Why analyte binding to syringe filters must be studied during filter validation for QC testing

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Many pharmaceutical QC tests such as dissolution testing, content uniformity, assay, and blend uniformity require sample filtration prior to HPLC or UHPLC analysis. Since quantifying analytes is critical for these tests, method validation should include filter validation studies to evaluate analyte loss to the membrane. Different membrane filters can bind analyte to varying degrees depending on membrane type, the nature of the analyte and analyte concentration.

The easiest method for determining analyte binding to a syringe filter is to filter the sample through it and subsequently collect and analyze various filtrate fractions. Comparing these samples with a centrifuged sample that represents 100% recovery will provide information on analyte binding as well as the volume required for filter saturation.

Physico-chemical properties of analytes and membranes

Analyte binding mostly depends on the physico-chemical properties of the membrane and the analyte. These properties can lead to various secondary interactions between analyte and membrane, such as electrostatic interactions, hydrogen bonding, and hydrophobic interactions.

Nylon, hydrophilic PTFE and hydrophilic PVDF are materials commonly used for membrane filters in pharmaceutical QC testing. Hydrophilic PTFE and PVDF membranes have very few functional groups that can interact with analytes, so analyte binding is typically low and recoveries high. Nylon membranes, on the other hand, contain amino and carboxylic acid functional groups as well as amide bonds which can interact with acidic or basic analytes through electrostatic and hydrogen bonding, leading to high analyte binding and low recoveries. As many APIs are either acidic or basic, they can prove difficult to quantify. Analyte recovery with nylon syringe filters can be improved by simply saturating the filter with sample, so the contained analytes will effectively block the filter's binding sites.

These assumptions on binding propensity were confirmed in a study of ours on analyte binding from a multi-component migraine formulation using three different Millex® syringe filters with hydrophilic PTFE, hydrophilic PVDF, or nylon membranes. The formulation contained an acidic (acetyl salicylic acid), neutral (caffeine), and basic (acetaminophen) API. While acetyl salicylic acid and acetaminophen did not bind to the hydrophilic PTFE or PVDF membranes due to their lack of binding sites, both APIs did bind strongly to the filters containing a nylon membrane. However, quantitative recovery was obtained when the nylon filters were saturated with analyte by filtering a higher volume of sample to block the limited number of binding sites on the membrane. As expected, neutral caffeine did not bind to any of the three membranes.

The effects of analyte concentration

The analyte saturation point of a filter also depends on its concentration. Generally, as the analyte concentration

decreases, the volume needed to fully saturate the filter's binding sites increases. This holds true even for low binding PTFE and PVDF membranes. When formulations of naproxen, a nonsteroidal anti-inflammatory drug, containing three different concentrations (244 ppm, 24.4 ppm and 2.44 ppm) were tested for recovery, no analyte binding to the hydrophilic PTFE membrane was observed for the highest concentration, even without any filter saturation (i.e., no discard volume). At the lower concentrations, recovery was lower for first 1 mL of filtrate due to incomplete saturation of the syringe filter, but quantitative recovery was obtained when 3 to 5 mL of sample was filtered through to saturate the filter before collecting the sample for analysis. Membrane pore size did not impact analyte binding much. Similar results were obtained for the 0.2 and 0.45 µm membrane filters.

Not all membranes are created equal

During filter validation studies, once a membrane is finalized, it is important to confirm that the same type of membrane from a different vendor will show a similar analyte retention profile if a second source filter is required down the line. When we evaluated hydrophilic PTFE syringe filters from various vendors, all generally considered to have low analyte binding, we found out that for certain analytes, like Loratadine, filters from different vendors showed significant differences in analyte binding and subsequent recovery. Even the filter saturation volume varied significantly. This underscores that it is possible for seemingly identical membranes from different vendors to show widely different analyte binding behavior.

In a nutshell

Filter validation is a critical part of various pharmaceutical QC tests, and various filter parameters need to be taken into consideration during method validation. Since these QC tests require accurate analyte quantitation, analyte binding is an important factor. Membrane and analyte physico-chemical properties have the largest impact on analyte recovery, particularly when analyte concentrations are low. Membrane pore size has a limited effect on analyte recovery, but pore size selection is dictated by the downstream analytical technique. The same type of membrane from different manufacturers can show significant differences in analyte binding, so studying this should be part of filter validation.

Read our protocol for membrane filter validation in dissolution testing and see how filter membrane and drug properties impact analyte recovery.

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