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Analytical Method Development and Validation for Estimation of Perindopril Erbumine in Bulk Drug and Dosage Form by Uv Spectroscopy

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ABSTRACT:
Perindopril, an angiotensin-converting enzyme (ACE) inhibitor, is a vital therapeutic agent in cardiovascular diseases. This study presents a robust method for the development and validation of perindopril bulk by UV spectroscopy. The method was validated following International Conference on Harmonization (ICH) guidelines. The UV method was performed using a UV-Vis spectrophotometer. The optimized conditions involved the use of methanol as the solvent. The calibration curve was found to be linear over the concentration range of X to Y µg/ml with a correlation coefficient (r^2) of Z. The method exhibited good precision with a relative standard deviation (RSD) of less than 2%. The accuracy of the method was confirmed with a recovery study yielding results within the acceptable range. The proposed method was successfully applied for the determination of perindopril bulk in pharmaceutical formulations. The results indicate that the developed UV spectroscopic method is accurate, precise, and sensitive for the estimation of perindopril bulk. Thus, the developed method can be used for routine quality control analysis of perindopril bulk in pharmaceutical industries, ensuring its efficacy and safety.

Keywords: International Conference on Harmonization, Angiotensin-Converting Enzyme (ACE), Ultra Violet Spectroscopy, Relative Standard Deviation

1. INTRODUCTION:

1.1 PERINDOPRIL:

Perindopril is a member of the angiotensin-converting enzyme (ACE) inhibitor class of medications, renowned for its efficacy in managing hypertension, heart failure, and other cardiovascular conditions. Its pharmacological profile encompasses potent inhibition of ACE, an enzyme pivotal in the renin-angiotensin-aldosterone system (RAAS), thereby impeding the conversion of angiotensin I to angiotensin II, a vasoconstrictor, and aldosterone release, ultimately leading to vasodilation, reduced blood pressure, and ameliorated cardiac workload. Introduced as an antihypertensive agent, perindopril has evolved into a multifaceted therapeutic option, demonstrated by extensive clinical trials and real-world data corroborating its utility in various cardiovascular pathologies. Its mechanism extends beyond blood pressure control, encompassing anti-remodeling, anti-inflammatory, and endothelial protective effects, contributing to its role in preventing cardiac remodeling, attenuating atherosclerosis, and improving

endothelial function. Perindopril's efficacy is not only attributed to its pharmacological actions but also to its favorable pharmacokinetic properties, including high oral bioavailability, prolonged duration of action, and low potential for drug interactions. Its prodrug nature, requiring enzymatic conversion to the active metabolite perindoprilat, provides sustained inhibition of ACE, facilitating once-daily dosing regimens and enhancing patient adherence. Clinical trials and observational studies have elucidated perindopril's superiority in cardiovascular risk reduction, evidenced by reductions in mortality, myocardial infarction, stroke, and heart failure hospitalizations, underscoring its indispensable role in primary and secondary prevention strategies. Furthermore, its safety profile, encompassing a low incidence of adverse effects such as cough and angioedema, enhances its tolerability and suitability for long-term therapy. In summary, perindopril stands as a cornerstone in the management of hypertension and cardiovascular diseases, owing to its robust pharmacological profile, proven clinical efficacy, and favorable safety profile. Its multifaceted benefits extend beyond blood pressure control, encompassing cardioprotective, Reno protective, and vasculoprotective effects, positioning it as a pivotal component in the armamentarium against cardiovascular morbidity and mortality.

1.2 UV SPECTROSCOPY: -

UV spectroscopy, or ultraviolet-visible spectroscopy, is a powerful analytical technique used to study the absorption, transmission, and emission of ultraviolet and visible light by molecules. It provides valuable insights into the electronic structure and properties of molecules, making it a cornerstone in analytical chemistry, biochemistry, and various scientific disciplines. When a molecule absorbs UV or visible light, it undergoes electronic transitions, moving from one energy state to another. These transitions are specific to the molecular structure and the types of atoms involved. By measuring the absorption of light at different wavelengths, UV spectroscopy allows scientists to identify molecules and to quantify the concentration of absorbing species in a sample. The UV-visible spectrum ranges from approximately 200 nm to 800 nm. UV light has higher energy and shorter wavelengths than visible light. The energy of the absorbed light corresponds to the energy required to promote an electron from a lower energy orbital to a higher energy orbital. UV spectroscopy is widely used in various fields such as pharmaceuticals, environmental monitoring, biochemistry, and materials science. Its applications include determining the concentration of a compound in a sample, studying the kinetics of chemical reactions, and investigating the stability of molecules under different conditions. In summary, UV spectroscopy plays a crucial role in understanding the electronic structure and properties of molecules, making it an indispensable tool in modern scientific research and analysis.

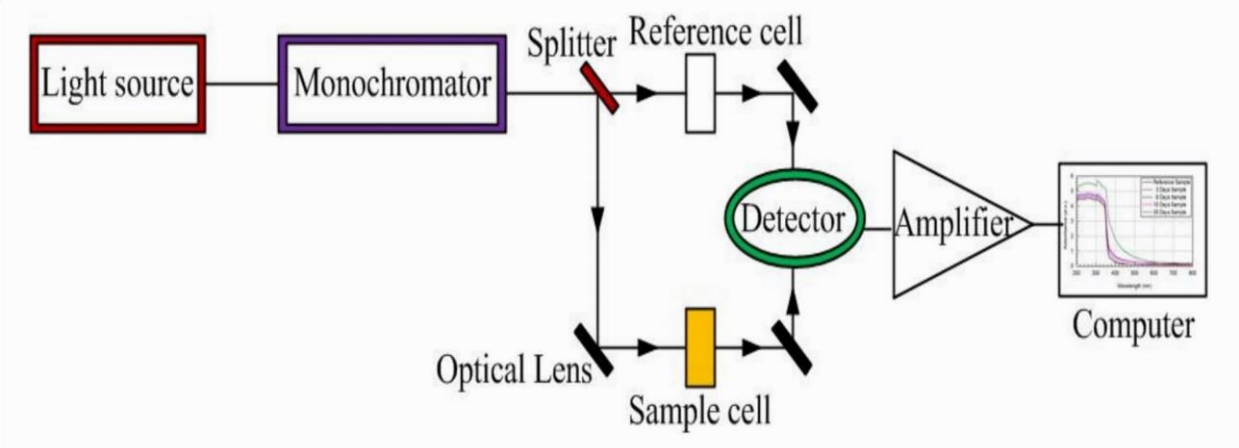


Fig.no 1 – UV-Spectroscopy

1.3 DEFINITIONS: -

UV Spectroscopy-

UV spectroscopy, or ultraviolet-visible spectroscopy, is a technique used to analyse the absorption and transmission of ultraviolet and visible light by molecules, providing valuable information about their electronic structure and properties.

Fig.no 2 – UV-Spectroscopy Instrument

Assay: An assay refers to a quantitative analysis method used to determine the concentration, potency, or purity of a drug or its active pharmaceutical ingredient (API). It ensures that the drug product meets the required specifications and standards for efficacy, safety, and quality.

Wavelength: Wavelength is the distance between successive crests, troughs, or any other equivalent points on a wave. In simpler terms, it's the length of one complete cycle of a wave, typically denoted by the symbol λ (lambda).

Validation: Validation in the pharmaceutical industry refers to the process of establishing documented evidence that a system or process, such as manufacturing, packaging, cleaning, or testing, will consistently and reliably produce results meeting predetermined specifications and quality attributes. This process ensures that pharmaceutical products are manufactured to meet their quality standards.

Method development: Method development in the pharmaceutical industry refers to the process of creating and optimizing analytical methods to accurately and precisely analyse the quality, purity, and potency of drugs and pharmaceuticals. This involves the identification and optimization of parameters such as solvents, columns, and detection methods to ensure reliable and reproducible results.

1.4 Advantages and Benefits-

Developing a UV spectroscopy method for perindopril can offer several benefits and advantages:

1. High Sensitivity: UV spectroscopy is highly sensitive, allowing for the detection of perindopril at low concentrations.
2. Cost-Effectiveness: UV spectroscopy is a relatively inexpensive method compared to other analytical techniques, making it cost-effective for routine analysis.
3. Rapid Analysis: UV spectroscopy offers quick and straightforward analysis, reducing the time required for testing and analysis.

4. Non-Destructive: UV spectroscopy is non-destructive, preserving the sample for further analysis or other tests.
5. Wide Availability: UV spectroscopy is a widely available technique in most laboratories, making it accessible for method development and routine analysis.
6. Selectivity: With careful method development, UV spectroscopy can be highly selective for perindopril, minimizing interference from other components in the sample.
7. Validation: UV spectroscopy methods are well-established and can be easily validated according to regulatory requirements, ensuring the reliability and accuracy of the results.
8. Environmental Friendliness: UV spectroscopy is an environmentally friendly technique, as it doesn't require the use of harmful chemicals.

1.5 Disadvantages and Limitations-

Limitations and disadvantages of UV spectroscopy include:

1. Lack of Selectivity: UV spectroscopy is not highly selective, as many compounds absorb in the UV range. It can be challenging to differentiate between different compounds based solely on UV absorption.
2. Sensitivity: UV spectroscopy may lack sensitivity, especially when analysing compounds with low molar absorptivity.
3. Interference: UV spectroscopy can be affected by interference from impurities, which can lead to inaccurate results.
4. Limited Application: UV spectroscopy is limited to compounds that absorb UV light.

Compounds that do not absorb UV light cannot be analysed using UV spectroscopy.

5. Sample Preparation: Sample preparation for UV spectroscopy can be time-consuming, and the method may require a larger sample size compared to other analytical techniques.
6. Overlapping Absorption: Overlapping absorption bands can complicate the analysis and make quantification difficult.
7. Solvent Dependency: UV spectroscopy can be sensitive to the solvent used, which may lead to variability in results.
8. Lack of Structural Information: UV spectroscopy does not provide detailed structural information about the compound being analysed.
9. Limited Quantification: It may be challenging to quantify complex mixtures accurately using UV spectroscopy.
10. Complexity of Analysis: UV spectroscopy may require sophisticated instrumentation and skilled operators for accurate and reliable results.

2. RATIONAL OF WORK:

Aim: Analytical Method Development and Validation for Estimation of Perindopril Erbumine in bulk drug and Dosage form by UV-Spectroscopy

Objectives:

1. Develop a reliable and accurate UV spectrophotometric method for the determination of perindopril in bulk form.
2. Validate the developed UV method to ensure its suitability for its intended purpose.
3. Establish the specificity of the method to ensure that there is no interference from excipients or other impurities.

4. Determine the linearity of the method to ensure accurate quantification over the expected concentration range.
5. Assess the precision and accuracy of the method to ensure consistent and reliable results.
6. Determine the robustness of the method to ensure its reliability under a variety of conditions.

CHAPTER 3 LITERATURE REVIEW

3. LITERATURE REVIEW:

Fox KM *et al.* (2000) – Management of coronary artery discase: implications of the EUROPA trial. Br J Cardiol. 13655 low-risk patients with stable coronary heart disease but no apparent heart failure was recruited between October 1997 and June 2000. After a 4-week runin period, 12218 patients were randomly assigned to receive perindopril 8 mg once daily (n=6110) or matching placebo (n=6108). Over a mean follow-up of 4.2 years, perindopril showed a 20% relative risk reduction (95% CI 9-29, p=0.0003) for the primary endpoint of cardiovascular death, myocardial infarction, or cardiac arrest. Perindopril was well-tolerated.

Chobanian AV *et al.* (2003) :- The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure emphasizes evidence-based approaches. Key points: In those over 50, systolic BP over 140 mm Hg poses higher CVD risk; from 115/75 mm Hg, each 20/10 mm Hg increase doubles CVD risk; normotensive at 55 have 90% risk of developing hypertension; prehypertensive need lifestyle changes; thiazide diuretics are recommended; high-risk conditions require specific drugs; for some, two or more medications may be needed; patient motivation is crucial for effective control.

Henine N et al. (2016): - They screened 2367 hypertensive treated patients referred for uncontrolled hypertension. Among them, 35.6% had suboptimal treatment, 15.4% poor adherence, and 8.5% a white-coat effect. Excessive salt intake and drug-related hypertension were identified in 11.9% and 1.5% of patients, respectively. A secondary cause of hypertension was diagnosed in 19.8% of subjects. Finally, only 173 patients showed true resistant hypertension, with a prevalence of 7.3% (CI 95%: 6.3-8.3). Less than one patient from ten referred had true resistant hypertension.

Kirk JK et al. (2017): - A systematic review of hypertension outcomes and treatment strategies in older adults. A systematic review of six trials (2006–2016) on hypertension management in patients over 70 found varied blood pressure goals (<120 to <160 systolic, <80 diastolic). Trials from Europe, China, Australia, Tunisia, US, and Japan showed treating to tight goals (<140/80) or lower, if tolerated, resulted in improved cardiovascular outcomes. Many trials were stopped early due to significant findings in mortality and cardiovascular outcomes.

Bounhoure JP et al. (2000): - Cardiac decompensation occurred in three patients of the placebo group, but not in the perindopril group. The effectiveness of perindopril in heart failure was demonstrated by the improvement observed in exercise test and severity score and by the decrease of cardiothoracic ratio. Changes in SAP, and serum creatinine levels, in particular, showed that the drug was well tolerated. A randomised double blind multicentre trial versus placebo was carried out over 3 months following a 15 days pre-inclusion period in 125 patients with NYHA Stage II and III cardiac failure stabilised by digitalis and diuretic therapy. Perindopril was administered at a dosage of 2 or 4 mg according to initial systolic blood pressure and efficacy was evaluated at 1 and 3 months according to the NYHA classification, a score of clinical severity, the duration of two exercise stress tests on a bicycle ergometer or treadmill and the cardiothoracic ratio.

Morgan T and Anderson A et al. (1992): - Morgan was an Australian scientist and he was at the University of Melbourne. He was from department of physiology. He worked on the clinical efficacy of Perindopril. Perindopril's effectiveness in mild to moderate hypertension was evaluated in three studies. Perindopril was more effective than sodium restriction in reducing blood pressure, and the effects were additive. Perindopril was as effective as atenolol in reducing blood pressure, and was well tolerated. Perindopril lowered blood pressure to the same extent as enalapril at peak drug levels but had a greater effect at the trough level of the drugs. Perindopril is an effective antihypertensive agent with an acceptable side-effect profile in people with hypertension.

Myers MG : - Myers studied "A dose-response study of perindopril in hypertension: effects on blood pressure 6 and 24h after dosing." To examine the dose-response characteristics of perindopril at the time of its peak and trough antihypertensive effects, with the primary outcome measure being changes in diastolic blood pressure. After a four-week, single-blind placebo run-in, patients were randomly allocated to four doses of perindopril or placebo using a parallel group design. Sixteen specialist centres in the United States. Of 483 patients entered into the run-in phase, 293 were eligible for randomization to perindopril or placebo therapy, with 260 patients included in the final efficacy analysis. Eligible patients were randomized to 12 weeks of therapy with perindopril 2, 4, 8 or 16 mg or placebo.

4. PLAN OF WORK:

Literature Review: - Review existing UV-visible spectrophotometric methods for perindopril assay. - Identify suitable wavelengths and sample preparation techniques.

Instrumentation and Reagents: - Calibrate UV-visible spectrophotometer. - Procure highquality reagents including perindopril standard solution.

Method Development: - Determine absorption spectrum of perindopril. - Select optimal wavelength(s) for maximum absorbance. - Adjust parameters for sensitivity and selectivity.

- Develop sample preparation methods.

Method Validation: - Assess specificity with placebo samples. - Construct calibration curve for linearity. - Determine accuracy through spiked samples. - Evaluate precision (repeatability and intermediate precision). - Determine LOD and LOQ. - Assess robustness under varied conditions.

Documentation and Reporting: - Prepare detailed protocol and experimental data documentation. - Summarize results in validation report with recommendations.

Regulatory Compliance: - Ensure compliance with relevant guidelines (e.g., ICH).

Method Transfer and Training: - Transfer validated method to lab and provide training if needed.

Continuous Monitoring and Maintenance: - Regularly monitor method and spectrophotometer performance. - Perform maintenance for accuracy and reliability.

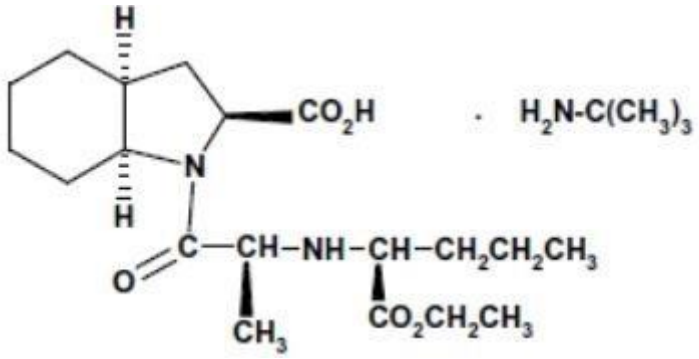
5. DRUG PROFILE:

5.1 Perindopril:

Perindopril is a nonsulfahydryl prodrug that belongs to the angiotensin-converting enzyme (ACE) inhibitor class of medications. It is rapidly metabolized in the liver to perindoprilat, its active metabolite, following oral administration. Perindoprilat is a potent, competitive inhibitor of ACE, the enzyme responsible for the conversion of angiotensin I (ATI) to angiotensin II (ATII). ATII regulates blood pressure and is a key component of the renin-angiotensinaldosterone system (RAAS). Perindopril may be used to treat mild to moderate essential hypertension, mild to moderate congestive heart failure, and to

reduce the cardiovascular risk of individuals with hypertension or post-myocardial infarction and stable coronary disease.

5.2 Molecular structure:



Perindopril Erbumine structure

5.2 Molecular formula: C23H43N3O5
5.3 Molecular weight: 441.613 g/mol
5.4 IUPAC name: (2S ,3a S, 7a S)-1-{(2S)-2-[(1S)-1-(ethoxycarbonyl) butylamino] propanoyl} octahydro-1H indole-2- carboxylic acid-2-methylpropan-2-amine (1/1).
5.5 Melting and boiling point: 126-128℃ and 537.4℃

6. EXPERIMENTAL WORK:

6.1 PRELIMINARY CHARACTERIZATION OF DRUG

6.1.1 Color, odor and appearance

Perindopril erbumine was evaluated for parameters like color; odor & appearance are shown in result**.**

6.1.2. Determination of solubility

The solubility was determined in Water at a concentration of 3 mg/mL as follows and are given in results**.**
Water: Weighed approx. 30 mg of Perindopril erbumine and sonicated for 5-10 minutes to dissolve in 10 ml of Water.

6.2 Selection of analytical wavelength

6.2.1. Selection of solvent

Water was selected as the solvent for dissolving Perindopril erbumine

6.2.2. Preparation of standard stock solutions

In order to prepare stock solution, weighed accurately 10 mg Perindopril erbumine and transferred into 20 ml volumetric flask, added 15 ml of Water and sonicated to dissolve the standard completely and diluted up to the mark with Water (500 PPM).
Further diluted 1 mL to 25 mL with Water. (20 PPM)

6.2.3. Selection of test concentration

When we analyzed 20 ppm of Perindopril erbumine between 400 nm to 200 nm. 20 ppm solution showed 0.3965 absorbance at its absorption maxima (215 nm). Hence, we select 30 ppm as a test concentration.
30 ppm of Perindopril erbumine will shows absorbance about 0.5948 and when we will perform the linearity from 50% to 150% on UV, the absorbance of 150% level will not go above 1, because absorbance should not be more than 1 in UV-spectroscopy as it is one of the limitations of UV-spectroscopy.

6.2.4. Preparation of System suitability test (Perindopril erbumine standard solution): Weighed about 10 mg of Perindopril erbumine and transferred in 50 mL volumetric flask, added 35 mL of water, sonicated to dissolve it, made volume up to the mark with water. Pipette out 1.5 ml from standard stock solution and transferred into 10 ml volumetric flask and made volume up to the mark with water **(30 µ/mL= working concentration).**

System suitability is a Pharmacopeial requirement and is used to verify, whether the UV system is adequate for analysis to be done. The tests were performed by collecting data from Five replicate of standard drug solution and the results are recorded.

Acceptance criteria

1. % RSD should not be more than 2.0 % for five replicates of standard solution.

6.3. Analysis of marketed Test sample:

Marketed test sample Having Name Coversyl 4 mg tablet are selected for analysis and for doing validation.

Average weight of test sample (Coversyl 4 mg tablet):

Weighed the 20 tablets at a time and calculated average weight of tablet by following formula:

Average weight (mg) = Weight of 20 tablets (mg) / 20

Fig.no 3 – Coversyl Tablet

Sample preparation of Marketed test sample:

Weighed 20 tablets transferred in mortar pestle and crushed to fine powder. Mixed the contents with butter paper uniformly. Weighed the powder material equivalent to 10 mg of Perindopril erbumine (343.0 mg) and transferred to clean and dried 50 mL of volumetric flask. Added 3035 mL of water, sonicated for 15 minutes with intermittent shaking. After 15 minutes allow the solution to cool at room temperature and made volume up to the mark with water. Filtered the solution through suitable 0.45 µ syringe filter discarding 3-5 mL of initial filtrate. Further diluted 1.5 ml of filtered stock solution to 10 ml with water. (30 mcg of Perindopril erbumine), and analyzed solution at 215 nm on UV-spectrophotometer.

Sample Prepared in duplicate. Summary of sample preparation as follows:

Sample	Sample (mg)	Diluted to (mL)	Volume taken	Diluted to (mL)
Sample 1	343.8	50	1.5	10
Sample 2	343.1	50	1.5	10

Formula for % Assay calculation:

$$\% \text{ Assay} = \frac{\text{Sample abs of Perindopril erbumine}}{\text{Standard abs Perindopril erbumine}} \times \frac{\text{Perindopril erbumine STD wt. (mg)}}{50} \times \frac{1.5}{10} \times \frac{50}{\text{Sample wt.}} \times$$

$$10 \times \frac{\text{Avg. wt. (mg)}}{1.5 \times \text{LC of Perindopril erbumine (mg)}} \times 100$$

6.4. VALIDATION OF UV METHOD

The developed method for estimation of Perindopril erbumine was validated as per ICH guidelines for following parameters.

FILTRATION STUDY:

Filtration study of an analytical procedure checks the interference of extraneous components from filter, deposition on filter bed and compatibility of filter with sample.

This study was conducted with Perindopril erbumine Test sample (Test solution).

Filtration study carried out with unfiltered and filtered test solution. During filtration activity 0.45 µm PVDF and 0.45 µm Nylon syringe filters used by discarding 5 mL of Initial aliquot sample.

STABILITY OF ANALYTICAL SOLUTION

Stability study was conducted for standard and test sample solution. Stability study was performed at normal laboratory conditions.

The solution was stored at normal illuminated laboratory conditions and analyzed at Initial and after 12 and 24 hours.

Standard and Test solution stability study was performed by calculating the difference between results of test solution at each stability time point to that of initial.

SPECIFICITY:

Specificity is the ability to access unequivocally the analyte in the presence of components which may be expected to be present.

Following solution prepared and analysed to prove the specificity nature of the method.

(Scanned each solution from 400 nm to 200 nm)

1. Blank (Water)
2. Placebo solution

Analysing marketed test sample contains excipients (additives) which are totally unknown. So Placebo prepared at lab level by using formula as follows:

Sr. No.	Ingredients	Role	Qty (mg)
1	Lactose	Filler	80
2	Starch	Binder	5
3	Magnesium stearate	Lubricant	5
4	Talc	Glidant	5
5	Crospovidone	Disintegrants	5
	Total		100 mg

Total 5 gm of placebo prepared:

Placebo Sample solution preparation:

Weighed 333.0 mg of placebo material (Which is equivalent to 10 mg of Perindopril erbumine) and transferred to clean and dried 50 mL of volumetric flask. Added 30-35 mL of water, sonicated for 15 minutes with intermittent shaking. After 15 minutes allowed the solution to cool at room temperature and made volume up to the mark with water. Filtered the solution through suitable 0.45 µ syringe filter discarding 3-5 mL of initial filtrate.

Further dilute 1.5 ml of filtered stock solution to 10 ml with water, scanned from 400 nm to 200 nm.

Acceptance criteria:

Blank: % Interference at Absorption maxima of Perindopril erbumine is NMT 1.0%

Placebo: % Interference at Absorption maxima of Perindopril erbumine is NMT 2.0%

4) LINEARITY AND RANGE

Preparation of linearity solution

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

5 levels of Linearity was performed from 50% to 150% of test concentration **Linearity Perindopril erbumine stock solution:**

Weighed 10 mg of Perindopril erbumine and dissolved in 10 mL with Water. Further diluted 5 mL to 50 mL with water (100 ppm)

Linearity level preparation for HPLC:

Sr. No.	Level (%)	mL of stock solution	Diluted to with water (mL)	Perindopril erbumine Concentration (µg/mL)
1	50%	1.50	10	15.00
2	75%	2.25	10	22.50
3	100%	3.00	10	30.00
4	125%	3.75	10	37.50
5	150%	4.50	10	45.00

Determination

Each level analyzed in triplicate and mean absorbance calculated. Calibration curve was plotted graphically as a function of analyte concentration in µg/mL on X-axis Vs mean absorbance on y-Axis as given in results. **Acceptance criteria**

Correlation Coefficient: NLT 0.98

Intercept: To be report
Slope: To be report
% RSD at each level: NMT 2.0%

5) Limit of Detection (LOD) and Limit of Quantitation (LOQ):

Detection limit:

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Quantitation limit:

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. As per ICH Q2R1 guidelines LOD and LOQ was determined by using the approach Based on the Calibration Curve in which residual standard deviation of a regression line was calculated and determined the LOD and LOQ by using following formula:

LOD = 3.3 σ / S

LOQ = 10 σ / S Where,

σ = residual standard deviation of a regression line

S = Slope of regression line

6) ACCURACY (% RECOVERY)

The accuracy of the analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value of the value found,

Accuracy will be conducted in the range from 50 % to 150 % of working concentration. Solution of each accuracy level was prepared in triplicate. Calculated % Recovery for each sample, Mean % recovery for each level and overall recovery and also calculated % RSD for each level and % RSD for overall recovery.

Accuracy levels details:

Refer Following table for each sample:

Level (%)	Perindopril erbumine Std (mg)	Placebo (mg)	Diluted to (mL)	Volume taken (mL)	Diluted to (mL)	Perindopril erbumine Concentration (µg/mL)
50	5.2	333.7	50	1.5	10	15.60
	5.3	332.8	50	1.5	10	15.90
	5.1	333.4	50	1.5	10	15.30
100	10.3	333.1	50	1.5	10	30.90
	10.2	332.9	50	1.5	10	30.60
	10.2	334.1	50	1.5	10	30.60
150	15.3	333.7	50	1.5	10	45.90
	15.1	333.4	50	1.5	10	45.30
	15.1	334.2	50	1.5	10	45.30

Procedure for preparation of Accuracy sample solution:

Take clean and dried 9 volumetric flasks of 50 mL. Weighed approx. 333.0 mg of placebo and transferred

in each 50 mL volumetric flask. Weighed Perindopril erbumine API as per accuracy level and transferred in same 50 ml volumetric flask. Added 30-35 ml of Water sonicated it for 15 minutes with intermittent shaking. Allowed the solution to cool at room temperature and made the volume up to the mark with Water. Filter the solution through 0.45 µ PVDF syringe filter discarding 3-5 mL of filtrate. Further dilute 1.5 ml of filtrate to 10 ml with water.

Acceptance criteria

1. % Recovery for each sample and Mean recovery should be in the range of 98-102%.
2. The Relative Standard Deviation should not be more than 2.0%.

7) PRECISION

Precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous test under the prescribed conditions. Precision is of two types, Repeatability and Intermediate precision. It is performed on tablet test sample.

I. Repeatability:

Preparation of sample solution (6 Samples prepared):

Weighed 20 tablets transferred in mortar pestle and crushed to fine powder. Mixed the contents with butter paper uniformly. Weighed the powder material equivalent to 10 mg of Perindopril erbumine (343.0 mg) and transferred to clean and dried 50 mL of volumetric flask. Added 30-35 mL of water, sonicated for 15 minutes with intermittent shaking. After 15 minutes allow the solution to cool at room temperature and made volume up to the mark with water. Filtered the solution through 0.45 µ PVDF syringe filter discarding 3-5 mL of initial filtrate. Further diluted 1.5 ml of filtered stock solution to 10 ml with water. (30 mcg of Perindopril erbumine), and analyzed solution at 215 nm on UV-spectrophotometer.

Six samples prepared.

Precision (Repeatability) Sample details are as follows:

Sample No.	Test powder material (mg)	Diluted to (mL)	Volume taken (mL)	Diluted to (mL)
1	343.7	50	1.5	10
2	343.1	50	1.5	10
3	342.7	50	1.5	10
4	343.8	50	1.5	10
5	344.2	50	1.5	10
6	343.2	50	1.5	10

Acceptance criteria:

% Assay: 90-110% for each sample and mean assay value

% RSD for % assay of 6 samples: NMT 2%

I. Intermediate precision

It is performed by doing analysis on another day to check reproducibility of results. Samples prepared in same manner as that of Repeatability parameter (6 Samples prepared).

Intermediate Precision Sample details are as follows:

Sample No.	Test powder material (mg)	Diluted to (mL)	Volume taken (mL)	Diluted to (mL)
1	342.9	50	1.5	10
2	343.6	50	1.5	10
3	343.2	50	1.5	10
4	344.3	50	1.5	10
5	343.6	50	1.5	10
6	343.7	50	1.5	10

Acceptance criteria:
% Assay: 90-110% for each sample and mean assay value
% RSD for % assay of 6 samples of Intermediate precision**:** NMT 2
% RSD for Total 12 samples: NMT 2% for test results (6 of Repeatability and 6 of Intermediate precision)

8) ROBUSTNESS
The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.
Determination: Test solution and Standard solution were analysed under different conditions as shown below.
1. Changes in Sonication time for test sample preparation by ±5 min
2. Change in wavelength (± 2 nm)

Acceptance criteria:
Abs difference for assay value of test solution by change in method parameter: NMT
2.0 w.r.t. Mean assay value of precision study

7. RESULT AND DISCUSSIONS:
7.1. PRELIMINARY CHARACTERIZATION AND IDENTIFICATION OF DRUG
7.1.1. Color, odor and appearance
Colour, odour and appearance of Drug

Sr. No	Name	Colour, odour and appearance of drug
1	Perindopril erbumine	White, odourless and Amorphous powder

7.1.2. Solubility study
Solubility study of Perindopril erbumine

Sr. No.	Name of Solvent	Observation	Conclusion	Summary
1	Water	No Drug Particles seen after sonication	Drug was found soluble in water.	Water used as a diluent for preparing stock solution.

7.2. Selection of solvent

Water was selected as the solvent for dissolving Perindopril erbumine

7.2.1. Selection of analytical wavelength 1) Blank:

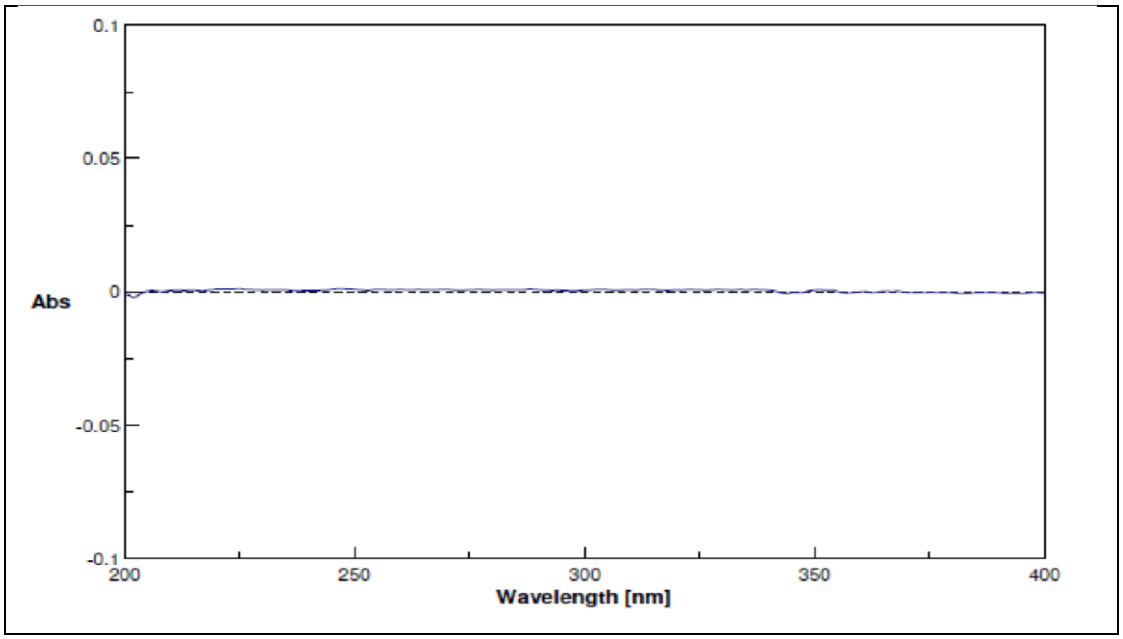


Fig. No. 1 UV spectrum of blank

2) Perindopril erbumine STD solution: (20 PPM)

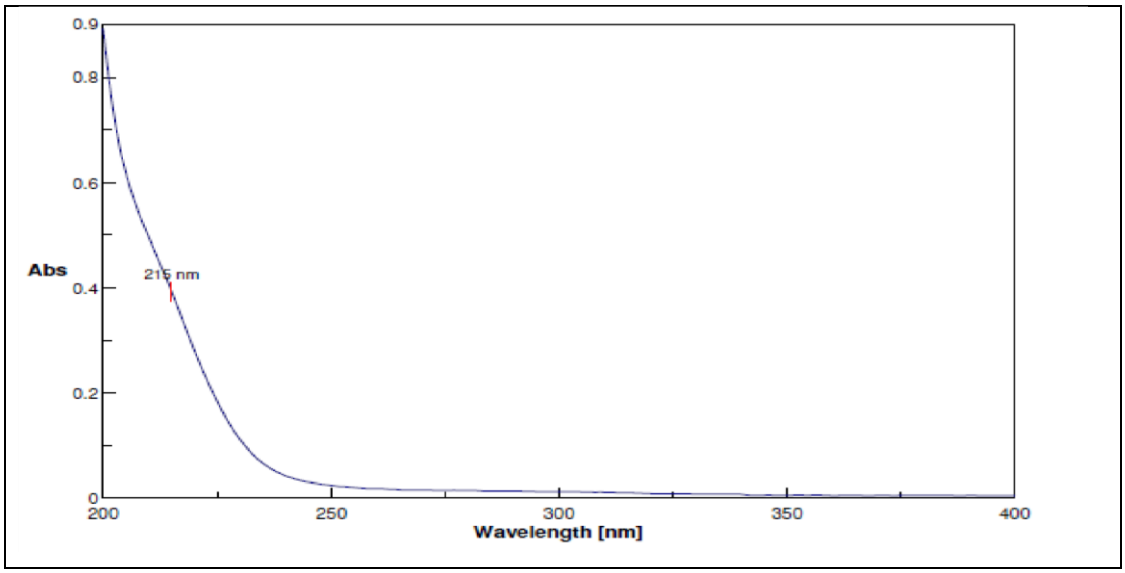


Fig. No. 2 UV spectrum of Perindopril erbumine

Observation: The standard solution was scanned between 200 nm to 400nm. Wavelength of maximum absorption was determined for drug. Perindopril erbumine showed maximum absorbance at 215 nm. It is shown in **Figure No.2**. Therefore 215 nm considered as an analytical wavelength for further determination.

7.3. System suitability test

Results for System Suitability Test of Perindopril erbumine.

Sr No.	Standard solution	Absorbance
1	Standard_1	0.5946
2	Standard_2	0.5942
3	Standard_3	0.5939
4	Standard_4	0.5949
5	Standard_5	0.5948
	Mean	**0.5945**
	STD Dev	**0.00042**
	% RSD	**0.07**

System Suitability Acceptance Criteria:

% Relative standard deviation for the absorbance of standard solution should not be more than 2.0 % for five replicates.

Data interpretation: It was observed from the data tabulated above; the method complies with system suitability parameters. Hence, it can be concluded that the UV method is adequate for intended analysis.

7.4. Analysis of Marketed Test samples (Assay)

Weight of 20 tablets: 2.7440 gm

Average weight = 2.7440 / 20 = 0.1372 gm = 137.2 mg **Assay of Coversyl 4 mg Tablet:**

Sample	Absorbance	% Assay	Mean Assay
Sample 1	0.5865	98.42	98.90
Sample 2	0.5909	99.37	

Acceptance criteria:

1) % Assay found should be in the range of 90-110%.

Data interpretation: From the above results, it can be concluded that the assay result is within the limit for selected marketed test sample and sample can be used for validation.

7.5. VALIDATION OF UV METHOD

1) FILTRATION STUDY:

Filtration study of an analytical procedure checks the interference of extraneous components from filter, deposition on filter bed and compatibility of filter with sample.

Performed on tablet test sample.

Results of Filter study

Sample description	Area	% Absolute difference
Unfiltered	0.5912	NA

0.45 µ PVDF filter	0.5871	0.69
0.45 µ Nylon filter	0.5856	0.95

Acceptance criteria: % Absolute difference of filtered samples NMT 2.0 w.r.t. Unfiltered sample.
Data interpretation: Both filters PVDF and Nylon passes the criteria for filter study, hence both filters can be used. We used PVDF filter because it showed less absolute difference as compare to Nylon filter.

2) SOLUTION STABILITY: Stability study was conducted for Standard as well as Test Sample. Stability study was performed at normal laboratory conditions. The solution was stored at normal illuminated laboratory conditions and analysed at initial, after 12 hours and 24 hours.
Results of Solution stability:

Sample solution			**Standard solution**		
Time point	**Area**	**% Absolute difference**	**Time point**	**Area**	**% Absolute difference**
Initial	0.5910	NA	**Initial**	0.5938	NA
12 Hours	0.5872	0.64	**12 Hours**	0.5909	0.49
24 Hours	0.5846	1.08	**24 Hours**	0.5892	0.77

Acceptance criteria: % Absolute difference of Stability solution: NMT 2.0 w.r.t. Initial solution.
Data interpretation: Standard and Test solution was found stable up to 24 Hrs. Hence both solutions can be used up to 24 Hrs.

3) SPECIFICITY: Specificity is the ability to access unequivocally the analyte in the presence of components which may be expected to be present.
Blank and placebo solution prepared and scanned from 400 nm to 200 nm.
Results of Specificity:

Description	**Observation**
Blank	No interference at Absorption maxima of Perindopril erbumine due to blank
Placebo	No interference at Absorption maxima of Perindopril erbumine due to placebo solution

UV-spectrum:

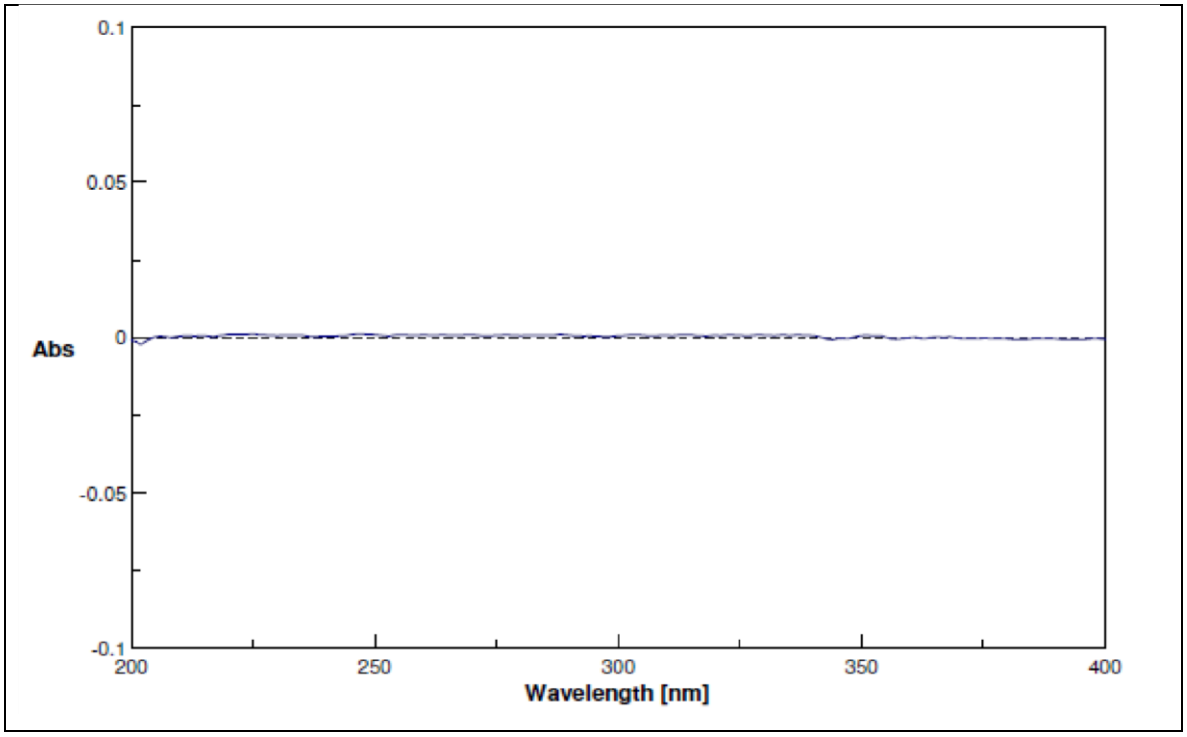


Fig. No. 3 Typical UV-spectrum of Blank solution.

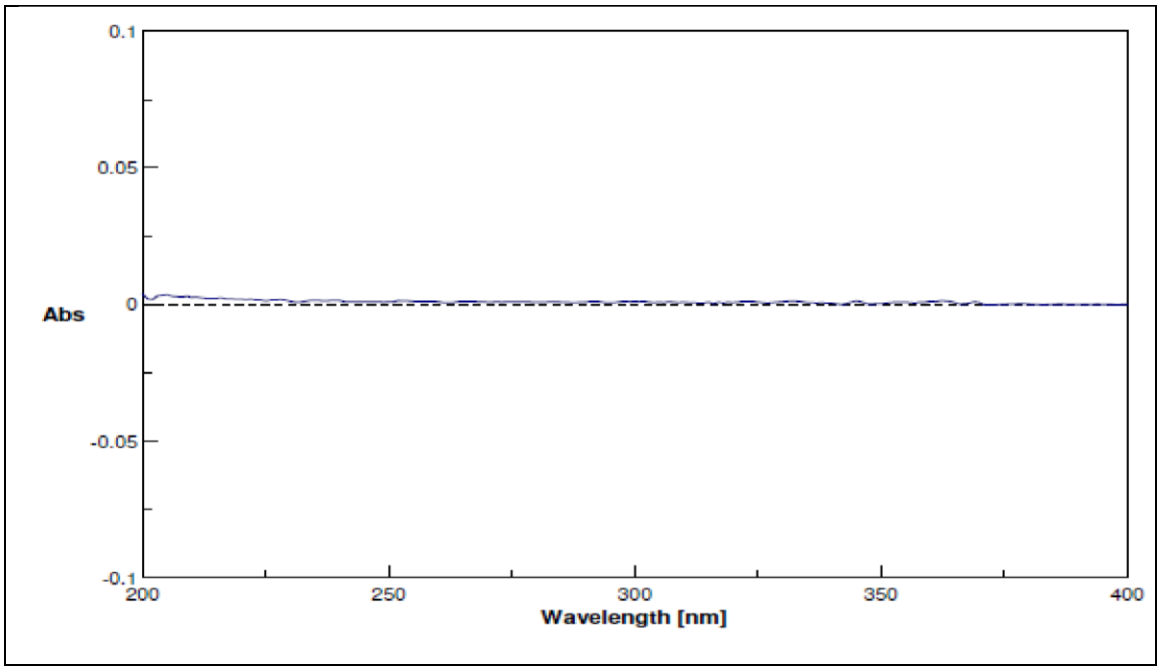


Fig. No. 4 Typical UV-spectrum of Placebo solution.

Acceptance criteria:

Blank: % Interference at Absorption maxima of Perindopril erbumine is NMT 1.0%

Placebo: % Interference at Absorption maxima of Perindopril erbumine is NMT 2.0% Data interpretation: Blank and placebo were not having interference at absorption maxima of Perindopril erbumine. Hence developed UV method passed the criteria for specificity.

4) Linearity on UV spectrophotometer:

Linearity of an analytical method is its ability to elicit test results that are proportional to the concentration of analyte in samples within a given range.

Results of UV Linearity for Perindopril erbumine:

Level	Conc (µg/mL)	Absorbance	Mean	% RSD
50%	15.00	0.2988	0.2986	0.070
		0.2984		
		0.2985		
75%	22.50	0.4437	0.4437	0.034
		0.4439		
		0.4436		
100%	30.00	0.5941	0.5943	0.042
		0.5943		
		0.5946		
125%	37.50	0.7465	0.7464	0.041
		0.7467		
		0.7461		
150%	45.00	0.8846	0.8846	0.023
		0.8848		
		0.8844		

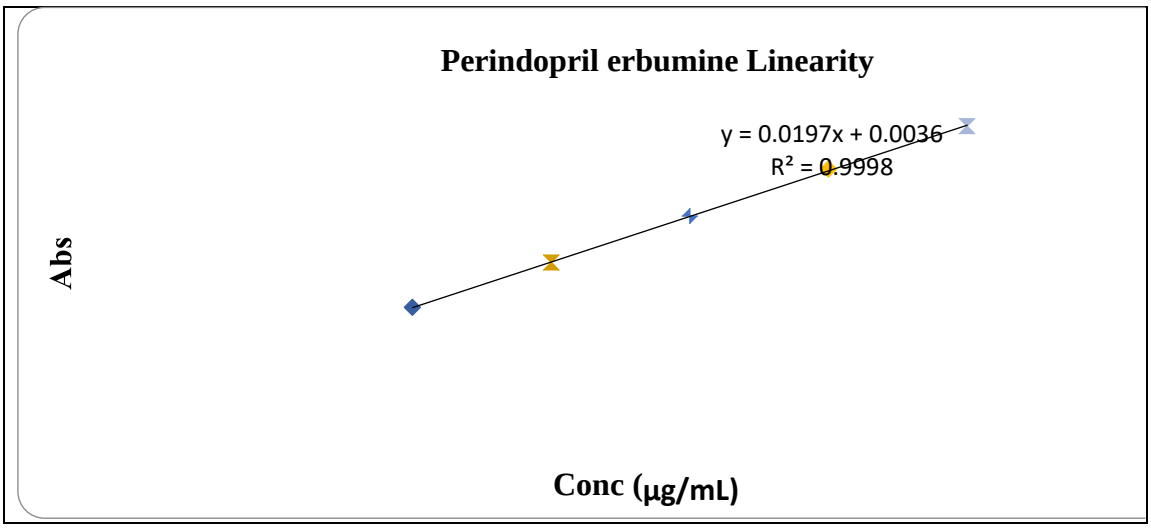


Fig. No. 7 Calibration curve of Perindopril erbumine on UV

Summary of UV-linearity of Perindopril erbumine:

Sr no.	Parameter	Result value	Acceptance criteria
1	Beer's linearity range	15.0-45.0 µg/mL	NA
2	Correlation coefficient (R^2)	0.99988	NLT 0.98
3	Intercept	0.00364	To be report
4	Slope	0.01966	To be report
5	% RSD for area at each level	NA	NMT 2.0

The respective linear equation for Perindopril erbumine was
Y = M X + C
Y = 0.01966 x + 0.00364
Where, x = concentration of Analyte in µg/mL
Y = is Absorbance.
M = Slope
C= Intercept

Conclusion:

From the calibration curve it was concluded that the Perindopril erbumine shows linear response in the range of 15.0-45.0 µg/ml. The Regression value was found well within the limit.

4) Limit of Detection (LOD) and Limit of Quantitation (LOQ):

σ = 0.00355 (Residual standard deviation of a regression line) s = 0.01966 (Slope)

Detection limit (LOD):

LOD = 3.3 σ / S
LOD = 3.3 x 0.00355 / 0.01966
LOD = 0.060 µg/mL

Quantitation limit (LOQ):

LOQ = 10 σ / S
LOQ = 10 x 0.00355 / 0.01966
LOQ = 1.81 µg/mL

6) ACCURACY (RECOVERY):

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of an analytical method is determined by applying the method to analysed samples to which known amounts of analyte have been added.

Result and statistical data of Accuracy of Perindopril erbumine

Level (%)	Absorbance	Recovered conc (µg/mL)	Added conc (µg/mL)	% Recovery	Mean Recovery	% RSD
50	0.3054	15.41	15.60	98.78	98.85	0.600
	0.3098	15.63	15.90	98.30		
	0.3016	15.22	15.30	99.48		

100	0.6087	30.72	30.90	99.42	99.58	0.751
	0.5998	30.27	30.60	98.92		
	0.6088	30.72	30.60	100.39		
150	0.8945	45.14	45.90	98.34	99.47	1.001
	0.8996	45.40	45.30	100.22		
	0.8963	45.23	45.30	99.85		

Overall Recovery: 99.30 %

% RSD for Overall Recovery: 0.774

Acceptance criteria:

% Recovery for each level and overall recovery: 98.0 to 102.0%

% RSD for each level and overall recovery: NMT 2.0

Data interpretation: Recovery of analytical procedure was found well within acceptance criteria at all 3 levels. % Recovery not gets hampered by changed in analyte concentration.

7) PRECISION

Precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogenous sample. Precision of an analytical method is usually expressed as standard deviation or relative standard deviation. Precision was performed on Test sample.

Result of Intra- day and Inter- Day Precision for Perindopril erbumine test sample assay:

	Sample	**Test Sample (mg)**	**Absorbance**	**% Assay**
Repeatability	Sample 1	343.7	0.5912	99.24
	Sample 2	343.1	0.5883	98.93
	Sample 3	342.7	0.5848	98.45
	Sample 4	343.8	0.5813	97.55
	Sample 5	344.2	0.5993	100.46
	Sample 6	343.2	0.5806	97.60
	Mean			**98.71**
	STD DEV			**1.0988**
	% RSD			**1.113**
Intermediate precision (Inter-Day)	Sample 1	342.9	0.5831	98.11
	Sample 2	343.6	0.5967	100.19
	Sample 3	343.2	0.5902	99.22
	Sample 4	344.3	0.5793	97.08
	Sample 5	343.6	0.5913	99.29
	Sample 6	343.7	0.5890	98.87
	Mean			**98.79**

	STD DEV	**1.0759**
	% RSD	**1.089**
Repeatability Plus Inter-day	**Mean**	**98.749**
	STD DEV	**1.0378**
	% RSD	**1.051**

Acceptance criteria:

% Assay: % Assay value for each sample (Individual sample) and mean assay value for precision (6 samples), mean assay value intermediate precision (6 samples), and mean assay value for precision plus intermediate precision sample (12 samples): 90-110%

% RSD: % RSD for precision study samples (6 samples), Intermediate precision study samples (6 samples) and precision plus intermediate precision sample (12 samples): NMT 2.0 **Data interpretation:** % Assay and % RSD was found well within acceptance limit and hence method is precise (Reproducible).

8) ROBUSTNESS:

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

Following changes made under Robustness:

- Change in Sonication time
- Change in Wavelength

A. Changes in Sonication time for test sample preparation by +5 min (20 minutes) Two samples prepared by change in this parameter. Summary as follows

Sample	Powder wt. (mg)	Diluted to (mL)	Volume taken	Diluted to (mL)
Sample 1	342.8	50	1.5	10
Sample 2	343.2	50	1.5	10

Results of Change in sonication time by + 5 minutes (20 minutes):

Sample	Absorbance	% Assay	Abs difference w.r.t. Precision assay value
Sample 1	0.5912	99.50	0.43
Sample 2	0.5876	98.78	
Mean		**99.14**	
STD DEV		**0.5091**	
% RSD		**0.514**	

B. Changes in Sonication time for test sample preparation by - 5 min (10 minutes) Two samples prepared by change in this parameter. Summary as follows

Sample	Powder wt. (mg)	Diluted to (mL)	Volume taken	Diluted to (mL)
Sample 1	343.2	50	1.5	10

Sample 2	343.6	50	1.5	10

Results of Change in sonication time by - 5 minutes (10 minutes):

Sample	Absorbance	% Assay	Abs difference w.r.t. Precision assay value
Sample 1	0.5837	98.13	
Sample 2	0.5884	98.80	
Mean		**98.47**	0.24
STD DEV		**0.4738**	
% RSD		**0.481**	

C. Changes in wavelength by -2 NM

Note: First two samples of Precision study analyzed at this wavelength and calculated its assay value. Abs difference calculated for assay value w.r.t. Precision assay value (Mean value)

Results of change in wavelength by -2 NM System suitability at 213 nm:

Sr No.	Standard solution	Absorbance at 213 nm
1	Standard_1	0.6232
2	Standard_2	0.6229
3	Standard_3	0.6235
4	Standard_4	0.6225
5	Standard_5	0.6237
	Mean	0.6232
	STD Dev	0.0005
	% RSD	0.08

Results of Test samples by change in – 2 nm wavelength:

Sample	Absorbance	% Assay	Abs difference w.r.t. Precision assay value
Sample 1	0.6210	99.44	
Sample 2	0.6136	98.43	
Mean		**98.94**	0.23
STD DEV		**0.7164**	
% RSD		**0.724**	

D. Results of change in wavelength by +2 NM

Note: First two samples of Precision study analyzed at this wavelength and calculated its assay value. Abs difference calculated for assay value w.r.t. Precision assay value

(Mean value)

System suitability at 217 nm:

Sr No.	Standard solution	Absorbance at 217 nm
1	Standard_1	0.5487
2	Standard_2	0.5482
3	Standard_3	0.5484
4	Standard_4	0.5481
5	Standard_5	0.5493
Mean		**0.5485**
STD Dev		**0.00048**
% RSD		**0.09**

Results of Test samples by change in +2 nm wavelength:

Sample	Absorbance	% Assay	Abs difference w.r.t. Precision assay value
Sample 1	0.5359	97.50	0.53
Sample 2	0.5424	98.86	
Mean		**98.18**	
STD DEV		**0.9583**	
% RSD		**0.976**	

Data interpretation: From the above results, it was concluded that the abs difference was found well within the limits and analytical method was robust.

8. SUMMARY AND CONCLUSION:

Summary:

The project focuses on developing and validating a method for the analysis of perindopril bulk using UV spectroscopy. Perindopril, an angiotensin-converting enzyme (ACE) inhibitor, is widely used to treat hypertension and heart failure. The objective is to establish a reliable and accurate method for determining the concentration of perindopril in bulk form using UV spectroscopy, which is a cost-effective and widely accessible technique. The study includes method development, which involves optimizing parameters such as wavelength, solvent, and pH, and validation, ensuring the method's accuracy, precision, linearity, robustness, and specificity. Successful validation confirms the method's suitability for determining perindopril concentration in bulk.

Conclusion:

In conclusion, the method development and validation of perindopril bulk by UV spectroscopy is a crucial undertaking in the pharmaceutical industry to ensure the quality, safety, and efficacy of the drug. Throughout this project, a robust, precise, and accurate method for the analysis of perindopril bulk has been developed and validated. The significance of this project lies in its contribution to the pharmaceutical field, providing a reliable technique for the analysis of perindopril, an essential antihypertensive drug. The successful development and validation of the UV spectroscopic method for perindopril bulk analysis signify a substantial advancement in pharmaceutical quality control and assurance. This method offers a cost-effective, rapid, and reliable means for quantifying perindopril in bulk samples. By employing UV spectroscopy, the pharmaceutical industry can ensure the consistency and quality of perindopril formulations. Throughout the project, various parameters were optimized to enhance the accuracy and precision of the UV spectroscopic method. By systematically investigating the method's specificity, linearity, accuracy, precision, robustness, and detection and quantification limits, a thorough understanding of the method's capabilities has been achieved. The method's specificity to perindopril ensures that other components do not interfere, while the linearity of the method allows for accurate quantification across a defined range of concentrations.

9. FUTURE SCOPE:

The project topic "Method Development and Validation of Perindopril Bulk by UV Spectroscopy" has significant future scope. Here are some potential areas to explore:

1. Advanced Analytical Techniques: Investigate and implement more advanced analytical techniques beyond UV spectroscopy, such as HPLC, LC-MS, or GC-MS, to enhance the accuracy and sensitivity of the analysis.
2. Method Automation: Develop automated methods to streamline the process, improve efficiency, and reduce human error.
3. Pharmacopeial Compliance: Ensure compliance with various pharmacopeias such as USP, BP, or EP and explore the possibility of expanding the validation according to their guidelines.
4. Stability Indicating Methods: Develop stability-indicating methods to determine the stability of perindopril bulk over time and in different environmental conditions.
5. Impurity Profiling: Extend the study to identify and quantify impurities present in perindopril bulk, providing a more comprehensive understanding of the substance.
6. Method Transferability: Investigate the transferability of the developed method to different laboratories or analytical instruments.
7. Application in Formulation: Apply the developed method to the analysis of perindopril in pharmaceutical formulations, such as tablets or capsules.
8. Biopharmaceutical Studies: Conduct biopharmaceutical studies to understand the pharmacokinetics and pharmacodynamics of perindopril, aiding in drug development.
9. Multivariate Data Analysis: Utilize multivariate data analysis techniques to extract more information from UV spectroscopy data, enhancing the method's robustness.
10. Green Analytical Chemistry: Investigate the possibility of developing the method using green analytical chemistry principles to reduce the environmental impact and enhance sustainability.

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