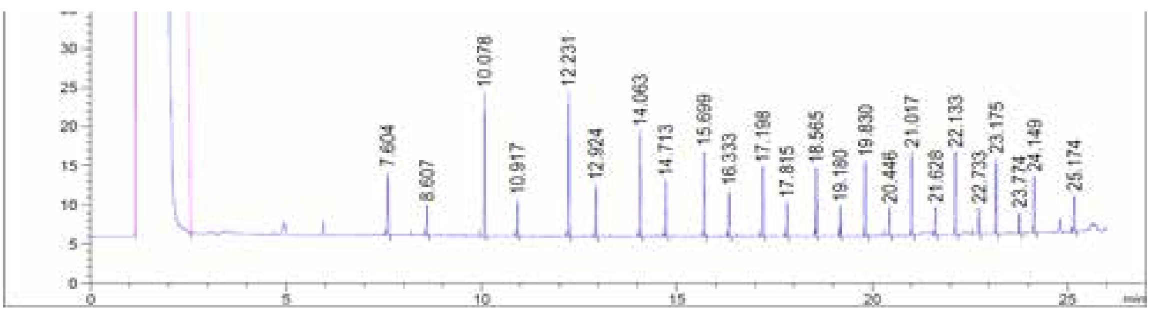

Figure 2. Leading manufacturer PTFE/silicone septa

The two chromatograms show the impact that raw materials and the curing process can have regarding impurities. Because septa are used in a wide variety of analytical workflows, the importance of manufacturing the highest level of purity in septa is crucial the highest quality of data generated.

Evaporation

Measuring evaporative loss over time is one of the hallmark performance tests of a sample handling system.

In this test, vial, cap, and septa all play an important role in maintaining the integral seal of the vessel over time.

If any of the components are poorly manufactured, evaporation can occur and will have significant impact on the integrity of the sample and thus its data.

The evaporation test is straight forward, measuring a before and after weight of the vial containing a low boiling point solvent and sealed with a cap and septa.

For the purposes of this comparative study, the 'after' weighing occurred at 48hrs. The hope is that the sample handling system chosen will provide similar results to the data below.

Sample	48 hr Dichloromethane retained in %
Trajan septa	99.97%
Sample	**48 hr Methanol retained in %**
Trajan septa	100%

Appearance

While most think of appearance of the septa as merely a cosmetic factor, the appearance of the septa can provide a telling indication of how the septum may perform.

With the images below, you will see the Trajan septa with a smooth, taught PTFE film across the entirety of the septa. Whereas in the second picture, you will see a non-Trajan septa where the PTFE film is uneven or perhaps not completely bonded to the silicone layer.

This type of appearance can be indicative of where the matrix may move past the PTFE protective film and begin to interact with the silicone layer thus creating several contamination and sample integrity issues. Chromatographers should take time to inspect the physical appearance of the septa upon opening a new bag. The time spent to avoid using septa with wrinkles and waves across the PTFE liner will more than pay for itself in avoidance of chromatographic issues later on.

Trajan septa

Leading septa manufacturer

Strength PTFE/silicone bond

As indicated in the previous section, the integrity of the bond between the PTFE and silicone is of utmost importance.

Without a strong bond between the two layers, delamination can occur creating an unintended exposure of the silicone layer to the sample matrix.

In the images below you can see the differences in the strength of PTFE/silicone bonding between Trajan manufactured septa and a leading manufacturer of septa.

With the Trajan septa, it is impossible to separate the PTFE layer from the silicone after soaking for 21hrs in dichloromethane. Whereas with non-Trajan septa, separates into two distinctive layers. Failure of this sort indicates a bonding process that is inferior and can lead to serious contamination issues.

Trajan septa | *Leading septa manufacturer*

Resealing

The last item studied was the impact of a septa to reseal itself after being pierced with a syringe. This is an important component within chromatography because from reanalysis it is common and septa are routinely pierced more than once. Because of the nature of the PTFE film, when a syringe pierces a PTFE/silicone septum, the PTFE layer does not reseal itself but the silicone layer will.

Syringe selection is important in this case as the chromatographer should select the smallest gauge syringe needle that will successfully pierce through both layers of septa.

As you can see in the images below, the two septa do not reseal comparatively. The Trajan syringe injection site is nearly imperceptible indicating the silicone septa resealed after injection and evaporative impact is minimized.

Silicone injection sites:

Trajan septa

Leading septa manufacturer

However, on the non-Trajan septa syringe injection site, the hole that is created by the syringe is easily noticed indicating there is a very likely chance that sample evaporation will occur creating quality issues for the sample.

Conclusion

There are 6 key performance tests that should be considered when selecting which septa is right for your analytical workflow. Too often cost is the leading driver for consumable selection as laboratories look for areas to reduce operating costs. However, focusing on cost alone may prove to be short-sighted as the potential impact to the chromatography far outweighs any potential savings an inferior septum may provide. As has been pointed out, the analytical workflow is delicate and eliminating all potential sources of quality issues should be a focus. Particular attention should be given to materials that come in contact with the sample including the selection of septa as a critical component in the workflow.