

Qbd Guided Central Composite Design Optimized Rp-Hplc Method for Doxazosin Assay in Dosage Forms

Varaprasada Rao Kollabathula¹, Udaya Kumar Thummala², Sheik Jakir Hussain Mustaq³, Nookaraju Chintala⁴, Jaithri Challa⁵ and Bhagavan Rajesh Babu Koppisetty^{1*}

¹ Department of Pharmaceutical Analysis, Vignan Institute of Pharmaceutical Technology (A), Duvvada, Visakhapatnam, AP – 530049.

² Dept of Pharmaceutics, School of Pharmacy, Aditya University, Surampalem, Andhra Pradesh, India-533437

³ Department of Pharmaceutical Sciences, JNTU-GV College of Pharmaceutical Sciences, JNTU-GV, Vizianagaram-535003

⁴ Department of Radiology, Centurion University of Technology and Management, Vizianagaram, Andhra Pradesh, India-535003

⁵ Department of Pharmaceutical Sciences, Sri Sivani College of Pharmacy, Etcherla, Srikakulam, Andhra Pradesh 532410.

*Corresponding Author: Bhagavan Rajesh Babu Koppisetty,
koppisettybrbabu@gmail.com

Received: 28th Feb, 2026; Revised: 6th March 2026; Accepted: 7th April, 2026; Available Online: 20th April, 2026

ABSTRACT

In the current research, a efficient, and consistent RP-HPLC technique was developed to the precise quantification of doxazosin in commercial pharmaceutical preparations. Method development entailed a systematic optimization of chromatographic conditions to develop an efficient separation and a precise estimation of the analyte in the dosage form. They were separated chromatographically using a C18 column (250 mm × 4.6 mm, 5 µm). Detection was done at 254 nm and the elution of doxazosin was at a retention time of 4.513 minutes. The method has been validated according to regulatory guidelines and has been shown to possess good validation parameters. Altogether, the designed RP-HPLC technique can be used in the routine analysis of doxazosin in the pharmaceutical formulation and drug substance.

Keywords: Doxazosin; RP-HPLC; Stability indicating; Forced degradation.

How to cite this article: Kollabathula VR, Thummala UK, Mustaq SJH, Chintala N, Challa J, Koppisetty BRB. Qbd Guided Central Composite Design Optimized Rp-Hplc Method for Doxazosin Assay in Dosage Forms. Int J Drug Deliv Technol. 2026;16(46s): 417-422. DOI: 10.25258/ijddt.16.46s.44

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Doxazosin mesylate is an antagonist of selective alpha-1-adrenergic receptor, which is used in the treatment of hypertension and benign prostatic hyperplasia. It decreases the blood pressure by decreasing the peripheral vascular resistance and enhances the urinary flow by relaxing the smooth muscles of the bladder neck and prostate. Due to its pharmacological significance, it is critical to develop effective analytical techniques to determine its content in pharmaceutical dosage forms [1]. The determination of doxazosin in different matrices has been reported to use some analytical methods. Saldanha Krai J et al. [2] developed an LC-UV technique to analyse the tablets and Nageswara Rao R et al. [3] reported a RP-LC method with PDA and ESI-MS to profile the impurities. Sripalakit P et al. [4] and Erceg M et al. [5] developed bioanalytical techniques to estimate plasma, and Rao KS et al. [6]

developed a stability-indicating RP-HPLC technique. But the vast majority of these methods are based on conventional optimization and are not systematic robust enough to be QbD-based methodologies.

In order to address these constraints, a QbD-based methodology was used to develop systematic approaches to method development using CCD to define the design space and optimize critical parameters. An RP-HPLC system was optimized to doxazosin separation using a QbD-based RP-HPLC system with the mobile composition of the phase and flow rate optimal to achieve efficient separation. The method has been shown to have good robustness and sensitivity and has been validated following to guidelines of ICH and is therefore deemed to be suitable to be used in routine quality control.

*Author for Correspondence: koppisettybrbabu@gmail.com

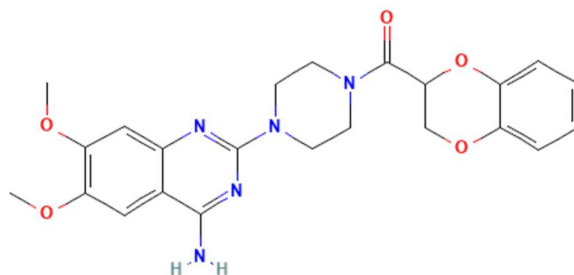


Figure 1 Structure of doxazosin

MATERIALS AND METHODS

Reagents

DOXA was received as a gift sample of Synpure Labs Pvt. Ltd., India. The purchase was of Cardura (4 mg) tablets (Pfizer, India) that were bought in a local pharmacy. Analytical-grade reagents, such as acetic acid and ammonium acetate were purchased through Fisher Scientific. Acetonitrile (ACN) and water of HPLC grade were obtained at Merck (India) and used without further purification.

Statistical assessment

Analysis of the data was done using Microsoft Excel 2007 and ANOVA, 3D response surface plot and optimization were done using Design-Expert.

Instrumentation

Analysis Data were analyzed using LC Solution and chromatographic analysis was performed on an HPLC system (Shimadzu, LC-20AD, Japan) with a PDA detector. Weighing was done using a Mettler Toledo analytical balance (ML303T) and UV-Vis measurements were taken with the help of a Lab India spectrophotometer.

Chromatographic parameters

The chromatographic separation was done with Phenomenex Luna C18 (250 x 4.6 mm, 5 mm) using isocratic mobile phase of ACN and 15 mM ammonium acetate buffer (63.5:36.5, v/v) at 0.95 mL/min with detection at 254 nm under ambient temperature.

Preparation of buffer system

A 15 mM ammonium acetate buffer was made by dissolving 1.156 g of Ammonium acetate in HPLC-grade water, adjusting the pH to 6 with dilute acetic acid, making up to 1000 mL, and filtering (0.45 μ m) followed by degassing before use.

Standard solution preparation

A stock solution was made by dissolving 25 mg of DOXA

in ACN in a 25 mL calibrated flask (sonicated 15 min), diluted to volume, further diluted with mobile phase to 30 μ g/mL, and passed through a 0.45 μ m membrane before analysis.

Preparation of sample solution

Ten Cardura tablets (4 mg) were powdered, and an amount equivalent to 25 mg of DOXA was transferred to a 25 mL volumetric flask, dissolved in ACN (sonicated 12 min), diluted to volume to obtain 30 μ g/mL, and clarified through a 0.45 μ m membrane prior to analysis.

Analytical method development based on Quality by Design (QbD) principles.

Analytical target profile (ATP)

Development of the ATP guided method was done to ensure the interference-free HPLC quantification of DOXA, targeting ≥ 3000 theoretical plates (N) and tailing factor (Tf) of 1.0-1.5 to ensure accurate analysis in formulations.

Risk assessment

HPLC performance was measured as severity and likelihood to guarantee consistency in results and adherence to good laboratory practice.

Experimental design

The application of the CCD was used after risk assessment to optimize two factors (flow rate and proportion of ACN) that influence N, and Tf. Initial experiments and QbD software defined the critical parameters, and 13 randomized runs were studied with the help of Design-Expert (Table 1).

Table 1. Experimental runs based on CCD

Run	F1	F2	R1	R2
	A:ACN (mL)	B:Flow rate (mL/min)	DOXA (Tf)	DOXA (N)
01	63.5	0.95	1.2478	5737
02	63.5	0.80	1.3374	4536
03	63.5	0.95	1.2421	5797
04	50	0.70	1.1101	2547
05	85	0.85	0.9237	1369

06	63.5	0.95	1.2412	5744
07	63.5	1.0	1.2368	5835
08	63.5	1.0	1.2311	5634
09	85	0.95	0.8310	1639
10	50	1.0	1.0623	3921
11	63.5	0.95	1.2421	5774
12	85	1.0	0.8126	1864
13	35	0.95	0.8334	955

F1: Factor 1; F2: Factor2; R1: Response 1; R2: Response 2

Results Assessment and Optimization

The experimental data were analyzed with the help of Design-Expert, and responses were evaluated with the help of ANOVA, 3D contour plots and equations of predictive models.

Method Validation

In line with ICH guidelines and relevant literature⁷⁻¹⁹, system suitability tests and method validation parameters were evaluated using established standard procedures.

RESULTS AND DISCUSSION

Development of Analytical Method using QbD Approach

A QbD strategy had been used to make sure that the development of the methodology was systematic by defining the targets, assessing the risks, and optimizing the variables. The optimal ACN: buffer ratio of 63.5:36.5

(v/v) was found to give rapid separation with good peak symmetry and was considered to be a key factor in determining retention. Ishikawa analysis and preliminary experiments identified ACN content and flow rate as the most important variables. Their effects on tailing factor (Tf, R1) and theoretical plates (N, R2) were studied using 13 runs using CCD. Model validation using ANOVA in Design-Expert (**Table 2**) confirmed significant quadratic models with adequate precision (>4), high Adj. R², low %CV, and good agreement with Pred. R². Regression equations were obtained of R1 and R2. **Figure 2** shows perturbation plots of Tf and N in response to flow rate and ACN proportion and **Figure 3** presents the chromatogram. **Figure 4(A)** show effect of flow rate and ACN proportion on the tailing factor of DOXA, while **Figure 4(B)** shows their influence on the theoretical plates.

Table 2. ANOVA results

Response	SD	Mean	r ²	C.V(%)	r ² (adj)	r ² (pred)	Adeq. Precision	Lack of fit p value	Sequential p value
R1	0.0008	1.12	1.0000	0.082	1.0000	0.9999	932.84	0.2836	<0.0001
R2	63.09	3871.52	0.9993	1.52	0.9982	0.9967	124.28	0.0871	<0.0001

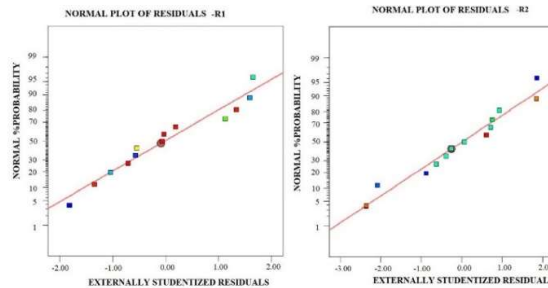


Figure 2 Residual Normal Plots from R1 and R2

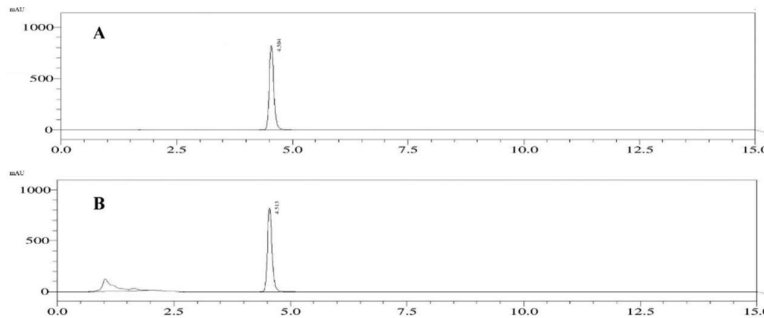


Figure 3 Chromatogram from the standard solution of DOXA(A) and Sample(B)

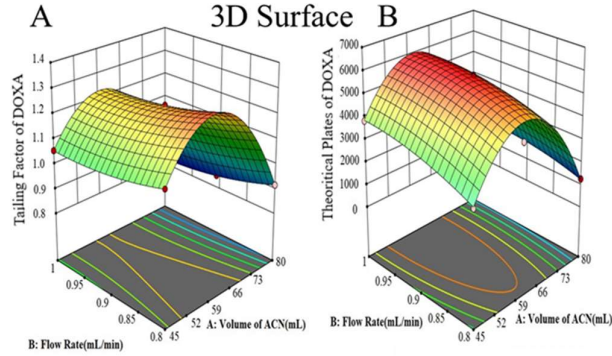


Figure 4 3-D plots of flow rate and ACN on T_f of DOXA(A), N of DOXA(B)

Model Prediction and Verification

The best conditions that were predicted in CCD and response surface methodology are 63.5% (v/v) ACN and 0.95 mL/min flow rate and the $T_f = 1.2011$ and $N = 5639$

with desirability = 1 (Figure 5). Experimental validation revealed that there were only a few deviations between prediction and experimental validation, indicating that model-driven optimization was validated (Table 3).

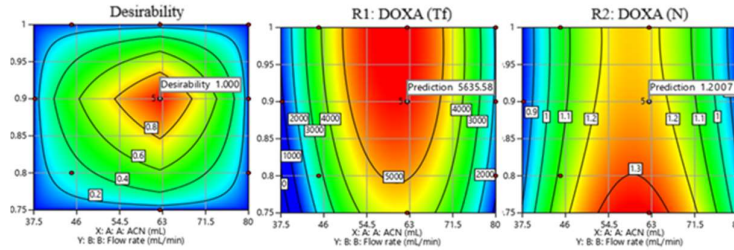


Figure 5 2-D plots for responses and desirability

Table 3: Predicted mean relative to Actual value

Response	Actual	Predicted Mean
R1	1.2478	1.2011
R2	5737	5639.27

3.2 System Suitability

System suitability parameters are within acceptance limits, confirming method reliability. The method was validated following ICH Q2 (R1) guidelines, and 254 nm was selected as the detection wavelength based on UV absorbance of DOXA.

Method Validation

DOXA exhibited maximum absorbance at 254 nm, selected for detection. The method, validated following ICH Q2 (R1), exhibited >3000 theoretical plates, $T_f < 1.5$, and %RSD < 2% for key parameters, confirming system suitability (Table 4).

Table 4. Results of validation studies

Validation	DOXA
Range limits	6.25-50 ($\mu\text{g/mL}$)
r^2 value	0.9981
Calibration equation	$Y = 341514x - 2493.8$
Repeatability	1.04 – 1.63 (%RSD)
Interm. precision	0.92 – 1.41 (%RSD)
Accuracy	
50% level	99.64%
100% level	99.78%
150% level	100.18%
Robustness (%RSD)	
Wavelength Variation	1.56
Flow Variation	1.64
Mobile phase Variation	1.31

Linearity

DOXA exhibited linearity over 6.25–50 $\mu\text{g/mL}$ with $Y = 341514x - 2493.8$ and $R^2 = 0.9981$, indicating reliable

quantification.

Precision

Precision was determined by evaluating repeatability and

intermediate precision at 15, 30, and 45 µg/mL (n = 3). The %RSD values were all below 2%, indicating good method reliability.

Precision was ascertained by assessing the repeatability and intermediate precision at 15, 30 and 45 µg/mL (n = 3). All of the values of the %RSD were less than 2 which means the reliable method.

Accuracy

Recovery studies were used to determine the accuracy of the method by using the standard addition method at the levels of 50,100 and 150 (n =3). Percent recovery of DOXA ranged between 99.64% and 100.18% which validated the accuracy and validity of the method.

Robustness

The method was resistant to small changes in the flow rate (0.93-0.97 mL/min), ACN content (62.5-64.5 mL), wavelength (252-256 nm) and sonication time (10-30 min) with all the system suitability parameters falling within the range and the %RSD value being less than 2%.

Assay of pharmaceutical formulation

The proposed method was used to analyse DOXA tablet formulations and the resulted mean recovery and %RSD values were close to the labelled content (**Table 5**), which is why the proposed method can be considered suitable to the quantification of DOXA in marketed products.

Table 5. Analysis of DOXA in formulation

Drug product	Labelled quantity (mg)	Exp. quantity (mg)	Recovery ± RSD
Cardura (4)	4	3.988	99.72±0.12

CONCLUSION

In this work, RP-HPLC procedure was designed based on the principles of quality by design and verified in terms of the quantification of DOXA in pharmaceutical formulations. The method showed good performance in terms of accuracy, precision, specificity, robustness, and reproducibility. The assay of DOXA was carried out using chromatographic separation with a C18 column using an optimised mobile phase. The technique also showed sufficient sensitivity to detect and quantify DOXA at low concentrations which makes it suitable in QC analysis and also in ensuring the safety and efficacy of the formulation throughout its shelf life.

Conflict of Interest

The authors report that there are no competing interests to declare.

Funding

No funding source applicable

Acknowledgements

The authors express their gratitude to the college management for providing the necessary facilities.

REFERENCES

- Fulton B, Wagstaff AJ, Sorkin EM. Doxazosin: an update of its clinical pharmacology and therapeutic applications in hypertension and benign prostatic hyperplasia. *Drugs*. 1995 Feb;49(2):295-320.
- Saldanha Krai J, Fontana de Andrade D, Carlos Ruver Beck R. Development and validation of a simple LC-UV method to assay doxazosin in polymeric nanocapsules and tablets. *Current Pharmaceutical Analysis*. 2017 May 1;13(3):264-70.
- Nageswara Rao R, Nagaraju D, Das AK, Jena N. Separation, characterization, and quantitation of process-related substances of the anti-hypertensive drug doxazosin mesylate by reversed-phase LC with PDA and ESI-MS as detectors. *Journal of chromatographic science*. 2007 Feb 1;45(2):63-9.
- Sripalakit P, Nermhom P, Saraphanchotiwiththaya A. Validation and pharmacokinetic application of a method for determination of doxazosin in human plasma by high-performance liquid chromatography. *Biomedical Chromatography*. 2006 Aug;20(8):729-35.
- Erceg M, Cindric M, Frketic LP, Vertzoni M, Cetina-Cižmek B, Reppas C. A LC-MS-MS method for determination of low doxazosin concentrations in plasma after oral administration to dogs. *Journal of chromatographic science*. 2010 Feb 1;48(2):114-9.
- Rao KS, Deb KB, Rao ME, Patro SS, Patnaik AK. Development and validation of stability indicating analytical method for doxazosin mesylate and its application to kinetic studies. *Chemical Sciences Journal*. 2012 Jan 1.
- Srinivasarao Y, Kumar TH, Chiranjivi P, Rao KV. Simultaneous Estimation of Solifenacin Succinate and Tamsulosin Hydrochloride in Combined Dosage Form by Using First Order Derivative Spectrophotometric Method. *Indian Journal of Pharmaceutical Sciences*. 2021 Mar 1;83(2).
- Kumar TH, Asha CH, Sankar DG. Estimation of amlodipine besylate and irbesartan in pharmaceutical formulation by RP-HPLC with forced degradation studies. *International Journal of Applied Pharmaceutics*. 2019 May 7;11(33):159-67.
- Panda M, Dadi V, Yarraguntla SR, Rao KV. RP-HPLC method for determination of azelnidipine and telmisartan in pharmaceutical dosage form. *Research Journal of Pharmacy and Technology*. 2023 Feb 1;16(2):509-13.
- Vidyadhara S, Rao YS, Ramu A, Sasidhar RL, Ramya AJ. Method development and validation for the simultaneous estimation of cinitapride and pantoprazole in solid dosage forms by RP-HPLC. *Oriental Journal of Chemistry*. 2013;29(3):1213.
- Swathi S, Kumar HT, Rao PK. Validated RP-HPLC

- method for simultaneous determination of rosuvastatin calcium and ezetimibe in pharmaceutical dosage form. *Int. J. Pharm. And Pharm. sci.* 2015;7(4):209-13.
12. Lakshmi GT, Rao YS, Rao K, Prasada V, Kumar TH. RP-HPLC method for estimation of atomoxetine hydrochloride in bulk and pharmaceutical dosage form. *Research Journal of Pharmaceutical Biological and Chemical Sciences.* 2015 Mar 1;6(2):1208-14.
 13. Koppisetty BR, Kollabathula VR, Challa GN, Yarraguntla SR. Quality by Design-Assisted RP-HPLC Method for Determination of Ritonavir and Darunavir in Pharmaceutical Formulation Using Central Composite Design. *Separation Science Plus.* 2024 Oct;7(10):e202300210.
 14. Koppisetty BR, Tatapudi HK, Dadi V, Gayathri PR, Komali P, Challa GN, Kollabathula VR, Yarraguntla SR. QbD based RP-HPLC method for simultaneous determination of a emtricitabine, tenofovir diproxil fumarate and efavirenz in tablet dosage form-an application to stability indicating assay. *Analytical Chemistry Letters.* 2023 May 4;13(3):267-88.
 15. Koppisetty BR, Yejella RP, Pawar AK, Yarraguntla SR, Kollabathula VR, Dadi V, Naidu CG. Development of a validated RP-HPLC assay method for quantitative separation of Teriflunomide and its process-related impurities in bulk drugs. *Journal of Applied Pharmaceutical Science.* 2023 Sep 20;13(1):028-33.
 16. Karanam SR, Poojitha N, Vasudha D, Rao YS, Rao KV. Estimation of Sotagliflozin in Tablet Dosage Form Using UV-Spectrophotometric and RP-HPLC Techniques: A Comparative Study. *Journal of Applied Spectroscopy.* 2025 Jul;92(3):629-36.
 17. Karanam SR, Prasad YR. Development And Validation Of New Method For The Simultaneous Estimation Of Ceftriaxone And Tazobactam Using RP-HPLC. *International Journal of Pharmaceutics and Drug Analysis.* 2019;2(3):189-94.
 18. Vasudha D, Reddy NV, Karanam SR, Manjusha KG, Rao YS. Comparison of UV-Spectrophotometric and RP-HPLC Methods for Quantification of Abemaciclib in Solid Dosage Form.
 19. Vallamkonda B, Yarguntla SR, Challa RR. Development and Validation of a Highly Sensitive LC-MS/MS Method for Quantification of Mutagenic N-Nitroso Desmethyl Citalopram in Drug Formulations. *Separation Science Plus.* 2025 Feb;8(2):e70011.