Technical Brief

Benzyl alcohol clearance from Protein A resins

Introduction

Chromatography media have traditionally been stored and shipped in 20% ethanol aqueous solution. However, ethanol's limited microbial-kill effectiveness and potential hazard has led to the search for alternative solutions. At 1-2.2% concentration, benzyl alcohol's antimicrobial effectiveness has been extensively studied.[1] The added benefit of low flammability of benzyl alcohol aqueous solutions has made it even more desirable as a key component in shipping, storage and sanitization for chromatography resins in biopharmaceutical applications, especially for Protein A affinity resins.

Selection considerations for shipping and storage solutions

The main consideration for the selection of shipping and storage solutions is the effectiveness in preventing microbial growth during the entire shelf life period (up to 5 years) without impacting the functionality of the media. In addition, the shipping and storage solution should have a low hazard profile to humans and the environment, be safe to handle and be easy to dispose of. The mixture of benzyl alcohol and acetate buffer fits this profile. It has been widely used in the biopharmaceutical industry.[2]

Selection considerations for sanitization solutions

Sanitization is typically practiced after a down-stream purification campaign and before storing chromatography columns and related devices. for future use. This step ensures the elimination of potential contaminants introduced by the process feed, such as undesirable microorganisms or spore forming species. An effective and fast sanitization method is critical in extending resin and column lifetime with minimal impact on the entire process train. A benzyl alcohol containing solution, PAB (120mM phosphoric acid, 167mM acetic acid, 2.2% benzyl alcohol), has been established as a superior sanitant in its efficiency and effectiveness as a sanitant for biopharmaceutical chromatography applications.[1] Differentiating itself from commonly used NaOH sanitization solution, PAB has no detectable impact on resin binding capacity after repeated sanitization. This makes PAB even more attractive for sanitization of protein affinity resins.

Practical considerations

From process and ease of operation point of view, the shipping/storage and sanitization solution components should also be easily cleared off the chromatography media prior to use. Compared with the commonly used aqueous ethanol solution or sodium hydroxyl solution, buffers containing benzyl alcohol are much more effective and safe for shipping/storage and sanitization. However, an understanding of the clearance of the organic solvent, benzyl alcohol, from each resin product is important. This clearance not only ensures consistency on subsequent chromatography operations but also minimizes safety concerns over residual benzyl alcohol.

EMD Millipore is a division of Merck KGaA, Darmstadt, Germany

General properties of benzyl alcohol

Benzyl alcohol is often used as a preservative in the food, cosmetic and pharmaceutical industry. This is mostly due to its high bacteriostatic effectiveness and fast metabolic clearance in human liver to benzoic acid.[3] Benzyl alcohol has limited solubility in water. At 1-2.2% typical aqueous solution, it is reported to be equal or more effective in its antimicrobial capability than that of 20% ethanol. Benzyl alcohol aqueous solution is non-flammable and can be easily disposed of in compliance with most local environmental regulations.

Benzyl alcohol clearance study from Protein A affinity chromatography resins

Objective: Determine clearance and flush volumes of benzyl alcohol from Protein A resins. ProSep® Ultra Plus resin (based matrix: controlled pore glass) and Eshmuno® A resin (base matrix: highly crosslinked polyvinylether) are used in this study.

Experimental: ProSep® Ultra Plus and Eshmuno® A resin were each packed into an OmniFit® column with 1 cm inner diameter x 5 cm bed height. Both columns were conditioned and then held in 2.2% benzyl alcohol with 0.1M sodium acetate pH 5.2 for > 72 hrs. The columns were then flushed with a typical Protein A equilibration buffer, 50 mM Tris, 25 mM NaCl, *p*H 7.2 at 3 min residence time on a chromatography system. Fractions of 0.5 CV were collected for for analysis for HPLC-UV analysis with a C-18 column. The experiment was repeated to confirm reproducibility (test 1 and test 2).

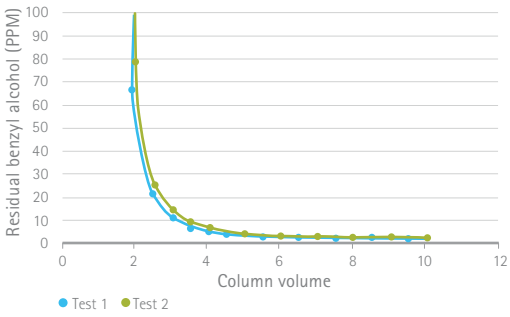

Figure 1.
Eshmuno® A media benzyl alcohol clearance with 50 mM Tris, 25 mM NaCl, *p*H 7.2

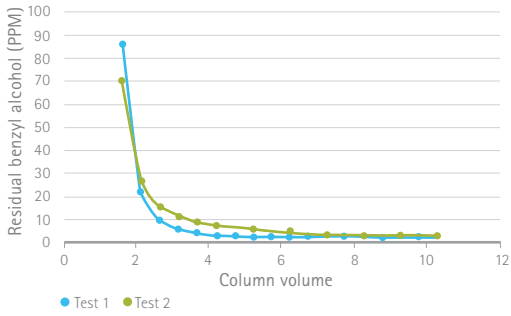

Figure 2.
Benzyl alcohol clearance data for ProSep® Ultra Plus media

Conclusion

For both ProSep® Ultra Plus and Eshmuno® A resins, it has been demonstrated that benzyl alcohol can be cleared in less than 5-6 column volumes using a common equilibration buffer. Since a typical chromatography operation includes equilibration with 5-6 column volumes of buffer before loading, there are no additional steps required to remove residual benzyl alcohol for ProSep® Ultra Plus or Eshmuno® A resin.

References

1. M. Rogers, et. Al. *J. of Chromatography A*, 1216 (2009) 4589–4596.
2. EMD Millipore Technical Brief TB0011EN00, Rev A 03/08
3. J. J. Lucchini, et. al. *Res. Microbiol.*, 141 (1990) 449-510.

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