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DEVELOPMENT AND VALIDATION OF NEW ANALYTICAL METHOD FOR OLMESATRON BY RP-HPLC

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ABSTRACT

Olmesarton is an angiotensin antagonist used in treatment of hypertension. The goal of present work was planned to develop accurate, precise, specific, and reproducible. A rapid, specific stability indicating reverse phase high performance liquid chromatography method has been developed and validated for olmesarton. The HPLC analysis used a reversed phase C18 (150 x 4.6mm, 5µm) column and a mobile phase constituted of acetonitrile, buffer pH 4.5 and methanol (55:40:5), flow rate was 0.8 ml/min. The method was validated according to the regulatory guidelines with respect to precision, accuracy, linearity and limit of detection (LOD) and Limit of Quantitation (LOQ). The percentage RSD for precision and accuracy of the method was found to be less than 2%.The method was found to be linear from 10-50 ppm for Olmesartan.

KEYWORDS: Olmesarton, HPLC, validated.

INTRODUCTION

Olmesartan (5-methyl-2-oxo-2H-1,3-dioxol-4-yl)methyl4-(2-hydroxypropan-2-yl)-2-propyl-1-({4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl)-1H-imidazole-5-carboxylate is a potent and selective angiotensin AT1 receptor blocker [1] which has been approved for the treatment of hypertension in the united states, Japan and European countries. The drug contains a medoxomil ester moiety and is cleaved rapidly by an endogenous esterase to release the active metabolite Olmesartan. [2] Due to the fact that hydrolysis of Olmesartan in human plasma is extremely rapid. [3] The drug works by inhibiting the effects of angiotensin II, a potent vasoconstrictor and one of the key contributors to cardiovascular and renal disease. [4]

Validation is a concept that has been evolving continuously since in first formal appearance in the United States in 1978. Validation is a rapidly growing and evolving subject. Validation in a requirement that has always made sense from both a regulatory and quality perspective. [5,8] It extended to those process steps determined to be critical to the quality purity of the final products. Analytical methods rely on scrupulous attention to cleanliness, sample preparation accuracy and precision. A standard method for analysis of concentration involves the creation of calibration curve. In the concentration of elements of compound in a sample is too high for the detection range of a technique, it can simply be diluted in a pure solvent. If the amount in sample is below an instruments range of measurement, the method of addition can be used. In this method a known quantity of the elements or compound under study is added, and the concentration observed in the amount actually in the sample. Analytical chemistry research is largely driven by performance of sensitivity, selectivity, robustness, linear range, accuracy, precision, speed, cost of purchase, operation, training, time and space. Validation is founded on but not specifically prescribed by regulatory requirements and is best viewed as an important and integral part of cGMP. Validation is therefore one element of quality assurance programmed associated with particular process. As the process differs so widely there is no universal approach to validation regulatory bodies such as FDA and EC for medicinal products have developed general non-mandatory guidelines. [6,9] The most compelling reason for validation should is to guarantee as for as possible that all processes and machinery in the Pharmaceutical manufacturing process are being used in a way which will ensure safety, integrity, quality and strength of the product for use by the general public. [7,10] Pharmaceutical validations among these methods undergo the world 'Validation' means 'Assessment' of validity or action of providing effectiveness, and validation as per ICH guidelines. [13]

MATERIALS AND METHODS

Chemicals and reagents: An active pharmaceutical ingredient sample of Olmesartan was received from bulk manufacturer Sura labs (Hyderabad, India). The HPLC grade Acetonitrile (ACN) was purchased from Merck (Darmstadt, Germany). Triethyl amine HPLC grade and Orthophosphoric acid AR grade were used.

Instrumentation: The HPLC system was composed of WATERS system fitted with Prominence PDA detector with Empower 2 Solution software. Analytical column used for this method was C18 (150 mm x 4.6 mm) 5µm.

Sample preparation: weigh accurately 10mg of drug in to the 10ml of volumetric flask and dilute with the methanol (stock solution 1000ppm) Further dilutions take 0.1ml of the above stock solution in to the 10ml of volumetric flask and make up the volume with methanol (10ppm).

CHROMATOGRAPHIC CONDITIONS

Instrument used	:	Waters HPLC with auto sampler and PDA detector 996 model.
Temperature	:	Ambient
Column	:	Symmetry C18 (4.6 x 150mm, 5µm)
Buffer	:	Take 6 ml of triethylamine in to a 1000ml of HPLC water and set the pH to 4.5 using Orthophosphoric acid.
pH	:	4.5
Mobile phase	:	ACN: TEA buffer (pH 4.5): Methanol (55:40:5 v/v)
Flow rate	:	0.8ml per min
Wavelength	:	255 nm
Injection volume	:	10 µl
Run time	:	10min.

PREPARATION OF BUFFER AND MOBILE PHASE

Preparation of Triethylamine buffer (pH-4.5): Take 6 ml of triethylamine in to a 1000ml of HPLC water and set the pH to 4.5 using Orthophosphoric acid.

Preparation of mobile phase: Accurately measured 550 ml (55%) of ACN and 400 ml of Triethylamine buffer (40%) and 5ml of Methanol(5%) were mixed and degassed in an digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

RESULTS AND DISCUSSION

Method development and optimization

Initially the mobile phase tried was Methanol: Water and Methanol: Phosphate buffer and ACN: Tri ethyl amine buffer with varying proportions. Finally, the mobile phase was optimized to ACN: TEA buffer (pH 4.5): Methanol in proportion 55:40:5 v/v respectively. Wavelength is 255 nm, Injection volume 10 µl.The method was performed with various C18 columns X- bridge column, X-terra, and ODS, Phenomenex Luna C18 column. Symmetry C18 (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

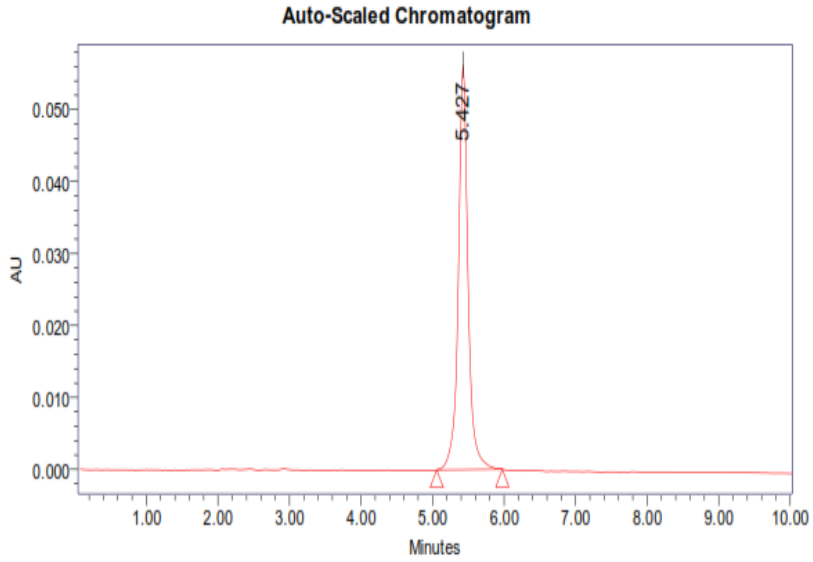

Figure: 1. Chromatogram for optimized trail 7

After conducting total 7 trials 7th trail shows proper peak, tailing, plate count and baseline in the chromatogram. So it's optimized chromatogram.

SYSTEM SUITABILITY

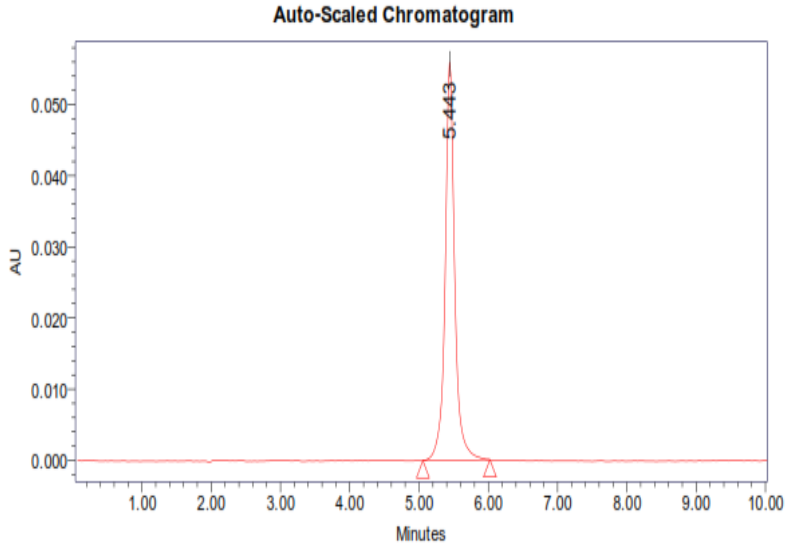

Figure: 2. Chromatogram for system suitability

Table: 1 Results of system suitability parameters for Olmesartan

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP tailing	USP plate count
1	Olmesartan	5.443	533969	55991	1.05	9186

Theoretical plates must be not less than 2000.Tailing factor must be not less than 0.9 and not more than 2.It was found from above data that all the system suitability parameters for developed method were within the limit.

METHOD VALIDATION

Linearity: The linearity study was performed for concentration range of 10-50ppm of Olmesartan of and the correlation coefficient was found to be 0.999 (NLT 0.999).

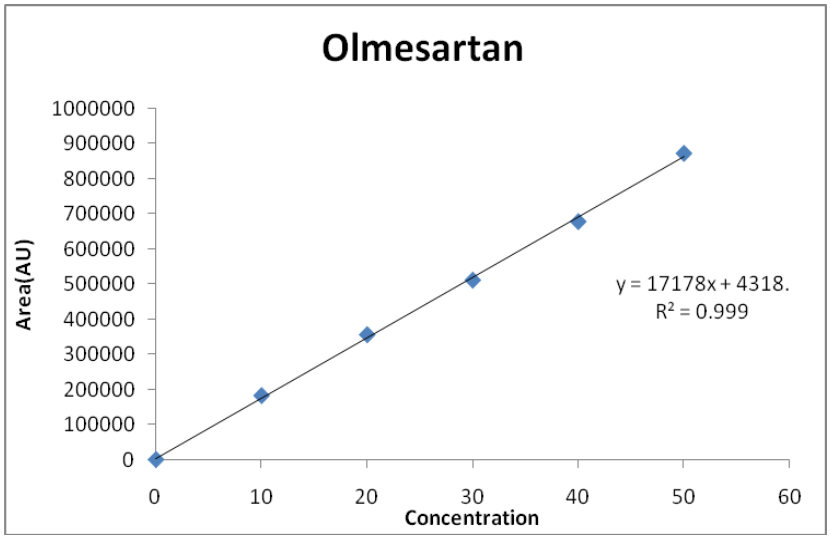

Figure: 3. Calibration curve of Olmesartan

Precision: The repeatability of the method was checked by repeated analysis of the formulation for six times with the same concentration. The amount of drug present in the formulation was calculated. The percentage RSD value was calculated. %RSD for sample should be NMT 2

Table:2 Results of method precision for Olmesartan

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Olmesartan	5.419	507837	54219	8931.7	1.1
2	Olmesartan	5.405	510468	54508	8957.7	1.1
3	Olmesartan	5.478	514561	55259	8764.6	1.1
4	Olmesartan	5.466	515381	55552	9037.7	1.1
5	Olmesartan	5.466	516416	55653	8972.4	1.1
6	Olmesartan	5.474	518217	55506	8953.2	1.1
Mean			513813.4			
Std.dev			3901.1			
%RSD			0.8			

Accuracy: Accuracy of the method was confirmed by recovery studies. To the pre-analyzed formulation a known quantity of the standard drug (50%, 100%, 150%) solution was added and the amount of drug recovered was calculated. The percentage RSD value was calculated. The percentage recovery was found to be within the limit (98-102%).The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Intermediate Precision/Ruggedness: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. %RSD of five different sample solutions should not more than 2.The %RSD obtained is within the limit, hence the method is rugged.

Table:3 The accuracy results for Olmesartan

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	89181	5	4.94	98.8%	98.3%
100%	172784	10	9.8	98%	
150%	257863	15	14.7	98.3%	

Table: 4 Results of ruggedness for Olmesartan

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate countCount	USP Tailing
1	Olmesartan	5.452	516091	54804	9009.0	1.1
2	Olmesartan	5.446	518221	54903	9131.5	1.1
3	Olmesartan	5.493	519536	55996	9015.7	1.0
4	Olmesartan	5.484	519881	56102	8987.3	1.0
5	Olmesartan	5.419	519895	55577	9070.5	1.0
6	Olmesartan	5.406	522826	55808	9047.6	1.0
Mean			519408.3			
Std. Dev.			2216.8			
% RSD			0.4			

Robustness

The robustness was performed for the flow rate variations from 0.7 ml/min to 0.9ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Olmesartan. The method is robust only in less flow condition and the method is robust even

by change in the Mobile phase ±10%. The standard and samples of Olmesartan were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count. The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

Table: 5 Results for variation in flow

S.no	Drug name	Flow (ml/min)	Area	Height	USP plate count	USP Tailing
1	Olmesartan	Less (0.7)	566441	58074	9364	1.02
		Actual(0.8)	530023	56127	9118	1.03
		More (0.9)	459187	53155	7559	0.98

Limit of detection and limit of quantification

The LOD and LOQ were determined by kD/S where k is constant (3.3 for LOD and 10 for LOQ), SD is the standard deviation of the analytical signal, and s is the slope of the concentration/response graph. LOD is 2.2µg/ml, LOQ is 6.8µg/ml.

Effect of Variation of mobile phase organic composition: The sample was analyzed by variation of mobile phase i.e. ACN: TEA buffer: Methanol was taken in the ratio and 65:30:5, 45:50:5 instead of 55:40:5 remaining conditions are same. 10µl of the above sample was injected twice and chromatograms were recorded.

Table: 6 Results for variation in mobile phase composition

S.no	Drug name	Organic (ml/min)	Area	Height	USP plate count	USP Tailing
1.	Olmesartan	Less	24366	2297	12009	1.00
		Actual	530023	56127	1.03	9118
		More	93382	12093	6274	1.07

CONCLUSION

The HPLC method developed for quantitative and related substance determinations of Olmesartan in both bulk drugs and pharmaceutical dosage forms were precise, accurate and specific. From the results of system suitability parameters it was observed that the peak was sharp and symmetrical with satisfactory capacity factor and column efficiency. The developed method was validated by means of Accuracy, Precision, Linearity and Range, and LOD and LOQ as per the guideline prescribed by ICH. The method was completely validated showing satisfactory data for all the method validation parameters tested. The

developed method for the routine analysis of production samples and also to check the stability of Olmesartan samples. hence it can be employed for routine analysis in quality control laboratories.

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