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Comparative Assessment of Levodopa and Carbidopa Cleanability from Stainless Steel Surfaces: A Risk-Based Approach for Pharmaceutical Cleaning Validation

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Abstract

Background: Cleaning validation represents a critical component of pharmaceutical quality assurance, requiring scientific evidence that manufacturing equipment can be consistently cleaned to predetermined acceptance criteria. The selection of worst-case products for validation studies necessitates comparative cleanability assessments based on physicochemical properties and empirical data.

Objective: To systematically evaluate and compare the cleanability profiles of Levodopa and Carbidopa from stainless steel surfaces using multiple cleaning methodologies, establishing a scientifically justified worst-case product for cleaning validation protocols.

Methods: Stainless steel coupons (316L, 50×50mm) were contaminated with 1000 µg of each compound and subjected to three cleaning procedures: purified water submersion, 0.1N sodium hydroxide solution, and 1% detergent solution, each with 2-minute contact time. Residual contamination was quantified using validated HPLC-UV methodology with swab sampling. Statistical analysis included two-sample t-tests and effect size calculations (Cohen's d).

Results: Water cleaning demonstrated significant differences in removal efficiency between Levodopa (93.00±0.14%) and Carbidopa (86.05±0.21%; p=0.0012, Cohen's d=40.83). Alkaline cleaning improved both compounds' removal (Levodopa: 99.58±0.04%, Carbidopa: 98.25±0.07%; p=0.0018), while detergent achieved optimal cleaning (>99.6% for both). Carbidopa consistently exhibited lower cleanability across all methods, with residuals exceeding acceptance limits (10 µg/coupon) under water-only cleaning (138-141 µg/coupon).

Conclusions: Carbidopa represents the worst-case product for cleaning validation due to significantly lower removal efficiency, attributed to its reduced aqueous solubility (0.2 mg/mL versus 2.0 mg/mL for Levodopa). These findings support risk-based cleaning validation approaches and emphasize the necessity of alkaline or detergent-based cleaning for adequate contamination control in multi-product facilities.

Keywords: Cleaning Validation; Levodopa; Carbidopa; Worst-Case Determination; Cleanability Comparison; HPLC Analysis; Risk Assessment

1. Introduction

Pharmaceutical manufacturing facilities producing multiple products face significant challenges in preventing cross-contamination between production campaigns. Regulatory authorities worldwide, including the FDA, EMA, and ICH, mandate robust cleaning validation programs demonstrating that residual contamination levels remain below

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scientifically justified limits (FDA, 2011; EMA, 2014). The complexity of these requirements intensifies when manufacturing products with varying physicochemical properties, necessitating comprehensive understanding of compound-specific cleaning behaviors.

Levodopa (L-3,4-dihydroxyphenylalanine) and Carbidopa (L-α-hydrazine-α-methyl-β-3,4-dihydroxybenzene propanoic acid monohydrate) represent critical therapeutic agents in Parkinson's disease management, commonly co-formulated to optimize therapeutic efficacy. Despite their frequent combination in pharmaceutical preparations, these compounds exhibit distinct physicochemical properties that potentially influence their cleanability profiles. Levodopa demonstrates higher aqueous solubility (2.0 mg/mL) compared to Carbidopa (0.2 mg/mL), suggesting differential cleaning challenges.

The concept of worst-case product selection remains fundamental to efficient cleaning validation strategies. Rather than validating cleaning procedures for every product combination, regulatory guidance supports scientifically justified worst-case approaches, wherein the most challenging product validates the cleaning process for all products manufactured on shared equipment (ISPE, 2016). However, determining worst-case scenarios requires systematic evaluation beyond theoretical solubility considerations, incorporating factors such as surface adhesion, chemical stability during cleaning, and practical removal efficiency under standardized conditions.

Previous investigations have explored individual compound cleanability but lack direct comparative assessments under identical experimental conditions. Zhang et al. (2019) demonstrated solubility-cleanability correlations for various pharmaceutical compounds, while Kumar and colleagues (2020) emphasized the importance of surface interaction mechanisms. However, no published studies specifically compare Levodopa and Carbidopa cleanability, despite their widespread co-manufacturing.

This investigation addresses this knowledge gap through systematic comparative assessment of Levodopa and Carbidopa cleanability from stainless steel surfaces. Our objectives encompass: (1) quantitative determination of cleaning efficiency using multiple methodologies, (2) statistical validation of cleanability differences, (3) correlation of removal efficiency with physicochemical properties, and (4) establishment of scientifically justified worst-case determination for cleaning validation protocols.

2. Materials and Methods

2.1. Materials

2.1.1. Test Compounds and Reagents

Levodopa USP (≥98% purity) and Carbidopa USP (≥98% purity) were obtained from certified suppliers with accompanying certificates of analysis. HPLC-grade methanol and acetonitrile were sourced from Fisher Scientific. Phosphoric acid (85%) and sodium hydroxide pellets (ACS grade) were acquired from Sigma-Aldrich. CP200 cleaning solution was acquired from Steris. Purified water meeting USP specifications (conductivity <1.3 μS/cm, TOC <500 ppb) was generated using a Millipore Milli-Q system.

2.1.2. Equipment and Surfaces

Stainless steel coupons (316L grade, 50×50 mm, #4 brush finish) were manufactured to specifications matching pharmaceutical processing equipment (ASME BPE SF4 Designation (Ra ≤ 0.38 am)). Surface characterization was performed using profilometry to ensure consistency. An Agilent 1260 Infinity II HPLC system equipped with diode array detector was employed for analytical quantification. Additional equipment included calibrated analytical balance (Mettler Toledo XPE205, ±0.1 mg), ultrasonic bath (Branson 5510), and environmental chamber maintaining 22±3°C, 35±5% relative humidity.

2.2. Experimental Design

2.2.1. Study Design and Sample Size

A randomized complete block design was implemented with compound type (Levodopa, Carbidopa) and cleaning method (water, sodium hydroxide, detergent) as primary factors. Sample size calculations indicated n=2 per treatment provided 80% power to detect 5% differences in cleaning efficiency (α=0.05). Six coupons per compound were allocated across three cleaning methods, with additional controls for method validation.

2.2.2. Contamination Protocol

Stock solutions (1000 mg/L) were prepared by dissolving accurately weighed compounds in purified water. Solution pH was monitored to assess ionization states. Contamination involved applying 1.00 mL solution via calibrated micropipette in a standardized droplet pattern (10-15 drops) across coupon surfaces. Environmental conditions were maintained at 22.3±0.5°C, 35±2% RH during application and subsequent 24-hour drying period, simulating worst-case manufacturing scenarios.

2.2.3. Cleaning Procedures

Three cleaning methodologies were evaluated

- **Water Submersion**: Complete immersion in purified water (22±1°C) for 2 minutes
- **Alkaline Cleaning**: Submersion in 0.1N sodium hydroxide (pH 13.0) for 2 minutes, followed by water rinse
- **Detergent Cleaning**: Submersion in 1% CP200 solution (pH 2.0) for 2 minutes, followed by water rinse

Post-cleaning, coupons underwent controlled air-drying for minimum 30 minutes before sampling.

2.3. Analytical Methodology

2.3.1. Sample Collection and Extraction

Swab sampling employed polyester-tipped swabs (TX714A-Alpha) using validated technique covering entire coupon surface. Swabs underwent extraction in 5.0 mL methanol: water (60:40, v/v) with 30-second vertexing and 10-minute sonication. Extracts were filtered through 0.45 mm PTFE membranes before analysis.

2.3.2. HPLC Analysis

Chromatographic separation utilized Phenomenex Luna C18(2) column (250×4.6 mm, 5 mm) maintained at 30°C. Gradient elution employed mobile phase A (0.1% phosphoric acid) and B (acetonitrile): 0-2 min: 5% B; 2-10 min: 5-25% B; 10-12 min: 25-40% B; 12-13 min: 40-5% B; 13-15 min: 5% B (re-equilibration). Flow rate was maintained at 1.0 mL/min with 20 all injection volume. Detection wavelengths were optimized at 280 nm (Levodopa) and 290 nm (Carbidopa).

2.3.3. Method Validation

Analytical method validation encompassed linearity (1-500 µg/mL, $R^2 \geq 0.999$), precision (RSD<2%), accuracy (recovery 98-102%), and sensitivity determination. Limits of detection were 0.3 mg./mL (Levodopa) and 0.4 mg./mL (Carbidopa), with corresponding quantification limits of 1.0 and 1.2 µg/mL, respectively.

2.3.4. Statistical Analysis

Data analysis employed two-sample t-tests for pairwise comparisons between compounds within each cleaning method. Effect sizes were calculated using Cohen's d to assess practical significance. Statistical significance was established at $\alpha=0.05$. Confidence intervals (95% CI) were calculated for mean differences. All analyses were performed using validated statistical software with independent verification.

3. Results

3.1. Method Validation and System Performance

Analytical method validation confirmed suitable performance characteristics for residue quantification. Calibration curves demonstrated excellent linearity for both compounds (Levodopa: $y=24.32x+0.85$, $R^2=0.9998$; Carbidopa: $y=18.68x+0.62$, $R^2=0.9999$). System suitability parameters remained within specifications throughout the study, with resolution >3.8, tailing factors <1.3, and retention time RSDs <1.3%. Control samples showed no detectable contamination, confirming absence of environmental interference.

3.2. Visual Assessment and Residue Characteristics

Pre-cleaning visual assessment revealed distinct residue morphologies. Levodopa formed discrete white crystalline deposits covering 82-88% of surface area. Carbidopa produced off-white film-like residues with embedded crystals,

covering 75-80% of surfaces. These morphological differences persisted through cleaning, with Carbidopa demonstrating greater surface adhesion evidenced by post-cleaning staining patterns under water-only conditions.

3.3. Cleaning Efficiency Quantification

3.3.1. Water Cleaning Performance

Water submersion achieved markedly different removal efficiencies between compounds (Table 1). Levodopa demonstrated 93.00±0.14% removal, leaving 69-71 μg residual contamination. Carbidopa showed significantly lower efficiency at 86.05±0.21% (p=0.0012), with 138-141 μg residuals exceeding acceptance limits by >13-fold.

Table 1 Cleaning efficiency comparison across methodologies

Cleaning Method	Levodopa (%)	Carbidopa (%)	Difference (%)	p-value	Cohen's d
Water	93.00±0.14	86.05±0.21	6.95	0.0012	40.83
NaOH	99.58±0.04	98.25±0.07	1.33	0.0018	23.67
Detergent	99.83±0.04	99.68±0.04	0.15	0.0421	4.29

3.3.2. Alkaline Cleaning Enhancement

Sodium hydroxide treatment substantially improved removal for both compounds. Levodopa achieved near-complete removal (99.58±0.04%), with residuals (4.0-4.5 μg) below acceptance criteria. Carbidopa removal improved to 98.25±0.07%, though residuals (17-18 μg) marginally exceeded limits. The efficiency difference remained statistically significant (p=0.0018) with large effect size (Cohen's d=23.67).

3.3.3. Detergent Cleaning Optimization

Detergent solution provided optimal cleaning for both compounds, achieving >99.6% removal efficiency. Residual levels (Levodopa: 1.5-2.0 μg; Carbidopa: 3.0-3.5 μg) remained well below acceptance limits. While statistically significant difference persisted (p=0.0421), the practical significance diminished (Cohen's d=4.29).

3.4. Statistical Analysis and Effect Sizes

Statistical evaluation confirmed significant cleanability differences across all methodologies (Figure 1). Effect size analysis revealed very large effects for water (d=40.83) and alkaline (d=23.67) cleaning, with moderate effect for detergent (d=4.29). Confidence intervals for mean differences excluded zero in all comparisons (Water: 95% CI [6.32, 7.58]; NaOH: [0.98, 1.67]; Detergent: [0.02, 0.28]), confirming robust statistical significance.

3.5. Correlation with Physicochemical Properties

Cleaning efficiency demonstrated strong correlation with aqueous solubility ($r=0.94$, $p<0.001$). The 10-fold solubility difference between Levodopa (2.0 mg/mL) and Carbidopa (0.2 mg/mL) translated to consistent cleanability advantages across all methods. pH-dependent ionization effects were evident in alkaline cleaning improvements, where both compounds exist predominantly in ionized forms, enhancing aqueous interactions and reducing surface adhesion.

4. Discussion

4.1. Mechanistic Insights into Differential Cleanability

This investigation provides comprehensive evidence establishing Carbidopa as the worst-case product for cleaning validation between these critical pharmaceutical compounds. The consistent cleanability disadvantage across all methodologies indicates fundamental physicochemical factors governing removal efficiency rather than method-specific artifacts.

The primary determinant appears to be aqueous solubility differential. Levodopa's 10-fold higher solubility facilitates more rapid dissolution and removal during aqueous-based cleaning. This aligns with established dissolution-rate theories where surface concentration gradients drive mass transfer processes. Carbidopa's limited solubility creates localized saturation at the surface-solution interface, impeding further dissolution and necessitating mechanical action or chemical modification for effective removal.

Surface interaction mechanisms contribute secondary effects. The observed film formation by Carbidopa suggests stronger intermolecular interactions, possibly through hydrogen bonding networks between the hydrazine moiety and stainless-steel surface oxides. Levodopa's simpler catechol structure may form weaker, more reversible surface interactions, facilitating removal. These hypotheses warrant further investigation using surface analytical techniques such as XPS or AFM.

4.2. pH Effects and Chemical Considerations

The dramatic improvement observed with alkaline cleaning reflects pH-dependent ionization effects. At pH 13, both compounds exist predominantly in anionic forms, increasing hydrophilicity and reducing hydrophobic surface interactions. The greater relative improvement for Carbidopa (12.2% increase) versus Levodopa (6.6% increase) suggests that ionization particularly benefits compounds with inherently lower aqueous solubility.

Interestingly, despite similar pika values (Levodopa: 2.3, 8.7, 9.7; Carbidopa: 2.3, 8.9), the compounds respond differently to pH modification. This differential response may reflect structural differences in ionizable group accessibility or steric effects influencing surface desorption kinetics. The hydrazine functionality in Carbidopa potentially creates additional pH-dependent interactions absent in Levodopa.

4.3. Implications for Cleaning Validation Strategies

These findings have significant implications for cleaning validation protocol development in facilities manufacturing both compounds. The identification of Carbidopa as worst-case product enables streamlined validation approaches, eliminating redundant studies while maintaining scientific rigor. Organizations can justify using Carbidopa contamination for all cleaning validation studies, providing conservative demonstration of cleaning effectiveness.

The inadequacy of water-only cleaning for both compounds (failing 10 μg/coupon acceptance criteria) necessitate implementation of enhanced cleaning procedures. While alkaline cleaning achieved acceptable Levodopa removal, marginal Carbidopa results suggest detergent-based procedures as preferred methodology. The minimal difference observed with detergent cleaning (0.15%, $p=0.0421$) indicates this approach effectively negates solubility disadvantages, achieving consistent high-efficiency removal.

4.4. Risk Assessment Considerations

From a quality risk management perspective, these data inform several critical decisions. The 6.95% efficiency difference under water cleaning represents substantial contamination risk, particularly for high-potency manufacturing where even minimal residues pose patient safety concerns. The large effect sizes (Cohen's $d > 20$ for water and alkaline cleaning) emphasize this is not merely statistical significance but practical, meaningful differences requiring risk mitigation.

Temperature effects, while not systematically evaluated here, warrant consideration. Our ambient temperature conditions (22±3°C) represent conservative scenarios. Elevated temperatures might enhance removal through increased solubility and diffusion rates, potentially reducing compound-specific differences. However, validation under worst-case (ambient) conditions provides appropriate safety margins.

4.5. Study Limitations and Future Directions

Several limitations merit acknowledgment. The study employed static submersion rather than dynamic cleaning (spray devices, CIP systems), potentially underestimating removal achievable under manufacturing conditions. Surface roughness effects were not evaluated; different finishes might alter relative cleanability. Additionally, aged residues (>24 hours) might exhibit different removal characteristics due to potential degradation or polymerization.

Future investigations should explore several avenues. Mechanistic studies using surface analytical techniques could elucidate binding mechanisms and guide rational cleaning agent selection. Evaluation of binary mixtures would assess potential synergistic or antagonistic effects during co-contamination scenarios. Investigation of alternative cleaning agents, including enzymatic cleaners or novel surfactant systems, might identify more efficient, environmentally sustainable approaches.

4.6. Regulatory Compliance and Documentation

This study design aligns with current regulatory expectations for cleaning validation scientific justification. The systematic comparison, statistical rigor, and correlation with physicochemical properties provide robust

documentation supporting worst-case selection. The data package demonstrates due diligence in understanding product-specific cleaning challenges, facilitating regulatory submissions and inspections.

The quantitative nature of our findings enables calculation of cleaning validation limits using health-based exposure assessments. With Carbidopa established as worst-case, organizations can apply appropriate safety factors calculating maximum allowable carryover (MACO) limits, ensuring patient safety while avoiding unnecessarily stringent specifications that compromise operational efficiency.

5. Conclusion

This comprehensive investigation establishes Carbidopa as the worst-case product for cleaning validation in manufacturing scenarios involving both Levodopa and Carbidopa. The significantly lower cleaning efficiency observed across all methodologies ($p<0.05$), with particularly pronounced differences under water-only conditions (6.95% difference, Cohen's d=40.83), provides robust scientific justification for this determination.

Key findings supporting this conclusion include

- **Consistent cleanability disadvantage**: Carbidopa demonstrated lower removal efficiency across all cleaning methods, with residuals exceeding acceptance limits under water-only conditions (138-141 µg versus 10 µg limit)
- **Physicochemical correlation**: The 10-fold solubility difference (0.2 versus 2.0 mg/mL) strongly correlates with observed cleanability differences, providing mechanistic understanding
- **Statistical robustness**: All comparisons achieved statistical significance with large to very large effect sizes, confirming practical relevance beyond statistical artifact
- **Risk-based implications**: Water-only cleaning proves inadequate for either compound, necessitating alkaline or detergent-based cleaning procedures for acceptable contamination control

These findings enable science-based cleaning validation strategies, supporting regulatory compliance while optimizing resource utilization. Organizations can confidently employ Carbidopa for worst-case validation studies, ensuring cleaning procedures adequate for both compounds. Implementation of detergent-based cleaning, achieving >99.6% removal for both compounds, represents the optimal approach balancing efficiency, consistency, and regulatory compliance.

Future investigations should explore mechanistic aspects of differential surface interactions and evaluate additional cleaning parameters to further optimize pharmaceutical cleaning processes. The methodology presented here provides a template for systematic cleanability assessments applicable to other compound combinations, advancing the scientific foundation of cleaning validation practices.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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