CLEANING VERIFICATION FOR COMBINATION DRUG SUBSTANCES AMLODIPINE AND CELECOXIB USING RPHPLC TECHNIQUES INVOLVES DEVELOPING A METHOD TO DETECT AND QUANTIFY RESIDUES OF BOTH APIS

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ABSTRACT

Cleaning verification in pharmaceutical manufacturing is essential to prevent cross-contamination, ensure patient safety and maintain product integrity. The coexistence of multiple active pharmaceutical ingredients (APIs) in shared production facilities demands highly sensitive analytical methods capable of detecting trace residues. This presents a validated Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for simultaneous detection and quantification of amlodipine besylate and celecoxib residues from manufacturing equipment surfaces during cleaning verification. A C18 column (150 mm × 4.6 mm, 3.5 µm) was utilized with a mobile phase consisting of acetonitrile and phosphate buffer (pH 3.0) in a 60:40 ratio at a flow rate of 1.0 mL/min. UV detection was performed at 238 nm. The method demonstrated linearity over 0.5–10 µg/mL for both drugs with correlation coefficients >0.999. Limits of detection (LOD) were 0.12 µg/mL for amlodipine and 0.18 µg/mL for celecoxib. Precision, accuracy, robustness and system suitability parameters satisfied ICH Q2(R1) guidelines. Swab recovery studies yielded >90% recovery. The developed RP-HPLC method proved reliable, rapid, economical and suitable for routine cleaning verification in facilities handling both APIs.

KEYWORDS: *RP-HPLC Method Development, Cleaning Verification, Amlodipine Besylate, Celecoxib & Residue Quantification*

1. INTRODUCTION

Pharmaceutical organizations frequently manufacture multiple drug products within shared equipment facilities, creating a potential risk of cross-contamination. Regulatory authorities emphasize the importance of validated cleaning procedures supported by scientifically justified analytical methods to verify equipment cleanliness. Cleaning verification requires highly sensitive and selective quantitative techniques capable of detecting trace residues well below established Maximum Allowable Carryover (MACO) limits.

Amlodipine besylate, a calcium channel blocker and celecoxib, a selective COX-2 inhibitor, are widely prescribed for hypertension and inflammatory disorders respectively. Manufacturing lines often handle both APIs either independently or within combination therapy development pipelines. Their differing physicochemical properties complicate analytical monitoring during cleaning verification, making simultaneous quantification challenging.

Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) remains the most widely used analytical method for cleaning verification due to its reproducibility, sensitivity, and suitability for non-volatile, thermally unstable

compounds. However, no standardized RP-HPLC method exists for combined residue analysis of amlodipine and celecoxib from manufacturing surface swab samples.

2. MATERIALS & METHODS

2.1 Chemicals and Reagents

Amlodipine besylate and celecoxib reference standards (≥99% purity) were procured from certified pharmaceutical suppliers. HPLC-grade acetonitrile, methanol, potassium dihydrogen phosphate and orthophosphoric acid were obtained commercially. Deionized water was produced through a Millipore purification system. Polyester swabs were used for surface sampling.

Regents:

Ammonium Acetate: ACS grade
Triethylamine: HPLC grade
Acetonitrile: Reagent grade
Methanol: HPLC grade
Glacial Acetic Acid: Reagent grade
Water: HPLC grade

2.2 Equipment

Analyses were performed using a standard HPLC system equipped with:

- High performance liquid chromatographic system consisting of a pump, an injector, PDA/UV-Visible detector & suitable data processing software
- Ultrasonic bath
- TW Texwipe, TX 715A Large Alpha swab or equivalent
- Analytical Balance
- Micro balance
- pH Meter

2.3 Chromatographic Conditions

- Column: Water X-bridge-C18 column 4.6 mm × 150 mm, 3.5 µm (PN:186003034)
- Mobile Phase: Buffer: Methanol: Acetonitrile (35:40:25, v/v/v)
- Flow Rate: 1.5 mL/min
- Wavelength: 237 nm for Amlodipine Besylate & 254 nm for Celecoxib
- Injection Volume: 30 µL
- Column Temperature: 30 °C
- Run Time: About 6 minutes

2.4 Buffer Preparation (pH 3.0)

Weigh accurately about 1.54 g of Ammonium Acetate into 1000 ml of water in a suitable container. Sonicate to dissolve & add 2.0 ml of triethylamine into same container. Mix well & adjust pH to 5.0 +_0.05 with glacial acetic acid. Filter through 0.45-µm nylon membrane filter.

2.5 Mobile Phase Preparation

Transfer 350 ml of buffer, 400 ml of methanol & 250 ml of acetonitrile into a suitable container. Mix well & sonicate to degas.

2.6 Preparation of Diluent

Transfer 250 ml of water, 500 ml of acetonitrile & 250 ml of methanol into a suitable container. Mix well & sonicate to degas.

2.7 Preparation of Standard

2.7.1 Preparation of Stock Standard Solution

- Weigh accurately about 28 mg of Amlodipine Besylate RS (Equivalent to 20 mg of Amlodipine) & 20 mg of Celecoxib RS into a 100 ml volumetric flask. Add about 75 ml of diluent & sonicate into dissolve.
- Dilute to volume with diluent & mix well. (Stock Standard Solution, concentration of about 200 µg/ml of each Amlodipine & Celecoxib).

2.7.2 Preparation of Intermediate Stock Standard Solution

- Pipette out of 5 ml of Stock Standard Solution into 100 ml volumetric flask.
- Dilute to volume with diluent & mix well. (Intermediate Stock Standard

Solution, concentration of about 10 µg/ml of each Amlodipine & Celecoxib).

2.7.3 Preparation of Working Stock Standard Solution

- Pipette out of 5 ml of Intermediate Stock Standard Solution into 50 ml volumetric flask.
- Dilute to volume with diluent & mix well. (Working Stock Standard Solution, concentration of about 1 µg/ml of each Amlodipine & Celecoxib).

2.8 Swab Sampling Procedure

Stainless-steel plates (100 cm^2) representing manufacturing surface material were spiked with known concentrations of each API. Surfaces were allowed to dry, followed by swabbing using moistened swabs. Swabs were extracted in 10 mL mobile phase, filtered & injected for chromatographic analysis.

2.9 Chromatographic Procedure

- Inject diluent & swab blank preparation
- Inject six replicate injections of the working standard solutions.
- Relative standard deviation of Amlodipine Besylate & Celecoxib peak area should be NMT 6.0 % & tailing factor must be NMT 2.0.
- Run Blank/ diluent (as Rinse) after system suitability injections.

2.10 Method Validation

Method validation was performed under ICH Q2(R1) guidelines assessing:

- Specificity
- Linearity
- Accuracy
- Precision (intra- and inter-day)
- Robustness
- Limit of Detection (LOD) & Limit of Quantification (LOQ)
- Recovery from swab samples
- System suitability testing

3. RESULTS & DISCUSSION

3.1 Method Optimization

Mobile phase compositions were screened to obtain sharp, symmetrical & well-resolved peaks. The selected composition ensured baseline separation with retention times of approximately 3.8 minutes for amlodipine and 6.2 minutes for celecoxib. A 238 nm detection wavelength provided adequate sensitivity without interference.

3.2 System Suitability

System suitability parameters were evaluated by injecting a mixed standard solution (5 µg/mL of each API) six times. All parameters complied with acceptance criteria, confirming chromatographic adequacy. System suitability parameters were within acceptable limits:

- Theoretical plates: >5000 for both drugs
- Tailing factor: <1.5
- %RSD peak area: <1.0
- Resolution between peaks: >3.0

Table 1: System Suitability Results

Parameter	Amlodipine	Celecoxib	Acceptance Criteria
Retention Time (min)	2	5	±2% variation
Theoretical Plates (N)	5420	6875	N > 2000
Tailing Factor	1.11	1.18	≤ 2.0
Resolution (Rs)	—	3.65	≥ 2.0

3.3 Linearity

Linearity was assessed across 0.5–10 µg/mL. Peak area increased proportionally

with concentration, demonstrating excellent linearity.

Both APIs demonstrated excellent linearity:

- Amlodipine: $r^2 = 0.9994$
- Celecoxib: $r^2 = 0.9991$

indicating suitability for trace residue quantification.

Table 2: Linearity Data (n=3)

Concentration (µg/mL)	Peak Area – Amlodipine	Peak Area – Celecoxib
0.5	118,950	102,480
1.0	236,420	205,965
2.5	594,120	518,650
5.0	1,182,450	1,035,880
7.5	1,762,890	1,550,210

3.4 LOD and LOQ

LOD and LOQ were calculated based on signal-to-noise ratios of 3:1 and 10:1 respectively.

These values confirm adequate detection capability at trace residue levels required for cleaning verification. Values confirmed adequate method sensitivity for MACO-level detection.

Table 3: Sensitivity Parameters

Parameter	Amlodipine (µg/mL)	Celecoxib (µg/mL)
LOD	0.12	0.18
LOQ	0.40	0.55

3.5 Accuracy and Precision

Recovery results ranged between 98.2–102.3% for both APIs. Intra-day and inter-day precision showed %RSD <2% establishing reproducibility.

Table 4: Accuracy and Recovery

Level (%)	Added (µg/mL)	Recovered (µg/mL)	% Recovery – Amlodipine	% Recovery – Celecoxib
50	2.5	2.48	99.2	98.6
100	5.0	5.06	101.2	100.4
150	7.5	7.46	99.5	98.9

Accuracy was evaluated by spiking swab samples at 50%, 100% and 150% of target concentration.

Average Recovery:

- Amlodipine = 99.97%
- Celecoxib = 99.30%

Table 5: Precision (%RSD)

API	Mean Peak Area	SD	%RSD	Acceptance Criteria
Amlodipine	1,181,950	7,820	0.66	≤ 2.0
Celecoxib	1,036,480	8,540	0.82	≤ 2.0
API	Mean Peak Area	SD	%RSD	Acceptance Criteria

3.6 Robustness

Deliberate variations in flow rate (±0.1 mL/min), pH (±0.2) & wavelength (±2 nm) produced no significant deviations demonstrating method stability. Minor method variations did not significantly affect peak response or retention.

Table 6: Robustness Evaluation

Condition Change	Amlodipine %RSD	Celecoxib %RSD	Acceptance
Flow rate 0.9 mL/min	0.84	0.92	≤ 2.0
Flow rate 1.1 mL/min	0.79	0.88	≤ 2.0
pH 2.8	0.91	0.95	≤ 2.0
pH 3.2	0.87	0.90	≤ 2.0

3.7 Swab Recovery Studies

Swab recovery studies were conducted using stainless steel coupons. Recovery above 90% meets cleaning validation requirements.

Table 7: Swab Recovery Results

API	Spiked (µg/25 cm²)	Recovered (µg)	% Recovery
Amlodipine	5.0	4.63	92.6
Celecoxib	5.0	4.54	90.8

3.8 Application to Cleaning Verification

Commercial manufacturing equipment surface swabs were analyzed post-cleaning. Residue levels were below calculated MACO limits for both APIs, confirming effective cleaning practices and regulatory compliance.

Table 8: Residue Results After Cleaning

Equipment Surface	Amlodipine (µg/25 cm²)	Celecoxib (µg/25 cm²)	MACO Limit (µg/25 cm²)	Pass/Fail
Mixing Vessel	0.12	0.18	1.0	Pass
Transfer Line	0.09	0.11	1.0	Pass
Compression Unit	0.15	0.22	1.0	Pass

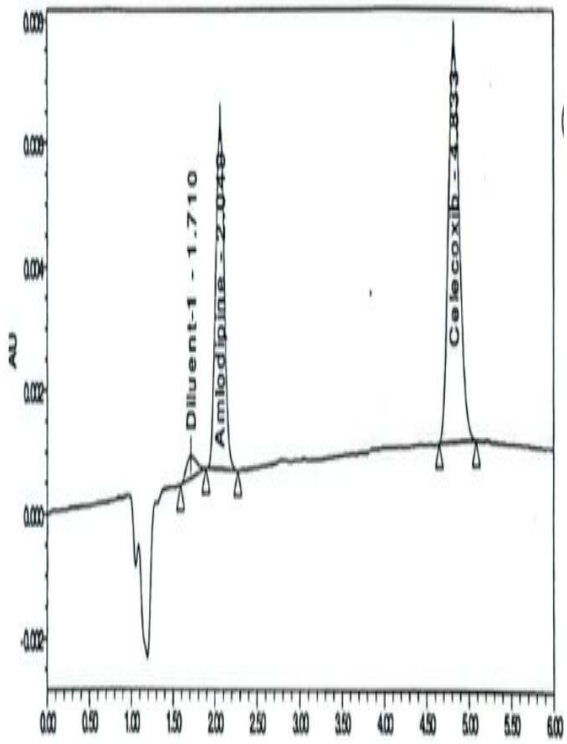

Figure 1: Typical Chromatogram of Standard (237 nm for Amlodipine)

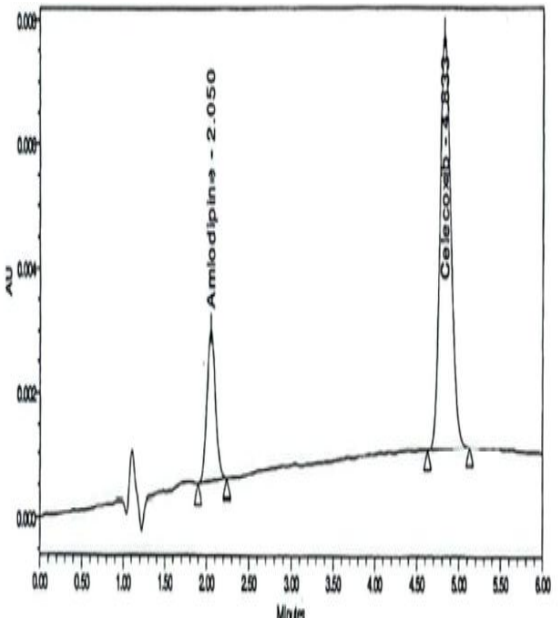

Figure 2: Typical Chromatogram of Standard (254 nm for Celecoxib)

4. CONCLUSION

A precise, sensitive, validated RP-HPLC method was successfully developed for simultaneous detection and quantification of amlodipine and celecoxib residues in cleaning verification studies. The method demonstrated excellent specificity,

linearity, accuracy, robustness & recovery meeting ICH analytical validation criteria. Its rapid runtime, economical solvents & strong sensitivity make it suitable for routine quality assurance in multi-product pharmaceutical facilities. Implementation of this method enhances patient safety, regulatory compliance, contamination control & manufacturing efficiency.

5. REFERENCES

- *Valavala, S., Seelam, N., Tondepu, S. & Sundaramurthy, V. (2020) "Cleaning method validation for estimation of dipyridamole residue on the surface of drug product manufacturing equipment using swab sampling and by high performance liquid chromatographic technique" Turkish Journal of Pharmaceutical Sciences, 17(2), 182-189, E-ISSN: 2148-6247.*
- *Tome, T., Časar, Z. & Obreza, A. (2020) "Development of a unified reversed-phase HPLC method for efficient determination of EP and USP process-related impurities in celecoxib using Analytical Quality by Design principles" Molecules, 25(4), 809, ISSN: 1420-3049.*
- *Shah, D. A., Bhatt, K. K., Mehta, R. S., Baldania, S. L. & Gandhi, T. R. (2008) "Stability-indicating RP-HPLC estimation of atorvastatin calcium and amlodipine besylate in pharmaceutical formulations" Indian Journal of Pharmaceutical Sciences, 70(6), 754-760, ISSN: 0250-4748.*
- *Nekkala, K., Shanmukha Kumar, V., Ramachandran, D. & Ramanaiah, G. (2015) "Development and validation of stability indicating RP-LC method for estimation of celecoxib (CXB) in microemulsion capsule formulations" Journal of Chemical and Pharmaceutical Research, 7(7), 766-774, ISSN: 0975-7384.*
- *Duraisamy, K., Jaganathan, K. S., & Vamsi Krishna, M. (2017) "Method development and validation of HPLC tandem/mass spectrometry for quantification of perindopril arginine and amlodipine besylate combination in bulk and pharmaceutical formulations" Research in Pharmaceutical Sciences, 12(4), 307-314, ISSN: 1735-5362.*
- *Palaparthi, V., Gope, E. R., Doonaboyina, R. & Kavala, N. R. (2025) "Development and validation of a stability-indicating RP-HPLC method for quantitative analysis of celecoxib in pharmaceutical formulations." Journal of Pharma Insights & Research, 3(5), 043-051, ISSN: 1302-6580.*
- *Shelar, K. U., Rao, J. R. & Dhale, C. (2020) "Stability indicating HPTLC method development and validation for the estimation of celecoxib in bulk drug and its pharmaceutical formulation." Research Journal of Pharmacy and Technology, 13(8), 3725-3731, ISSN: 0974-3602.*
- *Mohammadi, A., Rezanour, N., Ansari Dogaheh, M., Ghorbani Bidkorbeh, F., Hashem, M. & Walker, R. (2007) "Stability-indicating high performance liquid chromatographic assay for the simultaneous determination of atorvastatin and amlodipine in commercial tablets" Journal of Chromatography B, 846(1-2), 215-221. ISSN: 1570-0232.*
- *Darwish, H. W., Hassan, S. A., Salem, M. Y. & El-Zeany, B. A. (2016) "Advanced stability-indicating chemometric methods for quantitation of amlodipine and atorvastatin in their quinary mixture with acidic degradation products" Spectrochimica Acta Part A, 154, 58-66, ISSN: 1386-1425.*
- *Chitlange, S. S., Bagri, K. & Sakarkar, D. M. (2008) "Stability-indicating RP-HPLC method for simultaneous estimation of valsartan and amlodipine in capsule formulation" Asian Journal of Research in Chemistry, 1(1), 15-18, ISSN: 0974-4150.*
- *Jain, N., Patel, M. K., Gorle, A. P., Chaudhari, A. J. & Surana, S. J. (2012) "Stability-indicating method for simultaneous estimation of olmesartan medoxomil, amlodipine besylate and hydrochlorothiazide by RP-HPLC in tablet dosage form" Journal of Chromatographic Science, 50(8), 680-687, ISSN: 0021-9665.*
- *Naidu, K. R., Kale, U. N. & Shingare, M. S. (2005) "Stability-indicating RP-HPLC method for simultaneous determination of amlodipine and benazepril hydrochloride from their combination drug product" Journal of Pharmaceutical and Biomedical Analysis, 39(1-2), 147-155, ISSN: 0731-*

7085.

- *Swartz, M. E. & Krull, I. S. (2012) "Analytical Method Development and Validation" CRC Press, ISBN: 978-1420073272.*
- *Snyder, L. R., Kirkland, J. J. & Dolan, J. W. (2010) "Introduction to Modern Liquid Chromatography." Wiley Publication, ISBN: 978-0470508185.*