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METHOD DEVELOPMENT AND VALIDATION OF OLMESARTAN AND HYDROCHLOROTHIAZIDE BY UV SPECTROSCOPY

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ABSTRACT

In the present work a simple, inexpensive, rapid, easy, sensible and reproducible UV Spectrophotometric method has been developed and validated for determination of Olmesartan (OLM) and Hydrochlorothiazide (HCT) in bulk drug and pharmaceutical dosage form. A simple double beam UV spectrophotometric method has been developed using area under curve method and validated with different parameters such as linearity, precision, Accuracy, limit of detection (LOD), Limit of Quantification (LOQ), accuracy as per ICH guidelines. UV-visible spectrophotometric method, measurement of absorption at maximum wavelength in 10 ml methanol and volume make with water solvent system as reference OLM and HCT were found to be at 256 nm and 276 nm respectively. The drug obeyed the Beer's law and showed good correlation. Beer's law was obeyed in concentration range 2-32 µg/ml for Olmesartan and Hydrochlorothiazide respectively with correlation coefficient was 0.999. LOD values for OLM and HCT were found to be 0.056 µg/mL and 0.1050 µg/mL respectively and LOQ values for OLM and HCT were found 0.1698 µg/mL and 0.3183 µg/mL respectively. The proposed method is precise, accurate and reproducible and can be used for routine analysis of OLM and HCT in bulk and pharmaceutical dosage form.

Keywords: Olmesartan, Hydrochlorothiazide, Area under curve method, Method development, Validation, UV spectrophotometry, ICH guidelines.

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1. INTRODUCTION

Hypertension is the most common cardiovascular disease. As many as 50 million people in the world have systolic and/or diastolic blood pressure above 140/90. Uncontrolled hypertension can lead to dramatic risks in patients such as stroke, cardiac failure with myocardial infarction, renal insufficiency, and dissecting aneurysm of the aorta. Hence, effective lowering of blood pressure became a must in hypertensive patients. Patients with high blood pressure of the moderate to severe type require a combined therapy, of two or more drugs of different mechanisms of action, to achieve adequate blood pressure control over 24 hr/day. Therefore, antihypertensive drug combinations are formulated in tablets for once daily administration, leading to a comparable patient compliance as mono-therapy, with the advantage of increased effectiveness during treatment. This work involves the analysis of widely used Angiotensin receptor combinations with diuretic. The significant feature of these combinations lies in the fact that diuretic is present in minute amount compared to angiotensin receptor drugs which makes its analysis more complicated and tedious.

Looking to this factor it was proposed to develop analytical methods for the simultaneous estimation of fix dose combination drugs important drugs belonging to antihypertensive and diuretic categories which may be simpler and cost effective for future analysis.

The main objective of the study is to develop a rapid, specific and economic UV spectrophotometric method by using Methanol and distilled water as a solvent for simultaneous determination of Olmesartan (OLM) and Hydrochlorothiazide (HCT) content in bulk and pharmaceutical dosage formulations.

MATERIALS AND METHOD

MATERIALS

The drug samples, Olmesartan (OLM) and Hydrochlorothiazide (HCT) working standards were obtained as gift sample by Cipla Ltd Indore (MP) India. Olmesar-H marketed tablets manufactured by Macleods Pharmaceutical was procured from local market. Methanol, and water used were analytical grade and were purchased from Merck Specialties Private Limited, Mumbai, India.

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Impact Factor: 3.426

INSTRUMENTATION:

Variable wavelength programmable UV detector UV1800 double beam UV-Visible spectrophotometer was used to carry out spectral analysis and the data was recorded by Hitachi software. Sonicator (1.5L), FTIR spectrophotometer of Shimadzu 00203.Ultrasonicator was used to sonicating the mobile phase and samples. Standard and sample drugs were weighed by using Denver electronic chemical balance (SI-234).

2. EXPERIEMENTALS

2.1 PREPARATION OF STANDARED STOCK SOLUTION:

Accurately weighed (10.001 mg) each of standard OLM (10.001 mg) and HCT (10.002 mg) were transferred to two separate 100 mL calibrated volumetric flasks (100 mL) dissolved in methanol which were further diluted with the methanol to obtain standard solutions of OLM and HCT (100 µg/mL).

2.2 DETECTION OF WAVELENGTH

The λ max of OLM and HCT was obtained at 256 and 276nm. This found to be similar as given in the reference. Which shows that drug is pure. The UV spectrum of drug samples of OLM and HCT are shown in the fig. 1 and 2.

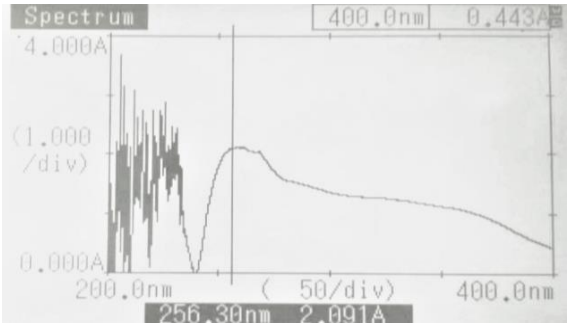

Fig. 1 UV Spectra of Olmesartan

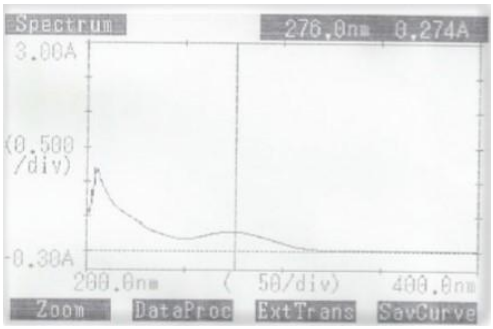

Fig. 2 UV Spectra of Hydrochlorothiazide

2.3 Fourier transform infrared (FTIR) spectroscopy

IR spectra of OLM and HCT were taken by FT-IR (Brucker FT-IR). The results obtained are found to be similar as given in the references[58, 59].Thespecifications of peaks are shown in **Fig. 3 and 5**

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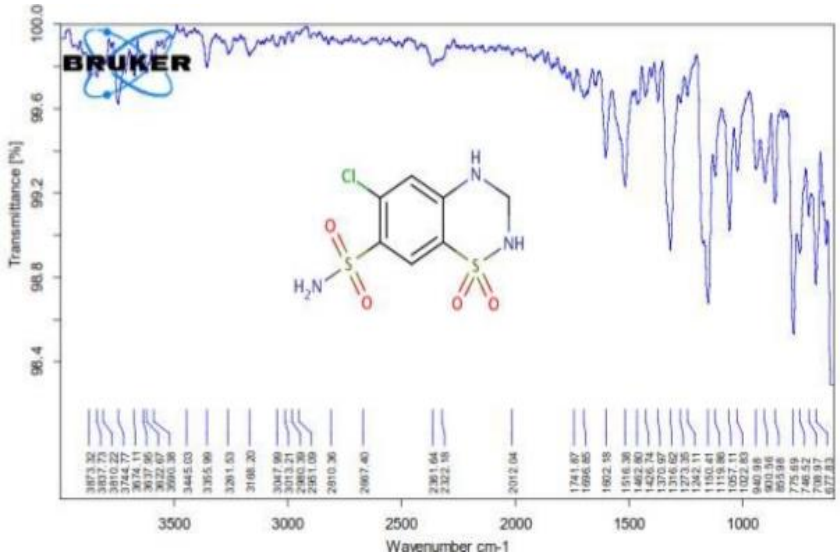

Fig. 3 FTIR Spectra of Olmesartan

Table 4: IR frequency (cm^{-1}) for Olmesartan

IR absorption bands (cm^{-1})	Inference
3672.58	O-H stretching
3058.71	C-H stretching
1645.74	C=O stretching
1515.84	C=N stretching
1455.86	C-H bending
1319.04	C-O stretching
1217.24	C-N stretching,

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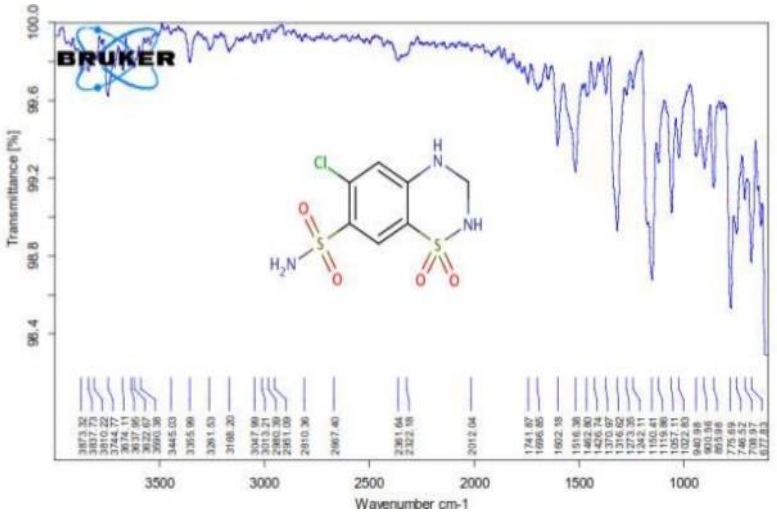

Fig. 5 FTIR Spectra of Hydrochlorothiazide

Table 6: IR frequency (cm^{-1}) for Hydrochlorothiazide

IR absorption bands (cm^{-1})	Inference
3810.22	NH_2 stretching
3637.95	N-H stretching
3261.53, 3168.20	C-H stretching
1462.80, 1426.74	C-H bending
1370.97	C-N stretching
1057.11	S=O stretching
855.98	C-Cl stretching

2.4 PREPARATION OF CALIBRATION CURVE OF OLM AND HCT

Calibration curves of Olmesartan and Hydrohlorothiazide were prepared in concentration range of 2, 4, 8, 12, 16, 20, 32 µg/ml with Methanol. The absorbance of each solution was measured at the wavelengths 256-267 nm and 268-276 nm. The absorbance values (mean of five determinations) with

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Impact Factor: 3.426

their standard deviation at different concentration in the range 2, 4, 8, 12, 16, 20, 32 are tabulated[62]. The drug obeys Beer's Lambert law in the concentration range. Linear regression analysis for all calibration curves of Olmesartan and Hydrochlorothiazide are given in Table 7 and 9.

Table 7: Data of standard calibration curve of Olmesartan

	Wavelength range (256-267)		Wavelength range (268-274)	
S.No.	**Concentration (µg/ml)**	**Absorbance (nm)**	**Concentration (µg/ml)**	**Absorbance (nm)**
1.	2	0.005	2	0.0006
2.	4	0.010	4	0.0007
3.	8	0.0175	8	0.0008
4.	12	0.0235	12	0.0009
5.	16	0.0284	16	0.0011
6.	20	0.0345	20	0.0015
7.	32	0.0543	32	0.0017
	Mean absorptivity ax1=0.12032		Mean absorptivity ax2=0.000654	

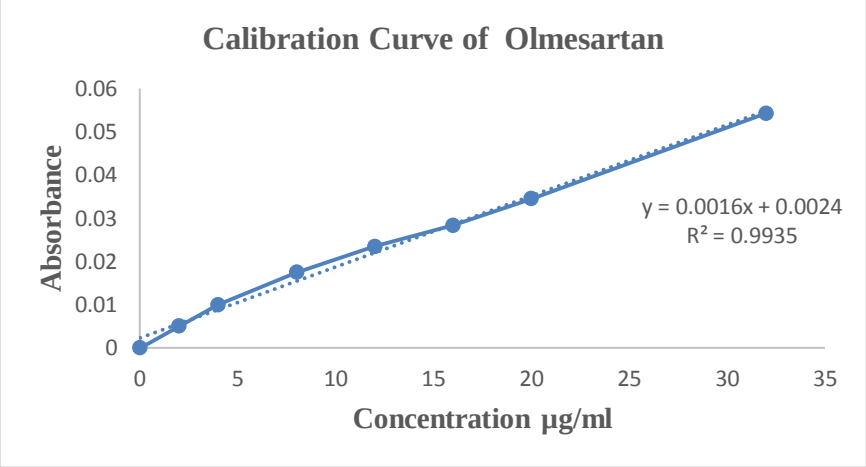

Fig. 8 Calibration Curve of Olmesartan

ISSN: 2519-9889
Impact Factor: 3.426

Table 9: Data of standard calibration curve of Hydrochlorothiazide

	Wavelength range (256-267)		Wavelength range (268-274)	
S.No.	**Concentration (µg/ml)**	**Absorbance (nm)**	**Concentration (µg/ml)**	**Absorbance (nm)**
1.	2	0.0025	2	0.0120
2.	4	0.0045	4	0.0120
3.	8	0.0081	8	0.0128
4.	12	0.0119	12	0.0138
5.	16	0.0152	16	0.0145
6.	20	0.019	20	0.0150
7.	32	0.0299	32	0.0178
	Mean absorptivity ay1=0.0048		Mean absorptivity ay2=0.0136	

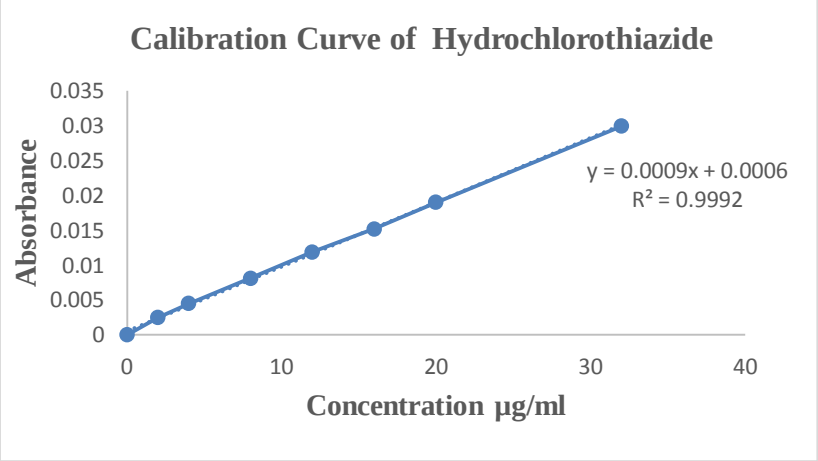

Fig. 10 Calibration Curve of Hydrochlorothiazide

ISSN: 2519-9889
Impact Factor: 3.426

3. METHOD DEVELOPEMENT

3.1 SIMULTANEOUS ESTIMATION OF OLM AND HCT BY AREA UNDER CURVE METHOD

Area under curve method

In the simultaneous equation using AUC method, the area under curves of the recorded spectrums were measured at the selected wavelength ranges, 256- 267 nm and 268- 276 nm and calibration curves were plotted by taking concentration on x axis and AUC at 256- 267nm or 268- 276 nm on Y-axis and the regression analysis of calibration curves and absorptivity values (X) of both these drugs are presented in Table 4.7 and Table 4.8. The 'X' values were determined as, X= Area under curve of component (from 256 - 267 nm or 268 - 276 nm)/concentration of the component in µg/ml. A set of two simultaneous equations framed using these 'X' values as follows,

$A1 = 0.0120\ C_{OLM} + 0.0448\ C_{HCT}$ ---------- (at λ 256- 267.0 nm) ---------- (1)

$A2 = 0.0006\ C_{OLM} + 0.0136\ C_{HCT}$ ---------- (at 268- 276. 0 nm) ---------- (2)

Where, C_{OLM} and C_{HCT} are the concentrations of OLM and HCT measured in µg/mL, in the sample solutions. A1 and A2 are the area under curve of sample solutions at the wavelength range 256 to 267 nm and 268 to 276.0 nm, respectively

Selection of working wavelength

From the overlay spectra (Fig. 6.2.1) of the selected drugs in wavelength range of 256 -267, 268-276 nm were selected for the analysis. The obtained result are similar as given in the references[61]. The overlay spectra of OLM and HCT are shows in fig. 11.

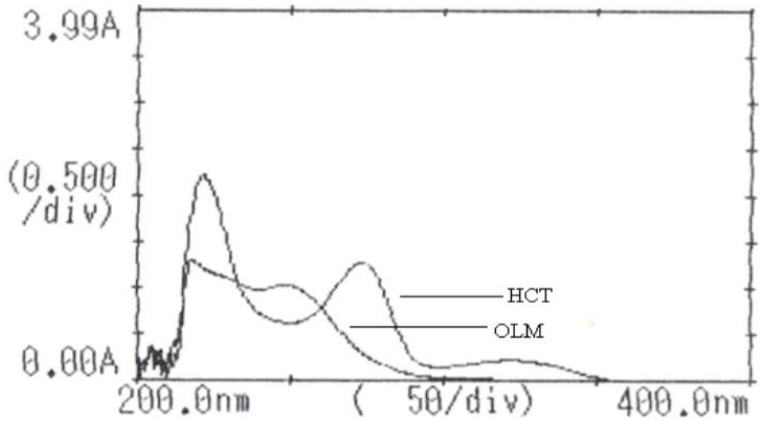

Fig. 11 Overlay Spectra of Olmesartan and Hydrochlorothiazide

ISSN: 2519-9889
Impact Factor: 3.426

4. VALIDATION

Validation of the developed method was done according to the USP 2006, Asian edition.

A. Linearity

Linearity was evaluated by analysis of working standard solution of Olmesartan and Hydrochlorothiazide at six different concentrations. Olmesartan found to be linear within conc. range of 2-32 µg/ml with regression coefficient of 0.998 and Hydrochlorothiazide was found to be linear within conc. range of 2-32 µg/ml with regression coefficient of 0.999 the results of regression analysis are summarized in (Table 12). Results shows that within the concentration range mentioned above, there was an excellent correlation between absorbance and concentration of Olmesartan and Hydrochlorothiazide (Fig. 13 and 14).

Table 12: Regression analysis of calibration graphs of Olmesartan and Hydrochlorothiazide for proposed UV Spectrophotometric method

Sr. No.	Parameters	Results (Olmesartan)	Results(Hydrochlorothiazide)
1.	Absorption maxima	256 nm	276 nm
2.	Beer's range	2-32 µg/ml	2-32 µg/ml
3.	Regression equation	y = 0.0016x + 0.0024	y = 0.0009x + 0.0006
4.	Correlation coefficient	0.998	0.999
5.	Slope	0.0016	0.0009
6.	Intercept	0.024	0.0006

ISSN: 2519-9889
Impact Factor: 3.426

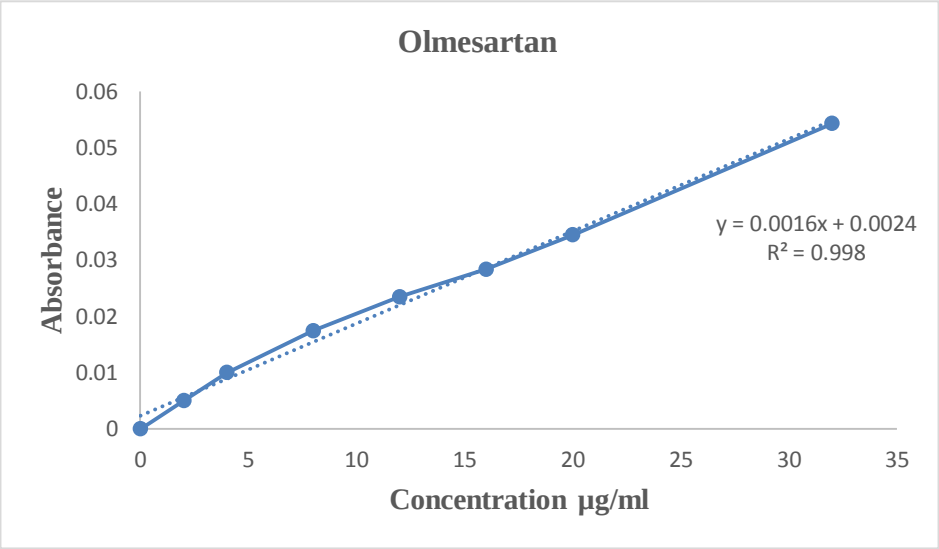

Fig. 13 Regression (Linearity) analysis for Olmesartan

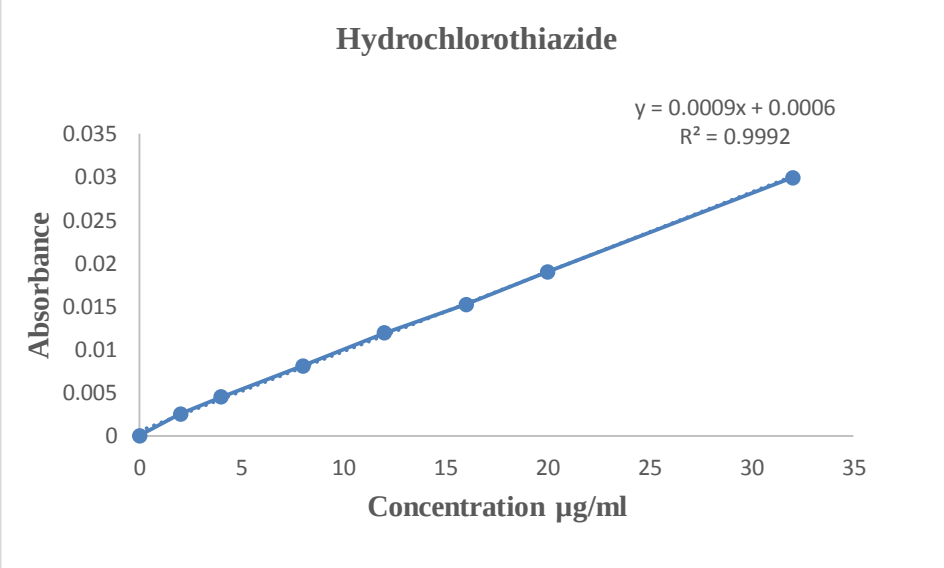

Fig. 14 Regression (Linearity) analysis for Hydrochlorothiazide

ISSN: 2519-9889
Impact Factor: 3.426

B. Accuracy

This parameter was evaluated by the percent recovery studies at concentration levels of 80, 100, and 120%, which consisted of adding known amounts of OLM and HCT working standard solution to a prequantified sample solution.

The recoveries were verified by estimation of drugs in triplicate preparations at each specified concentration level.

Results of recovery study were obtained in the range of 99.22-100.56% for both drugs as per ICH guidelines. Results are shown in **Table 15.**

Table 15: Recovery study of Olmesartan and Hydrochlorothiazide

% Level	Sample conc. (µg/mL)	Std added (µg/mL)	Total conc.	Amt. recovered	SD	%RSD	% recovery	SD	%RSD
Olmesartan									
80	6.0	4.8	10.8	10.77	0.0655	0.0688	99.7222	0.6071	0.6088
100	6.0	6.0	12.0	11.90	0.1365	1.1375	99.2222	1.1375	0.1464
120	6.0	7.2	13.2	13.24	0.1081	0.8128	100.3031	0.8291	0.8169
Hydrochlorothiazide									
80	8.0	6.4	14.4	14.29	0.0305	0.2137	99.2591	0.2121	0.2137
100	8.0	8.0	16.0	15.91	0.0305	0.1919	99.4583	0.1909	0.1919
120	8.0	9.6	17.6	17.61	0.0798	0.0983	100.056	0.9841	0.09835

C. Precision

The result of intraday, intraday precision for OLM, HCT are shown in **Table 16** respectively. The developed method was found to be precise as the %RSD valuefor intermediate precision studies was less than 2% as recommended by ICH Guidelines.

ISSN: 2519-9889
Impact Factor: 3.426

Day 1	**Hydrochlorothiazide (4 µg/mL)**			**Olmesartan (8 µg/mL)**		
	0h	**2h**	**4h**	**0h**	**2h**	**4h**
1	3.9800	3.9800	4.0500	8.0960	8.0100	7.9200
2	3.9900	3.9800	4.0200	8.0161	8.0500	7.9400
3	4.0100	3.9700	4.0300	8.0559	7.9800	8.0100
Mean	3.9900	3.9800	4.0300	8.0500	8.0300	7.9900
SD	0.0152	0.0057	0.0723	0.0399	0.0351	0.0472
RSD (%CV)	0.3825	0.1451	1.8423	0.4959	0.4382	0.5939
Mean (Day-1)	3.9900			8.0200		
SD (Day-1)	0.0346			0.0498		
RSD (Day-1)	0.8748			0.6222		
Day 2	**Hydrochlorothiazide (4 µg/mL)**			**Olmesartan (8 µg/mL)**		
	0h	2h	4h	0h	2h	4h
1	3.8800	3.9800	4.0100	7.9200	7.9000	8.0100
2	3.9800	3.8700	3.9700	7.9300	7.8900	7.9200
3	4.0100	4.0100	3.9800	8.0100	7.9000	7.9300
Mean	3.9500	3.9500	3.9800	7.9533	7.8900	7.9500
SD	0.0680	0.0737	0.0493	0.0493	0.0057	0.0493
RSD (%CV)	1.7203	1.8645	1.2562	0.0493	0.0057	0.0493
Mean (Day-2)	3.9800			7.9200		
SD (Day-2)	0.0218			0.0327		
RSD (Day-2)	0.5533			0.4123		
Day 3	**Hydrochlorothiazide (4 µg/mL)**			**Olmesartan (8 µg/mL)**		
	0h	2h	4h	0h	2h	4h
1	3.8700	3.9800	3.9700	7.9300	7.8800	7.8900
2	3.8800	3.9700	3.9000	7.8800	7.8700	7.9100
3	4.0100	4.1000	3.9300	7.8900	7.8000	7.9400
Mean	3.9200	3.9700	3.9500	7.9000	7.9000	7.9000
SD	0.0781	0.0723	0.0173	0.0264	0.0435	0.0251
RSD (%CV)	1.7924	1.8010	0.4384	0.3349	0.5552	0.3180
Mean (Day-3)	3.9300			7.9910		

ISSN: 2519-9889
Impact Factor: 3.426

SD (Day-3)	0.0494	0.0333
RSD (Day-3)	1.2487	0.4232

Table 16 Intra-day and inter-day precision data for drug

D. LOD and LOQ

LOD values for OLM and HCT were found to be 0.056 µg/mL and 0.1050 µg/mL respectively. LOQ values for OLM and HCT were found 0.1698 µg/mL and 0.3183 µg/mL respectively. The calculated data for LOD and LOQ are shown in **Table 17**

Table 17 limit of detection (LOD) and limit of quantification (LOQ) of Olmesartan and Hydrochlorothiazide

S.no	Parameters	Method(Area under curve method)	
		Olmesartan	Hydrochlorothiazide
1.	LOD(µg/ml)	0.0560	0.1050
2.	LOQ(µg/ml)	0.1698	0.3183

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CONCLUSION

It can be concluded that the Method development and Simultaneous estimation for both the drugs was performed and it gave good results. The best result was given by methanol solvent.

As method development procedure, validation studies were also performed for the same, but due to limited quantity of the compound only few parameters were observed as Linearity, limit of detection, Limit of quantitisation, Precision and linearity.

ISSN: 2519-9889
Impact Factor: 3.426

The validation procedure followed were as per the ICH guidelines. Olmesartan and Hydrochlorothiazide both gave excellent results. Since there were no reference results for this study, so the results were not compared to any standard.

The linearity was achieved with methanol solvent, Linearity, Accuracy and precision were satisfactory and the limit of detection (LOD), limit of quantitation achieved was also satisfactory. Hence we conclude that the simple, rapid, less-time consuming, cost effective and precise method was developed and validated by UV-spectroscopy with the simultaneous estimation of Olmesartan and Hydrochlorothiazide.

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