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Short communication

Cleaning validation of sampling tools

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Introduction

Cleaning validation and continuous cleaning monitoring are critical elements in ensuring the quality, safety, and compliance of pharmaceutical manufacturing processes (ECA Academy, 2022; ISPE, 2020,). Sampling tools used in Quality Control for sampling raw materials and active pharmaceutical ingredients (APIs) sampling are in direct contact with pharmaceutical substances and therefore require effective cleaning to prevent cross-contamination. For this reason, validated and reproducible cleaning procedures are essential to ensure that residues of APIs, excipients, cleaning agents, and microbial contamination are reduced to levels below predefined acceptance criteria (QbD Group, 2025).

The concept of a "worst-case" approach is commonly applied in cleaning validation, where the most critical substances, based on toxicity, solubility, and cleanability, are selected to represent the greatest potential risk (HPRA, 2018). In this study, Propyphenazone and Cefixime were identified as worst-case APIs for sampling tools used in the raw materials warehouse and the production of cephalosporins, respectively. The evaluation of cleaning effectiveness was performed through multiple cleaning cycles, followed by sampling and analytical testing using both specific (HPLC) and non-specific (pH, conductivity, TOC) methods, as well as microbiological assessment.

In addition, to verify the efficiency of the cleaning procedure, this study also evaluates the impact of holding times on equipment cleanliness. Dirty Hold Time (DHT) assesses the effect of delayed cleaning after use, while Clean Hold Time (CHT) evaluates the ability to maintain cleanliness during storage after cleaning. These parameters are essential for ensuring consistent equipment performance under routine conditions.

Materials and methods

Cleaning procedure

Sampling tools are made by inert material – stainless steel, easy to clean and sterilize which does not cause change in the sample that would affect the results of the further analysis.

Raw materials sampling tools are washed in Riebesam PH-26/03 pharma grade washing machines, using alkaline detergent and neutralization agent.

The used detergent is Deconex CIP power-x, a highly alkaline cleaning agent, intended for cleaning residues of active components, excipients and reagents. The detergent is used at concentration of 0.7% (*V/V*). After the alkaline detergent, neutralization is performed with neutralizer Deconex CIP acid. The neutralizer is used at concentration of 0.3% (*V/V*).

For this cleaning validation study three consecutive cleaning cycles were performed following predefined cleaning procedure.

Sampling methods

Sampling was carried out according to a defined sampling plan using swab and rinse sampling techniques. Samples were collected from predefined

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locations after cleaning, first for microbiological, then for chemical analysis.

Analytical methods

Physico-chemical analysis included specific HPLC methods and non-specific methods (pH, conductivity, and total organic carbon – TOC). Microbiological quality was assessed as total aerobic microbial count (bioburden).

Results and discussion

Physico-chemical results

HPLC analysis demonstrated that residues of the selected worst-case APIs (Propyphenazone and Cefixime) were either below the limit of detection or well below the calculated Maximum Allowable Carryover (MACO) limits at all sampling locations, confirming the effectiveness of the cleaning procedure.

Non-specific analytical parameters (pH, conductivity, and TOC) were within the predefined acceptance criteria, confirming the effective removal of residues of cleaning agents and pharmaceutical ingredients.

Microbiological results

Microbiological analysis demonstrated that bioburden levels for both swab and rinse samples remained within the acceptance criteria at all sampling points. Low microbial counts were observed after use, after cleaning, and during the evaluated holding times, indicating adequate control of microbiological contamination.

Hold time evaluation

The evaluation of Dirty Hold Time (3 days) showed no significant impact on the cleanliness of the sampling tools prior to cleaning. Similarly, Clean Hold Time (15 days) confirmed that the equipment maintained an acceptable microbiological quality during storage.

The obtained results demonstrate that the applied cleaning procedure is effective and reproducible, ensuring consistent removal of chemical residues and microbiological contamination. The analytical methods used were suitable for verifying the cleaning performance and confirming compliance with the predefined acceptance criteria (Moura et al., 2025).

Conclusion

The results of this study confirm that the validated cleaning procedure for sampling tools is effective and reproducible in removing API residues, excipients, cleaning agents, and microbiological contamination to levels below the established acceptance criteria.

All physico-chemical and microbiological parameters were within predefined limits, demonstrating consistent cleaning performance across all sampling tools and cleaning cycles.

The study also confirms that a Dirty Hold Time (DHT) of 3 days and a Clean Hold Time (CHT) of 15 days are acceptable under defined conditions, ensuring maintained cleanliness during use and storage.

The validated cleaning procedure can therefore be routinely applied in Quality Control, ensuring compliance with GMP requirements and minimizing the risk of cross-contamination.

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