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# ANALYTICAL METHOD VERIFICATION BY RP-HPLC FOR CLEANING VALIDATION OF EQUIPMENT USED IN MANUFACTURING IBUPROFEN TABLETS

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## ABSTRACT

**Background:** Effective cleaning validation and reliable analytical methods are critical components of pharmaceutical quality assurance, particularly in multi-product facilities. Residual drug presence on manufacturing equipment poses risks of cross-contamination, making method verification and cleaning validation essential for regulatory compliance. This study aimed to verify a reverse-phase high-performance liquid chromatography (RP-HPLC) method for the assay of Ibuprofen in Ibuprofen Tablets BP 800 mg and to validate the cleaning procedure for equipment used in its manufacture. **Methods:** Method verification was conducted for key parameters including specificity, precision, ruggedness, and solution stability as per ICH Q2(R1) guidelines. The validated method was then applied to analyze swab and rinse samples collected post-cleaning from three consecutive manufacturing batches. Microbial monitoring and rinse water quality assessment were also performed. **Results:** The RP-HPLC method demonstrated high specificity with no placebo interference, precision with %RSD < 0.3%, and solution stability up to 24 hours. Cleaning validation data showed that residual ibuprofen levels were below the Maximum Allowable Carryover (MACO) limit of 7 µg/4 sq. inch. Microbial contamination and rinse water parameters remained within acceptable regulatory limits across all equipment surfaces. **Conclusion:** The verified RP-HPLC method is suitable for routine assay determination of Ibuprofen Tablets BP 800 mg. The cleaning procedure effectively eliminates chemical and microbial residues and can be reliably implemented in ongoing and future manufacturing batches to ensure GMP compliance and product safety.

**KEYWORDS:** RP-HPLC, Ibuprofen, Cleaning Validation, Assay, Method Verification, GMP, Cross-contamination, Pharmaceutical Quality.

## INTRODUCTION

Pharmaceutical manufacturing requires stringent hygiene and cross-contamination control measures, especially when the same equipment is used for multiple products. Cleaning validation ensures that the cleaning procedures used for equipment are robust and effective in removing active pharmaceutical ingredients (APIs), excipients, microbial residues, and cleaning agents. Regulatory authorities including the US FDA and EMA mandate documented evidence demonstrating the reproducibility and effectiveness of cleaning procedures in compliance with Good Manufacturing Practices (GMP).[1,2]

Analytical method verification is a crucial part of the cleaning validation process. It confirms that an established analytical procedure—when transferred to a new laboratory or new matrix—performs reliably and accurately under actual experimental conditions. Reverse-phase high-performance liquid chromatography (RP-HPLC) is widely recognized as a powerful tool in analytical quality control, offering high sensitivity, specificity, and reproducibility for detecting trace levels of drug residues on manufacturing equipment surfaces.[3,4]

Ibuprofen is a widely used non-steroidal anti-inflammatory drug (NSAID) employed for its analgesic, antipyretic, and anti-inflammatory properties. Due to its frequent manufacture in tablet form, ensuring effective cleaning of production equipment is essential to avoid contamination in subsequent batches. Moreover, residual ibuprofen, even at low levels, may pose toxicological risks or lead to unwanted interactions with other products, particularly in a multiproduct facility.[5,6]

The present study aimed to verify an RP-HPLC analytical method for detecting residual ibuprofen and to validate the cleaning procedures used in manufacturing 800 mg ibuprofen tablets. The study includes method verification parameters such as specificity, precision, ruggedness, and solution stability. Swab and rinse samples collected after equipment cleaning were analyzed for chemical and microbiological residues. This research supports regulatory compliance and strengthens the quality assurance framework in pharmaceutical manufacturing environments.[7,8]

## MATERIALS AND METHODS

### 1. Chemicals and Reagents

The following reagents and solvents were used:

- **Ibuprofen Working Standard** (Analytical grade)
- **Methanol** (HPLC Grade, Merck)
- **Water** (HPLC Grade, Milli-Q system)
- **Orthophosphoric Acid** (AR Grade, Merck)

All chemicals and solvents were used without further purification. The mobile phase was filtered through a 0.45 µm membrane filter and degassed before use.

### 2. Instrumentation

Chromatographic analysis was performed using a **Shimadzu LC-2010 CHT** HPLC system equipped with a UV detector. Data acquisition was done using Lab Solutions software.

**3. Chromatographic Conditions**

| Parameter | Condition |
|---|---|
| Column | C18 (250 mm × 4.6 mm, 10 µm, L1 packing) |
| Mobile Phase | Orthophosphoric acid: Water: Methanol (3:247:750 v/v/v) |
| Flow Rate | 1.5 mL/min |
| Detection Wavelength | 264 nm |
| Injection Volume | 20 µL |
| Run Time | 1.5 minutes |
| Column Temperature | Ambient |

**4. Preparation of Solutions**

**4.1 Mobile Phase**

A mixture of 3 volumes of orthophosphoric acid, 247 volumes of water, and 750 volumes of methanol was prepared, filtered, and degassed prior to use.

**4.2 Standard Solution**

Accurately weighed 100 mg of Ibuprofen working standard was transferred to a 50 mL volumetric flask. About 30 mL of the mobile phase was added, sonicated for 5 minutes, and then made up to volume with mobile phase.

**4.3 Sample Solution**

Twenty Ibuprofen tablets were weighed and crushed into a fine powder. A quantity equivalent to 200 mg of Ibuprofen was accurately weighed and transferred to a 100 mL volumetric flask. About 30 mL of mobile phase was added, shaken mechanically for 30 minutes, sonicated for 5 minutes, and made up to volume with mobile phase. The solution was filtered through Whatman No.1 filter paper, discarding the first 2–3 mL of filtrate.

**4.4 Placebo Solution**

Placebo powder equivalent to 200 mg of Ibuprofen was processed similarly to the sample solution.

**5. System Suitability Criteria**

Before sample analysis, the system suitability was evaluated based on the following parameters:

- **Theoretical plates (N)**: ≥ 2000
- **Tailing factor (T)**: ≤ 2.0
- **% RSD of peak area from six injections**: ≤ 2.0%

**6. Validation Parameters (per ICH Q2(R1) guidelines)**

**6.1 Specificity**

Assessed by comparing chromatograms of blank, placebo, standard, and sample solutions to identify any potential interferences at the retention time of ibuprofen.

**6.2 Precision**

- **System Precision**: Six replicate injections of the standard solution were analyzed and % RSD of peak areas was calculated.
- **Method Precision**: Six sample preparations from the same batch were analyzed to determine % assay and % RSD.

### 6.3 Ruggedness

Assay results were evaluated by two different analysts under similar conditions. The % assay, % RSD, and overall variability were calculated.

### 6.4 Solution Stability

Standard and sample solutions were stored at room temperature and analyzed at 0, 6, 12, and 24 hours. The percentage change in area and assay values were calculated to assess stability.

## 7. Cleaning Validation Procedure

### 7.1 Equipment

Cleaning validation was conducted on the following equipment: Dispensing scoop, Sifter, Octagonal blender, Compression machine, Auto coater, Inspection conveyor, Metal detector, and Blister packing machine.

### 7.2 Swab Sampling (Chemical Residue)

Swabs were taken from critical surfaces post-cleaning using pre-moistened swabs over a 4 square inch area. The swabs were extracted with mobile phase and analyzed for residual ibuprofen content.

### 7.3 Rinse Sampling

Final rinse water from cleaned equipment was collected and tested for:

- **pH**
- **Conductivity**
- **Presence of residual ibuprofen** (by HPLC)

## 8. Microbial Analysis

### 8.1 Swab Sampling for Microbiological Analysis

Sterile swabs were used to sample 2 × 2 cm² surface areas. Samples were placed in sterile tubes and sent for microbial analysis. Following tests were performed:

- **TAMC** (Total Aerobic Microbial Count)
- **TYMC** (Total Yeast and Mold Count)
- **Pathogen detection** (where required)

### Acceptance Criteria

- TAMC: NMT 100 CFU/4 sq. inch
- TYMC: NMT 10 CFU/4 sq. inch

## 9. Acceptance Limits

| Parameter | Limit |
|---|---|
| Residual Ibuprofen | NMT 7 µg/4 sq. inch (MACO) |
| Visual Cleanliness | No visible residue |
| TAMC | NMT 100 CFU/4 sq. inch |
| TYMC | NMT 10 CFU/4 sq. inch |
| Rinse Water pH | ± 1 unit from blank |
| Rinse Water Conductivity | ± 5 µS/cm from blank |

## RESULTS

### 1. Method Verification Results

### 1.1 Specificity

The method showed no interference from excipients or placebo at the retention time of ibuprofen (RT ~6.74 min). Peak purity analysis confirmed that the ibuprofen peak was spectrally pure.

**Table 1: Specificity Results.**

| Sample | Retention Time (min) |
|---|---|
| Ibuprofen Standard | 6.740 |
| Ibuprofen Sample | 6.757 |

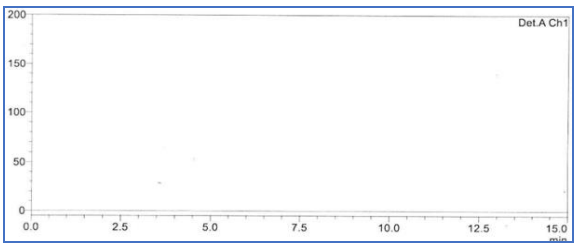


**Figure 1: Chromatogram of Blank.**

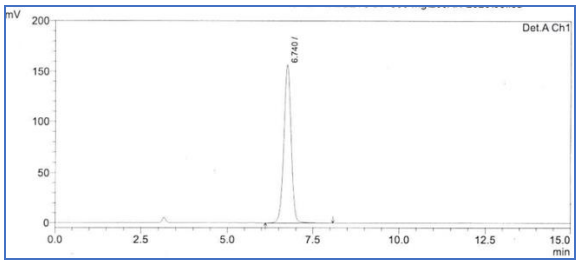


**Figure 2: Chromatogram of Standard.**

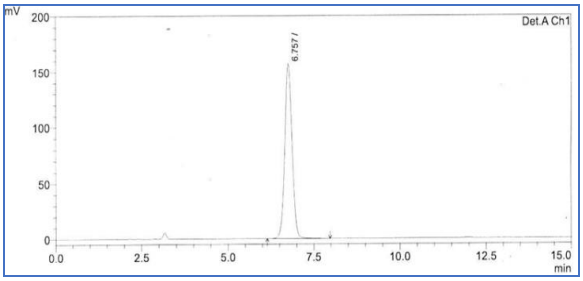


**Figure 3: Chromatogram of Sample.**

### 1.2 Placebo Interference

**Table 2: Placebo Interference.**

| Sample Type | RT (min) | Placebo Interference | Diluents Interference |
|---|---|---|---|
| Standard Solution | 6.740 | Nil | Nil |
| Sample Solution | 6.757 | Nil | Nil |

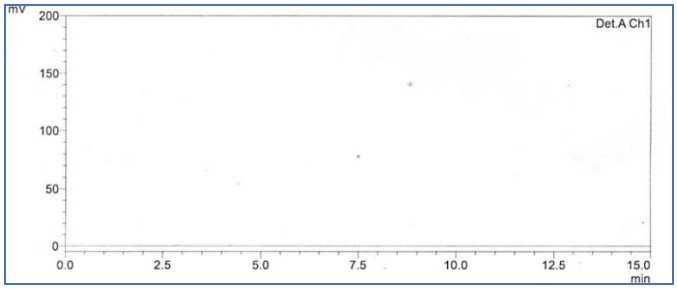


**Figure 4: Chromatogram for placebo interference.**

### 1.3 System Precision

Six replicate injections of the standard showed consistent area response.

**Table 3: System Precision.**

| Injection No. | Peak Area |
|---|---|
| 1 | 2,321,624 |
| 2 | 2,321,672 |
| 3 | 2,322,299 |
| 4 | 2,322,880 |
| 5 | 2,320,063 |
| 6 | 2,319,822 |

Mean: 2,321,443; % RSD: 0.05%

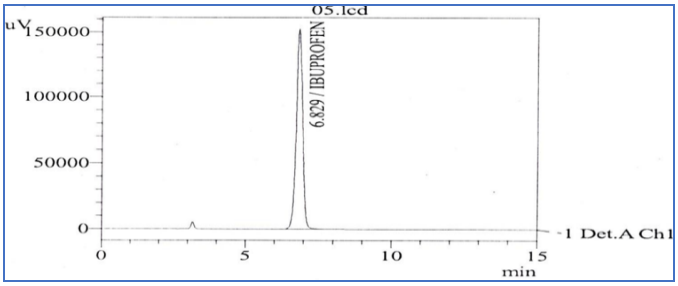


**Figure 5: Precision chromatogram for standard.**

### 1.4 Method Precision (Repeatability)

Six independent sample preparations were assayed.

**Table 4: Method Precision.**

| Sample ID | % Assay |
|---|---|
| 1 | 99.73 |
| 2 | 99.37 |
| 3 | 99.76 |
| 4 | 99.73 |
| 5 | 99.36 |
| 6 | 99.13 |

Mean: 99.51%; %RSD: 0.26%

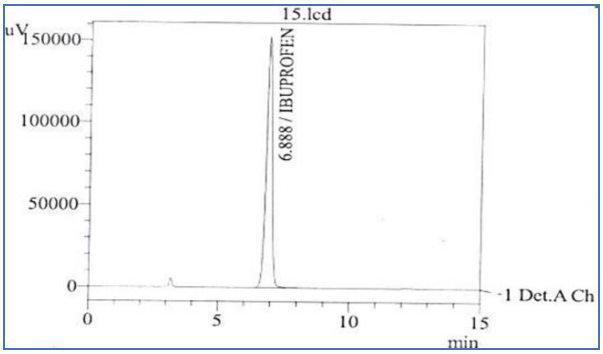


**Figure 6: Precision Chromatogram for sample with retention time 6.888.**

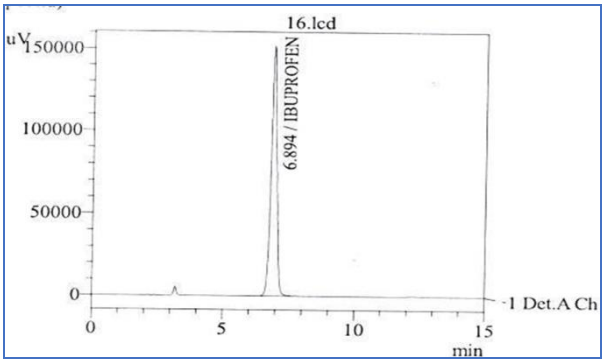


**Figure 7: Precision Chromatogram for sample with retention time 6.894.**

### 1.5 Ruggedness

Two analysts on different days performed the analysis.

**Table 5: Ruggedness Study.**

| Sample ID | Analyst 1 (% Assay) | Analyst 2 (% Assay) |
|---|---|---|
| 1 | 99.73 | 99.26 |
| 2 | 99.37 | 99.62 |
| 3 | 99.76 | 99.61 |
| 4 | 99.73 | 99.68 |
| 5 | 99.36 | 99.78 |
| 6 | 99.13 | 99.80 |

Overall % RSD: 0.23%

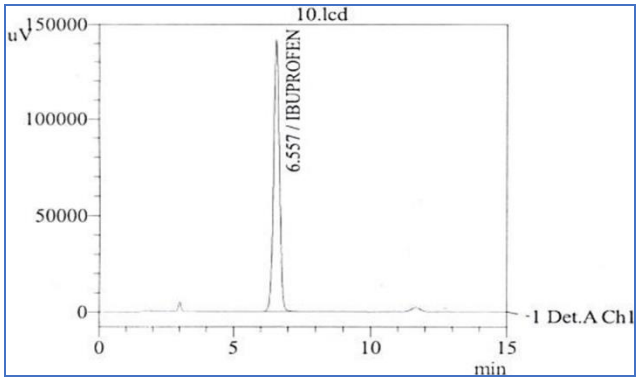


**Figure 8: Ruggedness chromatogram for sample at retention time 6.557.**

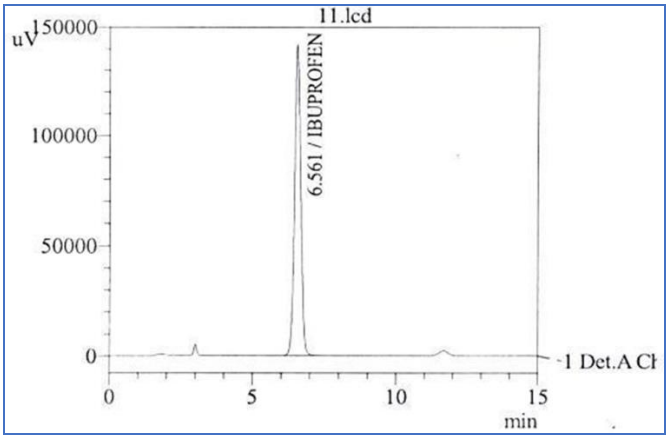


**Figure 9: Ruggedness chromatogram for sample at retention time 6.561.**

### 1.6 Solution Stability

Ibuprofen standard and sample solutions were stable for 24 hours at room temperature.

**Table 6: Solution Stability.**

| Time Point | Sample (% Assay) | % Correlation (Sample) | Standard Area | % Correlation (Std) |
|---|---|---|---|---|
| 0 hr | 99.73 | NA | 2,321,443 | NA |
| 6 hr | 99.32 | 99.59 | 2,303,577 | 99.23 |
| 12 hr | 99.41 | 99.68 | 2,299,750 | 99.07 |
| 24 hr | 98.22 | 98.48 | 2,320,703 | 99.97 |

### 2. Swab Sampling for Residual Drug (Ibuprofen)

Acceptance criteria: NMT 7 µg/4 sq. inch.

**Table 7: Traces of Ibuprofen (Batch W121G19).**

| Equipment | Part Swabbed | Residual (µg/4 sq.inch) |
|---|---|---|
| Sifter | Delivery chute | 9.95 |
| Octagonal Blender | Butterfly valve | 18.83 |
| Octagonal Blender | Rectangular door | 34.08 |
| Others | Multiple parts | BDL |

(BDL = Below Detection Limit)

### 3. Microbial Contamination Results

Acceptance criteria:

- TAMC: NMT 100 CFU/4 sq. inch
- TYMC: NMT 10 CFU/4 sq. inch

**Table 10: Microbial Contamination (W121G19 - Selected Results).**

| Equipment | Part | Bacterial CFU | Fungal CFU |
|---|---|---|---|
| Octagonal Blender | Butterfly valve | 12 | <10 |
| Sifter | Powder feeder | 10 | <10 |
| All other parts | Multiple | <10 or 0 | <10 |

**4. Rinse Water Quality**

**Table 11: Rinse Water Parameters (All Batches).**

| Parameter | Observed Value | Acceptance Range |
|---|---|---|
| pH | 6.8 – 7.2 | ±1 of blank |
| Conductivity (µS/cm) | <5 | ±5 µS/cm of blank |
| Residual Drug | BDL | < 7 µg/4 sq.inch |

**5. Maximum Allowable Carryover (MACO)**

**Table 12: MACO Calculations.**

| Criteria | Result |
|---|---|
| Dose-Based MACO | 920 µg/4 sq. inch |
| 10 ppm-Based MACO | 7 µg/4 sq. inch |
| Final Acceptance | 7 µg/4 sq. inch |

**DISCUSSION**

The validated RP-HPLC method proved to be highly specific, precise, and robust for the quantitative determination of residual ibuprofen on manufacturing equipment surfaces. The retention time for ibuprofen remained consistent (~6.74 minutes), with no observable interference from placebo or excipients, confirming method specificity. These findings are in alignment with ICH Q2(R1) guidelines, which emphasize specificity as a critical validation parameter for analytical methods in complex matrices.

System and method precision were evaluated through six replicates of standard and sample injections, respectively. The low %RSD values (0.05% for system precision and 0.26% for method precision) demonstrated excellent repeatability and reliability of the analytical system. Furthermore, ruggedness testing involving two independent analysts confirmed inter-analyst reproducibility (%RSD = 0.23%), suggesting the method's suitability for routine quality control applications in a GMP-compliant environment.

The solution stability study revealed that ibuprofen remained stable in solution for at least 24 hours under ambient conditions, supporting the method's robustness for batch-wise or time-separated sample analysis. This is particularly important in industrial settings where analytical throughput and time delays can vary.

Cleaning validation across three manufacturing batches (W121G19, W122G19, W123G19) revealed that most equipment surfaces had ibuprofen residue levels below the MACO threshold of 7 µg/4 sq. inch. While a few surfaces in the first batch exceeded this limit—most notably the butterfly valve and door of the octagonal blender—subsequent batches demonstrated complete compliance, suggesting that cleaning improvements were effective after the initial assessment. This reinforces the importance of ongoing monitoring and corrective actions in cleaning validation programs.

Microbiological swab tests indicated acceptable Total Aerobic Microbial Count (TAMC ≤ 100 CFU/4 sq. inch) and Total Yeast and Mold Count (TYMC ≤ 10 CFU/4 sq. inch) across all sampled equipment. These findings confirm that the cleaning process is not only chemically effective but also microbiologically sound, meeting regulatory requirements for non-sterile manufacturing.

Rinse water analysis provided further confirmation of cleaning effectiveness, with pH and conductivity measurements well within acceptable limits and no detectable drug residue. The combined chemical and microbiological data provide

strong evidence that the validated cleaning procedure reliably prevents cross-contamination and ensures compliance with cGMP.

Taken together, the data establish that the verified RP-HPLC method is an effective tool for monitoring residual ibuprofen and supports a robust cleaning validation protocol in a multi-product manufacturing facility. This study also highlights the critical role of method verification when adopting compendial methods for real-world industrial applications.

## CONCLUSION

The RP-HPLC method verification for the determination of assay in Ibuprofen Tablets BP 800 mg was successfully completed by evaluating critical analytical parameters including specificity, precision, ruggedness, and solution stability. The method demonstrated excellent specificity with no interference, high precision (%RSD < 0.3%), and stability over 24 hours, confirming its reliability. Therefore, the method is suitable for routine quality control testing of Ibuprofen Tablets BP 800 mg.

In line with a worst-case approach to cleaning validation, Ibuprofen 800 mg tablets were identified as the most challenging product within the tablet and capsule manufacturing chain. Cleaning validation performed on three consecutive manufacturing batches demonstrated that all tested surfaces met the predefined acceptance criteria for residual ibuprofen content, microbial proliferation, and rinse water quality. The consistent results confirm the effectiveness and reproducibility of the validated cleaning procedure.

Based on these findings, it is recommended that the verified RP-HPLC method be implemented for routine assay testing and that the validated cleaning procedure be adopted for future batches to ensure ongoing compliance with Good Manufacturing Practices (GMP) and to mitigate the risk of cross-contamination in multi-product manufacturing environments.

## CONFLICT OF INTEREST

None.

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