RESEARCH ARTICLE

Analytical Method Development and Validation for the Simultaneous Estimation of Olmesartan and Hydrochlorothiazide by RP-HPLC in Bulk and Tablet Dosage Forms

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Received: 27th December, 2022; Revised: 17th January, 2023; Accepted: 14th February, 2023; Available Online: 25th March, 2023

ABSTRACT

New medication combinations are introduced every day. As a result, various diseases and disorders are treated using a combination of several therapeutic medicines that each have a somewhat distinct mechanism of action. Therefore, it is crucial to develop methods of analyzing medicines employing a range of methods that may be utilized. A UV 730d (dad) absorbance detector, a 20 L injection loop, a sp 930d pump, a 4.6 by 100 mL C18 column (Agilent), and Chemstation software are all included in the setup: approximately 60 water and 40% methanol (pH 3.0 adjust with OPA). Maximum efficiency was achieved when the system was operated at a wavelength of 233 nm. The procedure's efficacy was confirmed by testing it against ICH guidelines. These techniques were found to be linear, precise, broad, and stable. The procedure was found to be easy, accurate, exact, affordable, and easy to use again and again. This means that olmesartan and hydrochlorothiazide, in both bulk form and finished products, can be tested for quality using the proposed methodologies.

Keywords: HPLC, Hydrochlorothiazide, Method development, Olmesartan, Validation.

International Journal of Pharmaceutical Quality Assurance (2023); DOI: 10.25258/ijpqa.14.1.07

How to cite this article: Ahmad S, Mujawar T, Pawara J, Sable V, Patil K, Tare H. Analytical Method Development and Validation for the Simultaneous Estimation of Olmesartan and Hydrochlorothiazide by RP-HPLC in Bulk and Tablet Dosage Forms. International Journal of Pharmaceutical Quality Assurance. 2023;14(1):30-35.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Analysis of pharmaceuticals is crucial to guarantee the quality and viability of both finished pharmaceutical products and the raw materials used to make them. Isolating, identifying, and quantifying chemicals in a sample matter are all part of pharmaceutical analysis, a subfield of analytical chemistry. Its focus is on the quantitative and qualitative chemical characterization of materials. Many new methods of analysis have emerged in recent decades. The analytical method is a specific application of a technique for the purpose of issue solving. The development and assessment of new products, as well as the safeguarding of consumers and the natural environment, all rely heavily on analytical instrumentation. This equipment can only ensure the safety of our food, medicine, water, and air because it gives the lower detection thresholds needed.

Validation of an analytical method is showing that the method meets certain criteria through controlled laboratory experiments, that the technique's performance characteristics fulfill the needs of its intended analytical applications. Assay validation is critical in the pharmaceutical sector for two main reasons. The first and most crucial is assay, an essential aspect

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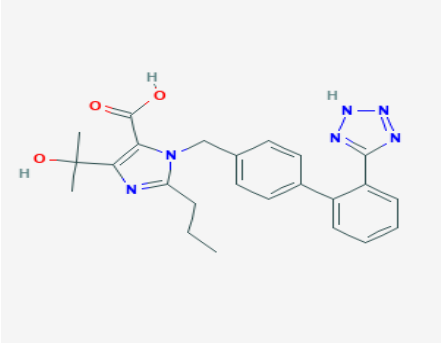

Figure 1: Olmesartan structure

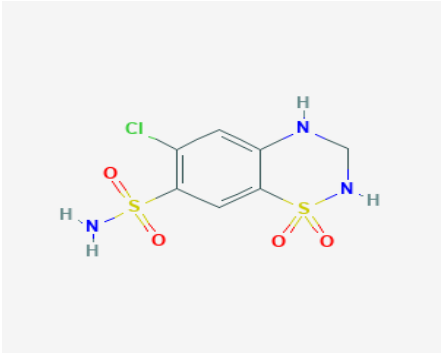

Figure 2: Hydrochlorothiazide structure

of any effective quality assurance strategy. To add to that, assay validation is mandated by current good manufacturing practise legislation.

Olmesartan, or OLM, is a medication used to treat high blood pressure. An innovative 1H-imidazole-5-carboxylic acid (Figure 1) has been shown to be effective in treating hypertension. It is a prodrug in the form of an ester that is hydrolyzed entirely and very quickly to the active form, olmesartan. Reducing vasoconstriction, olmesartan acts by preventing angiotensin II from binding to AT1 receptors in vascular smooth muscle. In hypertensive patients, this reduces total peripheral resistance and thus, blood pressure.[1,2] Olmesartan medoxomil is a white, crystalline powder. Hydrochlorothiazide (HCT) is diuretic (Figure 2). HCT blocks Cl and Na+ from being reabsorbed in the kidney's distal tubule, leading to more water being passed in urination.[3,4] Almost white in appearance, very little of this crystalline powder dissolves in water, and even less in methanol; ether and chloroform are utterly ineffective. It has been shown that OLM combined with HCT is a successful therapeutic approach for treating hypertension, although it has some undesirable side effects.

The USP outlines an RP-HPLC method for determining HCT in tablet formulation. No pharmacopeia has yet included a description of OLM. There are a number of reported analytical methods for its determination in biological samples such as plasma.[5-8] From my review of the literature, it seems that only a handful of reports of analytical methods can detect both HCT and OLM in a single sample of a combination medicinal dosage form. Therefore, we have designed RP-HPLC method.

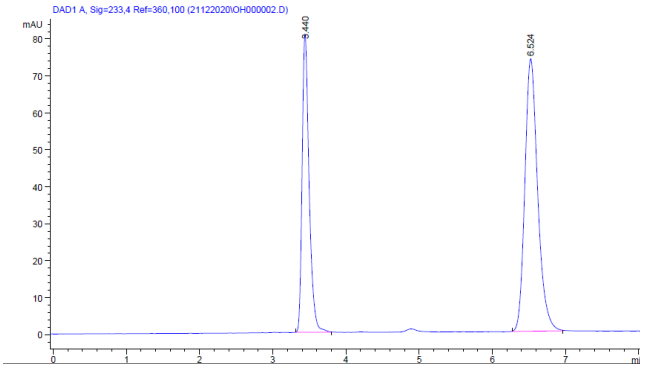

Figure 3: Standard combination chromatogram of olmesartan and hydrochlorothiazide

Table 1: Information about a chromatogram for a reference mixture that includes OLM and HTZ

Drug	*R.T*	*Area*	*Symm*	*Th.Plates*
Htz	3.440	534.36	0.66	7114
Olm	6.524	897.91	0.78	7277

MATERIALS AND TECHNIQUES

Raw Materials

The drug testing was done using an Agilent (S.K.) gradient system UV detector. Set up using Chemstation software and a UV 730d (dad) absorbance detector, as well as a reverse phase (Agilent) C_{18} column (4.6 x 100 mm, 2.5 m), an SP930d pump, an injection loop holding 20l, and a UV 730d (dad).

Medications olmesartan and hydrochlorothiazide were obtained from R.S.I.T.C. Jalgaon and other HPLC Grade reagents from Millipore, Bangalore. Mankind Pharmaceuticals Ltd. in Mumbai was contacted, and a pill containing olmesartan (20/40 mg) and hydrochlorothiazide (12.5 mg) was purchased. (Make: H 20:12.5 mg, Olm Time).

Chromatographic Requirements

A 2.5 m particle packing, 233 nm detection wavelength, 0.7 mL/min flow rate, 20 µL sample volume, ambient temperature, mobile phase, and a C_{18} column (100 mm 4.6 mm). It takes 15 minutes to run on a 60:40 methanol:water mixture (pH 3.0; if necessary, modify with OPA).

Making a stock Solution According to Established protocols

Using a 20 mL volumetric flask, we dissolved 20 mg of olmesartan (OLM) and 12.5 mg of hydrochlorothiazide in methanol to make a stock solution of 1000 µg/mL Olmesartan and 625 µg/mL hydrochlorothiazide. Then we diluted it to 20 mL with the mobile phase methanol + 0.1% OPA water with TEA(60) (Table 1 and Figure 3).

Method Development and Validation[9-15]

Assay Preparation for Commercial Formulation

After averaging the weight of 20 tablets containing olmesartan and hydrochlorothiazide, a sample containing 45 mg of olmesartan and hydrochlorothiazide should be added to a 10 mL volumetric flask after weighing for analysis. Sonicate to

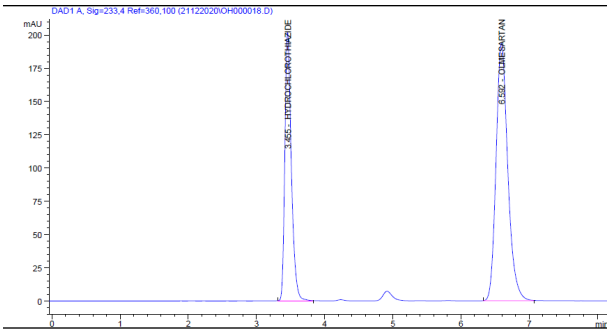

Figure 4: Marketed formulation chromatogram

Table 2: Marketed formulation's analysis

Assay method	*Drug Name*	*Amount found*	*Percent label claim*	*S.D.*	*Rsd (%)*
Rp-hplc method	Olm	29.5500	98.5283	0.008	0.14
	Hcz	1332.8979	99.8338	0.94	0.94

dissolve fully, then add enough diluent (10 mL methanol) to bring the volume up to the correct level. Combine and strain after passing it through a 0.45 millimeter-thick nylon membrane filter. Methanol water containing 0.1% OPA and TEA (60 + 40% v/v) in order to reach the necessary level. In the case of the test mixture of olmesartan and hydrochlorothiazide, the straightforward chromatogram presented in (Figure 4). The quantities per tablet were calculated by extrapolating the area value from the calibration curve. Five separate analyses were performed on the tablet variant. Table 2 presents the results of an assay for the labelled percentage of RSD.

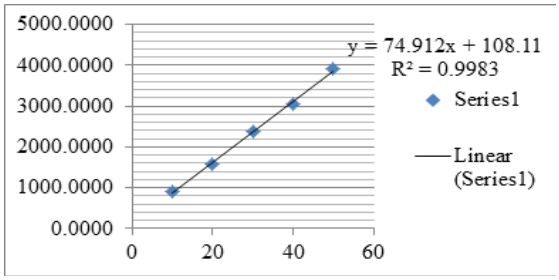

Figure 5: Olmesartan medoxomil calibration curve

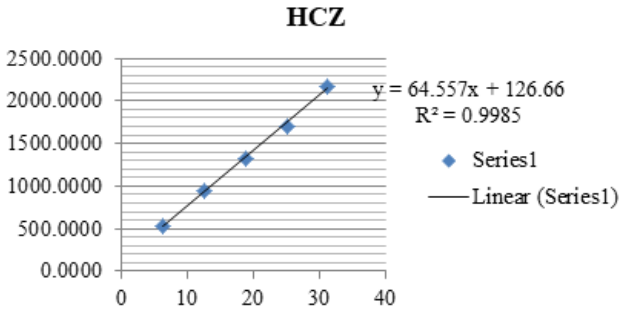

Figure 6: Hydrochlorothiazide calibration curve

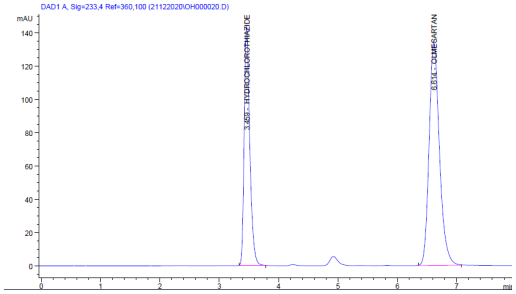

Figure 7: Accuracy chromatogram 80%

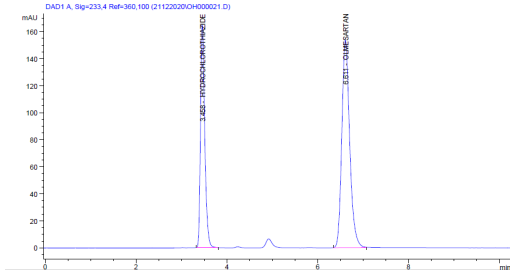

Figure 8: Accuracy chromatogram 100%

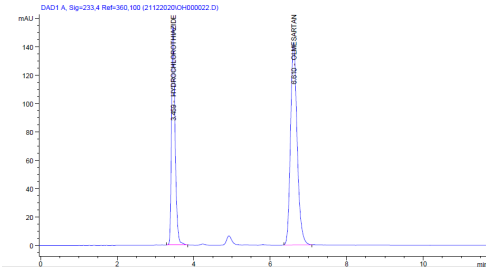

Figure 9: Accuracy chromatogram 120%

Table 3: Olmesartan medoxomil (Linearity data)

Name of method	Conc (µg/mL)	Peak area (µV. sec)		Peak area (µV. sec) average	Peak area S.D.	Peak area %RSD
		1	2			
Rp-HPLC method	10	905.1388	897.212	901.1754	5.6051	0.6220
	20	1565.6330	1562.6010	1564.1170	2.1439	0.1371
	30	2372.1630	2365.5263	2368.8447	4.6929	0.1981
	40	3046.4912	3042.9101	3044.7007	2.5322	0.0832
	50	3906.1020	3898.9765	3902.5393	5.0385	0.1291
	Equation		y =74.912 x +108.11			
	R2		0.999			

Table 4: Hydrochlorothiazide (Linearity data)

Method	Conc µg/mL	Peak area (µV.sec)		Peak area (µV.sec) average	Peak area S.D.	Peak area %RSD
		1	2			
UHPLC method	6.25	533.8121	534.366	534.0891	0.3917	0.0733
	12.5	944.3520	950.4490	947.4005	4.3112	0.4551
	18.75	1325.3540	1328.5530	1326.9535	2.2620	0.1705
	25	1702.2468	1705.2476	1703.7472	2.1219	0.1245
	31.25	2170.9528	2175.6945	2173.3237	3.3529	0.1543
	Equation		y = 64.557 X + 126.66			
	R2		0.998			

Table 5: Recovery data Result for OLM and HCT

Drug	*Level (%)*	*Amt. taken (µg/mL)*	*Amt. added (µg/mL)*	*Absorbance Mean* ± S.D.*	*Amount recovered Mean* ± S.D.*	*Percent recovery Mean* ± S.D.*
OLM	80	10	8	17.89 ± 0.10	7.89 ± 0.10	98.64 ± 1.32
	100	10	10	19.83 ± 0.001	20.58 ± 0.001	98.41 ± 0.042
	120	10	12	21.89 ± 0.002	20.58 ± 0.007	99.17 ± 0.061
HCZ	80	6.25	5	11.34 ± 0.006	5.09 ± 0.07	101.92 ± 1.47
	100	6.25	6.25	12.59 ± 0.01	6.34 ± 0.011	101.53 ± 0.17
	120	6.25	7.5	13.89 ± 0.02	7.64 ± 0.019	101.96 ± 0.28

*mean in triplicate

Table 6: Statistical validation of recovery studies OLM and HCT

Method	*Level of recovery (%)*	*Drug*	*%RSD*	*Standard deviation**	*Mean% Recovery*
Rp-HPLC Method	80	OLM	0.32	0.001	98.64
		HCZ	0.22	0.007	101.92
	100	OLM	0.42	0.001	98.41
		HCZ	0.17	0.002	101.53
	120	OLM	0.61	0.001	99.17
		HCZ	0.28	0.007	101.96

* Indicates the mean of three separate RP-HPLC analyses

RESULTS

Linearity and Range

Calibration data for olmesartan medoxomil and hydrochlorothiazide are shown in Tables 3 and 4, respectively. Linear regression analysis of these data revealed a linear connection between peak areas and concentrations in the ranges of 10 to 50 µg/mL and 6.25 to 31.25 µg/mL, respectively. Olmesartan medoxomil's linear equation for area of peak was y = 74.912 x + 108.11, while hydrochlorothiazide's was y = 64.557 x + 126.66. Correlation values ranged from 0.998 to 0.9999. Hydrochlorothiazide and olmesartan medoxomil calibration curve (Figure 5 and 6).

Accuracy

The effectiveness of the new approach was tested using recovery experiments. Pre-analyzed tablet solutions had different concentrations of standard drug added to them (80, 100, and 120%), and the results were compared to those from the original analysis. There was a 1% margin of error, placing the range of recovery at 98–101%. Studies on recoveries have been statistically validated in (Table 5, 6 and Figure 7, 8 and 9).

Table 7: OLM and HCT RP-HPLC repeatability studies

Method	*Conc. of OLM and HCT (mg/mL)*	*Peak area*	*Amount found (mg)*	*% Amount found*
HPLC OLM method	40	3044.154	39.22	98.06
	40	3048.5962		
		Mean	3046.3752	
		SD	0.14	
		%RSD	0.10	
HPLC HCT method	25	1706.8811	24.5164	98.09
	25	1711.3975		
		Mean	1709.14	
		SD	0.19	
		%RSD	0.16	

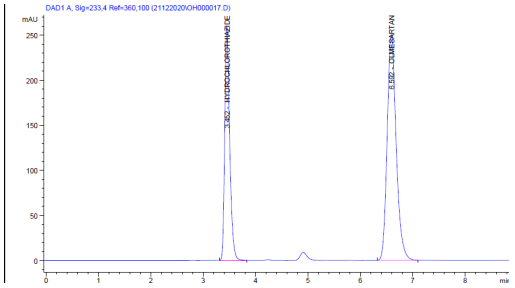

Figure 10: System suitability chromatogram 1

System Suitability Parameters

The parameters of the proposed chromatographic system for the determination of OLM and HCT were investigated to establish its adequacy in terms of resolution and reproducibility. Evidenced by the (Figure 10 and Table 7).

Table 8: Studies of RP-intra-HPLC's and inter-day precision for measuring OLM, HCT

Method	*Drug*	*Conc (µg/mL)*	*Interday precision*		*Intraday precision*	
			Mean ± SD	*Amount found (%)*	*Mean ± SD*	*Amount found (%)*
RP-HPLC method	HTZ	12.5	956.79 ± 0.96	102.89	950.79 ± 0.65	102.89
		18.75	1327.18 ± 0.13	99.19	1328.84 ± 0.69	99.19
		25	1707.29 ± 0.09	97.92	1717.22 ± 1.60	97.92
	OLM	20	1596.73 ± 0.65	99.36	1594.20 ± 0.52	100.87
		30	2383.87 ± 0.92	101.24	2382.46 ± 1.39	98.84
		40	3041.73 ± 0.68	97.90	3040.85 ± 0.75	94.96

*Mean 3 readings

Table 9: Robustness Study of OLM and HCT Results

Parameters	Conc. (µg/mL)	(Mean ± SD) amount of detected	Percent RSD	(Mean ± SD) amount of detected	Percent RSD
		For OLM		For HCT	
Flow change chromatogram 0.6 mL	25+40	12419.75 ± 0.78	0.01	1996.93 ± 0.36	0.12
Flow change chromatogram 0.8 mL	25+40	2575.72 ± 2.15	0.08	1260.09 ± 2.11	0.17
Wavelength change chromatogram 234 nm	25+40	2892.5 ± 1.58	0.05	1324.3 ± 0.88	0.14
Wavelength change chromatogram 232 nm	25+40	3043.53 ± 0.66	0.28	2174.33 ± 0.95	0.04
Mobile phase change chromatogram 59+41 mL	25+40	12505.2 ± 0.95	0.04	1723.9 ± 0.50	0.15
Mobile phase change chromatogram 61+39 mL	25+40	2945.28 ± 0.76	0.09	1710.49 ± 1.23	0.007

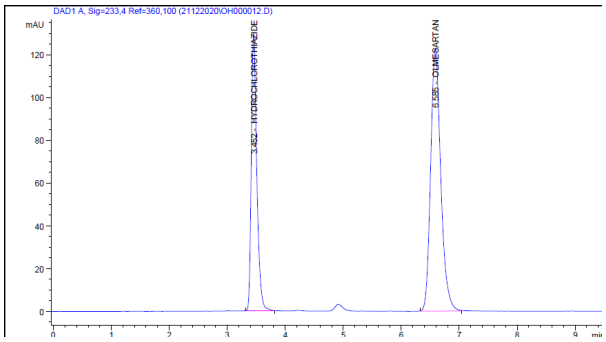

Figure 11: Precision Chromatogram

Precision

Different OLM and HCT benchmarks were analysed to build the methodology. In order to document any daily or weekly shifts in the solution's performance, it was analysed three times. The interday and intraday variation results (Table 8).

Robustness

Optimal method parameters were tweaked somewhat in order to assess the proposed approach's stability. Both the retention period and the tailing factor of the drug peak were measured and compared across a range of mobile phase composition and flow rates. Less variation was seen in retention time and tailing factor, as shown by the data (Table 9).

DISCUSSION

The proposed OLM and HCT estimation methods in tablet dose forms was straightforward, accurate, cost-effective, and rapid. This approach's effectiveness was demonstrated per ICH Q2 (R1) standards. OLM and HCT, after standard calibration, demonstrated a correlation coefficient (r2) of 0.999 for all of the observed wavelengths. Results of percent RSD were well within the allowed range of 2%, displaying extraordinary precision of procedures, and both medications improved symptoms by about 100%. All of this proves that the aforementioned techniques are applicable for simultaneous assessment of OLM and HCT in finished goods.

CONCLUSION

HPLC-developed methods were found to be more reliable than those developed using other means, including with respect to linearity, precision, range, and longevity. It was determined that the processes were straightforward and effective. The aforementioned methods could be used routinely in quality control labs.

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