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METHOD DEVELOPMENT AND VALIDATION OF FOSINOPRIL AND HYDROCHLOROTHIAZIDE IN TABLET DOSAGE FORM USING RP-HPLC

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Abstract : This research paper describes the development and validation of a robust, accurate, and reproducible Reverse Phase High-Performance Liquid Chromatographic (RP-HPLC) method for the simultaneous estimation of Fosinopril and Hydrochlorothiazide in a combined tablet dosage form. The study aimed to establish a validated analytical procedure in accordance with the guidelines set by the International Council for Harmonisation (ICH Q2B) to ensure reliability and consistency of results for routine pharmaceutical quality control.

The chromatographic separation was optimized using a Cosmosil C18 column (250 mm × 4.6 mm, 5 µm particle size), employing a mobile phase composed of methanol and water in a 60:40 (v/v) ratio. The flow rate was maintained at 0.8 mL/min, and detection was performed using a UV detector at 218 nm. The retention times of Fosinopril and Hydrochlorothiazide were observed at approximately 5.963 minutes and 4.198 minutes, respectively.

The method was subjected to comprehensive validation studies including specificity, linearity, accuracy, precision (intra-day and inter-day), robustness, ruggedness, and system suitability testing. Results confirmed the method's linearity across a concentration range of 10–50 µg/mL for Fosinopril and 25–125 µg/mL for Hydrochlorothiazide, with correlation coefficients (R^2) of 0.9992 and 0.9995, respectively. Recovery studies confirmed the method's accuracy within the range of 98–102%, and %RSD values for precision studies were within acceptable limits (<2%). This method is suitable for the simultaneous quantification of Fosinopril and Hydrochlorothiazide in routine analysis and quality assurance laboratories.

Introduction

Pharmaceutical analysis serves as a cornerstone for the assessment of quality, efficacy, and safety of drug formulations throughout their lifecycle [1, 2]. With the increasing complexity of combination drug formulations, the need for highly sensitive and selective analytical methods has become paramount [3, 4].

Fosinopril is a phosphonate-containing angiotensin-converting enzyme (ACE) inhibitor, commonly prescribed for the management of hypertension and heart failure [11, 12]. It functions by inhibiting the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor, thereby reducing blood pressure [13, 14]. Hydrochlorothiazide, on the other hand, is a thiazide diuretic that promotes the excretion of sodium and water, further aiding in the reduction of blood pressure and fluid retention [12, 13].

The fixed-dose combination of these two agents offers synergistic antihypertensive effects and is widely used in clinical practice [10, 15]. However, simultaneous estimation of both active pharmaceutical ingredients (APIs) in a single dosage form poses analytical challenges due to their differing chemical structures and physicochemical properties [16, 17]. Reverse phase high-performance liquid chromatography (RP-HPLC) has emerged as a powerful analytical tool in pharmaceutical analysis due to its high resolution, reproducibility, and versatility in separating and quantifying structurally diverse compounds [4, 5, 26, 30].

The importance of method development lies in optimizing detection, retention, and separation of analytes while minimizing interference from excipients or degradation products [6, 7]. A validated analytical method ensures regulatory compliance and provides confidence in the quality of the dosage form [2, 22, 23]. ICH Q2B guidelines outline essential parameters such as specificity, linearity, accuracy, precision, robustness, and system suitability that must be addressed in analytical method validation [2, 29, 39].

Fosinopril and Hydrochlorothiazide have been studied individually and in combination for their pharmacological effects, but literature on their simultaneous determination using RP-HPLC remains limited [20, 21, 37]. Hence, there is a critical need to establish a validated, reliable, and efficient method for their combined estimation, particularly in routine quality control settings [16, 19, 25, 28].

This study focuses on developing a validated RP-HPLC method that enables the efficient, simultaneous quantification of Fosinopril and Hydrochlorothiazide in tablet dosage form with suitable accuracy, sensitivity, and reproducibility [16, 20, 22, 33].

2. Materials and Methods

2.1 Chemicals and Reagents

- Fosinopril and Hydrochlorothiazide
- Methanol (HPLC grade)
- Potassium dihydrogen phosphate, orthophosphoric acid
- HPLC-grade water

2.2 Equipment

- HPLC: Analytical Technologies Ltd. (HPLC 3000 Series)
- Column: Cosmosil C18, 250 mm × 4.6 mm, 5 µm
- UV Detector: 218 nm
- Analytical Balance, pH Meter, Sonicator

2.3 Preparation of Mobile Phase

The mobile phase consisted of methanol and water in a 60:40 (v/v) ratio. The solution was filtered through a 0.45 µm membrane filter and degassed by sonication for 15 minutes to remove air bubbles and dissolved gases that might interfere with the chromatographic process.

2.4 Preparation of Standard Stock Solutions

Accurately weighed 100 mg each of Fosinopril and Hydrochlorothiazide were separately transferred to 100 mL volumetric flasks. Each was dissolved in a small volume of mobile phase, sonicated to aid dissolution, and then diluted to volume with the same mobile phase to yield a 1000 µg/mL (1000 ppm) stock solution. Working standard solutions of desired concentrations were freshly prepared by suitable dilution.

2.5 Sample Preparation

Twenty tablets were finely powdered, and an amount equivalent to 10 mg each of Fosinopril and Hydrochlorothiazide was weighed accurately and transferred to a 25 mL volumetric flask. About 10 mL of mobile phase was added, and the solution was sonicated for 30 minutes. After cooling, the volume was made up to 25 mL with the mobile phase. The solution was filtered through a 0.45 µm membrane filter and diluted appropriately for analysis.

3. Method Development

3.1 Chromatographic Conditions (Optimized)

The method was optimized after several trials with varying mobile phase compositions and flow rates. The finalized conditions were:

- Column: Cosmosil C18 (250 mm × 4.6 mm, 5 µm)
- Mobile Phase: Methanol:Water (60:40 v/v)
- Flow Rate: 0.8 mL/min
- Column Temperature: 25°C
- Wavelength: 218 nm
- Injection Volume: 20 µL
- Run Time: 8.75 min
- Retention Times: Fosinopril – 5.963 min, Hydrochlorothiazide – 4.198 min

These conditions offered symmetrical peaks with minimal tailing, acceptable resolution, and satisfactory theoretical plate counts.

Optimized Trial

Optimized method for the simultaneous estimation of Fosinopril and Hydrochlorothiazide by RP-HPLC was finally achieved by using the following chromatographic conditions.

Preparation of Mobile Phase:

Prepared a mixture of methanol and water in the ratio of 60:40 v/v mix well. Filter through 0.45µ membrane filter and degas it.

Chromatographic Condition

Column	: Cosmosil C18(250mm x 4.6ID,Particle size: 5 micron)
Mobile Phase	: Methanol:Water (60:40)
Flow Rate	: 0.8 ml/min
Column Temperature	: 25ºC
Wavelength	: 218 nm
Injection Volume	: 20 µl
Run Time	: 8.75 min
Retention Time	: 5.963min & 4.198 min Fosinopril and Hydrochlorothiazide respectively

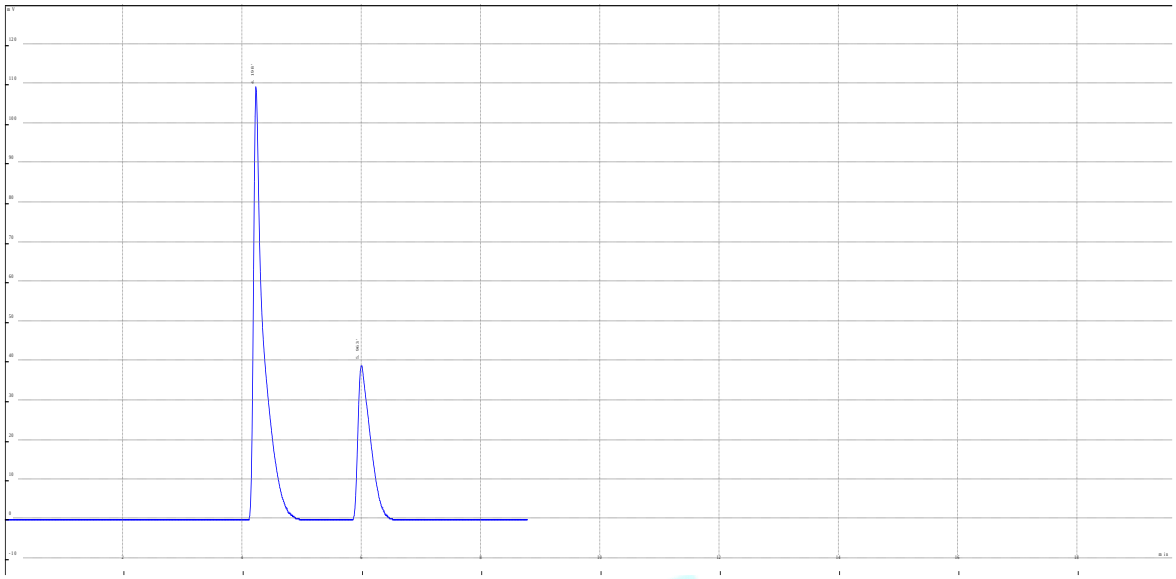

Figure : Typical Chromatogram of Finalized Trial

4. Method Validation

The optimized method was validated as per ICH Q2B guidelines covering the following parameters:

4.1 Specificity

The specificity was assessed by comparing chromatograms of blank, placebo, standard, and sample solutions. No interfering peaks were observed at the retention times of the analytes, confirming the method's specificity.

4.2 Linearity

The linearity of the method was demonstrated over a concentration range of:

- Fosinopril: 10–50 µg/mL (R^2 = 0.9992)
- Hydrochlorothiazide: 25–125 µg/mL (R^2 = 0.9995)

Calibration curves plotted as concentration versus peak area showed a linear relationship, with regression equations and correlation coefficients within the acceptable range.

4.3 Precision

Precision was evaluated in terms of repeatability (intra-day) and intermediate precision (inter-day). Six replicates were analyzed for each drug. The %RSD values for peak areas were consistently <2%, indicating high precision.

4.4 Accuracy (Recovery Studies)

Recovery studies were performed by the standard addition method at three concentration levels (80%, 100%, and 120%). The mean recovery was within the range of 98–102% for both drugs, affirming the method’s accuracy.

4.5 Robustness

To assess robustness, deliberate variations in method parameters such as wavelength (±2 nm) and mobile phase pH (±0.2) were introduced. The method remained unaffected, and results were within acceptable limits, confirming robustness.

4.6 Ruggedness

Ruggedness was tested by analyzing samples using two different analysts and instruments. The results showed no significant variations, and %RSD remained <2%, indicating the method’s reproducibility across different operators.

4.7 System Suitability

System suitability tests were conducted to ensure performance of the chromatographic system. Parameters such as retention time, tailing factor (<2), theoretical plates (>2000), and %RSD of peak area (<2%) met the acceptance criteria.

5. Results and Discussion

The method developed provides a sensitive, accurate, and robust approach for the simultaneous estimation of Fosinopril and Hydrochlorothiazide in tablet dosage form. Chromatographic separation was efficiently achieved with satisfactory resolution, and the method demonstrated excellent linearity and specificity. Validation parameters confirmed that the method is reliable for use in quality control and routine analysis. The low %RSD values in precision studies and high recovery rates reaffirm the consistency and reliability of the method. The use of a simple mobile phase (methanol:water) further adds to the method's practicality and cost-effectiveness.

6. Conclusion

A novel RP-HPLC method was successfully developed and validated for the simultaneous estimation of Fosinopril and Hydrochlorothiazide in combined tablet dosage form. The method offers several advantages including simplicity, sensitivity, reproducibility, and economy. All validation parameters fulfilled the requirements outlined by ICH Q2B guidelines, proving the method's suitability for routine quality control analysis in pharmaceutical industries.

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