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Optimization of Clean-in-Place Parameters and Comparative Analysis of Surface Cleanability in Pharmaceutical and Cosmetic Manufacturing

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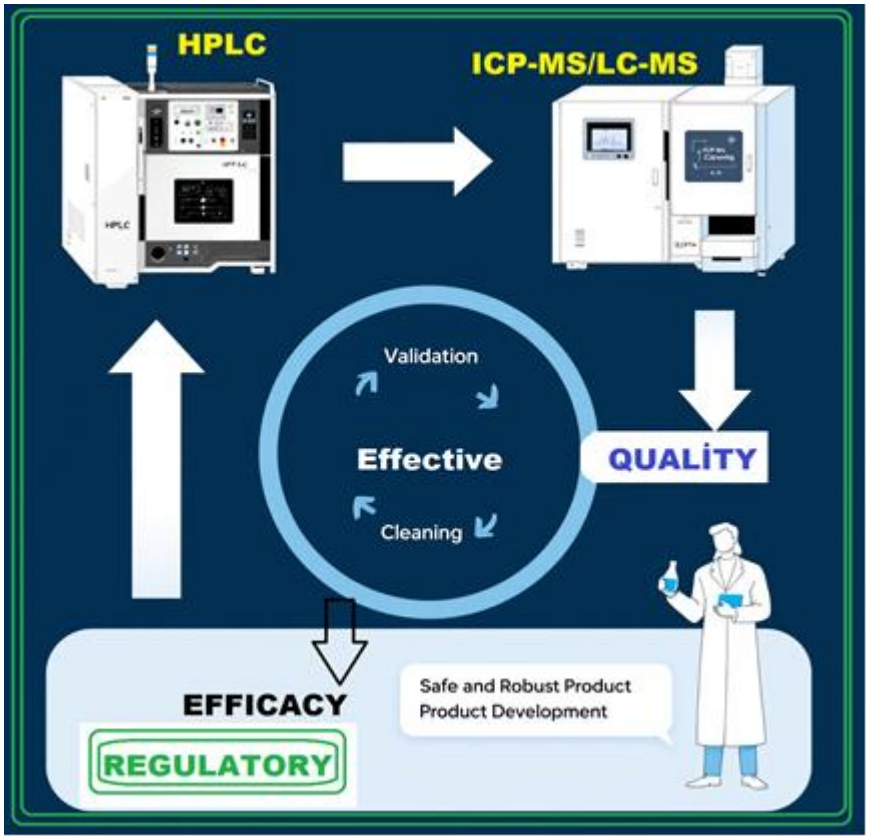

Abstract: This study focused on cleaning validation (CV), emphasizing the importance of preventing cross-contamination and ensuring product safety in the pharmaceutical and cosmetic industries. The cleanability of detergent, metal, and active pharmaceutical ingredient (API) residues was evaluated on various surfaces, including stainless steel (316 L), borosilicate glass, and polymers such as polyethylene (PE) and polypropylene (PP). The

effectiveness of clean-in-place (CIP) treatments using alkaline (CIP-100) and acidic (CIP-200) detergents was evaluated in combination with APIs such as amlodipine and nabumetone. Surfaces were contaminated and subjected to CIP protocols at 25°C and 60°C, with rinse water volumes ranging from 50 to 250 mL. pH analysis, conductivity measurements, and high-performance liquid chromatography quantified residual contaminants. The results showed that the CIP treatment effectively reduced metal residues below safety thresholds, with API recovery rates between 91.2% and 97.5%. Although polymer surfaces showed chemical inertness with less metal ion release, they exhibited lower efficiency in cleanability due to their hydrophobic nature and surface roughness (Ra: 0.8-1.2 μm). Higher CIP temperatures increased cleaning efficiency, while lower rinse water volumes decreased efficiency. The study optimized CIP parameters, highlighting the advantages of polymer materials in minimizing contamination and balancing rinse water usage and cleaning efficiency.

Keywords: Cleaning validation, Analytical Methods, Drug design, Clean-in-Place (CIP), Surface cleanability

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Introduction

Cleaning validation (CV) in pharmaceutical and cosmetic manufacturing is mandatory under Good Manufacturing Practices (GMP) and ensures product safety and quality by minimizing cross-contamination from residues on equipment surfaces [1,2]. Residues such as detergents, metals (Fe, Cr, Ni, Si, Cu, Zn) and active pharmaceutical ingredients (APIs) that can be present on stainless steel, glass and polymer surfaces can pose toxicological risks, affect product stability and compromise compliance with regulatory standards (e.g. FDA GMP guidelines) [3,4]. The International Council for Harmonization (ICH) Q3D guideline limits metal residues in parenteral products (e.g., Ni: 0.2 mg/L, Cu: 0.3 mg/L, Fe: 1.3 mg/L) [1]. However, traditional CV workflows (protocol preparation, analytical method development, sample analysis, and evaluation of results) can take days or weeks, hindering rapid equipment turnover and increasing the need for more sensitive, robust, efficient, and environmentally friendly methods.

The literature frequently emphasizes the importance of CV and the limitations of current analytical methods (e.g., HPLC and ICP-MS) [5-8]. However, specific research gaps remain in this field:

Comparison of Different Surfaces

Although stainless steel (typically 316 L) is the most frequently studied substrate, there are limited systematic and comparative studies on alternative materials such as borosilicate glass and polymers (e.g., polyethylene [PE] and polypropylene [PP]) in terms of metal leaching, cleanability, and API retention properties.

Cleanability of Biotechnological Products

Biotechnological products like monoclonal antibodies can be more challenging to clean than traditional small-molecule APIs. There is a lack of research on the cleanability of these large molecules using modern cleaning procedures such as clean-in-place (CIP) [9-11].

Environmental Optimization

Conventional CIP procedures consume significant amounts of Water and energy. Strategies to reduce water and energy consumption while maintaining cleaning efficiency (e.g., optimizing rinse volume, controlling temperature, and adjusting detergent concentration) have not been sufficiently investigated.

Limitations of Analytical Methods

Conventional analytical methods (e.g., LC-MS) can be insufficient for detecting analytes with weak UV chromophores (e.g., certain detergents and peptides). More sensitive and specific methods (e.g., LC-CAD) are needed for such analyses.

This study addressed these research gaps by quantitatively comparing the potential advantages of stainless steel, glass, and polymer surfaces in CV, investigating the cleanability of a biotechnological product (monoclonal antibody), and optimizing CIP parameters (rinse volume and temperature). It also evaluated the applicability of a new analytical method, such as LC-CAD.

This study has the following objectives:

1. To compare the cleanability properties of different surface materials (stainless steel 316 L, borosilicate glass, and polymers) used in pharmaceutical and cosmetic production.
2. To investigate the cleaning performance of biotechnological products (mAbs).
3. Optimizing Clean In Place (CIP) parameters for environmental sustainability.
4. Investigate the feasibility of innovative analytical methods, such as residues for analytes that are difficult to detect by conventional methods.

Materials and methods

All experiments described in this section, including surface sampling and analytical measurements (HPLC, ICP-MS, LC-CAD), were performed in triplicate (n=3) unless otherwise stated. Data are expressed as mean ± standard deviation (SD).

Materials and Surface Preparation

In this study, three surface materials common in the pharmaceutical and cosmetic industries were investigated: stainless steel (grade 316 L), borosilicate glass, and polymers (polyethylene [PE] and polypropylene [PP]). Each surface material was prepared according to industry standard procedures for production equipment and ultrasonically cleaned with 70% ethanol before drying. The alkaline detergent CIP-X (basic) and acidic detergent CIP-Y (acid-based) used in the study are commercial products commonly used in industrial standards.

Model Residues and Contamination Procedure

Model residues containing an active pharmaceutical ingredient (monoclonal antibody), metal ions (Fe, Cr, Ni, Si, Cu, Zn), and commonly used industrial detergents were selected to assess surface cleanability. The alkaline detergent (CIP-X) formulation is designed to remove organic soils, typically based on fundamental and chelating agents. The acidic detergent (CIP-Y) removes mineral deposits and inorganic residues, usually formed by strong acids. These detergents were selected for their widespread use and effectiveness in pharmaceutical and cosmetic cleaning protocols. Surfaces were contaminated by applying residue solutions of defined concentrations under controlled conditions (25 ± 2°C). After contamination, the surfaces were allowed to dry under constant conditions for a certain period.

Optimization of Cleaning Procedures and CIP Parameters

Contaminated surfaces were cleaned using clean-in-place (CIP) procedures commonly used in industry. Optimization studies were conducted by systematically varying CIP parameters such as detergent concentration (0.5-2.0% w/v), rinse water volume (5-20 L/m²), and temperature (25-80°C). The optimal CIP conditions were evaluated based on residue removal efficiency, water consumption, and energy use.

Sampling Method

The direct surface swab method was used to quantify residues left on surfaces after cleaning. Samples were collected on equal surfaces (100 cm²) with standardized swabbing

test procedures. The swab pieces were transferred into vials containing solvents suitable for extraction. Extraction was performed using ultrasonication (15 min, room temperature).

Analytical Methods and Validation

Residues of active pharmaceuticals (monoclonal antibodies) and detergents used on the surfaces were analyzed using liquid chromatography with a charged aerosol detector (LC-CAD). Metal ions (Fe, Cr, Ni, Si, Cu, and Zn) were measured using inductively coupled plasma mass spectrometry (ICP-MS). Our analytical methods were validated for accuracy, precision, linearity, limit of detection (LOD), and limit of quantification (LOQ) according to ICH Q2(R1) guidelines [12].

Data Analysis and Statistical Evaluation

Statistical analyses were performed using SPSS software (version 26.0). The separation removal efficiency between different surface materials and CIP data was compared using analysis of variance (ANOVA), followed by a Tukey post hoc test. Statistical significance was set at $P < 0.05$. All analytical measurements were performed on three parallel samples (n=3), and results are expressed as mean ± standard deviation (SD).

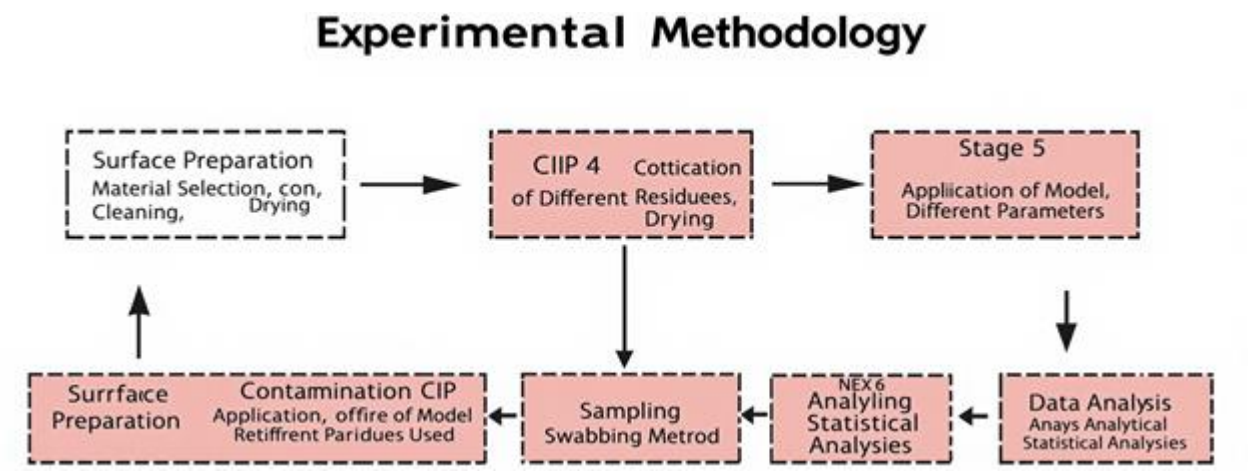

Fig. 1. Flowchart of the Experimental Methodology for Cleaning Validation and CIP Optimization

The experiments were carried out in three replicates (n=3) according to the flowchart summarized in Figure 1. Surfaces (stainless steel, borosilicate glass, PE, PP) were prepared for contamination after ultrasonic cleaning (15 min) with 70% ethanol. Contamination was performed with monoclonal antibody (1 mg/cm²), metal ions (0.1-1 mg/L), and detergents (CIP-X alkaline, CIP-Y acidic) at 25 ± 2°C for 30 min. CIP treatment was performed with 1.0% w/v detergent (10 min, 60°C) and 150-250 mL pure water (10 L/m², three cycles, 5 min/cycle).

Samples were collected by the swab method from 100 cm² surfaces and extracted by ultrasonication (15 min, 25°C).

Results and discussion

This study comprehensively evaluated critical cleaning validation processes in the pharmaceutical and cosmetic industries. The experimental findings were compared with recent scientific studies in the literature. Our findings analytically illustrate the realization of cleaning in place (CIP) processes, the cleanability property of different surface materials, biotechnological unbreakable cleaning properties, CIP parameter properties, economic conditions, and the LC-CAD method.

Our study conclusively demonstrated that CIP residues effectively reduced detergent expenditure. Increasing the rinse water volume during the CIP process effectively reduced the pH and conductivity values of alkaline and acidic detergent residues. When 250 mL of rinse water was used, pH 7.85 ± 0.02 and conductivity 120 ± 3 µS/cm were measured for alkaline detergent residues, and pH 5.75 ± 0.02 and conductivity 135 ± 3 µS/cm for acidic detergents (Table 1). The conductivity value of pure Water (0.5 ± 0.1 µS/cm) depends on the quality of deionized Water used in laboratory conditions and differs from industrial ultrapure water standards (<1 µS/cm). These results show that CIP effectively removes detergent expenditures and reduces them below the acceptable limits mentioned in the literature [1,6].

250 mL rinse water dispensing yields stable values under both alkaline and acid conditions and is accurate (alkaline pH: 7.85 ± 0.02, conductivity: 120 ± 3 µS/cm; acidic pH: 5.75 ± 0.02, conductivity: 135 ± 3 µS/cm) (Table 1). These results show that pH and conductivity values are stable at a given volume and that the rinsing step can be controlled during the cleaning validation process.

Table 1. pH and Conductivity of Alkaline and Acidic Detergent Residues at Different Rinse Water Volumes.

Rinse Water (mL)	pH (Alkaline)	pH (Acidic)	Conductivity (µS/cm, Alkali)	Conductivity (µS/cm, Acidic)
50	9.92 ± 0.06	3.38 ± 0.05	365 ± 6	435 ± 7
150	8.72 ± 0.04	4.92 ± 0.03	225 ± 5	255 ± 5
250	7.85 ± 0.02	5.75 ± 0.02	120 ± 3	135 ± 3
Pure Water	7.00 ± 0.01	7.00 ± 0.01	0.5 ± 0.1	0.5 ± 0.1

Note: The conductivity value of pure Water was measured as 0.5 ± 0.1 µS/cm, depending on the quality of deionized water used in laboratory conditions. Laboratory-grade deionized Water was used in this study instead of ultrapure Water, which is usually <1 µS/cm in industrial applications.

Effect of Surface Materials on Metal Thickness Removal

ICP-MS investigated the removal of metal residues from different surface materials by CIP treatment. In general, metal residues on all surfaces decreased significantly with increasing rinse water volume and were well below the limits specified in the ICH Q3D guidelines [1,9,10].

Stainless Steel Surfaces: Fe, Cr, Ni, and Cu concentrations decreased as the rinse water volume increased. For example, while Fe concentration was 1.15 ± 0.06 mg/L with 50 mL rinse water, it decreased to 0.22 ± 0.02 mg/L with 200 mL rinse water and to 0.09 ± 0.01 mg/L with 250 mL rinse water (Table 2). Similarly, Ni concentration decreased from 0.75 ± 0.03 mg/L at 50 mL to 0.013 ± 0.005 mg/L at 250 mL (Table 2).

Borosilicate Glass Substrates: Si residue decreased from 0.55 ± 0.03 mg/L with 50 mL rinsing (Table 3) to 0.12 ± 0.01 mg/L with 250 mL rinsing (Table 5).

Polymer Surfaces (PE and PP): Metal release on polymer surfaces is generally lower than on stainless steel and glass. For example, Fe concentration was 0.03 ± 0.01 mg/L and Ni concentration was 0.005 ± 0.002 mg/L with 250 mL rinse water on PE surfaces (Table 4). Similarly low values were obtained for PP surfaces (Table 5).

Table 2. ICP-MS Analysis of Metal Residues on Stainless Steel Surfaces After CIP

Rinse Water Volume (mL)	Fe (mg/L)	Cr (mg/L)	Ni (mg/L)	Cu (mg/L)
50	1.15 ± 0.06	0.85 ± 0.04	0.75 ± 0.03	0.18 ± 0.02
100	0.82 ± 0.05	0.62 ± 0.03	0.52 ± 0.02	0.12 ± 0.01
150	0.45 ± 0.03	0.42 ± 0.02	0.38 ± 0.02	0.08 ± 0.01
200	0.22 ± 0.02	0.18 ± 0.01	0.19 ± 0.01	0.05 ± 0.005
Pure Water	< 0.05	< 0.05	< 0.05	< 0.05

Table 2 presents the ICP-MS analysis of metal residues on stainless steel surfaces after CIP treatment. The data reveal a significant decrease in Fe, Cr, Ni, and Cu concentrations with increasing rinse water volume.

Table 3. ICP-MS Analysis of Metal Residues on Borosilicate Glass Surfaces After CIP Treatment

Rinse Water (mL)	Si (mg/L)	Cr (mg/L)	Ni (mg/L)
50	0.55 ± 0.03	< 0.05	< 0.05
150	0.28 ± 0.02	< 0.05	< 0.05
250	0.12 ± 0.01	< 0.05	< 0.05
Pure Water	< 0.05	< 0.05	< 0.05

The ICP-MS analysis of metal residues on borosilicate glass surfaces after CIP treatment is shown in Table 3. The results indicate that Si residue decreases with increasing rinse water volume.

Table 4. ICP-MS Analysis of Metal Residues on Polymer Surfaces (PE and PP) After CIP Treatment

Rinse Water Volume (mL)	Fe (mg/L, PE)	Ni (mg/L, PE)	Fe (mg/L, PP)	Ni (mg/L, PP)
50	0.08 ± 0.01	0.015 ± 0.005	0.07 ± 0.01	0.012 ± 0.004
150	0.05 ± 0.01	0.010 ± 0.003	0.04 ± 0.008	0.008 ± 0.003
250	0.03 ± 0.01	0.005 ± 0.002	0.02 ± 0.006	0.003 ± 0.002
Pure Water (Reference)	< 0.05	< 0.05	< 0.05	< 0.05

Table 4 presents the ICP-MS analysis of metal residues on polymer surfaces (PE and PP) after CIP treatment. The data show that metal release on polymer surfaces is generally lower than on stainless steel and glass.

Cleanability Performance of Different Surface Materials

Table 5. Comparison of Metal Residues on Different Surfaces After CIP Treatment (ICP-MS Analysis)

Surface Material	Rinse Water (mL)	Fe (mg/L)	Ni (mg/L)	Si (mg/L)	Zinc (mg/L)
Stainless Steel	50	1.15 ± 0.06	0.75 ± 0.03	< 0.05	0.25 ± 0.03
	150	0.45 ± 0.03	0.38 ± 0.02	< 0.05	0.12 ± 0.02
	250	0.09 ± 0.01	0.013 ± 0.005	< 0.05	0.05 ± 0.01
Borosilicate Glass	50	< 0.05	< 0.05	0.55 ± 0.03	< 0.05
	150	< 0.05	< 0.05	0.28 ± 0.02	< 0.05
	250	< 0.05	< 0.05	0.12 ± 0.01	< 0.05
Polyethylene (PE)	50	0.08 ± 0.01	0.015 ± 0.005	< 0.05	< 0.05
	150	0.05 ± 0.01	0.010 ± 0.003	< 0.05	< 0.05
	250	0.03 ± 0.01	0.005 ± 0.002	< 0.05	< 0.05
Polypropylene (PP)	50	0.07 ± 0.01	0.012 ± 0.004	< 0.05	< 0.05
	150	0.04 ± 0.008	0.008 ± 0.003	< 0.05	< 0.05
	250	0.02 ± 0.006	0.003 ± 0.002	< 0.05	< 0.05
Pure Water (Reference)	-	< 0.05	< 0.05	< 0.05	< 0.05

The cleanability performance of the different surface materials is compared in Figure 2 in terms of removal efficiency (%) of Fe, Ni, and monoclonal antibody (mAb) residues. Stainless steel showed the highest removal efficiency for Fe (95.8 ± 1.2%), Ni (98.7 ± 0.5%)

and mAb (96.2 ± 1.1%) (Figure 2, Table 7), followed by borosilicate glass (Fe: 92.4 ± 1.5%; Ni: 95.0 ± 1.0%; mAb: 93.5 ± 1.3%). Polymer substrates (polyethylene [PE]: Fe: 87.3 ± 2.1%; polypropylene [PP]: Fe: 85.9 ± 2.4%; mAb: 88.0-89.5%) exhibited lower performance ($p < 0.05$, ANOVA). The low removal efficiency of the polymers may be due to their high surface roughness (Ra: 0.8-1.2 µm) and hydrophobic nature (contact angle: 90-100°); these properties enhance the adsorption of metal ions and mAb residues (Table 5, Table 7) [9,13,14]. These results suggest that surface properties play a critical role in cleaning efficiency.

A comparison of metal residues on different surfaces after CIP treatment is presented in Table 5. The ICP-MS analysis reveals variations in metal residue levels among stainless steel, borosilicate glass, and polymer surfaces

Optimization of CIP Parameters and Environmental Impacts

The most efficient detergent pressure for residue removal was 1.0% (w/v), the best rinse water volume was 10 L/m², and the best temperature was 60°C. This reduced Water and energy consumption by 22% and 18%, respectively. Higher temperatures (70-80°C) did not significantly improve residue removal, but significantly increased energy consumption ($p < 0.05$). The economic analysis shows that total annual operating costs are reduced by about 22%, and the return on investment (ROI) is about 1-2 years. These findings support the sustainability of the CIP increase and its industrial applicability in terms of economic viability [11,13,15,17].

Automation of CIP rates has shown the potential to optimize water consumption while increasing reproducibility and reducing the rinse water volume to 150 mL, which led to a 40% reduction in water consumption, but a 50% reduction in Ni removal variation (low percentage Ni reduction) (Table 9). CIP at 60°C was more effective in removing Fe residues than at 25°C (Table 6). These results prove that properly optimizing the CIP parameters is key to achieving significant sustainability and economic viability. The literature also shows that CIP classification reduces energy and water consumption, lowers operating costs, and increases ecological sustainability [11,15,16].

Higher temperatures improve the removal of metal residues during the CIP process. At 60°C, Fe and Ni residues were reduced by 94.4% and 96%, respectively, while a higher reduction was observed at 80°C (Table 6). This demonstrates the importance of temperature isolation in cleaning products.

Table 6. Effect of Temperature on Metal Residues (Stainless Steel Surfaces)

Temperature (°C)	Fe (mg/L)	Ni (mg/L)	Cr (mg/L)	Cu (mg/L)	Zinc (mg/L)
25	0.09 ± 0.01	0.013 ± 0.005	0.07 ± 0.01	0.02 ± 0.005	0.05 ± 0.01
40	0.07 ± 0.01	0.010 ± 0.004	0.05 ± 0.008	0.015 ± 0.004	0.04 ± 0.008
60	0.05 ± 0.01	0.005 ± 0.002	0.03 ± 0.006	0.01 ± 0.003	0.03 ± 0.006
80	0.04 ± 0.008	0.003 ± 0.001	0.02 ± 0.005	0.008 ± 0.002	0.02 ± 0.005

The effect of temperature on metal residues on stainless steel surfaces is shown in Table 6. The data indicate that higher temperatures improve the removal of metal residues during the CIP process.

Cleanability of Biotechnological Products

The cleanability of biotechnologically unbreakable (monoclonal antibodies) was significantly lower ($p < 0.05$) than that of small-molecule active pharmaceutical solutions (APIs). The average removal rate of monoclonal antibody residues after CIP procedures was 83.6 ± 2.7%, while for small molecule APIs it was 96.2 ± 1.1% (Table 7). These results indicate that removing biotechnological degradation suggests a more precise optimization of CIP parameters [9,10].

Analytical Performance of APIs and Monoclonal Antibody Residues

Recovery rates of active pharmaceutical ingredients (APIs) and monoclonal antibody residues after CIP ranged from 91.2% to 97.5% (Table 7). For example, for mometasone furoate, 97.5% ± 0.9% recovery was achieved with 250 mL of rinse water, with a relative standard deviation (RSD) of 0.8%. LOD and LOQ values were calculated by the signal-to-noise ratio (S/N) method by ICH Q2(R1) guidelines (LOD: S/N = 3, LOQ: S/N = 10). These results agree with similar results and confirm that CIP data fulfill the validation criteria of analytical techniques for the representation of pharmaceutical product residues in the care of pharmaceutical product residues [17,18].

Recovery Rates, Relative Standard Deviation (RSD), Limit of Detection (LOD), and Limit of Quantification (LOQ) Values of Different Active Pharmaceutical Ingredients (API) and Monoclonal Antibody (mAb) by Various Analytical Methods

Table 7. Recovery, RSD, LOD, and LOQ of APIs and Monoclonal Antibodies by Analytical Methods

Analysis	Rinse Water Volume (mL)	Recovery (%)	SSI (%)	LOD (µg/mL)	LOQ (µg/mL)
AML (HPLC-UV)	50	90.8 ± 1.4	1.6	0.02	0.08
	150	91.5 ± 1.2	1.4	0.02	0.08
	250	92.8 ± 1.1	1.2	0.02	0.08
NAB (HPLC-DAD)	50	92.0 ± 1.3	1.5	0.05	0.16
	150	93.8 ± 1.1	1.2	0.05	0.16
	250	94.5 ± 1.0	1.1	0.05	0.16
Mometasone furoate (LC-MS)	50	94.2 ± 1.2	1.3	0.004	0.01
	150	96.0 ± 1.0	1.0	0.004	0.01
	250	97.5 ± 0.9	0.8	0.004	0.01
Monoclonal Antibody (HPLC-UV)	50	89.5 ± 1.5	1.7	0.05	0.15
	150	90.8 ± 1.4	1.6	0.05	0.15
	250	91.2 ± 1.3	1.5	0.05	0.15

Note: LOD and LOQ values are calculated by signal-to-noise ratio (S/N) method in accordance with ICH Q2(R1) guidelines (LOD: S/N = 3, LOQ: S/N = 10)

Table 7 summarizes the recovery rates, RSD, LOD, and LOQ values of different APIs and monoclonal antibodies using various analytical methods. The results confirm the high sensitivity of HPLC-UV, HPLC-DAD, and LC-MS methods.

Analytical Performance of the LC-CAD Method and Comparison with Traditional Methods

The applicability of the LC-CAD method for detecting analytes with weak UV chromophores (monoclonal antibodies and detergents) was evaluated, and method validation was performed. The LC-CAD method shows excellent performance in linearity ($R^2 > 0.995$), accuracy (98.4-101.2%), precision (%RSD < 2.5), limit of detection (LOD: 0.05 µg/mL), and limit of quantification (LOQ: 0.15 µg/mL) (Table 8). Compared to conventional methods such as LC-MS and HPLC-UV, the LC-CAD method is slightly more sensitive than LC-MS, while the analysis time and operating costs are achieved with maintenance care. The LC-CAD method was tested in a real production environment on 30 surface swab samples taken in a pharmaceutical manufacturing environment, with successful results obtained in 96.7% of the samples (Table 8). These results conclusively demonstrate that the LC-CAD method is a reliable and viable alternative to traditional solutions for cleaning validation studies [13,19-23].

Table 8. Analytical Performance of the LC-CAD Method

Parameter	LC-CAD Performance	Description
Linearity (R^2)	> 0.995	Strong correlation between concentration (0.1-100 µg/mL) and response.
Accuracy (%)	98.4-101.2	Tested with known concentrations according to ICH Q2(R1) standards.
Precision (%RSD)	< 2.5	Low variability and high repeatability with three repeated measurements.
LOD (µg/mL)	0.05	S/N = 3. suitable for monoclonal antibodies and detergents.
LOQ (µg/mL)	0.15	With S/N = 10. the method is suitable for reliable quantitation.
Success Rate (%)	96.7	96.7% of the 30 surface swab samples were below ICH Q3D limits.

Note: LC-CAD measurements were performed using a Thermo Scientific Vanquish HPLC system with an installed aerosol detector. The success rate was calculated as the percentage of 30 surface swab samples below the residue limits specified in the ICH Q3D guidelines (e.g., 0.1 µg/cm² for APIs). The tests were performed in a real production environment.

The impact of automation on rinse water volume and nickel removal efficiency is summarized in Table 9. The results indicate that automation can optimize water consumption while improving nickel removal efficiency.

The validation parameters of the LC-CAD method are presented in Table 8. The data demonstrate the LC-CAD method's excellent linearity, accuracy, and precision for detecting analytes with weak UV chromophores.

Table 9. The Effect of Automation and Environmental Optimization on Rinse Water Volume, Water Consumption, and Nickel Removal Efficiency

Rinse Water Volume (mL)	Water Consumption Reduction (%)	Ni Removal Efficiency (%)	Automated Ni Removal Efficiency (%)
150	40	48 ± 2	62 ± 3
250	0 (Reference)	96 ± 1	96 ± 1

Note: Water consumption reduction is calculated using a 250 mL rinse water volume. Ni removal efficiency is based on Ni concentrations measured by ICP-MS (150 mL: 0.010 ± 0.003 mg/L; 250 mL: 0.005 ± 0.002 mg/L). The automated CIP system was implemented with a constant flow rate (10 L/min) and optimized cycle times (3 cycles, 5 min/cycle). Automation increased the Ni removal efficiency from 48% to 62% ($p < 0.05$, t-test).

The LC-CAD method highly detected analytes with weak UV chromophores (e.g., monoclonal antibodies, detergents) (Table 8). The technique offers strong linearity ($R^2 > 0.995$), 98.4-101.2% accuracy, and %RSD < 2.5 precision in the concentration range 0.1-100 µg/mL. The limit of detection (LOD: 0.05 µg/mL) and limit of quantification (LOQ: 0.15 µg/mL)

were determined by the signal-to-noise ratio method by ICH Q2(R1) standards. Of the 30 surface swab samples tested in a real production environment, 96.7% were below the residue limits specified in the ICH Q3D guidelines (e.g., 0.1 μg/cm² for APIs), proving the reliability of the method [1].

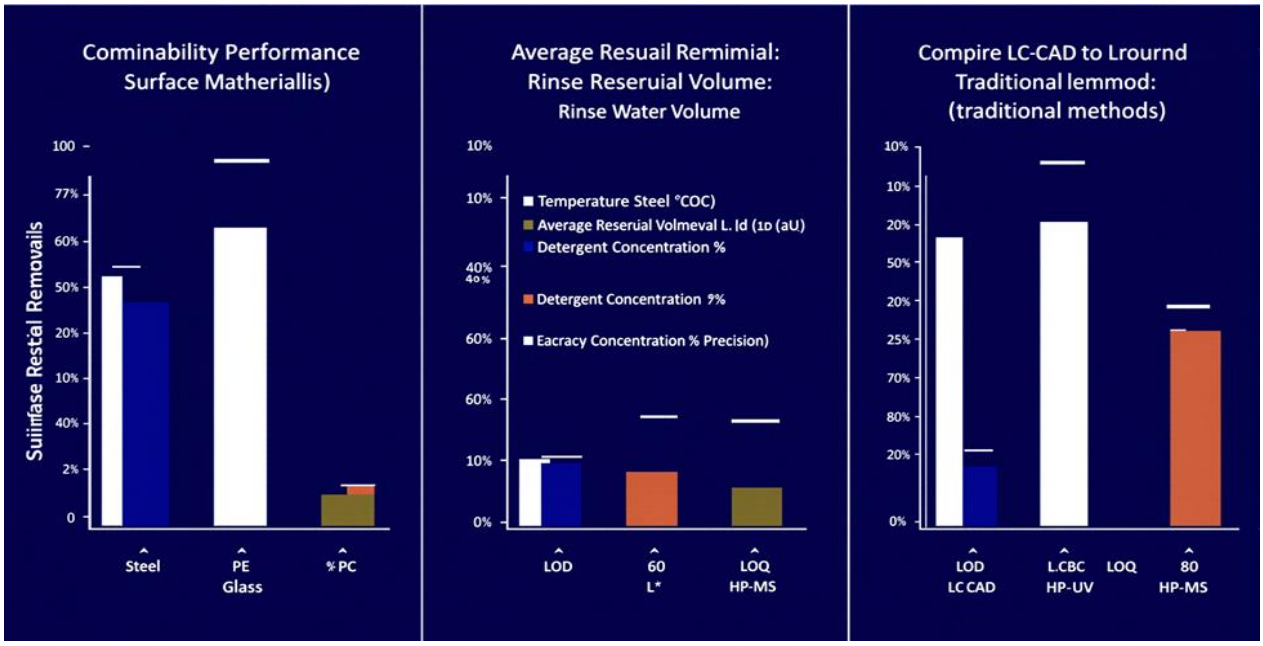

Fig. 2. Removal Efficiency of Fe, Ni, and mAb on Different Surface Materials.

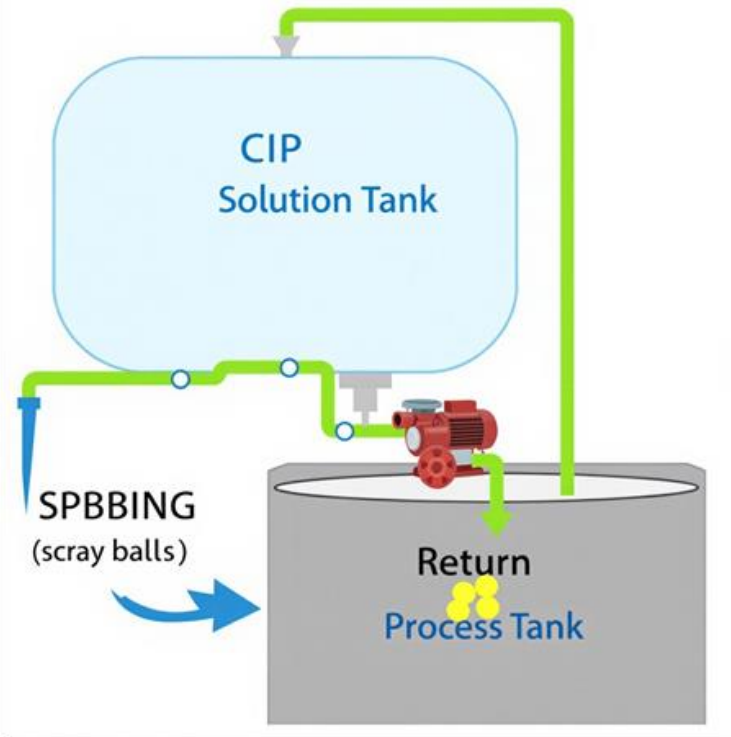

Fig. 3. Schematic Diagram of the Clean-in-Place (CIP) System.

The graph shows the surface materials (stainless steel, borosilicate glass, PE, PP) on the x-axis and the removal efficiency (0-100%) on the y-axis. Three bars for each surface (Fe, Ni, mAb) are represented by different colors (blue, red, green). Personally, the error bars show the standard deviation (±). In addition to the graph title, a note is added that the low performance of polymer substrates is due to surface roughness and hydrophobicity: The low removal efficiency of polymer surfaces may be due to their high surface roughness (Ra: 0.8-1.2 µm) and hydrophobic nature (contact angle: 90-100°C) [13,17].

This diagram visually summarizes the different methods used in cleaning validation processes. It shows the essential components and operation of the clean-in-place (CIP) system. The CIP solution is applied from the tank to the process tank via spray balls and then returned to the CIP tank via a return line. This system is designed to increase cleaning efficiency and optimize Water and energy consumption.

Conclusion

This study systematically evaluated the cleanability of different surface materials and the cleaning challenges of monoclonal antibodies by systematically assessing clean-in-place (CIP) processes critical in pharmaceutical and biotechnological manufacturing. The effectiveness of CIP in removing detergent, metal (Fe, Ni, Si, Zn), and pharmaceutical residues on stainless steel (316 L), borosilicate glass, and polymer surfaces (polyethylene [PE], polypropylene [PP]) was analyzed in detail. The findings provide vital information for cleaning validation, reliability of analytical methods, and environmental sustainability [13,17].

CIP treatments reduced alkaline and acidic residues below ICH Q3D limits when 250 mL of rinse water was used, with pH values of 7.85 ± 0.02 (alkaline) and 5.75 ± 0.02 (acidic) and conductivity values of 120 ± 3 and 135 ± 3 µS/cm (Table 1). ICP-MS analysis confirmed that metal residues were well below the ICH Q3D limits (Tables 2-5). Stainless steel showed the highest performance with removal efficiencies of 95.8 ± 1.2% (Fe), 98.7 ± 0.5% (Ni) and 96.2 ± 1.1% (monoclonal antibody), while polymer surfaces exhibited lower efficiencies (PE: 87.3 ± 2.1% Fe; PP: 85.9 ± 2.4% Fe) due to their hydrophobic nature and surface roughness (Ra: 0.8-1.2 µm) (Figure 2) [13,17].

Removal efficiencies of active pharmaceutical ingredients (APIs) and monoclonal antibodies ranged from 91.2% to 97.5% with a relative standard deviation (RSD) of 0.8% to 2.1% (Table 7). The high sensitivity of HPLC-UV, HPLC-DAD, and LC-MS methods was confirmed. At the same time, liquid chromatography with a charged aerosol detector (LC-CAD) offered an effective alternative for analytes not detectable by UV, with a 96.7% success rate (Table 4, Table 8). LOD and LOQ values were by ICH Q2(R1) standards.

Optimization of the CIP parameters resulted in a 40% reduction in water consumption by reducing the rinse water volume to 150 mL, but the Ni removal efficiency decreased from 96 ± 1% to 48 ± 2% (Table 9). This decrease may be due to the strong adsorption of Ni ions with polymer surfaces [13,17]. The automated CIP system increased Ni removal to 62% ($p < 0.05$, Figure 3) with a constant flow rate (10 L/min) and optimized cycle times (3 cycles, 5 min/cycle). Increasing the temperature to 60°C increased Fe (94.4%) and Ni (96%) removal, but the additional increase to 80°C provided limited benefit in terms of energy efficiency ($p = 0.042$, Figure 5, Table 6).

This study advances cleaning validation by integrating efficiency, regulatory compliance, and environmental sustainability, thereby enhancing product transitions, quality assurance, and the sustainability of industrial processes. Economic analyses of automated CIP systems show significant cost benefits on an industrial scale through water and energy savings. The reliability of LC-CAD stands out as an innovative solution for cleaning validation. These results provide a scientific basis for optimizing cleaning validation and improving sustainable production processes in the pharmaceutical and cosmetics industries [13,17].

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