INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF DOXAZOSIN FOR THE PRESENCE OF DEGRADATION PRODUCTS AND RELATED COMPOUNDS IN ITS TABLET DOSAGE FORM

K.S. Nataraj *, A. Srinivasa Rao, G.M. Kiranmai, U.L. Bhargavi and G. Karishma
Shri Vishnu College of Pharmacy, Bhimavaram, West Godavari (Dt), Andhrapradesh, India
*Corresponding Author Email: kalakondan@yahoo.com

Article Received on: 13/07/18 Approved for publication: 12/08/18

DOI: 10.7897/2230-8407.098169

ABSTRACT

A novel, simple, accurate and reproducible reverse phase high performance liquid chromatographic (RP-HPLC) method has been developed for the development and validation of Doxazosin Mesylate in Presence of its degradation Products & Related compounds in its tablet dosage form. Chromatography was performed on a 150×4.6 mm, Thermo Hypersil BDS C_8-column within a runtime of 55.00 min under gradient elution by a mixture of buffer and Acetonitrile and water at a flow rate of 0.8ml/min. A photodiode array (PDA) detector set at 210nm was used for detection. The method was validated according to the ICH guidelines with respect to specificity, precision, accuracy linearity and robustness. All the validation parameters have come under the limits.

Keywords: Doxazosin Mesylate, RP-HPLC, Gradient elution, Validation

INTRODUCTION

Reversed-Phase Chromatography is the reverse of Normal-Phase Chromatography in the sense that it involves the use of a non-polar stationary phase and a polar mobile phase[1]. As a result, a decrease in the polarity of the mobile phase results in a decrease in solute retention[2]. Modern Reversed-Phase Chromatography typically refers to the use of chemically bonded stationary phases, where a functional group is bonded to silica, for this reason, Reversed-Phase[3]. Polymeric stationary phases such as polymethacrylate or polystyrene[4], or solid stationary phases such as porous graphitic carbon, are used[5].

The aim of the present work was to develop a RP-HPLC method for the presence of its degradation products and related compounds in its tablet dosage form.

Doxazosin Mesylate[6-8] is an antihypertensive agent and its chemical name is 1-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-4-(1,4 benzodioxan- 2-ylcarbonyl) piperazine Mono methane sulfonate and is used to treat hypertension and benign prostatic hyperplasia. The structure was shown in figure 1.

MATERIALS AND METHODS

Instrumentation

An HPLC instrument of Shimadzu, weighing balance (MettlerToledo), P^H meter (Digeson), Sonicator (Fast Clean),

Chemicals and Reagents

Methanol (HPLC grade), Acetonitrile (HPLC grade), Orthophosphoric acid (EMPLURA grade), Hydrochloric acid (EMPLURA grade), Water (Milli Q grade).

Chromatographic Conditions

Column**:** Thermo Hypersil BDS C_8 150×4.6 mm, 3.5µm, flow rate of 0.8ml/min at detector wavelength of 210 nm and injection volume of 10µl at column oven temperature of 35 ^{0}C and at runtime of 55mins of gradient pump mode.

Preparation of Solutions

Preparation of Buffer
25g of Ortho phosphoric acid (84%-86%) was dissolved in 500 ml of water. It was filtered through 0.45µm nylon membrane filter.

Preparation of Mobile Phase-A
100% buffer was mobile phase-A.

Preparation of Mobile Phase-B
Acetonitrile was mobile phase-B.

Preparation of Mobile Phase-C
Milli Q water (Filter through 0.45µm Nylon filter) was mobile phase-C.

Preparation of Diluent
Mixed the composition of 0.1N HCl and Methanol in the ratio of 1:9 v/v

Preparation of standard stock solution

Weighed accurately 20.0mg of Doxazosin Mesylate working standard and it was transferred into a 200ml volumetric flask, to this 140ml of diluent was added and sonicated to dissolve the drug. Allow the solution to equilibrate at room temperature and diluted up to the volume with diluent and mix well. (This solution

contains about 100.0 µg/ml of Doxazosin). The chromatogram was shown in Fig 3.

Preparation of diluted standard solution

0.5ml of above standard stock solution was pipette out and transferred into a 100mL volumetric flask and dilute up to the volume with diluent and mix well. (This solution contains about 0.5µg/ml of Doxazosin.)

Preparation of Placebo Solution

Weighed accurately a portion of the placebo powder equivalent to 5mg of Doxazosin and it is quantitatively transfer into a 20ml volumetric flask. Added 15 ml diluent and it is sonicated for 30 minutes with intermittent shaking, the solution was cooled at room temperature. It is making up to the mark with diluent and it is mixed well. It is filtered through 0.45µm Millipore filter.

Preparation of sample solution

Weighed and powdered 10 tablets and weighed accurately a portion of the tablet powder equivalent to 5mg of Doxazosin and was quantitatively transferred into a 20ml volumetric flask. Then 15ml of diluent was added and it was sonicated for 30 minutes with intermittent shaking, then the solution was cooled to room temperature. It is making up to the mark with diluent and it is filtered through 0.45µm Millipore filter. (This solution contains about 250.0µg/ml of Doxazosin).

***Preparation* of *Terazosin Related Compound-A* standard stock solution *(TRC-A*)**
Weighed accurately 10.0 mg of Terazosin Related Compound-A standard and it was transferred into a 100 ml volumetric flask. Then 70ml of diluent was added and it was sonicated to dissolve and dilute up to the volume with diluent and mix well. (This solution contains about 100.0 µg/ml of Terazosin Related Compound-A).

***Preparation* of *Terazosin Related Compound-A Intermediate* standard solution (TRCIS-A)**
5.0 ml of Terazosin Related compound-A standard solution was taken into a 100ml of volumetric flask dilute up to volume with diluent and mix well. (This solution contains about 5.0 µg/ml of Terazosin Related Compound-A).

***Preparation* of *Terazosin Related Compound-A* standard solution at specification level**
5.0ml of Terazosin Related Compound-A Intermediate standard solution was taken into a 20ml of volumetric flask dilute up to volume with diluent and mix well. (This solution contains about 1.25 µg/ml of Terazosin Related Compound-A).

Preparation* of *Doxazosin Related Compound-D* standard stock solution *(TRC-D)
Weighed accurately 10.0mg of Doxazosin Related Compound-D standard and it was transferred into a 100ml volumetric flask. Then add 70ml of diluent was added and it was sonicated to dissolve and dilute up to volume with diluent and mix well. (This solution contains about 100.0µg/ml of Doxazosin Related Compound-D).

***Preparation* of *Doxazosin Related Compound-D Intermediate* standard solution (TRCIS-D)**
5.0 ml of Doxazosin Related Compound-D standard stock solution was taken into a 100 ml of volumetric flask and dilute up to volume with diluent and mix well. (This solution contains about 5.0 µg/ml of Doxazosin Related Compound-D).

***Preparation* of *Doxazosin Related Compound-D* standard solution at specification level**
5.0 ml of Doxazosin Related Compound-D Intermediate standard solution was taken from into a 20 ml of volumetric flask and dilute up to volume with diluent and mix well. (This solution contains about 1.25 µg/ml of Doxazosin Related Compound-D).

Method development

Several trials have been made until getting good peak resolution, acceptable plate count and tailing factor. Method was optimized with chromatographic conditions like 150×4.6 mm, Thermo Hypersil BDS C_8-column within a runtime of 55.00 min under gradient elution by a mixture of buffer and Acetonitrile and water at a flow rate of 0.8ml/min. A photodiode array (PDA) detector set at 210nm was used for detection. And the retention time was reported as 5.42 minutes for Terazosin impurity-A and 16.06 minutes for Doxazosin impurity-D and 20.56 for Doxazosin Mesylate respectively. The gradient program of optimized chromatogram is shown in Table 1 & 2 and the chromatogram are shown in Fig 2.

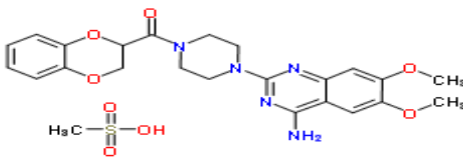

Fig 1: Structure of Doxazosin Mesylate

Table 1: Gradient program for Optimized Chromatogram

Time (min)	Mobile Phase A % V/V	Mobile Phase B % V/V	Mobile phase C % V/V
0.01	20	10	70
0.8	20	22	58
15.0	20	50	30
25.0	20	55	25
35.0	20	55	25
45.0	20	10	70
55.0	20	10	70

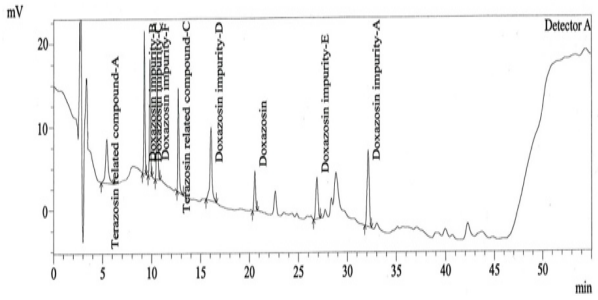

Fig 2: Optimized Chromatogram

Table 2: Results for Optimized Chromatogram

Peak Name	Retention Time	Area	Resolution	R R T
Terazosin RC-A	5.42	78160	...	0.26
Doxazosin Impurity-B	9.24	103937	11.80	0.45
Doxazosin Impurity-C	9.81	90307	2.61	0.48
Doxazosin Impurity-F	10.55	84045	3.09	0.51
Terazosin RC-C	12.69	88323	8.80	0.62
Doxazosin Impurity-D	16.06	101970	11.74	0.78
Doxazosin	20.56	41551	14.10	1.00
Doxazosin Impurity-E	26.87	60869	17.66	1.31
Doxazosin Impurity-A	32.13	109850	12.80	1.56

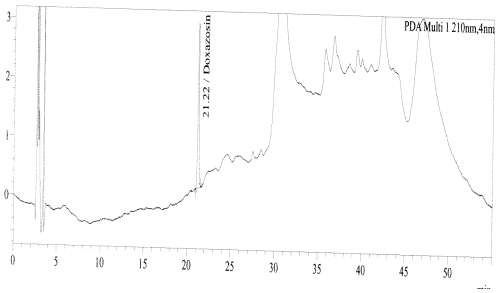

Fig 3: Chromatogram for standard solution

Table 3: System Suitability Parameters

Parameter	Result	Acceptance criteria
Theoretical plate count	83821	NLT 2000
Tailing factor	1.1	NMT 2.0
% RSD	1.9	NMT 10.0
System performance	0.7	NMT 10.0%

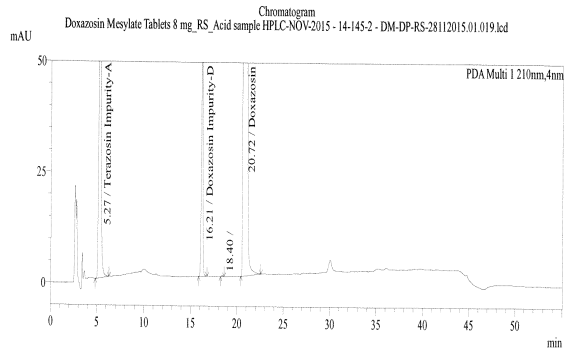

Fig 4: A typical chromatogram of the Acid treated sample solution

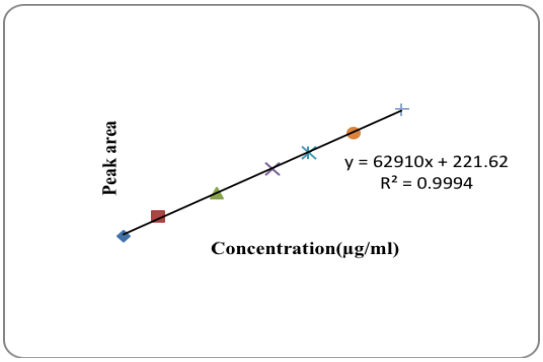

Fig 5: Calibration curve of Doxazosin

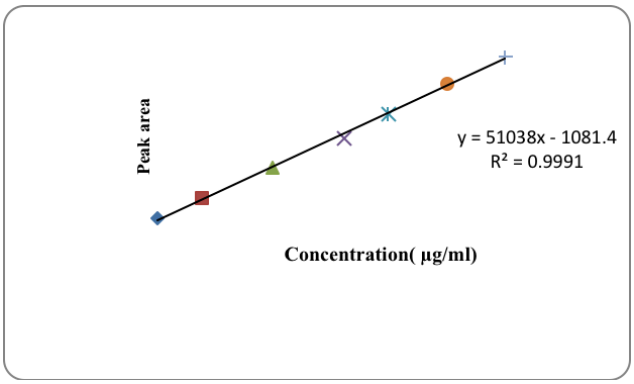

Fig 6: Calibration curve of Terazosin – A

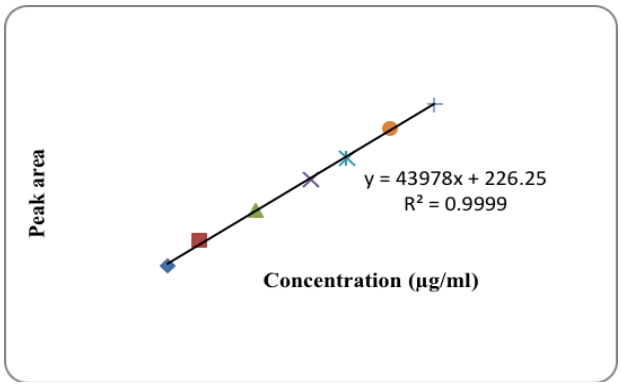

Fig 7: Calibration curve of Doxazosin impurity-D

RESULTS AND DISCUSSIONS

According to ICH guidelines the method was validated for parameters such as system suitability, accuracy, precision, linearity, robustness, ruggedness specificity and LOD and LOQ.

System suitability

The standard solutions of Doxazosin Mesylate were prepared in 5 replicates as per test method and injected into HPLC system. The system suitability parameters like Retention time, Peak area, USP Plate count, USP tailing factor were evaluated as per the test method and checked for acceptable limits.

From the system suitability studies, it was observed that % RSD of retention time of Terazosin Impurity-A was found to be 1.9, and Doxazosin Impurity-D was found to be 1.9. % Theoretical plates were found to be more than 2000. USP tailing factor of Terazosin Impurity-A was found to be1.1, and Doxazosin Impurity-D was found to be 1.1. All the parameters were within the limits. The results are shown in Table 3.

Accuracy

Accuracy of the test method was carried out by spiking known amounts of drug substance of Doxazosin Mesylate with placebo at 50%, 100% and 150% of target concentration in triplicate for each level. Calculated amount recovery, % recovery, mean % recovery and % RSD at each level and the results were checked for acceptable limits.

Accuracy for the average of triplicate from each concentration levels of Terazosin Impurity-A and Doxazosin Impurity-D were within 98.0 to 102.0 %, which shows that the method was accurate.

Linearity

Linearity was performed by preparing Doxazosin Mesylate, Terazosin Impurity-A and Doxazosin Impurity-D standard solution in the range of about 50-150% of test concentration and injected into the HPLC system. Chromatograms were recorded and measured the peak responses. Linearity of detector response was established by plotting a graph between concentration and response of Doxazosin Mesylate, Terazosin Impurity-A and Doxazosin Impurity-D. The squared correlation coefficient was calculated.
From the Linearity data it was observed that the method was showing linearity in the concentration range of 50-150 %. Correlation coefficient was found to be 0.999 shown in Fig 5.

Precision

To evaluate the precision, six samples were prepared and analyzed as per test method and % RSD of six samples are calculated.
The % RSD of 10 Standard injections of Terazosin Impurity-A and Doxazosin Impurity-D were found to be 0.8 respectively were shown in Fig 6 & 7. Hence the method is precise.

Specificity

Blank interference
Blank was prepared and injected as per test method and it was analyzed for any interference with analytical peaks of Doxazosin Mesylate, Terazosin Impurity-A and Doxazosin Impurity-D.at their Retention times.

Placebo interference
Placebo sample was prepared by taking the placebo equivalent to about the weight in portion of test preparation and injected into the HPLC system. No peak should be found at the retention time of Doxazosin Mesylate, Terazosin Impurity-A and Doxazosin Impurity-D.
The Chromatograms of Standard and Sample are identical with same Retention time. No interference due to Placebo and Sample at the retention time of analyte which shows that the method was specific.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Based on the Standard Deviation of the Response and Slope method the LOD and LOQ values were calculated from Linearity data and the LOD and LOQ level solutions were prepared by using standard stock solutions and injected in to HPLC individually as per the sequence.

The Limit of Detection and Limit of Quantitation was calculated from the linearity curve method using slope, and standard deviation of intercepts of calibration curve.

Ruggedness (Intermediate Precision)

To evaluate Ruggedness diluent (blank), placebo solution, plain sample solution and six Accuracy as recovery sample were injected as per sequence and calculated the amount of known impurities was recovered in percent from the each of Accuracy as Recovery sample and also Calculated the mean recovery and the %RSD. The % Average recovery at 100% level and %RSD for Terazosin Impurity-A is 94.5 and0.8 respectively and for

Doxazosin Impurity-D is 92.1 and 0.2 respectively. Hence the method is said to be rugged.

Robustness

Effect of variation in flow rate

A study was conducted to determine the effect of variation in flow rate. The system suitability parameters were evaluated at the flow rate of 0.7ml/min and 0.9ml/min. the system suitability results were checked for limits of higher and lower flow rates.

Effect of variation in wavelength

A study was conducted to determine the effect of variation in wavelength. The system suitability parameters were evaluated at 208nm and 212nm wavelengths.

As the percentage Recovery and within limits for variation in flow rate (± 0.1 of the specified flow). Hence the allowable flow rate should be within 0.6 ml to 0.8 ml. The results for robustness of change in wavelength and variations of columns with different lot numbers were illustrated.

Forced degradation studies

Forced degradation studies were conducted in acid, base and peroxide, Thermal, Humidity and homogeneity of the peak was assessed in terms of peak purity. Highly degradation was observed for 2 impurities i.e., Terazosin impurity-A and Doxazosin impurity-D when subjected to acid treatment. The chromatogram is shown in Fig 4.

No degradation was observed for any impurities when subjected to base, peroxide, Humidity, Thermal degradation studies.

CONCLUSION

A new gradient reverse Phase High Performance Liquid chromatographic method development for the determination of Doxazosin Mesylate in presence of its degradation products and related compounds in its tablet dosage form. The chromatographic separation was achieved on a Thermo Hypersil BDS C_8, 150×4.6mm, 5µ, column within a runtime of 55.01 min under gradient elution by a mixture of buffer and acetonitrile and water at a flow rate of 0.8ml/min. A photodiode array (PDA) detector set at 210 nm was used for detection. The method was validated according to the ICH guidelines with respect to specificity, precision, accuracy and linearity, and showing satisfactory data for all the method validation parameters tested. The % RSD for peak area response was found to be within the limit. The proposed method was found to be reproducible and convenient for the analysis of Doxazosin Mesylate, the method can be used for routine quality control analysis.

REFERENCES

1. Urban, Jiří, et al. "Preparation and characterization of polymethacrylate monolithic capillary columns with dual hydrophilic interaction reversed-phase retention mechanism for polar compounds." Journal of separation science 32.15-16; 2009: page numbers 2530-2543.
2. P.Jandera and J.Churacek Gradient elution in column liquid chromatography theory and practice.1985 Vol. 31. Elsevier. https://doi.org/10.1016/S0301-4770(08)60235-3
3. Csaba Horvath and Wayne Melander. "Liquid chromatography with hydro carbonaceous bonded phases; theory and practice of reversed phase chromatography." Journal of Chromatographic Science 15.09.1977: page numbers 393-404.
4. Sander, Lane C., and Stephen A. Wise. "Synthesis and characterization of polymeric C18 stationary phases for liquid chromatography." Analytical Chemistry.1984 (56.3): page numbers: 504-510.
5. Pereira, Luisa. "Porous graphitic carbon as a stationary phase in HPLC: Theory and applications." Journal of Liquid Chromatography & Related Technologies® 31.11.12 (2008): page numbers: 1687-1731.
6. Sanjay BB, Bharathi KR, Yogini JS, Atul SA. Impurity profile: Significance in Active Pharmaceutical Ingredient. Eurasian Journal of Analytical Chemistry. 2007; page numbers: 33-53.
7. Alabaster VA, Davey MJ. The alpha-1 adrenoceptor antagonist profile of doxazosin; preclinical pharmacology. British journal of Clinical Pharmacology volume 21 February 1986(issue S1) page numbers: 95-175.
8. Kasture A.V., Wadodkar S.G., Mahadik K.R., More H.N. Pharmaceutical Analysis. 2nd edition 1997.

Cite this article as:

K.S. Nataraj *et al*. RP-HPLC method development and validation of doxazosin for the presence of degradation products and related compounds in its tablet dosage form. Int. Res. J. Pharm. 2018;9(8):81-85 http://dx.doi.org/10.7897/2230-8407.098169

Source of support: Nil, Conflict of interest: None Declared