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Rp-Hplc Method Development And Validation Determination Of Simultaneous Estimation Of Olmesartan And Hydrochlorothiazide In A Combined Tablet Dosage Form

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Abstract: *A simple , precise and accurate RP-HPLC method was developed and validated for simultaneous estimation of Olmesartan and hydrochlorothiazide in tablet dosage form. Separation was achieved on a reversed-phase Symmetry C18 (4.6 x 150mm, 5µm, Make: XTerra) or equivalent using a mobile phase consisting of methanol / acetonitrile (pH 2.5, 65:35, v/v) at a flow rate of 0.8 ml per min and UV detection at 258 nm . The method was validated as per ICH guidelines for linearity , accuracy, precision and robustness. The developed method shows good linearity over the concentration range of 20-80 µg/mL (r^2=0.999) for both olmesartan and hydrochlorothiazide . The average percentage recoveries were in the range of 100.0-100.04% and 100.0-100.06% for olmesartan and hydrochlorothiazide, respectively . The limits of detection (LODs) were 0.04 µg/mL and 0.13 µg/mL for olmesartan and hydrochlorothiazide, and limits of quantification (LOQs) were 0.01 µg/mL and 0.05 µg/mL, respectively. Therefore, the proposed method can be applied for routine analysis of the bulk drugs as well as combined pharmaceutical dosage forms of olmesartan and hydrochlorothiazide.*

Keywords: *Olmesartan , Hydrochlorothiazide , Limits of quantification , limits of detection .*

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I. Introduction

Olmesartan is an antihypertensive agent, which belongs to the class of medications called angiotensin II receptor blockers . It is indicated for the treatment of high blood pressure and is marketed under the name Olmetec. Chemical formula is $C_{29}H_{30}N_6O_6$ and its Molecular mass 558.585 g/mol. Solubility is practically insoluble in water and sparingly soluble in methanol belongs to category Angiotensin II Type 1 Receptor Blockers Olmesartan is an ARB that selectively inhibits the binding of angiotensin II to AT1, which is found in many tissues such as vascular smooth muscle and the adrenal glands. Olmesartan is selective for AT1 and has a 12,500 times greater affinity for AT1 than the AT2 receptor. It reduces the reabsorption of electrolytes from the renal tubules. This results in increased excretion of water and electrolytes, including sodium, potassium, chloride, and magnesium. It has been used in the treatment of several disorders including edema, hypertension, diabetes insipidus, and hypoparathyroidism. IUPAC Name: 6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide Chemical formula: $C_7H_8ClN_3O_4S_2$ Solubility Slightly or very slightly soluble in water; sparingly soluble in alcohol; soluble in acetone; freely soluble in dimethylformamide; n-butylamine; and solutions of alkali hydroxides; insoluble in ether, chloroform, and dilute mineral acids.Category Antihypertensive Agents, Diuretics, Sodium Chloride Symporter Inhibitors Mechanism of action Hydrochlorothiazide, a thiazide diuretic, inhibits water reabsorption in the nephron by inhibiting the sodium-chloride symporter (SLC12A3) in the distal convoluted tubule, which is responsible for 5% of total sodium reabsorption. Normally, the sodium-chloride symporter transports sodium and chloride from the lumen into the epithelial cell lining the distal convoluted tubule. The energy for this is provided by a sodium gradient established by sodium-potassium ATPases on the basolateral membrane. Once sodium has entered the cell, it is transported out into the basolateral interstitium via the sodium-potassium ATPase, causing an increase in the osmolarity of the interstitium, thereby establishing an osmotic gradient for water reabsorption. By blocking the sodium-chloride symporter, hydrochlorothiazide effectively reduces the osmotic gradient and water reabsorption throughout the nephron.

II. Materials and Methods

Preparation of Phosphate buffer: Weighed 7.0 grams of KH_2PO4 into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. Adjusted the pH to 2.5with Orthophosporic acid.

Preparation of mobile phase.

Mix a mixture of above buffer 400 ml (40%) and 600 ml of Acetonitrile HPLC (60%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Diluent Preparation: Use the Mobile phase as diluent.

Preparation of the Hydrochlorothiazide & Olmesartan Standard & Sample Solution:

Standard and Sample Solution Preparation: Accurately weigh and transfer 10 mg of Hydrochlorothiazide and 10mg of Olmesartan working standard into a 10ml clean dry volumetric flask add about 7ml of Diluent and sonic ate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).Further pipette 0.4ml of Hydrochlorothiazide & Olmesartan the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.Inject 20 micro L of the standard, sample into the chromatographic system and measure the areas for the Hydrochlorothiazide and Olmesartan peaks .

VALIDATION SUMMARY:

PRECISION and ACCURACY :

Preparation of stock solution:

Accurately weigh and transfer 10mg of Hydrochlorothiazide and Olmesartan working standard into a 10ml clean dry volumetric flask add about 7ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.4ml of Hydrochlorothiazide & Olmesartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of Sample solutions:

For preparation of 50% ,100 % and 150 % solutions (With respect to target Assay concentration):

Accurately weigh and transfer 5.3mg,9.9mg,15.0mg of Hydrochlorothiazide and Olmesartan working standard into a 10m clean dry volumetric flask add about 7ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock Solution).

Further pipette 0.4ml of Hydrochlorothiazide & Olmesartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions.

LINEARITY:

Preparation of stock solution:

Accurately weigh and transfer 10mg of Hydrochlorothiazide and Olmesartan working standard into a 10ml clean dry volumetric flask add about 7ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Preparation of Level – I,II,III,IV,V and VI :

0.2ml,0.3ml,0.4ml,0.5ml and 0.6 ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluents for the preparation of 20 ppm to 60 ppm(Hydrochlorothiazide & Olmesartan)

Procedure:

Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

LIMIT OF DETECTION and LIMIT OF QUANTIFICATION:

(for Hydrochlorothiazide and Olmesartan)

Preparation of 40µg/ml solution:

Accurately weigh and transfer 10mg of Hydrochlorothiazide working standard into a 10ml clean dry volumetric flask add about 7ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.4ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 1.0% and 0.35%solution at Specification level (0.04µg/ml and 0.14µg/ml solution):

ROBUSTNESS:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition Temperature Variation was made to evaluate the impact on the method.

a). The flow rate was varied at 0.7 ml/min to 0.9ml/min. Standard solution 40ppm of Hydrochlorothiazide & Olmesartan was prepared and analysed using the varied flow rates along with method flow rate.

b). The Organic composition in the Mobile phase was varied from 55% to 65% Standard solution 40 µg/ml of Hydrochlorothiazide & Olmesartan was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

III. Results And Discussion

Table: 1 The results of Precision are summarized

Sl.No	Injections	Area of Hydrochlorothiazide	Area of Olmesartan
1	Injection 1	11999723	1572878
2	Injection 2	1208865	1576676
3	Injection 3	1207896	1578267
4	Injection 4	1207573	1580584
5	Injection 5	1211377	1579792
6	Injection 6	1207087	1577640
	Std.Deviation	4378.4	3053.3
	%RSD	0.36(less than one)	0.19(less than one)

Table: 2 The accuracy results for Hydrochlorothiazide and Olmesartan

%Concentration (at specification Level)	Area of HCT	Area of OLM	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	665659	862672	5.3	5.35	100.9%	100.3%
100%	1222077	1594725	9.9	9.89	99.9%	
150%	1851398	2424504	15.0	15.03	100.2%	

Table: 3 Linearity Results: (for Hydrochlorothiazide and Olmesartan)

S.No	Linearity Level	Concentration	Area of HCT	Area of OLM
1	I	20ppm	674644	868569
2	II	30ppm	953517	1240821
3	III	40ppm	1216843	1584141
4	IV	50ppm	1561020	2039735
5	V	60ppm	1841281	2408104
Correlation Coefficient			0.999	

Table: 4 System suitability results for Hydrochlorothiazide and Olmesrtan:

S.No	FlowRate (ml/min)		System Suitability Results	
		USP Plate Count	USP Plate Count	SP Tailing
1	0.7	2195.0	2574.4	1.3
2	0.8	2181.7	2589.8	1.4
3	0.9	2083.5	2382.7	1.4

On evaluation of the above results, it can be concluded that the variation in 10% Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is not robust even by change in the Mobile phase ±1

Table: 5 System suitability results for Hydrochlorothiazide

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results		
		USP Plate Count	USP Plate Count	USP Tailing
1	10% less	2103.4	2461.3	1.6
2	*Actual	2181.7	2589.8	1.4
3	10% more	2016.6	2435.6	1.3

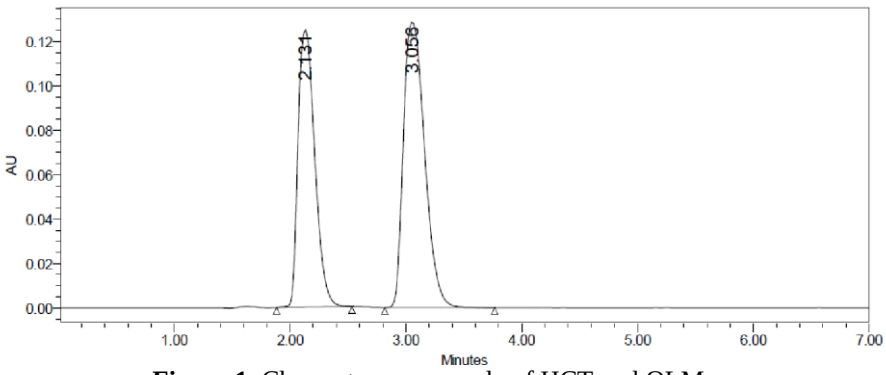

Figure.1. Chromatogram sample of HCT and OLM

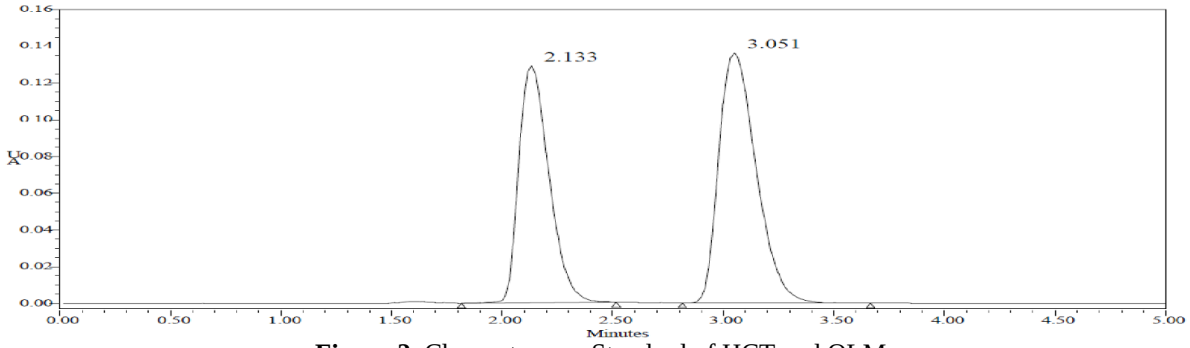

Figure.2. Chromatogram Standard of HCT and OLM

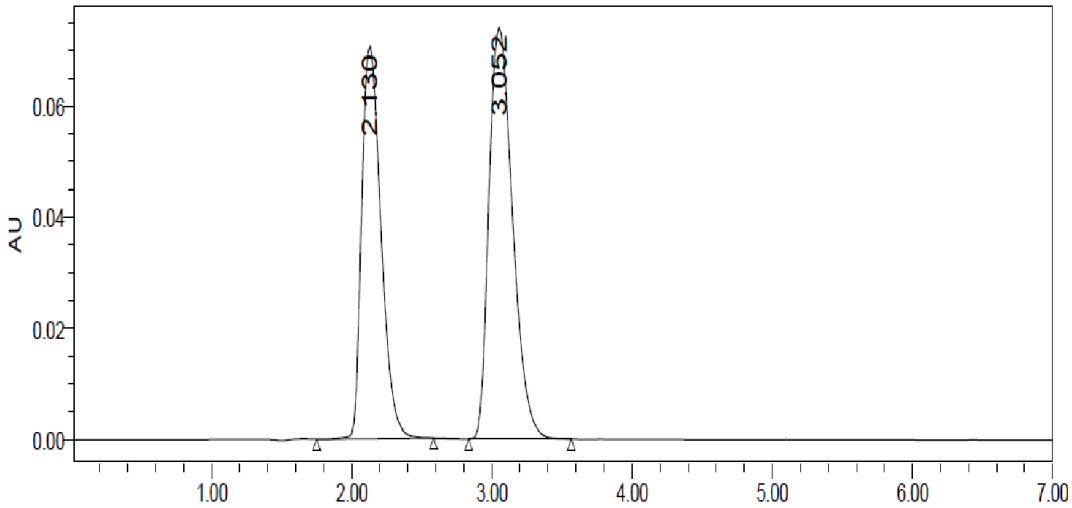

Figure3. Chromatogram Linearity of HCT and OLM

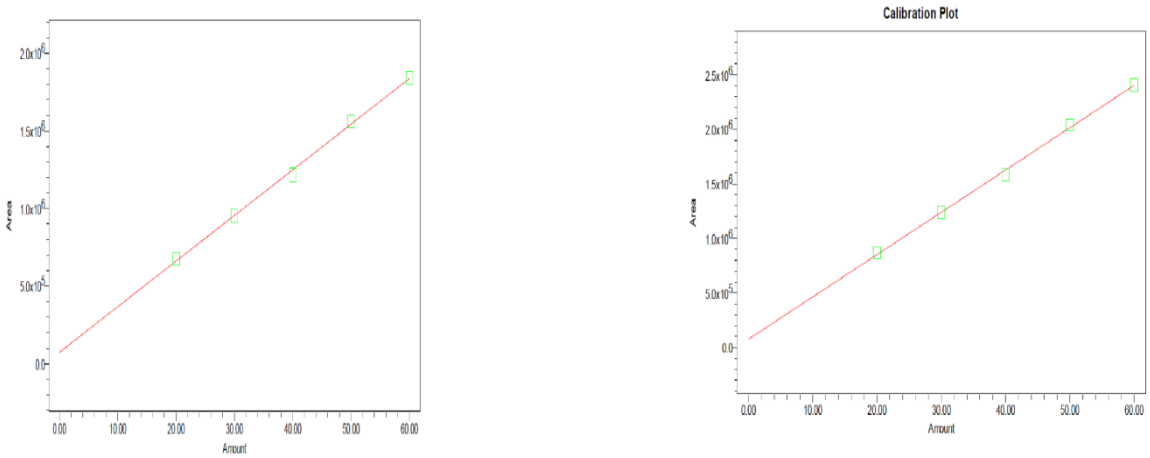

Figure.4. Calibration plot of HCT and OLM

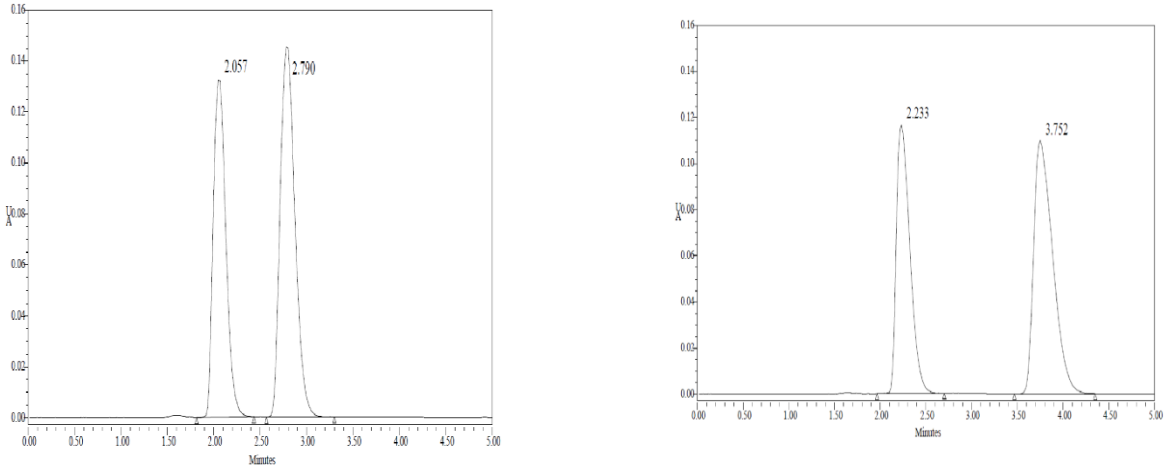

Figure.5. Chromatogram Robustness of less flow and more flow sample of HCT and OLM

IV. Results And Discussion

Hydrochlorothiazide is a thiazide diuretic Angiotensin 2 receptor antagonist, and their combination is used to treat high blood pressure. From the results shown in system suitability the % RSD for retention times,peak areas and number of theoretical plates and tailing factor were found to be within limits i.e.,%RSD for retention times not more than 2.0%,peak areas not more than 2.0% and number of theoretical plates not less than 2000 and tailing factor for not more than 2.0, so the had method passed system suitability.From the results shown in precision tables it was found that % RSD is not more than 2%; which indicates that the proposed method has good reproducibility.

In case of accuracy 50%,100%,150% of solutions with respect to target assay concentrations the percentage recovery for each levels are between 98.0 %to 102%. It indicates the method was accurate and also reveals that the commonly used excipients and additives present in the pharmaceutical formulations were not interfering the proposed method. From the results shown in Linearity table. It was found that the method was linear and the correlation coefficient is not less than the 0.9999. In case of the LOD and LOQ the S/N ratios are with in the limits for HCTZ.

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