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# Cleaning and Cleaning Validation of Glass Columns Laboratory and Pilot Scale

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# Overview

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# 1 Introduction

For pharmaceutical and biopharmaceutical LC-processes a validated and efficient cleaning method is essential. The optimum cleaning procedure removes the impurities reliably, is fast and compatible with the resin and the equipment. During cleaning procedure validation, this needs to be evaluated in order to guarantee product safety and to fulfil regulatory requirements.

The most time-efficient cleaning procedure is achieved with a packed and assembled column. Cleaning-in-place (CIP) is the preferred method in preparative chromatography. A robust and efficient CIP procedure ensures product integrity and increases process lifetime.
But how can column design help to establish an efficient cleaning-in-place method? The most important requirement for the column technology is an efficient distribution of the cleaning agent through the column in combination with reduced dead areas. In addition, easy to clean surfaces and frits influence the versatility of a column for CIP procedures.

In a recent in-house study, the influence of the column hardware on cleaning efficiency was investigated. The results show the excellent flow distribution of YMC glass columns allowing reliable, fast and efficient CIP procedures with any resin.



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# 2 Cleaning-In-Place (CIP)

In the manual of the Society of Dairy Technology from 1990, cleaning-in-place was defined as:

*"The cleaning of complete items of plant or pipeline circuits without dismantling or opening of the equipment and with little or no manual involvement on the part of the operator. The process involves the jetting or spraying of surfaces or circulation of cleaning solvents through the plant under conditions of increased turbulence and flow velocity."* [1]

This definition is still valid and generally describes the idea of CIP for all industries, where this method is used. For the selection of the correct cleaning agent, the nature of the possible contaminations need to be evaluated.

## 2.1 The substances which are to be removed

In preparative chromatography, CIP has different aims for every user. CIP is used to remove proteins, nucleic acids as well as to sanitize the column after contamination with bacteria, yeast, viruses and fungi. Most CIP procedures for cleaning and sanitization of chromatography resins use sodium hydroxide (NaOH) as this efficiently removes precipitated proteins, hydrophobic proteins, nucleic acids, endotoxins and viruses [2].

It is important to understand the basic chemistry of the substances which have to be removed and the surface from which they have to be removed. The characteristics of chromatographic interactions are very broad. For example, unfolded hydrophilic proteins are likely to have exposed hydrophobic binding sites that potentially interact with a resin crosslinking structure. Equipment surfaces may also affect cleaning efficiency.

One study demonstrated that an early liquid feedstock with multiple components bound to glass surfaces and resulted in poor recovery of the order of 55 %. On the other hand, removal of a relatively pure protein from stainless steel led to a recovery of about 89 % [3].

Continual evaluation of cleaning methods as the liquid feedstock changes, is essential for the early recognition of when changes are necessary.



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## 2.2 CIP Process Design

Cleaning efficiency, resin compatibility and equipment specifications are the three major aspects to consider for CIP process design. Before using any cleaning method it is crucial to check the resin compatibility. Besides the resin compatibility, the suitability of the associated equipment needs to be verified with the available and affordable cleaning agents.

### 2.2.1 Cleaning Solvents

The cleaning solvents for high cleaning efficiency are chosen depending on the characteristics of the impurities to be removed. A guideline of cleaning methods for CIP is described in the following table. In addition, the supplier of the used resin may have additional advice.

*Table 1: Typical cleaning solvents for CIP methods*

| Treatment | Contamination |
|---|---|
| **Pepsin, pH 1.5–2** | Hydrolysis of adsorbed proteins |
| **Non-ionic detergents (Triton X-100, Tween 80)** | Removal of hydrophobic proteins and lipids |
| **Cationic detergents, pH 9–11** | Removal of hydrophobic proteins and lipids |
| **Urea, 6–8 M** | Removal of protein aggregates |
| **1–100 mM EDTA, pH depending on the stationary phase** | Removal of metal complexes |
| **0.1–1 M NaOH** | Removal of bounded hydrophobic proteins and lipopolysaccharides |
| **0.5–1 M acetic acid in 60 % ethanol** | Removal of lipids, pigments, lipopolysaccharides and other lipophilic substances |

Depending on the nature of the contamination, three different procedures can be used: regeneration, sterilisation, depyrogenation.

**Regeneration** removes inorganic and organic contamination, which binds to the chromatography matrix and substantially affects the capacity and resolution of the column. Commonly these contaminants are: lipids, pyrogens, protein aggregates, pigments, polyphenols or metal complexes.

**Sterilisation** is the removal and/or destruction of microorganisms and spores that will contaminate the purified product. The most common method is the treatment with sodium hydroxide, acetic acid or ethanol solution.

**Depyrogenation** includes the removal of endotoxins, which can be bound to the chromatographic material or to the column hardware (frits, tubing, etc.) and which can contaminate the purified product.



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# 3 Materials and Methods

To demonstrate the importance of the most appropriate column hardware for high cleaning efficiency in CIP, a cleaning study was performed with two glass columns in different scales: a laboratory scale ECO glass column with an inner diameter of 25 mm and a pilot/production scale YMC Pilot glass column with an inner diameter of 140 mm. Both column dimensions were used for stepwise scale-up procedures with different kinds of resins. Detailed experiments were carried out in which the dye Coomassie Brilliant Blue G-250 was dissolved in 80 % isopropanol and injected on the column. The dye was subsequently rinsed with the same concentration of isopropanol. This whole process was monitored by UV detection.

## 3.1 Laboratory Scale Glass Columns

### 3.1.1 Method

The experimental conditions for the ECO glass column are shown in table 2.

*Table 2: Experimental conditions for a laboratory scale glass column (Method I)*

| | |
|---|---|
| **Glass Column** | ECO column, 25 mm ID, 200 mm length |
| **Packing Material** | YMC-Triart Prep C18-S (12 nm, 20 µm) |
| **Packed Bed Length** [mm] | 100 |
| **Sample** | Coomassie Brilliant Blue G-250 |
| **Sample Concentration** [mg/mL] | 0.01 |
| **Eluent** | Isopropanol/water 80/20 v/v% |
| **Temperature** [°C] | Room temperature |
| **Flow rate** [mL/min] | 10 |



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## 3.1.2 Results

The glass column was equilibrated with a flow of 10 mL/min for 5 minutes with 80 % isopropanol, which equals 30 column volumes (CV). In the next step, 20 CV of dye were injected (Figure 1 B).
The distribution of the colour in the glass column was clearly visible.

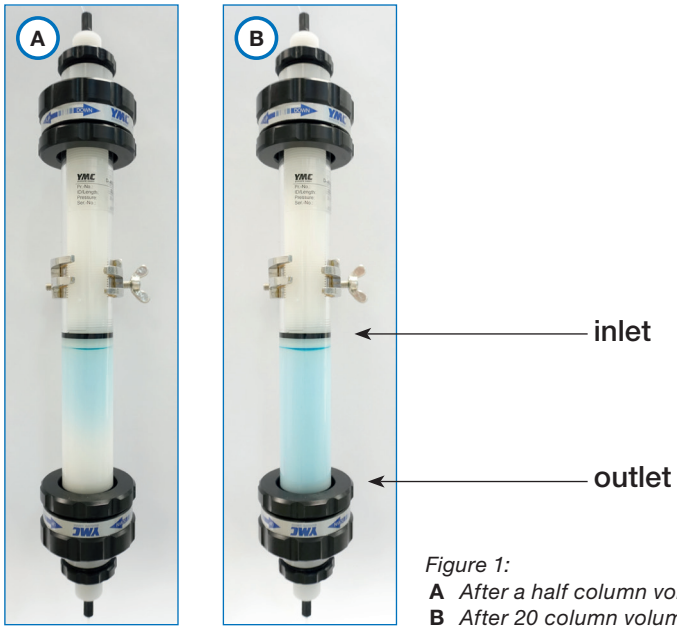


*Figure 1:*
**A** *After a half column volume of dye.*
**B** *After 20 column volume of dye.*

Figure 1A and 1B show that the sample is homogenously distributed via the frits. For the following cleaning validation, the same eluent was applied.
In Figure 2 it can be seen how the dye has been washed out, depending on the column volume. After 15 min and 3 CV, the dye was completely removed.


*Figure 2:*
**A** *After 2 CV (10 min) of cleaning.*
**B** *After 3 CV (15 min) of cleaning.*



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In addition to the optical inspection, the complete removal of the dye was confirmed by the chromatograms shown in Figure 3. The UV signal dropped abruptly after the beginning of the cleaning process started. After 3 column volumes have been applied, the signal reaches the baseline level of zero.

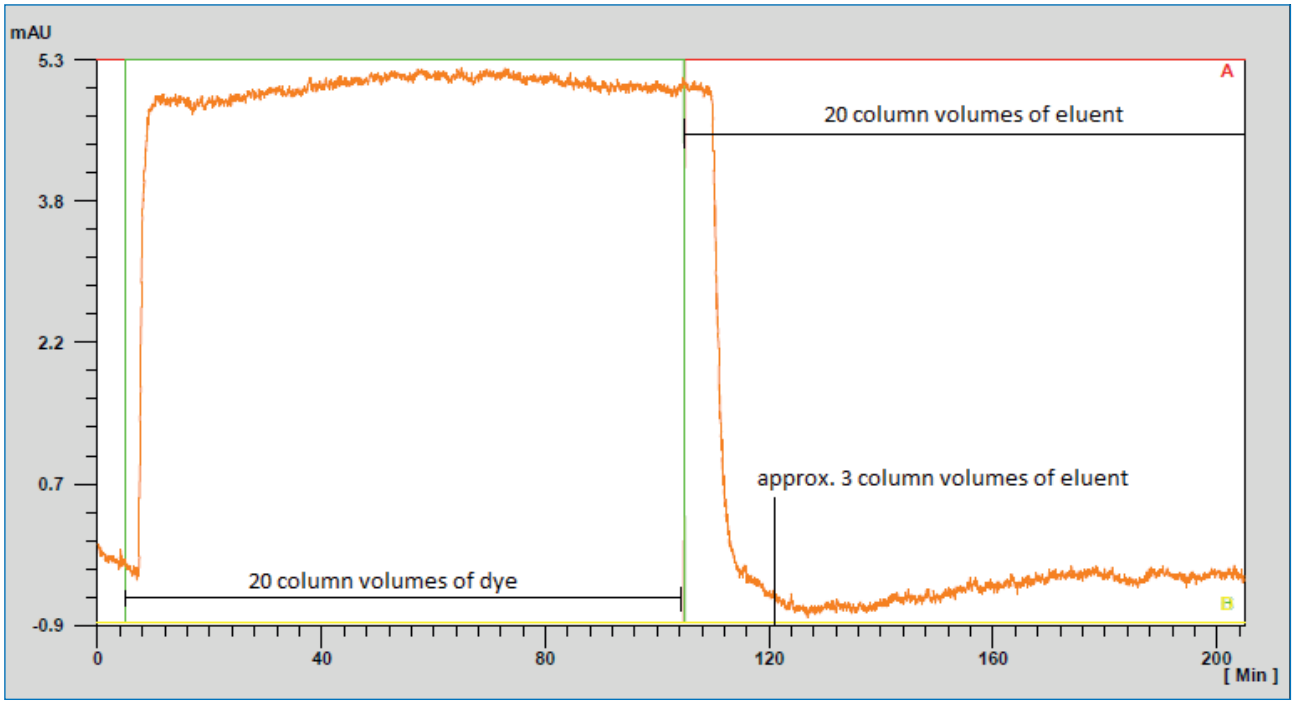


*Figure 3: Chromatogram of method I with the laboratory scale glass column.*

After visual and chromatographic cleaning validation, the column was disassembled. Optical inspection of the frits and the sealing rings shows a clean appearance without any visible residues (Figure 4–6).

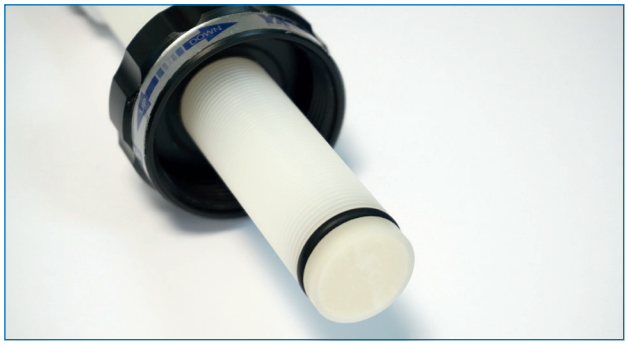

*Figure 4: Inlet of the column after CIP method.*

*Figure 5: Outlet of the column after CIP method.*

*Figure 6: Upper surface of frits for inlet (left) compared to a new one (right).*



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## 3.2 Pilot/Production Scale Glass Columns

### 3.2.1 Method

The same experiment was repeated using a YMC Pilot column to ensure appropriate cleaning on the pilot/production scale. The pilot scale glass column provides a higher column volume and has the larger ID, which requires an even better distribution at the frit. The experimental conditions are shown in table 3.

*Table 3: Experimental conditions for a pilot/production scale glass column (Method II)*

| | |
|---|---|
| **Glass Column** | YMC Pilot column, 140 mm ID, 500 mm length |
| **Packing Material** | YMC-Gel ODS-AM (12 nm, 50 µm) |
| **Packed Bed Length** [mm] | 120 |
| **Sample** | Coomassie Brilliant Blue G-250 |
| **Sample Concentration** [mg/mL] | 0.01 |
| **Eluent** | Isopropanol/water 80/20 v/v% |
| **Temperature** [°C] | Room temperature |
| **Flow rate** [mL/min] | 300 |



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## 3.3.2 Results

For complete distribution in the column, 20 CV of dye were injected to the column (Figure 7 A). In the next step the dye was washed out using 2.5 CV of eluent (Figure 7 B).


*Figure 7:*
**A** *After 20 column volumes of dye.*
**B** *After 2.5 column volumes of rinsing the column.*


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Analysis using UV detection also confirms the observed optical results (see Figure 8). The complete removal of the dye was achieved by washing with 2.5 CV of eluent as the signal returns to the baseline.

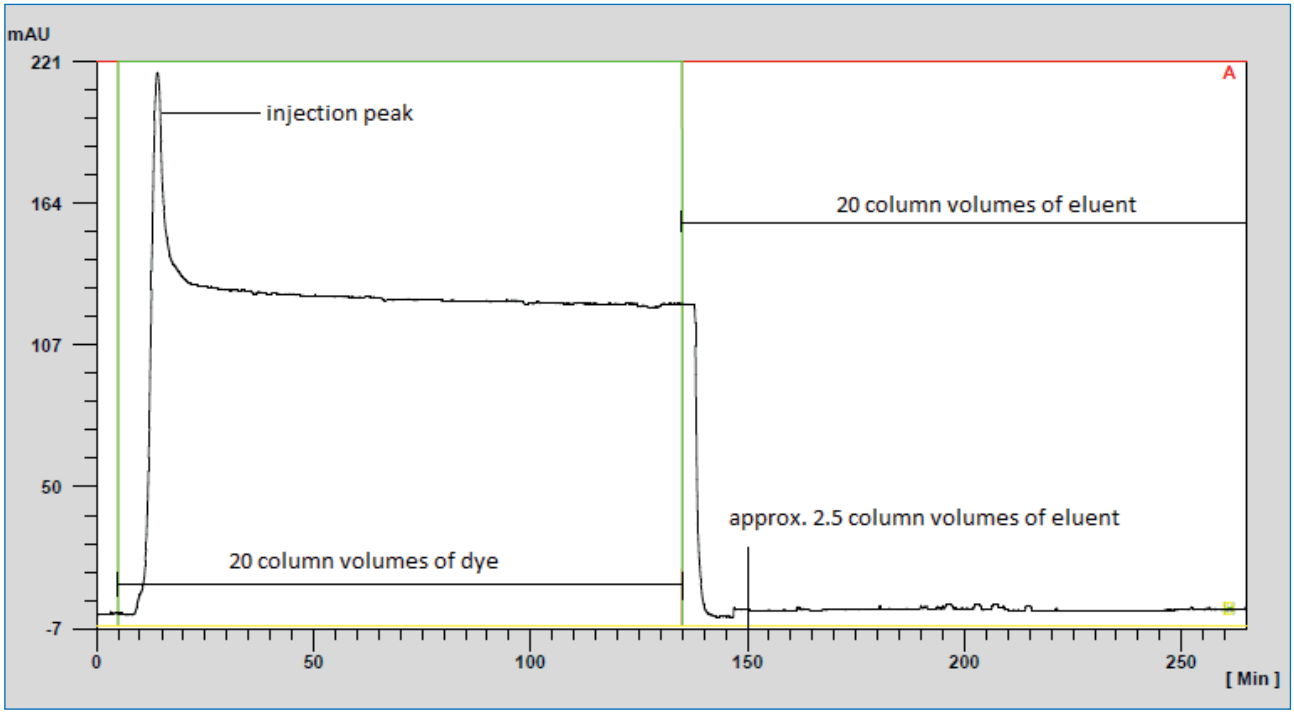
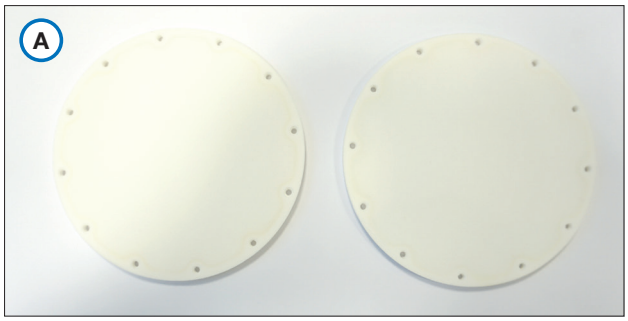



*Figure 3: Chromatogram of method II with the pilot/production scale glass column.*

The final inspection of the column frits after column disassembly showed no optical residues on the frits after the method had been carried out (Figure 9A and B).



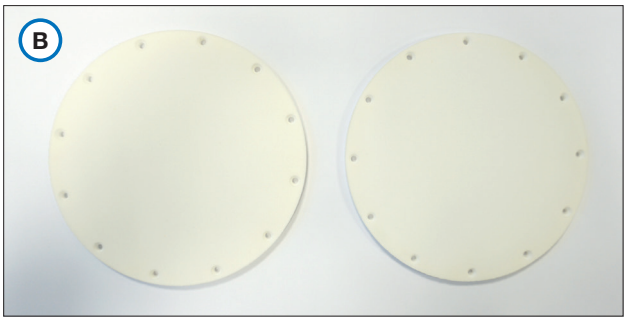




*Figure 9:*
*Frits after method:*
**A** *inlet.*
**B** *outlet.*



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# 4 Conclusion

This cleaning validation shows that a successful CIP process can be carried out with YMC glass columns due to the optimal distribution of the cleaning agent via the frits for laboratory and pilot/production scale. These results can be transferred to all CIP solvents.

A CIP process for cleaning chromatography columns is the simplest and best way as the column does not have to be unpacked or disassembled to clean it. This way, unnecessary work steps can be eliminated and through time and man-power savings, the efficiency of the overall process can be optimised.

# 5 Literature

[1] Tamime AY, Cleaning in Place: Dairy, Food and Beverage Operations. 3rd Edition, Oxford, UK: Backwell Publishing 2008

[2] Grönberg A., Eriksson M., Ersoy M., Johansson H. J. A tool for increasing the lifetime of chromatography resins, Landes Bioscience 2011: 192–202.

[3] Sofer G., Yourkin J. Cleaning and cleaning validation in process chromatography. BioProcess International 2007.



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