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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF SIMULTANEOUS ESTIMATION OF FOSINOPRIL SODIUM, HYDROCHLOROTHIAZIDE IN TABLET DOSAGE FORM BY RP-HPLC

K. Priyanka*, A. Ajitha, M. Sandhya Mamindla, V. Uma Maheswara Rao

Department of Pharmaceutical Analysis and Quality Assurance, CMR College of Pharmacy, Kandlakoya (v), Medchal road, Hyderabad – 501 401, A.P, India

***Corresponding author e-mail:** priyareddykothakapu@gmail.com

ABSTRACT

A simple, precise, rapid, specific and accurate reverse phase high performance liquid chromatography method was developed for simultaneous estimation of Fosinopril Sodium (FOS) and Hydrochlorothiazide (HCTZ) in pharmaceutical dosage form. Chromatographic separation was performed on X-terra(C_8) (4.6mm x 150mm, 3.5µm) column, with mobile phase comprising of mixture of buffer (pH 6.0, adjusted with ortho phosphoric acid), acetonitrile and methanol in the ratio of 80:10:10v/v, at the flow rate 0.8 ml/min. The detection was carried out at 226 nm. The retention times of FOS and HCTZ were found to be 2.1 and 3.3 mins respectively with a run time of 6 mins, theoretical levels for FOS and HCTZ were 2015 and 4034 respectively, with a resolution of 5.42. As per ICH guidelines the method was validated for linearity, accuracy, precision, limit of detection and limit of quantitation, robustness and ruggedness. Linearity of FOS was found in the range of 10-50 µg/mL and that for HCTZ was found to be 6.25-31.35 µg/mL. The correlation coefficient for FOS and HCTZ were 0.9992 and 0.9991 respectively. The LOD values for FOS and HCTZ were 0.88 and 1.11 µg/mL respectively. The LOQ values for FOS and HCTZ were and 2.96 and 3.7 µg/mL respectively. This demonstrates that the developed method is simple, precise, rapid, selective, accurate and reproducible for simultaneous estimation of FOS and HCTZ tablet dosage form.

Key words: Fosinopril Sodium (FOS), Hydrochlorothiazide (HCTZ), RP-HPLC, Validation

INTRODUCTION

Fosinopril Sodium (FOS) is a anti-hypertensive and is chemically-L-proline, 4-cyclohexyl-1-[[[2-methyl-1-(1-oxopropoxy) propoxy] (4-phenylbutyl) phosphinyl]acetyl] sodium salt. It is used in the treatment of Hypertension. It is an angiotensin converting enzyme inhibitor.[16, 18] Hydrochlorothiazide (HCTZ) is a diuretic and is chemically-6-chloro-1, 1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide. It is used in the treatment of hypertension. It acts by decreasing the sodium levels and water content in the body. [17]
Literature survey revealed that few analytical techniques are available for estimation of FOS alone as well as in combine dosage form such as UV, HPLC.[4-8] Similarly few analytical methods are available for estimation of HCTZ alone and its combination with drugs such as UV and HPLC.[1-8]

Keeping this objective in mind an attempt has been made to develop and validate the RP-HPLC method for the simultaneous estimation of Fosinopril Sodium and Hydrochlorothiazide which would be highly sensitive having good resolution, reproducible and cost effective. Various validation aspects of the analysis accuracy, precision, recovery, the limits of detection and quantification etc have been measured as per ICH guidelines. [15]

MATERIALS AND METHODS

Equipment: Chromatographic separation was performed on HPLC system - Water's 515 pump, UV Detector 2487 module, equipped with a solvent delivery pump, sample injector and column thermostats. N 2000 Chromatographic system software was applied for data collecting and processing.

Chemicals and reagents: Methanol, Acetonitrile (HPLC grade) was used. Buffer used was pH-6 (pH adjusted with orthophosphoric acid). (Reference standards Fosinopril Sodium and Hydrochlorothiazide were obtained from Pharmtech Solutions. Monopril hct Tablets of FOS (20mg) and HCTZ (12.5mg) manufactured by Bristol-Myers Squibb pharmaceuticals Ltd., were procured from local market.

Preparation of standard solutions: Accurately weighed and transferred 10 mg of FOS and 6.25 mg of HCTZ working standard into a 100mL clean dry volumetric flask and added about 70mL of diluent. It was sonicated to dissolve completely and made volume up to the mark with the same diluent. (Stock solution) (100, 62.5 µg/mL). From this, 3 ml of the solution was pipetted into another 10ml volumetric flask and diluted up to the mark with diluent (30,18.75 µg/mL).

Preparation of sample solution: Accurately weighed and transferred tablet powder equivalent to 10 mg of FOS and 6.25 mg of HCTZ into a 100mL clean dry volumetric flask and added about 70mL of diluent. It was sonicated to dissolve completely and made volume up to the mark with the same diluent. (Stock solution)(100, 62.5µg/mL) From this, 3 mL of the solution was pipetted into another 10ml volumetric flask and diluted up to the mark with diluent.

Preparation of buffer: Take 1000mL of HPLC grade water. The pH was adjusted to 6.0 with orthophosphoric acid.

Optimized chromatographic conditions:

Diluent **:** Water: Methanol (50:50)
Mobile phase : Buffer p^{H} - 6: Methanol: Acetonitrile (80:10:10)
Flow rate : 0.8mL/min
Column : X-terra (C_8) (4.6mm x 150mm, 3.5µm)
Detector wavelength : 226nm
Injection volume : 20 µL

METHOD VALIDATION

Linearity: Solutions were prepared containing 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, 50µg/ml, concentrations of Fosinopril Sodium and 6.25µg/ml, 12.5 µg/ml, 18.75µg/ml, 25µg/ml, 31.25µg/ml, concentrations of Hydrochlorothiazide which corresponding to 50, 75, 100, 125 and 150% respectively of the test solution concentration. Each solution was injected, linearity was evaluated by linear- regression analysis.

Accuracy:

Accuracy was determined by the recovery studies at three different concentrations (corresponding to 50, 100 and 150% of the test solution concentration) by addition of known amounts of standard to pre-analysed sample preparation. For each concentration, three sets were prepared and injected.

Precision:

Intraday and interday variations were determined by using six replicate injections of one concentration and analyzed on the same day and different days. Precision of an analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements.

Robustness:

The robustness was evaluated by assaying test solutions after slight but deliberate changes in the analytical conditions. The factors chosen for this study were the flow rate (±0.1ml/min), mobile phase composition.

Limit of detection (LOD) and Limit of quantification (LOQ):

LOD and LOQ was calculated from linear curve using formulae

LOD= 3.3 * σ / slope, LOQ= 10 * σ / slope

(Where σ = the standard deviation of the response and S = Slope of calibration curve).

Specificity: Specificity was checked for the interference of impurities in the analysis of blank solution and injecting sample solution under optimized chromatographic conditions to demonstrate separation of both FOS and HCTZ from impurities.

RESULTS AND DISCUSSIONS

Several mobile phase compositions were tried to resolve the peak of FOS and HCTZ. The mobile phase containing buffer: Methanol: Acetonitrile in proportion of 80:10:10v/v was found ideal to resolve the peak of FOS and HCTZ. Retention time of FOS and HCTZ were 2.1 and 3.3 min respectively (Figure 1&2). Result of assay is shown in Table-1. The proposed method was found to be linear in concentration range 10-50µg/ml for FOS and 6.25-31.25µg/ml for HCTZ. The data was shown in Table-2 and Figure-3 & 4. System suitability parameters were evaluated and results shown in (Table-3), which were within acceptance criteria. The mean percentage recovery for FOS and HCTZ was found to be 99.26% and 99.97% respectively, which are well within the limit and hence the method was found to be accurate (Table-4). LOD and LOQ values were 0.88µg/mL and 1.11 µg/mL for FOS and 2.96µg/mL and 3.7µg/mL for HCTZ (Table-5). Results of intraday and interday precision were shown in the (Table-6a&6b). The robustness of the method was investigated by varying experimental conditions such as changes in flow rate and mobile phase composition. The result obtained implies method is robust for routine qualitative analysis (Table-7).

CONCLUSION

The proposed RP-HPLC method was validated as per International Conference on Harmonization (ICH) guidelines, and found to be applicable for routine quality control analysis for the simultaneous estimation of FOS and HCTZ using isocratic mode of elution. The results of linearity, precision, accuracy and specificity, proved to be within the limits. The proposed method is highly sensitive, reproducible, reliable, rapid and specific.

ACKNOWLEDGEMENT

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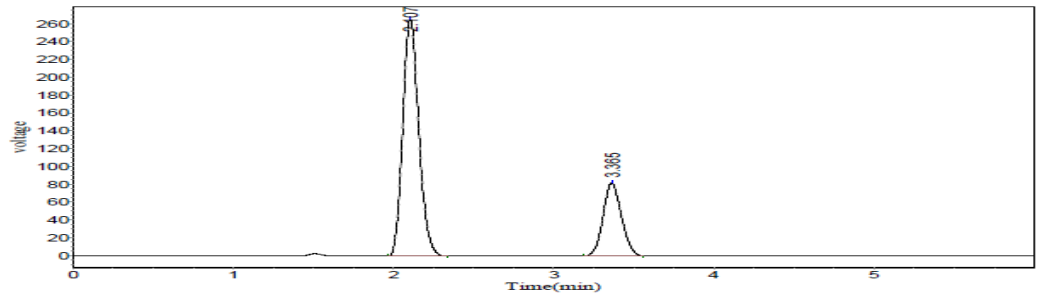

Figure-1: Chromatogram of FOS (30µg/mL) and HCTZ (18.75µg/mL) standard

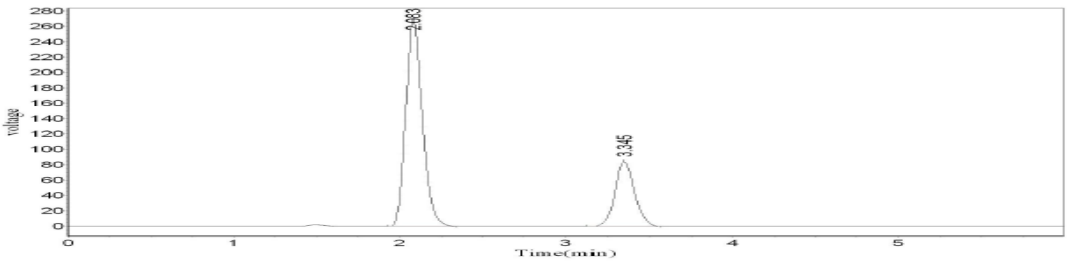

Figure-2: Chromatogram of FOS (30µg/mL) and HCTZ (18.75µg/mL) sample

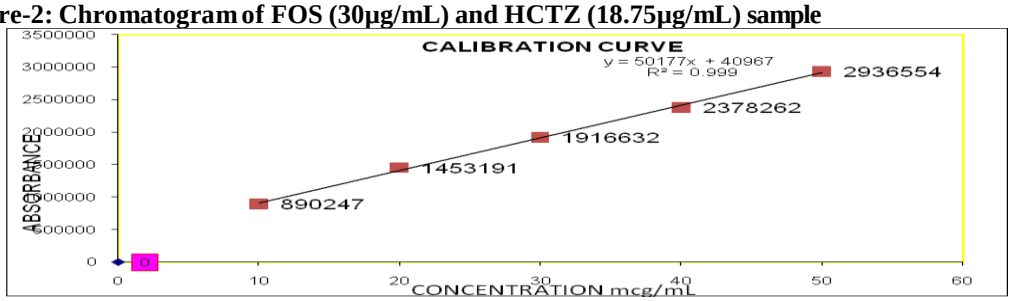

Figure-3: Calibration curve for Fosinopril Sodium

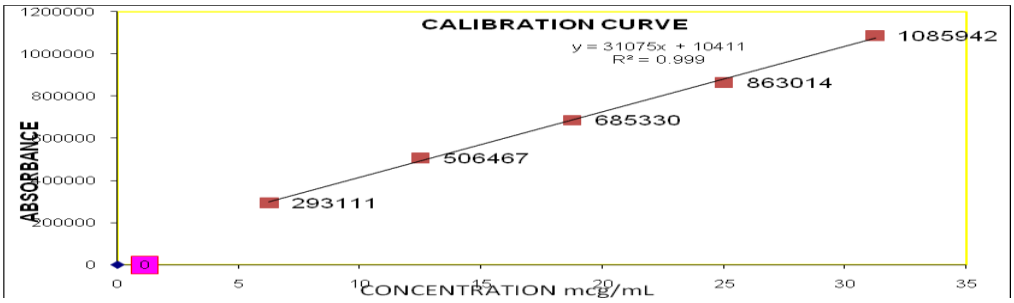

Figure -4: Calibration curve for Hydrochlorothiazide

Table -1 Analysis data of formulation (Monopril hct)

Injection	Label claim(mg)	Assay (%)
FOS	20	100.9
HCTZ	12.5	98.2

Table – 2: Result of Linearity

S. No	Fosinopril Sodium		Hyrochlorothiazide	
	Conc. (µg/ml)	Peak area	Conc. (µg/ml)	Peak area
1	10	890247	6.25	293111
2	20	1453191	12.5	506467
3	30	1916632	18.75	685330
4	40	2378262	25	863014
5	50	2936554	31.25	1085942

Table-3: System suitability studies

Parameters	Fosinopril Sodium	Hydrochlorothiazide	Acceptance criteria
Theoretical plates	2015	4034	Not less than 2000
Tailing factor	1.1	1.0	Not more than 2
Resolution	-	5.42	Not less than 2

Table-4: Recovery studies for Fosinopril Sodium and Hydrochlorothiazide

DRUG	Spiked level%	Amount taken (µg/ml)	Amount found (µg/ml)	Percent recovery n=3	Mean recovery
FOS	50	45	44.93	99.53	
	100	60	60.08	100.26	99.266
	150	75	74.08	98	
HCTZ	50	28.125	28.91	100.26	
	100	37.5	36.96	98.18	99.97
	150	46.87	47.29	101.47	

n- Number of replicate injections

Table-5: LOD and LOQ for Fosinopril Sodium and Hydrochlorothiazide

DRUG	LOD (µg/ml)	LOQ (µg/ml)
Fosinopril Sodium	0.88	2.96
Hydrochlorothiazide	1.11	3.7

Table-6(a): Results of Intraday Precision

DRUG	Conc. (µg/ml)	Peak area (n=6)	% RSD
FOS	30	1878699	1.73
HCTZ	18.75	656101	1.82

n- Number of replicate injections

Table-6(b): Results of Interday Precision

DRUG	Conc. (µg/ml)	Peak area (n=6)	% RSD
FOS	30	1812681	1.54
HCTZ	18.75	686325	0.54

Table-7: Results of Robustness study

S. no	Parameter	Condition	Theoretical levels		Tailing factor		Retention time	
			FOS	HCTZ	FOS	HCTZ	FOS	HCTZ
1.	Flow rate	0.7 ml/min	2244	4308	1.1	1.0	2.3	3.7
		0.9 ml/min	2015	3859	1.2	1.1	1.9	3.1
2.	Mobile	76:12:12 v/v	1965	3653	1.2	1.1	1.9	3.0
	phase	84:8:8v/v	2261	4204	1.1	1.0	2.2	3.6

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