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ORIGINAL ARTICLE

Cleaning Method Validation for Estimation of Herbal Tablet Residues on Manufacturing Equipment using High Performance Liquid Chromatography

J Kuber Singh[1], Sangeetha G[1]*, Kavitha AN[1], Raman Dang[1], Giridhar Balasubramanyam[2]

1 Department of Quality Assurance, Krupanidhi College of Pharmacy, Bengaluru, Karnataka, India
2 Himalya Wellness Company, Bangalore, Karnataka, India

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* **Corresponding author**.

sange2008@gmail.com

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Abstract

Objectives: This study focuses on developing and validating a cleaning procedure for the equipment used in manufacturing of herbal tablets containing boswellic acid as the target analyte. Due to its high concentration (66.8 mg/tablet) and limited water solubility which is a challenge for cleaning the parts of manufacturing equipments. **Methods:** A risk - based approach was adopted to identify key sampling points of the manufacturing equipment and samples were collected using rinse sampling and swab sampling methods. Rinse samples were analysed using UV spectrophotometry, while swab samples were examined using high-performance liquid chromatography method. **Findings:** The validation confirmed the method's reliability in terms of specificity, sensitivity, precision, accuracy, and robustness, compliance with regulatory standards. The findings showed that all equipment surfaces complied with the predefined limits for residual boswellic acid (NMT 0.000349% for swabs and 10 ppm for rinses). **Novelty:** This study successfully developed a method framework for cleaning validation, ensuring equipment cleanliness, regulatory compliance, and the production of high-quality and contamination-free herbal medicines.

Keywords: Boswellic Acid; Accuracy; Precision; Cleaning Validation; Regulatory Standards

1 Introduction

Cleaning validation is an essential component of pharmaceutical manufacturing designed to confirm that all production equipment is free from residual materials, contaminants, or cross-product traces that might compromise product safety and quality. With growing regulatory expectations, international guidelines such as ICH Q7 and WHO Technical Reports highlight the importance of risk-based validation

strategies, scientifically justified residue limits, and validated analytical methods to ensure the consistent cleanliness of manufacturing systems[1].

While extensive research supports cleaning validation in synthetic drug manufacturing, herbal and botanical formulations remain less thoroughly explored. Herbal products often present unique challenges due to their complex chemical profiles and limited solubility of active phytoconstituents. Boswellic acids, isolated from the resin of Boswellia serrata, are well-known for their anti-inflammatory and therapeutic properties and are routinely selected as standard marker compounds in herbal formulations[2]. However, their resinous and lipophilic character makes them strongly adherent to metal and glass surfaces, thereby complicating residue removal and increasing the risk of equipment contamination during batch manufacturing[3].

Most existing literature has focused on chemical drug residues, leaving a notable research gap in validated methods specifically developed for herbal products. Studies that address residue estimation of boswellic acids using advanced analytical tools such as high-performance liquid chromatography (HPLC) remain scarce. Furthermore, the integration of risk-based selection of sampling locations and the comparative evaluation of swab and rinse sampling methods has received limited attention in herbal manufacturing environments[4].

The present study aims to bridge these gaps by developing and validating a comprehensive cleaning method for equipment used in manufacturing herbal tablets containing boswellic acid. By combining HPLC and UV spectrophotometric techniques, the study proposes a scientifically reliable, reproducible, and regulatory-compliant approach to assure equipment cleanliness, avoid cross-contamination, and enhance the quality control framework in herbal pharmaceutical production[5].

2 Methodology

2.1 Identification of Sampling Point

Sampling points are the specific locations where samples are collected to assess the effectiveness of cleaning procedures. These points are strategically chosen to represent areas more likely to retain the residues on it.

The product's contact with the equipment's surface was taken into consideration when selecting the sampling points:

Dispensing booth (5 locations), vibratory sifter (8 locations), blender (4 locations), sampling thief (2 locations), compression machine (12 locations), dust extractor (3 locations), metal detector cum de-duster (14 locations), PIAB vacuum conveyor (5 locations), coating pan (8 locations), coating solution preparation vessel (3 locations), coating holding tank (4 locations), other accessories for coating (3 locations), neocota (8 locations), tablet inspection machine (5 locations), tablet counting machine (5 locations).

2.2 Sampling techniques

There are two sampling techniques which are rinse sampling technique and swab sampling technique.

2.2.1. Rinse sampling method

Rinse sample collection was carried out immediately following the final rinse of the relevant equipment or its product-contact parts to ensure accurate assessment of residual contaminants. To ensure complete covering of the whole internal surface area of the equipment that comes into direct touch with the product, a final washing was conducted using two litres of filtered water. This volume was chosen to optimize the possibility of finding any lingering residues of the active medicinal component and to accurately depict the whole contact surface[6].

Carefully, a 200 ml sample was extracted from the full rinse volume and put in a clean, dry glass bottle designed specifically for chemical analysis. Glass containers lower the risk of sample contact and contamination. The solvent of choice was filtered water, since it is a universal solvent that does not react or interact with the residues of cleaning agents and dissolves boswellic acid when boiled. The relevant identifying information was clearly visible on the label of every sample vial, including the equipment id, date, time, and sample number. Soon after, the collected samples and a correctly filled-out technical information sheet were forwarded to quality control[7].

2.2.2. Swab sampling method

Sterile cotton swabs were transported to the manufacturing area in a specially designated sampling toolbox. A validated sterile cotton swabs separately packaged in polypropylene tubes, including a cotton bud attached to a polypropylene stick was used to collect the swab samples[8]. This particular setup was chosen to avoid contamination from the swab like fibre shedding during collection and to be in compliance with the sampling process the sampling setup has been diagrammatically represented in Figure 1.

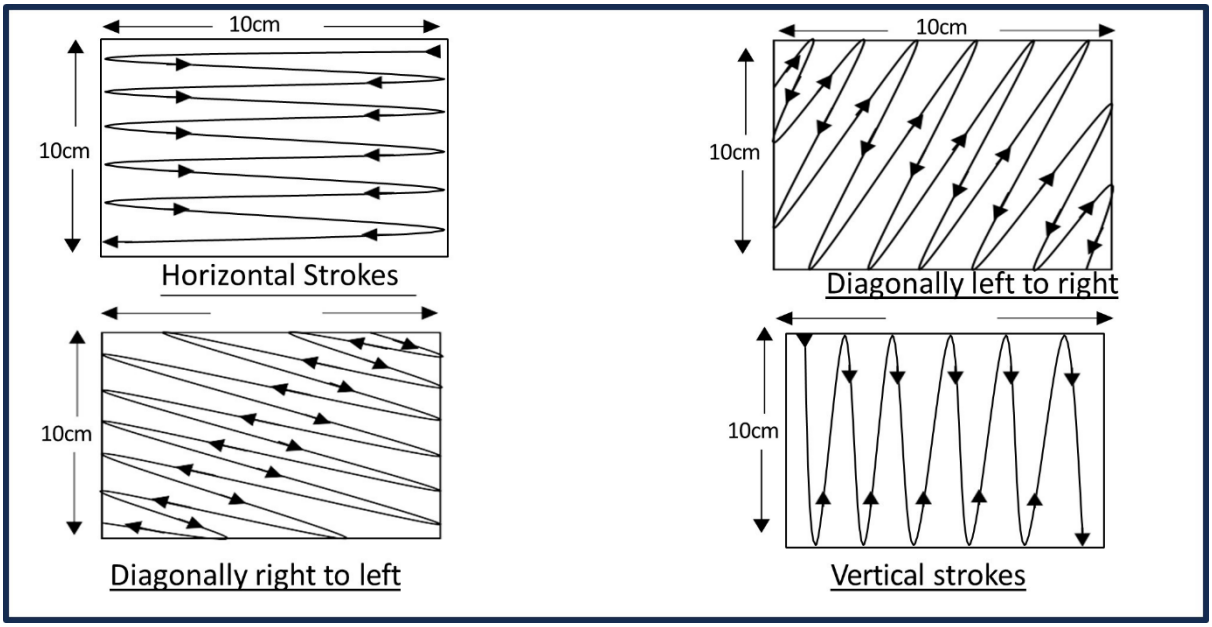

Fig 1. Swab sample collection strokes

The BSCP (buffered sodium citrate phosphate) solution was made in compliance with the applicable standard operating procedure (SOP) before sampling. Each polypropylene swab tube was precisely filled with 10 ml of the prepared and sterilized BSCP solution. After that, the tubes were capped and firmly sealed to preserve sterility and prevent spills.

When the swabs were ready, they were carefully assembled for field use. Each tube was labeled with accurate and thorough information, including the sample ID, equipment characteristics, date, and time, in order to ensure traceability and accurate identification throughout the analysis using HPLC, which is effective to use and capable of detecting very minute particles in ppm concentration.

2.3 Analytical Characteristics

2.3.1. Rinse sample analysis by UV – Visible Spectroscopy:

200 mL of filtered purified water was boiled for 15 minutes at 98 $\pm$ 2°C and was cooled to room temperature. The solution was filtered using Whatman No. 1 filter paper. This filtered solution was considered as the reference solution for baseline correction

A baseline correction was performed by scanning the reference solution across the wavelength range of 210 to 600 nm. After a baseline correction, absorbance of 100ppm and 10ppm standard solutions were recorded at 260nm. The rinse sample that had been boiled and cooled was then put into the cuvette and scanned at a wavelength of 260nm. The presence and relative amount of residual analyte were determined by comparing the rinse sample's absorbance values to those of the 10ppm reference solution.

2.3.2. Swab sample analysis by HPLC:

HPLC Analysis System had the following chromatographic conditions[9]:

Column: C18 Hypersil ODS (250 $\times$ 4.6) nm.
Particle Size: 5μ
Mobile phase: Acetonitrile:Buffer (90:10)
Flow rate: 1.0 mL/min
Wavelength: 248 nm
Injection volume: 20 μL
Column temperature: 25°C
Run time: 30 minutes

10 mL of the specified diluent were added straight into the swab tube to get the swab sample ready for analysis. To guarantee complete removal of any residue from the swab, the sample vial was subsequently sonicated for ten minutes. To prevent any possible contamination or interference, the solution was filtered using a 0.45 μm syringe filter after sonication. The first few mL of the filtrate was thrown away. After that, the filtrate was gathered and subjected to examination.

Before beginning the sample analysis, a blank chromatogram was performed to ensure that there was no carryover in the HPLC system. Then, baseline correction was performed using a prepared reference solution to eliminate background interference and ensure accurate spectral interpretation.

The blank solution was scanned in spectrum mode between 210 and 600 nm to provide a clean baseline. To generate a high-concentration reference spectrum, standard test solution A, which contained 100 ppm of analyte, was then scanned in the same range using a test cuvette. Standard test solution B, which was prepared at 10 ppm, was also scanned under the same conditions to demonstrate the reduced threshold of detection.

After cooling and refluxing, the extract from the swab sample was placed inside the cuvette and scanned in the same 210–600 nm spectral region. The absorbance spectra of the sample and the 10ppm reference solution were compared in order to determine the presence and concentration of any remaining analyte.

2.4 Preparation of Standard Solution

Stock Standard solution of Boswellic acids (1.0 mg/ml):

Accurately weighed 50 mg of standard Boswellic acids in a 50 ml volumetric flask. Add 30-40 ml of methanol and dissolve by sonication for about 5 minutes. Make the volume up to the mark with same solvent. Chromatogram as shown in Figure 2.

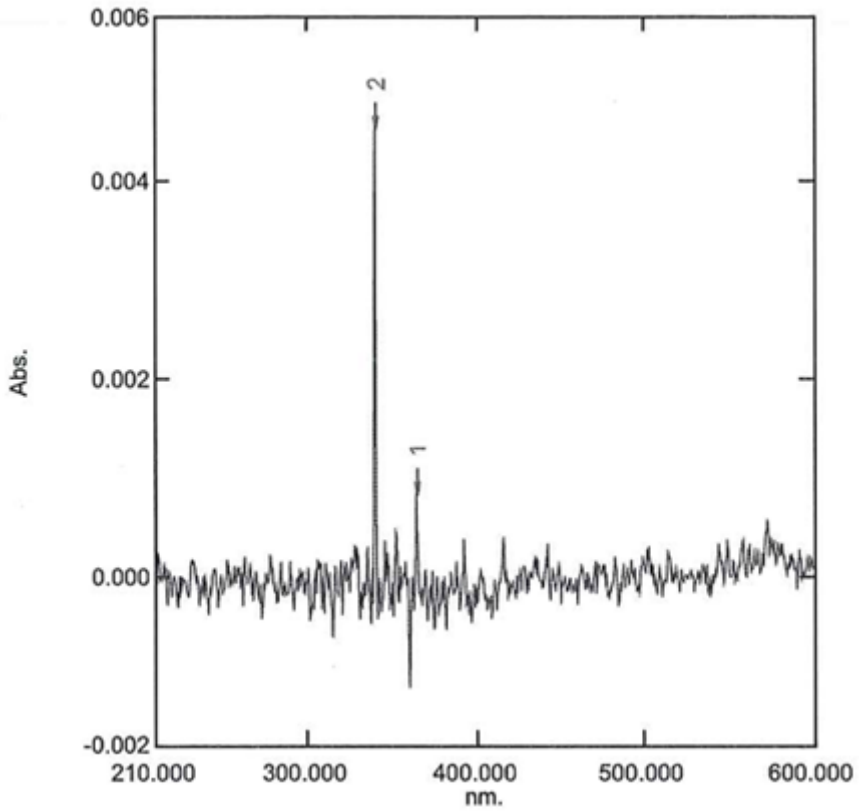

Fig 2. Blank Chromatogram

Working Standard solution of Boswellic acids (100 ppm):

Pipetted 5.0 mL of the above solution 50 mL volumetric flask diluted to 50 ml methanol and mixed well. Filtered through 0.45 μ syringe filter. Chromatogram as shown in Figure 3.

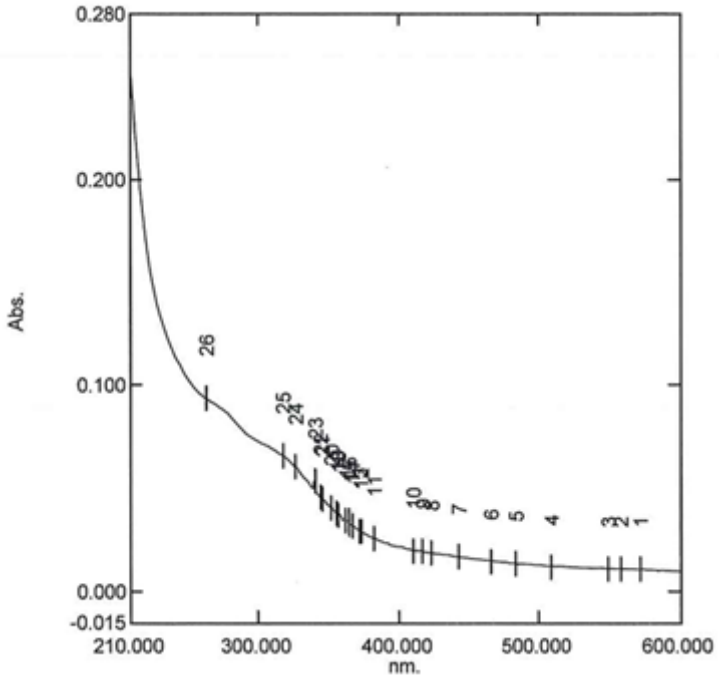

Fig 3. Chromatogram of 100ppm standard solution of boswellic acid

Second Working Standard solution of Boswellic acids (10 ppm):

Pipetted 5.0 mL of the above solution 50 mL volumetric flask, diluted to volume with diluent and mixed well. Filtered through 0.45 μ syringe filter (Figure 4).

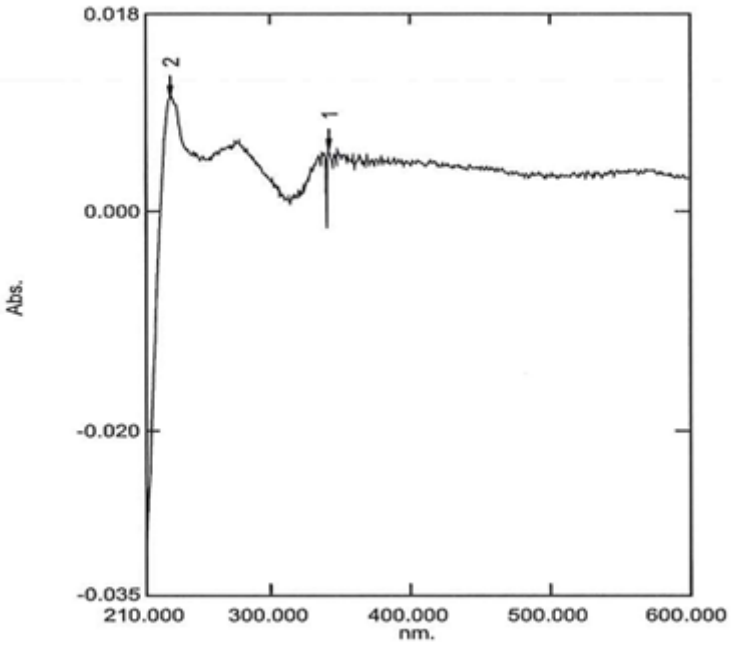

Fig 4. Chromatogram of 10ppm standard solution of boswellic acid

Preparation of Buffer:

Pipette out 1.0mL of Ortho Phosphoric acid into 900 mL of Milli-Q water and mix thoroughly. Adjust the volume to 1000 mL with purified water. Filter the buffer through 0.45 μm membrane filter.

2.5 Cleaning Validation

To validate the cleaning method, the following parameters were considered [10]

2.5.1. Demonstration of Accuracy:

Recovery tests at three concentration levels 50%, 100%, and 150% of the 5ppm, 10ppm and 15ppm concentrations of boswellic acid in 10mL were used to evaluate accuracy. To replicate real residual circumstances, pre-cleaned stainless-steel plates were injected with known volumes of Boswellic Acid standard solution. Following that, the spiked samples were diluted with the diluent to a final volume and subjected to analysis at each concentration level. By comparing the measured values with the known added amounts, the percentage recovery of boswellic acid was computed. The accuracy of the approach was assessed by calculating the average recovery and the relative standard deviation (%RSD).

2.5.2. Demonstration of Precision:

Analyzing replicate preparations of the Boswellic Acid standard solution under identical experimental circumstances allowed for the determination of precision. Six replicate samples at the desired concentration were made and examined the same day in order to assess repeatability and intra-day precision. In order to evaluate intermediate precision, the identical process was carried out again on a different day. To ascertain the method's consistency and reproducibility, the relative standard deviation (%RSD) of the peak area responses was computed in both scenarios.

2.5.3. Specificity:

To make sure that Boswellic Acid could be precisely measured even in the presence of any interfering chemicals, specificity was assessed. To verify that there were no peaks at the Boswellic Acid retention time, a blank solution made with the diluent was first introduced into the HPLC system. A photodiode array (PDA) detector was then used to observe the chromatograms after the standard and test solutions had been tested under the ideal chromatographic conditions. When the peak purity angle for boswellic acid in the test solution was compared to the predetermined purity threshold, the results demonstrated that there were no co-eluting contaminants because the purity angle was less than the threshold. As a result, the method's obvious specificity proved that it was appropriate for the accurate detection and measurement of boswellic acid residues.

2.5.4. Demonstration of Linearity:

Analysis was carried out, and the solutions were prepared by quantitative dilutions of the stock solution of Boswellic acids working standard to obtain solutions in the range LOQ to 150% of the 10μg/ml of Boswellic acids. Linearity was established over a minimum of 5 points.

2.5.5. Demonstration of LOD and LOQ:

Limit of Detection (LOD): The Limit of Detection (LOD) was determined to establish the lowest concentration of Boswellic Acid that could be reliably detected, though not necessarily quantified, under the proposed analytical conditions. A calibration curve was constructed using solutions prepared by serial dilutions of the Boswellic Acid working standard, covering concentrations from the LOQ up to 150% of the target level. The slope of the calibration curve (S) and the standard error of the regression line (STEYX) were calculated from the linearity data.

Using the formula LOD was obtained

Formula: $\mathbf{LOD} = \frac{\mathbf{3.3 \times STEYX}}{\mathbf{S}}$

Where,

STEYX = Standard error of regression

S = The slope of the calibration curve.

Limit of Quantitation (LOQ): The purpose of the Limit of Quantification (LOQ) was to identify the lowest Boswellic Acid concentration that could be quantitatively measured under the specified experimental circumstances with a satisfactory level of precision and accuracy. The calibration curve's slope (S) and standard error of regression (STEYX) were computed using the linearity research as a guide.

Then, using the formula, LOQ value was obtained.

Formula: $\mathbf{LOQ} = \frac{10 \times \mathbf{STEYX}}{\mathbf{S}}$
Where,
STEYX = Standard error of regression
S = The slope of the calibration curve

3 Result And Discussion

3.1 Identification of sampling points

Sampling locations were carefully chosen using a risk-based approach to guarantee a thorough evaluation of the equipment's cleanliness and pinpoint areas that are most likely to retain Boswellic Acid residues. Because of their greater potential for residue retention, product contact surfaces with complex structures, small spaces, or locations where material tends to gather such as hoppers, feeders, spray nozzles, and discharge chutes were given priority. To give representative results across a variety of surface types, flat, easily accessible surfaces were also included, such as the internal surface of the bin and the lid of the blender. Punches, dies, and turrets are pieces of equipment used in direct product transfer and compression, and they were selected because they are essential for preventing cross-contamination in later manufacturing cycles. As seen in Figure 5, Figure 6, Figure 7 and Figure 8.

Fig 5. Product discharge chute

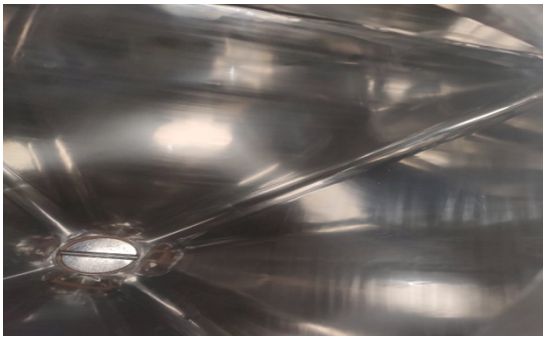

Fig 6. Inner surface of Blender (Bin)

3.2 Analytical Characteristics

Since the Blender (Bin), Compression Machine, and Neocota are the most crucial elements for potential residue retention and product carryover in the production process of herbal tablets based on boswellic acid, the data presented here focuses on them. All equipment units were used in the cleaning validation study. The Blender (Bin), which controls bulk powder mixing, was selected as the primary site for significant product contact and possible residue adherence. The compression machine was added because it has several complex surfaces, such as hoppers, feeders, punches, and dies, which are prone to retain small particles and

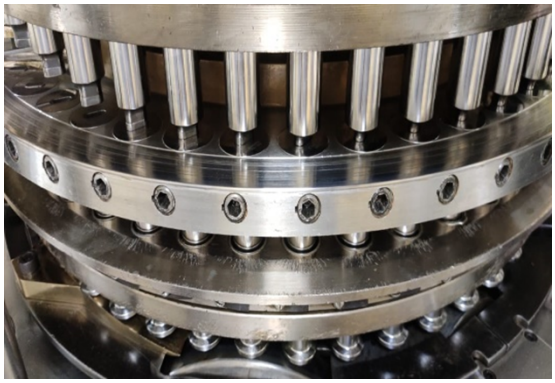

Fig 7. Surface of turret

Fig 8. Inner surface of Neocota

so provide a serious danger of cross-contamination. The internal surfaces, discharge chutes, and spray nozzles of the Neocota tablet coating system are difficult to clean and susceptible to coating solution buildup, which is why it was chosen.

3.2.1. Sample analysis by UV – Visible Spectrometry:

To evaluate the efficiency of the cleaning procedure at three crucial manufacturing stages—the blender (bin), the compression machine, and the neocota, a thorough rinse study was conducted. To guarantee a comprehensive assessment, samples of various contact surfaces from every piece of equipment were taken. The absorbance value of 0.104, or NMT 10 ppm, was the predetermined acceptable limit. To guarantee consistency and dependability, data were gathered from three consecutive batches: HWBB 1, HWBB 2 & HWBB 3; HWCB 1, HWCB 2 & HWCB 3; and HWNB 1, HWNB 2 & HWNB 3. Average values were taken into account.

3.2.1.1. Blender (Bin). Samples obtained from the blender lid (R11) and the inside surface of the bin (R10) in Table 1 had computed concentrations of 4.500 ppm and 3.000 ppm, respectively, with absorbance values ranging from 0.003 to 0.008. These outcomes, which are much below the acceptable limit, show that the cleaning procedure for these parts was both successful and fulfilling.

3.2.1.2. Compression Machine. The Y chute, feeders, the left and right hopper sections, and the in-process sample scoop were among the surfaces from Table 2 for the compression machine that were assessed. The absorbance values were found to be between 0.0053 and 0.0090, whereas the measured concentration levels varied from 3.975 ppm to 6.750 ppm. The Y chute (R14) and feeder-left (R17) had the highest residue levels, both measuring 6.750 ppm, however they were still well inside the acceptable range. The cleaning procedure turned out to be reliable enough.

3.2.1.3. Neocota. The inner surface, discharge chute, five spray nozzles, and sampling scoop were among the components of the Neocota system from Table 3 that were examined. With Spray Nozzle 2 (R47) having the highest level and the discharge chute (R45) having the lowest, the measured values ranged from 2.475 ppm to 6.225 ppm. Similar components, such as the spray nozzles, had consistent results, indicating that these various points of contact were cleaned uniformly.

Table 1. Rinse Sample of Bin Blender

Processing Stage	Sampling location	Sampling code for Rinse Analysis	Batch HWBB 1	Batch HWBB 2	Batch HWBB 3	Avg. Absorbance	Conc. (in ppm) Limit: NMT 10PPM
Blender (Bin)	Inner surface of bin Inner lower surface of bin Discharge valve	R10	0.007	0.001	0.004	0.0040	3.000 ppm
	Blender Lid	R11	0.008	0.007	0.003	0.0060	4.500 ppm

Table 2. Rinse Sample of Compression Machine

Processing Stage	Sampling location	Sampling code for Rinse Analysis	Batch HWCB 1	Batch HWCB 2	Batch HWCB 3	Avg. Absorbance	Conc. (in ppm) Limit: NMT 10PPM
Compression Machine	Inner surface of the Y Chute	R14	0.009	0.010	0.008	0.0090	6.750 ppm
	Inner surface of the Hopper-Left	R15	0.008	0.009	0.008	0.0083	6.225 ppm
	Inner surface of the Hopper-Right	R16	0.008	0.001	0.010	0.0063	4.725 ppm
	Feeder-Left	R17	0.009	0.009	0.009	0.0090	6.750 ppm
	Feeder-Right	R18	0.007	0.009	0.008	0.0080	6.000 ppm
	Scoop for in-process sample	R19	0.008	0.002	0.006	0.0053	3.975 ppm

Table 3. Rinse Sample of Neocota

Processing Stage	Sampling location	Sampling code for Rinse Analysis	Batch HWNB 1	Batch HWNB 2	Batch HWNB 3	Avg. Absorbance	Conc. (in ppm) Limit: NMT 10PPM
Neocota	Inner surface of Neocota	R44	0.009	0.004	0.000	0.0043	3.225 ppm
	Discharge chute	R45	0.006	0.003	0.001	0.0033	2.475 ppm
	Spray nozzle 1	R46	0.005	0.005	0.006	0.0053	3.975 ppm
	Spray nozzle 2	R47	0.008	0.010	0.007	0.0083	6.225 ppm
	Spray nozzle 3	R48	0.005	0.007	0.000	0.0040	3.000 ppm
	Spray nozzle 4	R49	0.004	0.008	0.001	0.0043	3.225 ppm
	Spray nozzle 5	R50	0.007	0.007	0.000	0.0046	3.450 ppm
	Scoop for Neocota	R51	0.011	0.000	0.003	0.0046	3.450 ppm

3.2.2. Swab sample analysis by HPLC:

The Blender (Bin), Compression Machine, and Neocota were the three main processing regions where a swab study was conducted to assess the cleaning process' efficacy. To guarantee consistency and dependability of results, a variety of product-contact surfaces were swabbed following cleaning from three consecutive batches: HWBB 1, HWBB 2 & HWBB 3; HWCB 1, HWCB 2 & HWCB 3; and HWNB 1, HWNB 2 & HWNB 3. The amount of residual product found, indicated in absorbance units, is reflected in the data.

3.2.2.1. Blender (Bin). Based on Table 4, Swab samples for the blender lid (S17), discharge valve (S16), and inner and lower surfaces of the bin (S14, S15) revealed incredibly low to undetectable residue levels. In batch HWBB 2, the lowest surface (S15)

had the highest value, 0.000216; all other measurements were either insignificant or zero. This suggests that the bin was cleaned effectively because there was no discernible residue buildup in any of the studied areas.

3.2.2.2. Compression Machine. Every area of the compression machine listed in Table 5 was evaluated, including the turret, die surface, feeders, discharge chutes, hopper sections, punches, Y chute, in-process sampling scoop, and turret. The greatest absorbance of 0.000298 was displayed by the Feeder-Left (S27) from batch HWCB 1, which is still within a safe and acceptable range. The Y chute (S20) and the in-process scoop (S31), which displayed trace amounts of residues in batch HWCB 2, were two other noteworthy values. Nevertheless, zero values were displayed throughout all batches at a number of locations, such as Feeder-Right, punches, and discharge chutes. These results support the comprehensiveness and consistency of the compression unit cleaning protocol.

3.2.2.3. Neocota. The inner surface, discharge chute, five spray nozzles, and sampling scoop were all examined by the Neocota swab study. Table 6 showed very low residue levels, with the majority of results falling between 0.000044 and 0.000117. There were trace amounts on the inner surface (S72) and spray nozzles (S74–S78), but none were high enough to be of concern. The discharge chute (S73) and scoop (S79) also showed no residue, which further supports the cleaning procedure's uniformity over all sampling locations.

Table 4. Swab Sample for Bin Blender

Processing Stage	Sampling location	Sampling code for Swab Analysis	Batch HWBB 1	Batch HWBB 2	Batch HWBB 3
Blender (Bin)	Inner surface of bin	S14	0.000	0.000068	0.000
	Inner lower surface of bin	S15	0.000	0.000216	0.000169
	Discharge valve	S16	0.000	0.000	0.000
	Blender Lid	S17	0.000	0.000080	0.000

Table 5. Swab Sample for Compression Machine

Processing Stage	Sampling location	Sampling code for Swab Analysis	Batch HWCB 1	Batch HWCB 2	Batch HWCB 3
Compression Machine	Inner surface of the Y Chute	S20	0.000269	0.000145	0.000
	Inner surface of the Hopper-Left	S21	0.000	0.000088	0.000
	Inner surface of the Hopper-Right	S22	0.000	0.000228	0.000
	Lower punch	S23	0.000	0.000	0.000159
	Upper punch	S24	0.000	0.000140	0.000
	Surface of the turret	S25	0.000	0.000053	0.000
	Surface of the Die	S26	0.000	0.000127	0.000118
	Feeder-Left	S27	0.000298	0.000074	0.000
	Feeder-Right	S28	0.000	0.000	0.000
	Discharge Chute-Left	S29	0.000	0.000	0.000
	Discharge Chute-Right	S30	0.000	0.000	0.000192
	Scoop for in-process sample	S31	0.000	0.000247	0.000

3.3 Validation

3.3.1. Demonstration of Accuracy:

The average recovery at the 50% spike level was 89.96% with a relative standard deviation (%RSD) of 3.06, indicating adequate repeatability, whereas individual recoveries ranged from 86.84% to 92.15%. The maximum level of precision was shown at the 100% level, with an incredibly low %RSD of 0.84, indicating little variability. Recovery results for the 150% level ranged

Table 6. Swab Sample for Neocota

Processing Stage	Sampling location	Sampling code for Swab Analysis	Batch HWNB 1	Batch HWNB 2	Batch HWNB 3
Neocota	Inner surface of Neocota	S72	0.000060	0.000117	0.000
	Discharge chute	S73	0.000	0.000072	0.000116
	Spray nozzle 1	S74	0.000	0.000067	0.000
	Spray nozzle 2	S75	0.000083	0.000085	0.000
	Spray nozzle 3	S76	0.000	0.000085	0.000
	Spray nozzle 4	S77	0.000066	0.000086	0.000
	Spray nozzle 5	S78	0.000078	0.000	0.000
	Scoop for Neocota	S79	0.000100	0.000044	0.000

from 90.90% to 93.45%, with an average of 92.50% and a percentage RSD of 1.51; this indicates that the method performed consistently at high concentrations (Table 7).

All recovery percentages across the tested range fell well within the regulatory acceptance limits of 75% to 125%, demonstrating that the method maintains accuracy across varying concentrations. These results collectively confirm that the procedure is both reliable and reproducible, making it fully suitable for determining trace levels of Boswellic Acid on manufacturing surfaces during cleaning validation protocols (Table 8).

Table 7. Accuracy

Accuracy Solution	Approx. concentration of Boswellic Acid (in ppm)	Vol. of accuracy stock solution spiked on to stainless steel plate	Final Volume with diluent (ml)
Accuracy Solution 50%	5.0	0.5	10
Accuracy Solution 100%	10.0	1.0	10
Accuracy Solution 150%	15.0	1.5	10

Table 8. Recovery

Level	Amount of Standard spiked in ppm	Recovery in ppm	% Recovery	Average % Recovery & RSD	Acceptance Criteria	% Remarks
Level 50%	5	4.54	90.88	89.96 & 3.06 %RSD	The % Recovery should be between 75% and 125%	Complies
	5	4.34	86.84			
	5	4.61	92.15			
Level 100%	10	9.98	99.78	99.27 & 0.84 %RSD		Complies
	10	9.97	99.71			
	10	9.83	98.31			
Level 150%	15	13.63	90.90	92.50 & 1.51 &RSD		Complies
	15	13.97	93.14			
	15	14.02	93.45			

3.3.2. Demonstration of Precision:

Six replicate preparations of boswellic acid at a standard concentration of 2.5 ppm were analyzed under similar conditions on the same day (Table 9) and on a different day (Table 10) in order to assess the method's precision. This evaluation aids in determining the method's repeatability and consistency over both brief and long time periods.

The area responses for intra-day precision varied from 4545 to 4767 for all six preparations, resulting in an average peak area of 4661 and a relative standard deviation (%RSD) of 1.65. With an average area of 4700 and a percentage RSD of 1.19, the area

values in the inter-day study ranged from 4602 to 4751. The %RSD results in both instances were much below the acceptable limit of 5.0%, indicating the method's outstanding repeatability and consistency.

These results demonstrate the method's great precision and reproducibility across various testing circumstances and time intervals. The method's resilience and usefulness for frequent usage in detecting and quantifying trace quantities of boswellic acid in cleaning validation applications are supported by the minimal variability in area response.

Table 9. Intra-day Precision

Preparation	Standard Conc. (ppm)	Area Response	Average Area & %RSD	Acceptance Criteria	Remarks
Sample Preparation 1	2.5	4687	4661 & 1.65 % RSD	The %RSD of area response should not be more than 5.0	Complies
Sample Preparation 2	2.5	4631			
Sample Preparation 3	2.5	4545			
Sample Preparation 4	2.5	4707			
Sample Preparation 5	2.5	4627			
Sample Preparation 6	2.5	4767			

Table 10. Inter-day Precision

Preparation	Standard Conc. (ppm)	Area Response	Average Area & %RSD	Acceptance Criteria	Remarks
Sample Preparation 1	2.5	4731	4700 & 1.19 % RSD	The %RSD of area response should be not more than 5.0	Complies
Sample Preparation 2	2.5	4730			
Sample Preparation 3	2.5	4602			
Sample Preparation 4	2.5	4751			
Sample Preparation 5	2.5	4665			
Sample Preparation 6	2.5	4720			

3.3.3. Specificity

3.3.3.1. Specificity – Interference Check. The diluent was evaluated to look for any peaks that appeared at the same retention time as the target compound in order to verify that the method could quantify Boswellic Acids accurately without the effect of outside factors. The method's specificity and applicability for regular analysis were demonstrated by the study, which showed the absence of interfering peaks at the Boswellic Acids retention time (Table 11).

3.3.3.2. Peak Purity – PDA Assessment. The spectral integrity of the Boswellic Acids peak in the test and standard samples was investigated using photodiode array (PDA) detection. The peak is clean and free of overlapping or co-eluting components, as shown by the peak purity angle being less than the predetermined threshold. This attests to the method's clean and dependable separation, which guarantees precise measurement of the sample's Boswellic Acids.

Table 11. Specificity

Sl. No	Analyte	Acceptance Criteria	Result / Remark
1	A	There should not be any interference at the retention time of Boswellic Acids from the diluent.	Complies
2	3D – PDA Data for standard and sample solution	Peak purity angle should be less than peak purity threshold for Boswellic Acids peak in standard and test solution	Complies

3.3.4. Demonstration of Linearity:

By examining Table 12 of Boswellic Acids at five concentration levels, from 2.5 ppm to 15.0 ppm (25% to 150% of the working range), linearity was confirmed. Analyte quantity and detector response are directly correlated, as seen by the corresponding average area responses, which increased steadily with concentration.

The correlation coefficient, as determined from Figure 9, is 0.996, exceeding the acceptability threshold of at least 0.990. This demonstrates strong linearity and dependability for quantitative analysis and validates that the method yields consistent and proportionate results over the studied range.

Table 12. Linearity

Level	Concentration of Boswellic Acids (in ppm)	Average Area Response	Result	Acceptance Criteria	Remarks
Sample- 1: 25%	2.50 ppm	4592	The correlation coefficient was found to be 0.996	The correlation coefficient should be NLT 0.990	Complies
Sample-2: 50%	5.00 ppm	10446			
Sample-3: 75%	7.50 ppm	14714			
Sample-4: 100%	10.00 ppm	21382			
Sample-5: 150%	15.00 ppm	29632			

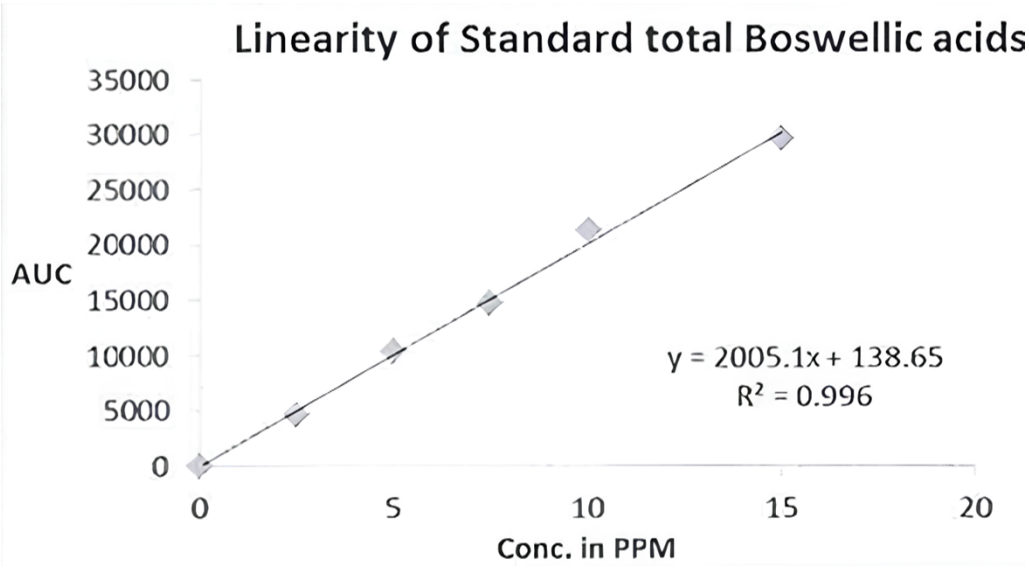

Fig 9. Standard graph of Boswellic Acid

3.3.5. Demonstration of LOD and LOQ:

By calculating the Limit of Detection (LOD) and Limit of Quantification (LOQ), the sensitivity of the approach was assessed. Table 13 shows that the method can accurately detect and measure low quantities of Boswellic Acids. The standard error of regression (STEYX) and the slope obtained from the linearity curve were used to compute these values. The calibration line's slope was 2005.061, and the resulting STEYX was 701.3684.

The LOD, or minimal amount that can be reliably detected but not necessarily measured, was estimated to be 1.15 ppm using these values. The lowest concentration that can be quantitatively identified with a satisfactory level of precision and accuracy is 3.49 ppm, which was judged to be the LOQ. These results support the method's usability in standard pharmaceutical analysis and quality assurance procedures by confirming that it is extremely sensitive and suitable for identifying even small levels of boswellic acids.

Table 13. LOD and LOQ

Sl. No	STEYX	Slope	LOD (ppm)	LOQ (ppm)
1.	701.3684	2005.061	1.15	3.49

4 Conclusion

The current study successfully created and validated a cleaning procedure for equipment used in the production of herbal tablets that contain boswellic acid as an active ingredient. This substance is well known for its poor water solubility and strong surface adherence. Critical sample locations were found throughout the three main stages of production and are Blender (Bin),

Compression Machine, and Neocota by using a risk-based methodology. UV-Visible spectrophotometric analysis of rinse samples showed that residual concentrations, which ranged from 2.475 ppm to 6.750 ppm, were far below the acceptability limit of 10 ppm on all tested surfaces. Likewise, HPLC analysis of swab samples revealed incredibly low residual levels, with the highest being 0.000298%, much below the 0.000349% swab limit. Study across all parameters was confirmed by method validation, where boswellic acid showed linearity between 2.5 ppm and 15.0 ppm, having a correlation coefficient of 0.996. Strong detection capability was indicated by the sensitivity analysis, which produced a LOD of 1.15 ppm and a LOQ of 3.49 ppm. Precision experiments showed %RSD values of 1.65 (intra-day) and 1.19 (inter-day), indicating strong reproducibility, while accuracy testing revealed recovery values ranging from 86.84% to 99.78%. These results demonstrate the method's scientific soundness, regulatory compliance, and suitability for routine cleaning validation to maintain equipment cleanliness and avoid cross-contamination in the production of herbal pharmaceuticals.

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