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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF TELMISARTAN AND INDAPAMIDE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A simple, rapid, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Telmisartan and Indapamide in tablet dosage form. An XBridge C_{18} column having (100x4.6mm, 5µ) in Isocratic mode with mobile phase containing Acetonitrile:Buffer(65:35) was used. The flow rate was 1.0mL / min and eluents were monitored at 210 nm. The retention times of Telmisartan and Indapamide were 5.268min and 2.366min respectively. The calibration curves were linear over a concentration range from 100-600 ppm for Telmisartan and over a concentration range from 3.75-22.5 ppm for Indapamide. Limit of detection (LOD) and Limit of quantification (LOQ) were 2.23 and 6.77 for Telmisartan and 0.32 and 0.98 for Indapamide respectively. The developed method was fast, accurate, precise and successfully applied to estimate the amount of Telmisartan and Indapamide in bulk sample and tablet dosage form so it can be used for regular quality control of the drug..

Key words: Telmisartan (TEL), Indapamide (IND), RP-HPLC, Simultaneous estimation.

INTRODUCTION

Telmisartan (TEL) is chemically 2-(4-{[4-methyl-6-(1-methyl-1H-1,3-benzodiazol-2-yl)-2-propyl-1H-1,3-benzodiazol-1-yl]methyl}phenyl)benzoic acid (fig-1). It is used in the management of hypertension. Telmisartan interferes with the binding of angiotensin II to the angiotensin II AT_1-receptor by binding reversibly and selectively to the receptors in vascular smooth muscle and the adrenal gland. [1]
Indapamide(IND) is chemically 4-chloro-N-(2-methyl-2,3-dihydro-1H-indol-1-yl)-3-sulfamoyl benzamide (fig-2) It is an hypertensive an diuretic. It is believed that Telmisartan's dual mode of action may provide protective benefits against the vascular and renal damage caused by diabetes and cardiovascular disease. Literature survey revealed few analytical techniques are available for estimation of TEL alone as well as in combine dosage form such as UV ,HPLC, HPTLC.[3-7] Similarly few analytical methods are available for estimation of IND alone and its combination with drugs such as UV and HPLC.[8-17] keeping this objective in mind an attempt has been made to develop and validate the RP-HPLC method for the simultaneous estimation of TEL and IND 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. [18]

MATERIALS AND METHOD

Equipment: Chromatographic separation was performed on HPLC system - Water's alliance 2695 with 2996 module Photo Diode Array (PDA) detector equipped with a solvent delivery pump, automatic

sample injector and column thermostats. Waters Empower2 software was applied for data collecting and processing.

Chemicals and reagents: Methanol, Acetonitrile (HPLC grade) was used. Buffer used was Potassium dihydrogen ortho phosphate. Reference standards Telmisartam and Indapamide were obtained from SPECTRUM PHARMA. INDITEL Tablets of TEL (40mg) and IND (3mg) manufactured by Cadila pharmaceuticals Ltd were procured from local market.

Preparation of standard solutions: Accurately weighed 1.5 mg of Indapamide and 40 mg of Telmisartan each was transferred into a clean and dry 100ml volumetric flask, dissolved with sufficient volume of diluent and sonicate for 5min. The volume made up to 100ml with diluent to obtain 30µg/ml Indapamide of and 400µg/ml of Telmisartan stock solutions. 1ml of standard stock solution of Indapamide (30µg/ml) and 1ml of standard stock solution of Telmisartan (400 µg/ml) are transferred in to a 10 ml volumetric flask and the volume made with diluent. The resulting solution was sonicated for 10 min.

Preparation of sample solution: 5 tablets of INDITEL containing 40mg of Telmisartan and 1.5mg of Indapamide were weighed and crushed into powder. From that powder weight equivalent to 20mg of Telmisartan and 3mg of Indapamide were transferred into a 500 mL volumetric flask, 300mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipetted out into a 10 ml volumetric flask and made up to 10ml with diluent.

Preparation of buffer: Accurately weighed 2.72gm of Potassium dihydrogen orthophosphate was transferred into a 1000ml of Volumetric flask, about 900ml of milli-Q water was added and sonicate to degassed and finally make up the volume with water. Finally pH is adjusted to 3.5 with dilute orthophosphoric acid solution.

Optimized chromatographic conditions:

Flow rate	:	1ml/min
Column	:	C_{18} 100x4.6 mm, 5µ, X Bridge.
Detector wave length	:	210nm
Column temperature	:	30°C
Injection volume	:	10µL
Run time	:	10 min
Diluent	:	Methanol (65:35)

METHOD VALIDATION

System suitability test: This parameter was evaluated before each stage of validation. Six replication injections of standard preparation were injected. Asymmetry, number of theoretical plates and relative standard deviation of peak area were determined.

Linearity: Solutions were prepared containing 100µg/ml, 200µg/ml, 300µg/ml, 400µg/ml, 500µg/ml, 600µg/ml concentrations of Telmisartan and 3.75µg/ml, 7.5µg/ml, 11.25µg/ml, 15µg/ml, 18.75µg/ml, 22.5µg/ml concentrations of Indapamide which corresponding to 25,50, 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 (buffer: methanol by 5%), temperature (±5°c).

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 IND and TEL from impurities.

RESULTS AND DISCUSSIONS

Several mobile phase compositions were tried to resolve the peak of TEL and IND. The mobile phase containing buffer: Acetonitrile in proportion of 65:35v/v was found ideal to resolve the peak of TEL and IND satisfactory. Retention time of TEL and IND were 5.268 and 2.366 min respectively (Figure 1&2). Result of assay is shown in Table-1. The proposed method was found to be linear in concentration range 100-600ppm for TEL and 3.75-22.5ppm for IND. 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 TEL and IND 100-101% was found to be between 97-101% and 99.12-100.95% respectively, which are well within the limit and hence the method was found to be accurate (Table-4). LOD and LOQ values were 2.23µg/ml and 6.67µg/ml for Telmisartan and 0.32µg/ml and 0.98µg/ml for Indapamide (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, mobile phase composition and temperature. 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 TEL and IND using isocratic mode of elution. The results of linearity, precision, accuracy and specificity, proved to be within the limits. The method provides selective quantification of TEL and IND without any interference. The proposed method is highly sensitive, reproducible, reliable, rapid and specific.

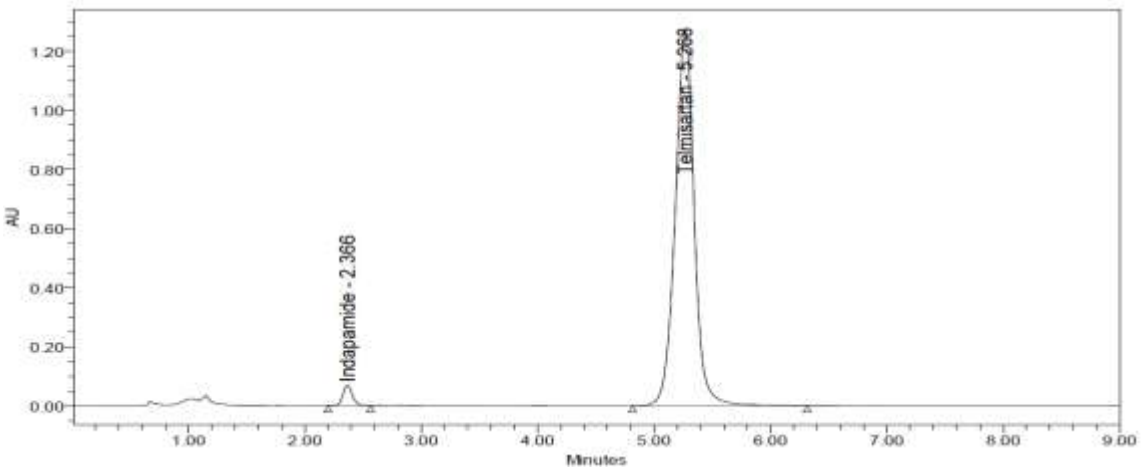

Figure-1: Chromatogram of TEL (40µg/ml) and IND (1.5µg/ml) standard

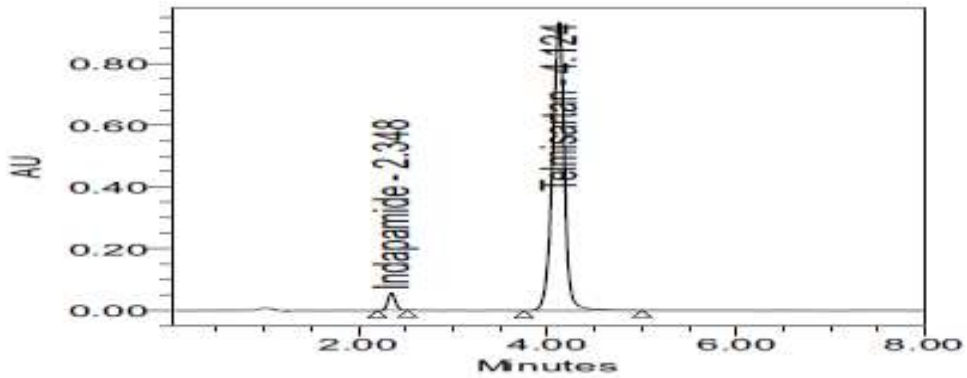

Figure-2: Chromatogram of TEL (40µg/ml) and IND (1.5µg/ml) sample

Table -1 Analysis data of tablet formulation (INDITEL-D)

TABLET	Label claim(mg)	Assay ± SD (% label claim)	%RSD
IND	1.5	99±0.88	0.88
TEL	40	100±0.43	0.43

RSD – relative standard deviation; SD – standard deviation

Table – 2: Result of Linearity

S. no	Telmisartan		Indapamide	
	Conc. (µg/ml)	Peak area	Conc. (µg/ml)	Peak area
1	100	1388724	3.5	72724
2	200	3000304	7.5	153967
3	300	4440446	11.25	224973
4	400	6083860	15	302485
5	500	7689934	18.75	369492
6	600	9260399	22.5	442839

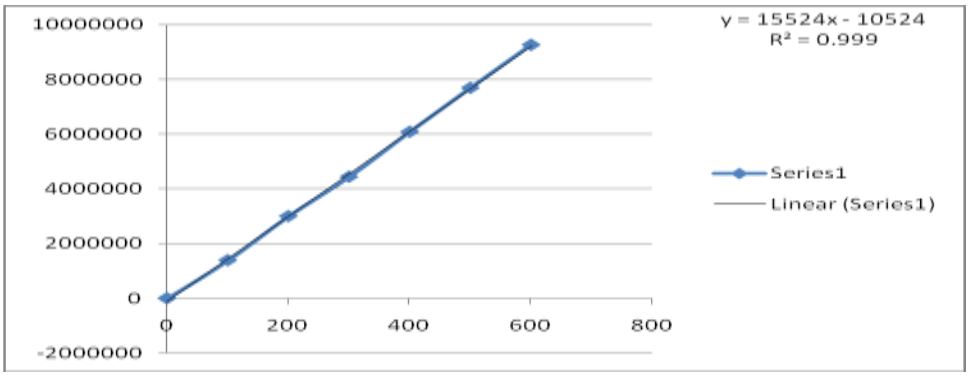

Figure-3: Calibration curve for Telmisartan

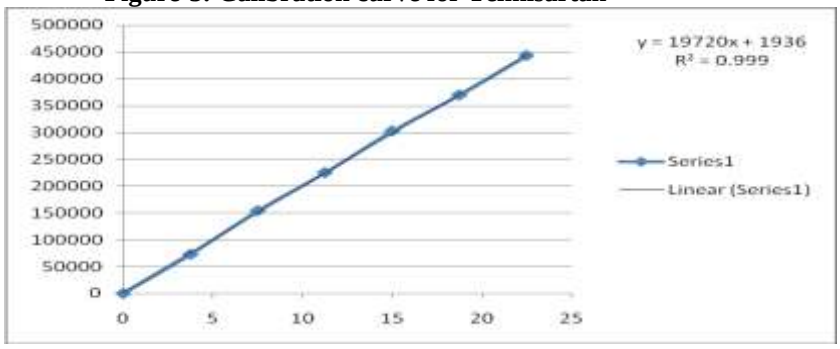

Figure -4: Calibration curve for Indapamide

Table-3: System suitability studies

Parameters	Telmisartan	Indapamide	Acceptance criteria
Theoretical plates	5375	4116	More than 2000
Tailing factor	0.99	1.14	Less than 2
Retention time	5.628	2.366	More than 2

Table-4: Recovery studies for Telmisartan and Indapamide

DRUG	Spiked level%	Amount taken (µg/ml)	Amount found (µg/ml)	Percent recovery n=3
IND	50	10.102	10.15	100
	100	19.80	19.22	97
	150	30.306	30.48	101
TEL	50	1.531	1.53	100
	100	3.00	2.97	101
	150	4.592	4.69	101

n- Number of replicate injections

Table-5: LOD and LOQ for Telmisartan and Indapamide

DRUG	LOD (µg/ml)	LOQ (µg/ml)
Telmisartan	2.03	6.7
Indapamide	0.19	0.9

Table-6a: Results of Precision

DRUG	Conc. (µg/ml)	Peak area (n=6)	% RSD
IND	3	305998	0.7
TEL	20	7842117	0.2

Table-7: Results of Robustness study

S. no	Parameter	Condition	Mean Peak area (n=2)		% change	
			IND	TEL	IND	TEL
1.	Flow rate	1.2 ml/min	256839	7024862	0.5	0.3
		0.8 ml/min	316641	8653852	0.3	0.2
2.	Temperature	35ºc	287392	7917494	0.1	0.2
		25ºc	289593	7957633	0.5	0.4

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