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"Method Development And Validation Of Simultaneous Estimation Of Olmesartan & Hydrochlorothiazide In Bulk & Formulation By RP- HPLC Method"

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A simple fast precise reverse phase isocratic high performance liquid chromatographic (HPLC) method has been developed for simultaneous estimation of Olmesartan & Hydrochlorothiazide in bulk and formulation by RP-HPLC method. The chemical name of Olmesartan is 5-(2-hydroxypropan-2-yl)-2-propyl-3-[[4-[2-(2H-tetrazol-5- yl)phenyl]phenyl]methyl]imidazole-4-carboxylic acid. The method was validated for several parameters such as accuracy precision robustness as per ICH guidelines. The RP-HPLC method for the estimation of Olmesartan and Hydrochlorthiazide in bulk and pharmaceutical dosage formwas developed. The quantification was carried out by using ZobraxSB-Aq column (250 mm × 4.6 mm, 5□m) as stationary

phase,0.1% Orthophosphoricacid:Acetonitrile [60:40] as mobile phase. Mobile phase was maintained at a flow rate of 1.0ml/min at 256nm. The drugsOlmesartan and Hydrochlorthiazide eluted at 4.93 and 3.63 minutes. Validation data demonstrates that, this method is accurate, precise, simple, economic and can be used in the routine analysis of Olmesartan and Hydrochlorthiazide in various formulations. Different mobile phase combination of acetonitrile and 0.1% orthophosphoric acid were tried for better resolution of the chromatogram.

Key words- Olmesartan, Hydrochlorthiazide, HPLC, Validation, ICH, Linearity.

INTRODUCTION

Quality is an important attribute for any pharmaceutical or chemical industry to make the safest, accurate and effective medication that reaches to patients. Any industry accredited under Food and Drug Administration carry's daily activity as per guidelines given by regulatory authority. The main objective of a pharmaceutical industry to produce and sell drugs that at safe, effective and have good quality properties. A quality product ensures that the patient attains his/her health as per the desired effects. For a Pharmaceutical industry to carry out regular quality check, there need to be a system that keeps a track of its internal quality control. Within this quality control there are specified Critical Quality Attributes that needs to be monitored. One of the attributes is Assay and Impurities of Active Pharmaceutical Ingredients (API). This is the most important attribute as this decides the safety, efficacy and purity of a drug. The Assay and related compound of most of the APIs are done using Chromatography technique. Chromatography technique included but are not limited to Liquids Chromatography, Gas Chromatography and paper chromatography. Liquid chromatography includes High Pressure Liquid Chromatography (HPLC) and Thin Layer Chromatography. HPLC techniques is the most widely used technique in industry for separation and isolation of analyte. The principles for developing chromatography methods for the analysis of assay and organic impurities/related substances/degradation products of pharmaceuticals (any drug product/drug substance/intermediate/raw material of pharmaceuticals) using ultra/high-performance liquid chromatography (UPLC/HPLC) techniques. [1] The findings may be used to statistically and qualitatively analyse final medicinal products and their components during the production process. This is accomplished by separating, quantifying, and identifying components in a mixture, and it may be used to expose a drug's identity and track the progress of a therapy on a condition. [2] One of the most important advantages of HPLC is its capacity to detect the structure and quantity of contaminants in pharmaceutical formulations. [3]

Mass spectrometry (HPLC/MS) is another technique that HPLC may be coupled with; the chromatograph is connected to a mass spectrometer through an interface. This type of study can look at a variety of components, including those that are thermally labile, polar, or have a large molecular mass.On the specialised interface, the components eluted from the column are introduced to the mass spectrometer. Electrospray ionisation and atmospheric pressure chemical ionisation interfaces are the two most frequent HPLC/MS interfaces. [4]

MATERIALS AND METHODS

Materials:

Olmesartan & Hydrochlorthiazide is a gift sample from Aadhaar Life Sciences Pvt. Ltd., Solapur, India. Acetonitrile LiChrosolv®, Water LiChrosolv® were procured from Merck Specialities Pvt. Ltd., Mumbai

Methods

Preliminary Analysis of Drug:

A. Olmesartan

a) Description: The sample ofOlmesartan was observed for its color and texture.

b) Solubility: The sample ofOlmesartan was taken in test tubes and observed for solubility in water, acetonitrile, and methanol.

c) Melting Point: The sample ofOlmesartan was taken in capillary tube and kept in melting point apparatus.

B. Hydrochlorthiazide

a) Description: The sample ofHydrochlorthiazidewas observed for its color and texture.

b) Solubility: The sample ofHydrochlorthiazide was taken in test tubes and observed for solubility in water, acetonitrile, and methanol.

c) Melting Point: The sample ofHydrochlorthiazide was taken in capillary tube and kept in melting point apparatus.

High Performance Liquid Chromatographic Method

I. Preparation of Standard Solution

a. Olmesartan Standard Stock Solution-I (OSSS-I):

Initially Prepare a Standard Stock Solution (SSS-I) of by adding 10 mg of Olmesartan in 10 ml volumetric flask & add 5 ml diluent, mix for 2 minutes and make the volume to 10 ml with diluent. (Conc. of Olmesartan = 1000 µg/ml).

b. Hydrochlorthiazide Standard Stock Solution-I (HSSS-I):

i. Then prepare a Standard Stock Solution (SSS-II) of Hydrochlorthiazide by adding 6.25 mg in 10 ml volumetric flask & add 5 ml diluent, mix for 2 minutes and make the volume to 10 ml with diluent.(Conc. of Hydrochlorthiazide = 625 µg/ml). Then add 2.0 ml of OSSS-I & 2.0 ml HSSS-II in 10 ml volumetric flask and add 5 ml diluent and vortex and make up the volume with diluent.(Conc. of Olmesartan=200 µg/ml &Hydrochlorthiazide =125 µg/ml).

II. Selection of Analytical Wavelength

To investigate the appropriate wavelength for determination of Olmesartan and Hydrochlorthiazide solution in the Water: ACN (50:50) were scanned in UV- Visible Spectrophotometer in the range of 190-400nm.

III. Selection of Mobile Phase and its Strength

The solution of Olmesartan(200 µg/ml) and Hydrochlorthiazide (125 µg/ml) was prepared in HPLC grade Water and ACN (50-50) and filtered through Millipore syringe filter, then injected into HPLC system. The chromatogram was analyzed using different combinations of

0.1% Orthophosphoric Acid: Acetonitrile [50:50, 40:60, 30:70, 70:30, 60:40] at a flow rate of 1ml/min for 10-20min at 256nm.

IV. Selection of column (stationary phase)

To get well resolved, symmetric peak with highest number of theoretical plates the solution of the Olmesartan and Hydrochlorthiazide; were analyzed using Polar C18 column as a stationary phase.

V. Chromatographic Conditions

- ✓ **Analytical Column:** : ZorbaxSB-Aq (250 x 4.6 mm, 5µ)
- ✓ **Mobile Phase:**0.1% Orthophosphoric acid: Acetonitrile (60:40)
- ✓ **Flow Rate:** 1 ml/min
- ✓ **Injection Volume:** 10 □l
- ✓ **Detection Wavelength:** 256 nm

Validation of RP-HPLC Method

I. Specificity

The chromatogram of blank and standard were compared to justify the specificity of the target analyte.

II. Linearity

Series of dilutions were made from OSSS-I (1000 µg/ml) of 160, 180, 200, 220 and 240 µg/ml solutions were prepared of Olmesartan. Series of dilutions were made from HSSS-I (625 µg/ml) of 100, 112.5, 137.5 and 150 µg/ml solutions were prepared of Hydrochlorthiazide. The solution were filtered using Millipore syringe filter and 10 µl injected in to the HPLC system and their chromatogram were recorded for 10 min. Under the chromatographic conditions as described after getting a stable baseline. Peak area was recorded for all the peaks. Calibration curve of Olmesartan and Hydrochlorthiazide was constructed by plotting the peak area v/s Conc. of Olmesartan and Hydrochlorthiazide. The correlation coefficient (r^2) of least square linear regression for Olmesartan and Hydrochlorthiazide was calculated.

III. Range

The range of analytical method was decided from the interval between upper and lower level of calibration curves by plotting the curve.

IV. Accuracy

1.6 ml, 2 ml and 2.4 ml from Standard stock solution OSSS-I and HSSS-I each were transferred to three different 10 ml volumetric flasks and volume adjusted up to 10 ml with diluent. All the solutions were filtered through Millipore syringe filter and injected into the HPLC system and chromatograms were recorded under the same chromatographic conditions after getting a stable baseline. Peak area was recorded for all the peaks. From above data percentage recoveries were calculated.

V. Precision

The precision of an analytical method was studied by performing Repeatability.

a) Repeatability:

200µg/ml of Olmesartan and 125 µg/ml of Hydrochlorthiazide solution were filtered through syringe filter and 10µl injected into the HPLC system and its chromatogram was recorded under the same chromatographic conditions after getting a stable baseline. Peak area was recorded. The procedure was repeated for five times and the % RSD was calculated.

b) Inter-day & Intra-day Precision:

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the intra-day studies 200µg/ml of Olmesartan and 125 µg/ml of Hydrochlorthiazide solution concentrations were analyzed on the same day and percentage RSD were calculated. For the inter day variation studies, Same sample were analyzed on 2nd consecutive day and percentage RSD were calculated.

VI. Limit of Detection

LOD calculated by the following formulae.

LOD = 2.98(SD/S)

Where, SD- Standard deviation; S- Slope of Curve.

VII. Limit of Quantitation

LOQ calculated by the following formulae.

LOQ = 9.03 (SD/S)

Where, SD- Standard deviation; S- Slope of Curve.

VIII. System Suitability

Chromatograms were studied for different parameters such as tailing factor, retention time, resolution and theoretical plates to see that whether they comply with the recommended limit or not.

IX. Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which column oven temperature and Mobile Phase composition was altered, and the effect on the area and percent assay were noted.

RESULTS

Preliminary Analysis of Drug
Olmesartan:

Table 1: Observations and Results of Preliminary Analysis of OLM

Sr. No.	Tests	Observations	Results

1.	Description	White to light-yellowish white powder	Complied
2.	Solubility	Practically insoluble in water and Sparingly soluble in methanol and freely soluble in acetonitrile	Complied
3.	Melting Point	180-182˚C	Complied

Hydrochlorthiazide:

Table 2. Observations and Results of Preliminary Analysis of HTZ

Sr. No.	Tests	Observations	Results
1.	Description	White to off-white powder	Complied
2.	Solubility	Soluble in water and Sparingly soluble in ethanol and freely soluble in acetonitrile	Complied
3.	Melting Point	273-275˚C	Complied

RP-HPLC Method for Olmesartan and Hydrochlorthiazide Selection of Wavelength

Fig.1. UV spectra of Olmesartan and Hydrochlorthiazide between 190-400 nm in diluent Olmesartan and Hydrochlorthiazide showed the appropriate intensity at 256 nm. So it was selected for analysis.

Selection of Mobile phase

Table 3. Optimization of Chromatographic Conditions

Mobile Phase Ratio	Olmesartan				Hydrochlorthiazide			
	Rt (min)	Tailing Factor	Theoretical Plates	Resolution	Rt (min)	Tailing Factor	Theoretical Plates	Resolution
50:50	-	-	-	-	3.09	9636	1.16	0.00

40:60	2.74	12144	1.06	2.87	2.45	9595	1.05	0.00
30:70	2.59	11776	1.14	2.79	2.32	8499	1.02	0.00
70:30	14.05	5504	2.46	20.76	4.59	14102	1.06	0.00
60:40	**4.93**	**7542**	**1.62**	**7.39**	**3.63**	**13483**	**1.07**	**0.00**

(Zorbax SB-Aq Column, at 1ml/min flow rate, detection wavelength is 256nm, mobile phase ratio 60:40 containing 0.1% Orthophosphoricacid : ACN respectively)
Different mobile phase combination of acetonitrile and 0.1% orthophosphoric acid were tried for better resolution of the chromatogram. After several combinations of mobile solvents, acetonitrile and 0.1% orthophosphoric acid was selected in ratio 40: 60 respectively using Zorbax SB-Aq column which has given good resolution, capacity factor, and acceptable system suitability. The drugs eluted within 07 mins.which will reduces the overall analysis time and cost.

Identification of Peak

Fig.2 Chromatogram of Blank in optimized chromatographic conditions

Fig. 3 Chromatogram of OLM (200 µg/ml) in optimized chromatographic conditions

Fig.4 Chromatogram of HTZ (125 µg/ml) in optimized chromatographic conditions
Validation of HPLC Method

1. Specificity

Fig.5 Chromatogram of Drug product
With above optimized conditions Olmesartan and Hydrochlorthiazide eluted at 5.36 min and 7.07 min respectively (Fig.5).Peak were sharp and well resolved.

2. Linearity

a. 80% Solution for Linearity of OLM & HTZ

b. 90% Solution for Linearity of OLM & HTZ

c. 100% Solution for Linearity of OLM & HTZ

d. 110% Solution for Linearity of OLM & HTZ

e. 120% Solution for Linearity of OLM & HTZ

Fig.6 Chromatograms of serial dilutions of OLM and HTZ in optimized chromatographic conditions

The peak response is directly proportional to the concentration of drug and was found to be linear in the range of 160-240 and 100-150 µg/ml for Olmesartan and Hydrochlorthiazide respectively (Fig.6.2.6). The correlation coefficient was found to be 0.9987 and 0.9968 for Olmesartan and Hydrochlorthiazide respectively which is well within the acceptance criteria.

Table 4 Response of OLM at various linearity levels

Conc. of OLM (µg/ml)	Peak Area (mAU)
160	5364312
180	5898351
200	6580390
220	7138429
240	7796468

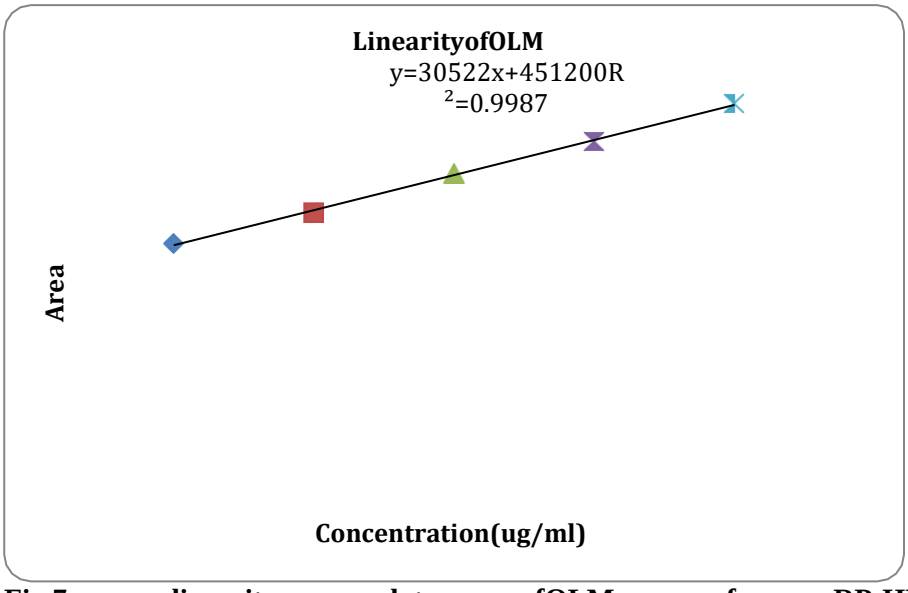

Fig.7. linearity plot ofOLM of RP-HPLC method

Table 5 Response of OLM at various linearity levels

Conc. of HTZ (µg/ml)	Peak Area (mAU)
100	2638370
112.5	2905291
125	3309212
137.5	3594013
150	3917054

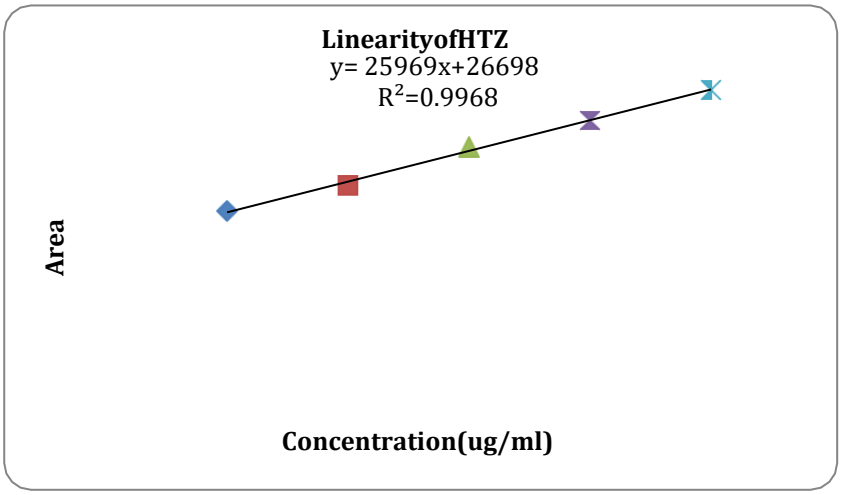

Fig.8 linearity plot ofHTZ of RP-HPLC method

Table 6 Linear Regression Analysis of Calibration Curves for OLM & HTZ

Parameters	OLM	HTZ
Slope	30522	25969
Intercept	451200	26698
Correlation Coefficient (r^2)	0.9987	0.9968

3. Range

Table 7 Range for RP-HPLC Method

Parameters	OLM	HTZ
Linearity Range (µg/ml)	160-240	100-150

4. Accuracy

Table 8 Accuracy of OLM for RP-HPLC Method

% Level	Reps	Spiked Conc (ug/ml)	Area	Amount Recovered (ug/ml)	% Recovery	AVG	STDEV	RSD
80%	Rep 1	159.52	5364312	162.52	101.88	101.84	0.067149	0.07
	Rep 2	159.52	5359312	162.37	101.79			
100%	Rep 1	199.4	6580390	199.37	99.98	99.96	0.032232	0.03
	Rep 2	199.4	6577390	199.28	99.94			
120%	Rep 1	239.28	7796468	236.21	98.72	98.71	0.007377	0.01
	Rep 2	239.28	7795644	236.19	98.71			

Table 9 Accuracy of HTZ for RP-HPLC Method

% Level	Reps	Spiked Conc (ug/ml)	Area	Amount Recovered (ug/ml)	% Recovery	AVG	STDEV	RSD
80%	Rep 1	99.70	2638370	99.32	99.62	99.53	0.122337	0.12
	Rep 2	99.70	2633788	99.15	99.45			
100%	Rep 1	124.63	3309212	124.58	99.96	99.93	0.045218	0.05
	Rep 2	124.63	3307095	124.50	99.90			
120%	Rep 1	149.55	3917054	147.46	98.60	98.96	0.508464	0.51
	Rep 2	149.55	3945620	148.53	99.32			

Percentage recoveries of the results indicate that the recoveries are well within the acceptance range, therefore, method was found to be accurate.

5. Precision

Repeatability

Table 10 Results of Repeatability Study for OLM and HTZ

Inj.	Peak Area (mV) of OLM	Peak Area (mV) of HTZ
1	6580390	3309212
2	6577390	3307095
3	6584488	3312352
4	6579810	3310474
5	6585234	3313339
SD	3310.745	2487.133
RSD	0.05	0.08

The percentage RSD (<2) values obtained showed that the method developed was precise at repeatability precision level**.**

Intra-day & Inter-day Precision:

Table 11 Results of Intra-day & Inter-day Precision Study for OLM and HTZ

Intra Day precision								
Day	**OLM**				**HTZ**			
	Area	**Assay**	**% RSD**	**Cumm % RSD**	**Area**	**Assay**	**% RSD**	**Cumm % RSD**
Morning	6580390	-	NA	NA	3310494	-	NA	NA
	6579543	99.99			3308850	99.01		
Afternoon	6741256	-	0.10	1.44	3324587	-	0.72	0.27
	6749823	100.13			3325464	100.03		
Evening	6847521	-	0.19	1.88	3487123	-	0.21	0.93
	6874254	100.39			3498527	100.33		
Inter Day precision								
Day	**OLM**				**HTZ**			
	Area	**Assay**	**% RSD**	**Cumm % RSD**	**Area**	**Assay**	**% RSD**	**Cumm % RSD**
Day 0	6580390	-	NA	NA	3310494	-	NA	NA
	6579543	99.99			3308850	99.01		

Day 2	6745218	-	0.42	1.6	3402571	-	0.73	1.61
	6784354	100.58			3403647	100.03		
Day 4	6903458	-	0.42	2.15	3455581	-	1.26	1.99
	6902147	99.98			3395383	98.26		

The % RSD was below 2 which showed that the method developed was precise for Olmesartan and Hydrochlorthiazide.

6. Detection Limit

Table 12 Limit of Detection data of OLM and HTZ

	OLM	**HTZ**
LOD (µg/ml)	13.87	13.71

Detection limit was calculated on basis of standard deviation of response and slope.

7. Quantitation Limit

Table 13 Limit of Quantitation data of OLM and HTZ

	OLM	**HTZ**
LOQ (µg/ml)	42.04	41.53

Quantification limit was calculated on basis of standard deviation of response and slope.

8. System Suitability Testing

Table 14 Results of System Suitability Parameters

Analyte	**RT(min)**	**Tailing Factor**	**Theoretical Plates (N)**	**Resolution**
OLM	4.93	1.64	7584	6.83
HTZ	3.63	1.10	13315	--
Required limits	--	T < 2	N > 2000	R > 2

Quantification limit was calculated on basis of standard deviation of response and slope.

9. Robustness:

Table 15 Results of OLM & HTZ for Robustness

Column Oven Temp Change					
Condition	**Sample**	**OLM**		**HTZ**	
		Area	**Assay**	**Area**	**Assay**
28^0 C	**WS**	6545720	-	3300157	-
	DP	6535481	99.84	3319754	100.59
30^0C	**WS**	6580390	-	6580390	-
	DP	6579543	99.99	6579543	99.99
32^0C	**WS**	6587459	-	3312436	-
	DP	6574982	99.81	3325418	100.39
Mobile Phase Change					
Condition	**Sample**	**OLM**		**HTZ**	
		Area	**Assay**	**Area**	**Assay**
MP-A Increase (52-48)	**WS**	6547821	-	3298472	-
	DP	6557489	100.15	3297458	99.97
Normal (50-50)	**WS**	6580390	-	3310494	-
	DP	6579543	99.99	3312145	100.05
MP-A Decrease (48-52)	**WS**	6538415	-	3458716	-
	DP	6597421	100.90	3468491	100.28

The Results showed that there was no much difference in area and the method developed was found to be robust for Olmesartan and Hydrochlorthiazide.

Conclusion:

This research was aimed to develop and validate a method for the estimation of Olmesartan and Hydrochlorthiazide in bulk and pharmaceutical dosage form. The proposed method was found to be appropriate due to its simplicity, reliability, sensitivity, rapidness and selectivity for detection at very low concentrations. The short chromatographic time makes this method suitable for processing of multiple samples in short time. The method showed no interference of the diluent peaks present in Olmesartan and Hydrochlorthiazide. The statistical parameters and recovery data reveals the good accuracy and precision of the proposed method. The RP- HPLC method developed for the estimation of Olmesartan and Hydrochlorthiazide was validated as per the ICH guidelines. Validation data demonstrates that, this method is accurate,

precise, simple, economic and can be used in the routine analysis of Olmesartan and Hydrochlorthiazide in various formulations.

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