

Analytical Method Development and Validation of Fosinopril Sodium and Hydrochlorothiazide in Bulk and Pharmaceutical Formulation by RP-HPLC

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ABSTRACT

The objective of this research was to develop and validate a robust RP-HPLC method for the simultaneous determination of Fosinopril Sodium (FNZ) and Hydrochlorothiazide (HCZ) in combined pharmaceutical dosage forms. Currently, pharmacopoeias lack an established procedure for the simultaneous estimation of this specific combination. Chromatographic separation was optimized using an Agilent C18 column (4.6 mm × 250 mm; 5 μm) at ambient temperature. The mobile phase consisted of a 50:50 v/v mixture of Methanol and Orthophosphoric acid, with the pH adjusted to 3.5 using Orthophosphoric Acid (OPA). The system operated at a flow rate of 0.7 mL/min, and detection was performed at a wavelength of 233 nm. The retention times were found to be 3.66 ± 0.02 min for HCZ and 4.94 ± 0.02 min for FNZ. The total analysis time was efficient, completed in less than 10 minutes. The method demonstrated excellent linearity in the concentration ranges of 12.5–62.5 μg/mL for HCZ and 10–50 μg/mL for FNZ, both yielding a correlation coefficient (R²) of 0.999. Recovery studies showed high accuracy, with results ranging from 99.02% to 99.83%. Precision was confirmed through intra-day and inter-day studies, with %RSD values generally remaining within acceptable limits. The Limit of Detection (LOD) was determined to be 0.0917, and the Limit of Quantitation (LOQ) was 0.2779. High theoretical plate counts (6938 for HCZ; 7000 for FNZ) and a resolution of 6.21 confirmed the efficiency and separation power of the system. The method was successfully applied to the analysis of a marketed capsule formulation (MONOPRIL HCT), with assay results showing 100.80% for HCZ and 101.56% for FNZ. This validated RP-HPLC method is simple, rapid, and precise, making it suitable for routine quality control and the simultaneous estimation of Fosinopril Sodium and Hydrochlorothiazide in tablet or capsule forms.

KEYWORDS: Fosinopril sodium, Hydrochlorothiazide, Method Development, Validation, RP-HPLC

I. INTRODUCTION

Fosinopril (Fos), an angiotensin-converting enzyme inhibitor used to treat hypertension which relax blood vessels, and hydrochlorothiazide (Hct), a diuretic agent, are together used in some commercial preparations [1]. One such combination of drug formulation is of Fosinopril Sodium and Hydrochlorothiazide which is used in treatment of hypertension. The body transforms it into fosinoprilat, its active metabolite [2,3]. Over 1.2 billion people worldwide suffer from hypertension, which is still the largest cause of cardiovascular morbidity and a major risk factor for myocardial infarction, stroke, and chronic kidney disease. This is especially noticeable in diverse patient groups where the effectiveness of monotherapy is influenced by physiological parameters such as baseline plasma renin activity (PRA) [4,5,6]. Fosinopril Sodium is chemically "Sodium;(2S,4S)-4-cyclohexyl-1-[2-[(2-methyl-1-propanoyloxypropoxy)-(4-phenylbutyl)phosphoryl]acetyl]pyrrolidine-2-carboxylate" (Fig.1) and Hydrochlorothiazide is chemically 6-chloro-1,1-dioxo-3,4-dihydro-2H-1,6,2,4-benzothiadiazinesulfonamide (Fig.2) [7,8,9]. Pharmacopoeias do not have an established procedure for the simultaneous determination of these substances [10]. Since Fosinopril Sodium and Hydrochlorothiazide is marketed in combination as tablets, there is a need to develop and validate a HPLC method for this combination of drugs. In the proposed project, an attempt shall be made to develop and validate a HPLC method and to apply the method for determination of Fosinopril Sodium and Hydrochlorothiazide in tablets [11-14].

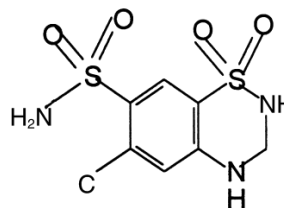
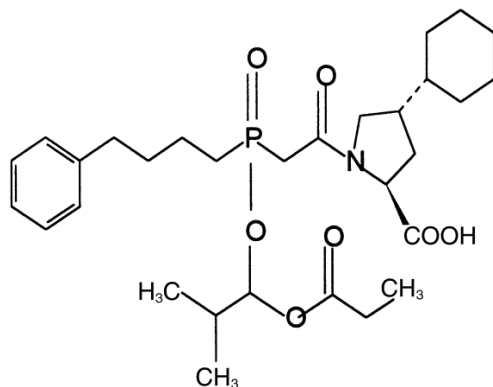


Fig No.1 Structure of Fosinopril Fig No.2 Structure of Hydrochlorothiazide

II. MATERIALS AND METHOD

2.1 Chemicals and Reagents

Fosinopril Sodium and Hydrochlorothiazide was purchased in market as MONOPRIL HCT 10/12.5mg (Bristol-Myers) and mobile phase is Methanol and Orthophosphoric acid were supplied by All other ingredients were used analytical grade

2.2 Instrumentation

HPLC Analysis was performed using AGILENT TECHNOLOGIES 1100 Series (Gradient system) and CHEMSTATION 10.1 software. AGILENT (1100) system equipped with G1311A solvent delivery system (Quaternary pump), auto sampler detector 1315D Diode array detector and column (AGILENT) C₁₈ (4.6 mm × 250 mm; 5 μm) used as stationary phase, a 20 μl injection loop and UV730D Absorbance detector.

2.3 Chromatographic Condition

The following chromatographic conditions were established by trial and error and were kept constant throughout the experimentation. Chromatographic separation was performed on, Column (4.6 mm × 250 mm) particle size with C-18 (AGILENT- ECLIPSA X DB) as stationary phase. Mobile phase was used as METHANOL: ORTHOPHOSPHORIC ACID (OPA) at 0.7 ml/min flow rate with 233 nm wavelength and particle size was 5 μm at ambient temperature with Run time is 15 min.

2.4 Preparation of Mobile Phase

Combination of mobile phase is Methanol and Orthophosphoric acid 50:50 filtered through 0.45 μm membrane filter and degassed by sonication.

Optimization of Mobile Phase Strength

With a view to separate out both the drugs simultaneously, various mobile phases consisting of methanol and water were tried, but tailing and low resolution of the chromatogram was observed.

Therefore, mobile phase consisting of methanol and potassium dihydrogen phosphate (50: 50 v/v) was tried and both these drugs were resolved properly. Well defined chromatograms were observed when the pH of the buffer was adjusted to 3.5 with OPA at flow rate of 0.7 mL/min; the retention time for HCZ and FNZ was found to be 3.66 ± 0.02 min and 4.94 ± 0.02 min respectively. The total time of analysis was less than 10 min.

2.5 Preparation of Stock Standard Solution

Accurately weighed quantity of 12.5 mg (HCZ) and 10 mg (FNZ) were transferred to 10 mL volumetric flask containing 10 mL methanol and volume was adjusted up to mark and sonicate for 15 mins to dissolve it and the resulting solution was further diluted to get concentration 50 μg/mL of HCZ and 40 μg/mL of FNZ.

UV-VIS Spectrophotometer

UV-VIS Spectrophotometer was selected as analytical technique for estimation of Fosinopril Sodium and Hydrochlorothiazide. UV absorbance range of 200-400 nm.

Instrument

Analytical Technologies Limited UV-VIS Spectrophotometer is double beam, high speed scanning spectrophotometer, the instrument needs about 1 minute for initialization. The light source used is Deuterium lamp of spectrophotometer, a computer is attached which helps in data processing and manipulation Quartz cuvette with path length 1 cm was used.

Study on the selection of UV spectrum use in UV-VIS spectrometer of Fosinopril sodium and Hydrochlorothiazide

Accurately weigh and transfer 12.5 mg of Fosinopril Sodium and 10 mg Hydrochlorothiazide working standard into 10 ml volumetric flask make volume up to the mark with the methanol. It was further

diluted to get concentration 50 µg/mL of HCZ and 40 µg/mL of FNZ and sonicate for 15 mins to dissolve it and from the resulting solution 0.5 ml was transferred to 10 ml volumetric flask and the volume was made up to the mark with methanol.

Study on the chromatographic conditions of Fosinopril sodium and Hydrochlorothiazide

Accurately weighed quantity of 12.5 mg (HCZ) and 10 mg (FNZ) were transferred to 10 mL volumetric flask containing 10 mL methanol and volume was adjusted up to mark and sonicate for 15 mins to dissolve it and the resulting solution was further diluted to get concentration 50 µg/mL of HCZ and 40 µg/mL of FNZ. Constant volume 20 µL was injected into column and peak area was recorded.

PRELIMINARY STUDIES ON FOSINOPRIL SODIUM AND HYDROCHLOROTHIAZIDE

Melting point

The procured reference standard of Fosinopril sodium and Hydrochlorothiazide were found to melt in the range of 197° C and 274° C respectively.

Solubility Studies

Various solvents were tried for checking solubility of Fosinopril Sodium and Hydrochlorothiazide. The drug was found to be Crystalline solid powder. From solubility studies it was concluded that of Fosinopril Sodium and Hydrochlorothiazide is poorly soluble in water however it is soluble in acetonitrile and methanol PH adjusted 0.1% Orthophosphoric Acid, Buffer pH 3.5.

UV Spectroscopy

UV absorption of mixed stock standard solution of HCZ (1250 mg/mL) and FNZ (1000 mg/mL) were prepared by dissolving 12.5 mg of HCZ and 10 mg of FNZ in 10 mL methanol. From the overlain spectra 233 nm was selected for the estimation of both these drugs simultaneously 20 mcg solution of Fosinopril sodium and Hydrochlorothiazide in MEOH was generated and absorbance was taken in the range of 200-400 nm.

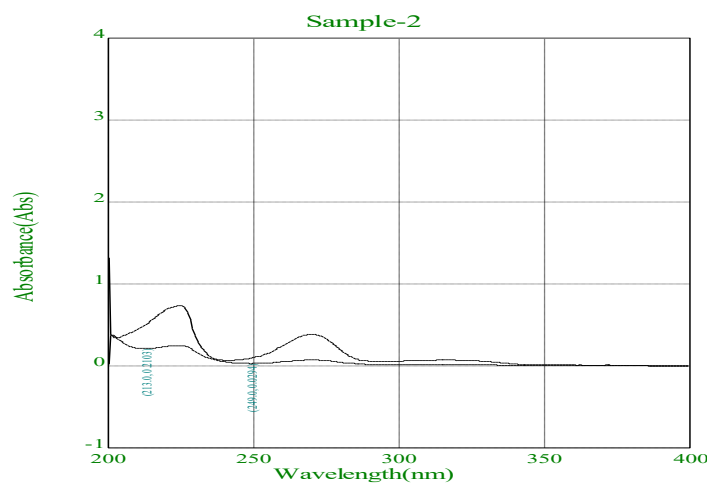


Fig No.3 An Overlain Spectra of HCZ and FNZ in Methanol

RP-HPLC Method

The absorption spectra were recorded in the wavelength region of 200 - 400 nm in UV-Spectrophotometric methods. Beer-Lambert's law was followed in the conc. range of 10-50µg/ml for Fosinopril sodium and Hydrochlorothiazide.

Linearity was observed with correlation co-efficient (r²) values 0.999 with linear equation for Y = 58.15x + 150.7 and Y = 14.51x + 10.18 for Fosinopril sodium and Hydrochlorothiazide respectively.

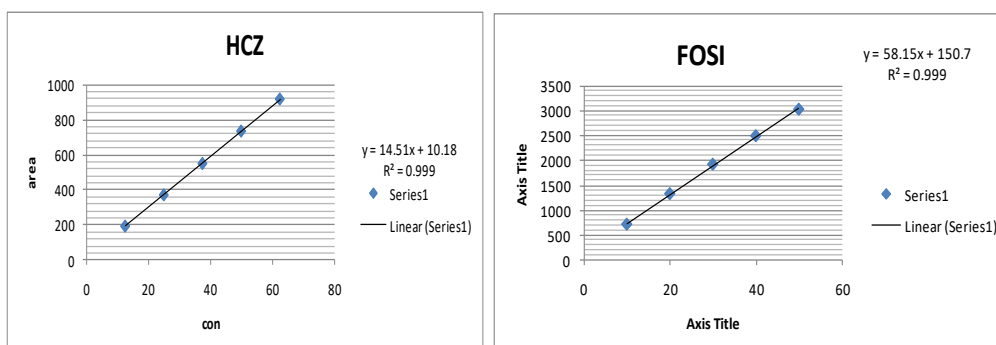


Fig No.4 Calibration curve for HCZ Fig No.5 Calibration curve for FNZ

The RP-HPLC method for respective linear equation $Y = 58.15x + 150.7$ and $Y = 14.51x + 10.18$ for Fosinopril sodium and Hydrochlorothiazide respectively, where x is the concentration and y is area of peak. The correlation coefficient was 0.999.

From the stock standard solution, aliquots portions (12.5-10 mL) were transferred into a series of 10 mL volumetric flasks and diluted up to the mark with mobile phase to obtain final concentration in the range of 12.5 – 62.5 µg/mL for HCZ and 10-50 µg/mL FNZ. A constant volume of 20 µL of each sample was injected with the help of Hamilton Syringe. Detection wavelength used was 233 nm.

ANALYTICAL METHOD VALIDATION

Linearity

Table No.1 Linearity Study of HCZ

Sr.No.	Concentration of HCZ [µg/mL]	Peak area [Mean ± SD; n = 5]	% RSD
1	12.5	194.85 ± 0.53	0.27
2	25	372.13 ± 2.56	0.69
3	37.5	551.38 ± 2.16	0.39
4	50	736.15 ± 0.97	0.13
5	62.5	919.06 ± 0.69	0.62

Table No.2 Linearity Study of FNZ

Sr.No.	Concentration of FNZ [µg/mL]	Peak area [Mean ± SD; n = 5]	% RSD
1	10	707.99 ± 2.57	0.36
2	20	1324.59 ± 3.57	0.27
3	30	1918.91 ± 1.05	0.05
4	40	2495.14 ± 0.85	0.03
5	50	3030.58 ± 1.44	0.05

Accuracy

The accuracy was determined by Fosinopril sodium and Hydrochlorothiazide (12.5 µg/mL of HCZ; 10 µg/mL of FNZ) were transferred to 10 mL volumetric flask containing 10 mL methanol and volume was adjusted up to mark and sonicate for 15 mins to dissolve it and the resulting solution was

further diluted to get concentration 50 µg/mL of HCZ and 40 µg/mL of FNZ (80 %, 100 % and 120 % of the label claimed, respectively) to quantity equivalent to average weight of marketed tablets. The % recovery of added drug was taken as a measure of accuracy.

Table No.3 Recovery studies

Drugs	Initial amount [µg/mL]	Excess drug added to the analyte [%]	Amount recovered ± S.D. [µg/mL]	Recovery [%]	%RSD [n = 3]
HCZ	24	80	19.08 ± 0.13	99.38	0.68
	24	100	23.82 ± 0.17	99.24	0.73
	24	120	28.60 ± 0.16	99.33	0.57
FNZ	06	80	4.79 ± 0.02	99.83	0.43
	06	100	5.94 ± 0.04	99.02	0.75
	06	120	7.18 ± 0.26	99.72	0.62

Precision

Intra-day precision

Sample solutions containing 25, 37.5 and 50 µg/mL of HCZ and 20, 30, 40 µg/mL of FNZ were analyzed three times on the same day and %R.S.D was calculated.

Inter-day precision

Sample solutions containing 25, 37.5 and 50 µg/mL of HCZ and 20, 30, 40 µg/mL of FNZ were analyzed three times on different concentration on different daysover a period of week. Repeatability was measured by analyzing 12.5 µg/mL of HCZ and 10 µg/mL of FNZ for six times.

Table No.4 Precision studies

Drug	Conc. [µg/mL]	Intra -day Amount Found [%] [n = 3]		Inter- day Amount Found [%] [n = 3]	
		Mean	% RSD	Mean	% RSD
HCZ	25	100.84	2.09	100.48	1.03
	37.5	102.80	1.99	100.72	1.03
	50	100.50	4.21	100.54	1.39
FNZ	20	101.56	0.83	101.62	3.38
	30	100.97	1.20	100.91	0.64
	40	101.02	0.41	100.90	1.78

Repeatability

Average weight of tablet sample (equivalent to 12.5 µg/mL of HCZ; 10 µg/mL of FNZ) was weighed and transferred to 10mL volumetric flask & diluents were added to make up the volume. Sonicated for 10 min with occasional swirling. The above solution was filtered through 0.45µm membrane filter and 0.4 ml of this solution diluted upto 10 ml with diluents. The results are shown in following table,

Table No.5 Repeatability studies

Drug	Concentration [µg/mL]	Peak Area Mean ± SD, [n = 6]	% RSD
HCZ	25	375.85 ± 1.82	0.49
FNZ	20	1331.84 ± 0.78	0.06

Robustness

The robustness is evaluated by the analysis of Tirzepatide under different experimental conditions such as making small changes in flow rate, wavelength and mobile phase concentration. In robustness overall %RSD

should not be more than 2.0% for the results obtained at the control and variable conditions. The results are discussed in following table,

Table No.6 Robustness Study of 25 µg/mL for HCZ and 20 µg/mL for FNZ

Parameters	Amount of detected (mean ±SD)		%RSD
	HCZ	FNZ	
Chromatogram of flow change 0.6 ml	679.32 ± 0.19	399.32 ± 0.19	0.05
Chromatogram of flow change 0.8 ml	769.65 ± 0.08	362.65 ± 0.08	0.02
Chromatogram at wavelength 232 nm	597.2 ± 0.39	367.2 ± 0.39	0.10
Chromatogram at wavelength 234nm chang57 nm	729.38 ± 0.19	424.38 ± 0.19	0.04
Chromatogram of mobile phase change 81+19 ml	614.6 ± 0.40	425.6 ± 0.40	0.10
Chromatogram of mobile phase change 70+21 ml	814.62 ± 0.35	425.62 ± 0.35	0.08

Ruggedness

From stock solutions, sample solutions of HCZ (24 µg/mL) and FNZ (6 µg/mL) were prepared and analyzed by two different analysts using similar operational and environmental conditions. Peak area was measured for same concentration solutions, six times; the results are shown in table,

Table No.7 Ruggedness study

Drug	Label Claim [mg]	% Amount Found [n = 6]		% RSD	
		Analyst I	Analyst II	Analyst I	Analyst II
HCZ	12.5	99.65	99.33	0.49	0.29
FNZ	10	99.59	99.24	0.34	0.32

LOD and LOQ

LOD and LOQ were calculated from the linearity curve by using the formula,

$$\text{LOD} = 3.3 \times \text{Avd. SD} / \text{Slope}$$

$$\text{LOQ} = 10 \times \text{Avd. SD} / \text{Slope}$$

Table No.8 LOD and LOQ Data of Tirzepatide

Parameters	Value
Slope	20.87
Intercept	0.58
Correlation coefficient R ²	R ² = 0.999
LOD	0.0917
LOQ	0.2779

System suitability test

System suitability testing is essential for the assurance of the quality performance of the chromatographic system. Earlier prepared solutions for chromatographic conditions were tested for system suitability testing.

Table no.9: System Suitability Test

System suitability Parameters	HCZ	FNZ
Retention time (t _R)	3.66 min	4.94 min
Theoretical plate (N)	6938	7000
Tailing factor (T)	0.73	0.61
Resolution	6.21	

Analysis of Capsule Formulation

Procedure

To determine the content of HCZ and FNZ in capsule formulation; twenty tablets (Label claim: HCZ 12.5 mg and FNZ 10 mg) were weighed accurately, their content removed and finely powdered. A quantity of powder equivalent to 12.5 mg of HCZ and 10 mg of FNZ was weighed and

transferred into 10 mL volumetric flask containing about 10 mL methanol. The solution was filtered through 0.45 µm membrane filter paper. The solution was further diluted with mobile phase to obtain concentration 50 µg/mL (HCZ) and 40 µg/mL (FNZ). Analysis of marketed formulation were also %Label Claim was found to be 100-101% Satisfactory are concluded.

Table No.9 Analysis of Capsule formulation

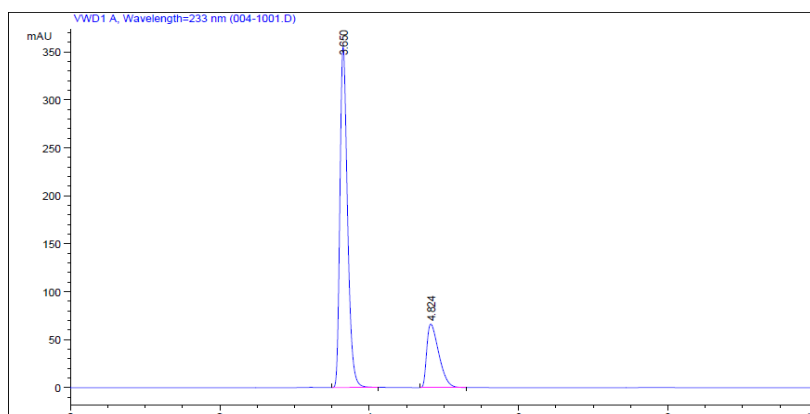
Brand Name: MONOPRIL HCT

Mfg. By: Bristol-Myers

Batch No.: NDC 0087-1492-01

Average weight :10/12.5 mg

Component	Label Claim [mg]	Amount Found mg ± SD [n = 6]	% Label Claim	% RSD
HCZ	25	375.85 ± 1.82	100.80	0.49
FNZ	20	20.31 ± 0.78	101.56	0.06



FigNo.6 Chromatogram of marketed formulation (30 mcg/ml)-1

III. CONCLUSION

A RP-HPLC method has been developed and validated for the simultaneous estimation of Fosinopril sodium and hydrochlorothiazide in bulk and pharmaceutical formulation. The HPLC analysis

was performed on the C₁₈ column (4.6mm×250mm), 5µm particle size in gradient mode, using Methanol : Orthophosphoric (50:50 %, v/v) pH adjusted to 3.5 with ortho-phosphoric acid as mobile phase filtered through 0.45µm; flow

rate was set at 0.7 mL/min. The detection was carried out at 233 nm. The retention time for Fosinopril sodium and Hydrochlorothiazide was found to be 3.66 ± 0.02 min and 6.64 ± 0.02 min, respectively. Fosinopril sodium and Hydrochlorothiazide followed linearity in the concentration range of 10-50 $\mu\text{g/mL}$ ($r^2 = 0.999$) and 12.5-62.5 $\mu\text{g/mL}$ ($r^2 = 0.999$) respectively. The method has successively been applied for the simultaneous determination of Fosinopril sodium and Hydrochlorothiazide in combined marketed formulation. Accuracy of the method was studied by the recovery studies at three different levels i.e. 80 %, 100 % and 120 % level. The % recovery was found to be within the limits of the range of 99.02 – 99.83 %. The precision of the method was studied as repeatability of sample application, intra-day and inter-day precision. The low value of % RSD (less than 2) indicates high precision of the method. The system suitability parameters were well within acceptable limits having Theoretical Plates is High values (6938 for HCZ and 7000 for FNZ) indicate high column efficiency and Resolution is A resolution of 6.21 confirms that the two drug peaks are well-separated. The low LOD (0.0917 $\mu\text{g/mL}$) and LOQ (0.2779 $\mu\text{g/mL}$) values further established its high sensitivity. The validated RP-HPLC method was applied to the analysis of MONOPRIL HCT capsules. The assay results showed 100.80% for HCZ and 101.56% for FNZ, which are within the satisfactory range for label claim. The proposed RP-HPLC method is simple, rapid, accurate, and precise. Because there was no established pharmacopoeial procedure for the simultaneous determination of these two substances in combination, this developed method provides a reliable tool for routine quality control analysis of Fosinopril Sodium and Hydrochlorothiazide in pharmaceutical dosage forms.

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