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Method Development and Validation of Antihypertensive Drug by Using UV Spectroscopy

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Abstract: *Analytical Chemistry is the branch of science that uses advance technologies in determining the composition by analytical technique. We can achieve both qualitative as well as quantitative results. Analytical instruments play a major role in the process to achieve high quality and reliable analytical data. Thus, everyone in the analytical laboratory should be concerned about the quality assurance of equipment.*

Keywords: UV spectroscopy

I. INTRODUCTION

Analytical Chemistry is the branch of science that uses advance technologies in determining the composition by analytical technique. We can achieve both qualitative as well as quantitative results. Analytical instruments play a major role in the process to achieve high quality and reliable analytical data. Thus, everyone in the analytical laboratory should be concerned about the quality assurance of equipment.
Instrumental technique like UV spectroscopy plays a vital role in analysis

UV-visible Spectroscopy

Spectroscopy as a science began with Isaac Newton splitting light with a prism and was called optics. Therefore, it was originally the study of visible light which we call color that later under the studies of James Clerk Maxwell came to include the entire electromagnetic spectrum. Spectroscopy is the branch of science dealing with the study of interaction of electromagnetic radiation with matter. The most important consequence of such interaction is that energy is absorbed or emitted by the matter in discrete amounts called quanta. The absorption or emission processes are known throughout the electromagnetic spectrum ranging from the gamma region (nuclear resonance absorption or the Mossbauer effect) to the radio region (nuclear magnetic resonance). When the measurement of radiation frequency is done experimentally, it gives a value for the change of energy involved and from this one may draw the conclusion about the set of possible discrete energy levels of the matter.The ways in which the measurements of radiation frequency (emitted or absorbed) are made experimentally and the energy levels deduced from these comprise the practice of spectroscopy. [1]

Principle of UV -Visible Spectroscopy

The UV-Visible Principle The absorption of ultraviolet or visible light by chemical compounds produces distinct spectra, which is the basis for spectroscopy. The interaction of light and matter is the foundation of spectroscopy. When matter absorbs light, it experiences excitation and de-excitation, which results in the formation of a spectrum.When an electromagnetic wave strikes a material, phenomena such as transmission, absorption, reflection, and scattering can occur, and the observed spectrum depicts the interaction of wavelengths with discretedimensional objects such as atoms, molecules, and macromolecules. Absorption occurs when the frequency of incoming light equals the energy difference between the ground and excited states of a molecule.
An electronic transition (Figure 1) describes the excitation of an electron from its ground state to its excited state. This is the fundamental concept of molecular spectroscopy.

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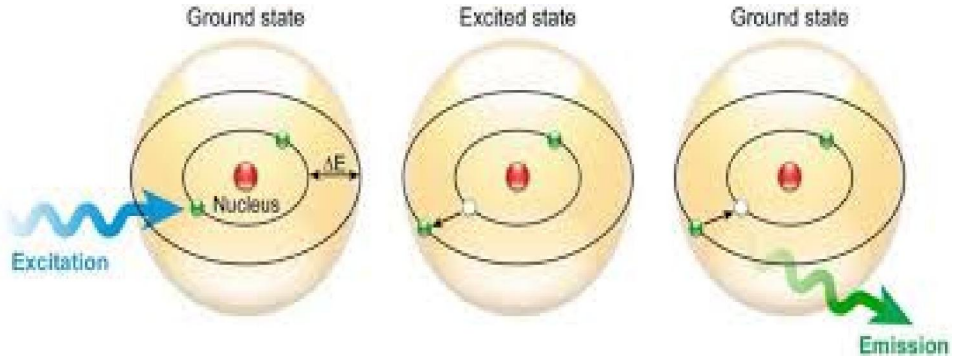

Fig (1): - Describes the excitation of an electron from its ground state to its excited state. This is the fundamental concept of molecular spectroscopy.

Instrumentation

Spectrophotometer

- Source of radiation
- Monochromators
- Recording system
- Detector

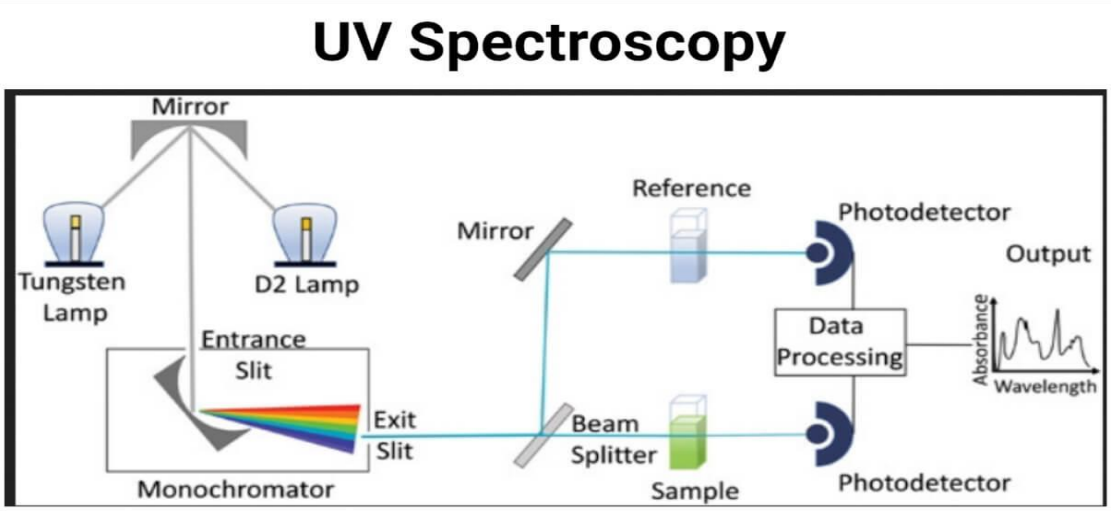

Fig (2): - Diagram of uv-visible spectrophotometer with instrumentation

1) Source of radiation-Requirements of an Ideal Source:

It should be stable and should not allow fluctuations.It should emit light of continuous spectrum of high and uniform intensity over the entire wavelength region in which it's used. It should provide incident light of sufficient intensity for the transmitted energy to be detected at the end of optic path. The best source of light is the one which is more stable, more intense and which give the range of spectrum from 200-800 nm.

Types of light source

a) Hydrogen discharge lamp

b) Deuterium lamp

b) Xenon discharge lamp

c) Mercury Arc

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• Monochromators are better and more efficient than filters in converting polychromatic light or heterochromatic light into monochromatic light.
• Monochromators are primarily designed for spectral scanning, i.e. a process of continuously varying the radiation wavelength over a considerable range.
• Mechanical construction of monochromators for UV, visible and IR radiation is similar in that all of them employ slits, lenses, mirrors, windows, and gratings or prisms

1) Prism Monochromator-Two types.
• Single-pass monochromators
• Double beam monochromators
2) Granting monochromator
It provides on alternative means of producing monochromators light. It consists of Series of parallel lines (groves) which reflected through highly polished surface of glass, quartz of alkyl halides.
3) Sample cells-
• The cells or cuvettes are used for handling liquid samples.
• The cell may either be rectangular or cylindrical in nature.
• For study in UV region; the cells are prepared from quartz or fused silica whereas color corrected fused glass is used for visible region.
• The surfaces of absorption cells must be kept scrupulously clean.
• No fingerprints or a touch should be present on cells.
• Cleaning is carried out washing with distilled water or with dialcohol, acetone.

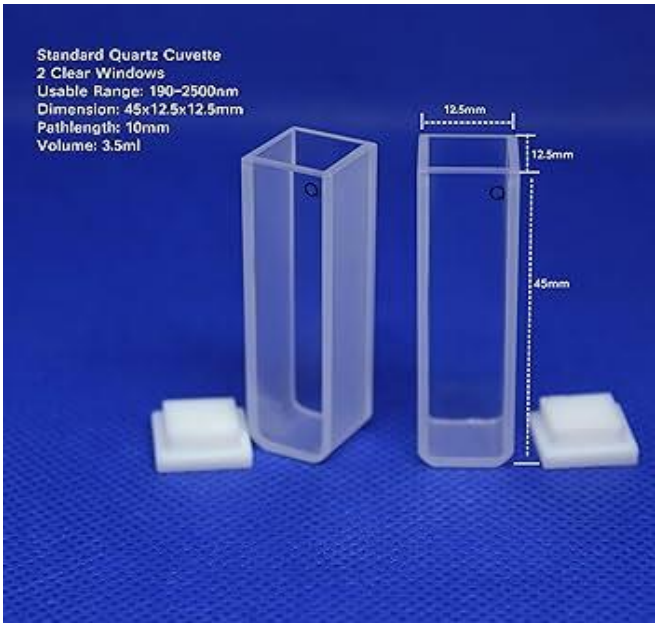

Fig (4):Diagram of cuvette

4) Detectors
• Device which converts light energy into electrical signals, that are displayed on readout devices.
• The transmitted radiation falls on the detector which determines the intensity of radiation absorbed by sample
• The following types of detectors are employed in instrumentation of absorption spectrophotometer
a) Barrier layer cell/Photovoltaic cell
b) Phototubes/Photo emissive tube
c) Photomultiplier tube.

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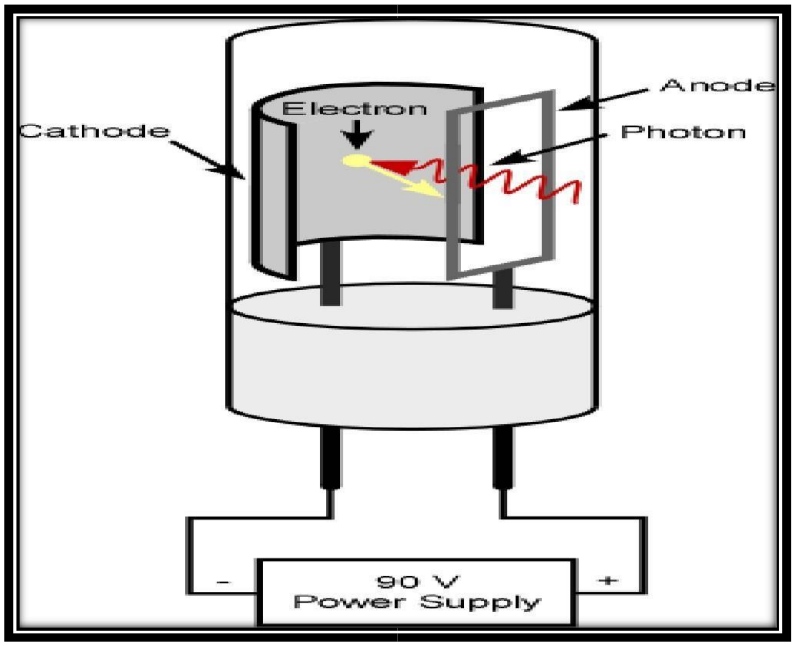

Fig (5): - Diagram of phototube photon detector

Application UV-Visible spectroscopy

1. It is useful in quantitative analysis.
2. It is used in drug identification
3. It is used for determination of different species
4. It is used for beverage analysis
5. It is used in DNA & RNA analysis.[2]

Introduction of Hypertension:

Hypertension, commonly referred to as high blood pressure, is a chronic cardiovascular disorder characterized by a sustained increase in the pressure exerted by circulating blood upon the walls of arteries. It is one of the most prevalent non-communicable diseases worldwide and a significant contributor to morbidity and mortality. Often asymptomatic in its early stages, hypertension has earned the title of "the silent killer," as it gradually damages vital organs such as the heart, kidneys, brain, and eyes without noticeable symptoms. The progressive impact of uncontrolled hypertension can lead to life-threatening complications including stroke, myocardial infarction, heart failure, renal failure, and even blindness. Given the extensive global burden of hypertension, effective strategies for its management are crucial for improving public health outcomes.

Antihypertensive drugs play a central role in the pharmacological management of elevated blood pressure. These agents are specifically designed to lower blood pressure by targeting various physiological mechanisms involved in its regulation. The pathophysiology of hypertension is complex and multifactorial, often involving increased vascular resistance, excessive sodium retention, overactivation of the renin-angiotensin-aldosterone system (RAAS), heightened sympathetic nervous system activity, and impaired endothelial function. Antihypertensive medications are therefore categorized based on their mechanisms of action, which include reducing blood volume through diuresis, decreasing heart rate and cardiac output, promoting vasodilation, and inhibiting hormonal pathways that contribute to vasoconstriction and sodium retention.

The major classes of antihypertensive drugs include diuretics, beta-blockers, calcium channel blockers, angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor blockers (ARBs), alpha-blockers, centrally acting agents, and direct vasodilators. Each class targets a distinct aspect of blood pressure regulation. For example, diuretics promote

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the excretion of sodium and water, thereby decreasing blood volume and pressure, while ACE inhibitors and ARBs inhibit the RAAS pathway to prevent vasoconstriction and fluid retention. Calcium channel blockers act on vascular smooth muscles to induce relaxation and lower peripheral resistance, and beta-blockers reduce the workload on the heart by decreasing the rate and force of cardiac contractions. The selection of a particular drug or combination therapy depends on individual patient factors such as age, race, the presence of comorbidities like diabetes or kidney disease, and the severity of hypertension.

Despite the availability of a wide range of antihypertensive agents, achieving optimal blood pressure control remains a global challenge. In many populations, especially in low- and middle-income countries, awareness, treatment, and adherence to antihypertensive therapy are still low. This underscores the importance of not only drug development but also healthcare access, patient education, and lifestyle interventions in the comprehensive management of hypertension. Furthermore, the trend towards fixed-dose combination therapies aims to simplify treatment regimens and improve compliance by minimizing the pill burden on patients. Advances in pharmacogenomics also hold promise for personalized antihypertensive therapy, where treatment can be tailored based on genetic profiles to enhance efficacy and reduce adverse effects.

In summary, antihypertensive drugs are indispensable tools in the management of hypertension and the prevention of cardiovascular diseases. Their effectiveness, however, is maximized when used in conjunction with lifestyle modifications such as dietary changes, physical activity, weight control, and cessation of smoking and alcohol. A deep understanding of these drugs—their mechanisms, classifications, and clinical uses—is essential for healthcare professionals, particularly pharmacists and clinicians, who are at the frontline of hypertension management and patient care.[3]

II. TYPE OF HYPERTENSIVE

1] Diuretics

Mechanism: Reduce blood volume by promoting the excretion of water and salt, thereby lowering blood pressure.

2] Beta-Blockers

Mechanism: Block beta-adrenergic receptors in the heart, reducing heart rate and cardiac output.

3] ACE Inhibitors

Mechanism: Inhibit the enzyme angiotensin-converting enzyme (ACE), reducing the production of angiotensin II, a vasoconstrictor.

4] Angiotensin II Receptor Blockers (ARBs)

Mechanism: Block the angiotensin II receptors, preventing vasoconstriction and the release of aldosterone.

5] Calcium Channel Blockers

Mechanism: Inhibit calcium ions from entering smooth muscle cells, causing vasodilation and reducing heart rate.

6] Alpha-1 Blockers

Mechanism: Block alpha-1 adrenergic receptors in the blood vessels, causing vasodilation and reduced peripheral resistance.

7] Alpha-2 Agonists

Mechanism: Stimulate alpha-2 receptors in the brain, reducing sympathetic nervous system activity and lowering blood pressure.

8] Direct Vasodilators

Mechanism: Directly relax the smooth muscle of blood vessels, causing vasodilation.

9] Renin Inhibitors

Mechanism: Inhibit the activity of renin, a precursor to angiotensin II, which lowers blood pressure.

10] Combined Alpha and Beta Blockers

Mechanism: Block both alpha and beta receptors, leading to vasodilation and reduced heart rate.

11] Vasopressin Receptor Antagonists

Mechanism: Block vasopressin receptors, reducing water retention and blood volume.

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12] Endothelin Receptor Antagonists
Mechanism: Block endothelin receptors, reducing vasoconstriction and promoting vasodilation.
13] Centrally Acting Antihypertensives
Mechanism: Act on the central nervous system to reduce sympathetic nervous system activity.
14] Neprilysin Inhibitors
Mechanism: Inhibit neprilysin, which breaks down natriuretic peptides, promoting vasodilation.
15] Phosphodiesterase Type 5 (PDE5) Inhibitors
Mechanism: Inhibit PDE5, leading to increased cyclic GMP levels, causing smooth muscle relaxation and vasodilation.
16] Sodium Nitroprusside
Mechanism: Releases nitric oxide, leading to vasodilation and reduction in blood pressure.
17] Mineralocorticoid Receptor Antagonists (Aldosterone Antagonists)
Mechanism: Block aldosterone receptors, reducing sodium and water retention.
18] Sympatholytic
Mechanism: Inhibit sympathetic nervous system activity, reducing heart rate and vasoconstriction.
19] Prostaglandin Analogues
Mechanism: Mimic the action of prostaglandins to cause vasodilation.
20] Aldosterone Synthesis Inhibitors
Mechanism: Inhibit aldosterone synthesis, reducing sodium and water retention.[4]

Causes of Hypertension (High Blood Pressure):
1. Primary (Essential) Hypertension:
No identifiable cause. It develops gradually over many years.
Risk factors:

- Genetics (family history)
- Age (increased risk with age)
- Obesity
- Lack of physical activity
- Excessive alcohol consumption
- High salt intake
- Stress
- Smoking

2. Secondary Hypertension:
Caused by an underlying medical condition or medication.
Causes:

- Kidney disease
- Hormonal disorders (e.g., hyperthyroidism, Cushing's syndrome)
- Sleep apnea
- Certain medications (e.g., birth control pills, decongestants)
- Drug use (e.g., cocaine, amphetamines)
- Chronic alcohol abuse

Signs and Symptoms of Hypertension (High Blood Pressure):
When symptoms do occur, they can include:

- Headaches (especially in the morning)
- Dizziness or lightheadedness
- Nosebleeds

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- Shortness of breath
- Chest pain

Classification OFANTIHYPERTENSIVE:

1] Diuretics
• Examples: Furosemide, Spironolactone, Bumetanide
2] Beta-Blockers
• Examples: Atenolol, Propranolol, Bisoprolol
3] ACE Inhibitors
• Examples: Ramipril, Enalapril, Captopril
4] Angiotensin II Receptor Blockers (ARBs)
• Examples: Valsartan, Olmesartan, Telmisartan
5] Calcium Channel Blockers
• Examples: Diltiazem, Verapamil, Nifedipine
6] Alpha-1 Blockers
• Examples: Doxazosin, Terazosin, Alfuzosin
7] Alpha-2 Agonists
• Examples: Methyldopa, Guanfacine, Clonidine
8] Direct Vasodilators
• Examples: Minoxidil, Diazoxide, Nitroprusside
9] Renin Inhibitors
• Examples: Aliskiren
10] Combined Alpha and Beta Blockers
• Examples: Labetalol, Carvedilol
11] Vasopressin Receptor Antagonists
• Examples: Conivaptan, Tolvaptan
12] Endothelin Receptor Antagonists
• Examples: Ambrisentan, Macitentan
13] Centrally Acting Antihypertensives
• Examples: Guanfacine, Guanabenz
14] Neprilysin Inhibitors
• Examples: Sacubitril (commonly combined with Valsartan in Entresto)
15] Phosphodiesterase Type 5 (PDE5) Inhibitors
• Examples: Tadalafil, Vardenafil
16] Sodium Nitroprusside
• Examples: Nitroprusside (used in hypertensive emergencies)
17] Alpha-1 and Beta Blockers Combination
• Examples: Labetalol, Carvedilol
18] Mineralocorticoid Receptor Antagonists (Aldosterone Antagonists)
• Examples: Eplerenone, Spironolactone
19] Sympatholytic
• Examples: Reserpine, Guanethidine, Methyldopa
20] Prostaglandin Analogues
• Examples: Misoprostol, Alprostadil
21] Aldosterone Synthesis Inhibitors
• Examples:Eplerenone,Spironolacton

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III. LITERATURE REVIEW

1. Patil P. et al. (2010)
Developed a UV spectrophotometric method for the estimation of Atenolol in bulk and tablet dosage form. The method showed λmax at 224 nm, linearity between 2–10 µg/mL, and good accuracy and precision.

2. Rao K.R. et al. (2012)
Designed a validated UV method for the determination of Amlodipine Besylate, with λmax at 238 nm. The method followed Beer-Lambert's law within the range of 5–25 µg/mL and was precise and accurate.

3. Pawar S.J. et al. (2011)
Developed a first-order derivative UV method for the simultaneous estimation of Losartan Potassium and Hydrochlorothiazide in combined dosage forms. Good resolution and recovery were obtained.

4. Jain N.K. et al. (2013)
Proposed a simple UV-visible spectrophotometric method for the determination of Enalapril Maleate. λmax was found at 215 nm with linearity between 5–25 µg/mL, showing good repeatability.

5. Mishra R. et al. (2014)
Developed and validated a UV method for Telmisartan estimation in pharmaceutical dosage form. λmax was observed at 296 nm with a linear range from 5–30 µg/mL.

6. Chitlange S.S. et al. (2012)
Developed a simultaneous UV method for Valsartan and Amlodipine in tablets using the absorbance ratio method. The method was validated as per ICH guidelines.

7. Singh R. et al. (2015)
Developed a validated UV spectrophotometric method for Ramipril, with a detection wavelength of 210 nm. The method was shown to be linear, accurate, and precise.

8. Kumar A. et al. (2016)
Designed a UV spectrophotometric method for Irbesartan, which demonstrated strong linearity ($r^2 = 0.999$) across a range of 2–20 µg/mL and met ICH validation parameters.

9. Bhalerao S. et al. (2018)
Developed a UV method for Lisinopril, with λmax at 214 nm. The method was linear in the range of 5–25 µg/mL and demonstrated acceptable robustness and reproducibility.

10. Mehta M. et al. (2017)
Conducted a comparative UV study on Olmesartan Medoxomil and Hydrochlorothiazide, using area under curve and dual wavelength methods. Both methods were successfully validated.

11. Kale V. and Patil M. (2019)
Developed a UV spectrophotometric method for the analysis of Perindopril Erbumine, with maximum absorbance at 214 nm. The method showed high specificity and minimal interference.

12. Gaikwad N. et al. (2020)
Established a stability-indicating UV method for Candesartan Cilexetil under various stress conditions, successfully separating degradation products and validating linearity and precision.

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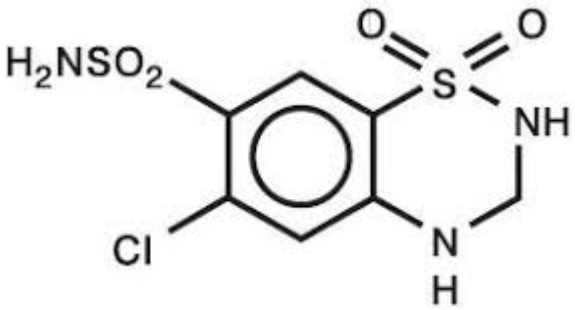
13. Roy A. et al. (2021)
Developed a novel UV spectroscopic method for Fosinopril Sodium with λmax at 231 nm. The method was validated for linearity, accuracy, and sensitivity, confirming its suitability for routine use.

14. Dey A. and Das P. (2022)
Established a UV method for Azilsartan Medoxomil and Chlorthalidone in fixed-dose combinations. The method was accurate, reproducible, and met ICH criteria for validation.

Aim:
To Develop and Validate Antihypertensive Drug for Assessing, Efficacy, Safety and Compatibility in Development by using UV Spectroscopy.

Objectives:
1. To Develop, Accurate, Simple, Precise & Rapid analytical Method for Metoprolol Hydrochloride.
2. To Validate analytical Method for Accuracy, Precision, Linearity, Robustness as per ICH guidelines.
3. Develop a UV Spectrophotometric method for Metoprolol Hydrochloride.
4. Optimizean analytical parameter like:
A] Solvent System
B] Wavelength
C] PH
D] Concentration Range
E] Absorption Spectrum of the drugs.

DRUG PROFILE
1. Generic & Brand Name
• Generic Name: Metoprolol Hydrochloride
• Brand Names: Lopressor, Toprol XL, Betaloc, Metolar, Betaloc, Metopro, Metolar-XL, Metopor, Vistaril (in combination formulations)
• Structure



2. Drug Class
• Class: Beta-blocker (Selective Beta-1 Adrenergic Antagonist)
• Mechanism: Metoprolol primarily blocks the β1-receptors in the heart, resulting in a decrease in heart rate, myocardial contractility, and blood pressure. At higher doses, it may also exhibit β2-receptor antagonism, which can lead to bronchoconstriction. It reduces the heart's demand for oxygen, which is particularly beneficial in heart-related conditions.

3. Uses / Indications

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• Approved Uses:

- Hypertension (High Blood Pressure): Lowering blood pressure reduces the risk of stroke, heart attack, and kidney problems.
- Angina Pectoris (Chest Pain): Reduces the workload on the heart and improves blood flow.
- Acute Myocardial Infarction (Heart Attack): Reduces mortality and recurrence by reducing heart rate and preventing arrhythmias.
- Heart Failure: Used as part of the treatment regimen for heart failure, particularly in combination with other drugs such as ACE inhibitors or diuretics.
- Arrhythmias (Irregular Heartbeat): Helps in controlling abnormal heart rhythms.

• Off-label Uses:

- Migraine Prophylaxis: Prevents migraines and reduces the frequency and severity of attacks.
- Thyrotoxicosis: Used to control symptoms like palpitations and tachycardia in hyperthyroidism.
- Essential Tremor: Reduces shaking or tremors, especially in conditions like Parkinson's disease.
- Anxiety Disorders: Sometimes prescribed for acute anxiety symptoms (e.g., performance anxiety).

4. Dosage and Administration

Immediate-Release (IR) Form

- Initial dose: 50 mg once daily
- Typical dose: 50–100 mg/day in divided doses (morning and evening)
- Maximum dose: 400 mg/day in divided doses (for severe cases like post-MI or heart failure)

Extended-Release (XR) Form

- Initial dose: 25–50 mg once daily
- Typical dose: 50–200 mg once daily
- Maximum dose: 400 mg/day (in patients with heart failure or post-MI)

Special Considerations:

- Start with a lower dose and increase gradually to minimize side effects.
- Taper off gradually when discontinuing to avoid rebound hypertension.

5. Mechanism of Action

- Beta-1 Selectivity: Metoprolol selectively inhibits β1-adrenoceptors, primarily found in the heart, reducing heart rate, cardiac output, and blood pressure.
- At higher doses: The β2-receptor antagonism may result in bronchoconstriction, making it less suitable for individuals with asthma or COPD.
- Effect on Heart and Circulation: Reduces myocardial oxygen demand, heart rate, and systolic blood pressure. This is beneficial for managing conditions such as hypertension, heart failure, and arrhythmias.

6. Advantages

- Beta-1 Selectivity: Unlike non-selective beta-blockers, metoprolol is less likely to cause bronchoconstriction, making it safer for patients with asthma or chronic obstructive pulmonary disease (COPD).
- Proven Mortality Benefit: Reduces the risk of death following a heart attack and improves survival rates in heart failure.
- Control of Heart Rate and Blood Pressure: Helps in stabilizing patients with arrhythmias, hypertension, and acute coronary syndrome.
- Extended-Release Formulation: Offers a once-daily dosing option, improving patient compliance.

7. Contraindications

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- Known hypersensitivity to metoprolol or any component of the formulation.
- Severe bradycardia (heart rate < 40 bpm).
- Second or third-degree heart block (unless a pacemaker is in place).
- Acute heart failure (unless stabilized by appropriate treatment).
- Severe hypotension (low blood pressure).
- Pheochromocytoma: Should not be used unless combined with alpha-blockers.
- Use in caution in patients with asthma or COPD due to the risk of bronchospasm.

8. Warnings and Precautions

• Bradycardia (slow heart rate): May worsen or cause symptomatic bradycardia. Careful monitoring is required, particularly in elderly or those with existing heart conditions.
• Hypotension: Can cause or exacerbate hypotension, particularly when starting treatment or increasing the dose.
• Heart Failure: May worsen symptoms if not properly adjusted. Start at a low dose and titrate gradually.
• Masking of Hypoglycemia Symptoms: May mask symptoms such as tachycardia in diabetic patients.
• Withdrawal: Abrupt discontinuation may lead to rebound hypertension, tachycardia, and angina. Gradual dose reduction is advised.

9. Common Side Effects

• Fatigue
• Dizziness
• Bradycardia (slow heart rate)
• Cold extremities (hands/feet)
• Hypotension (low blood pressure)
• Gastrointestinal disturbances: Nausea, constipation, diarrhea
• Headache
• Impotence or sexual dysfunction

Less Common or Serious Side Effects:

• Severe hypotension
• Severe bradycardia
• Bronchospasm (in asthmatic or COPD patients)
• Depression or mood changes
• Liver function abnormalities (rare)
• Hypoglycemia or masking of hypoglycemia symptoms

10. Drug Interactions

• Calcium Channel Blockers (e.g., verapamil, diltiazem): Risk of excessive bradycardia, hypotension, and heart block.
• Antiarrhythmic Drugs (e.g., amiodarone, digoxin): May potentiate the effects of beta-blockade leading to bradycardia or heart block.
• NSAIDs/Anticoagulants: NSAIDs may reduce the antihypertensive effect of metoprolol. Anticoagulants increase the risk of bleeding.
• CYP2D6 Inhibitors (e.g., fluoxetine, paroxetine): Increase the plasma concentration of metoprolol, potentially leading to enhanced side effects.
• Alcohol and CNS Depressants: May enhance the sedative effects of metoprolol, leading to excessive drowsiness or dizziness.
• Insulin or Oral Hypoglycemics: Can mask the symptoms of hypoglycemia (tachycardia).

11. Pharmacokinetics

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• Absorption: Well absorbed after oral administration but subject to first-pass metabolism.
• Bioavailability: Immediate-release form: 50–60%. Extended-release form: ~75%.
• Peak Plasma Concentration: Immediate-release form: 1–2 hours. Extended-release form: 6–12 hours.
• Half-life: Immediate-release form: 3–7 hours. Extended-release form: 10–20 hours.
• Metabolism: Mainly hepatic via CYP2D6 (partially dependent on genetic variations of CYP2D6).
• Excretion: Metabolites are excreted in the urine (kidneys).

12. Pregnancy & Lactation

• Pregnancy Category C: There is insufficient data on the use of metoprolol in pregnancy. It should be used only if the potential benefits justify the risks.
• Lactation: Metoprolol is excreted in breast milk, but the effects on a nursing infant are unknown. Use with caution.

13. Withdrawal (Discontinuation Syndrome)

• Symptoms upon abrupt withdrawal include:

- Rebound hypertension
- Tachycardia
- Angina
- Arrhythmias

Precaution: Always taper the dose gradually under medical supervision to avoid withdrawal symptoms and rebound effects.

14. Chemical Information

•IUPAC Name: (2S)-1-(Isopropylamino)-3-(p-tert-butylphenyl)propan-2-ol
• Molecular Formula: C15H25NO3
• Molecular Weight: 267.37 g/mol
• Solubility: Slightly soluble in water, soluble in alcohol, and ether.

III. PLAN OF WORK

Selection of Drug:

• Metoprolol Hydrochloride is selected as the antihypertensive drug for the spectrophotometric estimation.

Literature Survey:

• Review existing UV methods for antihypertensive drugs, specifically Metoprolol Hydrochloride, focusing on λmax, solvents, and analytical validation parameters.
• Study ICH guidelines on method validation criteria including linearity, accuracy, precision, sensitivity, and specificity.

Procurement of Materials:

• Procure Metoprolol Hydrochloride API and marketed tablet formulations.
• Prepare reagents and solvents, including methanol, ethanol, and 0.1 N NaOH.

Solubility Testing:

• Perform solubility testing of Metoprolol Hydrochloride in various solvents such as water, methanol, and 0.1 N NaOH to determine the best solvent for preparation.

Preparation of Stock Solutions:

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• Prepare standard stock and working solutions of Metoprolol Hydrochloride in the selected solvent, ensuring accurate concentrations.

Determination of λmax:

• Scan the solution in the UV range of 200–400 nm to determine the maximum absorbance wavelength (λmax) for Metoprolol Hydrochloride, typically found around 221 nm.

Calibration Curve Construction:

• Prepare a range of standard dilutions of Metoprolol Hydrochloride (e.g., 5–40 µg/mL).

• Measure absorbance of each dilution at the λmax and plot the calibration curve, correlating concentration to absorbance.

Method Development:

• Optimize key parameters such as solvent, wavelength, and concentration range for optimal absorbance and precise measurements.

Method Validation (According to ICH Guidelines):

Validate the developed method for:

- Linearity: Ensure a linear relationship between concentration and absorbance.
- Accuracy: Perform recovery studies by adding known quantities of Metoprolol Hydrochloride to the sample and calculating recovery.
- Precision: Assess intra-day and inter-day precision by analyzing samples on different days.
- LOD & LOQ: Determine the Limit of Detection and Limit of Quantification to evaluate the method's sensitivity.
- Specificity: Test for interference from excipients or other ingredients in formulations.
- Robustness & Ruggedness: Assess the method's reliability by altering experimental conditions (e.g., pH, temperature) and evaluating any effect on the results.

Data Interpretation & Conclusion:

Analyze the results from validation and tablet analysis.

Interpret the data to confirm the method's suitability for routine quality control of Metoprolol Hydrochloride in pharmaceutical formulations.

IV. MATERIALS & INSTRUMENTS

All the raw materials, reagents, solvents, and reference standards required for the development and validation of a UV-visible spectrophotometric method for Metoprolol Hydrochloride.[5]

A. Active Pharmaceutical Ingredient (API) / Procurement of Drug:

Drug Name	Supplied by	Quantity	Purity
Metoprolol Hydrochloride	www.yarrowpharma.com yarrowchemproducts@gmail.com	25g	99%

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B. Reagents and Solvents:

Reagent / Solvent	Grade	Purpose
Methanol	Analytical/HPLC Grade	Used as solvent/diluent; excellent UV transparency.
Distilled Water / Deionized Water	Lab-grade	Used for preparation of buffer and dilution of solutions.
Acetone/Ethanol	Analytical grade	Used for cleaning glassware and cuvettes

2. INSTRUMENTS:

The following instruments are essential for conducting spectrophotometric analysis and method validation in a pharmaceutical analysis laboratory for Metoprolol Hydrochloride.

A. Spectrophotometric Instrumentation:

Equipment	Specification	Purpose
UV-Visible Spectrophotometer	Shimadzu Spectrophotometer UV-1800, Double beam.	Quantitative analysis and wavelength absorbance study for Metoprolol.
Quartz Cuvettes	1 cm path length, UV compatible	Holding samples for spectrophotometric measurement.
Computer & Software	Connected to UV Probe 2.0.	For spectral data acquisition and analysis.

B. Glassware (All Class A Certified):

Glassware Item	Volume / Size	Purpose
Volumetric Flasks	10 ml, 25 ml, 50 ml, 100 ml	Preparation of standard and sample solutions.
Beakers	50 ml, 100 ml, 250 ml	Solution handling and dilution.
Conical Flasks (Erlenmeyer)	100 ml, 250 ml	Mixing and sample storage.
Pipettes (Graduated/Volumetric)	1 ml, 5 ml, 10 ml	Transfer of accurate volume of liquids.
Burettes	25 ml, 50 ml	Precise titration work (if required).
Funnels	Medium/Large	Used during filtration.
Glass Stirring Rods	Standard	Manual stirring of solutions.

C. Analytical Equipment:

Instrument	Specification	Purpose
Analytical Balance	Sensitivity ±0.1 mg	Weighing of drugs and reagents for accurate measurements.
Magnetic Stirrer	Adjustable RPM	Dissolution and uniform mixing of sample solutions.
pH Meter	Digital, 0.01 resolution	pH adjustment of buffer/media for proper conditions.
Ultrasonic Bath (optional)	40 kHz	Degassing and dissolution enhancement.

D. Filtration & Sample Preparation Tools:

Tool	Use
Whatman Filter Paper No. 41	Filtration of prepared samples to remove insoluble impurities.
Membrane Filter (0.45 µm)	For fine filtration and clarity of solution.
Syringe Filters	For sterile or particulate-free solution preparation.

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E. Laboratory Safety & Hygiene Materials:

Item	Purpose
Nitrile Gloves	Personal safety during chemical handling.
Lab Coats	Protection of clothing and skin from chemicals.
Safety Goggles	Eye protection from chemical splashes.
Fume Hood	Safe handling of volatile solvents or hazardous vapors.
Distilled Water Unit	For generation of lab-grade water.

1. SELECTION OF DRUG

The drug selected for the present study is Metoprolol Hydrochloride, chemically classified as a selective β1-adrenergic receptor antagonist (beta-1 blocker).

Rationale for Selection:

Therapeutic Importance:

Metoprolol Hydrochloride is widely prescribed for cardiovascular diseases, especially:

- Hypertension (primary and secondary forms),
- Angina pectoris,
- Acute myocardial infarction (heart attack),
- Chronic heart failure (in reduced ejection fraction patients),
- Cardiac arrhythmias (such as atrial fibrillation).

Control of hypertension and arrhythmias significantly reduces the risk of stroke, heart failure, renal failure, and mortality.

Thus, accurate and reliable drug analysis is essential for quality assurance of products containing Metoprolol.

Chemical and Physical Properties Favoring UV Analysis:

- Molecular Formula: C15H25NO3
- Molecular Weight: 267.37 g/mol
- Physical Appearance: White or almost white crystalline powder.
- Solubility Profile:
    - O Freely soluble in water.
    - O Slightly soluble in methanol.
- Stability:

Stable under normal conditions; sensitive to light and should be protected from prolonged exposure.

Metoprolol contains aromatic rings and conjugated systems which exhibit significant UV absorbance, especially between 220–230 nm.

2. PROCUREMENT OF MATERIALS

Metoprolol Hydrochloride (Analytical grade)

- Purpose: Active pharmaceutical ingredient (API) for developing the UV method.
- Theory: High purity ensures accurate absorbance measurements, free from impurities.

Methanol (HPLC grade)

- Purpose: Used in buffer/media preparation.
- Theory: Ensures purity and prevents interference in UV measurements.

Distilled Water (Laboratory grade)

- Purpose: Solvent for preparing drug solutions.
- Theory: Distilled Water dissolves Metoprolol Hydrochloride without significant UV absorbance at the selected wavelength.

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0.1 N NaOH Solution (Freshly prepared)
• Purpose: Solubility testing of Metoprolol Hydrochloride.
• Theory: NaOH helps identify the best solvent by altering solubility properties.
UV-Visible Spectrophotometer (Shimadzu UV-1800)
• Purpose: To measure absorbance at 221 nm.
• Theory: Essential for accurate UV spectrophotometric analysis.
The following materials were procured:
• Active Pharmaceutical Ingredient (API): Metoprolol Hydrochloride (analytical grade).
• Solvents and Reagents:
O Methanol (HPLC grade).
O Distilled Water (lab grade).
O 0.1N NaOH (freshly prepared).

3. SOLUBILITY TESTING

Objective
To determine the most suitable solvent for the UV spectrophotometric method by evaluating the solubility of Metoprolol Hydrochloride in different solvents.
Procedure
• 10 mg of Metoprolol Hydrochloride was added separately to 10 mL of Distilled Water, Methanol, and 0.1 N NaOH.
• Solutions were stirred using a magnetic stirrer to ensure complete dissolution.
• Visual inspection and UV measurements were done to assess solubility and clarity of the solutions.

Observations
• Methanol: Slight solubility observed with some undissolved particles, indicating poor solubility in water.
• 0.1 N NaOH: Moderate solubility with a clear solution, suggesting that the compound has some solubility in basic conditions.
• Water: Complete solubility, resulting in a clear, colorless solution.

TRIAL EXPERIMENTAL WORK OF METOPROLOL HYDROCHLORIDE

Spectrophotometric Condition	Result
Solvent: Distilled Water + Methanol	Result:Stock solution ofMetoprolol Hydrochloride has shown maximum absorbance of 2.671 at 215 nm.
Concentration: 100 µg/mL	Resion: Concentration of drug in the solvent was found to be more due to which the drug has not shown appropriateabsorbance and not given any particular peak on particular wavelength.
Ph of solution:6.8	
λmax: 215	

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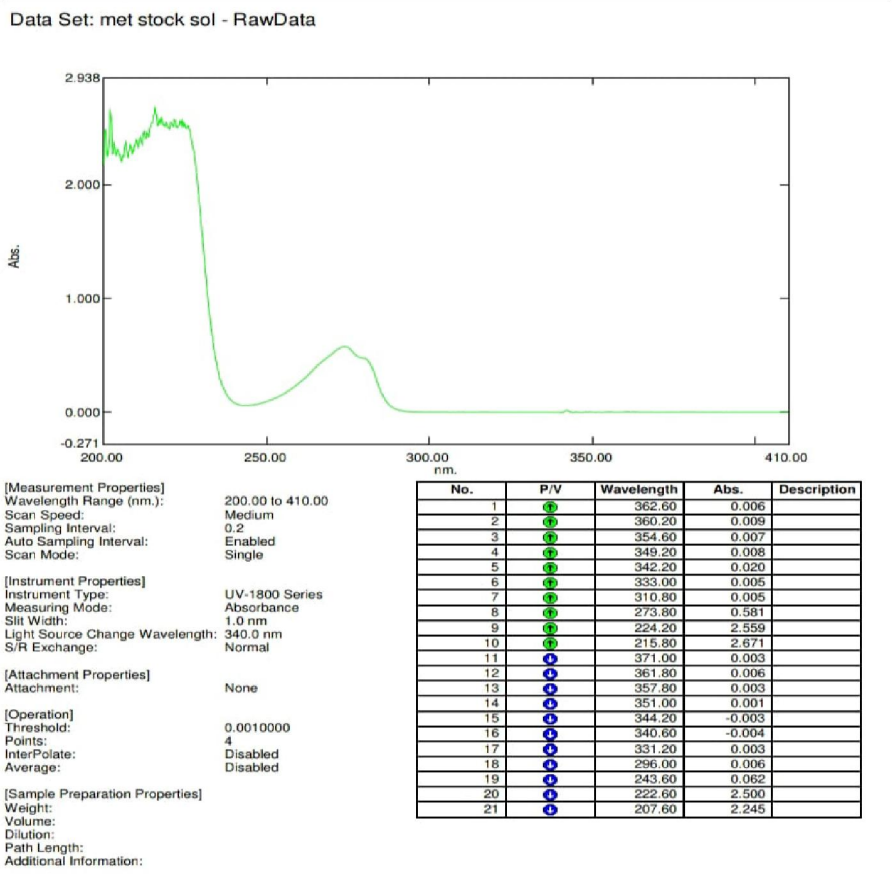
TRIAL GRAPH



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Light Source Change Wavelength: 340.0 nm
S/R Exchange: Normal

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Volume:
Dilution:
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2	↑	360.20	0.009	
3	↑	354.60	0.007	
4	↑	349.20	0.008	
5	↑	342.20	0.020	
6	↑	333.00	0.005	
7	↑	310.80	0.005	
8	↑	273.80	0.581	
9	↑	224.20	2.559	
10	↑	215.80	2.671	
11	↓	371.00	0.003	
12	↓	361.80	0.006	
13	↓	357.80	0.003	
14	↓	351.00	0.001	
15	↓	344.20	-0.003	
16	↓	340.60	-0.004	
17	↓	331.20	0.003	
18	↓	296.00	0.006	
19	↓	243.60	0.062	
20	↓	222.60	2.500	
21	↓	207.60	2.245	

Fig: UV Spectra of Metoprolol Hydrochloride (Trial Experimental Work)

V. PREPARATION OF STANDARD SOLUTIONS

5.1 Primary Stock Solution:

• Purpose: To prepare a concentrated solution of Metoprolol Hydrochloride for further dilution into working solutions.
• Procedure:
O Accurately weigh 0.01 mg of Metoprolol Hydrochloride.
O Dissolve in approximately 93 mL of distilled water+ 7 ml Methanol in a volumetric flask to make 100 ug\ml concentration
O Sonicate for 5 minutes to ensure complete dissolution.
O Store the solution in an amber bottle to protect it from light and prevent photodegradation.

5.2 Working Standard Solutions

• Purpose: To prepare solutions with varying concentrations for the construction of the calibration curve.
• Procedure:
O Prepare working solutions by diluting the primary stock solution with distilled water.
O The following concentrations were prepared:

5 µg/mL: Pipette 0.5 mL of stock solution and dilute to 10 mL with distilled water.
10 µg/mL: Pipette 1 mL of stock solution and dilute to 10 mL with distilled water.
20 µg/mL: Pipette 2 mL of stock solution and dilute to 10 mL with distilled water.

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30 µg/mL: Pipette 3 mL of stock solution and dilute to 10 mL with distilled water.
40 µg/mL: Pipette 4 mL of stock solution and dilute to 10 mL with distilled water.

VI. DETERMINATION OF λmax

Objective

To determine the wavelength of maximum absorbance (λmax) of Metoprolol Hydrochloride, which will be used for subsequent UV spectrophotometric analysis.

Procedure

- A 10 µg/mL solution of Metoprolol Hydrochloride was prepared using distilled water as the solvent.
- The solution was scanned in the UV-visible spectrophotometer between the wavelengths of 200–400 nm.
- Methanol was used as the blank to zero the instrument before measurements.
- The absorbance spectra were recorded, and the wavelength corresponding to the maximum absorbance was identified.

Observations

- The absorption spectrum revealed a sharp peak at 221 nm.
- This wavelength showed the highest absorbance for Metoprolol Hydrochloride.

VII. CALIBRATION CURVE CONSTRUCTION

Objective

To construct a calibration curve for Metoprolol Hydrochloride in the concentration range of 5–40 µg/mL, which will be used to quantify the drug in unknown samples.

Procedure

- The absorbance of the working standard solutions (5 µg/mL, 10 µg/mL, 20 µg/mL, 30 µg/mL, and 40 µg/mL) was measured at 221 nm using the UV-Visible spectrophotometer.
- Each measurement was performed in triplicate to ensure precision.
- The mean absorbance values for each concentration were calculated.
- A calibration curve was plotted with absorbance (y-axis) versus concentration (x-axis) using the mean absorbance values.

Result

- The calibration curve showed a linear relationship between absorbance and concentration over the range of 5–40 µg/mL.
- The linear regression equation for the calibration curve was:

$y=0.0397x+0.0409$

$R2 = 0.9905$

where:

o y = absorbance
o x= concentration (µg/mL)

- The correlation coefficient (R^2) was found to be 0.9905, indicating excellent linearity.

VIII. METHOD VALIDATION (ACCORDING TO ICH Q2 (R1))

8.1 Linearity

- Concentration range: 5–40 µg/mL.
- The calibration curve showed a direct proportional relationship.
- Linearity equation and R^2 confirmed method suitability.

8.2 Accuracy (Recovery Studies)

- Recovery was evaluated by spiking pre-analyzed samples with standard Metoprolol at three levels:

O 80%, 100%, and 120%.

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IX. RESULT WORK

1. λmax Determination:

Sample	λmax (nm)
Metoprolol Hydrochloride	221 nm

2. Linearity Table:

Concentration (µg/mL)	Absorbance
5	0.257
10	0.458
20	0.817
30	1.148
40	1.695

- Regression Equation: y=0.0397x+0.0409
- Correlation Coefficient (R^2): 0.9905

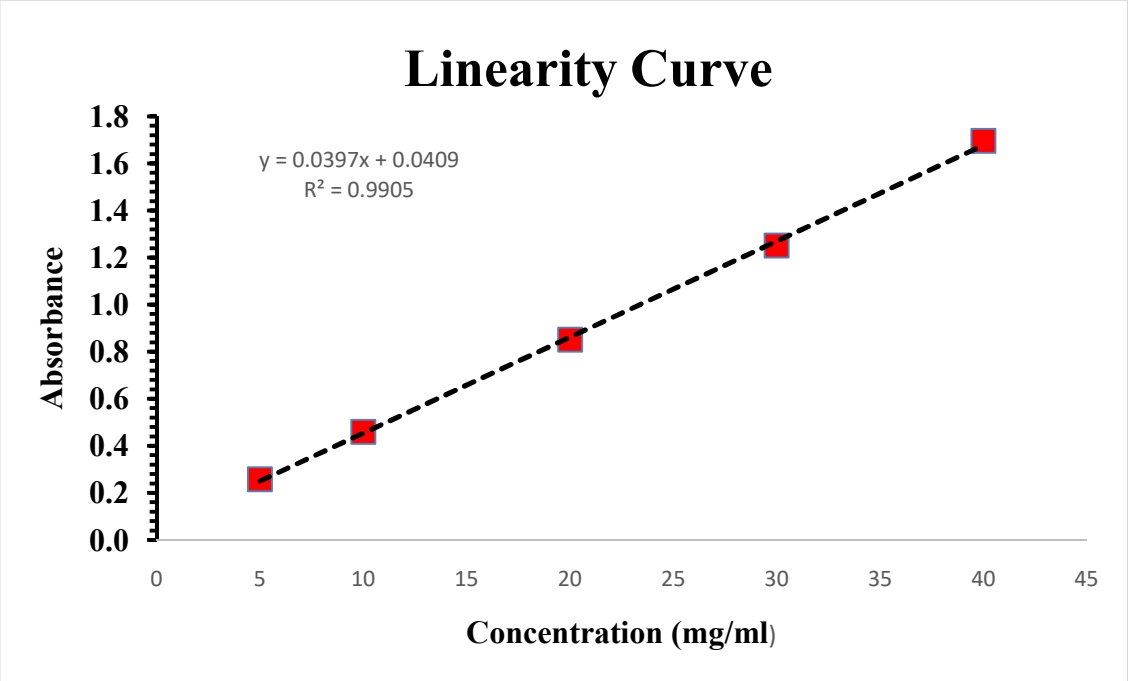

Fig. no. 6 Linearity plot for Metoprolol Hydrochloride

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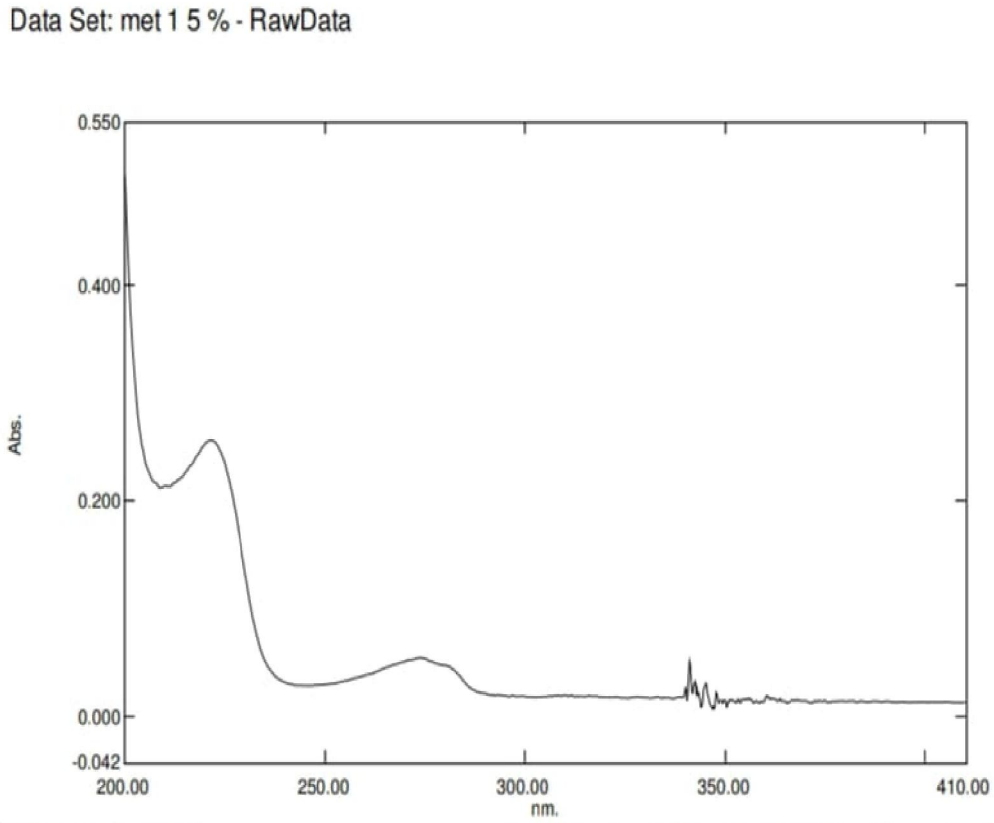

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3	↑	368.00	0.016	
4	↑	363.80	0.017	
5	↑	360.40	0.020	
6	↑	345.20	0.031	
7	↑	341.00	0.052	
8	↑	333.20	0.018	
9	↑	310.00	0.020	
10	↑	274.00	0.055	
11	↑	221.60	0.256	
12	↓	383.00	0.013	
13	↓	371.60	0.013	
14	↓	365.60	0.013	
15	↓	363.00	0.014	
16	↓	358.60	0.013	
17	↓	344.00	0.009	
18	↓	337.00	0.017	
19	↓	331.00	0.017	
20	↓	302.00	0.018	
21	↓	245.80	0.029	
22	↓	208.60	0.212	

Fig: UV Spectra of Metoprolol Hydrochloride Showing MaximumAbsorbance at (221nm). [5%]

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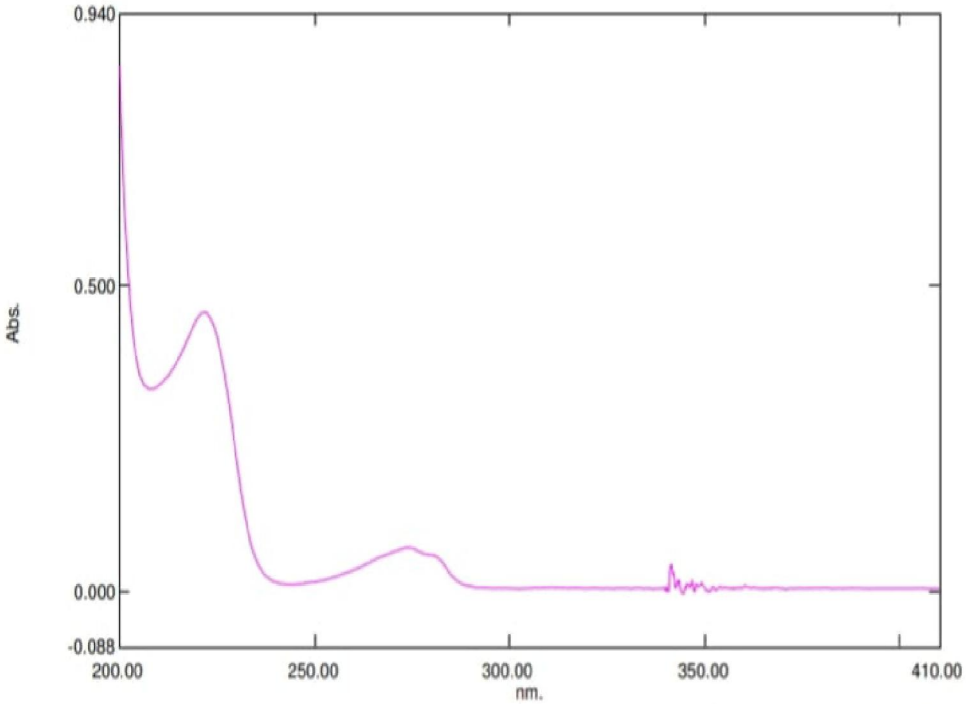

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6	↑	349.20	0.015	
7	↑	341.40	0.044	
8	↑	333.00	0.008	
9	↑	328.40	0.007	
10	↑	314.00	0.008	
11	↑	306.60	0.008	
12	↑	274.00	0.073	
13	↑	221.80	0.456	
14	↓	383.00	0.006	
15	↓	361.80	0.006	
16	↓	358.40	0.005	
17	↓	353.00	0.003	
18	↓	351.20	0.001	
19	↓	344.40	-0.002	
20	↓	340.60	0.001	
21	↓	331.20	0.006	
22	↓	326.60	0.005	
23	↓	319.40	0.006	
24	↓	307.40	0.007	

Fig: UV Spectra of Metoprolol Hydrochloride Showing Maximum Absorbance at (221nm). [10%]

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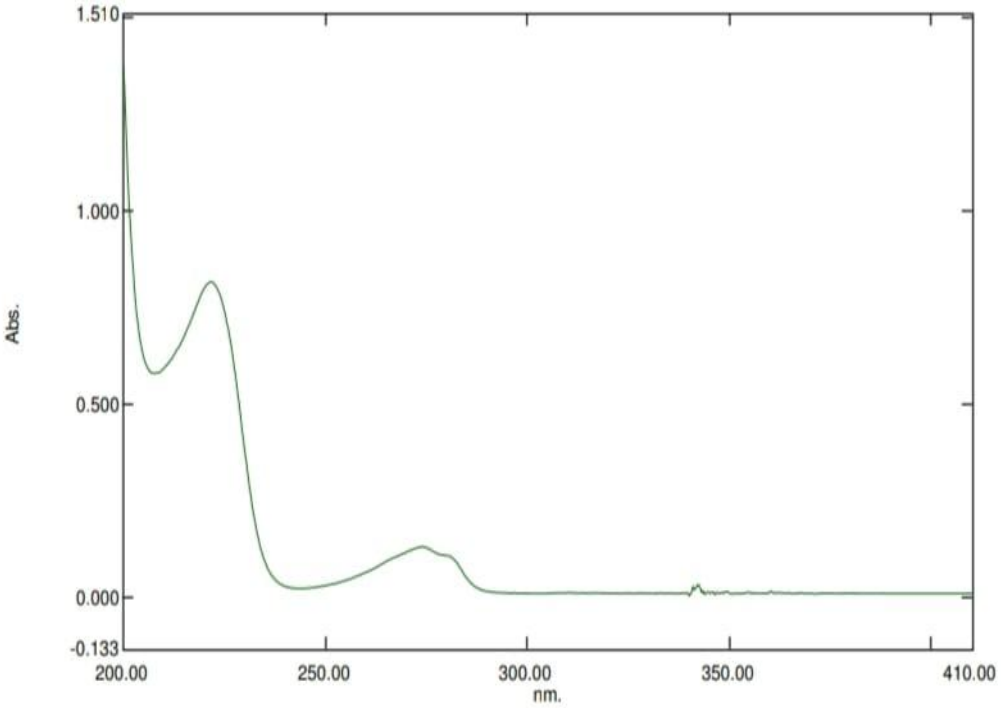

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7	↑	324.80	0.011	
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9	↑	274.00	0.130	
10	↑	221.60	0.817	
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13	↓	361.20	0.010	
14	↓	359.00	0.010	
15	↓	340.00	0.004	
16	↓	331.00	0.009	
17	↓	326.00	0.010	
18	↓	314.60	0.010	
19	↓	243.60	0.022	
20	↓	207.60	0.579	

Fig: UV Spectra of Metoprolol Hydrochloride Showing Maximum Absorbance at (221nm). [20%]

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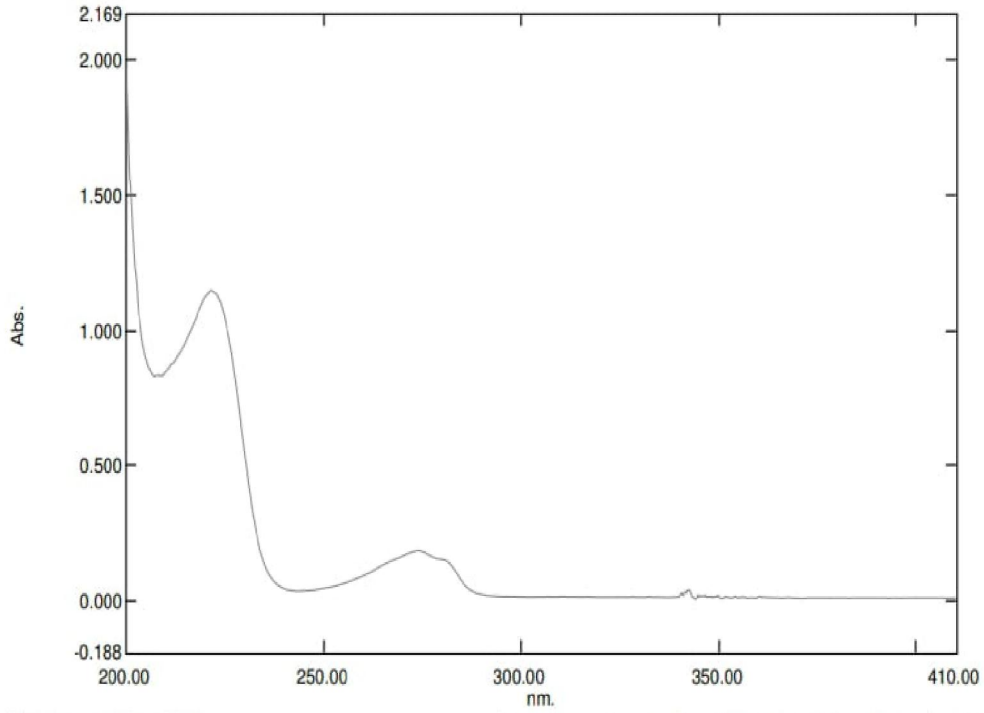

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9	↑	310.80	0.017	
10	↑	296.60	0.017	
11	↑	274.00	0.187	
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13	↓	383.00	0.012	
14	↓	371.00	0.010	
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16	↓	357.20	0.010	
17	↓	350.60	0.010	
18	↓	344.00	0.008	
19	↓	337.40	0.013	
20	↓	331.00	0.014	
21	↓	303.40	0.015	
22	↓	295.80	0.017	
23	↓	243.60	0.037	
24	↓	207.00	0.830	

Fig: UV Spectra of Metoprolol Hydrochloride Showing Maximum Absorbance at (221nm). [30%]

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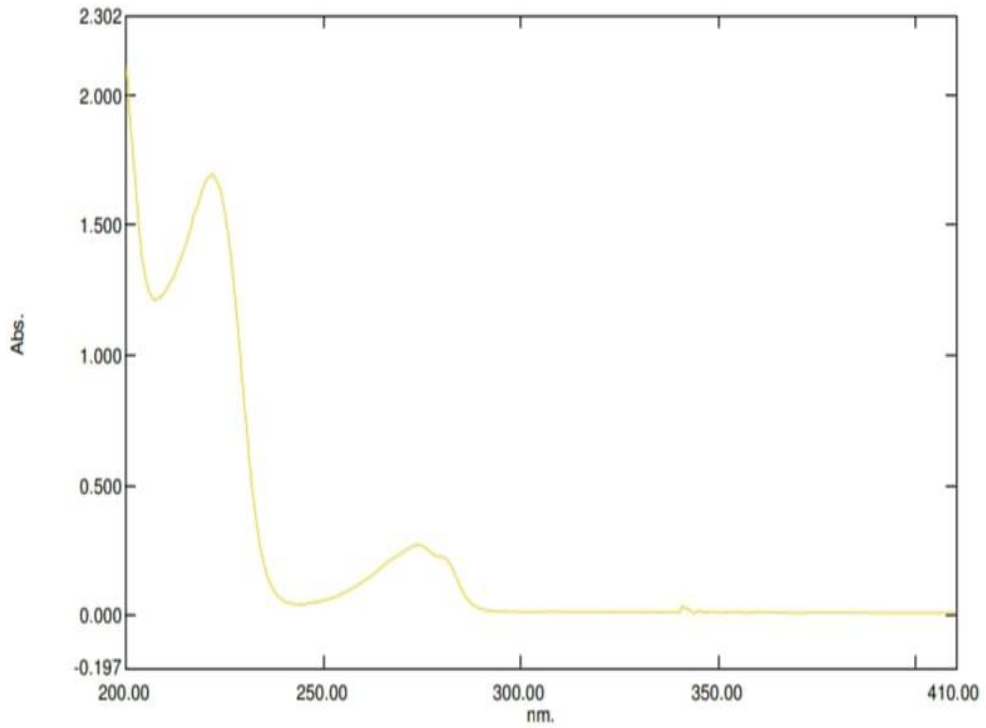

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[Instrument Properties]
Instrument Type: UV-1800 Series
Measuring Mode: Absorbance
Slit Width: 1.0 nm
Light Source Change Wavelength: 340.0 nm
S/R Exchange: Normal

[Attachment Properties]
Attachment: None

[Operation]
Threshold: 0.0010000
Points: 4
InterPolate: Disabled
Average: Disabled

[Sample Preparation Properties]
Weight:
Volume:
Dilution:
Path Length:
Additional Information:

No.	P/V	Wavelength	Abs.	Description
1	↑	384.00	0.013	
2	↑	374.80	0.013	
3	↑	362.00	0.015	
4	↑	360.40	0.019	
5	↑	345.20	0.021	
6	↑	341.00	0.039	
7	↑	333.00	0.016	
8	↑	310.60	0.017	
9	↑	274.00	0.274	
10	↑	222.00	1.695	
11	↓	383.00	0.012	
12	↓	371.00	0.012	
13	↓	361.20	0.014	
14	↓	358.20	0.012	
15	↓	343.80	0.011	
16	↓	340.00	0.011	
17	↓	321.20	0.015	
18	↓	296.00	0.017	
19	↓	243.60	0.046	
20	↓	207.20	1.212	

Fig: UV Spectra of Metoprolol Hydrochloride Showing Maximum Absorbance at (222 nm). [40%]

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Summary:

The development and validation of a UV-visible spectrophotometric method for the estimation of Metoprolol Hydrochloride involves several critical steps to ensure accuracy, precision, and reliability. Initially, the drug was selected for analysis based on its therapeutic significance as a beta-blocker for hypertension and cardiovascular conditions. A comprehensive literature survey was conducted to review existing UV methods, solvents, and validation criteria.

Materials like Methanol, 0.1 N NaOH, and distilled water were procured, and solubility testing was performed to select the best solvent for drug analysis. After preparing standard stock solutions, the optimal wavelength (λmax) was determined through scanning in the 200 – 400 nm range, with λ max $\approx$ 221 nm being identified. A calibration curve was constructed using standard dilutions (5 – 40 µg/mL), and method optimization was carried out to determine the best conditions for spectrophotometric analysis.

The validation of the method was carried out according to ICH guidelines, ensuring parameters like linearity, accuracy, precision, specificity, robustness, and limits of detection/quantification (LOD/LOQ) were met. The developed method was then applied to market formulations of Metoprolol Hydrochloride to evaluate the drug content, comparing the results with the label claim.

X. CONCLUSION

In conclusion, the UV-visible spectrophotometric method developed for Metoprolol Hydrochloride estimation has been successfully validated. The method demonstrated high accuracy, making it a reliable and cost-effective analytical technique for routine pharmaceutical analysis. The calibration curve showed excellent linearity, and the method proved to be specific, with no interference from common excipients in the tablets.

This UV spectrophotometric method provides an alternative to more complex and expensive techniques, offering a practical solution for quality control in pharmaceutical industries.

The method can be utilized for the routine analysis of Metoprolol Hydrochloride in quality control settings, ensuring the accurate and consistent production of pharmaceutical products.

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