

Research Article

Analytical method development and validation of doxazosin mesylate uncoated tablets by RP-HPLC

Santosh Kumar Sreevatsav. A^{*1}, Shyam sunder.K², Jayapal Maleraju¹ and Harish Racha³

¹MRR College Of Pharmacy, Nadergul, Saroornagar, R.R. Dist., India

²Department of Chemistry, S.V. University Tirupati, India

³Dept. of Pharmaceutical Chemistry, MLR Institute of Pharmacy, Dundigal (V), Hyderabad-43, A P, India.

***Correspondence Info:**

Santosh Kumar Sreevatsav. A,
Department of Pharmaceutical Analysis,
MLR Institute of Pharmacy,
Dundigal (V), Hyderabad-43, A P, India
Email: sreevatsav_a@yahoo.com

Abstract

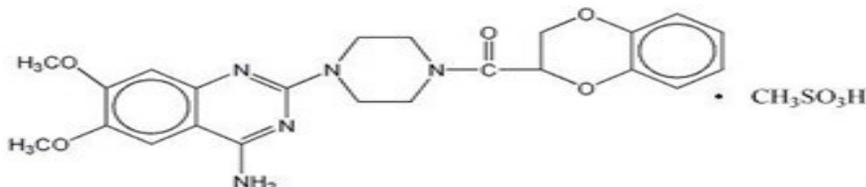
A Simple, Precise, Stability indicating reverse phase HPLC method has been developed and validated in order to determine Doxazosin mesylate uncoated tablet dosage form. A Chromolith RP-18 column made up of Stainless Steel has been used with dimensions 100mm x 4.6mm x 10µm. An Isocratic method with Mobile phase consisting of phosphate buffer: Methanol (40:60) ratio. A UV-Detector is used and the wavelength was set at 251nm. At the flow rate of 1.0ml/min the mobile phase was run for 10 minutes. The method was validated for Linearity, Precision, Accuracy, Robustness, and Stability of analytical solution. The Linearity range for the standard and the placebo was found to be between 50% and 150%. The method was validated for precision in method, system and intermediate. All the validation parameters have come under the limits.

Keywords: Chromolith column, Methanol, Phosphate buffer, Isocratic, Stability

1. Introduction

Doxazosin Mesylate is a quinazoline compound that is a selective inhibitor of the alpha1 subtype of alpha-adrenergic receptors. Doxazosin mesylate (4 amino-2-[4-(1, 4-benzodioxan-2-carbonyl)-piperazine-1-yl]-6,7-methoxy quinazoline mesylate, a quinazoline derivative, is effective and well known for treatment of hypertension and benign prostatic hyperplasia by selectively blocking α-1 adreno receptor^{1,2}. Several methods have been reported for the determination of Doxazosin in plasma and pharmaceutical formulations including HPLC, LC-MS, UV spectrophotometry and HPTLC^{3,4,5,6}.

Structure:



Doxazosin mesylate

2. Material and Methods

2.1 Instruments / Equipments Used

H.P.L.C- Waters - Alliance 510 with UV- 484 Data Ace software (Instrument I.D: AL-011)

HPLC - Agilent 1100 Series with Chromeleon software (Instrument I.D: AL-013)

HPLC Analytical column Chromolith RP - C18, 100mm x 4.6mm x 10 μ (C-098 & C-099)

Analytical weighing balance - Mettler Toledo B204S

Millipore membrane 0.45 μ m

Laboratory accessories

2.2 Chemicals Used

Doxazosin mesylate working standard (WRS No-WS-005; Purity – 99.98% on dry basis).

DOXACARD – 2 Tablets (B.No: - A93567)

Placebo or Excipients mixtures (about 100g)

Potassium Dihydrogen Ortho Phosphate - AR

Sodium Hydroxide-AR

Methanol -AR

Chromatographic conditions	
Column	Chromolith RP - C18, 100mm x 4.6mm x 10 μ
Buffer	Weigh accurately and transfer 6.8 g of Potassium dihydrogen orthophosphate to a 1000 ml volumetric flask. Add about 980 ml of water, dissolve and dilute to volume with water.
Mobile Phase	For isocratic system, prepare a mixture of Methanol, and buffer in the proportion 60 : 40 respectively. Mix well, adjust the pH to 5.0 \pm 0.05 with sodium hydroxide pellets. Filter through 0.2 μ Nylon membrane filter paper and degas prior to use.
Wavelength	251 nm
Flow Rate	1.0 ml / minute
Injection volume	20 μ l
Run time	10 minutes
Blank solution	Use Mobile phase as blank
Diluent	Use Mobile phase as blank

a) Preparation of Doxazosin mesylate Standard Solution: Weigh accurately about 20 mg of Doxazosin mesylate working standard and transfer to a 20 ml volumetric flask. Add 10 ml of diluent and sonicate to dissolve. Dilute to volume with diluent and mix. Transfer 1.0 ml of solution into a 10 ml of volumetric flask and dilute to volume with the diluent and mix.

(Dilution scheme: 20mg @ 20.0 ml @ 5 ml /10.0 ml)

b) Preparation of Test Solution: Weigh and transfer 139.7mg of sample powder into a 20 ml volumetric flask. Add about 10 ml of diluent and shake for 20 minutes by mechanical means or manually and further sonicate for 30 minutes. Dilute up to mark with diluent. Centrifuge this solution at 8000 rpm for 10 minutes. Decant the supernatant solution into another test tube and transfer 1.0 ml of supernatant solution into another 10 ml volumetric flask and make up the volume with diluent. Further transfer 1.0ml of solution into another 10 ml volumetric flask and make up the volume with diluent. Filter the solution through 0.45 μ m nylon membrane filter.

(Dilution scheme: 139.7mg @ 1 ml /10.0 ml @ 1 ml /10.0 ml)

c) System Suitability Solution: Use Doxazosin mesylate standard working solution as system suitability solution.

d) Procedure: Separately inject equal volumes of blank, five replicate injections of system suitability solution (Doxazosin mesylate standard working solution). Then inject two injections of test solution and record the chromatograms. Disregard any peak due to blank in the test solution. Calculate % RSD of five replicate injections of system suitability solution (Doxazosin mesylate standard working solution). Check tailing factor and theoretical plates of the peak in the

chromatogram obtained with 5th injection of system suitability solution (Doxazosin mesylate standard working solution). The limits are as below,

- 1) Theoretical plates should be not less than 2000.
- 2) Tailing factor should be less than 2.0.
- 3) % RSD should be not more than 2.0%.

2.3 Calculations:

$$\% \text{ Assay} = \frac{\text{AT} \times \text{WS} \times 1 \times 20 \times 10 \times 10 \times \text{AW} \times 100 - (\text{LOD})}{\text{AS} \times 20 \times 10 \times \text{WT} \times 1 \times 1 \times \text{L.C} \times \text{P}}$$

AT: Average Peak area of Doxazosin in test solution

AS: Mean peak area of Doxazosin in system suitability solution

WS: Weight of Doxazosin mesylate working standard taken in mg

WT: Weight of Tablet powder taken in mg

P: Assay of Doxazosin mesylate working standard in % on as is basis

L.C: Label Claim

LOD: Loss on drying

Express the results up to two decimals.

3. Results and discussions

3.1 Specificity / Selectivity: Selectivity was performed by injecting the diluent blank solution, excipient blend, system suitability solution, test solution

3.1.1 Acceptance criteria: The Doxazosin mesylate peak should be well resolved from any other peak and from each other. The diluent blank solution, excipient blend solution should not show any peak at the retention time of the Doxazosin mesylate.

3.1.2 Results: The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method.

Table-1 System Suitability - Selectivity

Sr. No.	Area of Doxazosin mesylate
1	2613.452
2	2668.983
3	2696.889
4	2602.931
5	2663.436
Mean	2641.22
Standard Dveiation	39.27
(%) Relative Standard Deviation	1.49

All the injections were processed at the wavelength provided in the method. There was no interference observed from diluent blank solution, excipient blend solution with Doxazosin mesylate peak.

3.2 Linearity

3.2.1 Linearity and Range for standard: For the linearity study five standard solutions of Doxazosin mesylate were prepared from the range starting from 50% to 150% of the theoretical concentration of assay preparation.

The system suitability solution and the linearity solutions were injected as per the protocol. The linearity graph of concentration against peak response was plotted and the correlation coefficient was determined.

3.2.2 Acceptance criteria: Correlation coefficient should be greater than or equal to 0.999.

3.2.3 Results: The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. (Refer to Table-2 for system suitability results).

Table 2: System suitability - Linearity of standard

Sr. No.	Area of Doxazosin mesylate
1	2731.58
2	2754.58
3	2749.44
4	2741.26
5	2761.21
Mean	2747.61
Standard Deviation	11.56
(%) Relative Standard Deviation	0.42

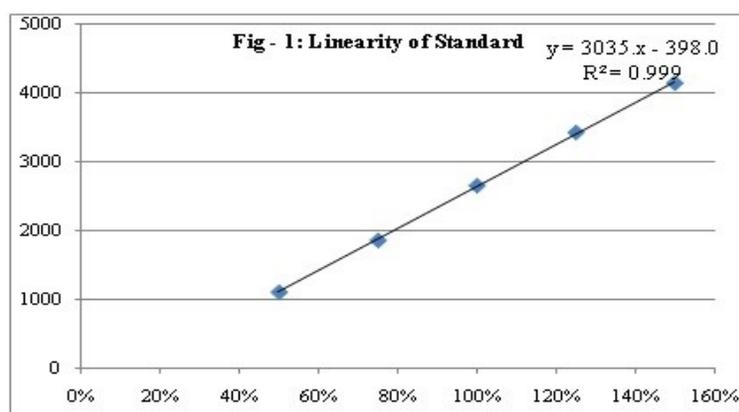
The average peak area of Doxazosin mesylate peak at each concentration level was determined and the linearity graph was plotted against the sample concentration in percentage. The results of linearity study are as given in Table 3.

Table 3: Results of linearity of standard

Linearity level	Sample Concentration (%)	Sample Concentration (in ppm)	Peak Area	Correlation coefficient
Level-1	50	50	1116.53	0.999
Level-2	75	75	1863.22	
Level-3	100	100	2653.81	
Level-4	125	125	3420.21	
Level-5	150	150	4132.10	

The linearity plot of peak area of **Doxazosin mesylate** Vs. standard concentration in percentage is presented in figure-1.

Figure 1: Linearity graph of Doxazosin mesylate standard



3.3 Linearity and Range for standard in presence of placebo:

3.3.1 Procedure: For the linearity study five standard solutions of Doxazosin mesylate were prepared from the range starting from 50% to 150% of the theoretical concentration of assay preparation.

The system suitability solution and the linearity solutions were injected as per the protocol. The linearity graph of concentration against peak response was plotted and the correlation coefficient was determined.

3.3.2 Acceptance criteria: Correlation coefficient should be greater than or equal to 0.999.

3.3.3 Results: The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. (Refer to Table 4 for system suitability results).

Table 4: System suitability - Linearity of standard in presence of placebo

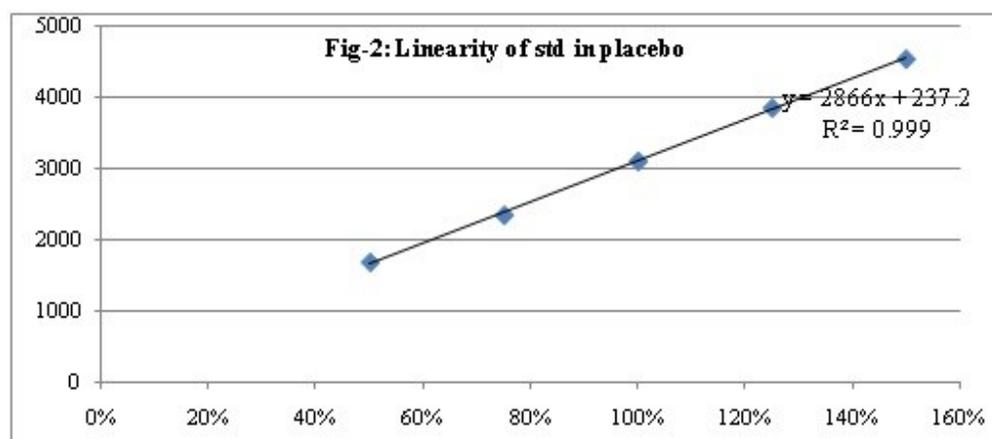
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5	2761.21
Mean	2747.61
Standard Deviation	11.56
(%) Relative Standard Deviation	0.42

The average peak area of Doxazosin mesylate peak at each concentration level was determined and the linearity graph was plotted against the sample concentration in percentage. The results of linearity study are as given in Table - 5.

Table 5: Results of linearity of standard in presence of placebo

Linearity level	Standard Concentration (%)	Standard Concentration (in ppm)	Placebo added to the Standard to the Standard solution	Peak Area	Correlation Coefficient
Level-1	50	50	137.2mg	1692.83	0.999
Level-2	75	75	137.2mg	2302.766	
Level-3	100	100	137.2mg	3100.247	
Level-4	125	125	137.2mg	3845.581	
Level-5	150	150	137.2mg	4527.554	

The linearity plot of peak area of **Doxazosin mesylate** Vs. standard concentration in presence of placebo in percentage is presented in figure - 2.



3.4 Precision:

3.4.1 System Precision:

3.4.1.1 Procedure: The system precision was performed by injecting 10 replicate injections of system suitability solution and the chromatograms are reviewed for the system suitability criteria.

3.4.1.2 Acceptance criteria: % RSD of peak areas of ten replicate injections of system suitability solution should not be more than 2.0% and system suitability criteria should pass as per analytical method.

3.4.1.3 Results: The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method.

Table 6: System precision

Sr. No.	Area of Doxazosin mesylate
1	2682.93
2	2720.06
3	2754.57
4	2775.52
5	2673.65
6	2680.66
7	2678.91
8	2694.05
9	2684.95
10	2685.40
Mean	2703.07
Standard Deviation	35.39
(%) Relative Standard Deviation	1.31

3.4.2 Method Precision:

3.4.2.1 Procedure: Six test solutions of Doxazosin mesylate in DOXACARD-2 and were prepared as per the analytical method. The % RSD of % assay of six test solutions was calculated.

3.4.2.2 Acceptance criteria: % RSD of the results of six test solutions should not be more than 2.0%.

3.4.2.3 Results: The system suitability criterion was found to meet the pre-established acceptance criteria as per the analytical method. The results of assay obtained from six test solutions preparations are presented in Table - 8

Table 7: System suitability - Method precision (Analyst 1)

Sr. No.	Area of Doxazosin mesylate
1	2685.40
2	2690.74
3	2661.86
4	2717.08
5	2714.88
Mean	2693.99
Standard Deviation	22.84
(%) Relative Standard Deviation	0.85

Table - 8: Results of method precision

Test Solution	% Assay of Doxazosin
1	99.97
2	102.10
3	99.64
4	100.16
5	100.36
6	97.89
Mean	100.02
Standard Deviation	1.35
(%) Relative Standard Deviation	1.35

3.4.2.4 Remark : The % RSD of the six assay results is found less than 2.0% and meets the pre-established acceptance criteria. Hence, it is concluded that the method is precise.

3.4.3 Intermediate Precision:

3.4.3.1 Procedure: Six test solutions of DOXACARD-2 Extended Release Tablets and were prepared as per the analytical method on different day. These test solutions were analyzed by a different analyst using different HPLC column of same

make but having different serial number and different HPLC system. The % RSD of % assay results of twelve test solutions (six samples from method precision and six samples from intermediate precision) was calculated.

3.4.3.2 Acceptance criteria: % RSD of the results of twelve test solutions (six of method precision and six of intermediate precision) should not be more than 2.0%.

3.4.3.3 Results: The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. (Refer to Table -10 for system suitability results). The results of assay obtained from six test solutions are presented in Table - 11. % RSD of assay results from method precision and intermediate precision (12 results) are presented in Table - 11.

Table - 9: System suitability - Intermediate precision (Analyst – 2)

Sr. No.	Area of Doxazosin mesylate
1	2613.46
2	2600.08
3	2615.63
4	2665.58
5	2598.26
Mean	2618.60
Standard Deviation	27.38
(%) Relative Standard Deviation	1.05

Table - 10: Results of intermediate precision

Test Solution	% Assay of Doxazosin mesylate
1	100.03
2	102.41
3	102.51
4	102.65
5	102.90
6	100.57
Mean	101.85
Standard Deviation	1.22
(%) Relative Standard Deviation	1.20

Table - 11: Results of twelve test solutions of Doxazosin mesylate in DOXACARD-2 (six of method precision & six of intermediate precision)

Analysis performed during method precision study By Analyst 1 on system 1 and on column 1 on day 1	
Same column	% Assay of Doxazosin mesylate
1	99.97
2	102.10
3	99.64
4	100.16
5	100.36
6	97.89
Analysis performed during intermediate precision study By Analyst 2 on system 2 and on column 2 on day 2	
Column sr. no.	015337030136 01
Test Solution	% Assay of Doxazosin mesylate
7	100.03
8	102.41
9	102.51
10	102.65
11	102.90
12	100.57
Mean	100.93
Standard Deviation	1.55
(%) Relative Standard Deviation	1.54

3.5 Accuracy (% Recovery):

3.5.1 Procedure: Accuracy study was performed by analyzing Doxazosin mesylate test solutions which were prepared by mixing Doxazosin mesylate API with excipient blend.

These test solutions were prepared by adding a quantity of Doxazosin mesylate API to excipient blend to produce three different concentration solutions equivalent to 50%, 75%, 100%, 125% and 150% of test concentration.

3.5.2 Acceptance criteria: Mean recovery at each concentration level should be between 98.0% and 102.0%.

3.5.3 Results: The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. (Refer to Table – 12 for system suitability result). The results of accuracy study obtained are presented in Table-13.

Table - 12: System suitability - Accuracy (%Recovery)

Sr. No.	Area of Doxazosin mesylate
1	2731.58
2	2754.58
3	2749.44
4	2741.26
5	2761.21
Mean	2747.61
Standard Deviation (\pm)	11.56
(%) Relative Standard Deviation	0.42

Table – 13 : Accuracy (%Recovery) – results

Level of addition	Amount of Doxazosin Mesylate added in mg	Amount of Doxazosin Mesylate found in mg	Recovery (%)
First Level (Rec-50%)	11.1	11.09	99.91
Second Level (Rec-75%)	15.2	15.09	99.28
Third Level (Rec-100%)	20.5	20.31	99.07
Fourth Level (Rec-125%)	25.3	25.19	99.57
Fifth Level (Rec-150%)	30.1	29.66	98.54
Mean	-	-	99.27
Standard Deviation	-	-	0.52
(%)Relative Standard Deviation	-	-	0.52

3.5.4 Acceptance criteria: System suitability criteria should pass as per analytical method and the % RSD between results obtained with changed condition and average result of method precision, should not be more than 2.0%.

3.6 Robustness:

3.6.1 Experiment: Prepare two test solutions of the same lot (as used in 7.0.a and 7.0.b) of Doxazosin mesylate in DOXACARD – 2 Tablets as per analytical method. Inject this solution along with diluent blank solution and system suitability solution along different chromatographic conditions as shown below:

Change in column lot (same make, different serial no.)

Change in flow rate (\pm 0.2 ml/minute)

Change in wavelength (\pm 2 nm)

Change in pH of mobile phase (\pm 0.2)

Change in Column Lot: [Normal Experimental Condition: Chromolith RP - C18, 100mm x 4.6mm x 10 μ)

The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. (Refer to Table - 14 for system suitability results).

Table - 14: System suitability - Robustness with Change in Column Lot

Sr. No.	Area of Doxazosin mesylate	
	Same column	Diff column
1	2685.40	3163.68
2	2690.74	3158.53
Mean	2688.07	3161.11
Standard Deviation	3.78	3.64
(%)Relative Standard Deviation	0.14	0.12

The assay results obtained with different flow rate conditions are as given in Table - 15.

Table - 15: Results for Change in Column Lot

Flow rate	Same column	Diff column
Sample	% Assay	
Test Solution	100.49	100.11
Average assay result from method precision	100.02	100.02
Mean	100.26	100.07
Standard Deviation	0.33	0.06
(%) Relative Standard Deviation	0.33	0.06

Change in Flow Rate (± 0.2 mL/minute): (Normal Experimental Condition: 1.0ml/minute)

The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. (Refer to Table - 16 for system suitability results).

Table - 16: System suitability - Robustness with change in flow rate

Sr. No.	Area of Doxazosin mesylate	
	0.8ml/min	1.2ml/min
1	3069.88	2638.01
2	3023.48	2666.13
Mean	3046.68	2652.07
Standard Deviation	32.81	19.88
(%) Relative Standard Deviation	1.08	0.75

The assay results obtained with different flow rate conditions are as given in Table 17.

Flow rate	0.8ml/min	1.2ml/min
Sample	% Assay	
Test Solution	100.97	100.30
Average assay result from method precision	100.02	100.02
Mean	100.50	100.16
Standard Deviation	0.67	0.20
(%) Relative Standard Deviation	0.67	0.20

Change in Wavelength (± 2 nm): (Normal Experimental Condition: 251nm)

The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. (Refer to Table - 18 for system suitability results).

Table - 18: System suitability - Robustness with change in wavelength

Sr. No	Area of Doxazosin mesylate	
	249nm	253nm
1	2615.63	2666.76
2	2672.46	2684.13
Mean	2644.05	2676.45
Standard Deviation	40.18	10.87
(%) Relative Standard Deviation	1.52	0.41

The assay results obtained with different wavelength conditions are as given in Table - 19.

Table - 19: Results for change in wavelength

Wavelength	249nm	253nm
Sample	% Assay	
Test Solution	100.41	99.20
Average assay result from method precision	100.02	100.02
Mean	100.22	99.61
Standard Deviation	0.28	0.58
(%) Relative Standard Deviation	0.28	0.58

Change in pH of Mobile Phase (± 0.2 units): (Normal Experimental Condition: pH = 5.0)

The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method (Refer to Table - 20 for system suitability results).

Table - 20: System suitability - Robustness with change in pH of mobile phase

Sr. No.	Area of Doxazosin mesylate	
	pH 4.8	pH 5.2
1	2590.08	2600.07
2	2678.11	2626.38
Mean	2634.10	2613.23
Standard Deviation	62.25	18.60
(%) Relative Standard Deviation	1.86	0.71

The assay results obtained with change in pH of mobile phase are as given in Table - 21.

Table - 21: Results for change in pH of mobile phase

pH	4.8	5.2
Sample	% Assay	
Test Solution	99.56	101.60
Average assay result from method precision	100.02	100.02
Mean	99.79	100.81
Standard Deviation	0.33	1.12
(%) Relative Standard Deviation	0.33	1.11

3.7 Stability of Analytical Solution:

3.7.1 Procedure: System suitability solution and test solution of DOXACARD-2 were prepared on 0th, 12th, 24th, 36th and 48th hour of experiment and stored these solutions at room temperature for every time interval up to 48 hrs and analyzed these solutions on 48 hrs with freshly prepared test solution. The system suitability solution was prepared freshly at the time of analysis. The assay of DOXACARD-2 in the sample was calculated.

3.7.2 Acceptance criteria: The analyte is considered stable if there is no significant change in % assay.

3.7.3 Results: The assay results obtained during solution stability experiment are as given in Table- 23

Table-22: System suitability for stability of Analytical Solution

Time	Std Area	Avg Std Area	Spl Area	Avg Spl area
0	2663.43	2666.05	2667.63	2674.19
	2668.66		2680.75	
12	2653.44	2665.94	2669.27	2670.99
	2678.44		2672.71	
24	2687.19	2693.48	2600.19	2610.03
	2699.78		2619.87	
36	2668.98	2682.93	2602.93	2602.11
	2696.88		2601.29	
48	2599.33	2590.25	2766.79	2777.32
	2581.18		2787.84	
Mean	2659.73	2659.73	2666.93	2666.93
Standard Deviation	39.62	40.57	66.58	70.17
(%) Relative Standard Deviation	1.49	1.53	2.50	2.63

Table - 23: Results for solution stability

% Assay results calculated against the freshly prepared system suitability standard	
Sample	% Assay of Doxazosin mesylate
0	100.79
12	100.67
24	97.37
36	97.45
48	99.12
Mean	99.08
Standard Deviation	1.66
(%) Relative Standard Deviation	1.68

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