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Traditionally, 20% ethanol has been the solution of choice for shipping and storage of chromatography media (resins). However, more stringent safety demands, for example, when large quantities of ethanol can be viewed as potentially hazardous, might require the use of alternative solutions. This application note evaluates benzyl alcohol as a shipping and storage solution. It includes data on clearance behavior and stability. Our internal investigation shows that 2% benzyl alcohol is an excellent alternative to 20% ethanol.

Criteria for storage solutions

The solutions used for shipping of chromatography media should meet a number of demands. The solution should be nontoxic to humans, inexpensive, and easily disposed of. For efficient process setup, the solution should be easy to wash out before use of the chromatography medium. It is also desirable that the same solution is compatible with a wide range of chromatography media.

In addition to 20% ethanol, the following solutions have been identified as fulfilling most of the above criteria:

- Sodium hydroxide down to 0.01 M
- Combinations of ethanol and sodium hydroxide (0.01 M sodium hydroxide in 10% ethanol)
- Benzyl alcohol (BnOH) concentrations of 1%–2%

One disadvantage of using sodium hydroxide as a storage solution is that it is incompatible with chromatography media with protein ligands. Benzyl alcohol, on the other hand, does not denature proteins and is often used by the biopharmaceutical industry for intermediate storage of chromatography media. Hence, benzyl alcohol is an attractive alternative to evaluate for shipping and storage of chromatography media.

Benzyl alcohol – general properties

Benzyl alcohol is popular in the cosmetic and pharmaceutical industries. It is soluble in water up to a concentration of about 4% but is usually used in a concentration of 0.9% in multiple-dose vials of solutions for parenteral therapy. For skin care products, concentrations between 0.5% and 1.5% are recommended. Benzyl alcohol is inexpensive, nonflammable, and can be disposed of relatively easy (local regulations may apply).

Benzaldehyde

Benzyl alcohol can be converted to benzaldehyde (and benzoic acid) by oxidation. The reaction rate in water solutions is slow and no significant decrease in the benzyl alcohol concentration was observed during the shelf life studies of the tested chromatograhy media.

The level of benzyl alcohol remaining after clearance has been used in a safety risk assessment. Although assuming a worst case scenario where all benzyl alcohol residues were converted into benzaldehyde, the level that potentially could contaminate a therapeutic protein was well below the limits set by the British Pharmacopoeia and the United States Pharmacopoeia for presence of benzaldehyde in benzyl alcohol when used in the manufacturing of parenteral formulations (0.05% and 0.2%, respectively). For preparation of solutions, use benzyl alcohol of high quality and from freshly opened containers. Avoid exposing the solution to excessive air (e.g., by vigorous stirring). Please read the material safety data sheet (MSDS) for benzyl alcohol before handling.

Precautions

Benzyl alcohol is only partially soluble in water, approximately 4 g/100 mL at room temperature. Benzyl alcohol will degrade some plastics, particularly at higher concentrations and temperatures. High-density polyethylene (HDPE) containers are suitable for storage of solutions containing 2% benzyl alcohol. Benzyl alcohol will, however, permeate into low-density polyethylene (LDPE).

Clearance of 2% benzyl alcohol

An important property of an effective storage solution is clearance behavior. Tests have been conducted in our laboratory to remove benzyl alcohol from a number of chromatography media with distilled water. The media were selected to be representative of different base matrices and ligand chemistries.

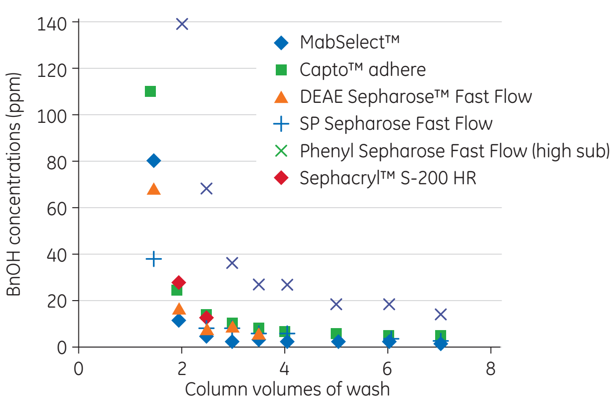

Fig 1. Removal (with distilled water) of 2% benzyl alcohol from chromatography media. The flow velocity was 600 cm/h.

A level of < 20 ppm benzyl alcohol after washing with five column volumes of water was chosen as an acceptable criterion for effective clearance. Typical clearance data obtained on a range of chromatography media are shown in Figure 1.

All tested media match the set criterion at a flow velocity of 600 cm/h, except for media based on Sepharose Big Beads (data not shown). Because of the larger bead size of Sepharose Big Beads media, a lower flow velocity (300 cm/h) is required. Also Phenyl Sepharose Fast Flow (high sub) shows better result at a lower flow velocity (300 cm/h), although within acceptance range at 600 cm/h.

When the same washing procedure was applied, 2% benzyl alcohol was removed from any tested chromatography medium just as easily and efficiently as 20% ethanol.

Long-term stability

A crucial parameter for a storage solution is the stability of chromatography media in the solution. The media should be stable not only for short periods in between use, but for the entire shelf life of the media. To prevent ligand degradation due to decreasing pH during long-term storage, sodium acetate (0.2 M) is added to the storage solution for some cation exchange (CIEX) and hydrophobic interaction chromatography (HIC) media.

A representative selection of chromatography media were subjected to a shelf life stability test (Table 1). The selected products represent different ligands as well as different base matrices. Based on the results for these products, storage stability can be estimated for other products with similar chemistry.

Table 1. Tests performed on selected chromatography media to evaluate the effects of storage in 2% benzyl alcohol (stability measures = minimum verified durations)

				Long-term stability	
Medium	**Storage solution**	**USP 51**	**Clearance***	**Accelerated (40°C)**	**Real-time**
Capto Q	2% BnOH		√		
Q Sepharose Fast Flow	2% BnOH	√	√	12 mo	3 yr
Q Sepharose XL	2% BnOH	√	√		
Capto DEAE	2% BnOH				2 yr
DEAE Sepharose Fast Flow	2% BnOH		√		
Capto S	2% BnOH + 0.2 M NaAc		√	9 mo	
SP Sepharose Big Beads	2% BnOH + 0.2 M NaAc		√		
SP Sepharose Fast Flow	2% BnOH + 0.2 M NaAc	√	√	12 mo	5 yr
SP Sepharose XL	2% BnOH + 0.2 M NaAc	√			4.5 yr
CM Sepharose Fast Flow	2% BnOH	√	√		3 yr
Phenyl Sepharose 6 Fast Flow (high sub)	2% BnOH + 0.2 M NaAc	√	√	4 mo	
Phenyl Sepharose 6 Fast Flow (low sub)	2% BnOH + 0.2 M NaAc		√		
MabSelect	2% BnOH	√	√		5 yr
MabSelect SuRe™	2% BnOH		√		5 yr
Protein A Sepharose 4 Fast Flow	2% BnOH	√	√		3 yr
Sephacryl S-200 High Resolution	2% BnOH	√	√	8 mo	3 yr

Note: In the USP 51 and Clearance columns an √ = media have been tested and fulfill requirements; a blank space = USP 51, clearance, or shelf life have not been tested on the actual product but the product is assumed to fulfill the criteria based on studies of products with similar chemistry. Only products that are recommended to be stored and shipped in 2% BnOH are shown here.

* < 20 ppm after 5 CV at 600 cm/h. Note: For Phenyl Sepharose 6 Fast Flow (high sub) and SP Sepharose Big Beads, a flow velocity of 300 cm/h is recommended.

Three batches of each chromatography medium were stored at the recommended temperature in 2% benzyl alcohol for three or five years. Stability indicating parameters for each medium were tested at 5 different time points with the quality control (QC) test methods and acceptance criteria used for product release.

Degradation under recommended storage conditions can be predicted based on the degradation under stressed conditions (e.g., at 40°C) and this is a commonly used procedure to shorten the storage time in stability investigations. Accelerated shelf life studies on several media (Table 1) were performed using temperature as the acceleration factor. The prediction of stability from 40°C to room temperature is complex, but one extensively used assumption is that the temperature dependence follows the Arrhenius equation, which states that a rise in temperature of 10°C doubles the speed of any degradation reaction that might take place. This assumption was used in these studies and one month storage at 40°C consequently corresponds to approximately three months storage at 25°C.

While small method variations and variations between batches were observed, no signs of decreased functionality were noted, indicating an estimated shelf life of 3 to 5 yr (depending on type of chromatography medium) when stored in closed original containers.

The storage studies were mainly performed on unused media stored directly in 2% benzyl alcohol. To prevent any potential changes during storage, pH should be measured during long-term storage of media in 2% benzyl alcohol, following replacement of 20% ethanol, or following other treatments prior to storage in 2% benzyl alcohol.

Column performance

It is important that a new storage solution does not affect the column performance in a negative way (e.g., resulting in larger equilibration volumes) when compared with 20% ethanol.

MabSelect and SP Sepharose XL media stored in 2% benzyl alcohol were packed in AxiChrom™ 50 columns according to a standard procedure. Peak efficiency was tested at a flow velocity of 60 cm/h. The acceptance criteria with respect to peak efficiency and peak asymmetry were fulfilled in all cases. This study confirmed that the change in storage solution does not affect the packed beds, when compared with 20% ethanol. 2% (v/v) benzyl alcohol is compatible with the acrylic material used in columns at room temperature for up to one year, see respective column's operating instructions for further details.

Conclusions

We have investigated benzyl alcohol as a possible alternative to 20% ethanol for shipping and storage of chromatography media. Although buffering might be needed for stability reasons for CIEX and HIC media, our investigation shows that 2% benzyl alcohol is an excellent alternative storage and shipping solution for all chromatography media tested.

References

1. United States Pharmacopoeia 51.
2. The British Pharmacopoeia, HMSO, London, England (2000).
3. The United States Pharmacopoeia 24 and the National Formulary 19 Rockville, MD, USA (2000).

Ordering information

Several GE Healthcare products are available in 2% benzyl alcohol upon customer request. Please contact your local GE Healthcare BioProcess™ sales representative/specialist for more information.

For local office contact information, visit
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