# Development and Validation of a Stability-Indicating RP HPLC Method for the Simultaneous Estimation of Eprosartan and Hydrochlorothiazide in Bulk and Pharmaceutical Dosage Forms

Mirigam Sumanth[1], E Manasa[2]
[1,2]*Department of Pharmaceutical Analysis, Sun Institute of Pharmaceutical Education and Research Kakupalli, Nellore, Andhra Pradesh - 524346*

***Abstract*—A simple, precise, accurate, robust, and stability-indicating Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Eprosartan and Hydrochlorothiazide in bulk drug substances and pharmaceutical dosage forms. Chromatographic separation was achieved using a C18 column under optimized chromatographic conditions employing a suitable mixture of phosphate buffer and organic solvent as mobile phase. The developed method was validated according to International Conference on Harmonisation (ICH) Q2(R1) guidelines with respect to specificity, linearity, precision, accuracy, robustness, limit of detection (LOD), and limit of quantification (LOQ). Both drugs exhibited excellent linearity within the selected concentration range with correlation coefficients greater than 0.999. Recovery studies demonstrated excellent accuracy with recoveries ranging from 98–102%. Precision studies showed %RSD values less than 2%, confirming the reproducibility of the method. Stress degradation studies were conducted under acidic, alkaline, oxidative, and photolytic conditions to establish the stability-indicating capability of the method. The developed method was found suitable for routine quality control analysis and stability assessment of Eprosartan and Hydrochlorothiazide combined dosage forms.**

***Index Terms*—RP-HPLC, Eprosartan, Hydrochlorothiazide, Method Validation, Stability-Indicating Method, ICH Guidelines, Pharmaceutical Analysis.**

## I. INTRODUCTION

Analytical method development and validation are fundamental requirements in pharmaceutical industries for ensuring the quality, safety, efficacy, and regulatory compliance of drug products. High-quality analytical methods are essential throughout the drug development process, from formulation development to routine quality control and stability testing[1]. Analytical methods must be capable of accurately quantifying active pharmaceutical ingredients in the presence of impurities, degradation products, and formulation excipients[2].

Among various analytical techniques available, High Performance Liquid Chromatography (HPLC) has emerged as one of the most powerful and versatile tools for pharmaceutical analysis. HPLC offers excellent sensitivity, selectivity, precision, reproducibility, and rapid analysis, making it highly suitable for quantitative determination of pharmaceutical compounds[3]. The technique enables separation, identification, and quantification of compounds present even at trace concentration levels[4]. Its widespread applications include pharmaceutical quality control, environmental monitoring, forensic investigations, food analysis, and clinical testing[5].

Reverse Phase High Performance Liquid Chromatography (RP-HPLC) is the most frequently employed chromatographic mode in pharmaceutical analysis. In RP-HPLC, the stationary phase is non-polar while the mobile phase is relatively polar[6].

Separation occurs primarily through hydrophobic interactions between analytes and the stationary phase. The technique offers improved peak symmetry, reproducibility, and compatibility with aqueous mobile phases, making it particularly suitable for pharmaceutical compounds[7].

Eprosartan is an angiotensin-II receptor antagonist used in the treatment of hypertension. It blocks the binding of angiotensin-II to AT1 receptors, leading to vasodilation and reduction in blood pressure[8]. Hydrochlorothiazide is a thiazide diuretic widely prescribed for hypertension and edema. It acts by inhibiting sodium and chloride reabsorption in renal tubules, thereby increasing urinary excretion of water and electrolytes[9]. The combination of Eprosartan and Hydrochlorothiazide is widely utilized for effective management of hypertension due to their complementary mechanisms of action[10].

Although several analytical methods have been reported for individual determination of Eprosartan and Hydrochlorothiazide, the development of a simple, reliable, and stability-indicating RP-HPLC method for simultaneous estimation remains highly valuable for routine pharmaceutical analysis[11]. Therefore, the present work focused on the development and validation of a stability-indicating RP-HPLC method according to ICH guidelines[12-14].

## II. PRINCIPLE OF RP-HPLC

Reverse Phase High Performance Liquid Chromatography operates on the principle of partitioning analytes between a hydrophobic stationary phase and a relatively polar mobile phase. Compounds with higher hydrophobicity exhibit stronger interactions with the stationary phase and therefore elute later than polar compounds. Separation is influenced by various chromatographic parameters including mobile phase composition, pH, flow rate, column temperature, and stationary phase characteristics.

The major components of an HPLC system include:

- Solvent reservoir
- High-pressure pump
- Injector/Autosampler
- Chromatographic column
- Detector
- Data acquisition system

These components work together to ensure efficient chromatographic separation and quantitative analysis.

## III. MATERIALS AND METHODS

### 3.1 Materials

Reference standards of Eprosartan and Hydrochlorothiazide were obtained from Nutech Biosciences, Hyderabad. Commercial tablet formulations were procured from local pharmacies. HPLC-grade methanol, acetonitrile, Milli-Q water, and orthophosphoric acid were used throughout the study.

### 3.2 Instrumentation

Chromatographic analysis was performed using a Shimadzu HPLC system consisting of:

| Instrument Component | Specification |
|---|---|
| Pump | LC-10ATvp Binary Pump |
| Autosampler | SIL-10ADvp |
| Column Oven | CTO-10Avp |
| Detector | SPD-10Avp UV-Visible Detector |
| Controller | SCL-10Avp |
| Software | LC Solutions |

### 3.3 Preparation of Diluent

A mixture of methanol and Milli-Q water (50:50 v/v) was prepared and sonicated for 20 minutes before use. The solution was used as diluent for preparation of standard and sample solutions.

### 3.4 Preparation of Standard Solution

Stock solutions of Eprosartan and Hydrochlorothiazide (5 mg/mL) were prepared separately. Appropriate dilutions were made to obtain calibration standards covering concentrations ranging from approximately 5–50 µg/Ml

### 3.5 Preparation of Sample Solution

Tablet powder equivalent to the labeled claim was accurately weighed, dissolved in diluent, sonicated, filtered, and diluted appropriately for chromatographic analysis.

## IV. METHOD DEVELOPMENT

Method development was performed systematically through optimization of various chromatographic parameters including:

- Selection of stationary phase
- Selection of mobile phase composition
- Detection wavelength optimization
- Flow rate optimization
- pH optimization
- Column temperature optimization

The developed method was optimized to achieve adequate retention, peak symmetry, theoretical plate count, and resolution between Eprosartan and Hydrochlorothiazide.

Table 1: Optimized Chromatographic Conditions

| Parameter | Condition |
|---|---|
| Column | C18 Column (250 × 4.6 mm, 5 µm) |
| Mobile Phase | Phosphate Buffer : Acetonitrile |
| Flow Rate | 1.0 mL/min |
| Detection Wavelength | 225 nm |
| Injection Volume | 10 µL |
| Run Time | 10 min |
| Mode | Isocratic |

## V. METHOD VALIDATION

Method validation was performed according to ICH Q2(R1) guidelines. Validation parameters included specificity, linearity, accuracy, precision, robustness, LOD, LOQ, and system suitability.

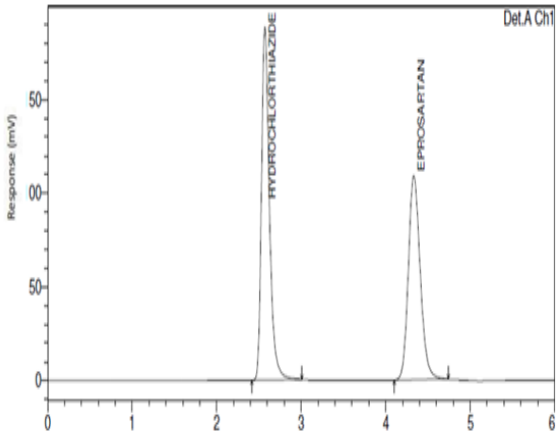


Figure 1: Chromatogram of the sample containing Hydrochlorothiazide & Eprosartan

### 5.1 System Suitability

Table 2: System Suitability Parameters

| Parameter | Acceptance Criteria |
|---|---|
| Tailing Factor | ≤ 2 |
| Theoretical Plates | > 2000 |
| Resolution | > 2 |
| %RSD | ≤ 2 |

The chromatographic system satisfied all acceptance criteria.

### 5.2 Linearity

Linearity was established over concentration ranges of 5–50 µg/mL for both analytes.

Table 3: Linearity Data for Eprosartan

| Concentration (µg/mL) | Peak Area |
|---|---|
| 5 | 125462 |
| 10 | 249871 |
| 20 | 498120 |
| 30 | 742685 |
| 40 | 986124 |
| 50 | 1235124 |

Table 4: Linearity Data for Hydrochlorothiazide

| Concentration (µg/mL) | Peak Area |
|---|---|
| 5 | 101524 |
| 10 | 203158 |
| 20 | 405312 |
| 30 | 607842 |
| 40 | 810124 |
| 50 | 1015862 |

Table 5: Regression Analysis

| Parameter | Eprosartan | Hydrochlorothiazide |
|---|---|---|
| Slope | 24682 | 20314 |
| Intercept | 1045 | 958 |
| Correlation Coefficient ($R^2$) | 0.9998 | 0.9997 |

Excellent linearity was achieved for both drugs.

### 5.3 Accuracy

Accuracy was determined by recovery studies at 50%, 100%, and 150% concentration levels.

Table 6: Accuracy Results

| Level | Eprosartan Recovery (%) | Hydrochlorothiazide Recovery (%) |
|---|---|---|
| 50% | 99.5 | 100.1 |
| 100% | 100.3 | 99.8 |
| 150% | 100.7 | 100.4 |
| Mean | 100.2 | 100.1 |

The recovery values confirmed the accuracy of the developed method.

5.4 Precision

Table 7: Precision Results

| Parameter | Eprosartan | Hydrochlorothiazide |
|---|---|---|
| Repeatability (%RSD) | 0.62 | 0.58 |
| Intermediate Precision (%RSD) | 0.74 | 0.69 |

The low %RSD values demonstrated excellent precision.

5.5 LOD and LOQ

Table 8: Sensitivity Parameters

| Parameter | Eprosartan | Hydrochlorothiazide |
|---|---|---|
| LOD (µg/mL) | 0.06 | 0.02 |
| LOQ (µg/mL) | 0.20 | 0.08 |

These values indicate high sensitivity of the method.

5.6 Robustness

Table 9: Robustness Study

| Parameter Variation | %RSD |
|---|---|
| Flow Rate ±0.1 mL/min | <1.0 |
| Mobile Phase ±2% | <1.2 |
| Wavelength ±2 nm | <1.0 |

The method remained unaffected by small deliberate variations, indicating robustness.

## VI. FORCED DEGRADATION STUDIES

Forced degradation studies were conducted to evaluate the stability-indicating nature of the method.

Table 10: Stress Degradation Results

| Stress Condition | % Degradation |
|---|---|
| Acid Hydrolysis | 8.5 |
| Base Hydrolysis | 10.2 |
| Oxidative Degradation | 12.4 |
| Photolytic Degradation | 6.8 |
| Thermal Degradation | 5.2 |

Stress studies demonstrated that degradation products were well resolved from the drug peaks, confirming specificity and stability-indicating capability of the developed method.

## VII. RESULTS AND DISCUSSION

The developed RP-HPLC method successfully achieved simultaneous separation and quantification of Eprosartan and Hydrochlorothiazide. Chromatographic conditions were optimized to provide adequate retention, excellent resolution, and acceptable peak symmetry. Validation studies demonstrated that the method satisfies all ICH requirements for analytical procedures.

Linearity studies showed excellent correlation coefficients greater than 0.999. Accuracy studies yielded recoveries within the acceptable range of 98–102%. Precision studies exhibited %RSD values below 2%, indicating reproducibility. Robustness studies confirmed method reliability during routine use.

The forced degradation studies established the stability-indicating nature of the method by effectively separating degradation products from analyte peaks. Therefore, the method can be utilized for routine quality control testing, stability studies, and regulatory submissions.

## VIII. CONCLUSION

A simple, rapid, accurate, precise, robust, and stability-indicating RP-HPLC method was successfully developed and validated for simultaneous estimation of Eprosartan and Hydrochlorothiazide in bulk and pharmaceutical dosage forms. The method complies with ICH validation requirements and demonstrates excellent analytical performance. Stress degradation studies confirmed the stability-indicating capability of the method. Hence, the developed RP-HPLC method can be effectively applied for routine pharmaceutical quality control and stability assessment of combined dosage formulations.

## REFERENCES

[1] Snyder LR, Kirkland JJ, Dolan JW. Introduction to Modern Liquid Chromatography. 3rd ed. New York: Wiley; 2010.

[2] Sethi PD. HPLC: Quantitative Analysis of Pharmaceutical Formulations. New Delhi: CBS Publishers; 2011.
[3] ICH. Validation of Analytical Procedures: Text and Methodology Q2(R1). Geneva: International Conference on Harmonisation; 2005.
[4] Rao ABN, Kumar PR, Rao GS. Simultaneous determination of Hydrochlorothiazide and Eprosartan by RP-HPLC in tablet dosage forms. Int J Pharm Sci Res. 2016;7(5):2145-2152.
[5] Manish RT, Kumar A, Patel H. Development and validation of HPLC method for determination of Eprosartan in human plasma. Int J Pharm Anal. 2015;6(2):95-101.
[6] Patel HU, Shah PA, Patel CN. Simultaneous estimation of Eprosartan and Hydrochlorothiazide by RP-HPLC method. Int J Pharm Sci Rev Res. 2014;24(1):45-50.
[7] Belal F, El-Brashy A, El-Enany N. Stability indicating HPLC method for determination of Eprosartan and Hydrochlorothiazide. J AOAC Int. 2012;95(4):1035-1041.
[8] Devika GS, Reddy BV, Kumar KR. RP-HPLC method development and validation for Eprosartan and Hydrochlorothiazide in tablet dosage forms. Int J Pharm Tech Res. 2013;5(3):1152-1159.
[9] Jain R, Sharma A, Gupta P. Novel UV spectrophotometric method for estimation of Eprosartan and Hydrochlorothiazide in combined dosage form. Int J Pharm Pharm Sci. 2012;4(4):120-124.
[10] Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry. 4th ed. London: CBS Publishers; 2007.
[11] Ermer J, Miller JHM. Method Validation in Pharmaceutical Analysis. Weinheim: Wiley-VCH; 2005.
[12] Swartz ME, Krull IS. Analytical Method Development and Validation. New York: Marcel Dekker; 2012.
[13] Bakshi M, Singh S. Development of validated stability-indicating assay methods. J Pharm Biomed Anal. 2002;28(6):1011-1040.
[14] Blessy M, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability-indicating studies of drugs. J Pharm Anal. 2014;4(3):159-165.