EJPMR, 2025, 12(12), 330-337 **Research Article** **Impact Factor 7.065**

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

ISSN (O): 2394-3211
ISSN (P): 3051-2573
Coden USA: EJPMAG

INTEGRATING ANALYTICAL METHOD DEVELOPMENT, SELECTION AND VALIDATION IN NDDS

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DOI: https://doi.org/10.5281/zenodo.17735722

How to cite this Article: Lavannya Fating*, Lalit Wange, Krutika Burange, Kritika Meshram, Krunal Takarkhede. (2025). INTEGRATING ANALYTICAL METHOD DEVELOPMENT, SELECTION AND VALIDATION IN NDDS. European Journal of Biomedical and Pharmaceutical Sciences, 12(12), 330–337.
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Article Received on 03/11/2025 **Article Revised on 24/11/2025** **Article Published on 01/12/2025**

ABSTRACT

UV-Visible Spectroscopy is a widely used analytical technique that measures the absorption of ultraviolet and light by chemical substances to determine their concentration and structural properties. It operates by passing light through a sample and recording the absorbance at specific wavelengths. The major components of a UV spectrophotometer include a light source, monochromator, sample cuvette, detector, and display system. The technique is fast, nondestructive, and suitable for routine quantitative and qualitative analysis in pharmaceutical research. The present study focuses on the development and validation of a simple, accurate, and precise UV–Visible spectrophotometric method for the estimation of Samples (Metronidazole). UV spectroscopy is based on the absorption of ultraviolet light (200–400 nm) by molecules, causing electronic transitions from lower to higher energy states. The analysis was carried out using a SHIMADZU UV–Visible double beam spectrophotometer (Model UV– 1900I) with quartz cuvettes. The standard solution of Metronidazole was prepared, and serial dilutions ranging from 0.5 to 2.0 µg/mL were analyzed. The wavelength of maximum absorbance (λ max) was observed at 321 nm. The calibration curve plotted between absorbance and concentration showed a linear relationship in accordance with Beer–Lambert's law, with absorbance increasing proportionally to concentration. The developed method was found to be accurate, reproducible, sensitive, and economical, making it suitable for the routine quality control and quantitative analysis of Metronidazole in bulk and pharmaceutical formulations.[1-2]

KEYWORD: UV Spectroscopy, Beer-Lambert' Law, Electronic Transition, Metronidazole.

INTRODUCTION

Spectroscopy is the technique which measures the Electromagnetic radiations (EMR) which is emitted or absorbed by molecules or atoms or ions of a sample when it moves from one energy state to another energy state and Electromagnetic radiation is a type of energy such as UV rays, Infrared rays, Micro-waves, Radio-waves, X-rays, Gamma rays and visible light etc.

Ultraviolet (UV) spectroscopy is one of the most widely employed analytical techniques in modern chemistry and pharmaceutical sciences. It is a type of absorption spectroscopy which involves the interaction of ultraviolet light, typically in the wavelength range of 200–400 nm, with a substance to study its electronic structure. When UV light passes through a sample, some of it is absorbed, causing the electrons in the molecules to move from a lower energy level (ground state) to a higher energy level (excited state), producing a distinct absorption spectrum. This spectrum serves as a molecular fingerprint that helps in the identification, purity assessment, and quantitative determination of chemical compound.

UV spectroscopy is simple, fast, and non-destructive, making it suitable for routine analysis and monitoring reactions. Key components include a light source, monochromator, sample holder, and detector.

Principle

The principle of UV-Vis spectroscopy is Based on the principle of absorption of UV light by chemical compounds, which result in production of different Spectra and the spectra arise from the transition of an electron within a molecule from ground state to excited

state. When the molecules absorb UV radiation frequency the electron in that molecule undergoes transition from ground level to higher energy level cause electronics transition.[3-4-5]

Electronic Transitions

When a molecule absorbs UV light, electronic transitions occur — electrons jump to higher energy orbitals. Molecules that have π-electrons (in double bonds) or non-bonding electrons (lone pairs) can easily absorb UV radiation. This absorption results in different types of transitions depending on the kind of electrons involved:

1. Σ → σ* (sigma to sigma star)
2. N → σ* (non-bonding to sigma star)
3. Π → π* (pi to pi star)
4. N → π* (non-bonding to pi star) These transitions differ in energy, arranged as:

Σ–σ > n–σ > π–π* > n–π***

(from highest to lowest energy required).

Beer-Lambert's Law

This absorption process is mathematically represented by the Beer–Lambert Law, which defines a direct proportionality between absorbance (A) and the concentration (C) of the absorbing species in the solution,

Beer – Lambert's law states that

When a beam of monochromatic radiation is passes through the absorbing medium, then the decrease in the intensity of the radiation is directly proportional to the thickness/pathlength as well as concentration of the solution.

The Beer -Lambert's law can be expressed as : A a c × l

A= ε ×c×l

Where, A=Absorbance ε=Molar absorptivity

c= concentration l= pathlength

According to this law, absorbance increases linearly with concentration within a specific range, which makes UV spectroscopy a reliable quantitative method for solution analysis.

Fig. 1 & 2: UV Spectrophotometer.

Instrumentation

UV - VIS SPECTROPHOTOMETER

A UV-Vis spectrophotometer is an analytical instrument used to measure the amount of light absorbed by a sample at different wavelengths in the ultraviolet (UV) region (190 to 400 nanometers) and the visible (Vis) region (approximately 400 to700 nanometers) of the electromagnetic spectrum. Its primary function is to determine the concentration of a substance in a solution or to help identify substances based on their unique absorption patterns. It works by passing a beam of light through a sample and measuring how much of that light passes through (transmittance) or is absorbed (absorbance).

The essential parts of a spectrophotometer are

1. Radiation Source
2. Monochromator
3. Sample cell
4. Detector
5. Recordings system

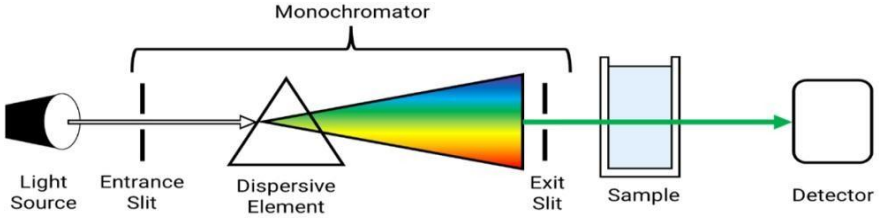

Fig. 3: Working of UV spectrophotometer.

Radiation Source

In UV-Vis spectroscopy, the selection of a light source is crucial for accurate and reliable measurements.

The light source used in UV-Visible spectroscopy should give a steady and uniform intensity of light for all wavelengths. However, maintaining this uniformity is challenging. Therefore, to cover the full range of wavelengths—from ultraviolet to visible and sometimes even near- infrared regions—spectrophotometers usually use a combination of two different light sources.[1-2]

Common light sources used in UV-Vis Spectrophotometers.

Light Source	Principle of Operation	Key Applications
Hydrogen Discharge Lamp	Gas discharge through low-pressure hydrogen; emits continuous UV (190–400 nm)	Historically used in UV spectrophotometry; now mainly for calibration due to less stability.
Deuterium Arc Lamp	Gas discharge using deuterium (isotope of hydrogen); continuous UV emission	Preferred UV source (approx. 190–400 nm) in UV-Vis spectrophotometers; offers continuous, stable, long-lasting output.
Xenon Arc Lamp	High-pressure gas discharge in xenon; broad spectrum across UV, visible, near-IR	High-intensity, sunlight-like output; used in fluorescence spectroscopy, solar simulators, and high-intensity UV-Vis applications.
Mercury Arc Lamp	Electric discharge through mercury vapor; emits sharp spectral lines	Valuable for instrument calibration using characteristic UV/visible lines (e.g., 254 nm, 365 nm); also employed in germicidal UV for sterilization.

Monochromator

Monochromator is also known as Wavelength selectors. Used to isolate the desired wavelength of radiation from wavelength of continuous spectra.

Components of Monochromator

- **Entrance Slit:** It narrows the incoming light beam to prevent overlapping of different wavelengths, ensuring a clean input for analysis.
- **Collimating Mirror (Concave):** It straightens the diverging light rays from the entrance slit into parallel beams, which is essential for accurate wavelength separation.
- **Prism or Grating:** This component disperses the parallel light into its individual wavelengths by bending (prism) or diffracting (grating) the light, separating colours like a rainbow.
- **Focusing Mirror or Lens**: It refocuses the dispersed light onto the exit slit, directing the selected wavelength precisely.
- **Exit Slit:** It allows only the desired narrow band of wavelengths to pass through, blocking the rest, to deliver pure monochromatic light.

TYPES OF MONOCHROMATORS

- **Prism Monochromator:** Prism works by refraction. Different wavelengths of Light are bent at It is a transparent container that holds the sample being measured They are typically constructed from materials like quartz, glass, or plastic different angles as they pass through the prism material. (e.g., quartz for UV, glass for visible)
- **Diffraction Grating Monochromator:** Diffraction Grating works by diffraction and interference. A grating has thousands of finely ruled parallel lines. Light striking these lines is diffracted, and different wavelengths interfere constructively at different angles, spreading the light into a spectrum.

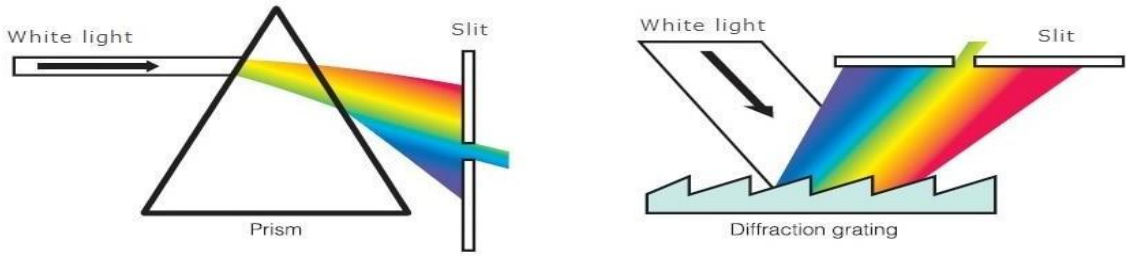

Fig. 4: Working of Monochromators.

Sample cell

In UV-Vis spectrophotometry, a sample cell, also known as a ***cuvette***, It is a transparent container that holds the sample being measured They are typically constructed from materials like quartz, glass, or plastic.

Fig. 5: Sample holder (Cuvette).

Detectors

A UV-Vis spectrophotometer, the detector is the component responsible for converting the light that has passed through the sample into a measurable electrical signal. This signal is then processed to determine the amount of light absorbed or transmitted by the samples.

Here are the common types of detectors used

1. Photovoltaic Cell (Photocell)
2. Phototube (Photocell)
3. Photomultiplier Tube (PMT)

Photovoltaic cell (photocell)

Working Principle: Based on the photovoltaic effect light falling on a semiconductor generates electron–hole pairs, producing an electric current.

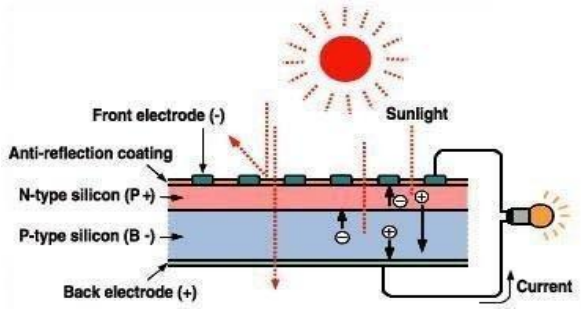

Fig. 6: Photovoltaic cell detector.

Photomultiplier tube (PMT)

Working Principle: Also based on the photoelectric effect but with electron Multiplication. The photoelectrons emitted from the cathode strike a series of Dynodes, each releasing multiple secondary electrons, resulting in a large Amplified current.

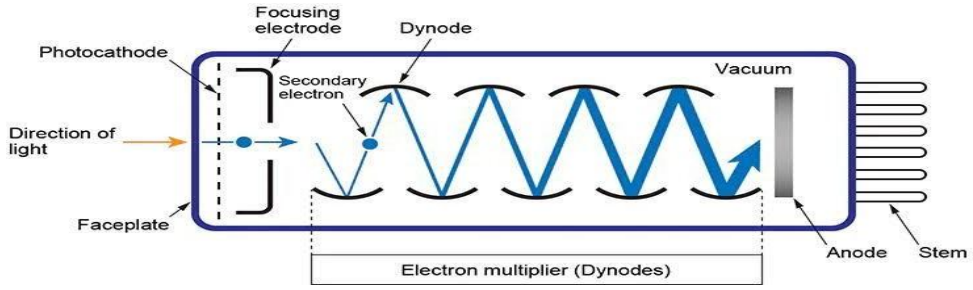

Fig. 7: Photomultiplier tube detector Phototube (Photo emissive Cell).

Working Principle: Works on the Working Principle: Based on the photovoltaic effect light falling on a semiconductor generates electron–hole pairs, producing an electric current. Effect – light photons strike a Photosensitive cathode, releasing electrons, which are collected at an anode.[3-4-5]

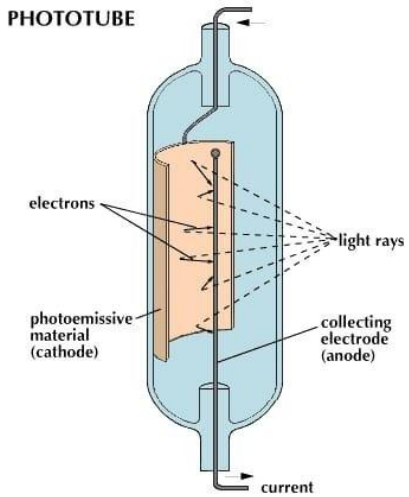

Fig. 8: Phototube detector.

Working

Working principle of a spectrophotometer is based on the following steps:

Blank (measure of the intensity of light transmitted through the solvent):

1. The solvent (e.g. water or alcohol) is added into a suitable, transparent and not absorbing container – a cuvette.
2. A light beam emitted by the light source passes through the cuvette with the solvent.
3. The intensity of the transmitted light at different wavelengths is then measured by a detector and recorded.

Sample determination

1. A sample is dissolved in the solvent and added into the cuvette.
2. A light beam emitted by the light source passes through the cuvette with the sample.
3. When passing through the cuvette, the light is partially absorbed by the sample molecules in the solution.
4. The transmitted light is then measured by the

detector.

5. The light intensity change at different wavelengths is calculated by dividing the transmitted intensity of the sample solution by the corresponding values of the blank.[6]

APPLICATIONS

A. Quantitative Chemical Analysis

In chemistry labs, UV spectrophotometry is used to measure how much of a substance (solute) is present in a solution. The Beer-Lambert Law explains that the amount of light absorbed by a solution is directly related to the concentration of the absorbing substance. This allows scientists to accurately determine concentrations.

B. Biochemical and Biomedical Research

In biology and medicine, UV spectrophotometers are used to study important molecules like proteins and nucleic acids (DNA and RNA). They help identify, measure, and monitor any structural changes in these biomolecules.

C. Pharmaceutical Analysis

In the pharmaceutical industry, UV spectrophotometry is used to check the concentration of active pharmaceutical ingredients (APIs) in drug formulations. This ensures that medicines contain the correct amount of each ingredient.

D. Environmental Monitoring

UV spectrophotometry is also used to test environmental samples, especially water. It helps detect pollutants such as heavy metals and organic compounds, ensuring that water quality.

AIM

To develop and validate a simple, accurate, precise, and sensitive UV-Visible spectrophotometric method for the estimation of sample and to study its analytical, physical, and chemical characteristics according to ICH guidelines.

OBJECTIVE

1. To understand the basic principle of UV–Visible Spectroscopy, which is based on the measurement of light absorbed by molecules in the ultraviolet and visible regions of the electromagnetic spectrum (190–700 nm).
2. To study the instrumentation and working mechanism of the UV–Visible Spectrophotometer, including its main components — light source, monochromator, sample cell (cuvette), detector, and display system.
3. To develop a simple and rapid UV-Visible spectrophotometric method for the estimation of the selected sample.
4. To ensure the developed method is accurate, precise, and sensitive for reliable analysis.
5. To validate the developed method in accordance with ICH (International Council for Harmonization) guidelines.
6. To evaluate the physical and chemical characteristics of the sample.

MATERIAL AND METHOD

Instrument and Material

SHIMADZU UV – visible double beam spectrophotometer model UV – 1900I with 1cm matched quartz cells were used for all the spectral measurements. Chemicals and reagents Distilled water, metronidazole tablets (METROGYL 400). Solvent mixture of methanol and water (80:20) was used as solvent for development of spectral characteristics. All the chemicals used were of analytical grade.

physical and chemical properties of Metronidazole

- Chemical Formula: C6H9N3O3
- Molecular Weight: 171.15 g/mol
- Physical Appearance: White to pale yellow crystalline powder
- Melting Point: Approximately 158–161°C
- Boiling Point: Estimated around 301 °C
- Solubility: Moderately soluble in water (about 1 g/100 mL at 20 °C), soluble in dilute acids and ethanol; very slightly soluble in ether and Chloroform pH of Saturated Solution: Around 5.8 to 6.5
- Odor: Slight odor, bitter and salty taste
- Stability: Darkens on exposure to light, stable under standard conditions but incompatible with strong oxidizing agents
- Density: Approximately 1.4 g/cm³

Structure of metronidazole

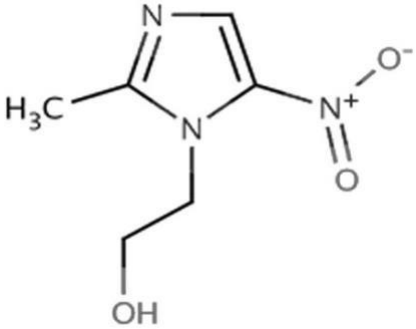

Fig. 9: Structure of Metronidazole.

Fig. 10: Metronidazole Tablets.

Clinical Uses of Metronidazole

- Treatment of amoebiasis (intestinal and hepatic)
- Treatment of trichomoniasis.
- Management of bacterial vaginosis.
- Treatment of anaerobic bacterial infections.
- Used in pseudomembranous colitis (Clostridium difficile infection). Part of ombination therapy for Helicobacter pylori in peptic ulcer disease

Side effects of metronidazole

- Vomiting, Nausea
- Loss of appetite
- Metallic taste in the mouth, Dry mouth
- Abdominal pain or cramps
- Mouth or tongue irritation
- Skin rash or itching[7-8-9]

PREPARATION OF SAMPLE

Preparation of standard stock solution

1. Twenty tablets were weighed accurately and ground into a fine powder.
2. A quantity of the powdered tablets, equivalent to 50 mg of metronidazole, was weighed accurately.
3. This powder was transferred into a 100 ml volumetric flask.
4. The contents were dissolved with sufficient amount of solvent and diluted up to the mark to obtain a sample stock solution
5. The solution was filtered through Whatman filter paper No. 41 to obtain a clear solution.

Preparation of Primary stock solution

6. From the stock solution, 10 ml was pipetted and transfer into a 100 ml volumetric flask and make up the volume with water.
7. Pipette out 1ml of solution and transfer it to the 10ml of volumetric flask. Make up the volume up to the mark by water to get the final dilutions.[10 -11]

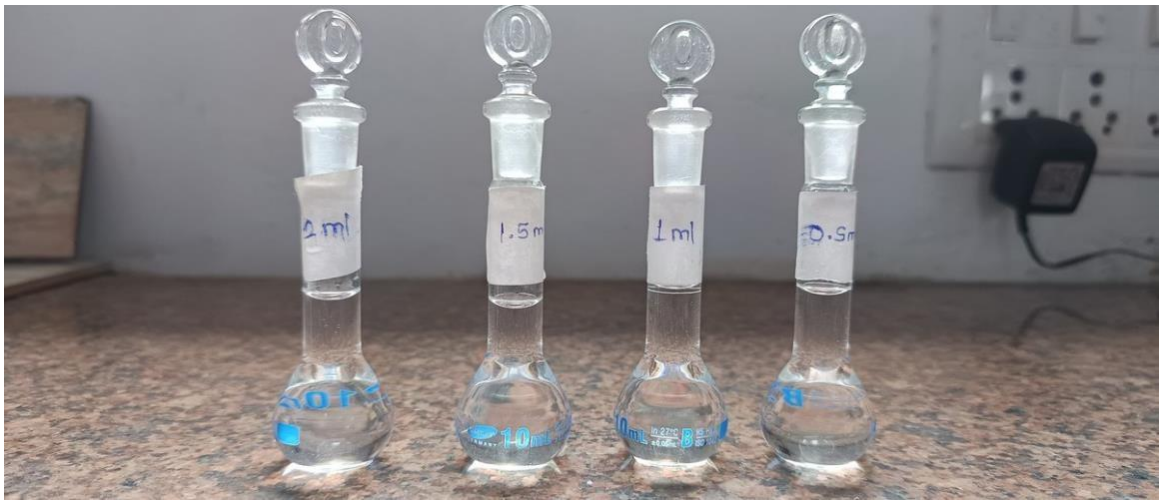

Fig. 11: Solution of different concentration.

Criteria for method selection for metronidazole

The criteria for method selection for metronidazole estimation by UV visible spectroscopy in novel drug delivery systems (NDDS) typically include:

- **Wavelength selection:** Metronidazole shows maximum absorbance (λ max) around 278-320 nm depending on the solvent and formulation type. This wavelength corresponds to its UV absorbance maximum for optimal sensitivity.
- **Linearity**: The method should obey Beer's law within a suitable concentration range (e.g., 5-30 µg/mL or larger ranges like 80%-120% of drug concentration). Correlation coefficient [R^2] values close to 0.999 indicate good linearity.
- **Accuracy and recovery**: Percentage recovery

should be within acceptable limits of around 98-102% to confirm accuracy.

- **Precision**: The method should demonstrate reproducibility and precision with low relative standard deviation (e.g., coefficient of variation below 2%) on replicate measurements.
- **Sensitivity**: The limits of detection (LOD) and quantification (LOQ) should be low enough for the required assay range.
- **Stability**: The sample solution should remain stable for a reasonable period (e.g., 24 hours) to allow consistent readings.
- **Specificity**: The method should clearly differentiate metronidazole from excipients or other formulation components without interference.[10]

RESULTS AND DISCUSSION

A series of standard solutions of Metronidazole was prepared in the concentration range of 0.5–2.0 µg/ml and scanned in the UV region (200–400 nm) to determine the wavelength of maximum absorbance (λ max). The λ max was found to be 321 nm.

A calibration curve was plotted between absorbance (Y-axis) and concentration (X-axis). The curve was found to be linear within the studied concentration range.

OBSERVATION

Sr. No	Concentration	Absorbance
1.	0.5	0.624
2.	1.0	1.246
3.	1.5	1.544
4.	2.0	2.262

Calibration Curve for Metronidazole

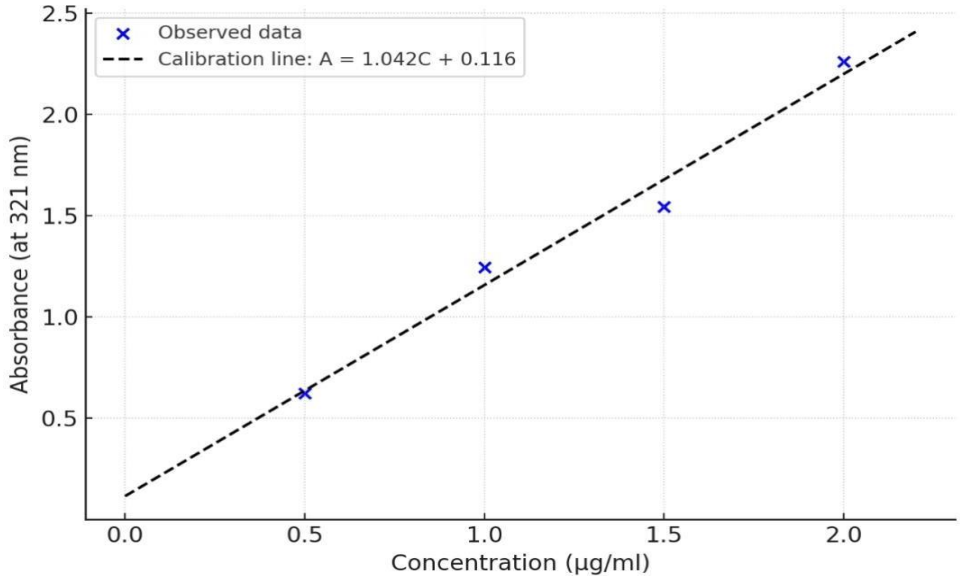

Fig. 12: Calibration Curve of Metronidazole.

Fig. 13: Spectrum Showing absorption maxima for metronidazole.

The UV spectrophotometric method developed for the estimation of Metronidazole showed a clear linear relationship between concentration and absorbance at 321 nm.

The linearity of the calibration curve suggests that absorbance increases proportionally with the concentration, validating the applicability of Beer–Lambert's law in this concentration range. The λ max at 321 nm corresponds to the characteristic absorption of Metronidazole, indicating no interference from excipients or solvent.

Thus, the method is suitable for the routine analysis of Metronidazole in bulk and pharmaceutical dosage forms using UV-visible spectrophotometry due to its simplicity, precision, and reproducibility.

SUMMARY AND CONCLUSION

The present study focused on the development and validation of a simple, accurate, precise, and sensitive UV–Visible spectrophotometric method for the estimation of Metronidazole in bulk and tablet dosage form according to ICH guidelines.

The λ max of Metronidazole was found to be 321 nm using a solvent system of methanol and water (80:20). Standard solutions with concentrations ranging from 0.5 to 2.0 µg/mL showed a linear relationship between absorbance and concentration, confirming adherence to Beer– Lambert's law. The calibration curve demonstrated excellent linearity, indicating the reliability of the method for quantitative analysis.

The developed method was found to be simple, rapid, and reproducible, with no interference from excipients or solvents. The accuracy and precision were within acceptable limits, making the method suitable for routine quality control analysis of Metronidazole in pharmaceutical.

ACKNOWLEDGEMENT

I wish to sincerely thank P. R. Patil Institute of Pharmacy, Talegaon for the valuable chance to participate in the Practice School session. This experience has significantly enhanced both my practical skills and understanding. I am very grateful to my teachers for their continuous encouragement, guidance, and support throughout my session.

I also deeply appreciate Prof. Kunal Takarkhede for providing me the opportunity to complete my project on "INTEGRATING ANALYTICAL METHOD DEVELOPMENT, SELECTION AND VALIDATION IN NDDS" at their respected institution. The ongoing support, expert advice, and willingness to share expertise made a substantial impact on the successful completion of my report.

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