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Development and Validation of Antihypertensive Drug (Fosinopril) in Bulk by Rp-Hplc Method

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Abstract

The methodology was set up for synchronous estimation of a Fosinopril by RP-HPLC system. The chromatographic conditions were viably created for the unit of Fosinopril by using Inertsil - ODS C18 (250 x 4.6 mm, 5µ), column by utilizing the versatile arrange Methanol: Acetonitrile (90:10% V/V), at a stream rate of 1.0 ml/min. The area was carried out at a wave length of 271 nm.

Keywords: Fosinopril, RP-HPLC, Acetonitrile, Methanol, Water.

1. Introduction to HPLC

High Performance Liquid Chromatography (HPLC) was derived from the classical column chromatography and, is one of the most important tools of analytical chemistry today.[1]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.[2] HPLC is the method of choice for checking peak purity of new chemical entities, monitoring reaction changes is in synthetic procedures or scale up, evaluating new formulations and carrying out quality control / assurance of the final drug products.[3]

The Goal of HPLC method is to try & separate, quantify the main drug, any reaction impurities, all available synthetic intermediates and any degradants.[4]High Performance Liquid Chromatography is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantify the compounds that are present in any sample that can be dissolved in a liquid. HPLC is the most accurate analytical methods widely used for the quantitative as well as qualitative analysis of drug product and used for determining drug product stability.[5]

1.1 Principle

HPLC principle is the solution of sample is injected into a column of porous material (stationary phase) and liquid phase (mobile phase) is pumped at higher pressure through the column. The principle of separation followed is the adsorption of solute on stationary phase based on its affinity towards stationary phase. (Figure-1) The technique of HPLC has following features.[6]

1.2 HPLC Method Development:

Methods are developed for new products when no official methods are available. Alternate methods for existing (Non-Pharmacopoeias) products are to reduce the cost and time for better precision and ruggedness. When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit/demerits are made available. The goal of the HPLC-method is to try &

separate, quantify the main active drug, any reaction impurities, all available synthetic inter-mediates and any degradants.[7]

Steps involved in Method development are. [6, 7]

Understanding the Physicochemical properties of drug molecule

Selection of chromatographic conditions

Developing the approach of analysis

Sample preparation

Method optimization

Method validation

2. Material and Methods

2.1 Instruments Used:

Table no. 1: Instruments and Apparatus

Sr. No.	Instruments and Apparatus	Make
1	HPLC Model NO.2690/5 series Compact System Consisting of Inertsil-C18 ODS column.	Waters
2	UV spectrophotometer	Systronics
3	Electronic balance	Sartorius
4	Sonicator	Fast clean
5	Hot Air Oven	Bio Technics India
6	Micropipette	Pipette
7	Cellulose Membrane Filter	Pall Corporation

2.2 Chemicals and reagents

The solvents utilized were of HPLC/ AR review. Immaculate medicate test of Fosinopril was gotten as a blessing test from MSN PVT LTD, HYD.

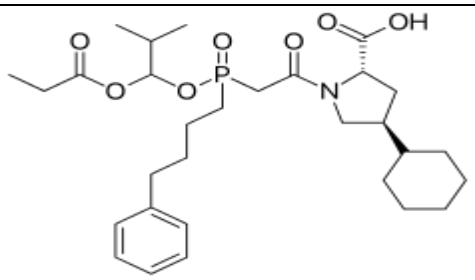
Methanol

Water

Acetonitrile

Drug Profile

Common Name	FOSINOPRIL
Brand Name	MONOPRIL
IUPAC Name	(2*S*,4*S*)-4-cyclohexyl-1-[2-[hydroxy(4-phenylbutyl)phosphoryl]acetyl]pyrrolidine-2-carboxylic acid
Molecular Formula	$C_{30}H_{46}NO_7P$
Molecular Weight	563.672 $g \cdot mol^{-1}$

Structural Formula		
Physicochemical Properties	**Appearance**	White, capsule shaped biconvex tablets with indents, engraved APO on one side and FOS-10 on the side
	Solubility	Soluble in Organic solvent methanol.
Melting Point		149-153
pKa Value	**Strongest Acidic**	3.87
	Strongest Basic	-4.4
Log P		4.3
Absorption		36% orally
Mechanism of action		Competes with ATI for binding to ACE and inhibits and enzymatic proteolysis of ATI to ATII.
Protein binding		87% fosinoprilate**.**
Half-life		12 hour fosinoprilate
Metabolism		Liver, gut, mucosa to fosinoprilate.
Route of administration		Oral route

3. Experimental Work:

3.1 Stock and standard Working solution

Fosinopril is used as working standard in method development

3.2 Stock Solution Preparation

Take 100 mg Fosinopril working standard in 100 ml volumetric flask add methanol sonicate it for 30 minutes, (That is 1000 ppm solution).

3.3 Further Dilution (or) Trials Solution:

Take 10 ml of above solution in 100 ml V.F add methanol up to mark sonicate it for 10 minutes (That 100 ppm solution).

3.4 Selection of Wave Length:

Scan standard solution in UV spectrophotometer between 200 nm to 400 nm on spectrum mode, using diluents as a blank. Fosinopril shows λ max at 271 nm.

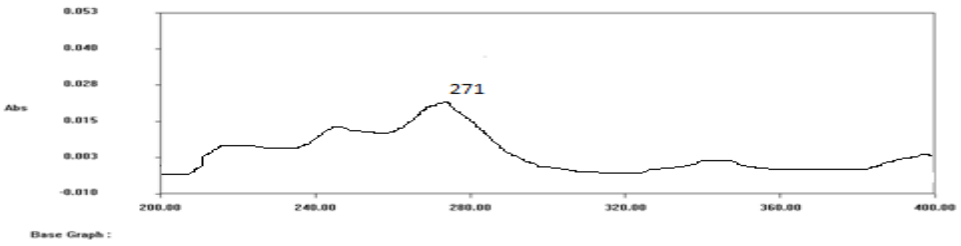


Fig. no. 1: UV Spectrum of Fosinopril at 271nm

3.5 Development of HPLC Method:

The goal of this study was to improve the assay technique for simultaneous quantification of Fosinopril on literature surveys. As a result, the trials detailed below show how the optimization was accomplished.

Table no. 2: Chromatographic Conditions

Sr. No.	Trial	Mobile Phase	Name of the peak	Retention time (min)	Flow rate	Time to run	Tempo in the column
1.	1	Methanol: Water 55:45.V/V	Fosinopril	3.145 min.	1.0ml/min	6min	Ambient
2.	2	Acetonitrile: methanol 30:70 V/V	Fosinopril	2.913 min.	1.0ml/min	6min	Ambient
3.	3	Acetonitrile: Water 40:60 V/V	Fosinopril	3.071 min.	1.0ml/min	6min	Ambient

4 Method Validation

4.1 System Suitability:

A Standard solution was prepared by using Fosinopril working standard as per test method and was injected Five times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from five replicate injections for Fosinopril**,** retention times and peak areas.

The % RSD for the retention times of principal peak from 5 replicate injections of each Standard solution should be not more than 2.0 %. The % RSD for the peak area responses of principal peak from 5 replicate injections of each standard Solution should be not more than 2.0%.The number of theoretical plates (N) for the Fosinopril peaks is NLT 3000.The Tailing factor (T) for the Fosinopril peaks is NMT 2.0.

4.2 SPECIFICITY:

Solutions of standard and sample were prepared as per the test method are injected into chromatographic system. Chromatogram of standard and blank should be identical with near Retention time.

4.3 PRECISION:

4.3.1 Repeatability:

System precision: Standard solution prepared as per test method and injected five times.

Method precision: Prepared five sample preparations individually using single as per test method and injected each solution.

The % relative standard deviation of individual Fosinopril, from the five units should be not more than 2.0%. The assay of Fosinopril should be not less than 98% and not more than 102.0%.

4.3.2 Intermediate precision

A study was conducted by two analysts as per test method. The individual assays of Fosinopril should be not less than 98% and not more than 102% and % RSD of assay should be NMT 2.0% by both analysts.

Table no. 3: Data of Repeatability

Conc. 40ppm	System precision			Method precision			Intermediate precision		
	Inj.	Peak Areas of Fosinopril	%Assay	Inj.	Peak Areas of Fosinopril	%Assay	Inj.	Peak Areas of Fosinopril	%Assay
	1	128470.52	100.12	1	128591.23	100.21	1	128563.22	100.19
	2	128532.12	100.18	2	128537.32	100.17	2	128501.99	100.14
	3	128544.85	100.17	3	128487.85	100.13	3	128580.64	100.20
	4	128420.34	100.08	4	128503.12	100.14	4	128603.55	100.22
	5	128455.09	100.11	5	128499.95	100.14	5	128582.56	100.21
Statistical Analysis	**Mean**	128484.58	100.13	**Mean**	128528.59	100.16	**Mean**	128571.28	100.20
	SD	52.646570	0.0410	**SD**	39.19785	0.0305	**SD**	36.6634	0.028607
	% RSD	0.0409750	0.0410	**% RSD**	0.030497	0.0305	**% RSD**	0.02851	0.028550

4.4 ACCURACY:

A study of Accuracy was conducted. Drug Assay was performed in triplicate as per test method with equivalent amount of Fosinopril into each volumetric flask for each spike level to get the concentration of Fosinopril equivalent to 50%, 100%, and 150% of the labelled amount as per the test method. The average % recovery of Fosinopril was calculated. The mean % recovery of the Fosinopril at each spike level should be not less than 98.0% and not more than 102.0%

Table no. 4: Data of Accuracy

Concentration % of spiked level	Area	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% Sample 1	64280.03	20	20.01	100.07		
50% Sample 2	64264.22	20	20.00	100.04	**MEAN**	100.06
50% Sample 3	64272.65	20	20.01	100.06	**%RSD**	0.01
100 % Sample 1	128582.12	40	40.08	100.20		
100 % Sample 2	128514.34	40	40.06	100.15	**MEAN**	100.18
100% Sample 3	128555.54	40	40.07	100.18	**%RSD**	0.02659
150% Sample 1	191220.35	60	59.63	99.38		
150% Sample 2	191256.55	60	59.64	99.40	**MEAN**	99.40
150% Sample 3	191270.56	60	59.64	99.41	**%RSD**	0.013

4.5 LINEARITY:

A Series of solutions are prepared using Fosinopril working standard at concentration levels from 20ppm to 70 ppm of target concentration. Correlation Coefficient should be not less than 0.9990. % of y-Intercept should be ±2.0. % of RSD for level 1 and Level 6 should be not more than 2.0%.

Table no. 5: Data of Linearity

Concentration (ppm)	Average Area	Statistical Analysis	
0	0	Slope	3204
20	64282.5	y-Intercept	153.1
30	96420.75	Correlation Coefficient	0.999
40	128565.25	----	---
50	160760.30	----	---
60	191225.25		
70	224988.80	----	---

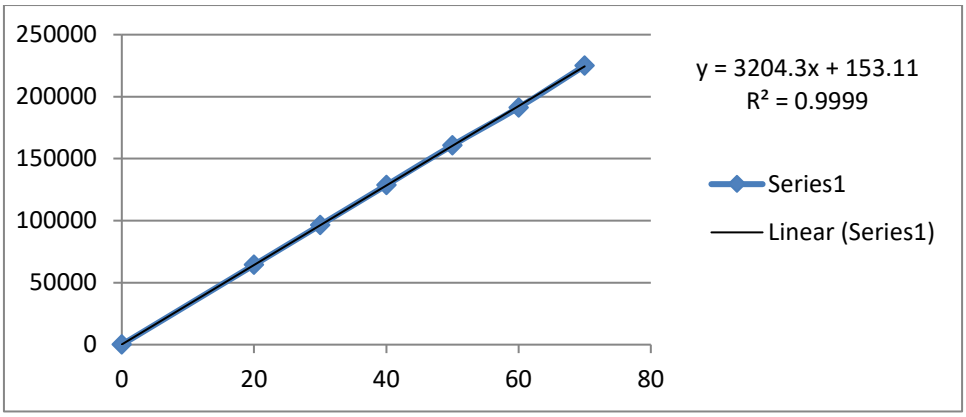


Fig. no. 2: Linearity Plot (Concentration Vs Response)

4.6 Ruggedness:

System to system variability:

System to system variability study was conducted on different HPLC systems, under similar conditions at different times. Six samples were prepared and each was analyzed as per test method. Comparison of both the results obtained on two different HPLC systems, shows that the assay test method are rugged for System to system variability. The % relative standard deviation of Fosinopril from the six sample preparations should be not more than 2.0%. The % assay of Fosinopril should be between 98.0%-102.0%.

Table no. 6: Data on System Variability

Sr. NO:	Peak area	Assay % of Fosinopril
1	128460.46	100.11
2	128495.56	100.14
3	128500.04	100.14
4	128470.54	100.12
5	128509.38	100.15

6	128525.12	100.16
Mean	128493.58	100.14
%RSD	0.0188	0.0188

4.7 ROBUSTNESS:

Effect of variation of flow rate:

A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into the HPLC system using flow rates, 1.0ml/min and1.2ml/min. The system suitability parameters were evaluated and found to be within the limits for 1.0ml/min and 1.2ml/min flow. Fosinopril was resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having flow rates 1.0ml/min. The Tailing Factor of Fosinopril standards should be NMT 2.0 for Variation in Flow.

Table no. 7: Data on Robustness

	Std Area	**Tailing factor**		**Std Area**	**Tailing factor**		**Std Area**	**Tailing factor**
Flow 0.8 ml	120156.32	1.106	**Flow 1.0 ml**	128564.02	1.110	**Flow 1.2 ml**	136289.32	1.123
	120200.35	1.110		128507.23	1.112		136264.32	1.125
	120185.56	1.112		128499.05	1.110		136311.24	1.124
	120225.62	1.118		128530.44	1.111		136301.56	1.124
	120201.53	1.117		128540.28	1.112		136296.96	1.123
Avg	120193.87	1.112	**Avg**	128528.20	1.111	**Avg**	136292.68	1.123
SD	25.4351	0.0049	**SD**	26.0934	0.001	**SD**	17.7286	0.0008
%RSD	0.0211	0.4475	**%RSD**	0.0203	0.090	**%RSD**	0.01300	0.0744

4.8 LOD AND LOQ (LIMIT OF DETECTION AND LIMIT OF QUANTITATION):

From the linearity plot the LOD and LOQ are calculated:

$$LOD = 3.3\ \sigma/S$$

$$3.3\times130.15611/3204$$

$$= 0.134$$

$$LOQ = 10\ \sigma/S$$

$$10\times130.15611/\ 3204 = 0.406$$

4.9 Market Sample:

Table no. 8: Market Sample Analysis

Drug Name	**Brand Name**	**Company**
Fosinopril	Fovas 10	Cadila

$$\%\ \text{Assay} = \frac{\text{Amount found(x)}}{\text{Amount added}} \times 100$$

X=y-c/m

Table no. 9: Data for Market Sample

Injection	Peak Areas of Fosinopril	%Assay
1	128543.04	100.18
2	128075.28	100.15
3	128491.75	100.16
4	128375.06	100.19
5	128347.97	100.14
6	128075.28	100.15
Mean	128366.6	100.164
SD	347.3655	0.020736
% RSD	0.022091	0.020702

4.10 FTIR: - (Fourier Transform Infrared Spectroscopy)

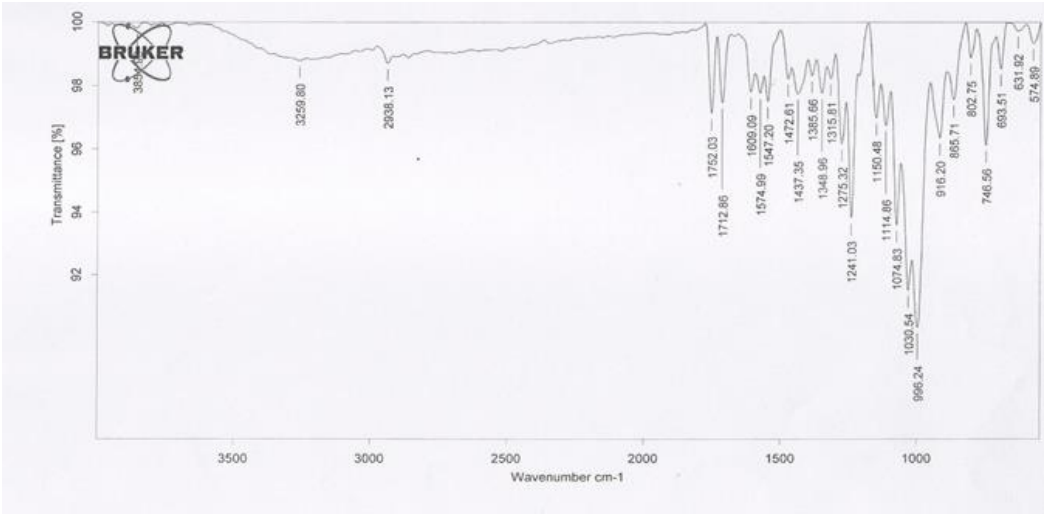


Fig. no. 3: FTIR Spectra for Fosinopril

CONCLUSION:

Different parameters were studied to create the analytical approach For starters, the maximum absorbance of Fosinopril was discovered to be 271 nm. The injection volume was set at 20µl, which resulted in a nice peak area. The Inertsil C18 column was employed in this work, and ODS picked a nice peak shape. The temperature of the ambient environment was determined to be adequate for the type of the medication solution. Because of the good peak area, adequate retention duration, and good resolution, the flow rate was set at 1.0ml/min. Different mobile phase ratios were investigated, however the mobile phase with a Methanol: Acetonitrile (90:10) ratio was chosen because to its symmetrical peaks and high resolution. As a result, the planned research made use of this mobile phase.

The accuracy of both the system and the procedure was determined to be precise and well within range. The correlation coefficient and curve fitting were discovered during the linearity investigation. For bulk drug and formulation, the analytical approach was shown to be linear throughout a range of 20-70ppm of

the target concentration. Both robustness and ruggedness tests were passed by the analytical. The relative standard deviation in both circumstances was excellent.

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