ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24JUL1770

Submitted to
Faculty of Pharmacy
Palamuru University

Analytical Method Development and Validation for Butylated Hydroxy Toluene by using UV- Visible Spectrophotometric Method

A Dissertation

Under the Guidance of

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In the partial fulfillment of the requirements for the award of the degree of

Bachelor of Pharmacy

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ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24JUL1770

Estd : 1998

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CERTIFICATE

This is to certify that the investigations described in this dissertation entitled "ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR BUTYLATED HYDROXYTOLUENE BY USING UV-VISIBLE SPECTROPHOTOMETRIC METHOD" Presented to Palamuru University to Partially fulfill the requirements for the degree of Bachelor of Pharmacy ALEEMUNISSA BEGUM, FABIHA FATHIMA, GOLLA BHAVAGNA, N.SHINU PRIYA, SENAPATHI HARSHAVARDHAN under my guidance and supervision.

The results embodied in this dissertation has not been submitted to any other University or Institute for the award of any degree or diploma.

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DECLARATION

We hereby declare that this dissertation entitled **"ANALYTICAL METHOD**

DEVELOPMENT AND VALIDATION FOR BUTYLATED HYDROXY TOLUENE BY UV-VISIBLE SPECTROPHOTOMETRIC METHOD" is based on the original work carried out by us in Smt. Sarojini Ramulamma College of Pharmacy, Mahabubnagar, under the guidance of **MR. YAKUB PASHA** M.Pharm, (Ph. D)., Assistant professor, Department of Pharmaceutical Analysis for submission to Palamuru University in partial fulfillment for the award of degree of Bachelor of Pharmacy.

The work is original and has not been submitted for any degree of this to any other University.

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ACKNOWLEDGEMENT

In forwarding this thesis entitled **"ANALYTICAL METHOD**

DEVELOPMENT AND VALIDATION FOR BUTYLATED HYDROXYTOLUENE BY USING THE UV- VISIBLE SPECTROPHOTOMETRIC METHOD" I would like to express my sincere thanks to my guide **Mr. YAKUB PASHA** M.Pharm, Ph. D, Assistant Professor, Smt. Sarojini Ramulamma College of Pharmacy, Sheshadri Nagar, Mahabubnagar.

I extended my sincere thanks to our H.O.D, **M BHASKAR** and, I pay my sincere gratitude to our principal **Dr. T. MANGILAL.**

My deep sense of gratitude to our Chairman **Sri. Dr. PANUGANTI**

BALRAJ, and academic director Smt. Sarojini Ramulamma College of Pharmacy.

I must have to recourse it, and express my deep depth of gratitude with honorable regards, to our esteemed teacher and guide, **Mr. YAKUB PASHA,** philosophical suggestions made us possible to submitmy dissertation.

I gratified to all of our **CLASSMATES** for their heart full cooperation and timely help in submission my thesis.

I extend my thanks to our teaching staff and non-teaching staff, lab technicians, lab attenders, and all counter part of our college, who directly and indirectly helped us to bring out this dissertation.

Last but not least, we express our deep sense of gratitude to my **PARENTS** for their co-operation offered in reaching everymilestone of our life.

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ABSTRACT

Upon utilizing the Prediction Activity Spectra of Substances (PASS) program, computer-aided predictions were conducted to assess the antioxidant activities. Compounds 1, 3, 4, and 5 underwent evaluation using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and lipid peroxidation assays to confirm the predictions obtained from the PASS program. Notably, compounds 3 and 5 exhibited higher inhibition of the DPPH stable free radical at 10 M compared to the widely recognized standard antioxidant, butylated hydroxytoluene (BHT). Of particular interest, compound 5 demonstrated significant in vitro inhibition of Fe-induced lipid peroxidation in the lipid-rich medium of essential egg yolk (83.99%, IC50 16.07 ± 3.51 µM/mL) in contrast to α-tocopherol (α-TOH, 84.6%, IC50 5.6 ± 1.09 µM/mL). Furthermore, the drug-likeness of these BHT analogues was assessed based on Lipinski's "rule-of-five" (RO5), revealing a violation of one parameter (Log P > 5) despite their solubility in protic solvents. The predictive polar surface area (PSA) and absorption percentage (% ABS) data imply a potential capacity for cell membrane penetration. Consequently, these new multipotent antioxidants (MPAOs) hold promise as effective agents for addressing oxidative stress and lipid peroxidation processes.

Keywords:- *Butylated Hydroxy Toluene, Drug-Likeness Properties, DPPH, Lipid Peroxidation, Multipotent Antioxidant, Rule-of-Five, PASS and Activity Prediction, Thiosemicarbazide, 1,2,4-Triazole, 1,3,4-Thiadiazole.*

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24JUL1770

ABBREVIATIONS

API : Active pharmaceutical ingredient

FIG : Figure

FDA : Food and Drug administration

HPLC : High performance liquid chromatography

HPTLC : High performance thin layer chromatography

UPLC : Ultra performance liquid chromatography

UV : Ultra visible

ICH : International conference on Harmonization

IUPAC : International union of pure and applied chemistry

LC : Liquid chromatography

LOD : Limit of detection

LOQ : Limit of quantitation

LC - MS : Liquid chromatographic - Mass spectrometer

Mg : Milligram

Nm : Nanometers

NMT : Not more than

NLT : Not less than

NO : Number

Ppm : Parts per million

p.s.i : Pounds per square inch

PKa : Dissociation constant

PH : Negative logarithm of hydrogen ion concentration

Sd : Standard derivation

USP : United states of Pharmacopoeia

Vis : Visible

RSD : Percentage relative Standard Deviation

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cGMP : Current Good Laboratory practices

SB : Standard derivation slope

ARB : Angiotensin Receptor blocker

BP : Blood Pressure

CKD : Chronic kidney Diseases

CV : Cardio Vascular

IV : Intra venous

ARB : Angiotensin converting Enzyme

BPS : Bromo Phenol Blue

BOG : Bromo Cresol Green

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24JUL1770

CHAPTER ONE
INTRODUCTION

➢ *UV-Vis Spectrometry*

The study of the interaction between matter and various types of radiation, particularly electromagnetic radiation across the electromagnetic spectrum, is at the heart of spectroscopy. Spectroscopic methods encompass a variety of analytical techniques deeply rooted in atomic and molecular spectroscopy. Spectrometry and its methods involve the measurement of radiation intensity using electronic devices such as photoelectric transducers.

UV-Vis spectrometry plays a crucial role in instrumental analysis, serving as the foundation for various methods used to determine minute quantities of analytes in a sample. The technique involves observing the effects of electromagnetic radiation in the UV and/or visible range on absorbing species such as atoms, molecules, or ions.

➢ *Origin of UV-Visible Spectrum*

The UV-Vis spectrum is the result of electromagnetic radiation interacting with molecules, ions, or complexes in the UV-visible region. This analysis method serves as the foundation for studying various substances, including inorganic, organic, and biomolecules. The insights gained from these determinations find applications in diverse fields such as research, industry, clinical laboratories, and environmental sample analysis. Therefore, it is crucial to comprehend the origin and characteristics of the UV-Vis spectrum.

➢ *Radiation and Energy*

Radiation is a type of energy that is transmitted. It is called electromagnetic radiation because it has electric and magnetic fields that oscillate at the same time in planes that are mutually perpendicular and in the direction of propagation through space. Electromagnetic radiation has a dual nature: it shows properties of waves and particles.

➢ *The Nature of Light*

Light, being a form of energy, can travel from one location to another through either particle or wave motion. Multiple theories have been proposed to explain the nature of light, with wave and particle theories being key contenders.

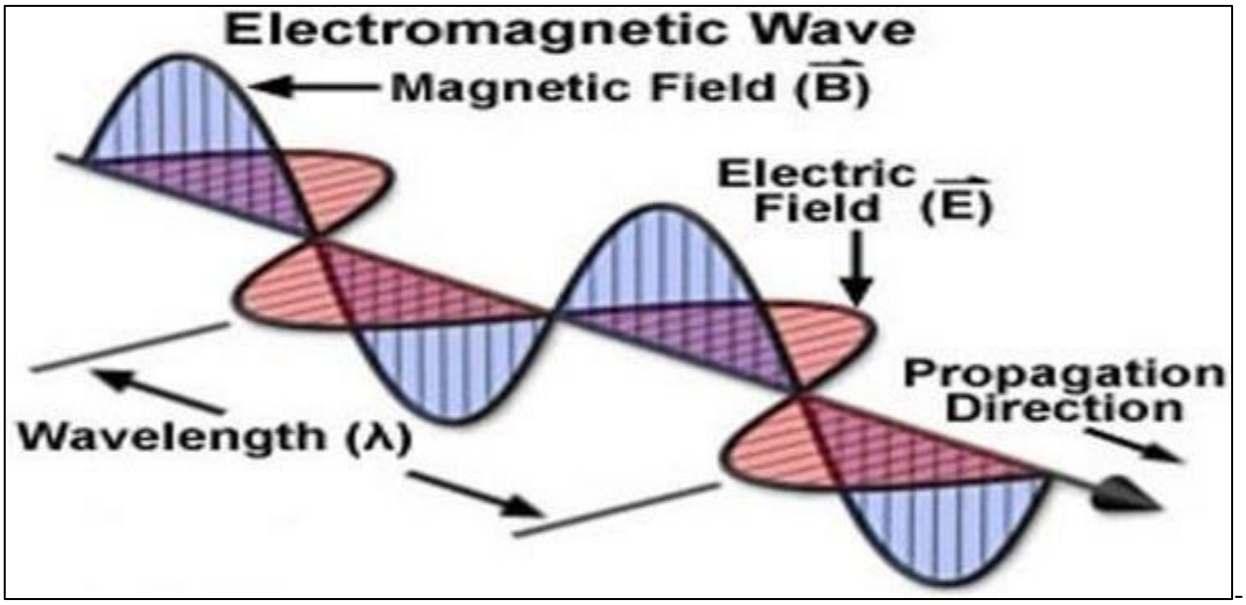

Fig 1 Figure showing Electromagnetic Wave

- *Wavelength (λ) [lambda]:*

The term "wavelength," represented by the symbol λ (lambda), indicates the distance between two consecutive peaks or troughs in a wave, signifying the spacing between any two adjacent identical points in a wave.

✓ *Units of Wavelength:*

- Angstrom (A): 1 A = 1x10^-10 m
- Nanometer (nm): 1 nm = 1x10^-9 m
- Micrometer (μm): 1 μm = 1x10^-6 m

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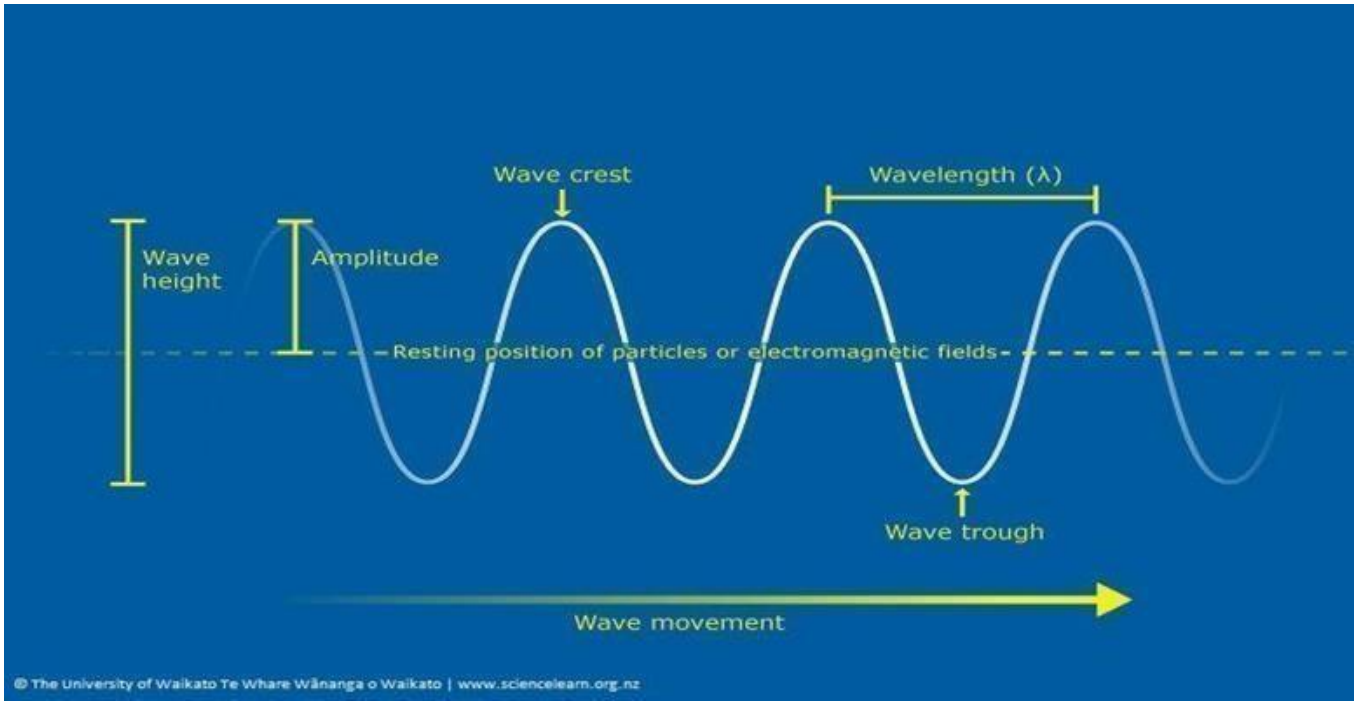

Fig 2 Figure Showing Wavelength

- *Frequency (f or ν [nu]):*

✓ *Frequency (f or [nu]):*

The concept of frequency refers to the number of oscillations occurring in a single second. It measures the complete cycles per second in the context of alternating current direction. The hertz (Hz) is the standard unit of frequency.

✓ ***"Frequency units:"***

- Hertz (Hz): Equal to 1 cycle/s.
- Fresnel: Equal to 10 - 12 cycles/s.

- *Wavenumber(ύ):*

The term "wavenumber (ύ)" represents the count of wavelengths that cross a particular point within a given time unit, usually per second. Alternatively, it can be viewed as the reciprocal of the wavelength, and wavenumber units are commonly denoted in cm-1**.**

- *Particle Properties:*

When considering the interaction of electromagnetic radiation with matter, it is helpful to envision the radiation beam as a stream of photons, where the energy of each photon is directly linked to the frequency of the radiation through specific relationships.

Where, **C =** velocity = 3.0 x 10 8 m/s in a vacuum

C remains unaffected by the presence or absence of matter in a vacuum.

E = h

When we talk about the energy of radiation, denoted as E, it is directly related to the frequency, denoted as v. The symbol h represents Planck's constant, which has a value of 6.626 x 10 -34 J s. We can express the relationship between these variables with the equation E = hν or E = hc/λ, where λ is the wavelength. This tells us that the energy is inversely proportional to the wavelength. In simpler terms, radiation with higher frequency carries more energy. Hence, a photon with a high frequency (short wavelength) contains more energy than one with lower frequency (longer wavelength).

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➢ *"The Characteristics of Electromagnetic Waves and their Different Spectral Bands."*

The electromagnetic spectrum covers a broad array of wavelengths and frequencies, indicating different energy levels from high-energy gamma rays to low-energy radio waves. This extensive range of radiation is commonly referred to as the electromagnetic spectrum**.** The spectrum is divided into major spectral regions, as illustrated in the figure below. These divisions are based on the specific methods required to generate and detect different types of radiations.

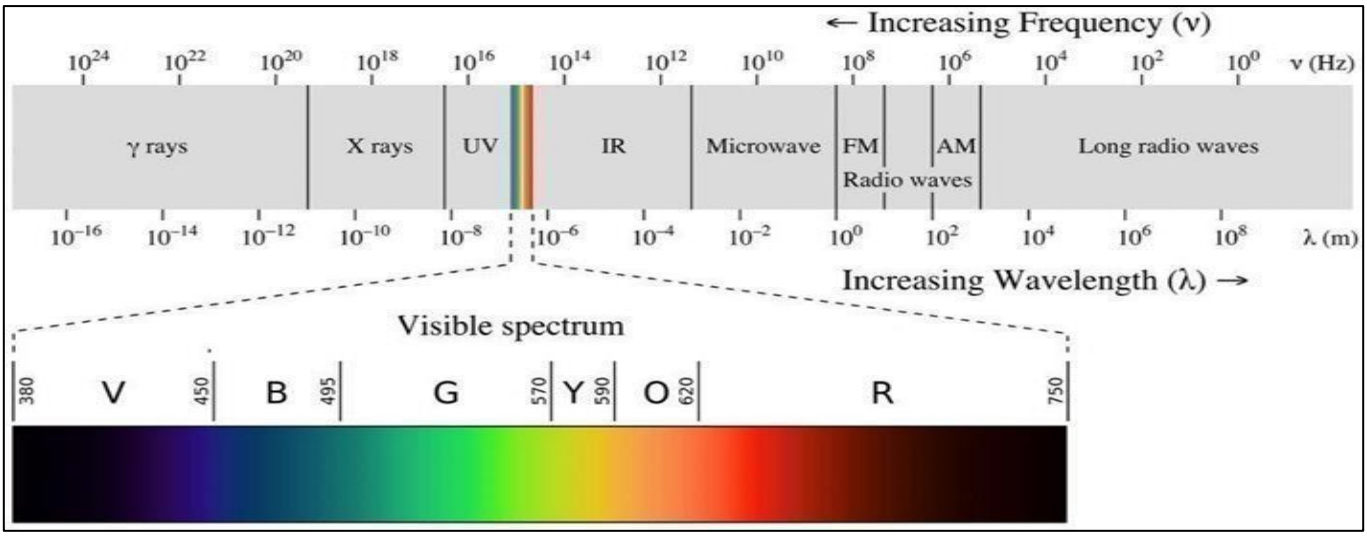

Fig 3 Figure showing Visible spectrum

Table 1 Different Types of Spectro Scopy

Type of spectroscopy	Type of transitions	Wavelength range
Gamma rays	Nuclear	(10-10-10-14)m
X rays	Inner k and L-shell Electrons	(10^{-9})-(6×10^{-12})m
Ultraviolet rays	Valence and middle shell electron	(3.8×10^{-7}) - (6×10^{10}) m
Visible	Valence electrons	$(7.8\text{-}3.8)\times10^{-7}$
Infrared	Molecular vibrations and rotations	(10^{-3})-(7.8×10^{-7})m
Microwave	Molecular rotations	0.3m-1mm
Radio waves		Few km-0.3m

➢ *Its Transitions and Wavelength Range:*

The field of spectroscopic methods encompasses a broad spectrum of electromagnetic radiation, ranging from visible light and gamma rays to x-rays, ultraviolet, infrared, microwave, and radiofrequency radiation. It's important to clarify that despite being referred to as optical methods, the human eye can only perceive visible light and is not sensitive to the other types of radiation mentioned. This naming convention may stem from similarities in how these different types of radiation interact with matter.

When electromagnetic radiation interacts with matter, it can cause either absorption or emission of energy. Absorption happens when radiant energy is transferred to matter, while emission involves the conversion of internal energy of matter into radiant energy. When particles in an excited state go through the emission process, they can release photons of specific energies as they transition back to lower energy states or ground states. Furthermore, when radiation interacts with matter, it may be absorbed, scattered, reflected, or re-emitted at the same or different wavelengths upon exiting the sample. Additionally, the passage of radiation through matter may cause changes in orientation or polarization.

- *Absorption of radiation:*

The process of radiation absorption involves the transfer of energy from the radiation to the atoms or molecules of the medium. This results in increased internal energy as well as increased rotational and vibrational energy of the absorbing medium. Although the absorption of radiation does not directly increase translational energy, it can indirectly do so through processes such as the conversion of rotational or vibrational energy or by the degradation of electronic energy due to intermolecular collisions.

Furthermore, atomic absorption spectra are produced when atoms absorb electromagnetic radiation. These spectra are utilized to study atomic reactions that necessitate the activation of an atom. The movement of an electron from a lower energy state to a higher one happens when it absorbs photons with a precise amount of energy. It's worth noting that the energy absorbed must match the gap between the two energy levels.

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- *Molecular Absorption:*

The absorption of molecules presents a significantly more intricate spectrum compared to atomic absorption, mainly due to the extensive range of potential energy states involved. In a molecular absorption spectrum, the energy is composed of three distinct energy components.

When we delve into the absorption spectrum of a material, we are essentially looking at how the material absorbs radiation across different frequencies, and this is heavily influenced by the material's atomic and molecular makeup. Typically, the material absorbs radiation at specific frequencies that correspond to the energy difference between two quantum mechanical states of the molecules. This absorption between two states is referred to as an absorption line, and a spectrum usually consists of several of these absorption lines.

- *Absorption:*

In UV-Vis absorption spectroscopy, energy is exchanged and transferred between the radiation field and an absorber like an atom, molecule, or solid due to energy level transitions. This process can lead to emission, where energy is transferred from a higher energy level back to the radiation field. Conversely, if no radiation is emitted, the transition from higher to lower energy levels is termed nonradioactive decay. The spectroscopy analysis produces what is referred to as a spectrum, which visually displays the energy intensity detected against the wavelength (or mass, momentum, frequency, etc.) of the energy.

UV-Vis spectroscopy is focused on examining how the intensity of a light beam changes as it travels through a sample or is reflected from its surface. Typically, the visible spectrum ranges from approximately 400 nm to 800 nm. Our perception of color relies on the wavelength of light, and the color of a substance is determined by the light it absorbs and the light it reflects or transmits, leading to the creation of complementary colors.

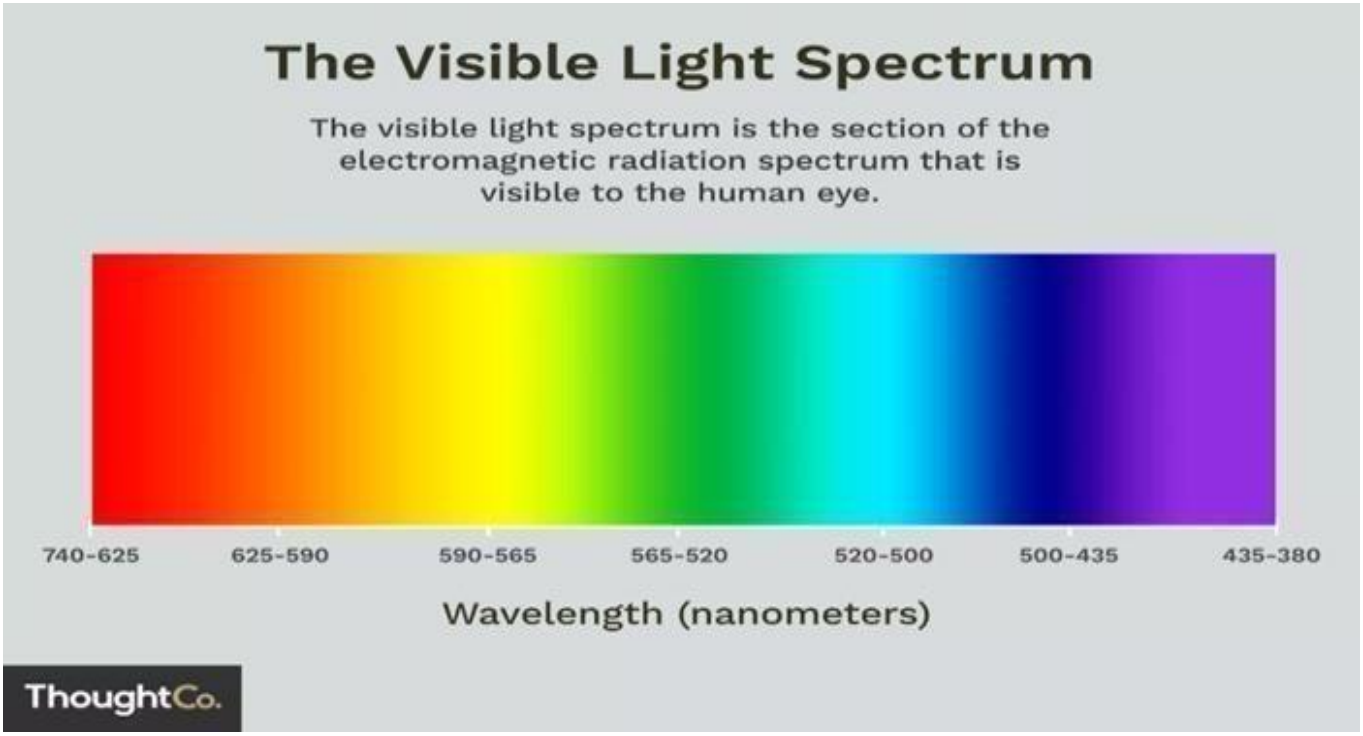

Fig 4 The Visible Light Spectrum

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24JUL1770

Fig 5The Wavelengths & Absorbed Color

The hue of a substance plays a crucial role in its properties. It is connected to how much light it absorbs or reflects. When it comes to what we see, our eyes perceive the color that is opposite to the one absorbed.

➢ *Instruments:*

- *UV Visible Spectrophotometer*

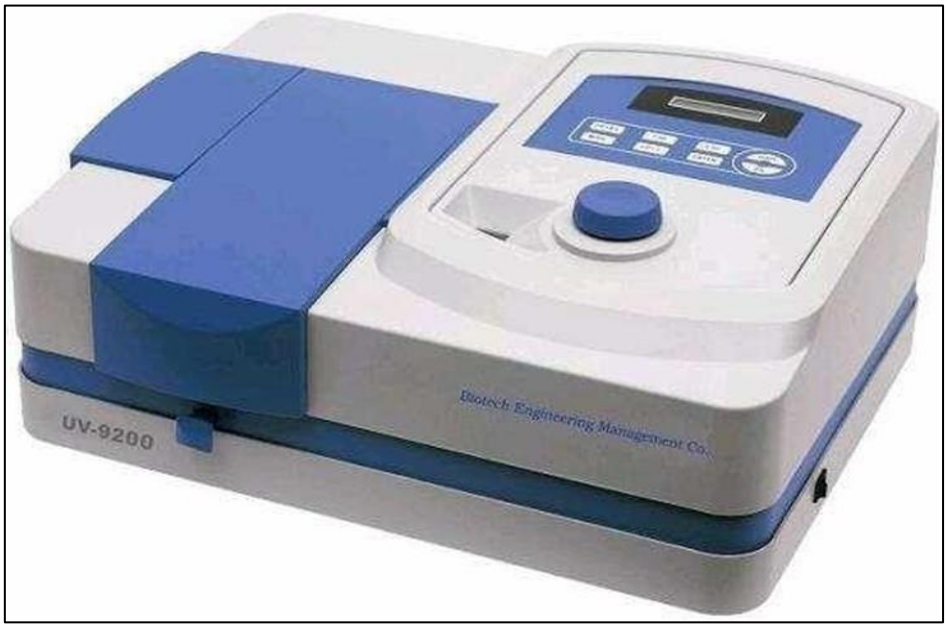

Fig 6 UV Visible Spectrophotometer

- *Instrumentation for UV Spectroscopy:*

✓ *The Equipment Comprises the Following Components:*

- Radiation source
- Monochromators
- Sample cells
- Detector
- Recorder (or) display.

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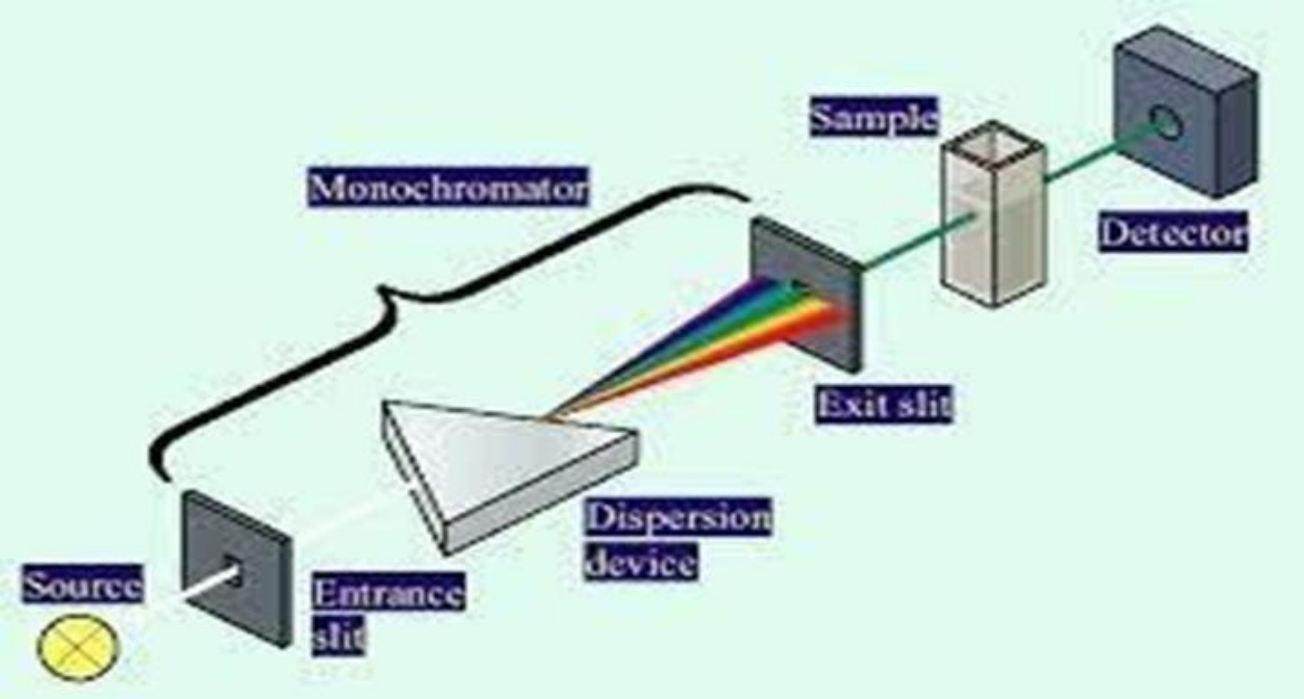

Fig 7 Instrumentation of UV – Vis Spectroscopy

- *Radiation Sources*

✓ *The Tungsten-Halogen Lamp ;*

Emits wavelengths from the red end of the visible spectrum (750-800 nm) to the near ultraviolet spectrum (300320 nm). It is designed with a quartz outer sheath to facilitate the use of the ultraviolet part of the spectrum.

On the other hand, the Hydrogen (or) deuterium lamp is primarily utilized for measuring far ultraviolet wavelengths (down to 200 nm). It comprises of two electrodes immersed in a deuterium-filled silica envelope.

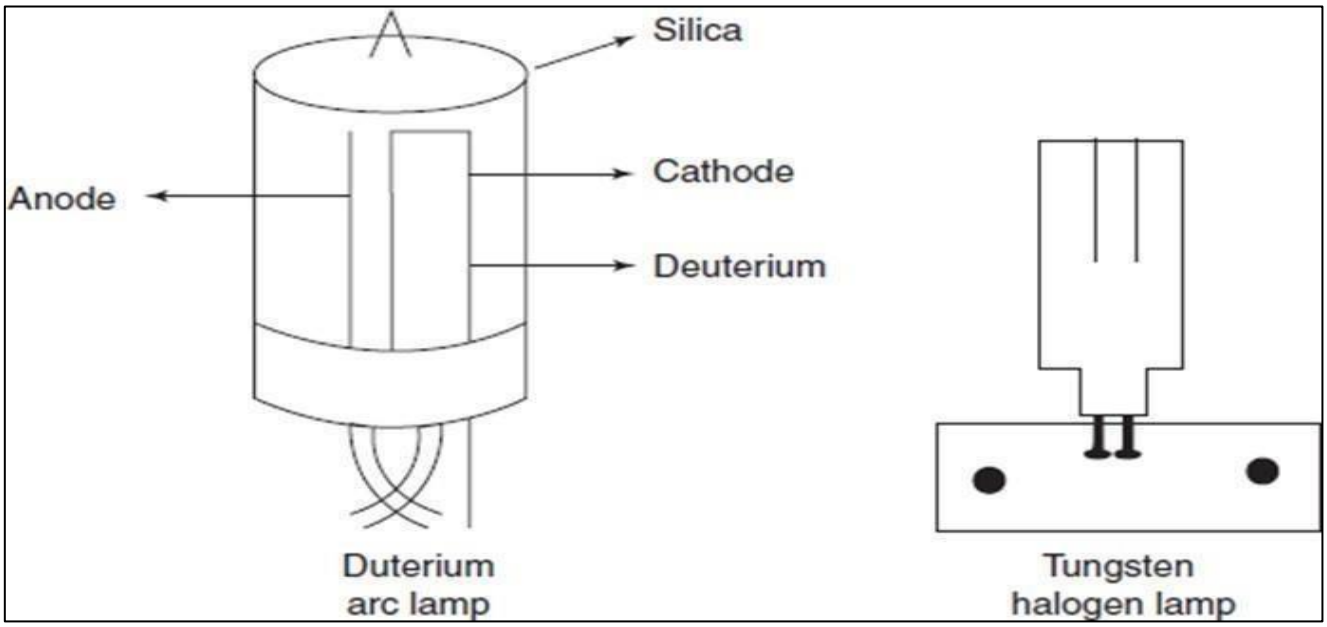

Fig 8 Deuterium Lamp, Tungsten Halogen Lamp

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- *Monochromator*

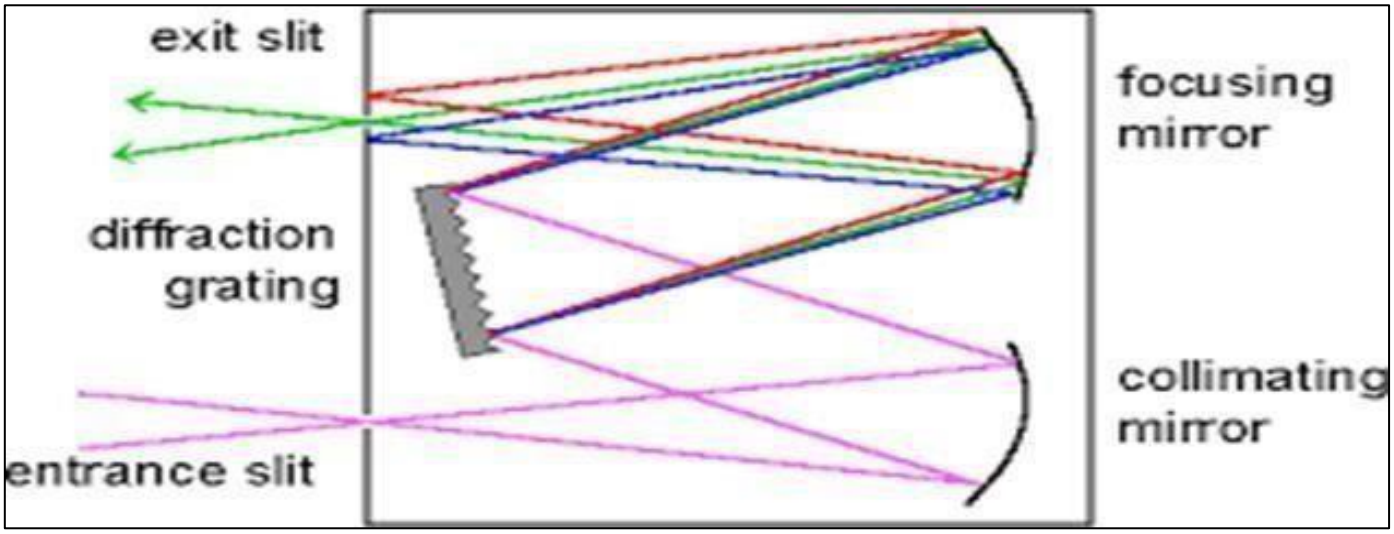

Fig 9 Monochromator Components

Two primary methods of wavelength selection can be observed: filters and a dispersing system (such as a prism or diffraction grating).

✓ *Regarding Filters:*

Color glass or gelatin filters represent the simplest form of selection, but their usefulness is quite limited because they are confined to the visible region and they possess wide spectral bandwidths. Typically, their bandwidths are rarely better than 30-40nm. Interference filters, which consist of a substrate (commonly glass, but may also be silica) with materials of different refractive indices deposited on it, can be created with band widths of approximately 10 nm or less. However, the relatively wide bandwidth and therefore limited resolution of filters, along with their inability to provide a continuous spectrum (except in special form such as wedge filters), make them unsuitable for routine laboratory spectrophotometry despite their low cost and technical simplicity.

✓ *In terms of Prisms:*

A prism made of suitable material and geometry will yield a continuous spectrum with the component wavelengths separated in space. It is customary to enhance the definition of the light between the source and the prism by using an entrance slit (to define the incident beam) and a collimator (to produce a parallel beam at the prism). Following dispersion, the spectrum is focused at the exit slit, which can be scanned across the beam to isolate the required wavelength. In practice, the prism is usually rotated to cause the spectrum to move across the exit slit.

- *Sample Cells*

The material presented for spectrophotometric analysis could exist in solid, liquid, or gaseous states. Ideally, the material containing the sample should be transparent at the wavelength of measurement to ensure accurate analysis.

Figure 10 illustrates sample cells used for the analysis of liquids and gases in the UV/visible region. For measurements above 320 nm, cells constructed with optically flat fused glass are suitable, while measurements below 320 nm require the use of more expensive fused silica cells, which are transparent to wavelengths below 180 nm. Standard path lengths of cells typically range from 10 nm, with 50 nm cells available for specialized applications.

- *Detectors*

In terms of detectors, photomultiplier tubes are commonly used in UV/visible spectrophotometers. These detectors employ the multiplication of initial photoelectrons by secondary emission to achieve greater sensitivity to very weak light intensities. Multiple anodes at increasing potential are utilized in one bulb for this purpose.

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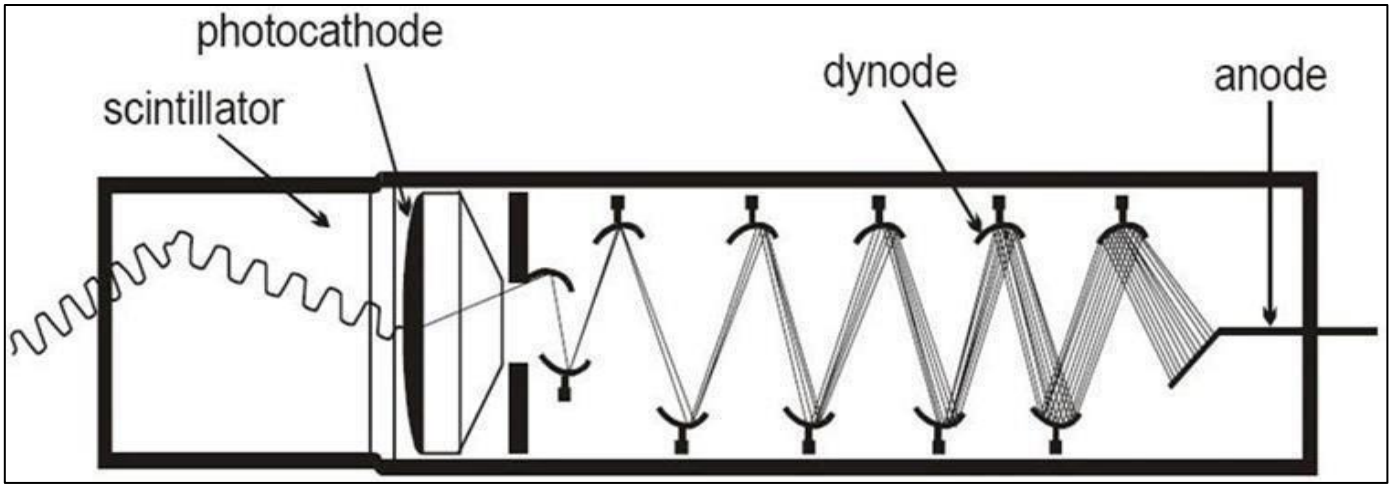

Fig 10 Mechanism of Detectors

When electrons are emitted from the photocathode, they are initially attracted to anode 1. At this point, more electrons are liberated, and they travel to anode 2 and subsequently to the final anode, resulting in a final current that is 106-108 times greater than the primary current.

In terms of recorders, the primary function of a spectrophotometer culminates in providing a signal, usually an electrical voltage, that is directly proportional to the absorption by a sample at a given wavelength. Depending on the application, the signal handling and measuring systems can range from a simple setup involving just an amplifier and a meter to a more complex arrangement involving a personal computer and printer. A meter can serve the purpose of indicating the absolute value of the output signal or, in some cases, the null point in a back-off circuit. Digital readouts, especially LED or LCD displays, are preferred for their clarity and lack of ambiguity. They may be linked to a microprocessor so that the readout is presented in preferred terms, such as directly in concentration units. Additionally, chart recorders or similar devices can be utilized with instruments equipped with wavelength scanning systems to directly provide an absorption spectrum. These recorders are also beneficial in studying reaction rates, especially when there is a need to plot absorption against time at a fixed wavelength.

When it comes to UV-visible spectrometers, single-beam spectrometers are just what they sound like - they work with a single beam of light. This same beam is used to both measure the absorption of the sample and obtain the reference. The light from the source goes through a filter or a monochromator to create a band or monochromatic light, which then passes through the sample or the reference. A photo detector detects the transmitted light, and the resulting signal is recorded as a readout.

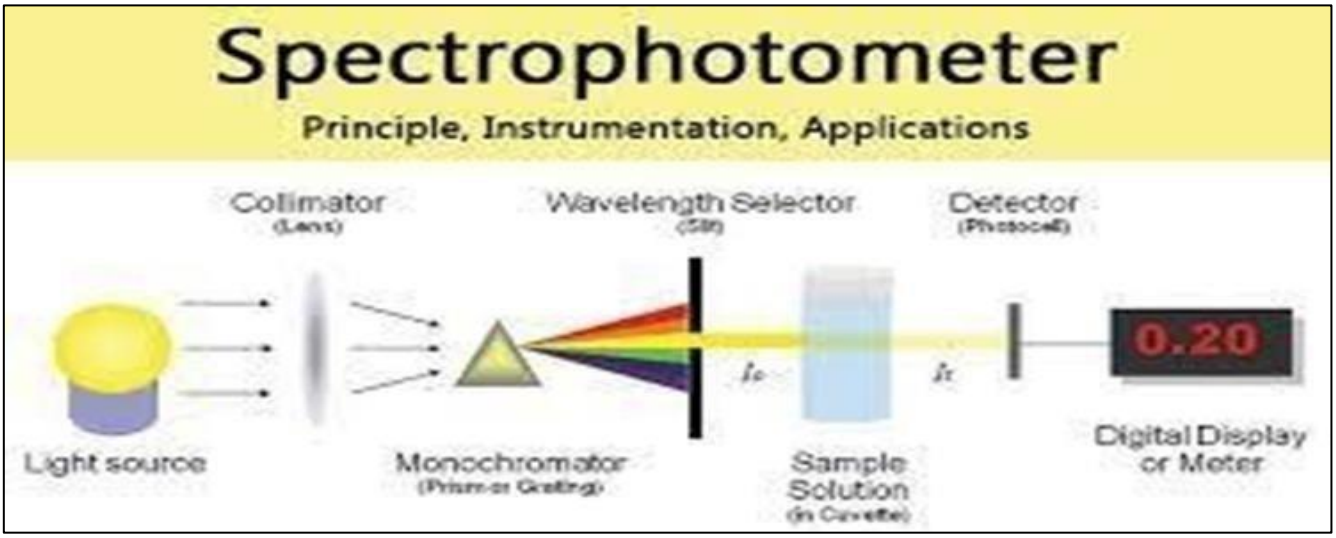

Fig 11 Single Beam Spectrometer

➢ *Double Beam Spectrometer*

In a double beam spectrometer, the radiation from the monochromator is split into two beams using a beam splitter. These beams are then simultaneously passed through the reference sample and the sample cell. The transmitted radiations are detected by the detectors, and the difference in the signal at the wavelengths is appropriately amplified and processed for the final output.

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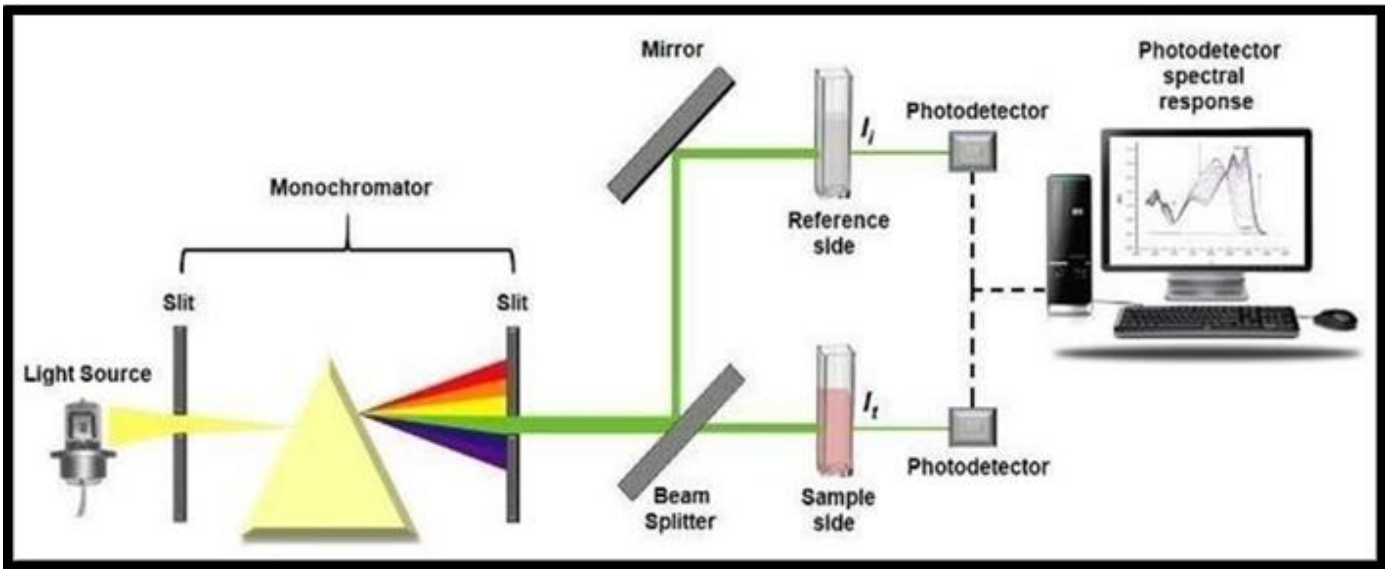

Fig 12 Double Beam Spectrometer

➢ *Selection Of Solvents*

When using UV/visible spectrophotometry, the choice of solvents is crucial. The selected solvents must meet the following criteria:

- They should not interfere with the solute.
- They should not exhibit significant absorption.

Thank you for considering this information.

Table 2 Example of Solvents

SOLVENT	^MAX (nm)
Water	190nm
Hexane	199nm
Ethanol	207nm
Methanol	210nm
Cyclohexane	212nm
Chloroform	247nm
Carbon tetra chloride	257nm
Benzene	280nm

➢ *Applications:*

- **I**n the investigation of the Absorption curve and concentration of a substance, concentration was employed. For instance, KNO3 determination was one of the applications.

- Additionally, it was utilized to examine the impact of substituents on the absorption spectrum. An example is the comparison of the absorption spectrum of benzoic acid with that of 4-hydroxyl benzoic acid and 4-aminobenzoic acid.
- Moreover, it was used for simultaneous spectrophotometric determinations, such as the simultaneous determination of manganese and chromium in steel and other ferro-alloys.
- Furthermore, it played a role in determining molar absorption coefficients.
- In the analysis of Binary mixtures, for instance, the Benzene-toluene mixture Binary analysis was conducted using this approach. It was also used in the determination of phenols in water.
- Furthermore, it aided in the determination of the constituents in a medical preparation through derivative spectroscopy. An example is the determination of pseudoephedrine and tripolidine in a medicinal preparation.
- Lastly, it was used in the determination of keto-enol tautomerism**.**

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CHAPTER TWO
LITERATURE REVIEW

- *Musakhanian, J., Rodier, J-D., and Dave*

M. conducted a review on the oxidative stability in lipid formulations. The article published in AAPS Pharm SciTech in 2022 delves into the mechanisms, drivers, and inhibitors of oxidation. The DOI is 10.1208/s12249-022- 02282-0.

- *Ayres, L. et al.'s*

research focuses on predicting antioxidant synergism using artificial intelligence and benchtop data. Their work was published in the Journal of Agricultural and Food Chemistry in 2023; the DOI is 10.1021/acs.jafc.3c05462.

- *Li, J., Chen, J., Bi, Y., and Yang, H.*

provide insight into synergistic antioxidation mechanisms of butyl hydroxy toluene with common synthetic antioxidants. Their article was published in Grain Oil Science and Technology in 2022, with a DOI of 10.1016/j.gaost.2022.06.004.

- *Bampidis, V., et al.*

discuss the safety and efficacy of a feed additive consisting of butylated hydroxy toluene (BHT) for all animal species in the EFSA Journal in 2022. The DOI for this publication is 10.2903/j.efsa.2022.7287.

- *Rychen, G., et al.*

assess the safety and efficacy of butylated hydroxy toluene (BHT) as a feed additive for all animal species. Their findings were published in the EFSA Journal in 2018, with a DOI of 10.2903/j.efsa.2018.5215.

- *VAkkbik, M., Assim, Z., & Ahmad, F. (2011)*

Optimization and validation of RP-HPLC-UV/Vis method for determination of phenolic compounds in various personal care products. International Journal of Analytical Chemistry, 2011(1). https://doi.org/10.1155/2011/858153

- *Galimany-Rovira, F., et al.*

"Development and validation of a new RP-HPLC method for simultaneous determination of hydroquinone, kojic acid, octinoxate, avobenzone, BHA and BHT in skin-whitening cream." In Anal. Methods, 2016, pp. 1170-1180. DOI: 10.1039/C5AY02207J. [CrossRef] [Google Scholar].

- *Hadjmohammadi, M.R., et al. "*

"Utilizing experimental design to extract BHA and BHT from edible vegetable oil and analyzing them using HPLC" was published in QScience Connect in 2012. The DOI for this publication is 10.5339/connect.2012.7. You can find more information about this research on CrossRef or Google Scholar.

- *9Yıldız, E., and Çabuk, H.*

"Determination of the synthetic antioxidants butylated hydroxy toluene (BHT) and butylated hydroxy toluene (BHT) by matrix acidity-induced switchable hydrophilicity solvent-based homogeneous liquid-liquid microextraction (MAI-SHS-HLLME) and high-performance liquid chromatography with ultraviolet detection (HPLC-UV)." In Anal. Lett., 2022, pp. 480-494..

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24JUL1770

CHAPTER THREE
DRUG PROFILE

DRUG NAME : Butylated Hydroxy Toluene.

STRUCTURE :

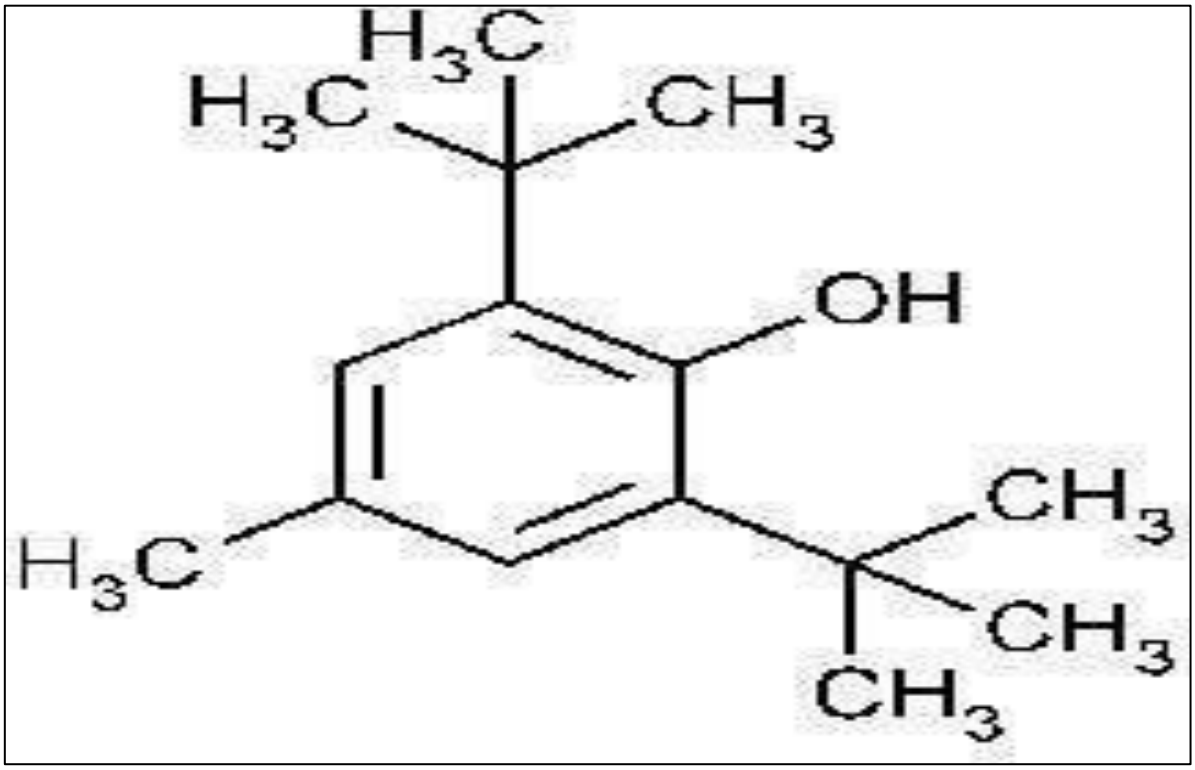

Fig 13 Structure of Butylated Hydroxy Toluene

➢ *Product Information:*

- *IUPAC Name: 2,6-bis(1,1-dimethylethyl)-4-methylphenol*
- *Molecular Formula: C15H24O*
- *Molecular Weight: 220.35g/mol*
- *Category: Antioxidants*
- *Solubility: It is soluble in organic solvents such as ethanol, methanol, and acetone, but only sparingly soluble in water.*
- *Absorbance: Falls within the range of 270 to 290 nm.*

➢ *Mechanism of Action:*

- *Free Radical Scarenging :*

BHT functions as an antioxidant by scavenging free radicals, which are highly reactive molecules with unpaired electrons. By donating hydrogen atoms to these radicals, it stabilizes them and prevents them from causing oxidative damage to other molecules.

- *Chain -breaking Antioxidant*

Additionally, BHT interrupts the chain reaction of lipid oxidation by reacting with lipid radicals, breaking the chain of free radical propagation.

- *Hydrogen Atom Donation*

The phenolic hydroxyl groups in BHT easily donate hydrogen atoms to free radicals, forming stable radicals themselves, which are less reactive and do not cause further damage to other molecules.

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- *Pharmacology :*

Upon absorption, BHT is swiftly distributed to the liver and body fat. The primary excretion route is through urine, with a smaller amount being excreted in the feces. It's worth noting that there are notable differences in BHT metabolism among different species. Contrary to rats, rabbits, and dogs, biliary excretion of BHT seems to play a less significant role in humans.

- *Pharmacodynamics :*

Butylated hydroxytoluene (BHT) is a lipophilic organic molecule widely employed in the food industry as a food additive due to its antioxidant properties. Its fat-soluble nature makes it an effective food preservative by reducing the autoxidation rate of various food components, consequently preventing changes in taste and color. Beyond its traditional use in food, BHT now finds applications in cosmetics, petroleum transformer oil, pharmaceuticals, rubber, and jet fuel.

BHT's antioxidant properties are harnessed to inhibit peroxide generation in edible products and exhibit antimicrobial activity.

- *Side Effects :*

The reported side effects of BHT include allergic reactions, endocrine disruption, liver toxicity, carcinogenicity, and the potential for accumulation. Furthermore, it has been observed to impact testosterone levels, affect sperm quality, lead to liver enlargements, cause inflammatory effects in the lungs, induce renal dysfunction, and decrease potassium levels.

- *Uses:*

This versatile compound is used for its antioxidant and antimicrobial properties, serving as a stabilizer in the pharmaceutical industry, as a deterrent for pests and insects, for treating cold sores, preserving skincare products, and as a preservative in the food industry.

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CHAPTER FOUR
AIM AND OBJECTIVE

- *Aim:*

The aim of this study was to create precise and accurate methods for estimating Butylated hydroxy toluene in pharmaceutical dosage forms using UV spectrophotometry. Additionally, we sought to develop an analytical method for Butylated hydroxy toluene using UV spectrophotometry and to validate the analytical method according to ICH guidelines.

- *Objective:*

The primary aim of our current work is to create new, uncomplicated, highly sensitive, precise, and cost-effective analytical techniques for determining Butylated hydroxy toluene. Additionally, we aim to validate these proposed methods in line with ICH guidelines for the specific analytical purpose of analyzing Butylated hydroxy toluene in pharmaceutical dosage forms using UV Spectrophotometry.

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CHAPTER FIVE
PLAN OF WORK

Selection and collection of literature for drugs for analysis.

➢ *Method Development by UV Spectrophotometry Method :*

We will be conducting literature selection and collection for drug analysis, focusing on UV spectrophotometry method development. The proposed plan consists of several key steps:

- Conduct an extensive literature survey to gather information on the physicochemical properties, pharmacological properties, and analytical methods of the drugs, forming the foundation for method development.
- Undertake solubility studies for drug identification, as well as the selection and collection of literature for analysis.
- Choose a suitable solvent for the quantitative extraction of drugs from the formulations, considering factors such as availability, cost-effectiveness, and analytical grade quality.
- Select the appropriate analytical wavelength.
- Establish initial spectrophotometric conditions for the assay of BUTYLATED HYDROXY TOLUENE.
- Perform an assay of pure mixed standards and spectrophotometric formulations.
- Validate the developed UV spectrophotometric analytical method according to the ICH method validation parameters.

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CHAPTER SIX
ANALYTICAL METHOD VALIDATION

➢ *Materials used for the Study:*

Table 3 List of Chemicals used

S. No	Chemicals	Company name
1.	Butylated hydroxy toluene	Gilead sciences
2.	Butylated hydroxy toluene (pure drug)	Micro labs ltd
3.	PH buffer 6.8	Torrent pharmaceuticals UTD
4.	Sodium hydroxide	S. D fine chem ltd
5	HCl	Surya fine chem ltd

Table 4 Equipments and Glass Wares used for the Study

Instruments	Company name
UV-Visible Spectroscopy	Lab India
Ultra sonicate clearance	Nano enterprise
Analytical Balance	Contech
Hot air oven	Dolphin
Standard flask 100ml	Borosil
Standard flask 50 ml	Borosil
Standard flask 25ml	Borosil
Standard flask 10ml	Borosil
Pipette 10 ml	Borosil
Pipette 5 ml	Borosil
Pipette 1ml	Borosil
Beakers	Borosil
Funnel	Borosil

➢ *Analytical method validation:*

Validation involves generating documented evidence to ensure that a particular method consistently yields the desired result or a product meeting its predetermined quality. The validation process encompasses various parameters such as linearity, specificity, selectivity, accuracy, precision, robustness, ruggedness, limit of quantification (LOQ), and limit of detection (LOD) in accordance with the guidelines outlined by the International Conference on Harmonization (ICH)..

➢ *Linearity:*

The sample was analysed using UV-VIS spectrophotometer, using phosphate buffer pH 6.8 as a blank. The linearity of the BUTYLATED HYDROXY TOLUENE was studied using range of dilution of 10, 20,30, 40, and 50 ug/mL. All experiments were performed in triplicate.

➢ *Accuracy:*

The accuracy of the method was determined by preparing solution of different concentrations, that is 50%, 100%, and 150%, in which the amount of marketed formulation was kept constant (30 mg) and the amount of pure drug was varied, that is, 24mg,30mg,36mg for 50, 100, and 150%, respectively. All experiments were performed in triplicate.

➢ *Precision:*

The precision of method was performed by intra-day and inter-day margination studies. In the intra-day variation study, six different solution of same concentration (30 ug/mL) were prepared and analyzed thrice a day (morning, afternoon, evening) and the absorbance were recorded. In inter-day variation study, the six different solution of same concentration (30 ug/mL) were prepared and analyzed thrice, for three consecutive days, and the absorbance were recorded. All experiments were performed in triplicate.

➢ *Specificity:*

Specificity method was determined by preparing 10 mg of BUTYLATED

HYDROXY TOLUENE prepared (10 solution mg), in and and phosphate 150% analyzed (15 buffer mg) for (pH of any excipients. 6.8) change along with All in the 50% absorbance solutions (5 mg), 100%were and percentage drug recovery at respective wavelengths.

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- *Oxidative degradation method:*

In a 10 mL volumetric flask, mix 1.5 mL of SSS with 1 mL of 30% (v/v) hydrogen peroxide and dilute up to the mark with methanol. Set it aside overnight at room temperature. For the blank solution, 1 mL of 30% w/v hydrogen peroxide was kept under normal conditions overnight in a 10 mL volumetric flask. Both solutions were heated for 15 minutes to remove excess hydrogen peroxide and then analyzed using UV.

- *Degradation by UV method:*

A sample of BUTYLATED HYDROXY TOLUENE was placed near a UV source in a stability chamber for 12 hours, ensuring the illumination reached not less than 1.2 million lux hours. From this sample, 10 mg of the drug was dissolved in a 10 mL volumetric flask with HCl and then brought up to volume with buffer solution. The solution was then diluted with methanol to a concentration of 30 ug/ml and analyzed using UV.

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CHAPTER SEVEN
RESULT AND DISCUSSION

➢ *UV Spectrum of Butylated Hydroxy Toluene*

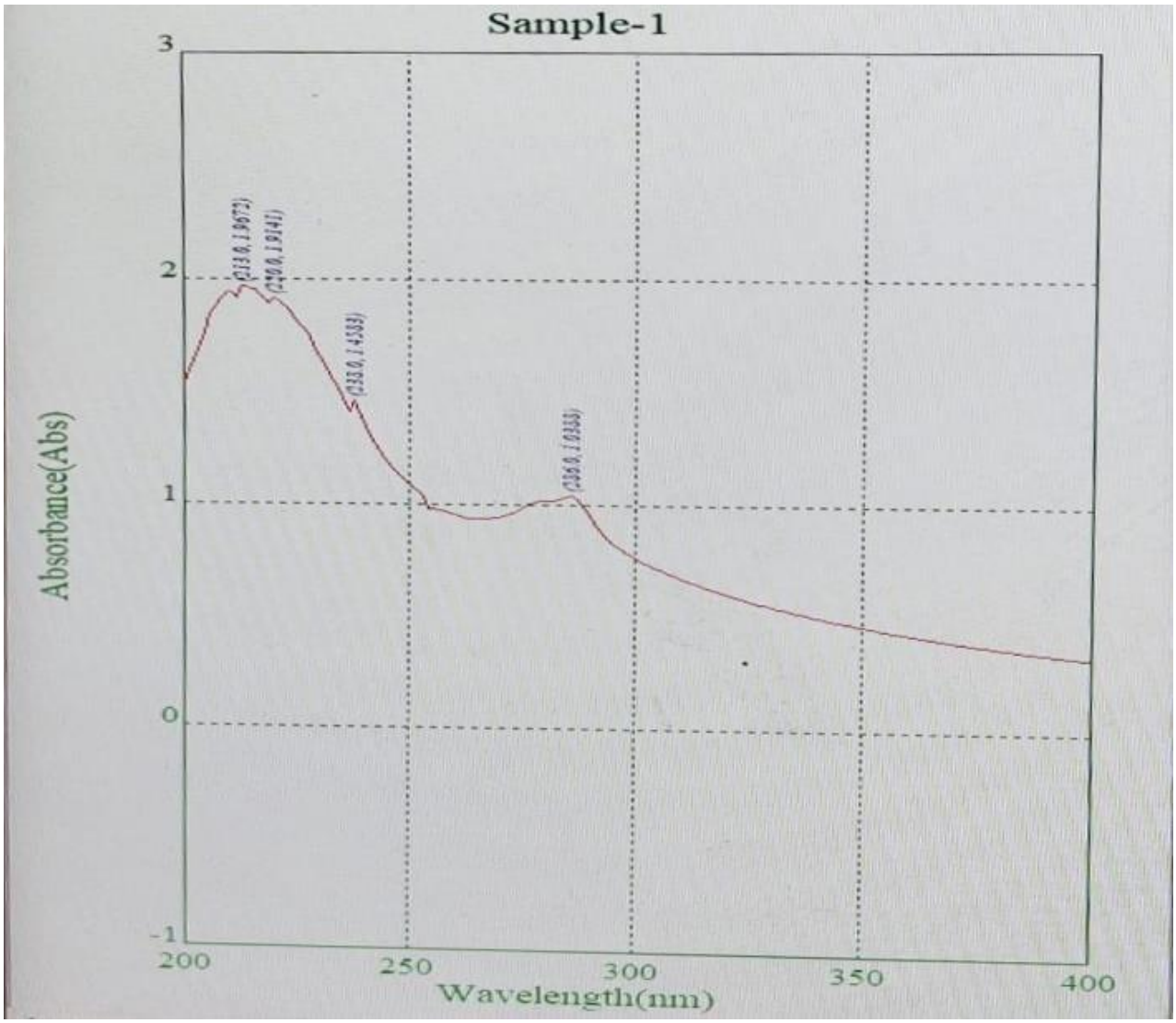

Fig 14 UV Spectrum of Butylated Hydroxy Toluene Standard

- *Calculation:*

% assay of Butylated hydroxy toluene =

$$\frac{AT}{AS} \rightarrow \frac{WS}{WT} \times \frac{DT}{DS} \times \frac{P}{100} \times \text{Avg Wt.}$$

$$\frac{0.412}{0.415} \rightarrow \frac{10}{900} \times \frac{10}{10} \times \frac{99.9}{100} \times \frac{900}{10}$$

0.99 x 99.9 = 99%

The % Purityof Butylated Hydroxy Toluene in Pharmaceutical dosage form was found to be 99%.

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➢ *Linerity and Range :*

Table 5 Absorbance of Various Concentrations of Drug Solution

S.NO	CONCENTRATION (µg/ml)	Absorbance
1	2	0.2538
2	4	0.5939
3	6	0.6406
4	8	0.7780
5	10	0.7478
6	12	0.9809

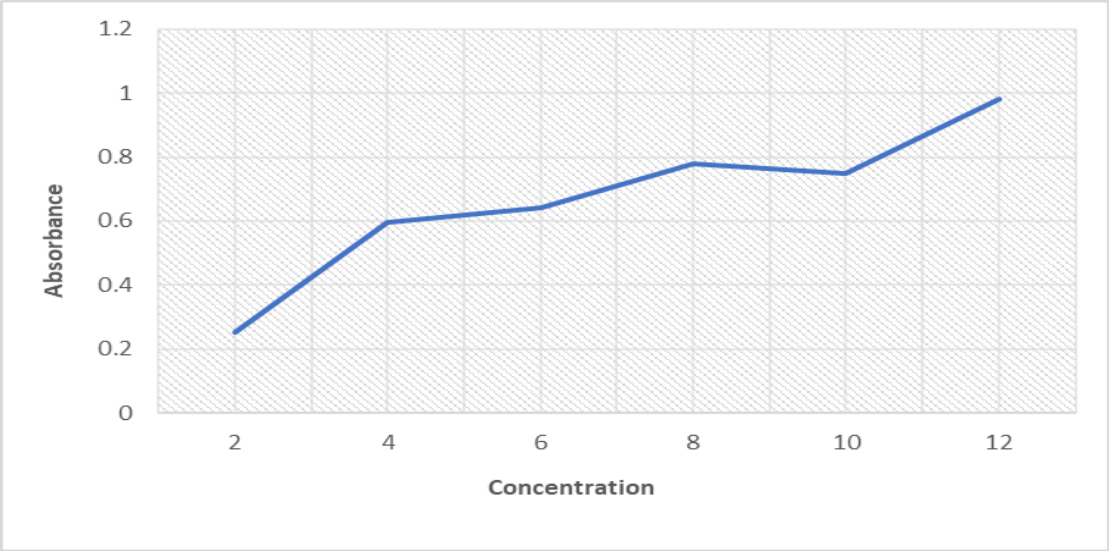

Fig 15 Linearity Graph Showing on Absorbance of Various Concentrations of Drug Solution and its Linearity.

➢ *Linearity Plot :*

- *Linearity Plot Assessment:*

The concentration [x] versus the absorbance [y] plot of Butylated hydroxy toluene demonstrates a linear relationship, as evidenced by the equation Y = mx + c. The correlation coefficient (r) is calculated to be 0.9998, exceeding the required acceptance criteria of not being less than 0.99.

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➢ *Precision:*

Table 6 Intra-Day Precision

Concentration Taken	Absorbance	Concentration Found	% Assay
6	**1.7019**	**0.596**	**59.6**
6	**1.9672**	**0.689**	**68.9**
6	**1.9502**	**0.683**	**68.3**
6	**1.9311**	**0.679**	**67.9**
6	**2.0541**	**0.720**	**72.0**
6	**2.4408**	**0.855**	**85.5**
AVERAGE	**2.00855**	**0.703667**	**10.36667**
SD	**0 .242144**	**0.084866**	**8.486617**
RSD	**12.05567**	**12.06056**	**12.06056**

Table 7 Inter – Day Precision

Concentration Taken	Absorbance	Concentration Found	%Assay
12	2.6894	0.942	94.2
12	2.6806	0.940	94.0
12	2.6806	0.939	93.9
12	2.6185	0.918	91.8
12	2.6699	0.936	93.6
12	2.6031	0.912	91.6
Average	2.657267	0.931167	93.11667
SD	0.036852	0.012813	1.281275
%RSD	1.386821	1.375989	1.375989

The relative standard deviation of the Assay of Butylated hydroxy toluene from 6 sample preparations should not exceed 2.0%. Each individual Assay of Butylated hydroxy toluene should be within the range of 98.0% to 102.0%.

➢ *Method Precision*

- *Analyst to analyst variability*

Table 8 Data Obtained for Analyst 1

S.NO	Concentration (µg/ml)	Absorbance	%Assay
1	**6**	**2.4166**	**114.09**
2	**6**	**2.0386**	**135.83**
3	**6**	**2.2625**	**122.39**
	MEAN	**2.23923**	**124.103**

TABLE 9 Data Obtained for Analyst 2

S.N0	Concentration (µg/ml)	Absorbance	%Assay
1	**6**	**2.4772**	**111.78**
2	**6**	**2.6643**	**103.93**
3	**6**	**2.2029**	**127.70**
	MEAN	**2.44813**	**114.47**

- *Acceptance Criteria :*
  The % Relative Standard Deviation should be not more than 2.0 %

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➢ *Accuracy*

Table 10 50% Accuracy

S.NO	Absorbance	Concentration found	% Recovery for 50%
1	0.0163	0.00571	93.6
2	0.0125	0.00438	93
3	0.0128	0.00448	93
AVERAGE	0.0138	0.00485	93.2
SD	0.0021	0.000740	0.3464
% RSD	15.2355	15.2511	37.684

AVERAGE = 93.6+93+93/3

= 279.6/3

= 93.2

Table 11 100% Accuracy

S.N0	Absorbance	Concentration Found	% Recovery for 50%
1	1.7019	0.596	96.5
2	1.9672	0.689	96.5
3	1.9502	0.683	96.5
AVERAGE	1.8731	0.656	96.5
SD	0.1345	0.0520	0
% RSD	7.1808	7.9342	0

AVERAGE = 96.5+96.5+96.5/3

= 289.5/3

= 96.5

➢ *Accuracy:*

Table 12 150 % Accuracy

S.NO	Absorbance	Concentration Found	% Recovery for 50%
1	0.12322	0.0432	89.53
2	0.1285	0.0450	89.5
3	0.1302	0.0456	89.5
AVERAGE	0.1273	0.0446	89.5
SD	0.003651	0.00124	0.01732
% RSD	2.8680	2.8004	0.01935

AVERAGE = 89.53+89.5+89.5/3

= 268.53/3

= 89.5

Table 13 Net Accuracy Data

S.NO	PERCENTAGES	ACCURACY	STANDARD DEVIATION	%RSD
1	50% ACCURACY	93.2	0.0007	15.2511
2	100% ACCURACY	96.5	0.0520	7.9342
3	150% ACCURACY	89.5	0.0012	2.8004

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➢ *Acceptance Criteria :*
The average Butylated hydroxy toluene recovery at each level should fall between 98.0% and 102.0%.

- *Optical Characterstics :*

TABLE 14 Optical Characteristic Results

S . NO	Parameter	Result
1.	Absorption Maxima	226nm
2.	Linearity Range	10-50µg/ml
3.	Standard Regression Equation	Y = 0.0101x + 0.1475 R^2 = 0.9998
4.	Correlation coefficient	0.9998
5.	Accuracy	50% - 93.2 100% - 96.5 150% - 89.5
6.	Precision	INTRA DAY INTER DAY
7.	Analyst – 1	1.70
8.	Analyst -2	2.60

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CHAPTER EIGHT
SUMMARY AND CONCLUSION

A highly efficient UV Spectrophotometer using the Butylated hydroxy toluene method has been developed and validated for estimating Butylated hydroxy toluene in both bulk and tablet dosage forms.

The method utilized 0.1 N NaOH as a solvent, and the λ max was identified at 226 nm. Validation was carried out for linearity, precision, accuracy, sensitivity, and ruggedness, and a calibration plot was constructed. Adhering to ICH guidelines and standards, the method was established. Accurate analysis of marketed formulations revealed a percentage recovery within acceptable limits, demonstrating high accuracy. The method also exhibited good precision, as evidenced by low interday and intraday relative standard deviation (RSD) values.

In conclusion, this UV spectrophotometer-based method is precise, accurate, simple to execute, and cost-effective. Unlike spectrophotometric methods, it does not require expensive or sophisticated chemicals.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24JUL1770

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