ejpmr, 2018,5(12), 316-321

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

DEVELOPMENT AND VALIDATION OF AN RP-HPLC METHOD FOR ESTIMATION OF MOXONIDINE IN BULK AND FORMULATION

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Article Received on 02/10/2018 **Article Revised on 22/10/2018** **Article Accepted on 12/11/2018**

ABSTRACT

A rapid and precise Reverse Phase High Performance Liquid Chromatographic method has been developed for the validated of Moxonidine, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Phenomenex Luna C18 (4.6 x 250mm, 5µm) column using a mixture of Methanol and Water (75:25% v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 223nm. The retention time of the Moxonidine was 2.7 ±0.02min. The method produce linear responses in the concentration range of 20-100ppm of Moxonidine. The method precision for the determination of assay was below 2.0% RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

KEYWORDS: Moxonidine, RP-HPLC, validation.

INTRODUCTION

Moxonidine is an antihypertensive agent whose site of action is the Central Nervous System (CNS), specifically involving interactions with I1- imidazoline and alpha-2-adrenergic rececptors within the rostral ventrolateral medulla (RSV). Mechanism of action is stimulation of central alpha 2-adrenergic receptors is associated with sympathoadrenal suppression and subsequent reduction of blood pressure. As this class was further explored it was discovered that sympathoadrenal activity can also be suppressed by a second pathway with a newly discovered drug target specific to imidazolines. Specifically, moxonidine binds the imidazoline receptor subtype 1 and to a lesser extent αlpha-2-adrenoreceptors in the RSV causing a reduction of sympathetic activity, reducing systemic vascular resistance and thus arterial blood pressure. The literature review reveals few analytical methods such as HPTLC,[1] Liquid Chromatography[2] and HPLC.[3] Only few methods were reported for RP-HPLC[4] for the estimation of this drug in bulk and in its formulation. Hence the present work targeted to develop a new precise, accurate and sensitive RP-HPLC[5-10] method for the determination of Moxonidine in API and formulation. The developed method validated as per ICH guidelines.[11,12]

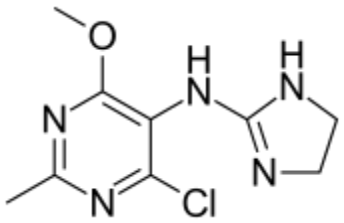

Figure 1: Structure of Moxonidine.

MATERIALS AND METHODS

Chemicals and reagents used

Moxonidine as pure standard reference drug was obtained from SURA LABS, Hyderabad, India. Acetonitrile, Methanol and Water used were of HPLC grade and purchased from MERCK specialties Private Limited, Mumbai, India.

Apparatus

HPLC analysis was performed on chromatographic system of water 2695 separation module with empower software liquid chromatography comprising water 996 photo diode array detector, Symmetry C18 (4.6×250mm)5µ was used and an equipped with auto sampler.

Preparation of standard solution

Accurately weigh and transfer 10 mg of Moxonidine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.6ml of the above Moxonidine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Preparation of Mobile Phase

Preparation of mobile phase

Accurately measured 250ml (25%) of HPLC Water and 750ml (75%) of HPLC Methanol in to a 1000ml of volumetric flask and degassed in a digital ultrasonicator for 10 minutes.

Diluent Preparation

The Mobile phase was used as the diluent.

Experimental conditions

Chromatographic separation achieved using an analytical Symmetry C18 (4.6×250mm)5µ. Mobile phase consisted of Methanol: Water (75:25% v/v). The elution was achieved isocratically at a flow rate of 1.0ml/min with injection volume of 10µl. the column temperature was set at ambient temperature and chromatograph was recorded at wavelength 223nm.

Method development

Trials showed that mobile phase with reverse phase Symmetry C18 (4.6×250mm)5µ column gives symmetric and sharp peaks. After the optimization of chromatographic conditions, estimation of Moxonidine as carried out by the developed RP-HPLC method. Standard solution of drug was injected separately and chromatogram of Moxonidine was recorded in Figure 2. Now the sample solution was injected separately and chromatogram was recorded until the reproducibility of the peak areas were satisfactory. The sample chromatogram was shown in figure 3.

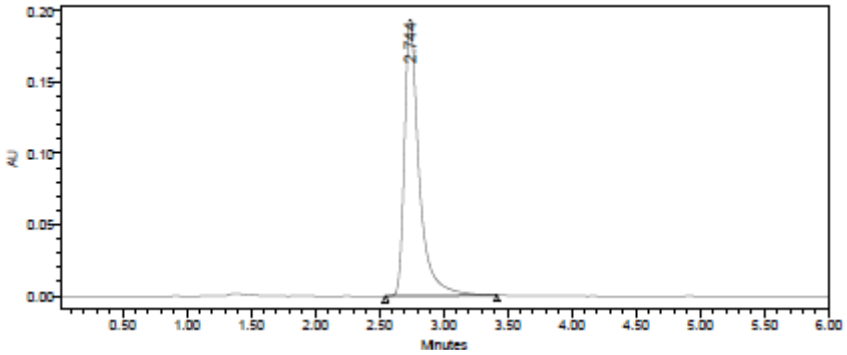

Figure 2: Standard Chromatogram of Moxonidine.

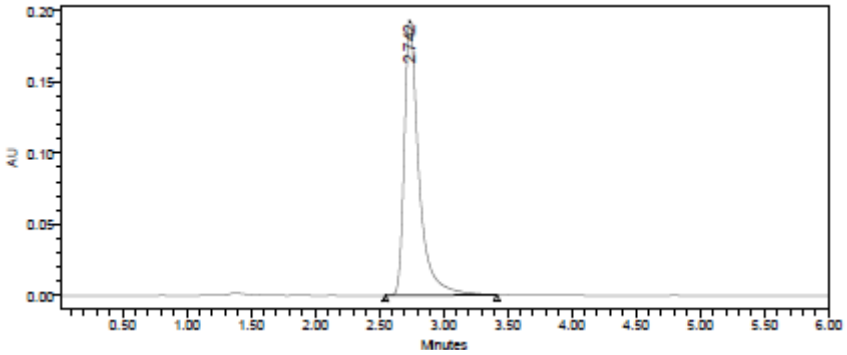

Figure 3: Sample Chromatogram of Moxonidine.

Analytical method validation

HPLC method was validated[13,14] according to the International Conference on Harmonization guidelines (ICH Q2B, validation of analytical procedures, methodology). The method was validated for parameters such as linearity, precision, accuracy, system suitability limit of detection, limit of quantification and robustness.

Linearity

Inject each level (20, 40, 60, 80, 100µg/mL) solutions (prepared from standard stock solution) into HPLC system and observed the linear relationship between concentration and peak area in the concentration range of 20 – 100µg/mL. Calibration curves were plotted with observed peak areas against concentration followed by

the determination of regression equations and calculation of the correlation coefficients.

Precision

Repeatability

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 calculated.

Intermediate precision

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions. For intermediate precision % RSD was calculated from repeated studies.

Accuracy

Inject the three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Moxonidine and calculate the individual recovery and mean recovery values.

Robustness

Robustness was done by changing the actual chromatographic conditions like mobile phase ratio and flow rate. Results were determined by calculating the %RSD for injections peak area values of each change in condition.

System suitability

This parameter used to know whether the HPLC system is suitable for actual chromatographic conditions or not. System suitability was estimated by injecting five standard solutions of Moxonidine and from the chromatograms %RSD, theoretical plates and peak symmetry were calculated.

Specificity

Specificity of a method was determined by testing standard substances against potential interferences. The method was found to be specific when the test solution was injected.

Limit of detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Quantitation limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

RESULTS AND DISCUSSION

Linearity and range

Linearity and range estimated by constructing the calibration curve by taking concentration on X-axis and peak area on Y-axis of (20, 40, 60, 80 and 100µg/mL) solutions (prepared from standard stock solution) and calculate the correlation coefficient. Correlation Coefficient (r) is 0.99, and the intercept 9423. These values meet the validation criteria as shown in Figure 4 and linearity values tabulated in Table 1.

Table 1: Chromatographic data for linearity study.

Concentration Level (%)	Concentration µg/ml	Average Peak Area
60	20	506172
80	40	1061027
100	60	1542964
120	80	2083016
140	100	2539881

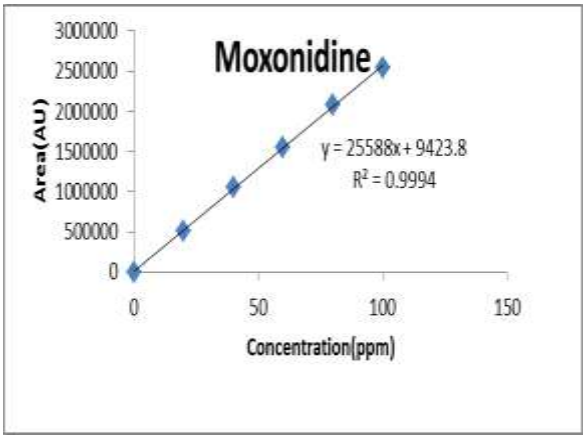

Figure 4: Calibration curve of Moxonidine.

Precision

Intermediate precision

Day 1

The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits. The results were reported in table 2.

Day 2

The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits. The results were reported in table 3.

Table 2: Results of Intermediate precision for Moxonidine.

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Moxonidine	2.744	1532019	193578	8937	1.27
2	Moxonidine	2.742	1532127	195358	8826	1.33
3	Moxonidine	2.745	1533916	194712	9174	1.3
4	Moxonidine	2.740	1536916	196617	6916	1.17
5	Moxonidine	2.740	1538575	196709	5582	1.2
6	Moxonidine	2.768	1547986	200278	6552	1.1
Mean			1536923			
Std. Dev.			6020.166			
% RSD			0.391702			

Table 3: Results of Intermediate precision Day 2 for Moxonidine.

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Moxonidine	2.744	1536490	193619	8927	1.0
2	Moxonidine	2.742	1536351	195397	7725	1.13
3	Moxonidine	2.745	1539021	194759	6816	1.27
4	Moxonidine	2.740	1539344	196639	7187	1.22
5	Moxonidine	2.740	1540984	196731	9917	1.18
6	Moxonidine	2.742	1540351	195505	7563	1.11
Mean			1538757			
Std. Dev.			1941.276			
% RSD			0.126159			

Repeatability

Multiple sampling from a sample solution was done and five working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas Standard Deviation and % Relative Standard Deviation are mentioned in Table 4.

Table 4: Results of repeatability for Moxonidine.

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Moxonidine	2.744	1537286	193619	8846	1.18
2	Moxonidine	2.742	1535366	195397	7927	1.3
3	Moxonidine	2.745	1536325	194759	7588	1.22
4	Moxonidine	2.740	1530184	196639	6817	1.12
5	Moxonidine	2.740	1547547	196731	9033	1.1
Mean			1537342			
Std.dev			5662.526			
%RSD			0.368332			

Accuracy

Inject the three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. The accuracy results for Moxonidine are recorded in Table 5.

Table 5: The accuracy results for Moxonidine.

% Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1524991	30	29.8	99.3	99.6%
100%	3017461	60	59.9	99.8	
150%	4576325	90	89.89	99.8	

Robustness

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Moxonidine. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Moxonidine 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 results were recorded in Table 6.

Table 6: Results for Robustness.

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.8 mL/min	1536490	2.744	7583	1.12
Less Flow rate of 0.7mL/min	1537522	3.009	8947	1.13
More Flow rate of 0.9mL/min	1529711	2.563	9917	1.11
Less organic phase (about 5% decrease in organic phase)	1502872	3.199	8771	1.22
More organic phase (about 5% Increase in organic phase)	1528472	2.467	9471	1.4

System suitability

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. The results were cited in table 7.

Table 7: Results of system suitability for Moxonidine.

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Moxonidine	2.744	1536490	193619	7836	1.1
2	Moxonidine	2.742	1536351	195397	8826	1.14
3	Moxonidine	2.745	1539021	194759	5928	1.14
4	Moxonidine	2.740	1539344	196639	7758	1.22
5	Moxonidine	2.740	1540984	196731	9573	1.1
Mean			1538438			
Std. Dev.			1777.251			
% RSD			0.115523			

Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately quantitate Moxonidine in drug product. The percentage purity was found to be 98.9%. The results for specificity of Moxonidine were cited in Table 8 and Table 9.

Table 8: Peak results for assay standard.

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Moxonidine	2.742	1540351	195505	1.7	3291.9	1
2	Moxonidine	2.745	1535453	194292	1.7	3370.6	2
3	Moxonidine	2.743	1530767	195279	1.7	3315.3	3

Table 9: Peak results for Assay sample.

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Moxonidine	2.768	1547986	200278	1.14	7554	1
2	Moxonidine	2.773	1546861	200103	1.22	8926	2
3	Moxonidine	2.771	1549654	200370	1.17	7748	3

Limit of detection for moxonidine

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The value was found to be 3.6µg/ml.

Quantitation limit for moxonidine

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined. The value was found to be 11.1µg/ml.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Moxonidine in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Moxonidine was freely soluble in acetonitrile ethanol, methanol and sparingly soluble in water. Water: Methanol (25:75% v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Moxonidine in bulk drug and in Pharmaceutical dosage forms.

ACKNOWLEDGEMENT

Authors express their sincere thanks to our principal Dr. M. Dhanalakshmi, M.Pharm, Ph.D. KLR Pharmacy College, Palvoncha and the Management of KLR Pharmacy College for providing necessary facilities to carry out the research work.

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