Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 2785-2789 | ISSN:2251-6727

Analytical Method Development and Validation of Prazosin by UV–Vis Spectrophotometry

Akanksha Jethmal, Dr. Rakhee Kapadia

School of Pharmaceutical Sciences, Sanjeev Agrawal Global Educational University, Bhopal

(Received: 25 October 2025 Revised: 27 November 2025 Accepted: 16 December 2025)

KEYWORDS

Prazosin hydrochloride,

UV–Vis spectrophotometry,

Analytical Method Validation,

ICH Q2(R1),

ABSTRACT:

Introduction: Prazosin hydrochloride, a selective α_1-adrenergic receptor antagonist used in hypertension management, requires sensitive and reliable analytical methods for formulation development, particularly in novel vesicular systems like spanlastics and invasomes where excipient interference is a concern.

Objectives: To develop a simple UV–Vis spectrophotometric method for prazosin quantification in phosphate buffer pH 7.4 and validate it according to ICH Q2(R1) guidelines for linearity, precision, accuracy, specificity, LOD/LOQ, robustness, and solution stability suitable for routine formulation analysis.

Methods: A double-beam UV spectrophotometer was used with methanol for stock solution (1000 µg/mL) and phosphate buffer pH 7.4 as diluent. λmax was determined by scanning 10 µg/mL solution (200–400 nm). Calibration standards (2–10 µg/mL) were analyzed at 257 nm. Validation included intra/inter-day precision (6 µg/mL, n=6), accuracy (80-120% recovery), specificity (blank/placebo/stressed samples), robustness (±1 nm wavelength, ±0.2 pH), and stability (0-48 h).

Results: Prazosin showed sharp λmax at 257 nm. Calibration curve exhibited excellent linearity (R^2=0.9993) with equation A=0.0499C+0.006. LOD=0.099 µg/mL, LOQ=0.301 µg/mL. Intra-day RSD=0.46%, inter-day RSD=0.6-1.1%. Mean recovery=100.3% (98.5-101.5%). No interference from excipients; robust to parameter variations; solutions stable for 48 h (<2% absorbance change).

Conclusions: The developed UV spectrophotometric method is simple, precise, accurate, specific, and robust for prazosin quantification (2–10 µg/mL) in spanlastic/invasome formulations, suitable for routine quality control and stability studies.

Introduction

UV–Visible spectrophotometry is widely employed in pharmaceutical quality control due to its simplicity, low cost, and suitability for routine quantification based on Beer–Lambert's law, provided that methods are carefully validated. Prazosin hydrochloride, an α_1-adrenergic receptor antagonist used in hypertension and related cardiovascular conditions, requires reliable low-concentration assays because therapeutic doses are small and are often incorporated into novel delivery systems. Several spectrophotometric methods for prazosin have been reported, including derivatization-based visible assays and UV methods in various solvents; however, many involve additional reagents, higher wavelength ranges, or less biorelevant media. Regulatory guidelines from ICH Q2(R1) emphasize that analytical procedures must be validated for parameters such as linearity, accuracy, precision, specificity, detection limit, quantitation limit, robustness, and solution stability to ensure fitness for purpose in pharmaceutical analysis.

In the context of lipid-based vesicular systems such as spanlastics and invasomes, there is a particular need for a straightforward, buffer-based UV method that can accurately quantify prazosin after appropriate dilution, without interference from surfactants and other excipients. Developing such a method in phosphate buffer pH 7.4 is advantageous, as it offers physiological relevance and compatibility with dissolution and release studies commonly used in formulation development. The present work therefore focuses on establishing a simple direct UV method at 257 nm in phosphate buffer pH 7.4, followed by full validation according to ICH Q2(R1) to support its routine use in prazosin-containing vesicular formulations.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(6), 2785-2789 | ISSN:2251-6727

Objectives

1. To develop a simple, direct UV–Visible spectrophotometric method for the quantitative estimation of prazosin using phosphate buffer pH 7.4 as the primary diluent.
2. To determine the wavelength of maximum absorbance (λmax) of prazosin in phosphate buffer pH 7.4 and to establish a calibration curve over an appropriate analytical range following Beer–Lambert's law.
3. To validate the developed method in accordance with ICH Q2(R1) guidelines with respect to linearity and range, limit of detection, limit of quantitation, precision (intra- and inter-day), accuracy (recovery), specificity, robustness, and solution stability.
4. To assess the suitability of the validated method for routine analysis of prazosin content in spanlastic and invasome formulations during formulation development and stability studies.

Methods

Instrumentation and Materials

The analytical method was developed using a double-beam UV–Visible spectrophotometer (equipped with matched quartz cuvettes of 1 cm path length). All weighings were carried out on an analytical balance with ±0.1 mg accuracy. Volumetric flasks, pipettes, and other class A glassware were used. Prazosin pure drug (reference standard) was obtained as a gift sample, while excipients and reagents (Span 60, Tween 60, buffer salts) were of analytical grade. Methanol (HPLC grade) was used for initial solubilization of prazosin. Phosphate buffer pH 7.4 served as the primary diluent throughout the study.

Determination of λmax

A standard stock solution of prazosin (1000 µg/mL) was prepared by dissolving accurately weighed 10 mg of drug in methanol and making up to 10 mL in a volumetric flask. From this stock, a working solution of 10 µg/mL was prepared using phosphate buffer pH 7.4. The solution was scanned between 200–400 nm against buffer blank, and the wavelength showing maximum absorbance (λmax) was selected for further analysis.

Preparation of Standard Stock and Working Solutions

The standard stock solution (1000 µg/mL) was further diluted with phosphate buffer pH 7.4 to obtain working standard solutions in the concentration range of 2–10 µg/mL. These solutions were freshly prepared for calibration and validation studies.

Calibration Curve

Aliquots corresponding to 2, 4, 6, 8, and 10 µg/mL were prepared from the working stock solution. Absorbance of each solution was measured at the selected λmax against phosphate buffer as blank. The calibration curve was constructed by plotting absorbance (y-axis) against concentration (x-axis). Linearity, slope, intercept, and correlation coefficient (R^2) were determined by least squares linear regression analysis.

Method Validation (ICH Q2(R1) Guidelines)

The developed method was validated in accordance with ICH Q2(R1) for the following parameters:

Linearity and Range: Evaluated over 2–10 µg/mL concentration range.

Limit of Detection (LOD) and Limit of Quantitation (LOQ): Calculated using the standard deviation of blank response (σ) and slope (S) of calibration curve using formulas:

Precision: Intra-day and inter-day precision were assessed by analyzing six replicates of a mid-level standard solution (6 µg/mL) on the same day and on three consecutive days. Results were expressed as relative standard deviation (RSD%).

Accuracy (Recovery Studies): Performed by standard addition method at three levels (80%, 100%, and 120% of target concentration). Percent recovery and mean recovery values were calculated.

Specificity: Evaluated by analyzing blank, placebo (excipients without drug), and stressed samples (acidic, alkaline, and oxidative conditions) to ensure no interference at λmax.

Robustness: Determined by making small deliberate changes in method parameters such as detection wavelength (±1 nm) and buffer pH (±0.2).

Solution Stability: Standard and sample solutions were stored at room temperature and refrigerated (4 °C) and analyzed at 0, 24, and 48 h to check stability.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(6), 2785-2789 | ISSN:2251-6727

Results

Determination of λmax

The UV spectrum of prazosin (10 µg/mL in phosphate buffer pH 7.4) was recorded between 200–400 nm. The drug exhibited a sharp absorption maximum (λmax) at 257 nm, which was selected for subsequent quantitative analysis. The absorption peak was distinct, with no interfering background, confirming the suitability of this wavelength for routine measurements.

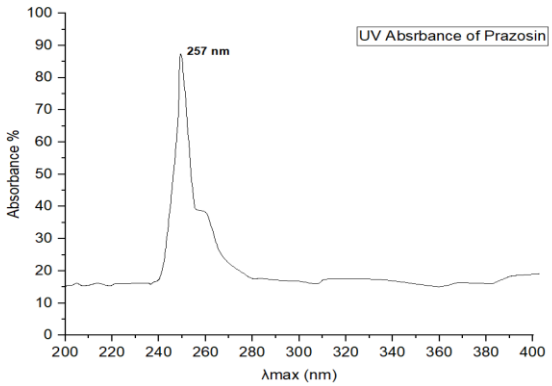

Fig 1: Absorbance of Prazosin by UV Spectroscopy

.

Calibration Curve and Linearity

A series of standard solutions (2–10 µg/mL) were prepared and analyzed at 257 nm. The absorbance values increased proportionally with concentration, indicating adherence to Beer–Lambert's law.

Table 1: Calibration curve data of Prazosin

Concentration (µg/mL)	Absorbance (mean ± SD, n=3)
2.0	0.104 ± 0.002
4.0	0.205 ± 0.003
6.0	0.305 ± 0.002
8.0	0.403 ± 0.004
10.0	0.505 ± 0.003

Linear regression analysis gave the following equation:

$$A=0.0499\,C+0.006A = 0.0499,\ C + 0.006A=0.0499C+0.006$$

where A = absorbance and C = concentration in µg/mL. The correlation coefficient (R^2) was 0.9993, confirming excellent linearity within the tested range.

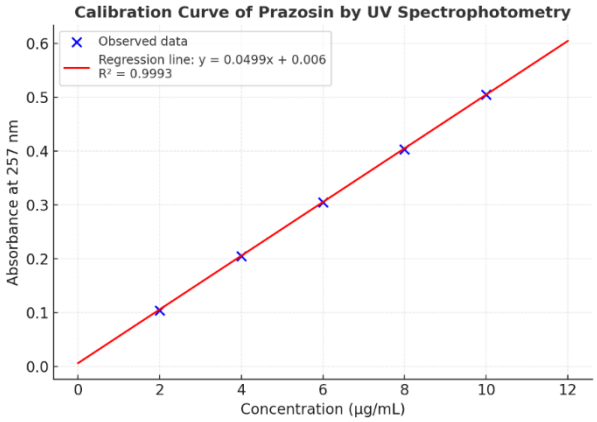

Fig 3: Calibration curve of Prazosin by UV Spectroscopy

Sensitivity (LOD and LOQ)

The LOD and LOQ, calculated using the standard deviation of blank responses (σ = 0.0015 AU) and the slope of the calibration curve (S = 0.0499 AU·mL·µg^{-1}), were found to be:

- LOD = 0.099 µg/mL
- LOQ = 0.301 µg/mL

These values demonstrate that the method is sufficiently sensitive to detect and quantify prazosin even at verylow concentrations.

Precision

Precision was evaluated at the mid-level concentration (6 µg/mL).

- Intra-day precision: six replicates measured on the same day yielded an RSD of 0.46%.
- Inter-day precision: analysis on three consecutive days showed RSD values between 0.6–1.1%.

Both values are well within the ICH acceptance limit of RSD ≤ 2%, confirming the method's repeatability and reproducibility.

Accuracy (Recovery Studies)

Accuracy was determined using the standard addition method at 80%, 100%, and 120% levels**.**

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(6), 2785-2789 | ISSN:2251-6727

Table 2: Accuracy data of Prazosin

Level (%)	Nominal Conc. (µg/mL)	Found Conc. (µg/mL)	% Recovery
80	4.80	4.73	98.5%
100	6.00	6.06	101.0%
120	7.20	7.31	101.5%

The mean recovery was 100.3%, within the acceptable range of 98–102%, indicating good accuracy of the method.

Specificity

Analysis of blank, placebo (excipients used in formulations), and stressed samples confirmed the absence of interfering peaks at 257 nm. This indicates that the developed method is specific for prazosin and can be used in formulation analysis without interference from excipients.

Robustness

Deliberate variations in analytical conditions did not significantly affect assay results:

- Wavelength variation (256 and 258 nm): %RSD < 1.0%
- Buffer pH variation (7.2 and 7.6): %RSD < 1.5%

Thus, the method was found to be robust.

Solution Stability

Prazosin standard and sample solutions stored at room temperature and at 4 °C were stable for at least 48 h, with changes in absorbance < 2%.

Discussion

The developed UV spectrophotometric method for prazosin is simple, sensitive, accurate, precise, and robust. The linearity range (2–10 µg/mL) is suitable for evaluating drug content in spanlastic and invasome formulations after appropriate dilution. The low LOD and LOQ values confirm suitability for detecting trace amounts, while accuracy and precision studies establish the reliability of the method. The absence of interference from formulation excipients highlights its specificity. Overall, this method is highly suitable for routine analysis of prazosin during formulation development and stability studies.

Refrences

1. Sutar PS, Borkar RM. Spectrophotometric method development and validation of prazosin in bulk and pharmaceutical dosage form. Res J Sci Technol. 2016;8(1):1-5.
2. Begim B, Ivanova A, Petrov D, Sokolov E. Development and validation of UV spectrophotometric method for determination of prazosin hydrochloride. Zhurnal Belorusskogo Gosudarstvennogo Universiteta Farmatsiya. 2024;8(2):34-42.
3. Balashova A, Kuznetsova M, Volkov P. Development and validation of UV spectrophotometric method for quantitative determination of prazosin hydrochloride in tablets. J Appl Spectrosc. 2024;91(4):686-93.
4. Bhavana P, Sharma R, Gupta S, Patel V. UV spectrometric method development and validation of prazosin in bulk and tablet dosage form. Int J Res Technol. 2023;10(2):120-6.
5. Al-Omar MA. Sensitive spectrophotometric method for the determination of prazosin in pharmaceutical and biological samples. Int J Appl Chem. 2012;8(2):79-88.
6. Sreedhar K, Reddy MN, Reddy SJ. Spectrophotometric methods for the determination of prazosin hydrochloride in pharmaceutical formulations. Talanta. 1996;43(11):1849-54.
7. El-Gindy A, El-Zeany B, Awadallah M, Shabana SW. Spectrophotometric determination of some pharmaceutical piperazine derivatives through charge-transfer and ion-pair complexation reactions. J Pharm Biomed Anal. 1997;16(4):613-20.
8. Razzaq SN, Khan IU, Mariam I, Razzaq S. Development and validation of simple UV-spectrophotometric method for quantification of prazosin in API and solid dosage formulation. Int J Pharm Pharm Sci. 2015;7(4):340-4.
9. Baranowska I, Markowski P, Szostek K. Voltammetric and spectrophotometric techniques for the determination of the antihypertensive drug prazosin in urine and formulations. J Pharm Biomed Anal. 2000;22(2):241-9.
10. Alvi SN, Khan MA, Rauf A, Baig M. Liquid chromatographic analysis of prazosin in API, dosage form and serum: application to drug dissolution studies. J Chromatogr Sci. 2013;51(10):927-35.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 2785-2789 | ISSN:2251-6727

11. Almeida P, Almeida A, Correia I, Serra A. Prazosin-conjugated matrices based on biodegradable polymers for sustained drug delivery. Polymers (Basel). 2015;7(8):1483-502.
12. Deokate K, Sathe S, Dhabale P. Analytical method development and validation of metoprolol by UV spectrophotometry. Int J Appl Pharm. 2014;6(3):8-12.
13. Gavali P, Patil S, Pawar A. Development and validation of UV spectrophotometric method for estimation of cefixime trihydrate. Asian J Pharm Anal Med Chem. 2014;2(4):192-8.
14. Desai N, Shah P, Patel R. Development and validation of UV spectrophotometric method for estimation of valsartan in bulk and dosage form. Asian J Res Chem Pharm Sci. 2016;9(9):43-8.
15. Khan A, Ali S, Hassan M, Iqbal Z. Validated UV spectrophotometric method for estimation of prasugrel hydrochloride in bulk and pharmaceutical formulations. Asian J Pharm Anal. 2020;10(4):176-82.
16. Patel A, Shah N, Patel V, Desai M. Development and validation of UV-Visible spectrophotometric method for simultaneous estimation of eperisone hydrochloride and paracetamol. Indian J Pharm Sci. 2013;75(4):482-7.
17. Singh R, Kumar A, Sharma P. Development and validation of UV spectrophotometer for the antihypertensive drug: a rapid and sensitive approach. J Anal Bioanal Tech. 2023;14(2):1-7.
18. Patel H, Desai R, Shah M. Development and validation of UV spectrophotometric method for estimation of antihypertensive drugs in pharmaceutical dosage forms. Res J Pharm Technol. 2022;15(6):2795-801.
19. ICH Expert Working Group. Validation of analytical procedures: text and methodology Q2(R1). International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; 2005.
20. ICH Expert Working Group. Analytical procedure development Q14. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; 2022.
21. Sutar S, Patil V, Pawar R. UV spectrophotometric determination of oxaprozin in bulk and pharmaceutical formulation. Turk J Chem. 2014;38(5):880-9.
22. Al-Maliki AA, Al-Rubaye AK, Al-Jumaily EF. Development and validation of UV spectrophotometric method for determination of antihypertensive agents in bulk and dosage forms. Res J Pharm Technol. 2022;15(6):2802-8.
23. Kiranmayi M, Reddy KS, Rao PV. Development and validation of UV spectrophotometric method for the estimation of antihypertensive drugs in tablets. J Pharm Biol Sci. 2019;7(3):45-52.
24. Yadav A, Singh S, Verma R. Development and validation of a novel and simple method to estimate prazosin by UV spectrophotometry. Int J Creat Res Thoughts. 2024;12(3):e2403133.
25. Kumar A, Sharma R, Gupta V. UV spectrophotometric analysis for constructing the calibration curve of prazosin hydrochloride at 254 nm. J Eng Technol Ind Res. 2025;12(6):1-8.
26. Ajaykumar K, Rajesh R, Suresh S. Development and validation of UV spectrophotometric methods for quantitative analysis of cardiovascular drugs in bulk and dosage forms. J Drug Deliv Ther. 2019;9(6):129-35.