# Original Article

## Development and Validation of a UHPLC Method for the Simultaneous Estimation of Hydrochlorothiazide and Amlodipine in Solid Dosage Forms

**Boopathyraja R[1], Ezhilkumar E[1], Krishna S[1], Nehamagimai Nisha A[1], Priyadharshini R[1], Sivaraj A[1], Thrisha S[1], Vinitha R[1], Ganesan S[2], Medapati Vijaya Vara Prasad[3], Kathiravan Mani[4]**

*From, [1]Department of Pharmaceutical Chemistry, KG College of Pharmacy and Research Institute, Ashokapuri, Villupuram, Tamil Nadu, India, [2]Principal, KG College of Pharmacy and Research Institute, Ashokapuri, Villupuram, Tamil Nadu, India, [3]Professor, Department of Pharmaceutical Chemistry, KG College of Pharmacy and Research Institute, Ashokapuri, Villupuram, Tamil Nadu, India, [4]Associate Professor, Department of Pharmaceutical Chemistry, KG College of Pharmacy and Research Institute, Ashokapuri, Villupuram, Tamil Nadu, India,*

## ABSTRACT

**Background:** Hypertension, a leading global cardiovascular risk factor, is commonly managed with combination pharmacotherapy. Hydrochlorothiazide (HCT), a thiazide diuretic, and Amlodipine (AML), a calcium channel blocker, are frequently co-prescribed in fixed-dose tablet formulations to achieve optimal blood pressure control. Despite their widespread co-administration, a validated Ultra-High-Performance Liquid Chromatography (UHPLC) method for the simultaneous quantification of both drugs in solid dosage forms was not available in the published literature at the time of this investigation. **Methodology:** A UHPLC method was developed and validated on an Agilent system using a C8 column (4.6 mm × 50 mm, 3 µm) operated under isocratic conditions. The mobile phase consisted of 0.02 M potassium dihydrogen orthophosphate buffer (pH 3.0) and acetonitrile (60:40, v/v) delivered at 0.5 mL/min. Detection was performed at 230 nm with an injection volume of 2 µL. Validation was executed in accordance with ICH Q2(R1) guidelines, encompassing system suitability, specificity, linearity, precision, accuracy, and robustness. **Results:** Hydrochlorothiazide and Amlodipine were resolved at retention times of 1.810 min and 2.911 min, respectively, within a 5-minute run. Linearity was established over 50–150% of the nominal concentration; correlation coefficients were $R^2$ = 1.0000 (HCT) and $R^2$ = 0.9993 (AML). Assay recovery values were 100.33% (HCT) and 100.07% (AML). Repeatability %RSD values were 0.16% and 0.07%, respectively. Mean accuracy recoveries ranged from 100.60–101.28% (HCT) and 99.57–100.97% (AML). All robustness parameters remained within acceptance criteria under deliberate variations in flow rate and detection wavelength. **Conclusion:** The proposed UHPLC method is rapid, precise, accurate, and specific for the simultaneous estimation of hydrochlorothiazide and amlodipine. It satisfies ICH validation requirements and is suitable for routine quality control analysis in pharmaceutical manufacturing settings.

***Key words:*** Hydrochlorothiazide; Amlodipine; UHPLC; Method Development; Method Validation; ICH Guidelines; Simultaneous Estimation; Solid Dosage Forms.

Hypertension remains one of the most prevalent non-communicable diseases worldwide and a major contributor to cardiovascular morbidity and mortality. Recent global estimates indicate that approximately 1.4 billion adults are affected, with a substantial proportion remaining undiagnosed or inadequately controlled, particularly in low- and middle-income countries [1]. Effective management of hypertension is therefore a critical public health priority, with current therapeutic strategies emphasizing combination pharmacotherapy to achieve optimal blood pressure control [2].

Among the commonly prescribed antihypertensive agents, thiazide diuretics and calcium channel blockers occupy a central role in treatment guidelines [3]. Hydrochlorothiazide (HCT), a thiazide diuretic, exerts its antihypertensive effect by inhibiting sodium reabsorption in the distal convoluted tubule, leading to reduced plasma volume and peripheral resistance [4]. Amlodipine (AML), a long-acting dihydropyridine calcium channel blocker, lowers blood pressure by inhibiting

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**Correspondence to:** M. Kathiravan, Department of Pharmaceutical Chemistry, KG College of Pharmacy and Research Institute, Ashokapuri, Villupuram, Tamil Nadu, India.

**Email:** kathirpharmacy@gmail.com

L-type calcium channels, resulting in vascular smooth muscle relaxation and sustained vasodilation [5].

The combination of these two agents is widely utilized due to their complementary mechanisms of action, improved therapeutic efficacy, and enhanced patient adherence [3]. Analytical determination of HCT and AML in combined pharmaceutical formulations is essential for quality control, regulatory compliance, and routine batch analysis. Several analytical methods have been reported for their estimation, including UV spectrophotometry and conventional reversed-phase high-performance liquid chromatography (RP-HPLC) [7]. However, these methods often suffer from limitations such as insufficient selectivity, longer run times, and higher solvent consumption, which reduce their suitability for high-throughput analytical environments [7].

In recent years, Ultra-High-Performance Liquid Chromatography (UHPLC) has emerged as a powerful alternative to conventional HPLC, offering significant improvements in chromatographic efficiency, resolution, and analysis speed through the use of sub-2 µm particle size columns and elevated operating pressures [8]. These advantages make UHPLC particularly attractive for routine pharmaceutical analysis, where rapid and reliable quantification is essential [8]. Despite the widespread clinical use of hydrochlorothiazide–amlodipine combination therapy and the analytical advantages of UHPLC, a comprehensive review of the literature indicates a lack of validated UHPLC methods for the simultaneous estimation of these two drugs in solid dosage forms.

Most reported methods are based on conventional HPLC systems with longer analysis times, limiting their practical applicability in modern quality control laboratories. Therefore, the present study aims to develop and validate a simple, rapid, precise, and robust UHPLC method for the simultaneous estimation of hydrochlorothiazide and amlodipine in tablet dosage forms in accordance with ICH Q2(R1) guidelines. The proposed method is intended to provide a time-efficient and reliable analytical tool suitable for routine pharmaceutical quality control applications.

## MATERIALS AND METHODS

### 2.1 Chemicals and Reagents

Working standards of hydrochlorothiazide and amlodipine besylate were obtained as in-house reference materials. Potassium dihydrogen orthophosphate ($KH_2PO_4$), orthophosphoric acid, and acetonitrile (HPLC grade) were procured from Rankem (India). Ultra-pure water (Milli-Q, resistivity ≥18 MΩ·cm) was used throughout the study. All reagents and solvents were of analytical or HPLC grade and used without further purification.

### 2.2 Instrumentation

Chromatographic analysis was performed using an Agilent UHPLC system equipped with a quaternary pump, autosampler, thermostatted column compartment, and variable wavelength UV detector, operated via Agilent ChemStation software. Sample preparation employed a semi-micro analytical balance (Radwag, readability 0.01 mg) and an ultrasonicator. The mobile phase was filtered through a 0.45 µm nylon membrane filter prior to use.

### 2.3 Chromatographic Conditions

Separation was achieved on a C8 column (4.6 mm × 50 mm, 3 µm particle size) under isocratic conditions. The mobile phase consisted of 0.02 M $KH_2PO_4$ buffer (pH 3.0, adjusted with orthophosphoric acid) and acetonitrile in a ratio of 60:40 (v/v), delivered at a flow rate of 0.5 mL/min. Detection was carried out at 230 nm with an injection volume of 2 µL. The total run time was 5 minutes, and the mobile phase was used as the diluent (Table 1).

**Table 1. Optimized UHPLC Chromatographic Conditions**

| Parameter | Condition |
|---|---|
| Column | C8, 4.6 mm × 50 mm, 3 µm particle size |
| Mode | Isocratic |
| Mobile Phase | Buffer : Acetonitrile = 60:40 (v/v) |
| Buffer | 0.02 M $KH_2PO_4$, pH 3.0 adjusted with $H_3PO_4$ |
| Flow Rate | 0.5 mL/min |
| Detection Wavelength | 230 nm |
| Injection Volume | 2 µL |
| Run Time | 5 minutes |
| Diluent | Mobile phase |

### 2.4 Preparation of Solutions

#### 2.4.1 Standard Stock Solutions

Hydrochlorothiazide stock solution (200 µg/mL) was prepared by dissolving an accurately weighed quantity (20 mg) in 100 mL of diluent. Amlodipine besylate stock solution (~110 µg/mL) was prepared by dissolving 22 mg in 200 mL of diluent. All solutions were sonicated until complete dissolution and brought to volume with diluent.

#### 2.4.2 Standard Working Solution

A combined standard working solution was prepared by transferring 5.0 mL of each stock solution into a 50 mL volumetric flask and diluting to volume with diluent to obtain the nominal concentration.

#### 2.4.3 Sample Preparation

Tablet powder equivalent to 5 mg of amlodipine (corresponding to 12.5 mg hydrochlorothiazide) was accurately weighed and transferred into a 25 mL volumetric flask. The sample was dissolved in diluent with sonication for 10 minutes and diluted to volume. The solution was filtered through a 0.45 µm nylon membrane filter. An aliquot of 2.0

mL of the filtrate was further diluted to 50 mL with diluent to obtain the final analytical concentration.

### 2.5 Method Validation

Method validation was performed in accordance with ICH Q2(R1) guidelines. The following parameters were evaluated:

- System suitability: Five replicate injections of the standard solution were analyzed, and parameters including retention time, peak area, tailing factor, and theoretical plates were assessed. The %RSD of peak areas was required to be ≤2.0%.
- Specificity: Blank and placebo solutions were analyzed to confirm the absence of interfering peaks at the retention times of the analytes.
- Linearity: Calibration curves were constructed using five concentration levels (50–150% of nominal concentration), and linear regression analysis was performed.
- Precision: Repeatability was evaluated using six independent sample preparations (intra-day), while intermediate precision was assessed on a different day by another analyst.
- Accuracy: Recovery studies were performed at 80%, 100%, and 120% levels, each in triplicate.
- Robustness: The effect of deliberate variations in flow rate (±0.1 mL/min) and detection wavelength (±2 nm) was examined.

## RESULTS

### 3.1 Method Development – Trial Chromatographic Conditions

Four chromatographic trial conditions were evaluated systematically prior to finalization of the optimized method. Table 2 summarizes the trial conditions and their outcomes (Figure 1).

**Table 2. Summary of Trial Chromatographic Conditions and Observations**

| Trial | Column | Mobile Phase (Buffer:ACN) | pH / Run Time | Observation |
|---|---|---|---|---|
| 1 | C8, 100 mm | 30:70 | 2.0 / 5 min | Poor separation |
| 2 | C8, 100 mm | 40:60 | 3.0 / 2 min | Poor peak shape & separation |
| 3 | C8, 100 mm | 50:50 | 3.0 / 3 min | Poor peak shape |
| 4 | C8, 100 mm | 60:40 | 3.0 / 10 min | Excessive run time |
| Optimized | C8, 50 mm | 60:40 | 3.0 / 5 min | Good peak shape, adequate separation |

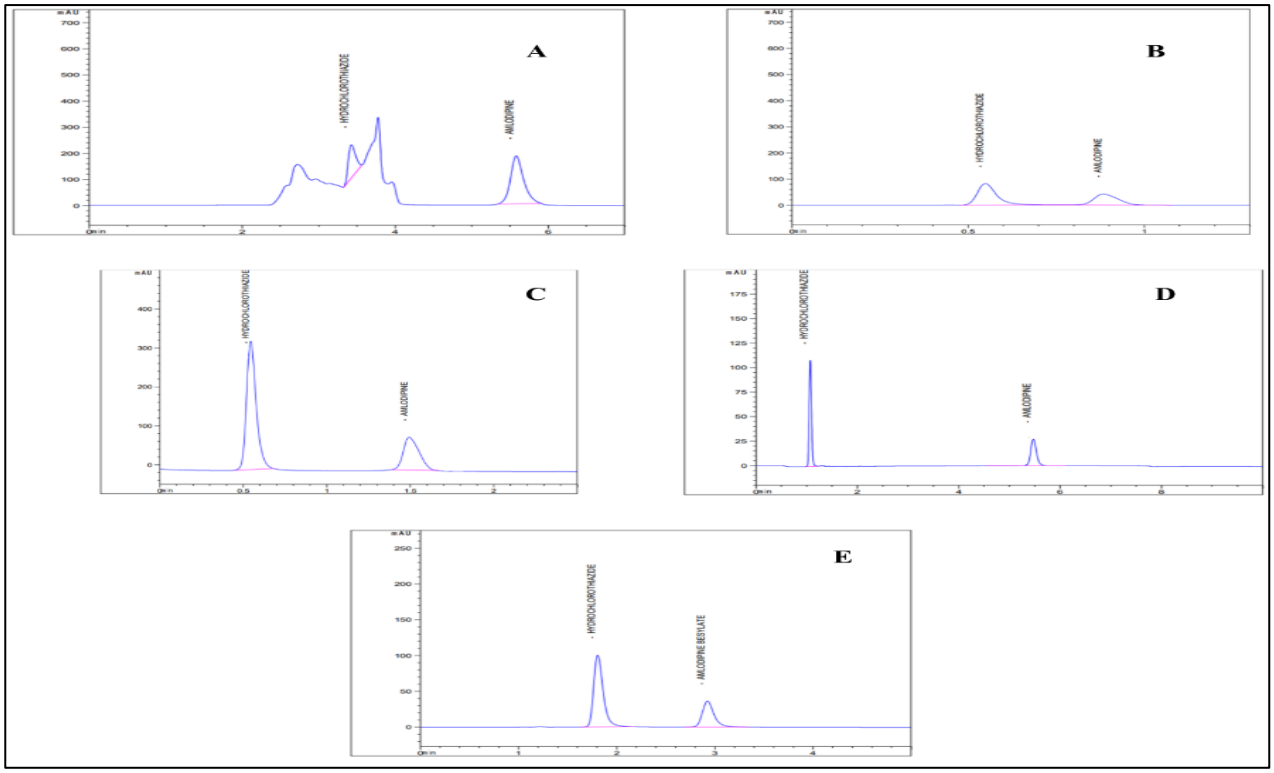


**Figure 1: Trial chromatograms (A–D) illustrate the stepwise optimization of chromatographic conditions, while chromatogram (E) shows the optimized method with well-resolved, sharp, and symmetrical peaks of Hydrochlorothiazide and Amlodipine suitable for analysis.**

The final optimized method employed a shorter C8 column (50 mm) with a 60:40 buffer-to-acetonitrile mobile phase ratio at pH 3.0, yielding satisfactory peak symmetry, adequate resolution, and a total run time of 5 minutes. Hydrochlorothiazide eluted at 1.810 min and amlodipine at 2.911 min.

### 3.2 System Suitability

System suitability was evaluated by performing five consecutive injections of the standard working solution under the optimized conditions. All parameters complied with ICH and USP acceptance criteria (theoretical plates >500; tailing factor <2.0; %RSD of area ≤2.0%) (Table 3 and 4) (Figure 2).

**Table 3. System Suitability Parameters for Hydrochlorothiazide**

| Injection | Retention Time (min) | Peak Area | Theoretical Plates | Tailing Factor |
|---|---|---|---|---|
| Standard 01 | 1.806 | 675.495 | 1807 | 1.381 |
| Standard 02 | 1.805 | 675.641 | 1811 | 1.357 |
| Standard 03 | 1.806 | 675.914 | 1835 | 1.363 |
| Standard 04 | 1.804 | 676.108 | 1822 | 1.359 |
| Standard 05 | 1.806 | 675.950 | 1824 | 1.349 |
| Mean | 1.805 | 675.822 | 1820 | 1.362 |
| SD | 0.00 | 0.248 | 11.12 | 0.01 |
| %RSD | 0.05 | 0.04 | 0.61 | 0.87 |

**Table 4. System Suitability Parameters for Amlodipine**

| Injection | Retention Time (min) | Peak Area | Theoretical Plates | Tailing Factor |
|---|---|---|---|---|
| Standard 01 | 2.924 | 300.149 | 3182 | 1.316 |
| Standard 02 | 2.925 | 301.452 | 3184 | 1.317 |
| Standard 03 | 2.925 | 300.485 | 3205 | 1.306 |
| Standard 04 | 2.931 | 300.380 | 3190 | 1.309 |
| Standard 05 | 2.952 | 301.251 | 3188 | 1.312 |
| Mean | 2.931 | 300.743 | 3190 | 1.312 |
| SD | 0.01 | 0.573 | 9.07 | 0.00 |
| %RSD | 0.40 | 0.19 | 0.28 | 0.35 |

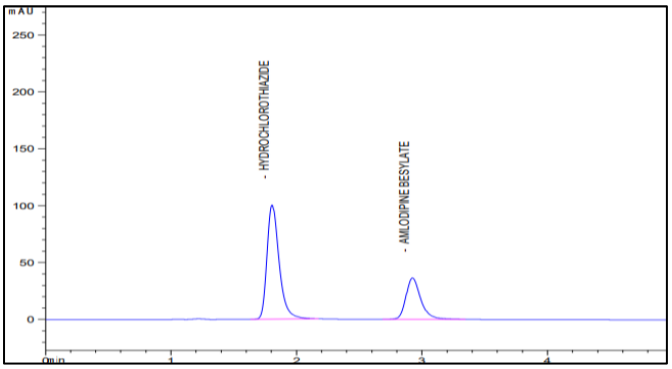


**Figure 2: System suitability chromatogram showing well-resolved, sharp, and symmetrical peaks of Hydrochlorothiazide and Amlodipine, confirming the adequacy of the chromatographic system.**

### 3.3 Specificity

Specificity was assessed by comparing the chromatograms of the diluent blank, standard, and sample solutions. No interfering peaks were observed in the blank at the retention times of HCT (1.854 min) or AML (2.510 min). The peak areas of the standard and sample solutions were reproducible, confirming the absence of matrix-related interferences. The method was deemed specific (Table 5) ( Figure 3).

**Table 5. Specificity Results for Hydrochlorothiazide and Amlodipine**

| Sample ID | Drug | Retention Time (min) | Peak Area | Inference |
|---|---|---|---|---|
| Blank | HCT | 1.854 | No peak | No interference |
| Standard | HCT | 1.854 | 675.845 | Specific peak |
| Sample | HCT | 1.877 | 676.098 | Specific peak |
| Blank | AML | 2.510 | No peak | No interference |
| Standard | AML | 2.510 | 300.105 | Specific peak |
| Sample | AML | 2.519 | 299.997 | Specific peak |

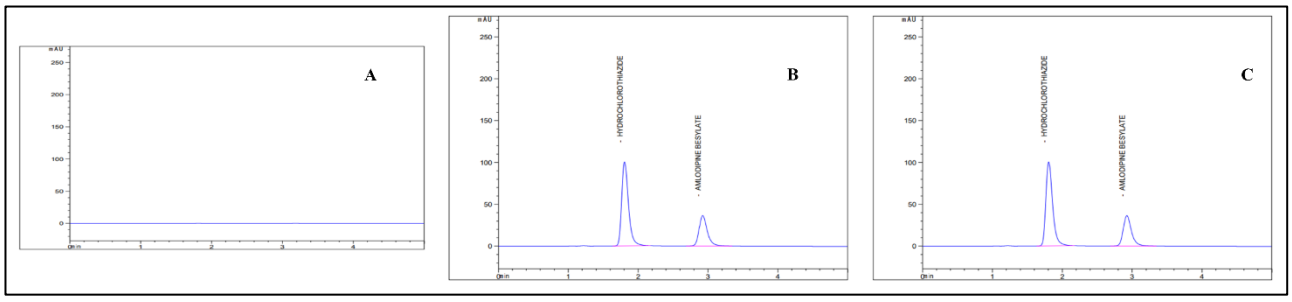


**Figure 3: Blank chromatogram (A), standard chromatogram (B), and sample chromatogram (C) showing no interference at the retention times of Hydrochlorothiazide and Amlodipine, confirming the specificity of the method.**

### 3.4 Assay

The assay of the combined tablet formulation was performed using the optimized method. The mean assay percentage for hydrochlorothiazide was determined to be 100.33% and for amlodipine was 100.07%, both falling within the pharmacopoeial acceptance range of 98.0–102.0% (Figure 4).

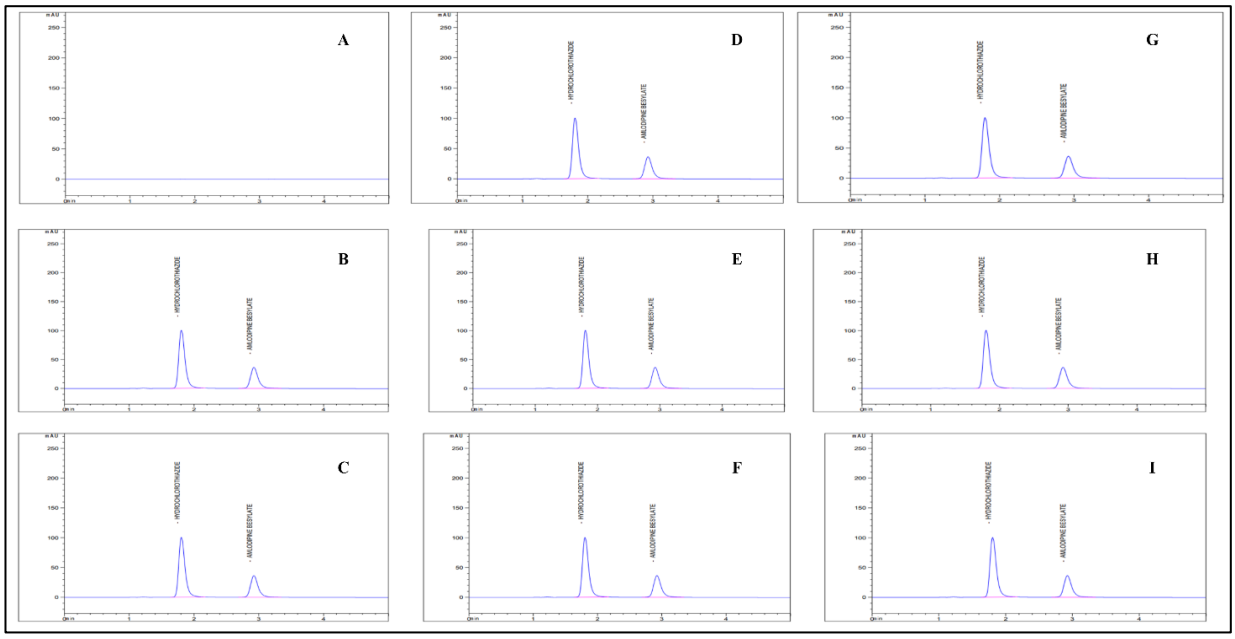


**Figure 4: Assay blank chromatogram (A), standard chromatograms (B–F), and sample chromatograms (G–I) showing consistent peak response and retention times of Hydrochlorothiazide and Amlodipine, confirming the reliability of the assay method.**

### 3.5 Linearity

Linearity was evaluated by preparing duplicate injections at five concentration levels spanning 50% to 150% of the nominal working concentration. The peak area responses were linearly related to concentration over the evaluated range for both analytes. The regression equations and correlation coefficients are presented in Table 6 (Figure 5 and 6).

**Table 6. Linearity Parameters for Hydrochlorothiazide and Amlodipine**

| Parameter | Concentration Range | HCT | AML |
|---|---|---|---|
| Linearity Range | 50% – 150% | 50% – 150% | 50% – 150% |
| Regression Equation | y = mx + c | y = 34.1228x − 1.1040 | y = 39.0443x − 11.4956 |
| Correlation Coefficient ($R^2$) | — | 1.0000 | 0.9993 |
| Acceptance Criterion ($R^2$) | — | ≥ 0.999 | ≥ 0.999 |
| Compliance | — | Complies | Complies |

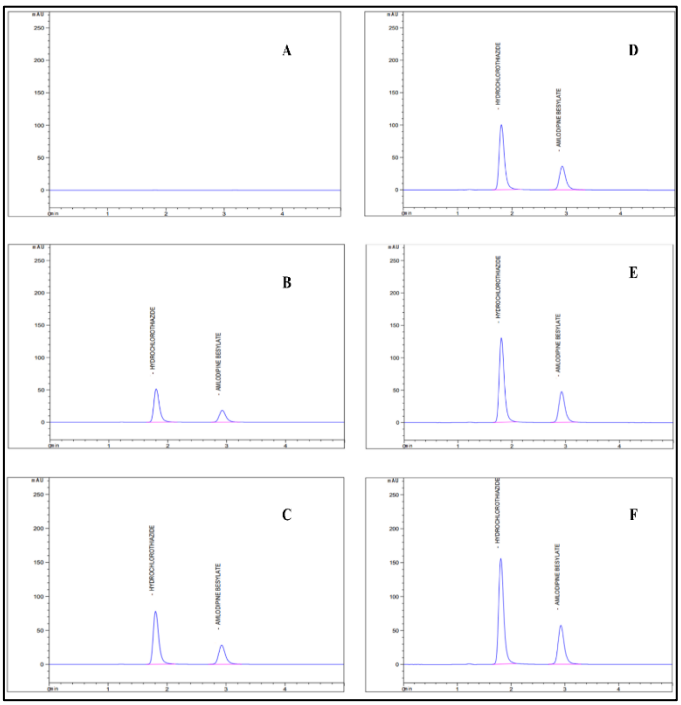


**Figure 5: Linearity chromatograms showing blank (A) and increasing concentration levels (B–F) with proportional peak responses of Hydrochlorothiazide and Amlodipine, confirming the linearity of the method.**

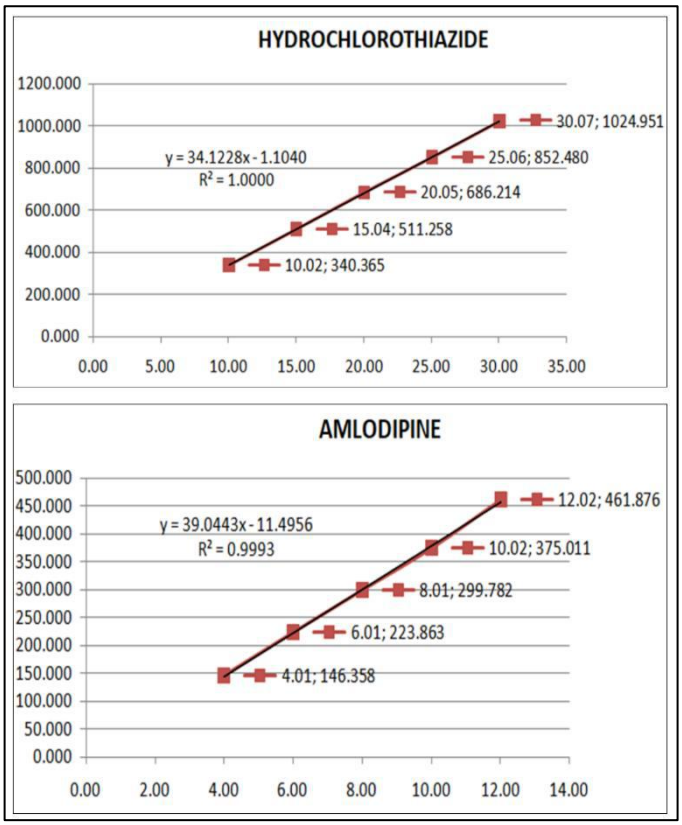


**Figure 6: Linearity calibration curves of Hydrochlorothiazide and Amlodipine showing a linear relationship between concentration and peak area with high correlation coefficients, confirming the linearity of the method.**

### 3.6 Precision

Repeatability (intra-day precision) was assessed by analysing six sample preparations of the same concentration on Day 1. Intermediate precision was evaluated by repeating the experiment on Day 2. %RSD values for both compounds at both time points were well within the ICH acceptance limit of 2.0%, as shown in Table 7 (Figures S1–S13).

**Table 7. Precision Data – Repeatability and Intermediate Precision**

| Parameter | Acceptance Criterion | HCT (Day 1) | AML (Day 1) |
|---|---|---|---|
| Repeatability %RSD | NMT 2.0% | 0.16% | 0.07% |
| Intermediate Precision %RSD (Day 2) | NMT 2.0% | 0.10% | 0.20% |

### 3.7 Accuracy

Accuracy was determined by spiking the formulation blank at three concentration levels (80%, 100%, and 120% of the nominal concentration) in triplicate. The mean % recovery values for HCT ranged from 100.60% to 101.28%, and for AML from 99.57% to 100.97%, all within the ICH acceptance range of 98.0%–102.0% (Table 8) (Figures S14–S28).

**Table 8. Accuracy (% Recovery) Data for Hydrochlorothiazide and Amlodipine**

| Accuracy Level | Acceptance Criterion (%) | HCT % Recovery Range | AML % Recovery Range | Compliance |
|---|---|---|---|---|
| 80% | 98.0 – 102.0 | 100.60 – 101.28 | 99.57 – 100.97 | Complies |
| 100% | 98.0 – 102.0 | 100.60 – 101.28 | 99.57 – 100.97 | Complies |
| 120% | 98.0 – 102.0 | 100.60 – 101.28 | 99.57 – 100.97 | Complies |

### 3.8 Robustness

The robustness of the method was evaluated by introducing deliberate, minor changes in the flow rate (±0.1 mL/min from 0.5 mL/min) and detection wavelength (±2 nm from 230 nm). System suitability parameters remained within acceptance criteria under all four altered conditions. All %RSD values were below 2.0% and assay results remained within ±2% of the nominal value, indicating that the method is robust under the tested variations (Table 9) (Figures S29–S76).

**Table 9. Robustness Results for Hydrochlorothiazide and Amlodipine**

| Condition Altered | HCT %RSD | HCT Assay Results (%) | AML %RSD | AML Assay Results (%) |
|---|---|---|---|---|
| Flow Rate (−): 0.4 mL/min | 0.05% | 100.35 – 100.47 | 0.15% | 100.14 – 100.53 |
| Flow Rate (+): 0.6 mL/min | 0.04% | 100.30 – 100.42 | 0.24% | 100.11 – 100.81 |
| Wavelength (−): 228 nm | 0.09% | 100.16 – 100.40 | 0.17% | 100.07 – 100.54 |
| Wavelength (+): 232 nm | 0.10% | 100.42 – 100.67 | 0.20% | 99.91 – 100.42 |

## DISCUSSION

The present study successfully established a rapid and reliable UHPLC method for the simultaneous estimation of hydrochlorothiazide (HCT) and amlodipine (AML) in solid dosage forms. Compared to previously reported RP-HPLC methods, which typically exhibit longer run times (10–20 min) and higher solvent consumption, the developed method significantly reduces analysis time to 5 minutes while maintaining excellent chromatographic performance [6,8].

The selection of chromatographic conditions played a critical role in achieving optimal resolution and peak symmetry. Initial trials demonstrated that lower buffer ratios and shorter run times resulted in poor separation and peak distortion, likely due to insufficient interaction between analytes and the stationary phase. Optimization using a C8 column (50 mm length) with a 60:40 buffer–acetonitrile composition at pH 3.0 provided improved retention behavior and peak shape. The acidic pH ensured proper ionization of

both analytes, enhancing reproducibility and minimizing peak tailing, which is consistent with earlier chromatographic findings for dihydropyridine and thiazide drugs [9].

The system suitability parameters confirmed the robustness of the chromatographic system, with %RSD values well below 2%, indicating excellent repeatability. Theoretical plate counts and tailing factors were within acceptable limits, reflecting high column efficiency and good peak symmetry. These results are comparable to or better than previously reported HPLC methods for similar drug combinations [5,10].

Specificity studies confirmed that there was no interference from excipients or diluent at the retention times of HCT and AML, demonstrating the selectivity of the method. This is particularly important for combination drug products where excipient interference is a common analytical challenge [11]. Linearity was established over a wide concentration range (50–150%), with correlation coefficients of 1.0000 for HCT and 0.9993 for AML, indicating an excellent linear relationship between concentration and detector response. These values meet and exceed ICH acceptance criteria and are consistent with other validated chromatographic methods [12].

Precision studies, including repeatability and intermediate precision, yielded %RSD values below 2%, confirming the method's reproducibility under normal operating conditions. Accuracy results showed recovery values within 98–102%, indicating that the method is both accurate and free from systematic error. These validation outcomes align with ICH Q2(R1) requirements and demonstrate the method’s suitability for routine quality control analysis [13].

Robustness testing further confirmed that small deliberate variations in chromatographic conditions, such as flow rate and detection wavelength, did not significantly affect analytical performance. This highlights the method’s reliability in real-world laboratory environments where minor variations are inevitable [14].

A key advantage of the developed UHPLC method is its efficiency. The reduced run time and lower solvent consumption not only improve throughput but also reduce operational costs and environmental impact. This is particularly beneficial for pharmaceutical industries engaged in high-volume quality control testing [7,15].

Overall, the method demonstrates clear advantages over conventional analytical approaches in terms of speed, precision, and resource efficiency, while maintaining compliance with regulatory validation standards. The absence of previously reported UHPLC methods for this specific combination further underscores the novelty and practical relevance of this work.

## CONCLUSION

A simple, rapid, precise, and accurate UHPLC method for the simultaneous estimation of hydrochlorothiazide and amlodipine in a combined tablet formulation was successfully developed and validated in compliance with ICH Q2(R1) guidelines. The method employs a C8 column (4.6 × 50 mm, 3 µm), an isocratic mobile phase of 0.02 M $KH_2PO_4$ buffer (pH 3.0) and acetonitrile (60:40, v/v) at a flow rate of 0.5 mL/min, with UV detection at 230 nm and a total run time of 5 minutes.

All validated parameters such as system suitability, specificity, linearity, precision (repeatability and intermediate precision), accuracy, and robustness complied with regulatory acceptance criteria. The reduced run time relative to conventional HPLC methods translates into improved laboratory productivity and reduced solvent consumption. The method is suitable for routine quality control testing of hydrochlorothiazide and amlodipine combination tablets in pharmaceutical manufacturing environments.

Future investigations should extend this work to include stress degradation studies for the development of a fully stability-indicating method, and to evaluate the method's applicability to other formulation types such as extended-release tablets and transdermal patches.

### Supplementary Section

Supplementary Material includes detailed chromatograms for precision (Figures S1–S13), accuracy (Figures S14–S28), and robustness studies (Figures S29–S76), supporting the validation of the developed UHPLC method.

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