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Research

New analytical method development and validation for estimation of eprosartan and hydrochlorothiazide in bulk and tablet dosage form by rp-hplc method

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Abstract

A new RP-HPLC method has been developed and validated for the simultaneous quantification of Eprosartan and Hydrochlorothiazide in both bulk and pharmaceutical formulations. The separation was achieved using a Phenomenex Luna C18 column (4.6×250mm, 5µm) with an isocratic mobile phase of Acetonitrile: Phosphate Buffer (pH 4.6) (45:55 v/v) at a flow rate of 1.0 mL/min. Detection was performed at 245 nm, with retention times of 2.102 and 3.537 minutes for Eprosartan and Hydrochlorothiazide, respectively. The method exhibited linear response over the concentration ranges of 6µg/mL to 14µg/mL for Eprosartan and 18µg/mL to 42µg/mL for Hydrochlorothiazide, with correlation coefficients of 0.999 for both analytes. The limits of detection (LOD) and quantification (LOQ) were determined to be 0.6 µg/mL and 1.8 µg/mL for Eprosartan, and 0.8 µg/mL and 2.4 µg/mL for Hydrochlorothiazide, respectively. Method validation according to ICH guidelines confirmed the method's accuracy, precision, specificity, and robustness. The percentage recoveries for Eprosartan and Hydrochlorothiazide were 100.351% and 100.93%, respectively, indicating high accuracy. Specificity testing demonstrated good correlation between retention times of standards and samples, ensuring the method's ability to differentiate and quantify analytes in the presence of tablet excipients. In conclusion, the developed RP-HPLC method offers a simple, precise, accurate, and reproducible approach for the simultaneous determination of Eprosartan and Hydrochlorothiazide in pharmaceutical formulations, making it suitable for routine quality control analysis.

Keywords: Eprosartan and Hydrochlorothiazide, High performance liquid chromatography, Validation.

INTRODUCTION

In the modern pharmaceutical industry, high-performance liquid chromatography (HPLC) is the major and integral analytical tool applied in all stages of drug discovery, development and production. It is ideal for the analysis of many drugs in both dosage forms and biological fluids due to its simplicity, high specificity and good sensitivity. High Performance Liquid Chromatography (HPLC) is a technique that has arisen from the application to liquid chromatography the use of an instrumentation that was originally developed for gas chromatography. High Pressure Liquid Chromatography was developed in the mid-1970 and was improved with the development of column packing material and the additional convenience of on-line detectors. The various components of HPLC are pumps (solvent delivery system), mixing unit, gradient controller and solvent degasser, injector (manual or automatic), guard column, analytical columns, detectors, recorders and/or integrators. Recent models are equipped with computers and software for data acquisition and processing. The mobile phase in HPLC refers to the solvent being continuously applied to the column or stationary phase at a flow rate of 1-5 cm3/min. The mobile phase acts as a carrier for the sample solution. The chemical interactions of the mobile phase and sample with the column determine the degree of migration and separation of components contained in the sample. The mobile phase can be altered in order to manipulate the interactions of the sample and the stationary phase.

Types of Chromatography [1]

Normal-phase chromatography

Mechanism: Retention by interaction with the polar surface of the stationary phase with polar parts of the sample molecules.

Stationary phase: SiO2, Al2O3, -NH2, -CN, -Diol, -NO2, etc.

Mobile phase: Heptane, hexane, cyclohexane, CHCl3, CH2Cl2, dioxane, methanol, etc.

Application: Separation of non-ionic, non-polar to medium polar substances. Disadvantage: Lack of reproducibility of retention times as water or protic organic solvents change the hydration state of the silica or alumina chromatographic media.

Reversed-phase chromatography

Mechanism: Retention by interaction of the stationary phase's non-polar hydrocarbon chain with non-polar parts of the sample molecules.

Stationary phase: n-octadecyl (RP-18), n-octyl (RP-8), ethyl (RP-2), phenyl, (CH2)n-CN, (CH2)n-diol, etc.

Mobile phase: Methanol, Acetonitrile, water, buffer (sometimes with additives of THF or Dioxane), etc.

Application: Separation of non-ionic and ion forming non-polar to medium polar substances (carboxylic acids, hydrocarbons). If ion forming substances (as carboxylic acids) are to be separated, a pH control by buffers is necessary.

Reversed-phase ion-pair chromatography

Mechanism: Ionic sample molecules are ionically bound to an ion-pair reagent. The ion- pair reagent contains an unpolar part suitable for interaction with the unpolar hydrocarbon chain of the stationary phase.

Stationary phase: Reversed phase materials (RP-18, RP-8, CN), etc.

Mobile phase: Methanol, Acetonitrile, buffer with added ion-pair reagent in the concentration range of 0.001 to 0.01 M, etc.

Application: Ionic substances often show very poor retention in reversed phase chromatography. To overcome this difficulty an ion-pair reagent is added to the eluent.

Applications of HPLC [2]

- Natural Products: HPLC is an ideal method for the estimation of various components in plant extracts which resemble in structure and thus demand a specific and very sensitive method e.g., analysis of digitalis, cinchona, liquor ice, and ergot extracts.
- Stability studies: HPLC is now used for ascertaining the stability of various pharmaceuticals. With HPLC the analysis of the various degradation products can be done and thus stability indicating HPLC systems have been developed.
- Bioassays and its complementation: Complex molecules as antibiotics and peptide hormones are mainly analyzed by bioassay which suffers from high cost, necessity replicates, poor precision and length of time required. Also, bioassay gives an overall estimate of potency and gives no guidance about the composition. Thus, HPLC can be used to complement bioassays and give an activity profile. It has been used for analysis of chloramphenicol, penicillins and clotrimoxazole, sulfas and peptides hormones.
- HPLC has also been used in the cosmetic industry for quality control of various cosmetics.

The basic components of HPLC are: [4-8]

1. Pumping System
2. Sample Introduction Device
3. Chromatographic Column
4. Detector
5. Data handling Device

MATERIALS AND METHODS

Instruments used

HPLC from WATERS Alliance 2695 separation module, software: Empower 2, 996 PDA detector.

Chemicals used

Eprosartan and Hydrochlorothiazide from Sura Pharma Labs, Water and Methanol for HPLC from LICHROSOLV (MERCK), Acetonitrile for HPLC from Merck.

Hplc method development

Trails

Preparation of standard solution

Accurately weigh and transfer 10 mg of Eprosartan and Hydrochlorothiazide working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.1ml of the above Eprosartan and 0.3ml of the Hydrochlorothiazide stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Validation

Preparation of buffer and mobile phase

Preparation of Potassium dihydrogen Phosphate (KH2PO4) buffer (pH-4.6)

Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 4.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra sonication.

Preparation of mobile phase

Accurately measured 450 ml (45%) of Methanol, 550 ml of Phosphate buffer (55%) were mixed and degassed in digital ultrasonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase : Acetonitrile: Phosphate Buffer (pH-4.6) (45:55 v/v)
Column : Phenomenex Luna C18 (4.6×250mm, 5µm) particle size
Flow rate : 1 ml/min
Wavelength : 245 nm
Column temp : 35ºC
Injection Volume : 10 µl
Run time : 7 minutes

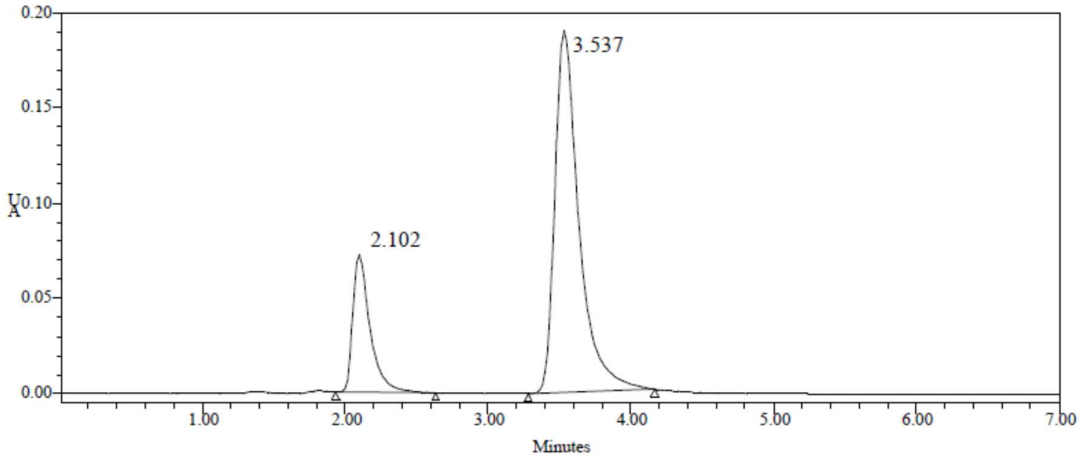

Fig 1: Optimized Chromatogram

Table 1: Peak results for Optimized Chromatogram

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Eprosartan	2.102	765789	69584		0.97	5587.0
2	Hydrochlorothiazide	3.537	2532158	190049	2.97	1.26	5398.0

From the above chromatogram it was observed that the Eprosartan and Hydrochlorothiazide peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

Mobile phase : Acetonitrile: Phosphate Buffer (pH-4.6) (45:55 v/v)
Column : Phenomenex Luna C18 (4.6×250mm, 5µm) particle size
Flow rate : 1 ml/min
Wavelength : 245 nm
Column temp : 35ºC
Injection Volume : 10 µl
Run time : 7 minutes

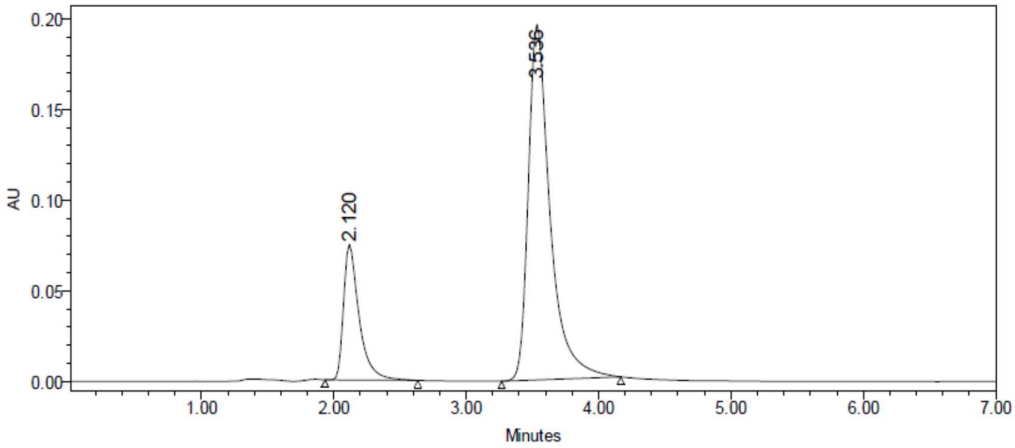

Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Eprosartan	2.120	775684	13124		0.99	6365.0
2	Hydrochlorothiazide	3.536	2658478	937405	5.06	1.23	7458.0

- *Resolution between two drugs must be not less than 2.; Theoretical plates must be not less than 2000.*
- *Tailing factor must be not less than 0.9 and not more than 2.*
- *It was found from above data that all the system suitability parameters for developed method were within the limit.*

Assay (Standard)

Table 3: Peak results for assay standard

Sno	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Eprosartan	2.102	759868	71255		1.7	5689	1
2	Hydrochlorothiazide	3.537	2458754	215654	2.04	1.6	5362	1
3	Eprosartan	2.105	759458	72541		1.7	5748	2
4	Hydrochlorothiazide	3.552	2465885	226565	2.00	1.6	5452	2
5	Eprosartan	2.112	759245	72584		1.7	5584	3
6	Hydrochlorothiazide	3.560	2489578	221542	2.04	1.6	5456	3

Assay (Sample)

Table 4: Peak results for Assay sample

S.no	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Eprosartan	2.120	756985	68958		0.98	7253	1
2	Hydrochlorothiazide	3.536	2569856	198564	2.06	1.23	8836	1
3	Eprosartan	2.120	758745	69857		1.05	6530	2
4	Hydrochlorothiazide	3.537	2598654	195682	2.04	0.99	7270	2
5	Eprosartan	2.102	756848	69588		1.7	7586	3
6	Hydrochlorothiazide	3.537	2587454	192541	2.04	1.6	8371	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Eprosartan and Hydrochlorothiazide in pharmaceutical dosage form was found to be 99.8%.

Linearity

Chromatographic data for linearity study

Eprosartan

Concentration µg/ml	Average Peak Area
6	205035
8	381239
10	561128
12	740162
14	909922

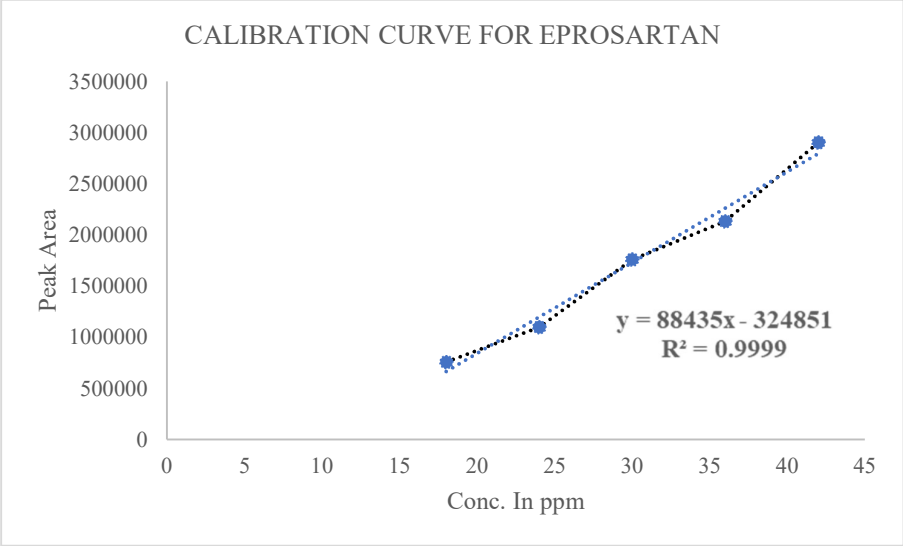

Fig 3: Calibration Graph for Eprosartan

The response linearity is verified if the Correlation Coefficient is 0.99 or greater. Correlation Coefficient (r) is 0.99, and the intercept is 1669. These values meet the validation criteria.

Hydrochlorothiazide

Concentration µg/ml	Average Peak Area
18	757881
24	757881
30	1458941
36	2132457
42	2901811

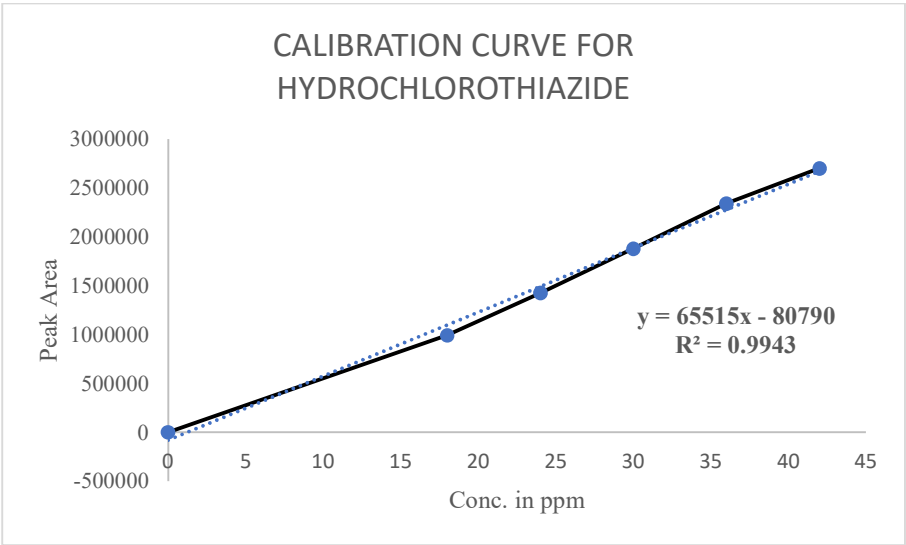

Fig 4: Calibration Graph for Hydrochlorothiazide

The response linearity is verified if the Correlation Coefficient is 0.99 or greater.Correlation Coefficient (r) is 0.99, and the intercept is 45591. These values meet the validation criteria.

Precision
Repeatability

Table 5: Results of Repeatability for Eprosartan:

S.no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Eprosartan	2.108	766854	702564	5685	1.6
2	Eprosartan	2.105	765884	698789	5584	1.4
3	Eprosartan	2.113	765842	701235	5521	1.6
4	Eprosartan	2.109	768985	700124	5525	1.9
5	Eprosartan	2.109	765845	698986	5578	1.7
Mean			766682			
Std. Dev			1357.973			
% RSD			0.177123			

- *%RSD for sample should be NMT 2*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Table 6: Results of method precision for Hydrochlorothiazide

S.no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Hydrochlorothiazide	3.552	2569865	2231111	5365	1.6
2	Hydrochlorothiazide	3.550	2578474	2674210	5425	1.6
3	Hydrochlorothiazide	3.564	2568985	2231261	5368	1.5
4	Hydrochlorothiazide	3.564	2586845	2421301	5359	1.5
5	Hydrochlorothiazide	3.565	2545898	2324710	5498	1.6
Mean			2570013			
Std. Dev			15309.45			
% RSD			0.595695			

- *%RSD for sample should be NMT 2*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Intermediate precision
Day 1

Table 7: Results of Intermediate precision for Eprosartan

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Eprosartan	2.108	758955	68986	5785	1.6
2	Eprosartan	2.105	759869	68957	5698	1.4
3	Eprosartan	2.113	758985	68545	5689	1.6
4	Eprosartan	2.109	756894	68952	5781	1.9
5	Eprosartan	2.109	759854	68595	5785	1.7
6	Eprosartan	2.102	756985	68952	5693	1.6
Mean			758590.3			
Std. Dev			1339.793			
% RSD			0.176616			

- *%RSD of Six different sample solutions should not more than 2.*

Table 8: Results of Intermediate precision for Hydrochlorothiazide

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Hydrochlorothiazide	3.552	2659852	190025	5485	1.5	2.04
2	Hydrochlorothiazide	3.550	2648574	190048	5421	1.6	2.03
3	Hydrochlorothiazide	3.564	2659865	190054	5468	1.6	2.01
4	Hydrochlorothiazide	3.564	2658547	190078	5487	1.6	2.05
5	Hydrochlorothiazide	3.565	2648981	190016	5492	1.6	2.02
6	Hydrochlorothiazide	3.537	2654652	190057	5463	1.6	2.03
Mean			2655079				

Std. Dev	5242.086
% RSD	0.197436

- *%RSD of Six different sample solutions should not more than 2.*
- *The %RSD obtained is within the limit, hence the method is rugged.*

Day 2

Table 9: Results of Intermediate precision Day 2 for Eprosartan

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Eprosartan	2.102	766895	69858	5586	1.5
2	Eprosartan	2.105	765988	69854	5636	1.6
3	Eprosartan	2.112	766532	69824	5432	1.6
4	Eprosartan	2.113	766214	69875	5468	1.6
5	Eprosartan	2.109	765897	69854	5546	1.9
6	Eprosartan	2.109	765245	69848	5507	1.7
Mean			766128.5			
Std. Dev			567.7234			
% RSD			0.074103			

- *%RSD of Six different sample solutions should not more than 2.*

Table 10: Results of Intermediate precision for Hydrochlorothiazide

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Hydrochlorothiazide	3.537	2653254	190110	5428	1.6	7.98
2	Hydrochlorothiazide	3.552	2648985	190058	5452	1.6	6.4
3	Hydrochlorothiazide	3.560	2658213	190142	5498	1.6	8.9
4	Hydrochlorothiazide	3.564	2653652	190031	5442	1.5	8.3
5	Hydrochlorothiazide	3.564	2648978	190058	5489	1.5	7.5
6	Hydrochlorothiazide	3.565	2658985	190047	5463	1.6	5.3
Mean			2653678				
Std. Dev			4313.355				
% RSD			0.162543				

- *%RSD of Six different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is rugged.*

Accuracy

Table 11: The accuracy results for Eprosartan

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	392891.7	5	5.027	100.540%	
100%	781996	10	10.026	100.260%	100.351%
150%	1171988	15	15.038	100.253%	

Table 12: The accuracy results for Hydrochlorothiazide

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	204962	15	15.156	101.040%	
100%	365018	30	30.378	101.260%	100.93%
150%	521064.3	45	45.218	100.484%	

- *The percentage recovery was found to be within the limit (98-102%).*

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness
Eprosartan

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	765789	2.102	5587	1.7
Less Flow rate of 0.9 mL/min	758698	2.330	5458	1.7
More Flow rate of 1.1 mL/min	7689584	1.950	5696	1.7
Less organic phase	758412	2.290	5586	1.4
More organic phase	769852	1.998	5355	1.5

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Hydrochlorothiazide

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	2532158	3.537	5398	1.6
Less Flow rate of 0.9 mL/min	2458692	3.885	5329	1.7
More Flow rate of 1.1 mL/min	2658642	3.263	5256	1.7
Less organic phase	2452148	4.435	5214	1.2
More organic phase	2653894	3.009	5524	1.0

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

SUMMARY

A new RP-HPLC method has been developed and validated for the simultaneous quantification of Eprosartan and Hydrochlorothiazide in both bulk and pharmaceutical formulations. The method utilizes a Phenomenex Luna C18 column with an isocratic mobile phase consisting of Acetonitrile: Phosphate Buffer (pH 4.6) (45:55 v/v) at a flow rate of 1.0 mL/min. Detection was performed at 245 nm, yielding retention times of 2.102 minutes for Eprosartan and 3.537 minutes for Hydrochlorothiazide. The method demonstrated excellent linearity over the concentration ranges of 6µg/mL to 14µg/mL for Eprosartan and 18µg/mL to 42µg/mL for Hydrochlorothiazide, with correlation coefficients of 0.999 for both analytes. The limits of detection (LOD) and quantification (LOQ) were found to be 0.6 µg/mL and 1.8 µg/mL for Eprosartan, and 0.8 µg/mL and 2.4 µg/mL for Hydrochlorothiazide, respectively.

CONCLUSION

The developed RP-HPLC method offers a robust and reliable approach for the simultaneous determination of Eprosartan and Hydrochlorothiazide in pharmaceutical formulations. The method's validation according to ICH guidelines confirmed its accuracy, precision, specificity, and robustness. The linear response, high correlation coefficients, and low LOD and LOQ values indicate the method's sensitivity and suitability for routine analysis. The successful application of the method to both bulk samples and pharmaceutical formulations underscores its versatility and practical utility in pharmaceutical quality control laboratories. Overall, this study provides a valuable analytical tool for the accurate quantification of Eprosartan and Hydrochlorothiazide, ensuring their quality and compliance with regulatory standards.

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