# Use of benzyl alcohol as a shipping and storage solution for chromatography media

**Traditionally, 20% ethanol has been the preservative of choice as a shipping and storage solution for chromatography media (resins). However, more stringent safety demands sometimes require the use of alternative solutions when large quantities of ethanol may be viewed as potentially hazardous. This Application note evaluates benzyl alcohol as a shipping and storage solution. It includes data on antimicrobial effectiveness, clearance behavior, and stability. Our investigation shows that 2% benzyl alcohol is an excellent alternative, with at least the same antimicrobial effectiveness as 20% ethanol.**

## Criteria for storage solutions

The preservatives used for chromatography media shipping solutions must meet a number of demands. Most importantly, they should be sufficiently effective at preventing growth of microorganisms during the entire shelf life, without affecting the functionality of the medium. In addition, the preservative should be non-toxic to humans, inexpensive, and easily disposed of.

For efficient process set-up, the preservative should be easy to wash out of the chromatography medium before use. It is also desirable that the same preservative 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 concentrations of 1%-2%

One disadvantage of sodium hydroxide as a preservative is that it is incompatible with chromatography media derived from 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. This makes benzyl alcohol an attractive alternative for evaluation as a shipping and storage solution.

## Benzyl alcohol – general properties

Benzyl alcohol is popular as a preservative 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% as a bacteriostatic preservative in multiple-dose vials of solutions or drugs for parenteral therapy. For skin care products, concentrations between 0.5% and 1.5% are recommended. Benzyl alcohol solutions are microbiocidal (i.e., they have an antimicrobial effect) but are not regarded as a sanitizing agent, which would require a faster reduction of microorganisms.

Benzyl alcohol is inexpensive, non-flammable, and can be disposed of relatively easily (local regulations may apply). In comparison with ethanol, a sufficient antimicrobial effect is obtained with a lower concentration of additive.

### *Benzaldehyde*

Benzyl alcohol may 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 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 as preservative in the manufacturing of parenteral forms (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).

### *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).

## Antimicrobial effectiveness

Several studies were performed in order to determine the optimal composition of the storage solution with respect to antimicrobial effectiveness. The effect of benzyl alcohol concentration (1-2%), buffering (buffered/non-buffered solutions), buffer salt (acetate/citrate) and pH-value (pH 5.5, pH 6.4 and pH~8) was evaluated. Test organisms were chosen according to USP 51 [1], and the concentration of microorganisms in the solutions was measured after 24 h and after 7 days.

No major differences between the tested conditions have been observed. 1% benzyl alcohol fulfills USP 51 criteria but is slightly less effective than 20% ethanol. Therefore, 2% benzyl alcohol was chosen for further studies. At this concentration, buffering or pH does not appear to affect the result.

For some chromatography media, 0.2 M sodium acetate, pH~8, is added to the storage buffer to stabilize the ligand.

The antimicrobial effectiveness has sometimes been reported to be lower at pH values > 7 and USP 51 tests were therefore performed on a newly prepared buffer solution (2% benzyl alcohol, 0.2 M sodium acetate, pH 7.7) and on a number of chromatography media that require buffering (Table 2).

After seven days, no microbial growth was detected. In fact, no tendency for less antimicrobial effectiveness can be seen in storage solutions above pH 7.

Full USP 51 tests were performed for a representative choice of chromatography media (see Table 2). All tested media completed this test successfully. After 7, 14, and 28 days, no colony-forming units (CFU) were detected, demonstrating a complete inactivation of biological activity. The result obtained for Q Sepharose™ Fast Flow is representative for all tested media (Table 1).

To ensure that the antimicrobial effect of the 2% benzyl alcohol solution would last for the entire shelf life of a chromatography medium, a sample of benzyl alcohol that had been used for storage for a four year period was tested. The result was the same as for a newly prepared solution, confirming that the anti-microbial effect lasts for the entire storage period.

**Table 1.** Results of antimicrobial effectiveness testing according to USP 51 for Q Sepharose Fast Flow in 2% benzyl alcohol

| Test organism | Initial (cfu/ml) | 7 days (cfu/ml) | 14 days (cfu/ml) | 28 days (cfu/ml) |
|---|---|---|---|---|
| *Staphylococcus aureus* ATCC 6538 P | $1.2 \times 10^5$ | < 1 | < 1 | < 1 |
| *Escherichia coli* ATCC 8739 | $1.9 \times 10^5$ | < 1 | < 1 | < 1 |
| *Pseudomonas aeruginosa* ATCC 9027 | $2.3 \times 10^5$ | < 1 | < 1 | < 1 |
| *Candida albicans* ATCC 10231 | $1.5 \times 10^5$ | < 1 | < 1 | < 1 |
| *Aspergillus niger* ATCC 16404 | $1.1 \times 10^5$ | < 1 | < 1 | < 1 |

## Antimicrobial headspace studies

The headspace area of a shipping container is the space between the liquid surface and the lid. There were concerns that the lower vapour pressure of 2% benzyl alcohol compared to 20% ethanol would pose a risk for microbiological growth in the headspace area of shipping containers containing chromatography media preserved with benzyl alcohol.

A study was performed in order to study the risk for microbial growth. Containers were filled with water solutions containing benzyl alcohol (1.7% or 2.0%), ethanol (20%), or no added preservative. Reference test organisms (*Aspergillus niger, Pseudomonas aeruginosa* and *Staphylococcus aureus*) were inoculated onto the inside of the caps. The caps were closed and the containers were stored at ambient temperature. The microbial growth on the caps was checked after predetermined intervals of time for up to 3 months

Essentially the same results (i.e., no growth) were obtained for containers preserved with benzyl alcohol and 20% ethanol. In containers without a preservative, *A. niger* showed a large increase during the test period. 20% ethanol has been the preservative for GE Healthcare's chromatography media for many years and according to this study, there is no increased risk for microbial growth in the headspace area in containers containing 2% benzyl alcohol. Another study showed that condensate droplets, which may be formed in the headspace area, contain enough benzyl alcohol to achieve a preserving effect. The risk for microbiological growth in the head space area of containers containing chromatography media preserved with benzyl alcohol thus appears to be low.

## Clearance of 2% benzyl alcohol

An important property of an effective storage solution is straightforward 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.

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.

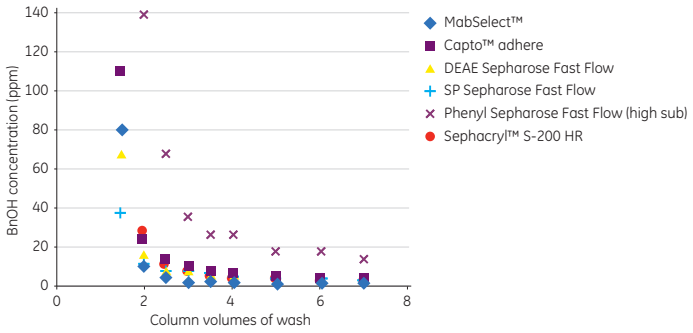


**Fig 1.** Removal (with distilled water) of 2% benzyl alcohol from chromatography media. The flow rate was 600 cm/h.

All the 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). Due to the larger bead size of the medium, a lower flow rate (300 cm/h) is required. Also Phenyl Sepharose Fast Flow (high sub) gives a better result with a lower flow rate (300 cm/h) even if it was approved at 600 cm/h. A list of tested media can be found in Table 2.

When the same washing procedure is applied, 2% benzyl alcohol is 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 between usage, but for the entire shelf life of the media. To prevent matrix degradation in the medium, sodium acetate buffer (0.2 M) is added to the storage solution of some cation exchange media and HIC media (see Table 2) in order to prevent pH from decreasing during long-term storage.

A representative selection of chromatography media were subjected to a shelf life stability test (Table 2). The selected products represent different ligands as well as different base matrices, and based on these products, storage stability can be estimated for other products with similar chemistry.

Three batches of each medium were stored at the recommended temperature in 2% benzyl alcohol for three or five years (equivalent to the shelf life when stored in 20% ethanol). Stability indicating parameters for each medium were tested at 5 different time points with the QC test methods and acceptance criteria used for product release.

The results obtained for MabSelect are shown in Figure 2. Three batches were stored for three years in 2% benzyl alcohol at 4°C to 8°C with no buffering substances added. The breakthrough capacity ($Q_{B,\ 10\%}$) and additional stability indicating parameters were measured at 0, 6, 12, 24, and 36 months. Although small variation between batches and a certain random variation due to the analysis methods can be seen, no trends indicating a decrease in functionality have been detected for any of the media tested.

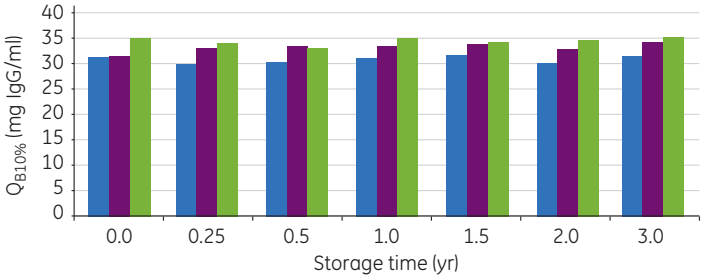


**Fig 2.** Breakthrough capacity ($Q_{B10\%}$), measured in mg human IgG/ml packed medium. Results of QC tests for three batches of MabSelect stored in 2% benzyl alcohol.

**Table 2.** Tests performed on selected chromatography media to evaluate the effects of storage in 2% benzyl alcohol. Stablity 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 | 2 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 | 3 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 | √ | √ | | 3 yr |
| MabSelect SuRe™ | 2% BnOH | | √ | | 1 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) a flow rate of 300 cm/h is recommended.

The same criteria used for establishing shelf life for products in 20% ethanol were used. Shelf life in 2% benzyl alcohol has been established to 3 yr for MabSelect and to 4.5 yr for SP Sepharose XL.

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 2) have been 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 seen, no signs of decreased functionality were noted, indicating an estimated shelf life for 3 to 5 yr (depending on type of chromatography media) when stored in closed original containers.

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

## 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 to 20% ethanol. MabSelect and SP Sepharose XL stored in 2% benzyl alcohol were packed in prototype AxiChrom™ 50 mm 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 to 20% ethanol.

## Conclusions

We have investigated possible alternatives to 20% ethanol for shipping and storage of chromatography media. After a brief inventorial investigation, benzyl alcohol at a concentration of 2% was chosen. At this concentration no buffering is needed to maintain the antimicrobial effectiveness of benzyl alcohol. However buffering may be needed for stability reasons for cation exchange and hydrophobic interaction chromatography media.

The antimicrobial effect appears to last for the entire shelf life of agarose-based media, which is 3 to 5 yr.

Our investigation shows that 2% benzyl alcohol is an excellent alternative storage and shipping solution, with at least the same antimicrobial effectiveness as 20% ethanol.

## 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
**www.gelifesciences.com/contact**

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