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Method development and validation for simultaneous estimation of telmisartan and hydrochlorothiazide in bulk and pharmaceutical dosage forms by RP-HPLC

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ABSTRACT

An economic, uncomplicated, selective, detailed, and accurate RP-HPLC procedure for simultaneous quantitative determination of telmisartan and hydrochlorothiazide in combined dosage forms was formulated and validated according to ICH guidelines. The method was developed using Agilent 1200 series HPLC and INERTSIL column C18(150x4.6 ID) 5µm in isocratic mode, with mobile phase comprising of sodium dihydrogen phosphate buffer: acetonitrile (30:70) the flow rate was 1.0 ml/min and the detection was carried at a wavelength of 293nm. The retention time and percentage assay of purity for telmisartan and hydrochlorothiazide was found to be 2.853 min and 4.090 min, 100.43% and 99.40% respectively. The method was successfully validated for accuracy, precision, ruggedness, linearity and range, specificity and robustness in accordance with ICH guidelines. The proposed method was found to be within the acceptance limits indicating that the method is accurate, specific and economical.

Keywords: Telmisartan, Hydrochlorothiazide, Precision, Accuracy, Linearity, HPLC.

INTRODUCTION

Telmisartan is an angiotensin II receptor antagonist used in the management of hypertension [1]. Telmisartan is indicated in the treatment of essential hypertension [2]. Telmisartan is an angiotensin II receptor blocker that shows high affinity for the angiotensin II receptor type 1, with a binding affinity 3000 times greater for AT1 than AT2. It acts as a selective modulator of peroxisome proliferator-activated receptor gamma, a central regulator of insulin and glucose metabolism. 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 [3, 4, 5, 6].

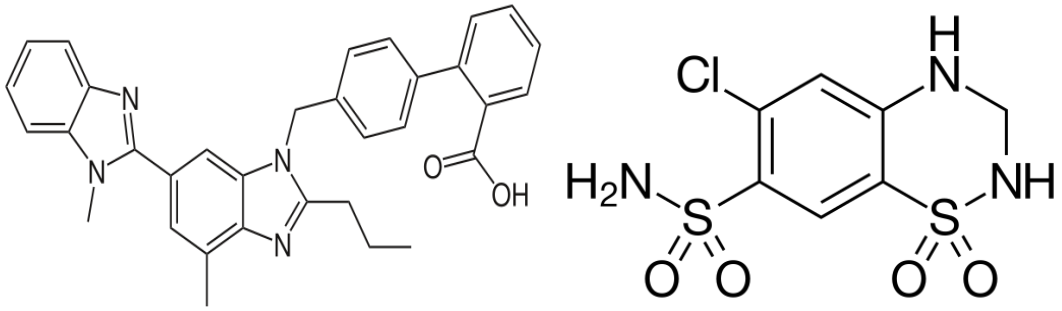

Figure 1: Structure of telmisartan and hydrochlorothiazide

Hydrochlorothiazide (HCTZ or HCT) is a diuretic drug used to treat hypertension and swelling due to fluid buildup. It is used for the treatment of hypertension, congestive heart failure, symptomatic edema, diabetes insipidus, renal tubular acidosis [7]. Hydrochlorothiazide belongs to thiazide class of diuretics. It reduces blood volume by acting on the kidneys to reduce sodium (Na+) reabsorption in the distal convoluted tubule. The major site of action in the nephron appears on an electro neutral NaCl co-transporter by competing for the chloride site on the transporter. By impairing Na+ transport in the distal convoluted tubule, hydrochlorothiazide induces a natriuresis and concomitant water loss. Thiazides increase the reabsorption of calcium in this segment in a manner unrelated to sodium transport [8, 9].

MATERIALS AND METHOD

Telmisartan and hydrochlorothiazide gift samples obtained from pharma industry were used for the study. All the solvents and reagents used were of HPLC grade.

Equipment

Agilent 1200 series HPLC system was provided. The chromatographic analysis was performed using INERTSIL C18 column (150 × 4.6mm; 5µm) as a stationary phase.

Chromatographic Conditions

Mobile phase was pumped at a flow rate of 1 mL/min using a binary mixture of sodium di hydrogen phosphate buffer: acetonitrile (30:70 v/v) in isocratic mode. The injection volume of 20 µL was given and the detection wavelength for telmisartan and hydrochlorothiazide was set at 293 nm and the separation was achieved at room temperature.

Mixed Standard Solution Preparation

10mg of hydrochlorothiazide and 12.5mg of telmisartan was weighed accurately in 10 ml of volumetric flask and dissolved in 10ml of mobile phase and volume made up with mobile phase. From the above stock solution 100 µg/ml of hydrochlorothiazide and 125µg/ml of telmisartan is prepared by diluting 1ml of hydrochlorothiazide and 1.25ml of TELM to 10ml with mobile phase. The chromatogram was recorded using the solution.

Preparation of sample solution

20 tablets (each tablet has 10mg of hydrochlorothiazide and 12.5mg of telmisartan) were weighed and crushed to fine powder taking into a mortar and and uniformly mixed. Tablet stock solutions of hydrochlorothiazide (100µg/ml) and telmisartan (125µg/ml) were prepared by dissolving weight equal to 10mg of hydrochlorothiazide and 12.5mg of telmisartan and were dissolved in mobile phase sufficiently. After that the solution was filtered using 0.45-micron syringe filter and sonication was done for 5 min and diluted to 100ml with mobile phase. Further dilutions were prepared in 5 replicates of 100 µg/ml of hydrochlorothiazide and 125µg/ml of telmisartan was made by adding up 1ml and 1.25ml of stock solution to 10 ml of mobile phase.

RESULTS AND DISCUSSION

Table 1: Optimized chromatographic conditions

Mobile phase	sodium di hydrogen phosphate buffer: Acetonitrile (30:70)
Column	INERTSIL column,C18(150x4.6 ID) 5µm
Flow rate	1.0 mL/min
Column temperature	Room temperature(20-25^{o}C)
Sample temperature	Room temperature(20-25^{o}C)
Wavelength	293nm
Injection volume	20 µL
Run time	6min
Retention time	2.853min for Hydrochlorothiazide and 4.090min for Telmisartan

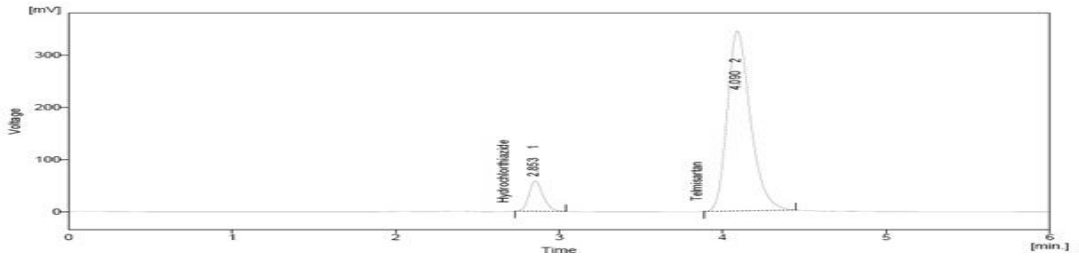

Figure 2: Typical Chromatogram of Hydrochlorothiazide and Telmisartan

Assay

Table 2: Assay Results

Hydrochlorothiazide			**Telmisartan**	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	337.476	347.226	3490.215	3406.047
Injection-2	335.597	348.496	3403.940	3483.819
Injection-3	351.238	352.868	3464.683	3403.609
Injection-4	352.701	364.739	3436.878	3499.205
Injection-5	366.228	351.533	3406.121	3426.737
Average Area	348.648	352.972	3440.367	3443.883
Tablet weight (mg)	125		125	
Standard weight	25		80	
Sample weight	250		250	
Label amount	12.5		40	
std. purity	99.2		99.3	
Amount, mg	**12.55**		**39.76**	
Assay(%purity)	**100.43%**		**99.40%**	

Validation of the HPLC Method: The proposed method was validated as per ICH guidelines [10].

Linearity and range

Linearity of detector response of assay method was found by injecting standard solutions with concentration ranging from 50 % to 150 % of the test concentration Peak area is measured and each level injected into the chromatographic system and the. A graph plotted of peak area versus concentration the correlation coefficient calculated. The results were shown in Table 3, 4 and Figures 2, 3.

Table 3: Linearity of Hydrochlorothiazide

S.No.	Conc.(µg/mL)	Area
1	15	212.082
2	20	274.081
3	25	351.359
4	30	423.469
5	35	492.695

Table 4: Linearity of Telmisartan

S.No.	Conc.(µg/mL)	Area
1	48	2124.641
2	64	2734.196
3	80	3614.224
4	96	4205.076
5	112	4959.097

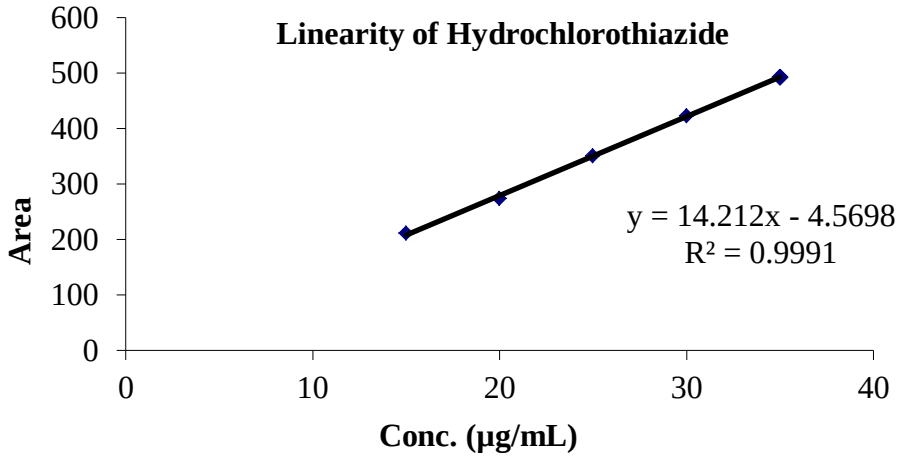

Figure 3: Linearity graph of Hydrochlorothiazide

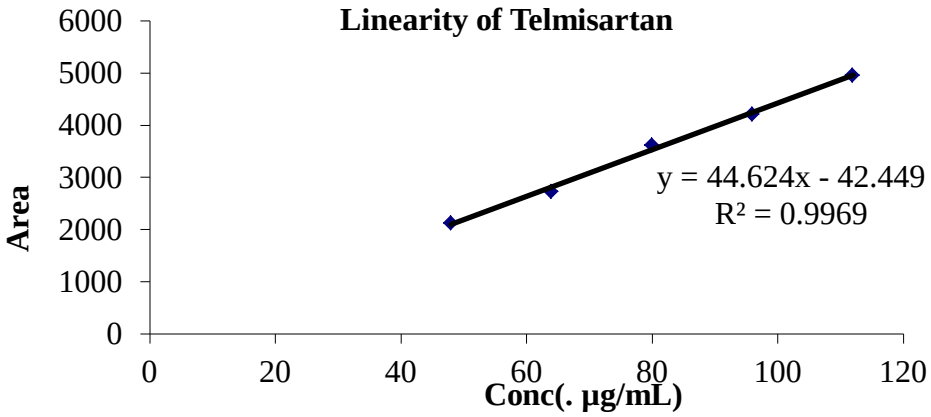

Figure 4: Linearity graph of Telmisartan

Accuracy

Recovery studies and accuracy of the method was determined. To the formulation which is a pre analyzed sample, at the level of 50%, 100%, 150% the reference standards of the drugs were added and the recovery studies were carried out three times. The results were shown in Table 5, 6.

Table 5: Recovery results for Hydrochlorothiazide

Accuracy Hydrochlorothiazide					**Average % Recovery**
Amount taken(mcg/ml)	**Area**	**Average area**	**Amount recovered(mcg/ml)**	**%Recovery**	
25	349.739	344.385	25.13	100.52	
25	339.096				
25	344.321				
30	422.535	424.709	30.22	100.73	**99.99%**
30	426.041				
30	425.551				
35	490.940	487.747	34.55	98.72	
35	488.872				
35	483.430				

Table 6: Recovery results for Telmisartan

Accuracy Telmisartan					**Average % Recovery**
Amount taken(mcg/ml)	**Area**	**Average area**	**Amount recovered(mcg/ml)**	**%Recovery**	
80	3418.869	3473.492	81.30	101.63	
80	3451.170				
80	3550.430				
96	4308.821	4276.872	94.67	98.61	
96	4281.189				
96	4240.607				**99.85%**
112	4858.126	4872.034	111.23	99.31	
112	4822.206				
112	4935.770				

Precision

Sample preparations of hydrochlorothiazide and telmisartan were prepared as per the method and injected 6 times into the column. And the relative standard deviation of assay results was calculated. The results were shown in Table 7.

Table 7: Results for Method precision of Hydrochlorothiazide and Telmisartan

Hydrochlorothiazide			**Telmisartan**		
S.No.	**Rt**	**Area**	**S.No.**	**Rt**	**Area**
1	2.983	358.060	1	4.457	3515.269
2	2.967	360.049	2	4.443	3513.118

3	2.943	354.429	3	4.423	3484.753
4	2.947	355.992	4	4.413	3501.980
5	2.953	358.513	5	4.387	3490.006
6	2.947	349.258	6	4.307	3483.064
avg	2.9567	356.050	**avg**	4.405	3498.032
stdev	0.0154	3.869	**stdev**	0.054	14.178
%RSD	**0.52**	**1.09**	**%RSD**	**1.22**	**0.41**

Intermediate Precision/Ruggedness

Precision was performed on a different day by using different columns of same dimensions and method evaluated. The area was measured in HPLC for the five times injected standard solution. The results were shown in Table 8.

Table 8: Results for Ruggedness

Hydrochlorothiazide	%Assay	Telmisartan	%Assay
Analyst 01	97.99	Analyst 01	99.96
Analyst 02	98.37	Analyst 02	97.59
%RSD	0.27	%RSD	1.69

Robustness

Chromatographic conditions variation

The prepared solution is injected at different variable conditions like Temperature and wavelength as per test method. System suitability parameters were compared with that of method precision. The results were shown in Table 9

Table 9: Result of Robustness study

Parameter	Hydrochlorothiazide		Telmisartan	
	Retention time(min)	Tailing factor	Retention time(min)	Tailing factor
Flow				
0.8ml/min	4.263	1.162	6.067	1.519
1.2ml/min	2.157	1.389	3.100	1.414
Wavelength				
290nm	2.897	1.333	4.133	1.472
293nm	2.900	1.292	4.137	1.444

Limit of detection (LOD)

The LOD for this method was found to be 2.53µg/ml (Telmisartan) and 7.95µg/ml (Hydrochlorothiazide).

$$\text{LOD} = \frac{3.3\sigma}{S}$$

= (3.3)*(11.302)/44.62
= 2.53µg/ml (TELMISARTAN)
=(3.3)* (11.306)/14.21
=7.95µg/ml (Hydrochlorothiazide)

Where, σ = the standard deviation of the response
S = the slope of the calibration curve
The slope S may be estimated from the calibration curve of the analyte.

Limit of quantification (LOQ)

The LOQ for this method was found to be 0.83µg/ml (Telmisartan) and 2.60µg/ml (Hydrochlorothiazide).

$$\text{LOQ} = \frac{10\sigma}{S}$$

= (10)*(11.302)/44.62
= 0.83µg/ml (TELMISARTAN)
=(10)* (11.306)/14.21
=2.60µg/ml (Hydrochlorothiazide)
Where
σ = the standard deviation of the response
S = the slope of the calibration curve
The slope S may be estimated from the calibration curve of the analyte.
The LOQ for this method was found to be 0.83µg/ml (Telmisartan) and 2.60µg/ml (Hydrochlorothiazide**).**

CONCLUSION

From the above it can be concluded that all validation parameters such as precision, accuracy, linearity and Ruggedness met the predetermined acceptance criteria as mentioned in ICH guidelines. The developed RP-HPLC method is simple, rapid, accurate, and precise and can be applied for routine analysis of telmisartan and hydrochlorothiazide in bulk and its dosage forms.

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