

Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF NSAID AND ANTACID

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ABSTRACT

NSAIDs are among the most often prescribed pain relievers. NSAIDs are a highly successful medicine class for pain and inflammation due to the ever-increasing number of medications and drug combinations on the market necessitates the development of analytical tools for monitoring their quality. The procedures must be developed in such a way that they take less time to design and produce the most accurate and robust results possible. Omeprazole, a widely used proton pump inhibitor (PPI), has become a cornerstone in the treatment of various acid-related gastrointestinal disorders such as gastroesophageal reflux disease (GERD), peptic ulcers, and Zollinger-Ellison syndrome. By inhibiting the proton pump in parietal cells, omeprazole effectively reduces gastric acid secretion, thereby promoting healing and alleviating symptoms associated with hyperacidity.

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INTRODUCTION

OVERVIEW OF UV-VISIBLE SPECTROSCOPY

UV-visible spectroscopy is a powerful analytical technique used to measure the absorbance of light by a sample in the ultraviolet (UV) and visible (Vis) regions of the electromagnetic spectrum. This technique is based on the principle that different substances absorb light at specific wavelengths. By analyzing the absorbance at these wavelengths, one can obtain valuable information about the concentration and properties of the substance being studied^[1]

Wavelength Range:

UV-visible spectroscopy typically covers the wavelength range from 200 nm to 800 nm. The UV region spans from 200 nm to 400 nm, while the visible region ranges from 400 nm to 800 nm^[2].

Absorption Peaks:

The technique detects peaks in the absorbance spectrum, which correspond to specific electronic transitions in the molecules of the sample. These peaks are used to identify and quantify different compounds^[3]

PRINCIPLE ABSORPTION OF LIGHT:

UV-visible spectroscopy is based on the interaction between light and matter. Here's a breakdown of the fundamental principles:

Electromagnetic Spectrum:

Light is composed of electromagnetic waves that vary in wavelength. UV-visible spectroscopy focuses on the UV (200-400 nm) and visible (400-800 nm) regions of the spectrum.^[4]

Electronic Transitions:

When light passes through a sample, certain wavelengths are absorbed by the sample. This absorption occurs due to electronic transitions within the molecules. Specifically, electrons in the sample's atoms or molecules move from lower energy levels to higher energy levels when they absorb photons.

$\pi \rightarrow \pi$ Transitions: Common in conjugated organic compounds, where electrons in a π -bond system are excited to a π^* anti-bonding orbital.

$n \rightarrow \pi$ Transitions: Involves non-bonding electrons (n) moving to π^* anti-bonding orbitals, typically seen in molecules with lone pairs of electrons.

Absorption Spectra:

The result of these interactions is an absorption spectrum, which plots absorbance versus wavelength. Peaks in the spectrum correspond to specific electronic transitions and can provide information about the molecular structure and concentration of the analyte ^[5].

Instrumental method:

UV-Visible spectroscopy, commonly known as UV-V is spectroscopy, is an effective method of analysis used for the study of compounds and materials based on their interaction with ultraviolet and visible light. This spectroscopic technique is used in many scientific fields, like chemistry, biochemistry, physics, and environmental science ^[6].

ULTRAVIOLET (UV) LIGHT AND ITS CLASSIFICATION.

Near Ultraviolet (NUV):

This range extends from approximately 300 to 400 nm. It's closest to visible light and includes UV-A, which is less harmful than other types of UV radiation but still can cause skin damage over time.

Middle Ultraviolet (MUV):

This range covers around 200 to 300 nm. It includes UV-B, which is more energetic than UV-A and can cause more damage to skin and eyes and is also responsible for sunburn.

Far Ultraviolet (FUV):

This range spans from about 100 to 200 nm. It's more energetic and can ionize atoms and molecules, which can be harmful. This includes UV-C, which is highly effective at killing bacteria and viruses but is absorbed by the Earth's atmosphere and doesn't reach the surface.

Extreme Ultraviolet (EUV):

With wavelengths shorter than 100 nm, EUV radiation is closer to X-rays. It's highly energetic and can penetrate materials deeply. This type is used in various scientific and industrial applications, including semiconductor manufacturing. ^[7]

According to ISO 21348:2007, the components and their corresponding wavelengths are defined in the table below.

There are two types of absorbance instruments used to collect UV-visible spectra:

- 1. Single beam spectrometer**
- 2. Double beam spectrometer**

SINGLE BEAM SPECTROMETER: -

A single-beam spectrometer is an analytical instrument utilized to measure the intensity of light across a spectrum of wavelengths. It is fundamental in fields such as chemistry, biology, and materials science for analyzing the absorbance and transmittance properties of samples.

Components of a Single-Beam Spectrometer:

The key components of a single-beam spectrometer include a light source, monochromator, sample holder, detector, and readout device. The light source is responsible for providing a continuous spectrum of light, which can range from ultraviolet to visible light. Common light sources include deuterium lamps for UV light (160-375 nm) and tungsten lamps for visible light (350-2500 nm). Stability in the lamp's voltage is crucial to ensure a consistent energy output. ^[5]

The monochromator plays a vital role in isolating specific wavelengths of light from the continuous spectrum emitted by the light source. It comprises an entrance slit to narrow the light beam, a dispersive element such as a prism or diffraction grating to separate the light into its component wavelengths, and an exit slit to select a narrow band of wavelengths to pass through to the sample. The sample holder, typically a cuvette made of quartz for UV measurements or glass/plastic for visible light measurements, holds the sample in the light path. The detector measures the intensity of light after it has passed through the sample. Common detectors include photodiodes and photomultiplier tubes (PMTs), the latter being particularly sensitive to low light levels. The readout device converts the signal from the detector into a readable format, such as a digital display or computer interface, facilitating the interpretation of the measured light intensity. ^[6]

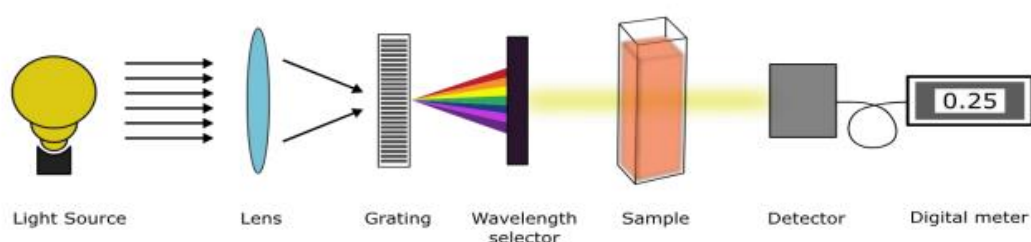


Figure 1. Single beam UV visible Spectrophotometer

DOUBLE-BEAM SPECTROMETER: -

A double-beam spectrometer is an advanced analytical instrument designed to measure light intensity across a spectrum of wavelengths with high accuracy and stability. This type of spectrometer enhances measurement precision by simultaneously comparing the light passing through a sample with a reference beam. This approach compensates for any fluctuations in the light source and other environmental factors, ensuring consistent and reliable results.

Components of a Double Beam Spectrometer:

A double-beam spectrometer consists of several key components: a light source, monochromator, beam splitter, sample and reference holders, detectors, and a readout device. The light source provides a continuous spectrum of light, which can range from ultraviolet to visible light. Typically, deuterium lamps are used for ultraviolet light (160-375 nm), and tungsten lamps are used for visible light (350-2500 nm). Maintaining a stable voltage for the light source is crucial to ensure consistent energy output. The monochromator is responsible for isolating specific wavelengths of light from the broad spectrum emitted by the light source. It consists of an entrance slit that narrows the incoming light beam, a dispersive element such as a prism or diffraction grating that separates the light into its component wavelengths, and an exit slit that selects the desired wavelength to be directed toward the sample. A beam splitter divides the monochromatic light into two beams: one directed toward the sample and the other toward the reference. This simultaneous measurement helps to compensate for any fluctuations in the light source intensity and other environmental factors. The sample holder contains the sample to be analyzed, while the reference holder contains a reference sample, often the solvent or blank. For UV measurements, quartz cuvettes are used, while glass or plastic cuvettes are suitable for visible light measurements. The detectors measure the intensity of light from both the sample and reference beams. Common detectors include photodiodes and photomultiplier tubes (PMTs), with PMTs being particularly sensitive to low light levels. The readout device converts the signals from the detectors into a readable format, such as a digital display or computer interface. This allows for precise interpretation of the measured light intensities. ^[8]

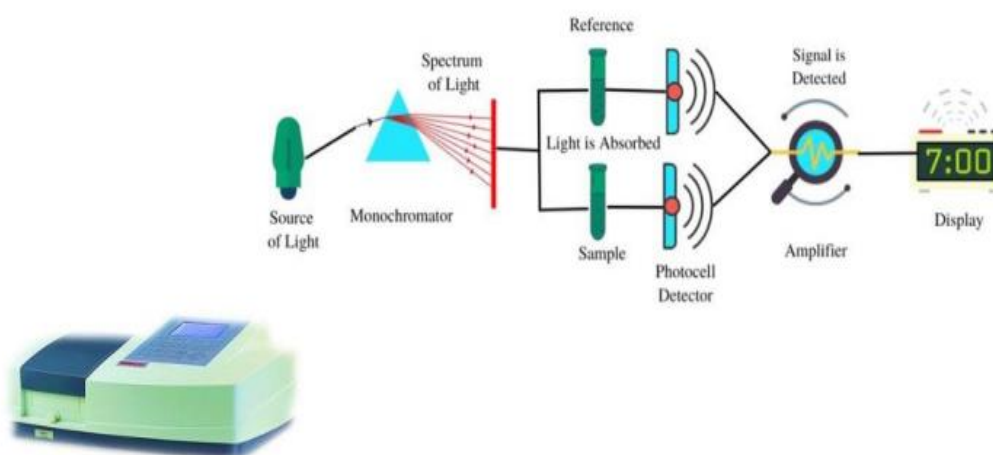


Figure 2. Double beam UV visible Spectrophotometer

INSTRUMENTATION:

UV-visible spectroscopy (UV-Vis) is a widely used analytical technique that measures the absorption or transmission of ultraviolet and visible light by a sample. Here's an overview of the instrumentation involved in UV-V is spectroscopy ^[9]

1. Source
2. Monochromator
3. Sample cell
4. Detector
5. Computer and Software

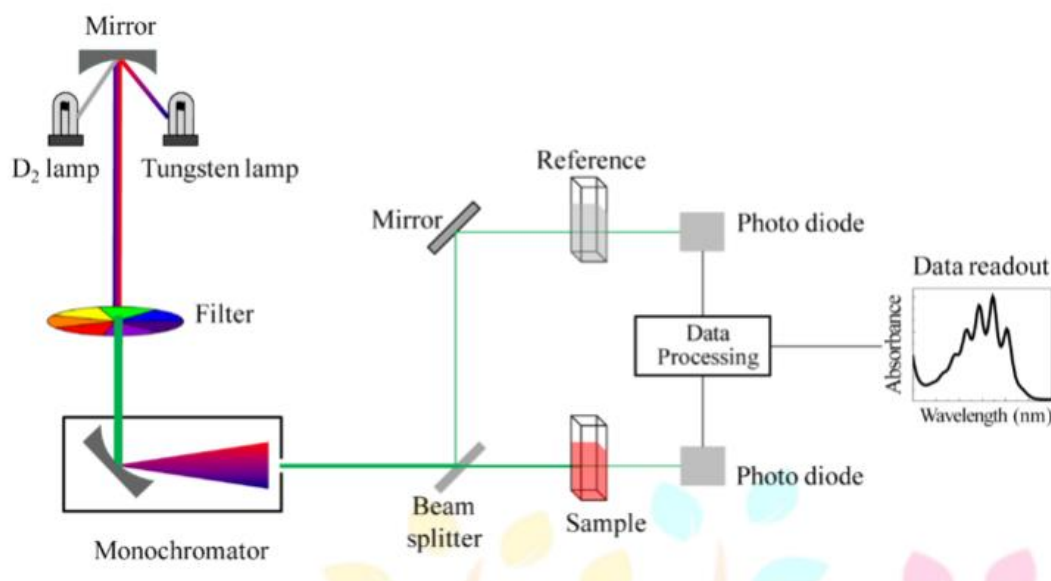


Figure 3. Instrumentation of UV visible Spectroscopy

LIGHT SOURCES

Deuterium Lamp Emission Range:

The deuterium lamp is used to provide continuous UV emission in the range of 160-375 nm. This range is particularly important for detecting compounds that absorb in the ultraviolet region.

Window Material:

Quartz windows are necessary for deuterium lamps because glass absorbs UV light below 350 nm, which would interfere with accurate measurements. Quartz, on the other hand, is transparent to UV light, allowing the emitted UV radiation to pass through without significant absorption.

Emission Range:

The tungsten-halogen lamp provides continuous visible light in the range of 350-2500 nm. This includes the entire visible spectrum and extends into the near-infrared region, making it versatile for various applications.

Voltage Stability:

Ensuring stable lamp voltage is critical for consistent light output. Fluctuations in voltage can cause variations in light intensity, which would affect the accuracy and precision of measurements.

MONOCHROMATOR

A monochromator is a key component in UV-visible spectrophotometry that isolates specific wavelengths of light from a broader spectrum.

Here's a detailed look at its structure and function

Function:

The primary function of a monochromator is to separate the light into its constituent wavelengths and allow only a narrow band of wavelengths to pass through to the sample. This enables precise measurement of absorbance or transmittance at specific wavelengths.

Key Components:

Entrance Slit:

Narrows the incoming light beam, which helps improve the spectral resolution by reducing the amount of stray light and focusing the beam onto the dispersive element.

Design: Typically, adjustable to control the width of the light beam entering the monochromator.

Operation

1. **Light Entry:** Light from the source enters through the entrance slit.
2. **Dispersion:** The dispersive element (grating or prism) separates the light into its constituent wavelengths.
3. **Wavelength Selection:** The separated light is then directed through the exit slit, which selects a specific wavelength.
4. **Transmission to Sample:** The chosen wavelength is directed towards the sample, and the intensity of the transmitted or absorbed light is measured by the detector.

Importance

Spectral Resolution:

The monochromator's design and the width of the entrance and exit slits influence the spectral resolution of the spectrophotometer. Narrow slits and high-quality dispersive elements provide higher resolution.

Wavelength Accuracy:

The precise control of the monochromator allows accurate measurement of absorbance or transmittance at specific wavelengths, essential for identifying and quantifying compounds based on their spectral characteristics.

SAMPLE CELL

Cuvettes:

Containers that hold the sample. They are typically made of quartz for UV measurements and glass or plastic for visible measurements. The path length is usually 1 cm.

Temperature Control:

Some instruments include temperature control for studies requiring precise temperature management.

DETECTOR:

Photomultiplier Tube (PMT):

PMTs are known for their high sensitivity and low noise, making them ideal for detecting very low light levels. They work by amplifying the signal through a series of dynodes that increase the number of electrons generated from the incident photons. This makes them suitable for applications where high sensitivity is crucial, such as in low-concentration analyses or in systems with weak signals.

Photodiode Array (PDA):

PDAs are composed of an array of photodiodes, each detecting light at a different wavelength simultaneously. This allows for rapid scanning and data acquisition over a range of wavelengths without moving parts. PDAs are commonly used in modern UV-Vis spectrometers for their speed and efficiency, particularly in applications requiring quick and comprehensive spectral analysis.

COMPUTER AND SOFTWARE

Data Acquisition:

This system converts analog signals from the detector (like a PMT or PDA) into digital data that can be processed by a computer. The conversion process involves sampling the signal and quantifying its intensity, which is crucial for accurate measurements and analysis. High-quality data acquisition systems ensure that the data captured is both precise and representative of the actual signal.

Software:

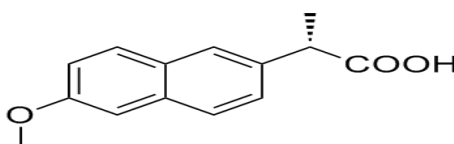
The software associated with spectrophotometers serves multiple functions. It controls the various components of the instrument, including the light source and detector. It also manages data collection, allowing users to set up experimental parameters and run analyses. Once data is collected, the software performs various calculations, such as absorbance, transmittance, and concentration, and can often generate spectral plots and perform other advanced analyses. Good software is essential for effective data interpretation and for making the most of the capabilities of the spectrophotometer.

Application of Absorption Spectroscopy (UV, Visible):

1. Detection of impurities
2. Structure elucidation organic compound
3. Quantitative analysis
4. Qualitative analysis
5. Dissociation constant of acid and bases
6. Chemical kinetics
7. Quantitative analysis pharmaceutical substances
8. Molecular weight determination

Drug Profile:

Structure of Naproxen:



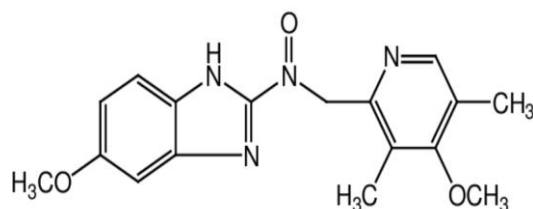
Physiochemical and Pharmacokinetic Properties of Naproxen:

Sr. No.	Parameter	Description
1.	Molecular Formula	C ₁₄ H ₁₄ O ₃
2.	Molecular Weight	230.26 g/mol
3.	Melting Point	153°C
4.	IUPAC Name	(+)-(S)-2-(6-Methoxynaphthalen-2-yl)propanoic acid
5.	Category	NSAID
6.	Description	White powder
7.	Solubility	DMSO, Methanol
8.	Adverse Effect	A frequent sore throat, nosebleeds and infections

Table no.1 Physiochemical and Pharmacokinetic Properties of Naproxen

Drug Profile:

Structure of Omeprazole:



Physiochemical and Pharmacokinetic Properties of Omeprazole:

Sr. No.	Parameter	Description
1.	Molecular Formula	C ₁₇ H ₁₉ N ₃ O ₃ S
2.	Molecular Weight	345.4 g/mol
3.	Melting Point	156 °C
4.	IUPAC Name	5-Methoxy-2-[(4-methoxy-3,5-dimethylpyridin-2-yl) methanesulfinyl]-1H-benzimidazole
5.	Category	proton pump inhibitors
6.	Description	white to pale brown
7.	Solubility	DMSO
8.	Adverse Effect	Diarrhea, Headache, Allergic reaction

Table no.2 Physiochemical and Pharmacokinetic Properties of Omeprazole

MATERIAL AND INSTRUMENTS

INSTRUMENT

Sr. No.	Name of Instrument	Model Specification	Manufacturer
1.	Weighing balance	AUY-220	Shimadzu
2.	UV-Visible Double beam spectrophotometer	V-630 sr.no. C256161148	Jasco v-630

Table No. 3 List of Equipment's Used

Chemicals and Reagents:

Sr. No.	Chemical	Grade	Manufacturer
1.	DMSO	UV	RXChemicals.Ltd. Mumbai

Table No. 4 List of Chemical Used

Drug Sample

Sr. No.	Drug Sample
1.	Naproxen
2.	Omeprazole

Table No. 5 List of Drug Sample

Working Standard:

Naproxen Sodium:

Molecular Formula: $C_{14}H_{14}O_3$

Molecular Weight: 230.26 g/mol

Omeprazole:

Molecular Formula: $C_{17}H_{19}N_3O_3S$

Molecular Weight: 345.4 g/mol

Preparation of Stock Solution:

10 mg of each Naproxen sodium and omeprazole were weighed separately and transferred into two different 100mL volumetric flasks. The drugs were dissolved in 10mL of DMSO by sonication and adjusted the volume to 100ml with distilled water to get a concentration of 100 μ g/mL of Naproxen sodium and Omeprazole.

Preparation of Standard Solution:

From the stock solution of Naproxen sodium and Omeprazole the standard solution of prepared in 5-25 μ g/ml concentration range of Naproxen sodium and 5-25 μ g/ml concentration range of Omeprazole respectively.

Preparation of sample solution:

The sample was prepared by taking 1ml of each Naproxen sodium and Omeprazole from stock solution in a 10ml volumetric flask, mix well and make volume upto the mark using distilled water, the measure the absorbances of the sample solution at respective wavelength using

double –beam UV-visible spectrophotometer.

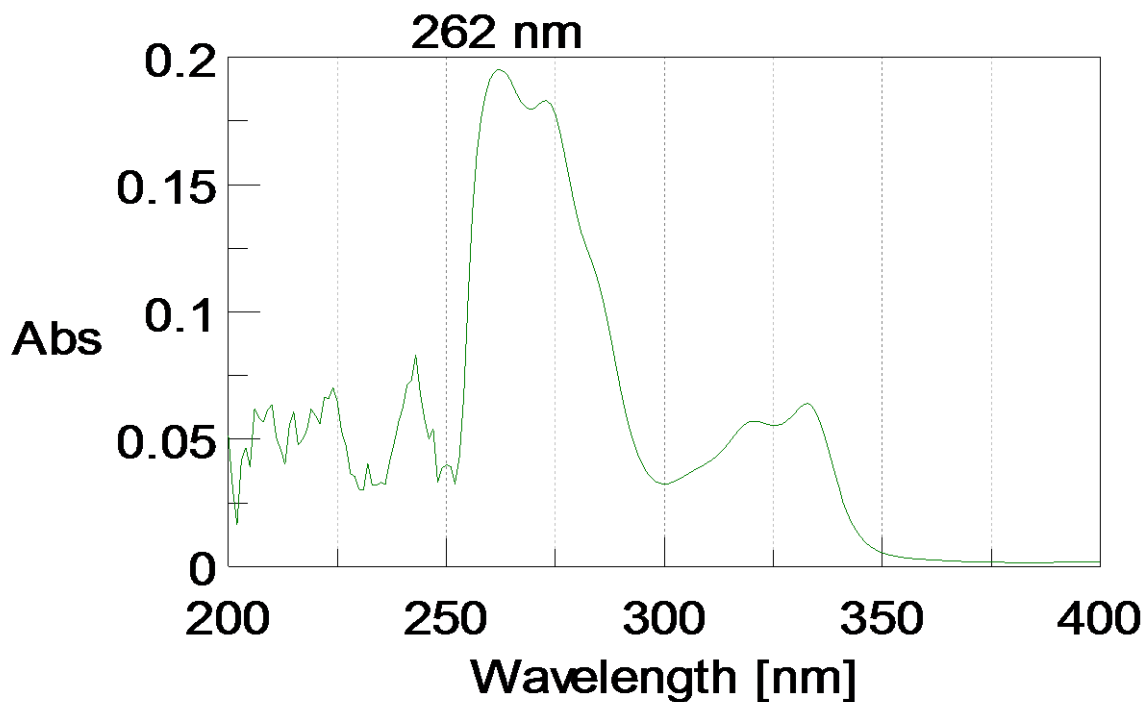


Figure No.4 UV spectra of Naproxen sodium in DMSO at 262nm(20ppm)

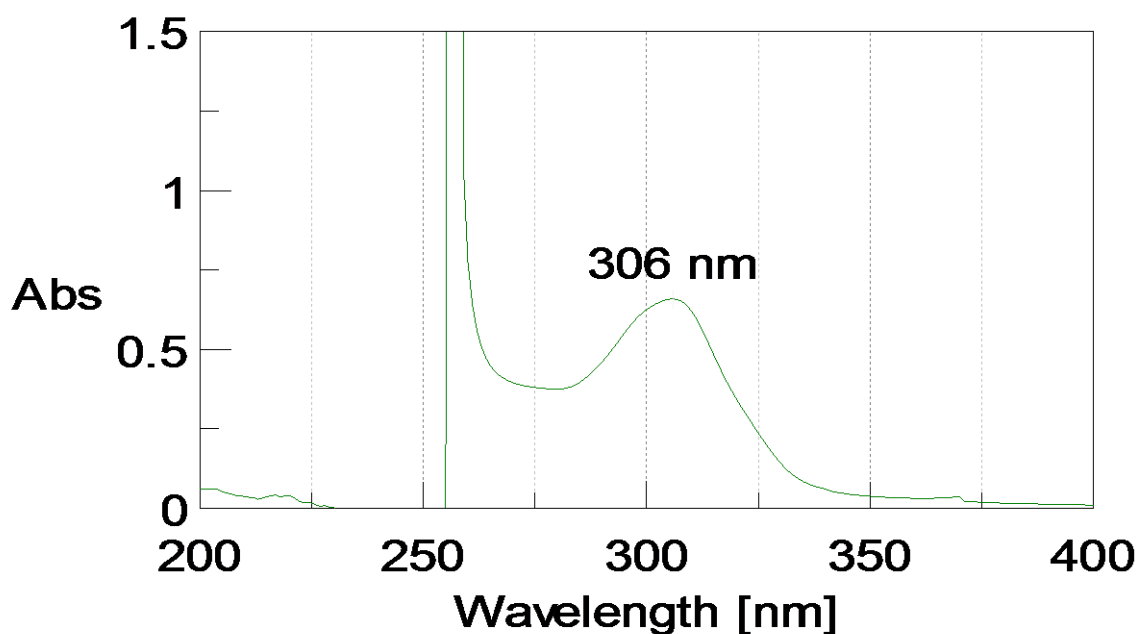


Figure No. 5 UV spectra of Omeprazole in DMSO at 306nm (20ppm)

RESULTS AND DISCUSSION

UV- VISIBLE SPECTROSCOPY METHOD DEVELOPMENT:

Linearity:

The calibration curve was plotted for both the Naproxen Sodium and Omeprazole at 262nm and 303nm. Both drugs show linearity and obey the beer's law in the concentration range of 5-25 µg/ ml for Naproxen Sodium and 5-25µg/ ml for omeprazole. The correlation coefficients of calibration curve were found to be 0.9875 for Naproxen Sodium and 0.9749 for Omeprazole respectively.

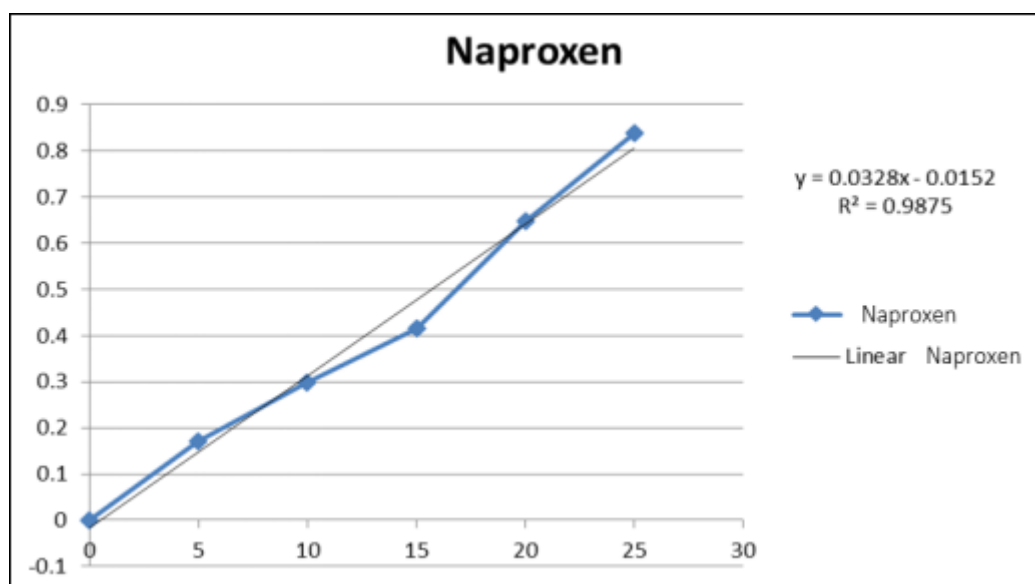


Figure 6 Calibration curve of Naproxen

Sr. No.	Concentration	Absorbance
1.	5	0.1702
2.	10	0.2983
3.	15	0.4144
4.	20	0.6473
5.	25	0.8386

Table No. 6 Linearity of Naproxen

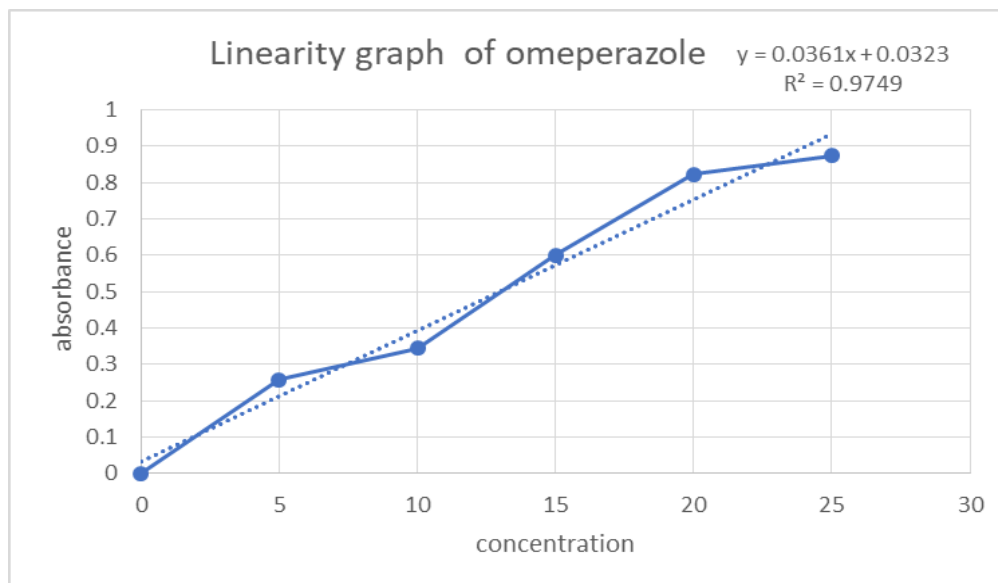


Figure 7 Calibration curve of Omeprazole

Sr. No.	Concentration	Absorbance
1.	5	0.2581
2.	10	0.3449
3.	15	0.6006
4.	20	0.8224
5.	25	0.8726

Table No. 7 Linearity of Omeprazole

Simultaneous Equation Method:

Concentration μ/ml	Naproxen			
	301 nm		262 nm	
	Abs	Absorptivity	Abs	Absorptivity
5	0.1821	0.0364	0.2521	0.0502
10	0.3602	0.0360	0.4574	0.0457
15	0.5436	0.0362	0.6981	0.0465
20	0.6996	0.0349	0.8998	0.0449
25	0.9102	0.0364	0.9821	0.0392
	Avg. Absorptivity ax ₁ =0.0359		Avg. Absorptivity ax ₂ =0.0453	

Table No.8 Simultaneous Estimation Method for Naproxen

Concentration μ/ml	Omeprazole			
	301 nm		262 nm	
	Abs	Absorptivity	Abs	Absorptivity
5	0.1720	0.0344	0.1450	0.029
10	0.3450	0.0345	0.2901	0.0290
15	0.4999	0.0333	0.4351	0.0290
20	0.6825	0.0341	0.575	0.028
25	0.8563	0.0342	0.7016	0.0280
	Avg. Absorptivity ay ₁ = 0.0341		Avg. Absorptivity ay ₂ = 0.0286	

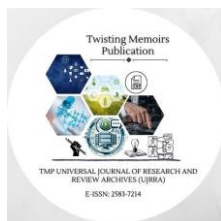
Table No.8 Simultaneous Estimation Method for Omeprazole

CONCLUSION

A simple, rapid accurate and precise UV Spectrophotometric method have been developed and validated for routine analysis of naproxen and omeprazole the method is suitable for simultaneous determination of naproxen and omeprazole in multi component formulation without interference of each other. The value of standard deviation and coefficient of variation calculated were satisfactorily low indicating the suitability of the propose method for the routine estimation of combined dosage forms. So, it can conclude that they can be successfully apply for the simultaneous estimation of naproxen and omeprazole.

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