# METHOD DEVELOPMENT AND VALIDATION OF PRAZOSIN HYDROCHLORIDE CAPSULES BY USING REVERSE PHASE -HPLC


**A Dissertation Submitted to**

**THE TAMILNADU DR.MGR.MEDICAL UNIVERSITY, CHENNAI**

**In partial fulfillment for the award of degree in**

**MASTER OF PHARMACY**

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**SAIDAPET, CHENNAI-15.**

**OCTOBER-2025**

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| LIST OF ABBREVIATIONS | |
|---|---|
| % | Percentage |
| %RSD | Percentage Relative standard |
| µ | Micron |
| µl | Micro litre |
| °C | Degree Celsius |
| AU | Absorption Unit |
| Gms | Grams |
| HPLC | High Performance Liquid Chromatography |
| ICH | International Conference on Harmonization |
| LC | Liquid Chromatography |
| LOD | Limit of Detection |
| LOQ | Limit of Quantification |
| ml | Milliliter |
| ml/ min | Milliliter/Minute |
| MS | Mass Spectrometry |
| ng/ ml | Nanogram Per Milliliter |
| Nm | Nanometer |
| pH | Negative Logarithm of Hydrogen Ion |
| Rpm | Rotations Per Minute |
| Rt or tR | Retention Time |
| SD | Standard Deviation |
| USP | United States Pharmacopoeia |
| UV-VIS | Ultraviolet – Visible |
| v/v/v | Volume / Volume/ Volume |
| %RSD | Percentage Relative Standard Deviation |

# INTRODUCTION

# 1. INTRODUCTION

Analysis with instruments 1–10 is separated into three groups based on the kind of property of the material being tested.

1. Spectral techniques
2. Methods of electroanalysis
3. Distinct techniques

The spectrum methods of analysis employ a device to quantify the quantity of radiation that the sample absorbs, emits, or scatters throughout the assay.

For Example Absorption spectroscopy of atoms

- Spectroscopy using nuclear magnetic resonance
- Spectroscopy using electron spin resonance
- The infrared spectrum
- Visible spectroscopy at UV
- Thermal Analysis and Refractometry
- Flame photometry and fluorimetry
- Turbidimetric Nephelometry

By applying an electrical signal to the sample, electro-analytical techniques track an electrical characteristic of the material. This approach is broken down as follows:

Electrogravimetry, polarography, conductometry, coulometry, amperometry, and potentiometry.

Separative methods depend on the components of a sample being separated before a property of the components is measured. The categories of instrumental separative approaches are as follows:

**TLC, GC, CC, PC,** and **HPLC** chromatography, mass spectroscopy, and electrophoresis

Several methods that integrate two or more instruments into one have been developed recently. Combining a mass spectrometer **(MS)** with gas chromatography **(GC), GC-MS, or HPLC (LC-MS)** is one of the first examples of these hybrid fields. Every **HPLC** device is connected to another instrument, such as a **UV-**visible detector, fluorimetry, etc. Thermospray, which functions well in **R.P.** chromatography, is one of the most used liquid insertion interfaces for **HPLC-MS**. One of the most advanced analytical techniques for identifying and detecting metabolites and medications in bodily fluids is **HPLC-MS** with Thermosprayinterface.

Occasionally, two similar instruments are used in the analysis. For example, **TANDEM** Mass Spectrometry involves coupling one mass spectrometer to another, where the first one isolates the molecular ions of different mixture components and the second one fragments the molecular ions the first one produces.

The most recent techniques use a potentially more potent GC-FTIR-MS combo, where the three instruments are used in series. Typically, the G.C. effluent is divided, with 98% going into the FTIR and 2% going straight to the more sensitive MS. The resulting MS and IR spectra are used for individual library searches.

## 1.1Drug Assay in Dosage Forms :

Usually, medications are created and produced in dosage forms before being administered to patients. To ensure therapeutic benefit, dosage forms need to pass a number of tests and standards. The development of control assays and tests is complicated by the complexities of delivery methods. Exam tablets may contain excipients, or substances other than the drug, such as dyes, antioxidants, buffers, resins, a lubricant (like magnesium stearate) to help move the granulated materials through tablet presses, a filter (like lactose) to add bulk, a disintegrate (like modified cellulose) to aid in the dissolution process after ingestion, and more. Procedures for drug recovery and extraction before quantitative analysis may be complicated by the drug delivery matrix.

New dosage forms, such controlled-release inserts and capsules, are frequently made to administer medications in vivo for at least 12 hours. There are difficulties in creating laboratory tests and protocols to regulate such performance.

## 1.2 Drug assay in combination dose forms :

For a variety of therapeutic reasons, it is necessary to administer two or more medications at once. Several drug combinations have been shown to be successful because of their combined mechanism of action on the body. When developing a dosage form assay, the intricacy of dosage forms—such as the inclusion of several pharmacological entities, like various vitamin tablets—presents difficulties. The assay should also indicate stability.

# 2. LIQUID CHROMATOGRAPHY WITH HIGH PERFORMANCE (HPLC)

When compared to traditional column chromatography, the high performance liquid chromatography technique performs better, hence its name. Since high pressure is employed in contrast to traditional column chromatography, it is also known as high pressure liquid chromatography. 11–21

Thanks to developments in column technology, high pressure pumping systems, and sensitive detectors, liquid column chromatography has evolved into HPLC, a high-speed, high-efficiency, precise, and high-resolution separation technique. The foundation of the column technology is the employment of small particles (3–50μ) of the stationary phase packing material and small pore (1.2mm i.d.) columns, which enable quick equilibrium between the stationary phase and mobile phase. To obtain a flow rate of a few milliliters per minute, the small particle column technique needs a high pressure pumping system to provide the mobile phase (4000 psi).

## 2.1 Benefits of HPLC

1. Speed: a lot of analyses may be completed in 20 minutes or under.
2. A greater range of stationary phases for better resolution.
3. Increased sensitivity (other detectors might be used).
4. A reversible column, which can be utilized for a variety of analyses despite its initial high cost.
5. Perfect for big molecules and ionic species (low volatility chemicals).
6. Simple sample retrieval.

7. Automatic injection, detection, or collection are possible with advanced devices.

8. Accurate and repeatable.

9. The integrator itself performs the computation.

10. Simple to manage, run, and maintain.

## 2.2 Chromatography in the normal and reverse phases

It is possible to distinguish between normal phase and reverse phase chromatography with the invention of the bonded phases.

## 2.2.a. Chromatography in the normal phase

A system with a polar stationary phase and a comparatively non-polar liquid mobile phase (such as hexane, benzene, CHCl3, etc.) is referred to as being in the normal phase. Silica gel is most likely the stationary phase employed in this technique. The "OH" groups affixed to silicon atoms serve as the active binding sites, and the silanol groups at the end of the silica structure are saturated. Plasticizers, dyes, steroids, amines, alkaloids, alcohols, phenols, aromatics, and metal complexes are among the best separating substances.

## 2.2.b. Chromatography in reverse phase

In the fields of chemical, biological, pharmaceutical, food, and biomedical sciences, this is the most widely used method for analytical and preparatory separations of substances of interest. With an octyl or octadecyl functional group linked to the silica gel, the stationary phase in this mode is a non-polar hydrophobic packing, while the mobile phase is a polar solvent. Secondary solute chemical equilibria (ionization control, ion separation, ion pairing, and complexation) can be used to regulate retention and selectivity in an aqueous mobile phase.

In this phase, non-polar molecules are held for a longer period of time while polar compounds elute first. The majority of medications and pharmaceuticals elute more quickly and are not maintained for longer since they are polar in nature. Octadecylsilane (ODS) or C18, C8, C4, etc. are the several columns that are utilized (in order of increase in the polarity of the stationary phase). Therefore, variable stationary phase polarities can be achieved by altering the organic moiety in the silanization reagent (dimethyl chlorosilane derivatives).

Through a chemical process, the highly polar Si-OH silanol group of silica gel becomes less polar and eventually non-polar. In HPLC, these stationary phases are referred to as bound stationary phases.

## 2.3 Polarity is important in HPLC

In chromatography, the term "polarity" refers to a measure of a compound's capacity for mutual interaction. The polarity of the mobile phase primarily determines its eluting power or solvent strength in both normal phase and reverse phase HPLC.

Poor separation is expected to emerge from identical solute-phase interactions if the polarities of the stationary phase and mobile phase are similar. As a result, distinct polarities are chosen for the stationary and mobile phases. Retention of the solute is often changed by altering the polarity of the mobile phase. A balance of intermolecular force between the sample, mobile phase, and stationary phase is necessary for effective chromatographic separation.

## 2.4. Equipment

The following are the various components of HPLC:

1. Systems for treating solvents and mobile phase reservoirs.
2. A pumping system for delivering solvents.
3. Injection mechanism for samples.
4. Column
5. Detector
6. Integrators and recorders

### 2.4.1. Reservoir for mobile phases

Depending on the chromatographic technique and detector being utilized, the mobile phase in HPLC might be an aqueous-organic mixture, a buffer solution, or a mixture of organic solvents. Reservoirs made of stainless steel or glass are frequently utilized. To ensure that there is no dust or other particle matter that could disrupt the pumping operation or harm seals or valves, the mobile phase is filtered before arriving at the pump. To get rid of dissolved air, degassing is necessary.

## 2.4.2. System for delivering solvents

A number of the solvent delivery system's characteristics need to be taken into account.

1. Accurate solvent delivery across a comparatively wide flow range,
II. The highest pressure that is possible
III. Compatibility with additional HPLC system components
IV. Compatibility with a variety of solvents
V. The amount of noise caused by any pulsations in the detector.

There are two primary types of HPLC pumps:

1. Pumps with constant pressure
2. Pumps with constant flow

The mobile phase is delivered at a steady pressure by constant pressure pumps. Mobile phase is delivered by constant flow pumps at a steady flow rate. This kind of pump maintains a steady flow rate while compensating for variations in the chromatographic system. Constant flow pumps are divided into two primary categories.

I. Pumps with reciprocating pistons.
2. Pumps for syringes

## 2.4.3. Column

Columns come in different diameters and lengths. Columns are made of thick walls, stainless steel tubing, or glass-lined metal tubing to handle the tremendous pressure involved. Zero empty volume must be incorporated into the design of connectors and end fittings.

In HPLC, columns typically have an interior diameter of 4 to 5 mm. Packing needs to be mechanically stable and consistent in size. The most widely used column has particle sizes ranging from 4 to 7 μm. The length of a column might vary from 5 to 30 cm.

Hydrocarbon groups are chemically bonded to the surface of silica particles to create bonded phases. The substance bound to the silica surface determines the classification of the columns.

### 2.4.4. System for injecting samples

There are primarily two kinds of injectable devices available. A septum injection device is one in which a self-sealing rubber is used to inject the sample solution. The other type is a Rheodyne injector, which is a rotary valve and loop type with a drilled stainless steel and teflon block that offers two different solvent flow routes that may be chosen by a revolving valve. A volume of 20 µl to 200 µl can be stored in the loops. Excellent precision and compliance with the pressure seen in HPLC are two benefits of valve injectors.

### 2.4.5. Sensors

The characteristics of the substances to be separated determine which detectors are employed. There are various kinds of detectors.

### a. Detectors of the refractive index

This detector is ubiquitous and non-specific. Due to its limited sensitivity and specificity, this is not frequently employed in analytical applications.

### b. A UV detector

This is predicated on the sample's properties related to light absorption. There are two kinds of detectors. The first is a fixed wavelength detector. The majority of pharmacological molecules absorb at 254 nm, where it acts. The variable wavelength detector, which operates between 190 and 600 nm, is the other.

### c. A conductivity detector

This detector records the response and is based on electrical conductivity. When the sample contains conducting ions, such as cations and anions, this detector is employed.

### d. A fluorimetric instrument

The excitation and emission wavelengths for each compound can be chosen based on the fluorescence radiation that a particular class of compounds emits. The sensitivity and specificity of this detector are higher. The fact that certain chemicals lack fluorescence is a drawback.

### e. Detector of photodiode arrays (PDA detector)

All wavelengths of radiation strike the detector at once. A three-dimensional representation of response vs time versus wavelength is the resultant spectrum. The benefit is that all of the compounds' responses are detected by the detector, therefore there is no need to choose a wavelength.

### 2.4.6. Integrators and recorders

The replies that detectors provide are recorded using recorders. In relation to time, they document the baseline as well as every peak that was attained.

Improved recorders with data processing capabilities are called integrators. The retention duration, peak height and width, peak area, percentage of area, and other details can be recorded for each unique peak. Currently, computers and printers are utilized to control various processes as well as to record and process the data that is obtained.

## 3. HPLC developed the method.

Drug analysis techniques can be created as long as one is aware of the characteristics of the sample, such as its wavelength, polarity, ionic character, and solubility parameter. However, since method development entails extensive trial and error methods, a specific recipe for HPLC cannot be offered. Choosing which column type to test with which mobile phase is typically the most challenging issue. Reversed phase chromatography is typically used to start with molecules that are water soluble and hydrophilic, meaning they have a lot of polar groups.

The gradient elution method can be used to estimate the concentration of the organic phase needed for the mobile phase. Gradient reversed phase chromatography is the most effective method to begin with for aqueous sample combinations. Within 20 to 30 minutes, the organic phase concentration (acetonitrile or methanol) can be raised to 100%. The gradient can begin with 5–10% organic phase.

By adjusting the gradient's slope and the initial mobile phase composition in accordance with the chromatogram from the preliminary run, separation may then be maximized. The location of the compounds' elution, or the composition of the mobile phase, can be used to determine the composition of the mobile phase.

The polarity of the mobile phase can be changed to modify the elution of drug molecules. A mobile phase's polarity determines its elution intensity; the stronger the polarity, the higher the elution. If ionic samples are in an un-dissociated state, they can be split into basic and acidic forms. By choosing the right pH, ionic sample dissociation can be inhibited.

It is necessary to choose the mobile phase's pH such that the chemicals do not ionize. The organic phase concentration in the mobile phase may drop by 5% in increments of 5% if the retention periods are too short. The organic phase concentration must be increased by 5% steps if the retention periods are excessively long.

Ionic sample separation may be impacted by the addition of peak modifier to the mobile phase. For instance, the addition of trace amounts of triethylamine to the mobile phase may have an impact on the retention of basic molecules. Likewise, a tiny quantity of acetic acid can be applied to acidic substances. This may result in beneficial sensitivity adjustments.

The presence of tailing or fronting indicates that the mobile phase and the solutes are not entirely compatible. Most of the time, protonation occurs because the pH is not chosen correctly.

Bad peak shapes might also result from the sample's poor solubility in the mobile phase. To prevent compound precipitation in the column or injector, it is always recommended to prepare the sample solution using the same solvent as the mobile phase. Controlling the pH of the mobile phase by adding a buffer is highly recommended whenever acidic or basic samples need to be separated. Before adding the organic phase, the buffer's pH can be changed. pH should be controlled within the range of ≈pKa±1.0 using the buffer that was chosen for that specific separation.

## 4. HPLC quantitative analysis:

For quantitative analysis, three techniques are typically employed. These three methods are the standard addition method, the internal standard method, and the external standard method.

## 4.1 External standard method:

This approach uses one standard or as many as three standard solutions. When calculating an unknown concentration, the slope of the calibration curve based on standards that include concentrations of the compounds of interest is taken into account, or the peak area or height of the sample and the standard are directly compared.

## 4.2 Internal standard method:

Adding an internal standard to account for different analytical mistakes is a common quantitation approach. This method compensates for the loss of the compounds of interest during sample preparation steps by adding a known compound at a controlled concentration to a given quantity of samples to produce distinct peaks in the chromatograms. An equivalent percentage of the internal standard will be lost along with any loss of the component of interest. The equivalency of the chemicals of interest and the internal standard determine how accurate this method is.

The requirements for an internal standard must;

- Give a completely resolved peak with no interferences,
- Elute close to the compound of interest,
- Behave equivalent to the compounds of interest for analysis like pretreatments, derivative formations, etc.,
- Be added at a concentration that will produce a peak area or peak height ratio of unity with the compounds of interest,
- Not to be present in the original sample,
- Be stable, unreactive with sample components, column packing, the mobile phase and be commercially available in high purity.

Before beginning the sample preparation process, the internal standard should be added to the sample and mixed with it. The concentration of a sample component in the original sample is ascertained using the response factor. The ratio of the internal standard **(AISTD),** which is derived by injecting the same amount, to the peak regions of the sample

component **(Ax)** is known as the response factor **(RF).** It can be computed using the following formula:

**RF = Ax/AISTD.**

The response factor for each molecule should be identified when multiple compounds from the sample need to be examined.

The standard addition approach involves adding a known quantity of the standard chemical to the solution that needs to be evaluated. This approach is more practical in that it permits calibration in the presence of excipients or other components, and it is appropriate if a sufficient quantity of the sample is available.

Analytical method validation: Validation22 is the process of assessing a measuring procedure's performance and confirming that it satisfies predetermined standards. Validation of an analytical procedure is, in essence, proving that the developed procedure is appropriate for its intended use and that it operates reproducibly when performed by the same or different individuals, in the same or different laboratories, with different brands of reagents, equipment, etc.

The following are typical validation attributes to take into account:

1. Precision
2. Accuracy
   a. First, reproducibility;
   b. second, intermediate precision;
   c. third, reproducibility
3. Linearity
4. Specificity
5. The range
6. Detection limit
7. Quantification limit
8. Sturdiness
9. Sturdiness
10. System appropriateness

## 5. Accuracy:

The degree to which test findings produced by an analytical procedure closely resemble the actual value is known as its accuracy. It is important to determine an analytical method's accuracy over its range. Accuracy can be defined as the difference between the mean and the recognized actual value, together with confidence intervals, or as the percentage of recovery by the assay of the known added amount of analyte in the sample. The percentage of analyte recovered by the assay is used to compute accuracy based on test results.

The degree of agreement between individual test results when an analytical procedure is applied repeatedly to multiple samplings of a homogenous sample is known as precision. The standard deviation or relative standard deviation (coefficient of variation) of a set of measurements is typically used to represent the accuracy of an analytical procedure. Assaying enough aliquots of a homogenous sample to compute a statistically sound estimate of standard deviation or relative standard deviation establishes the precision of an analytical procedure. Precision determinations, which typically fall between 0.3 and 3% for assays, allow one to evaluate the reliability of a single determination.

## 6. Specificity:

According to papers from the International Conference on Harmonization (ICH), specificity is the capacity to determine with certainty whether analyte components that may be anticipated to be present—such as contaminants, degradation products, and matrix components—are present.

For an assay to demonstrate specificity, the presence of excipients or contaminants must have an impact on the process. In actuality, this can be accomplished by adding suitable amounts of impurity or contaminants to the substance or product and proving that the presence of this extraneous material has no effect on the assay results. Specificity can be shown by comparing the test findings of samples containing impurities or degradation products to a well-characterized technique in the event that standards for these substances are not available. Samples kept under pertinent stress conditions, such as light, heat, humidity, acid/base hydrolysis, and oxidation, should be included in this comparison.

## 7. Selectivity:

The capacity of an analytical technique to precisely and precisely measure the target analyte in the presence of elements that may be anticipated to be present in the same matrix is known as selectivity.

## 8. Linearity and Range:

Linearity refers to how well an analytical method can produce results that are directly proportional to the concentration of the analyte in a sample, within a certain range. This should be confirmed across the full range the method is intended to cover. Usually, linearity is described by looking at the variation around the slope of a regression line, which is calculated from test results using samples with different analyte concentrations.

The range of the method is the span between the lowest and highest analyte levels that can be accurately and precisely measured using the procedure. This range should also show consistent linearity and is typically reported in the same unit as the test results, such as percent (%) or parts per million (ppm).

## 9. The detection limit:

The lowest amount of analyte in a sample that can be detected—albeit not always quantitatively—under the specified experimental circumstances is known as the limit of detection, or LOD. The analyte concentration (e.g., percent/ppm) in the sample is typically used to express the detection limits.

Quantification limit:

The lowest concentration of analyte in a sample that can be identified with reasonable precision and accuracy under the specified experimental conditions is known as the limit of quantification, or LOQ. It is stated as the analyte concentration (percent/ppb) in the sample.

The degree of reproducibility of test results obtained by analyzing the samples under various conditions, such as different laboratories, different analysts, different lots of reagents, different elapsed assay times, different assay temperatures, and different days, is known as the ruggedness of an analytical method. Ruggedness is typically defined as the absence of an impact on the analytical method's operational and environmental variables' test outcomes.

## 10. Robustness:

An analytical method's ability to withstand minor but intentional changes in its parameters is measured by its robustness, which also shows how reliable it is under typical operating conditions. It is a good idea to routinely change key method parameters and assess how they affect separation. These parameters include things like flow rate, column temperature, mobile phase additives, pH, and mobile phase composition.

## 11. Stability:

In order to produce repeatable and trustworthy findings, analytical procedures depend on the stability of the sample, standard, and reagents. For instance, solutions and reagents that must be produced for every assay must have 24-hour stability. Even the greatest LC column will ultimately deteriorate and lose its original performance, therefore long-term column stability is essential for method ruggedness.

## 12. System suitability tests:

These tests make sure the developed method can produce results with a sufficient level of precision and accuracy. Prior to analysis, system appropriateness can be established using the parameters defined by the USP. These metrics include the relative standard deviation (RSD) of the peak area and column efficiency (N).

# LITERATURE REVIEW

# 2. LITERATURE REVIEW

Prazosin hydrochloride, an $\alpha_1$-adrenergic receptor antagonist used in the treatment of hypertension and benign prostatic hyperplasia, has been widely studied for its analytical determination in bulk drugs and pharmaceutical formulations. The drug's susceptibility to degradation under various stress conditions has encouraged the development of stability-indicating analytical methods, particularly by reverse-phase high-performance liquid chromatography (RP-HPLC). The following section reviews ten key studies relevant to RP-HPLC method development and validation for prazosin hydrochloride.

**Bakshi, Ojha, and Singh (2004)** reported one of the earliest comprehensive stability-indicating HPLC methods for prazosin, terazosin, and doxazosin. Their study included forced degradation under acid, base, oxidative, thermal, and photolytic conditions as recommended by ICH guidelines. The authors demonstrated that prazosin and its degradation products could be effectively separated on a C18 column using an optimized mobile phase, confirming the method's specificity and suitability for stability testing.

**Shrivastava and Gupta (2012)** developed a stability-indicating RP-HPLC method for the simultaneous determination of prazosin, terazosin, and doxazosin in pharmaceutical formulations. The authors validated the method per ICH Q2(R1) guidelines, establishing linearity, precision, accuracy, and robustness. Their work reinforced the necessity of acidic pH conditions and adequate organic content to achieve sharp, symmetrical peaks for prazosin.

**Panigrahy et al. (2020)** proposed an RP-HPLC method for simultaneous estimation of prazosin and polythiazide in bulk and dosage forms. The method employed a phosphate buffer–acetonitrile mobile phase (pH 3.5) and was validated as per ICH guidelines. The study's findings emphasized the use of low pH buffers to prevent tailing and demonstrated good resolution and reproducibility, making it adaptable for routine quality-control assays.

**Earlier, Bhamra et al. (1986)** introduced a foundational HPLC method for prazosin, establishing key chromatographic parameters such as retention behavior and optimal UV

detection wavelength. This pioneering work laid the groundwork for subsequent method improvements and remains a historical reference for chromatographic conditions.

A related open-access report, "Liquid chromatographic analysis of prazosin in API, dosage form and serum," presented a rapid isocratic method using an acetonitrile–water mobile phase at pH 3.2. The study offered a simplified approach for both API and formulation assays, demonstrating acceptable accuracy and precision while highlighting the need for careful optimization of organic composition to balance retention and resolution.

**Mamina (2017)** summarized the analytical parameters of existing HPLC methods for prazosin hydrochloride and provided consolidated data on linearity, precision, and detection limits. Her work underscored the importance of validation parameters in accordance with regulatory expectations and recommended standard chromatographic conditions using C18 columns and acidified mobile phases for routine quality control.

**Eswarudu et al. (2019)** developed a bioanalytical RP-HPLC method for simultaneous determination of prazosin and polythiazide in plasma. While bioanalytical in nature, the study contributed valuable insights into sample preparation, extraction efficiency, and stability assessments that are equally applicable to dosage-form analysis.

**Rama Krishna et al. (2018)** applied a Quality by Design (QbD) approach to RP-HPLC method development for prazosin combinations. Their work demonstrated the benefit of systematic optimization of pH, mobile-phase composition, and flow rate to enhance robustness and reproducibility—principles directly applicable to formulation assays.

**Suma Pallavi and Prapulla (2021)** further advanced simultaneous estimation studies for prazosin and polythiazide. Their RP-HPLC method validated key analytical parameters, including linearity, accuracy, and precision, consistent with ICH guidelines, thereby confirming the reproducibility of previously developed methods across laboratories.

**Finally, Loh et al. (2021**) presented a fast LC–MS/MS method for the quantification of α-blockers in plasma, offering insights into sensitivity enhancement and degradation-product identification. Although not specifically designed for capsule assays, their approach

demonstrated how LC–MS can complement RP-HPLC in stability studies by identifying degradation products formed during stress testing.

Collectively, these studies confirm that most validated RP-HPLC methods for prazosin hydrochloride employ C18 columns, acidic mobile phases, and UV detection around 254–265 nm. Forced degradation under ICH conditions is a recurring feature across stability-indicating methods. However, literature gaps remain regarding capsule-specific placebo interference studies and detailed filter compatibility assessments. Addressing these limitations can strengthen the robustness and regulatory compliance of new methods for prazosin hydrochloride capsule formulations.

**Kunicki, Grymm, Pawiński, Szulczyk, Waligóra, and Kopeć (2025)** developed a straightforward isocratic HPLC-UV assay for monitoring adherence in pulmonary arterial hypertension by quantifying riociguat, bosentan, and macitentan in plasma. The method uses a Suplecosil LC-CN column (150 × 4.6 mm, 5 µm), liquid-liquid extraction from alkalized plasma, and detection at 245 nm. It is linear over 5-1000 ng/mL for riociguat, 10-2000 ng/mL for bosentan, 20-2000 ng/mL for macitentan, with precision and accuracy within acceptable limits (≤15 %, ≤20 % at LLOQ). Importantly, the method was applied to samples taken before dose ($C_0$) and 3 hours post-dose ($C_3$) to assess therapeutic adherence. While this method is accessible and suitable for monitoring, its sensitivity is limited compared to mass spectrometry-based detection, especially for ultra-trace or genotoxic impurities. For prazosin HCl polyhydrate, a similar HPLC-UV based adherence or monitoring method may work for higher concentration analytes or major impurities, but not for trace-level impurities (like nitrosamines).

**Tang, Shapiro, Mintz, Hollifield, & Reist (2020)** conducted a meta-analysis to assess the efficacy of prazosin versus placebo for improving overall PTSD symptoms, nightmares, and sleep quality (i.e., sleep disturbances) in patients with post-traumatic stress disorder (PTSD). In their synthesis of six randomized, placebo-controlled trials comprising 429 participants, prazosin showed statistically significant benefits: a moderate improvement in overall PTSD symptom scores (standardized mean difference [SMD] = –0.31; 95% CI: –0.62, –0.01), substantial reductions in nightmare frequency (SMD = –0.75; 95% CI: –1.24, –0.27), and enhancements in sleep quality (SMD = –0.57; 95% CI: –1.02, –0.13). Despite these positive findings, the authors caution that the most recent large trial included in the meta-analysis

exhibited a strong placebo effect, particularly in the measure of nightmares, which dampened the difference between treatment and placebo in that study. Limitations noted include heterogeneity in study populations, differences in prazosin dosing and duration, and relatively small sample sizes across the trials. The implications for treatment are promising; however, further research is encouraged to optimize dosing regimens, reduce heterogeneity, and better characterize long-term outcomes. Tang et al.'s findings provide useful insight into prazosin's therapeutic potential, particularly for sleep disturbances, though they also underscore considerations that are relevant when extrapolating to other contexts (e.g., different indications or formulations such as prazosin HCl polyhydrate).

**Illendula and Sharma (2024)** have proposed a rapid and cost-effective RP-HPLC method for quantifying Lorlatinib in its raw (API) form and commercial formulations. Using a Hypersil C18 (4.6 × 150 mm, 5 µm) column with a methanol:water (25:75 v/v) mobile phase at 1.0 mL/min, and detection set at 310 nm, their method yields a retention time of approximately 3.51 minutes under 38 °C. Validation results show that the assay has high accuracy (~98.96% recovery), acceptable precision (both repeatability and intermediate precision), and sensitivity (LOD 0.332 µg/mL, LOQ 1.0078 µg/mL) consistent with ICH guidelines. The authors emphasize its utility as a simple, economical tool for quality control, particularly for routine analysis. However, the applicability in detecting impurities (especially trace / genotoxic ones) was not addressed, highlighting a potential area of improvement for drugs similar to prazosin hydrochloride polyhydrate, where impurity profiling and ultra-trace detection are essential.

# DRUG PROFILE

## 3. DRUG PROFILE

**Name :** Prazosin Hydrochloride, USP

**Chemical Names :**

1. Piperazine, 1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(2-furanylcarbonyl)-,monohydrochloride

2. 1-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-4-(2-furoyl) piperazine monohydrochloride.

**Molecular Formula** : $C_{19}H_{21}N_5O_4 \cdot HCl$

**Molecular Weight:** 419.86 g/mol

**STRUCTURE :**

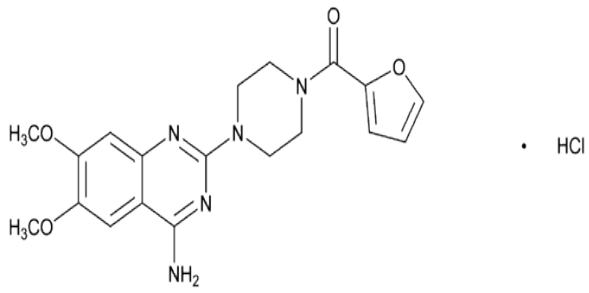


**Figure 1 Structure of Prazosin Hydrochloride**

**ADMINISTRATION :**

Prazosin hydrochloride is administered orally.

**SOLUBILITY :**

Prazosin hydrochloride's solubility varies significantly depending on the solvent, with high solubility in methanol and low solubility in water, especially at neutral to alkaline pH.

**HALF-LIFE :** Half-life is about 2-3 hours

**PROTEIN BINDING :**

Highly bound to proteins with 97% binding to albumin and alpha 1-acid glycoprotein. Prazosin is thought to be mostly (about 80-90%) bound to albumin.

## PHARMACOKINETICS :

Prazosin, a quinazoline derivative, is a peripheral vasodilator used in the treatment of arterial hypertension and more recently, congestive heart failure (CHF). Prazosin is extensively metabolised by the liver and has high first-pass metabolism and low oral bioavailability. In normal healthy volunteers, the time of peak concentration occurs between 1 and 3 hours after oral administration, with wide interindividual variations. The extent of oral absorption seems to be similar for different pharmaceutical forms and is not influenced by the presence of food in the digestive tract. Oral bioavailability of prazosin ranges from 43.5 to 69.3% (mean 56.9%). Prazosin is highly (92 to 97%) bound to human plasma proteins (albumin and alpha 1-acid glycoprotein) and the extent of binding is independent of the plasma concentration of the drug in the range of 20 to 150 ng/ml. Preliminary studies in humans indicate that pathways for biotransformation of prazosin are similar to those observed in the rat and dog. Only 6% of prazosin is excreted unchanged, mainly in the urine. The two main metabolites (0-demethylated) are almost completely excreted in bile. The time course of disappearance of prazosin from plasma after intravenous injecton indicates that prazosin disposition should be described by a model containing at least 2 kinetically distinct compartments. The mean elimination half-life is about 2.5 hours. After intravenous administration, the steady-state volume of distribution has been calculated to be 42.2 +/- 8.9L and the total body clearance 12.7 +/- 1.3L/h. In hypertensive patients with normal renal function, prazosin kinetics do not differ significantly from normals. However, prazosin disposition is modified in chronic renal failure and in congestive heart failure. In both cases, the plasma free fraction of prazosin is increased and plasma elimination half-life is longer. Prazosin kinetics may be expected to be altered in patients with liver diseases. Pharmacokinetic data do not suggest a mechanism to explain the disappearance of the first-dose effect during continued administration of prazosin. Although more investigation is needed to define prazosin kinetics in congestive heart failure and chronic renal failure, the available information about prolongation of elimination half-life, decreased protein binding and increased peak plasma concentrations suggest that prazosin dosage should be titrated cautiously in such patients

## PHARMACODYNAMICS :

The pharmacodynamic and therapeutic effect of this drug includes is a decrease in blood pressure as well as clinically significant decreases in cardiac output, heart rate, blood flow to the kidney, and glomerular filtration rate. The decrease in blood pressure may occur in both standing and supine positions.

Many of the above effects are due to vasodilation of blood vessels caused by prazosin, resulting in decreased peripheral resistance. Peripheral resistance refers to the level resistance of the blood vessels to blood that flows through them. As the blood vessels constrict (narrow), the resistance increases and as they dilate (widen), and peripheral resistance decreases, lowering blood pressure.

Effects on sleep disturbance related to post-traumatic stress disorder (PTSD)

Some studies have suggested that this drug improves sleep in patients suffering from insomnia related to nightmares and post-traumatic stress disorder, caused by hyperarousal. This effect likely occurs through the inhibition of adrenergic stimulation found in states of hyperarousal

## USES :

Prazosin hydrochloride is used alone or in combination with other classes of antihypertensive agents in the management of hypertension. However, because of established clinical benefits (e.g., reductions in overall mortality and in adverse cardiovascular, cerebrovascular, and renal outcomes), current evidence-based practice guidelines for the management of hypertension in adults generally recommend the use of drugs from 4 classes of antihypertensive agents (angiotensin-converting enzyme [ACE] inhibitors, angiotensin II receptor antagonists, calcium-channel blockers, and thiazide diuretics).

## TOXICITY :

**TDLO, LD50**: Oral TDLO (human): 285 µg/kg; Oral TDLO (woman): 10 µg/kg.

Oral LD50 (rat): 1950 mg/kg; Intraperitoneal LD50 (rat): 102 mg/kg.

## OVERDOSE INFORMATION :

Accidental ingestion of at least 50 mg of prazosin by a two-year-old child led to severe drowsiness with depressed reflexes. There was no fall in blood pressure, and the child recovered without complication.

# AIM

# AND

# PLAN OF WORK

# 4. AIM AND PLAN OF WORK

## AIM:

Prazosin Hydrochloride capsules is relatively used in the treatment of Hypertension. For quantification of Prazosin in Capsule formulation, very few HPLC methods were available.

Hence the aim of present work is to develop simple and validated RPHPLC method by isocratic mode for the quantification of Prazosin Hydrochloride Capsule in bulk and it's formulation.

## PLAN OF WORK:

The plan of present work is as follows:

### Method Development

- Selection of wavelength
- Selection of initial separation conditions
- Selection of mobile phase (pH, peak modifier, solvent strength, ratio and flow rate)
- Nature of the stationary phase
- Selection of separation method and agent

### Validation of the developed method

The developed method has to be validated by using the various validation parameters such as,

- System precision
- Linearity and range
- Accuracy
- Precision
- System Suitability
- Specificity
- Ruggedness
- Robustness

# METHODOLOGY

# 5. METHODOLOGY

## 1. MATERIALS AND INSTRUMENTS USED

### 1.1 DRUG SAMPLES

Prazosin Hydrochloride drug sample was obtained from NURAY Chemicals Pvt. Ltd and Capsules dosage form belongs to Novitium Labs

### 1.2 CHEMICALS AND SOLVENTS USED

| SNO | NAME | GRADE |
|---|---|---|
| 1 | Sodium Hydroxide | Merck (HPLC Grade) |
| 2 | Sodium Dihydrogen Phosphate | Merck (HPLC Grade) |
| 3 | Methanol | HPLC Grade |
| 4 | Acetonitrile | HPLC Grade |
| 5 | Hydrochloric Acid | Supelco (HPLC Grade) |

### 1.3 EQUIPMENTS & INSTRUMENTS USED

| SNO | NAME | MODEL |
|---|---|---|
| 1 | Analytical Column | |
| 2 | Digital Balance | Satorious |
| 3 | UV-Vis Spectrophotometer | Shimatzu |
| 4 | PH Meter | Thermofisher |
| 5 | HPLC | Waters Alliance |
| 6 | Sonicator | Ultrasonics |

## 2. Mobile Phase Preparation

**2.1.1 Preparation of 2 N Sodium Hydroxide Solution:** Dissolve about 8.0 g of Sodium hydroxide in 100 mL of water and mix well.

**2.1.2 Preparation of 0.1M Sodium Dihydrogen Phosphate Buffer:** Accurately weigh and transfer about 1.38 g of Sodium dihydrogen phosphate monohydrate into a suitable container. Dissolve in 1000 mL of water. Adjust to pH 7.5± 0.05 with 2 N sodium hydroxide solution. Filter through a 0.45µM membrane filter.

**2.1.3 Preparation of Organic Mixture:** Mix 900 mL of Methanol and 100 mL of Acetonitrile in a suitable container. Sonicate to degas.

**2.1.4 Preparation of Mobile phase:** Mix 550 mL of buffer and 450 mL of organic mixture in a suitable container. Sonicate to degas.

## 2.2 Diluent Preparation

**2.2.1 Preparation of Dilute Hydrochloric acid:** Dissolve 1.0 mL of Hydrochloric acid in 1000 mL of water.

**2.2.2 Diluent Preparation:** Mix 700 mL of Methanol and 300 mL of Dilute Hydrochloric acid in a suitable container. Mix well and sonicate to degas.

## 2.3 Standard Preparation

**2.3.1 Preparation of Standard Stock Solution:** Weigh accurately and transfer about 22 mg of Prazosin Hydrochloride reference standard (Equivalent to 20 mg of Prazosin) into a 100mL volumetric flask. Add diluent of about 60 mL and sonicate to dissolve and dilute to volume with diluent and mix well (concentration of about 200 µg/mL of Prazosin).

**2.3.2 Preparation of Working Standard Solution:** Pipette out 5.0 mL of standard stock preparation into 50 mL volumetric flask. Dilute to volume with diluent. Mix well. (Concentration of about 20 µg/mL of Prazosin).

## 2.4 Sample Preparation

**2.4.1 Stock Sample Preparation:** Randomly select the required number of capsules. Carefully open the capsules and transfer the contents along with the empty capsule shells using funnel in to suitable volumetric flask (100 mL for 1 mg Capsules and 250 mL for 5 mg Capsules). Rinse the funnel with diluent into volumetric flask and add diluent of about 50 mL for 1 mg and 125 mL for 5 mg strength and sonicate for about 30 minutes with intermittent shaking. Kept for mechanical shaking for about 10 minutes. Dilute to volume with diluent and mix well. Allow to settle down for about 10 minutes and centrifuge the solution for about 10 minutes.

**2.4.2 Working Sample Preparation:** Pipette out 5.0 mL of sample stock preparation into 25 mL volumetric flask. Dilute to volume with diluent. Mix well. (Concentration of about 20 µg/mL of Prazosin). Filter through 0.45µm PVDF filter by discarding at least first few mL of filtrate.

## 2.5. Chromatographic Parameters:

| | |
|---|---|
| Mobile Phase | Buffer: Organic Mixture (550:450) |
| Column | Waters X – Bridge C18 column; 100 mm X 4.6 mm, 5.0µm |
| Flow rate | 1.0 mL/min |
| Column Temp. | 30°C |
| Sample Temp. | Ambient |
| Wavelength | 254 nm |
| Injection Volume | 20 µL |
| Run Time | About 10 minutes |
| System Suitability | RSD from 5-replicate injections of the working standard should be NMT 2.0%, USP Tailing factor should be NMT 2.0 and USP plate count should be NLT 2000 for Prazosin peak. |

# 3. VALIDATION OF THE METHOD

## 3.1 SYSTEM PRECISION

### 3.1.1 Determination

A standard solution was prepared as per the method and injected. The tailing factor and relative standard deviation of peak area responses for 5-replicate injections of the standard solution was calculated.

| | Injection No. | Peak Area | USP Tailing | USP Plate Count |
|---|---|---|---|---|
| 1 | 1 | 3917967 | 1.1 | 2847 |
| 2 | 2 | 3919549 | 1.1 | 2847 |
| 3 | 3 | 3925037 | 1.1 | 2815 |
| 4 | 4 | 3922675 | 1.1 | 2825 |
| 5 | 5 | 3924534 | 1.1 | 2833 |
| Mean | | 3921952 | | |
| % RSD | | 0.1 | | |

**Table 1 System Precision-Prazosin**

### 3.1.2Conclusion

The % RSD of Prazosin peak area for 5-replicate injections of standard solution is less than 2.0, tailing factor is less than 2.0 and USP plate count is more than 2000.

### 3.1.3 Acceptance Criteria

a. The % relative standard deviation of Prazosin peak area for 5-replicate injections of standard should not be more than 2.0
b. The USP tailing factor should not be more than 2.0.
c. USP plate count should be NLT 2000.

## 3.2 LINEARITY AND RANGE

### 3.2.1 Determination

Solutions of Prazosin at varying concentrations ranging from 40% to 160% of the target concentration (about 20µg/mL of Prazosin) were injected into HPLC system. The linearity graph was plotted from 40% to 160%.

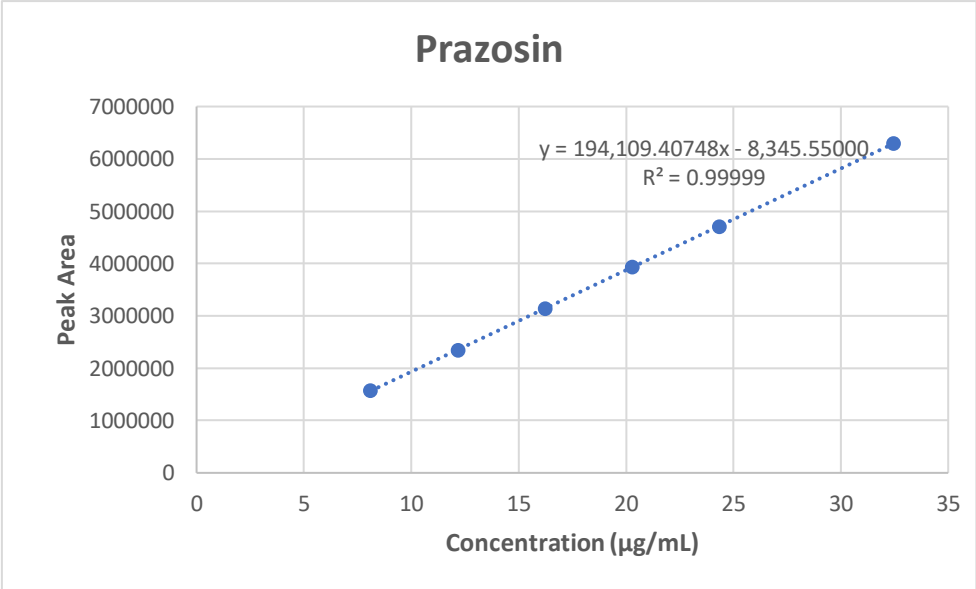


**Figure 2 Linearity Plot of Prazosin**

| Sample Name | Concentration (µg/mL) | Peak Area |
|---|---|---|
| Linearity-40% | 8.1164 | 1571670 |
| Linearity-60% | 12.1746 | 2351185 |
| Linearity-80% | 16.2328 | 3142030 |
| Linearity-100% | 20.2910 | 3933239 |
| Linearity-120% | 24.3493 | 4710430 |
| Linearity-160% | 32.4657 | 6297994 |

**Table 2 Linearity Study of Prazosin**

### 3.2.2 Conclusion

The correlation coefficient square ($r^2$) for Prazosin peak is 0.9999 which met the acceptance criteria of more than 0.999. The linear regression data shows that the method is linear over the entire concentration range (40% to 160% of the standard concentration of 20 µg/mL of Prazosin) of Prazosin and it is adequate for its intended concentration range.

### 3.2.3 Acceptance Criteria

The correlation coefficient square, $r^2$ must be not less than 0.999

## 3.3 METHOD PRECISION

### 3.3.1 Determination

Precision of the assay method was determined by injecting, 6-individual sample solutions of Prazosin Hydrochloride Capsules USP, 1mg and 5 mg. The samples were prepared as per the method.

Representative Chromatograms depicted below.

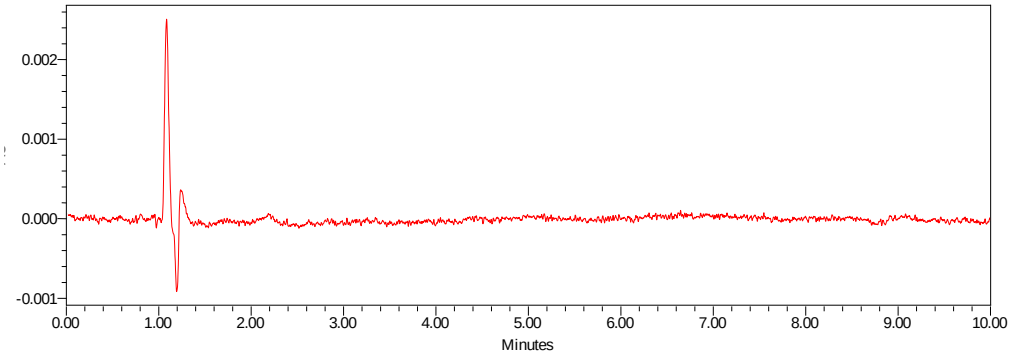


**Figure 3 Typical Chromatogram of Blank**

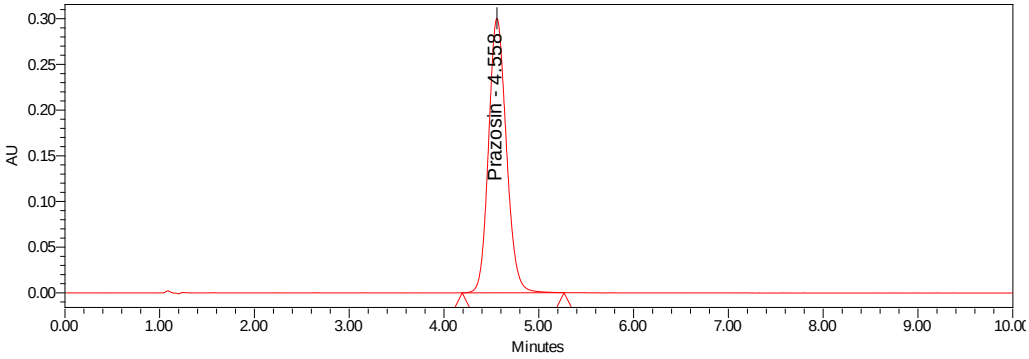


**Figure 4 Typical Chromatogram of Standard**

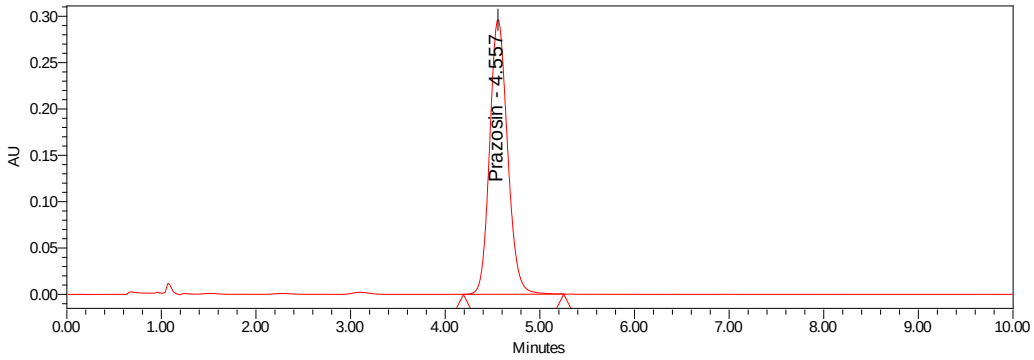


**Figure 5 Typical Chromatogram of Sample Solution**

| | SampleName | Peak Area | % Assay |
|---|---|---|---|
| 1 | Precision-1 | 3883241 | 100.4 |
| 2 | Precision-2 | 3909133 | 101.2 |
| 3 | Precision-3 | 3951962 | 102.2 |
| 4 | Precision-4 | 3926414 | 101.4 |
| 5 | Precision-5 | 3908927 | 101.1 |
| 6 | Precision-6 | 3839408 | 99.6 |
| Mean | | | 101.0 |
| % RSD | | | 0.9 |

| | SampleName | Peak Area | % Assay |
|---|---|---|---|
| 1 | Precision-1 (5 mg) | 3870315 | 99.9 |
| 2 | Precision-2 (5 mg) | 3863141 | 99.6 |
| 3 | Precision-3 (5 mg) | 3852245 | 99.7 |
| 4 | Precision-4 (5 mg) | 3837462 | 99.3 |
| 5 | Precision-5 (5 mg) | 3858501 | 99.9 |
| 6 | Precision-6 (5 mg) | 3867461 | 99.8 |
| Mean | | | 99.7 |
| % RSD | | | 0.2 |

**Table 3 Method Precision-1mg and 5mg Capsules**

### 3.3.2 Conclusion

The RSD for % assay of Prazosin from 6-samples is less than 2.0%. Therefore, the method is precise.

### 3.3.3 Acceptance Criteria

The % RSD for % assay from six (6)-sample preparations should not be more than 2.0.

## 3.4 INTERMEDIATE PRECISION (STANDARD)

### 3.4.1 Determination

The standard solution prepared by Analyst-2 was injected in a different HPLC system, on a different day, using a different column. The system suitability parameter calculated by Analyst-2 and compared with Analyst-1.

| | Injection No. | Peak Area | USP Tailing | USP Plate Count |
|---|---|---|---|---|
| 1 | 1 | 3917967 | 1.1 | 2847 |
| 2 | 2 | 3919549 | 1.1 | 2847 |
| 3 | 3 | 3925037 | 1.1 | 2815 |
| 4 | 4 | 3922675 | 1.1 | 2825 |
| 5 | 5 | 3924534 | 1.1 | 2833 |
| Mean | | 3921952 | | |
| % RSD | | 0.1 | | |

**Table 4 Intermediate Precision-System**

| | Injection No. | Peak Area | USP Tailing | USP Plate Count |
|---|---|---|---|---|
| 1 | 1 | 3781001 | 1.1 | 2496 |
| 2 | 2 | 3782311 | 1.1 | 2496 |
| 3 | 3 | 3784141 | 1.1 | 2504 |
| 4 | 4 | 3759707 | 1.1 | 2468 |
| 5 | 5 | 3784467 | 1.1 | 2497 |
| Mean | | 3778325 | | |
| % RSD | | 0.3 | | |

**Table 5 Intermediate Precision-System**

### 3.4.2 Conclusion

The %RSD of Prazosin peak area for 5-replicate injections of standard solution is less than 2.0%, tailing factor is less than 2.0 and USP plate count is less than 2000.

### 3.4.3 Acceptance Criteria

a. The % RSD for 5-replicate injections should be not more than 2.0 % of the standard solution.
b. The tailing factor should be not more than 2.0.
c. USP plate count should be NLT 2000.

## 3.5 INTERMEDIATE PRECISION (SAMPLE)

### 3.5.1 Determination

The method precision ruggedness (reproducibility) of the assay method was determined by preparing 6- individual samples by a second analyst using a different system and a different column on a different day. Samples were prepared as per the method.

| | SampleName | Peak Area | % Assay |
|---|---|---|---|
| 1 | Precision-1 | 3883241 | 100.4 |
| 2 | Precision-2 | 3909133 | 101.2 |
| 3 | Precision-3 | 3951962 | 102.2 |
| 4 | Precision-4 | 3926414 | 101.4 |
| 5 | Precision-5 | 3908927 | 101.1 |
| 6 | Precision-6 | 3839408 | 99.6 |
| Mean | | | 101.0 |
| % RSD | | | 0.9 |

| | SampleName | Peak Area | % Assay |
|---|---|---|---|
| 1 | Precision-1 (5 mg) | 3870315 | 99.9 |
| 2 | Precision-2 (5 mg) | 3863141 | 99.6 |
| 3 | Precision-3 (5 mg) | 3852245 | 99.7 |
| 4 | Precision-4 (5 mg) | 3837462 | 99.3 |
| 5 | Precision-5 (5 mg) | 3858501 | 99.9 |
| 6 | Precision-6 (5 mg) | 3867461 | 99.8 |
| Mean | | | 99.7 |
| % RSD | | | 0.2 |

**Table 6 Intermediate Precision-1mg & 5mg**

| | SampleName | Peak Area | % Assay |
|---|---|---|---|
| 1 | 1mg Intermediate Precision-1 | 3793330 | 101.8 |
| 2 | 1mg Intermediate Precision-2 | 3783452 | 102.1 |
| 3 | 1mg Intermediate Precision-3 | 3743036 | 100.4 |
| 4 | 1mg Intermediate Precision-4 | 3731141 | 100.7 |
| 5 | 1mg Intermediate Precision-5 | 3925787 | 105.7 |
| 6 | 1mg Intermediate Precision-6 | 3812212 | 102.4 |
| Mean | | | 102.2 |
| % RSD | | | 1.9 |

| | SampleName | Peak Area | % Assay |
|---|---|---|---|
| 1 | 5mg Intermediate Precision-1 | 3805198 | 102.7 |
| 2 | 5mg Intermediate Precision-2 | 3805388 | 102.5 |
| 3 | 5mg Intermediate Precision-3 | 3781929 | 102.0 |
| 4 | 5mg Intermediate Precision-4 | 3772848 | 101.6 |
| 5 | 5mg Intermediate Precision-5 | 3791688 | 102.3 |
| 6 | 5mg Intermediate Precision-6 | 3781757 | 101.6 |
| Mean | | | 102.1 |
| % RSD | | | 0.4 |

**Table 7 Intermediate Precision-1mg & 5mg Capsules**

Difference in mean % assay for 1 mg capsules is 1.2 and for 5 mg capsules is 2.4

### 3.5.2 Conclusion

The %RSD for % assay of Prazosin from 6-samples is less than 2.0%. Difference between two analysts is less than 3.0%. Therefore, the method is considered rugged.

### 3.5.3 Acceptance Criteria

a. RSD of Assay from 6-samples should not be more than 2.0%
b. Difference between two analyst: NMT 3.0%.

## 3.6 SOLUTION STABILITY

### 3.6.1 Determination

The sample and standard solutions were prepared and injected. Replicate injections of both standard and sample solutions were made at the following time intervals: Initial, 24 hours, 48 hours and 96 hours for Standard Solution and Sample solution when stored at room temperature. The % assay difference between the initial and time point for both standard and sample solution were generated.

| Time | Peak Area | % Assay | Mean % Assay | % Assay Difference |
|---|---|---|---|---|
| Initial | 3921952 | 85.3 | 85.3 | NA |
| 24 hours-inj1 | 3943420 | 85.6 | 85.6 | 0.3 |
| 24 hours-inj2 | 3941205 | 85.6 | | |
| 48 hours-inj1 | 3959939 | 85.8 | 85.7 | 0.5 |
| 48 hours-inj2 | 3951774 | 85.6 | | |
| 96 hours-inj1 | 3897551 | 85.6 | 85.7 | 0.5 |
| 96 hours-inj2 | 3908418 | 85.8 | | |

**Table 8 Solution Stability of Standard Solution at room temperature**

| Time | Peak Area | % Assay | Mean % Assay | % Assay Difference |
|---|---|---|---|---|
| Initial | 3883241 | 100.4 | 100.4 | NA |
| 24 hours-inj1 | 3901631 | 100.7 | 100.6 | 0.2 |
| 24 hours-inj2 | 3892534 | 100.5 | | |
| 48 hours-inj1 | 3906294 | 100.6 | 100.6 | 0.2 |
| 48 hours-inj2 | 3903639 | 100.6 | | |
| 96 hours-inj1 | 3897947 | 101.8 | 101.8 | 1.4 |
| 96 hours-inj2 | 3904172 | 101.9 | | |

**Table 9 Solution Stability of Sample Solution at room temperature**

## 3.6.2 Conclusion

All results met acceptance criteria. Based on above results its concluded that standard and sample solutions were stable up to 96 hours when stored at room temperature.

## 3.6.3 Acceptance Criteria

**a.** The difference in % assay between the initial and time point for standard and sample solution, respectively, should be NMT 2.0

**b.** Include an appropriate cautionary statement in the method based on the solution stability

## 3.7 SPECIFICITY

### 3.7.1 Determination

A forced degradation study is performed in order to prove that the method is stability indicating. Prazosin Hydrochloride capsules, USP, 1mg were stressed under the following conditions.

1. Acid Stress
2. Base Stress
3. Peroxide Stress
4. UV light Stress
5. Heat Stress

#### *3.7.1.a Control Sample Preparation:*

About 2000 mg of finished product was transferred into a 100mL volumetric flask, 50 mL of Diluent was added and kept for sonication for 30 minutes with intermittent shaking. Kept for mechanical shaking for about 10 minutes. Dilute to volume with diluent and mixed well. Centrifuged for about 10 minutes. Transferred 5.0 mL of supernatant sample stock preparation into 25 mL volumetric flask. Dilute to volume with diluent. Mix well. (Concentration of about 20 µg/mL of Prazosin). Filter through 0.45µM PVDF filter discarding 4 mL of filtrate.

#### *3.7.1.b Acid Degradation:*

About 2000 mg of finished product was transferred into a 100mL volumetric flask. Added 5.0 mL of 5N HCl and heated in a water bath at 80°C for 3 hours. After 3 hours, the sample was removed from water bath and allowed to cool to room temperature. Then neutralized with 5.0 mL of 5N NaOH solution. Added 50 mL of diluent and kept for sonication for 30 minutes with intermittent shaking. Kept for mechanical shaking for about 10 minutes. Dilute to volume with diluent and mixed well. Centrifuged for about 10 minutes. Transferred 5.0 mL of supernatant sample stock preparation into 25 mL volumetric flask. Dilute to volume with diluent. Mix well. (Concentration of about 20 µg/mL of Prazosin). Filter through 0.45µM PVDF filter discarding 4 mL of filtrate.

### *3.7.1.c Base Degradation:*

About 2000 mg of finished product was transferred into a 100mL volumetric flask, 5.0 mL of 0.1N NaOH was added and heated in a water bath at 80°C for 1 hour. After 1 hour, the sample was removed from water bath and allowed to cool to room temperature. Then neutralized with 5.0 mL of 0.1N HCl solution. Added 50 mL of diluent and kept for sonication for 30 minutes with intermittent shaking. Kept for mechanical shaking for about 10 minutes. Dilute to volume with diluent and mixed well. Centrifuged for about 10 minutes. Transferred 5.0 mL of supernatant sample stock preparation into 25 mL volumetric flask. Dilute to volume with diluent. Mix well. (Concentration of about 20 µg/mL of Prazosin). Filter through 0.45µM PVDF filter discarding 4 mL of filtrate.

### *3.7.1.d Oxidation:*

About 2000 mg of finished product was transferred into a 100mL volumetric flask, 5.0 mL of 3% Hydrogen peroxide solution was added and heated in a water bath at 80°C for 3 hours. After 3 hours, the sample was removed from water bath and allowed to cool to room temperature. Added 50 mL of diluent and kept for sonication for 30 minutes with intermittent shaking. Kept for mechanical shaking for about 10 minutes. Dilute to volume with diluent and mixed well. Centrifuged for about 10 minutes. Transferred 5.0 mL of supernatant sample stock preparation into 25 mL volumetric flask. Dilute to volume with diluent. Mix well. (Concentration of about 20 µg/mL of Prazosin). Filter through 0.45µM PVDF filter discarding 4 mL of filtrate.

### *3.7.1.e Heat Degradation*:

Required amount of finished product was transferred into a Petri dish and kept in an oven at 105°C for 24hours. After 24 hours, the sample was removed from oven and allowed to cool to room temperature. Accurately weighed and transferred about 2000 mg of finished product into a 100mL volumetric flask. Added 50 mL of diluent and kept for sonication for 30 minutes with intermittent shaking. Kept for mechanical shaking for about 10 minutes. Dilute to volume with diluent and mixed well.

Centrifuged for about 10 minutes. Transferred 5.0 mL of supernatant sample stock preparation into 25 mL volumetric flask. Dilute to volume with diluent. Mix well. (Concentration of about 20 µg/mL of Prazosin). Filter through 0.45µM PVDF filter discarding 4 mL of filtrate.

### *3.7.1.f UV-Light Degradation*:

Required amount of finished product was transferred into a Petri dish and kept in a UV chamber for 24hours. After 24 hours of exposure, accurately weighed and transferred about 2000 mg of finished product into a 100mL volumetric flask. Added 50 mL of diluent and kept for sonication for 30 minutes with intermittent shaking. Kept for mechanical shaking for about 10 minutes. Dilute to volume with diluent and mixed well. Centrifuged for about 10 minutes. Transferred 5.0 mL of supernatant sample stock preparation into 25 mL volumetric flask. Dilute to volume with diluent. Mix well. (Concentration of about 20 µg/mL of Prazosin). Filter through 0.45µM PVDF filter discarding 4 mL of filtrate.

Control, Acid, Base, Peroxide, Heat Stress, and UV stress placebo were prepared separately using placebo weight equivalent to in sample, solutions prepared in the similar way as sample. Similarly, control, acid, base, peroxide blanks were prepared in the similar way as done for sample. All the treated and control samples were injected.

The chromatograms of Control, Acid, Base, Peroxide, Heat, UV Stress samples and Spiked sample are represented below:

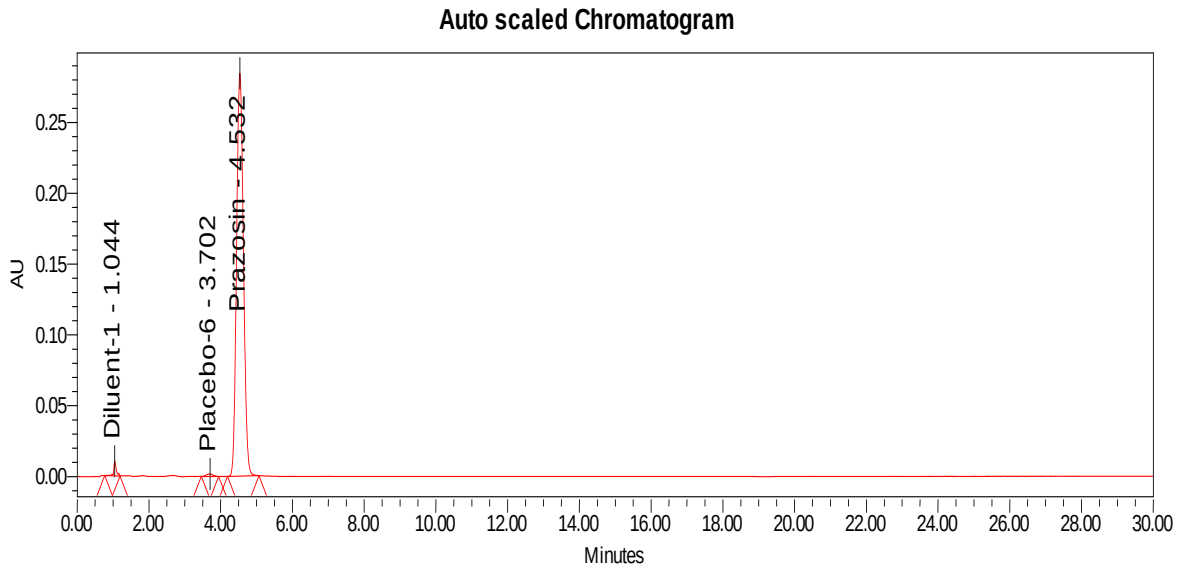


**Figure 6 Typical Chromatogram of Control Sample**

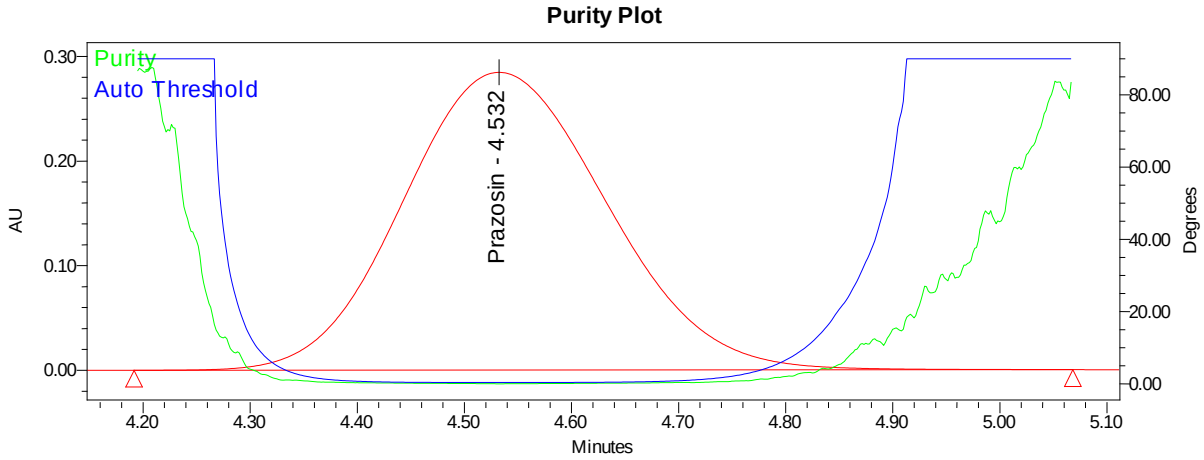


**Figure 7 Peak Purity Chromatogram of Control Sample**

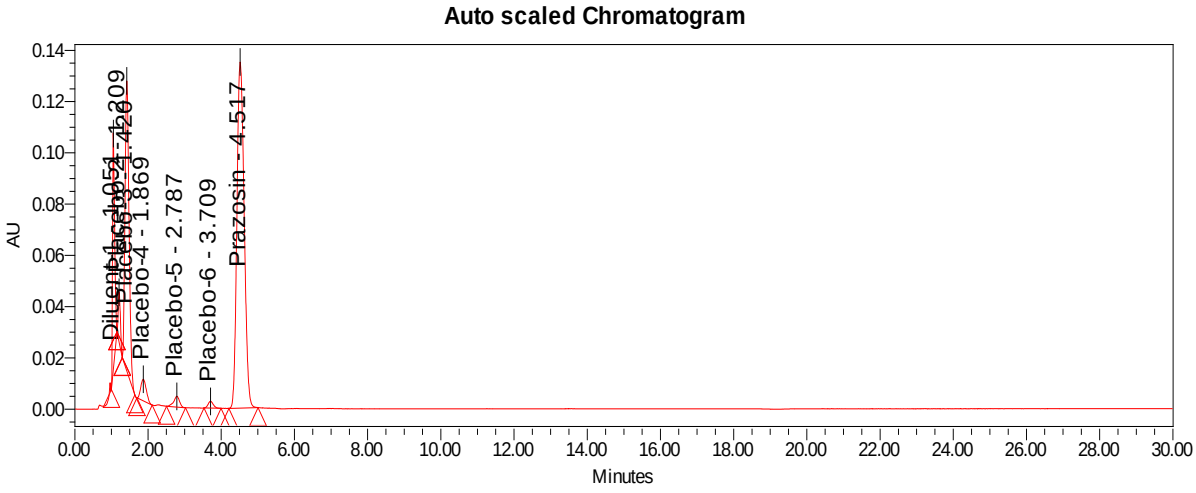


**Figure 8 Typical Chromatogram of Acid Stress Sample**

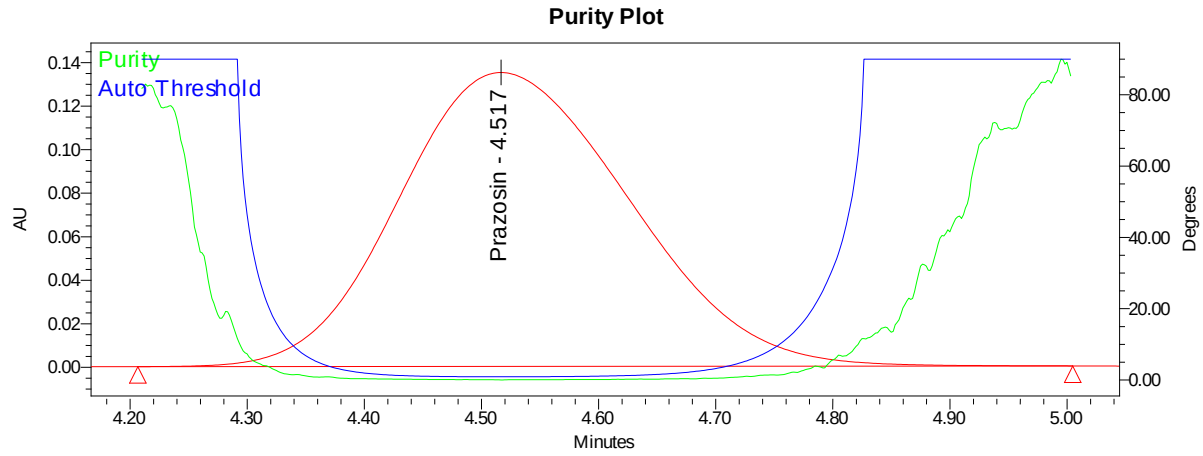


**Figure 9 Peak Purity Chromatogram of Acid Stress Sample**

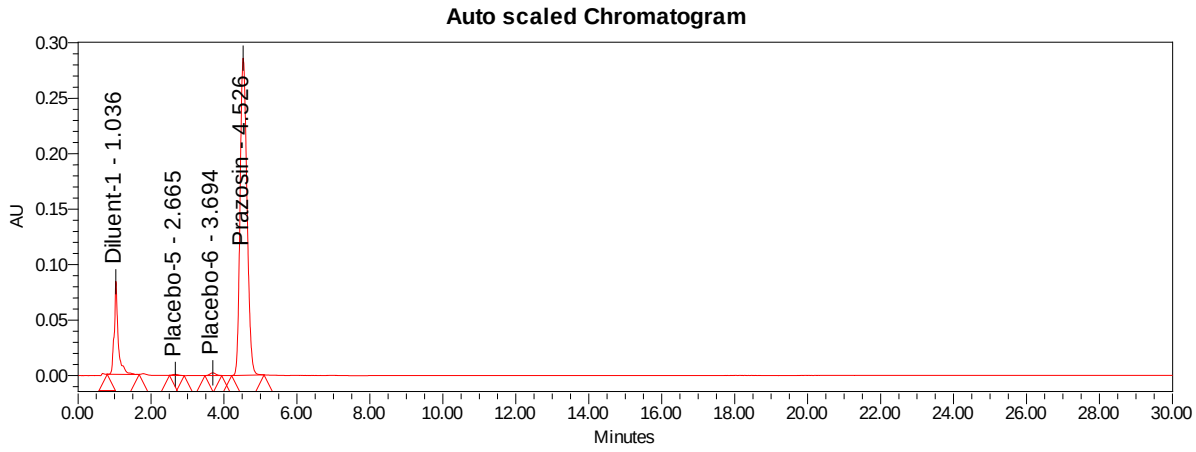


**Figure 10 Typical Chromatogram of Base Stress Sample**

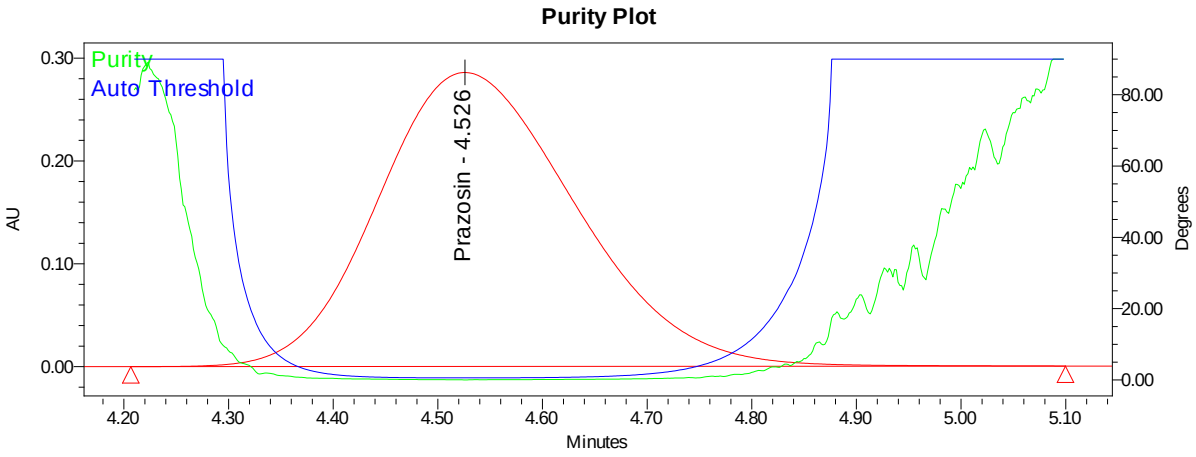


**Figure 11 Peak Purity Chromatogram of Base Stress Sample**

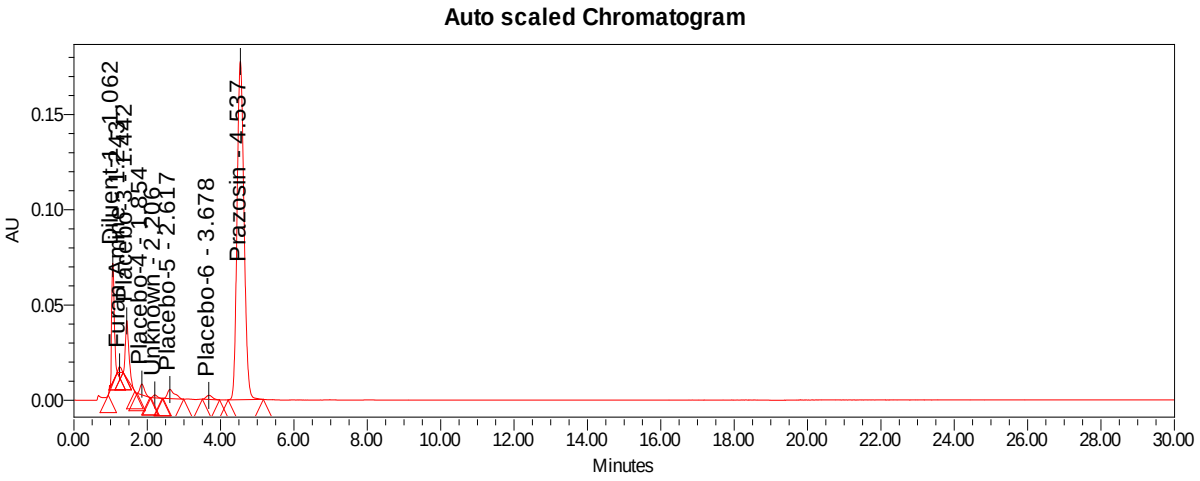


**Figure 12 Typical Chromatogram of Peroxide Stress Sample**

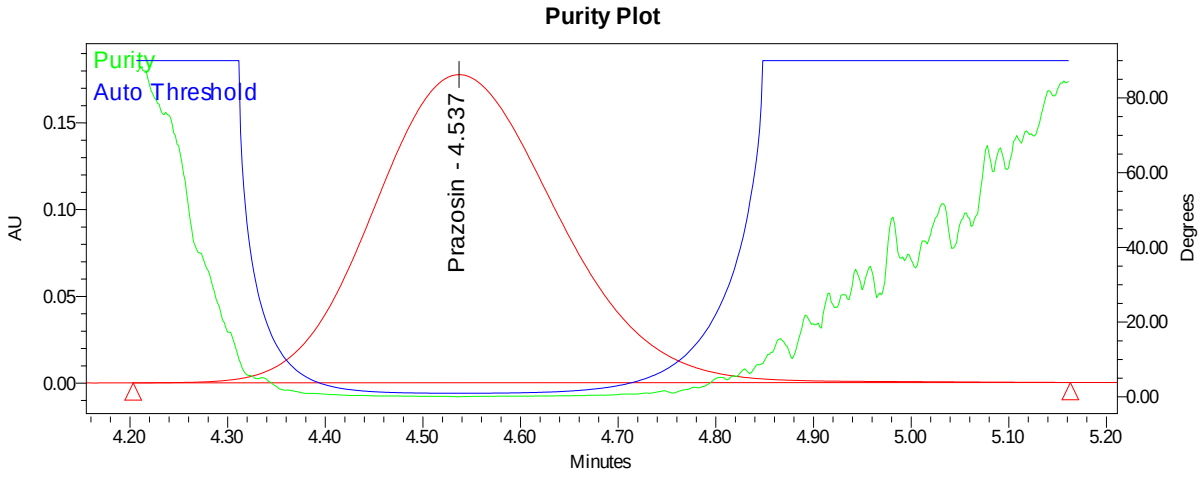


**Figure 13 Peak purity Chromatogram of Peroxide Stress Sample**

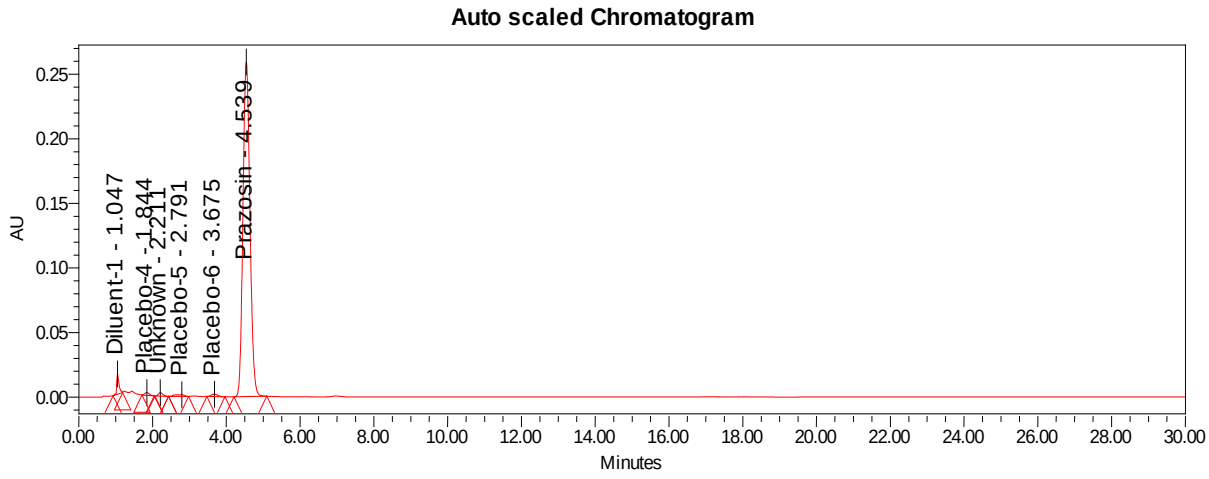


**Figure 14 Typical Chromatogram of Heat Stress Sample**

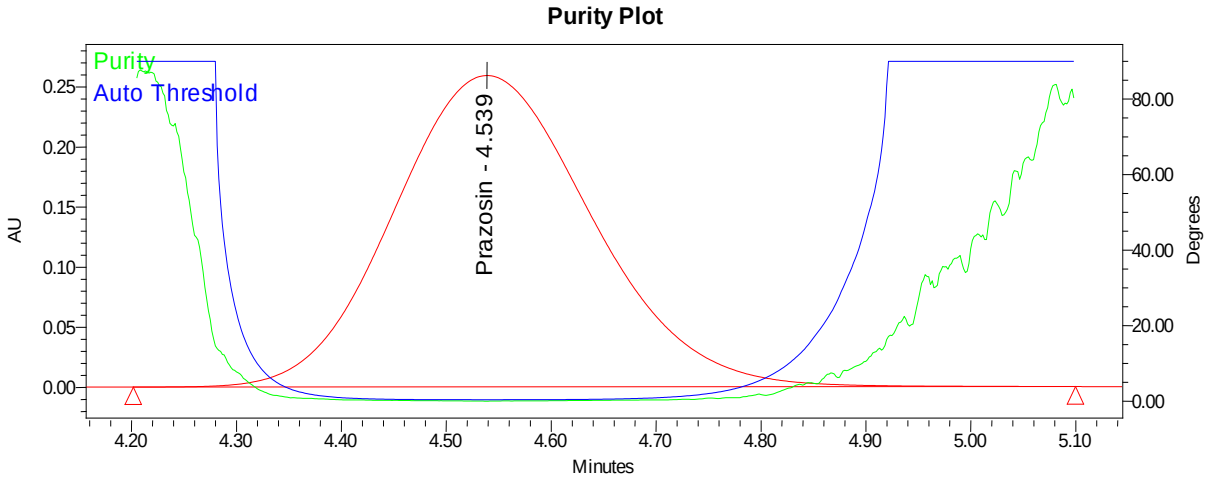


**Figure 15 Peak Purity Chromatogram of Heat Stress Sample**

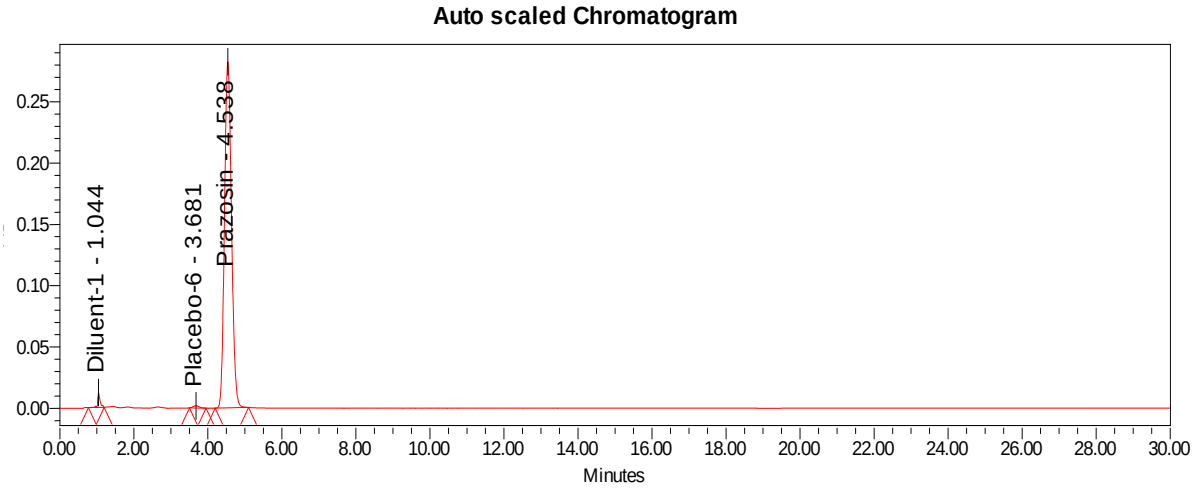


**Figure 16 Typical Chromatogram of UV Light Stress Sample**

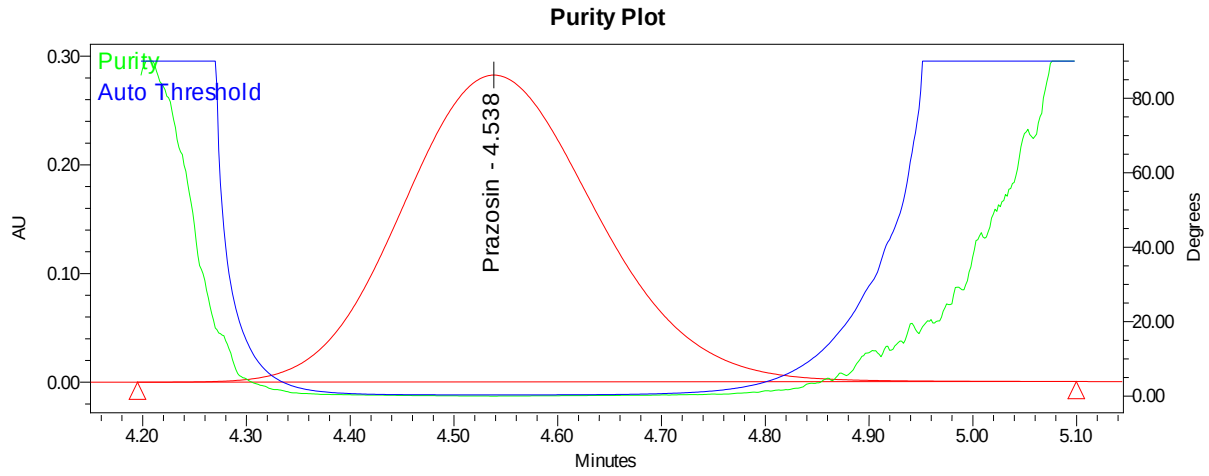


**Figure 17 Peak Purity Chromatogram of UV Light Stress Sample**

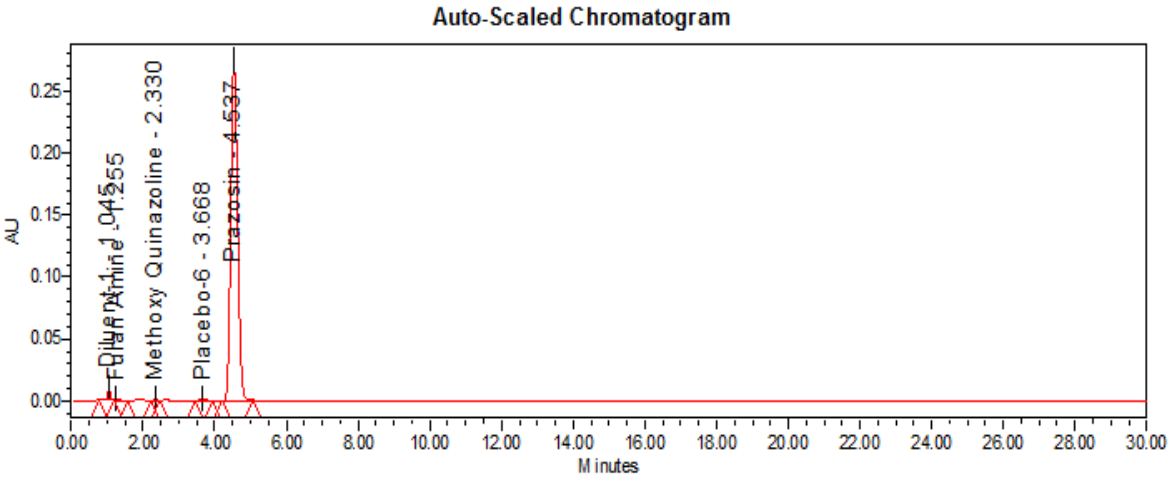


**Figure 18 Typical Chromatogram of Spiked sample**

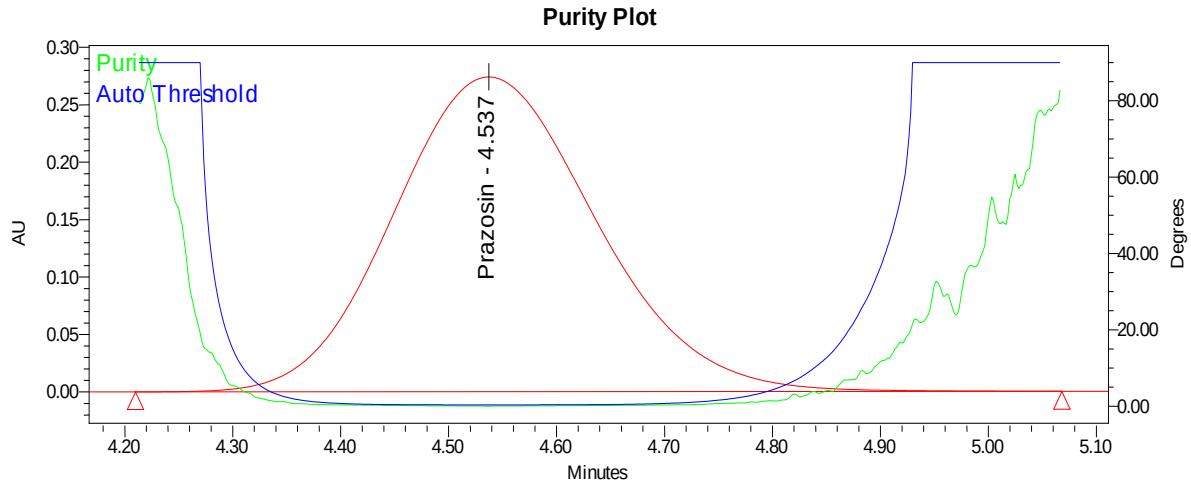


**Figure 19 Peak Purity Chromatogram of Spiked sample**

| | SampleName | % Assay | Purity Angle | Purity Threshold |
|---|---|---|---|---|
| 1 | Control Sample | 96.7 | 0.163 | 0.504 |
| 2 | 5N Hcl FP 3Hr 80°C | 48.1 | 0.266 | 1.443 |
| 3 | 0.1N NaoH FP 1hr 80°C | 96.9 | 0.202 | 0.928 |
| 4 | 3% Hydrogen FP 3Hr 80°C | 60.6 | 0.348 | 1.711 |
| 5 | UV FP 24 Hr | 94.2 | 0.156 | 0.447 |
| 6 | Heat_105°C FP 24 Hr | 88.3 | 0.187 | 0.567 |
| 7 | Spiked Sample | 92.9 | 0.160 | 0.456 |

## Table 10 Forced Degradation Results

| Sample Name/Condition | %Assay | %Total Impurity | Mass Balance (%Assay+ %Total Imp) |
|---|---|---|---|
| Control Sample/NA | 96.7 | N/A | 97 |
| Acid Sample/5 mL 5N | 48.1 | 28.560 | 77[1] |
| Base Sample/5 mL 0.1N | 96.9 | 0.266 | 97 |
| Peroxide Sample/5 mL 3% | 60.6 | 7.915 | 69[1] |
| UV Light Sample/UV light (254 | 94.2 | 0.131 | 94 |
| Heat Sample/105°C/24-hrs. | 88.3 | 1.570 | 90 |

## Table 11 Forced Degradation-Mass Balance

The mass balances calculated were less as the unknown impurities formed in Acid and Peroxide degradation conditions eluted in the RT range of 3.0-6.0 minutes were integrated as valley to valley instead of base to base integration that led to less value of % total impurity.

| Name of the Impurity | Retention time (min) | RT Ratio |
|---|---|---|
| Furan Amine | 1.255 | 0.28 |
| Furan Diamine | 1.407** | 0.31 |
| Furan Dimer | 1.960** | 0.43 |
| Methoxy Quinazoline | 2.330 | 0.51 |
| Prazosin | 4.537 | 1.00 |
| Bis Quinazoline | 11.360** | 2.50 |

**Table 12 Spiked Sample-RT and RRT**

** Retention times were considered from individual ID solutions of respective impurities

### 3.7.2 Conclusion-Forced Degradation and Specificity

1 The drug product is stable in base and UV Light degradation with very less amount of degradation
2 Finished product is sensitive to acid and peroxide degradation conditions and the % assay is about 48% and 61% respectively.
3 The degradation is found to be mild in heat stress condition.
4 During forced degradation it was observed that no secondary peak arising from any of the degradation conditions eluted at the retention time of Prazosin peak.
5 No interference observed from blank and Individual known impurities at the retention time of Prazosin peak in spiked sample and hence the method is specific.
6 Peak purity analysis using the photodiode array detector demonstrated Prazosin peak homogeneity.

### 3.7.3 Acceptance Criteria

**a.** Any Secondary peak arising from forced degradation study should not interfere with the Prazosin peak.
b. No interference should be observed from diluent and all known impurities at the retention time of Prazosin peak.

## 3.8 Robustness

The robustness is a measure of method capacity to remain unaffected by small, deliberate variations in method parameters and provides an indication of method reliability during normal use.

### 3.8.1 Determination

Standard solution was prepared and injected into the chromatographic system as per the conditions specified in the method. The same standard solution was re-injected by changing one parameter at a time, keeping other parameters constant. A set of system suitability data was calculated for standards injected under altered method conditions and compared against the values generated under normal method conditions Method Parameters:

1. Flow Rate (Procedural flow is 1.0 mL/min)
    a. Low flow → 0.8 mL/min
    b. High flow → 1.2 mL/min
2. Column Operating Temperature (Procedural temperature is 30°C)
    a. Low Temperature → Ambient
    b. High Temperature → 35° C
3. Mobile Phase Composition Variation (Buffer: Organic mixture (550:450)
    a. Low Organic Mixture → Buffer: Organic mixture (595:405)
    **b.** High Organic Mixture → Buffer: Organic mixture (505:495)

| Method Parameters | | Retention Time (min) | %RSD | USP Tailing (1st injection) | USP Plate count (1st injection) |
|---|---|---|---|---|---|
| Normal Condition | Flow Rate: 1.0mL/min<br>Column Temp:30°C<br>Mobile Phase: 550:450<br>(Buffer: Organic Mixture) | 4.558 | 0.1 | 1.0 | 2847 |
| Flow Rate Minus | 0.8 mL/min | 5.620 | 0.3 | 1.1 | 2566 |
| Flow Rate Plus | 1.2 mL/min | 3.812 | 0.2 | 1.1 | 2301 |
| Column Temp. Minus | Ambient | 5.175 | 0.1 | 1.1 | 2432 |
| Column Temp. Plus | 35°C | 4.113 | 0.2 | 1.1 | 2367 |
| Low Organic Mixture | Buffer: Organic mixture (595:405) | 7.689 | 0.0 | 1.1 | 3239 |
| High Organic Mixture | Buffer: Organic mixture (505:495) | 3.189 | 0.1 | 1.2 | 2197 |

**Table 13 Robustness Study (System Suitability)**

### 3.8.2 Conclusion

No significant change was observed in retention time after individually changing the conditions of flow rate of mobile phase, column operating temperature and mobile phase composition as mentioned above. Calculations for all other system suitability parameters met the acceptance criteria and the data generated are comparable with the normal conditions. Based on the above result, it is concluded that the method is unaffected by small, deliberate variations in flow rate, column temperature and mobile phase composition variation. Hence, method is robust.

### 3.8.3 Acceptance Criteria

All the system suitability requirements must be met. Include the cautionary statement based on the results.

## 3.9 METHOD ACCURACY

### 3.9.1 Determination

The Placebo was taken and varying amounts of Prazosin representing 40 to 160 % of standard concentration of Prazosin (20µg/mL) were added to the flasks. The spiked samples were prepared as per the method in triplicate and injected.

| | SampleName | Amount Added (µg/mL) | Amount Found (µg/mL) | % Recovered |
|---|---|---|---|---|
| 1 | Recovery_40%_Prep-1 | 7.2727 | 7.2874 | 100.2 |
| 2 | Recovery_40%_Prep-2 | 7.2727 | 7.3104 | 100.5 |
| 3 | Recovery_40%_Prep-3 | 7.2727 | 7.3484 | 101.0 |
| Mean | | | | 100.6 |

| | SampleName | Amount Added (µg/mL) | Amount Found (µg/mL) | % Recovered |
|---|---|---|---|---|
| 1 | Recovery_100%_Prep-1 | 18.1817 | 18.1734 | 100.0 |
| 2 | Recovery_100%_Prep-2 | 18.1817 | 18.3235 | 100.8 |
| 3 | Recovery_100%_Prep-3 | 18.1817 | 18.1628 | 99.9 |
| Mean | | | | 100.2 |

| | SampleName | Amount Added (µg/mL) | Amount Found (µg/mL) | % Recovered |
|---|---|---|---|---|
| 1 | Recovery_160%_Prep-1 | 29.0907 | 29.0066 | 99.7 |
| 2 | Recovery_160%_Prep-2 | 29.0907 | 28.8246 | 99.1 |
| 3 | Recovery_160%_Prep-3 | 29.0907 | 28.9743 | 99.6 |
| Mean | | | | 99.5 |

**Table 14 Accuracy Study (Prazosin)**

### 3.9.2 Conclusion

The % recovery of triplicate preparation at each level is between 98.0-102.0 for Prazosin and hence the method is accurate.

### 3.9.3Acceptance Criteria

Recovery at each level must be between 98.0-102.0% for Prazosin.

# RESULTS AND DISCUSSION

# 6. RESULTS AND DISCUSSION

An In-house method was developed for the determination of assay of Prazosin Hydrochloride Capsules USP, 1mg, 2mg and 5mg and the same was validated.

## Summary of Validation Results

<table>
<tr><th>Parameters</th><th>Acceptance Criteria</th><th>Result Summary</th></tr>
<tr><td>System precision</td><td>% RSD<br><br>USP Tailing<br><br>USP Plate count</td><td>0.1<br><br>1.1<br><br>2847</td></tr>
<tr><td>Linearity and Range</td><td>NLT 0.999</td><td>Passes</td></tr>
<tr><td>Accuracy</td><td>40%<br>100%<br>160%</td><td>100.6<br>100.2<br>99.5</td></tr>
<tr><td>Precision</td><td>RSD NMT 2.0%</td><td><table><tr><th>1mg Capsules</th><th>5mg Capsules</th></tr><tr><td>101.0</td><td>99.7</td></tr><tr><td>0.9</td><td>0.2</td></tr></table></td></tr>
<tr><td>System Suitability</td><td>NMT 2.0<br><br>Standard Solution<br><br>Sample Solution</td><td><table><tr><th colspan="3">Difference in % Assay</th></tr><tr><th>24H</th><th>48H</th><th>96H</th></tr><tr><td>0.3</td><td>0.5</td><td>0.5</td></tr><tr><td>0.2</td><td>0.2</td><td>1.4</td></tr></table></td></tr>
</table>

<table>
<tr><th>Parameters</th><th>Acceptance Criteria</th><th>Result Summary</th></tr>
<tr><td>Specificity</td><td>Any Secondary peak arising from forced degradation study should not interfere with the Prazosin peak.<br><br>No interference should be observed from the diluent and all known impurities at the retention time of Prazosin peak.<br><br>The peak purity analysis using photodiode array detector should demonstrate peak homogeneity in all degradation samples and spiked sample.</td><td>No interference<br><br>No interference<br><br>Passes</td></tr>
<tr><td>Ruggedness</td><td>% RSD<br><br>USP Tailing<br><br>USP Plate count<br><br>Mean Assay<br><br>% RSD<br><br>Difference in mean % Assay</td><td>0.3<br><br>1.1<br><br>2496<br><br><table><tr><th>1mg Capsules</th><th>5mg Capsules</th></tr><tr><td>102.2</td><td>102.1</td></tr><tr><td>1.9</td><td>0.4</td></tr><tr><td>1.2</td><td>2.4</td></tr></table></td></tr>
<tr><td>Robustness</td><td>All the System suitability requirements must be me</td><td>Passes</td></tr>
</table>

## DEVIATION SUMMARY

No Deviations were observed during the execution of method validation of assay for Prazosin Hydrochloride Capsules USP, 1mg and 5mg.

# CONCLUSION

# 7. CONCLUSION

Based on the results of the above studies, it is concluded that the method development for Prazosin Hydrochloride Capsules USP, 1mg and 5mg is specific, accurate, precise, rugged, robust and linear over the concentration range. Standard and sample solutions are stable up to 96 hours when stored at room temperature. It could be effectively applied for routine analysis.

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