



DEVELOPMENT AND VALIDATION OF UV-SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF COLCHICINE IN BULK AND PHARMACEUTICAL DOSAGE FORM.

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ABSTRACT: A Simple, Precise, Accurate and Economical UV spectrophotometric method was developed and validated for estimation of *Colchicine* in bulk and pharmaceutical dosage form. The drug was highly soluble in distilled water, so it was selected as the solvent system. The detection wavelength of *Colchicine* was found to be 246 nm. The linearity of *Colchicine* was found to be in range of 2-20 µg/ml with correlation coefficient value 0.995. The linear regression equation obtained by least square regression method, were $y = 0.0176x - 0.0112$. The absorbance was found to increase linearly with increasing concentration of *Colchicine*. The correlation coefficient of *Colchicine* was found to be 0.995. The LOD and LOQ were found to be 2.94 µg/ml and 8.92 µg/ml respectively. Mean recovery of *Colchicine* was found to be in the range of 98.06% to 100.77% signifies the accuracy of method. The method was also found to be precise as % RSD was less than 2. This method was validated as per ICH Q2 (R1) guidelines. The proposed methods were found to be accurate, specific and reproducible which can be effectively applied to pharmaceutical dosage form. Robustness obtained % RSD was found to be 0.827 and % Purity obtained by assay was 98.60%.

KEYWORDS: *Colchicine*, ICH Guideline, Distilled water, Method Development, Validation.

I. INTRODUCTION:

Colchicine is obtained from the fully developed dried whole corns, collected before flowering. The *Colchicine* has a specific clinical effect in the treatment of acute gout. *Colchicine* can be administered both orally and intravenously. Its anticancer properties are attributed to its ability to bind itself to tubulin, the protein subunit of microtubules. It also has anti mitotic activity. *Colchicine* [N-(5, 5, 7, 9-Tetrahydro-1, 2, 3, 10-tetramethoxy-9-oxobenzo [alpha] heptalen-7-yl) acetamide.] occur in the form of pale yellow colour, amorphous powder. It is freely soluble in water (45 mg/ml), chloroform, and benzene (10 mg/ml). *Colchicine* is slightly soluble in ether (4.5 mg/ml). It having 399.5 dalton molecular weight and melts at about 145°C. To bypass the first pass hepatic metabolism which degrades the potency of the molecule.⁽¹⁾

The genus *Colchicum* belongs to the *Colchicaceae* family. Molecular formula of *Colchicine* C₂₂H₂₅NO₆ with IUPAC name N-[(7S)-1,2,3,10-tetramethoxy-9-oxo-5,6,7,9-tetrahydrobenzo [a] heptalen-7-yl] acetamide and molecular weight is 399.44 g/mol. It is a highly poisonous alkaloid containing various species of *Colchicum*. COLC is the main alkaloid obtained from the bulb and seeds of *Colchicum*.⁽²⁾ It is used in human and veterinary medicine. The medicinal value of *colchicum* is due, to the presence of *Colchicine*, the main alkaloid, which was isolated from all species of *Colchicum*. It is widely used in breeding studies and as drug to treat gout but is also valuable for other diseases such as familial Mediterranean fever, primary biliary cirrhosis and breast cancer.⁽³⁾

This action alters mitosis and doubles the chromosome set. The resultant double chromosome set has been the basis for plant improvement using *Colchicine*. *Colchicine* is an alkaloid, found in the corm and seeds of various species of *Colchicum*. Chemically it is (S)-N-(5,6,7,9-tetrahydro-1,2,3,10-tetramethoxy-9-oxobenzo[a]heptalen-7-yl) acetamide.⁽⁴⁾ It is well known remedy for Gout and also an effective adjuvant to other drugs such as corticosteroids and immunosuppressive drugs for prevention and treatment of recurrent pericarditis. It is also used for certain dermatological conditions including psoriasis, actinic keratosis,

urticarial vasculitis, scleroderma, cystic acne, erythma nodosum leprosum, behcet's syndrome, sweet's syndrome, amyloidosis.⁽⁵⁾ It is a potent alkaloid obtained from the dried corns and seeds of plants of the genus *Colchicum* which belong to *Liliaceae* family. Among this family, the commonly used plants are *Colchicum autumnale* "meadow saffron" and *Colchicum autumn crocus*. *Colchicine* has been used for the treatment of acute gout since more than 2000 y ago.⁽⁶⁾ It was also used to treat pseudogout and familial Mediterranean fever for several decades. It is highly effective in the treatment of acute gout, especially when given in the first 12–36 h of the gouty attack. However, *Colchicine* for the treatment of acute gout was only approved by the United States food and drug administration (FDA) in 2009, although *Colchicine* tablets have been prescribed in the United States since the 19th century.⁽⁷⁾

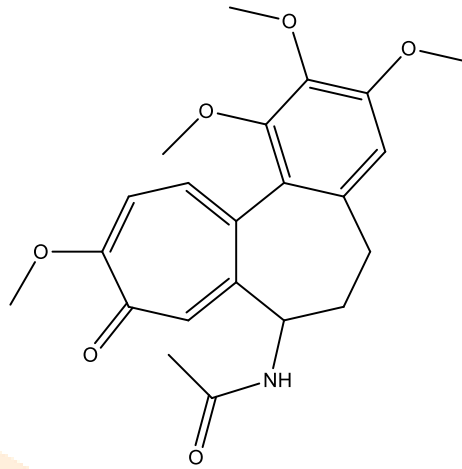


Fig. No. 1. Structure of Colchicine

Colchicine has FDA approval for gout prophylaxis and treatment of acute gouty flares. It also has approval for the treatment of familial Mediterranean fever. *Colchicine* has been used off- label to treat several other conditions, including hepatic cirrhosis, primary biliary cirrhosis, and pseudogout. *Colchicine* has primarily anti-inflammatory properties. *Colchicine* is a tricyclic alkaloid with anti-inflammatory properties extracted from the herbaceous *Colchicum autumnale* plant and first isolated and synthesized in the 19th century.⁽⁸⁾ It is one of very few drugs surviving from antiquity to modernity, since it was described in a 1550 BC Egyptian papyrus and used by ancient Greek, Byzantine, and Arabian physicians. Historically, *Colchicine* has been used in gout, and since the discovery in the 1970', for preventing attacks of the hereditary auto- inflammatory disease familial Mediterranean fever (FMF) and its most dreaded complication -AA amyloidosis. However, in recent years, most recently in the COVID-19 pandemic, several intriguing new applications for *Colchicine* are being established in large-scale randomized trials, leading to a steady rise in utilization and making it among the 200 most prescribed drugs in the USA.^{(9) (10)}



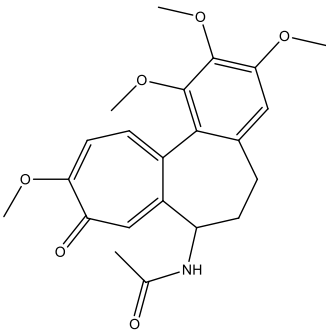
Fig. No. 2. Gloriosa spp. (Marker plant of Colchicine)

Gloriosa spp., a climbing herb, is widely distributed throughout the tropical areas of Africa and Asia. Other than its use as an ornament, it is also a source of medicinal agents. The essential well-known compound in *Gloriosa* spp. is *Colchicine*, which is a phytochemical alkaloid also found in other plants such as *Colchicum autumnale*.⁽¹¹⁾

Synonyms	Flame lily, Climbing lily, Glory lily.
Botanical name	<i>Glorisa superba</i>
Kingdom	Plantae
Class	Liliopsida
Order	Liliales
Family	<i>Colchiaceae</i>
Genus	<i>Astragulus</i>
Species	<i>Glorisa superba</i>

Table. No. 01: Taxonomical Characteristics of Gloriosa spp.

❖ **Drug Profile of Colchicine :**

Sr. No.	Properties	Inference
1	Structure	
2	IUPAC Name	N-[(7S)-1,2,3,10-Tetramethoxy-9-oxo-5,6,7,9- tetrahydrobenzo[a]heptalen-7-yl]acetamide
3	Molecular formula	C ₂₂ H ₂₅ NO ₆
4	Molecular Mass	239.437g/mol
5	BCS Class	Class III (High Solubility, Low Permeability)
6	Category	Anti-gout
7	Therapeutic use	<i>Colchicine</i> is used in the treatment of gout flares and Familial Mediterranean fever, and prevention of major cardiovascular events. It has also been investigated in other inflammatory and fibrotic conditions. ⁽¹²⁾
8	Toxicity	The precise dose of <i>Colchicine</i> that results in significant toxicity is unknown. Toxicity has occurred after the ingestion of a dose as low as 7 mg over four days, while other patients survived after taking more than 60 mg. ⁽¹³⁾
9	Metabolism	<i>Colchicine</i> is metabolized in the liver. It undergoes CYP3A4- mediated demethylation into major metabolites, 2-O- demethylcolchicine and 3-O-demethylcolchicine.
10	Protein binding	70%
11	Absorption	Well-absorbed
12	Trade Name	Colcrys, Mitigare ⁽¹⁴⁾
13	Half -life	20-40 hrs.
14	Melting point	163-165 ⁰ C
15	Appearance	Pale yellow colour
16	Dose	1.8 mg/day (maximum)
17	Solubility	Soluble in water, Freely soluble in alcohol and practically insoluble in cyclohexane.

18	Mechanism of action	<i>Colchicine</i> binds to tubulin, a microtubular protein, causing its depolymerization. This disrupts cellular function, such as the mobility of granulocytes, thus decreasing their migration into the affected area. Furthermore <i>Colchicine</i> blocks cell division by binding to mitotic spindles. <i>Colchicine</i> also inhibits the synthesis and release of the leukotriens. ⁽¹⁵⁾
19	Purity	98.60%
20	Marketed formulations	Zycolchin Tablet , Zydus Healthcare, India
21	pH value	Between 2 to 10.
22	Odour	Odourless ⁽¹⁶⁾
23	CAS No	64-86-8
24	Substitute	Indomethacin
25	Side effects	Stomach pain, diarrhea, swollen mouth, sore throat, bleeding, red or purple marks on the skin.
26	Contraindications	Biliary obstructions, Alcoholism, Dialysis, Bone marrow suppression, Dental disease, Geriatric, Neuromuscular toxicity, Renal impairment, Hepatic disease, Renal disease. ⁽¹⁷⁾

Table. No. 02: Drug Profile of *Colchicine*.

❖ Introduction to Analytical Chemistry:

Analytical chemistry can be defined as —The resolution of a chemical compound into its proximate or ultimate parts; the determination of its elements or of the foreign substances it may contain. This definition outlines the importance and scope of analytical chemistry in very broad terms. When an analyst being presented a completely unknown sample, the first objective is to ascertain the substances which are present in it. The fundamental problem in the analysis is finding out what impurities are present in the given sample, or finding out certain specified impurities which are absent.

Analytical method development and validation can be defined as a continuous and interconnected activity conducted throughout the drug development process to verify that a given method measures a parameter as intended and establishes the performance limits of the measurement. Validated methods always produce results within known uncertainties. The results of validated method are crucial for the continuation of drug development, as these methods define the budding knowledge base which supports the product. The time and effort that are put into developing transferrable, robust and scientifically-sound analytical methods have to be in association with the drug development stage.

General classification of analytical methods:

1. Spectral methods: These are depending on light absorption or emission characteristic of drugs. e.g.: UV, Visible, IR spectroscopy (infra-red spectroscopy, NMR (Nuclear magnetic resonance) spectroscopy, Fluorimetry, Colorimetry.
2. Chromatography methods: These are depended on the affinity or partition coefficient difference between drugs. e.g., Thin layer chromatography (TLC), High performance thin layer chromatography (HPTLC), Paper chromatography.
3. Electro analytical methods: These are based on electro-chemical properties of the drug. e.g. Potentiometry, Conductometry, Paper electrophoresis.
4. Microbiological and Biological methods: In these methods, animals and microorganisms are used and their activity is determined. e.g. Biological assay of specific vitamins, microbiological assay of vitamins.
5. Radioactive methods: In these methods the radioactivity is measured for analysing the drug.e.g. Radio Immuno Assay (RIA)
6. Physical methods: In these methods some physical characteristics of drugs are measured.e.g. Differential scanning calorimetry –DSC.

Factors affecting the choice of analytical methods:

The techniques described above have differing degrees of sensitivity, selectivity, cost of analysis, sophistication etc. For a given determination, analyst has to select the best procedure considering all these parameters. For this following criteria's are considered.

- a. The type of analysis required: molecular or elemental, occasional or routine.
- b. The nature of substance to be analyzed: Hygroscopic substances, radioactive substances, corrosive substances, etc.
- c. Interferences from other substances other than the analyte under investigation.
- d. Analytical instruments and other facilities available.
- e. The concentration range needed for the analysis. Accuracy needed for the method.
- f. Time required for the analysis. In case of analytical results required faster time should be monitored.

g. Which method of analysis will be preferred for the analyte of interest? Destructive or nondestructive?

h. Accuracy needed for the method.

i.

❖ **UV-Visible Spectroscopy:**

The study of the absorption of UV radiation with wavelengths spanning from 200 to 400 nanometers is known as ultraviolet spectroscopy. Any molecule containing 'n', 'π', or 'π-π*' electrons, or a combination of these electrons, absorbs the characteristic radiation and moves from the ground to the excited state. The characteristic absorption peaks can be used to determine the sort of electrons present, and consequently the molecular structure.

UV spectroscopy is the absorption or reflectance spectroscopy of the ultraviolet and adjacent visible regions of the electromagnetic spectrum. It is also known as UV-visible spectrophotometry (UV-Vis or UV/Vis). Because of its low cost and ease of implementation, this methodology is widely used in a wide range of applied and fundamental applications. The only requirement is that the sample absorb in the UV-Vis range, indicating that it is a chromophore. Absorption spectroscopy supplements fluorescence spectroscopy. Aside from the wavelength, the parameters of interest are absorbance (A), transmittance (%T), and reflectance (%R), as well as their variations over time.

UV-Visible spectroscopy is an analytical technique that measures the amount of discrete wavelengths of UV or visible light that are absorbed by or transmitted through a sample in comparison to a reference or blank sample. Ultraviolet-visible (UV-visible) spectrophotometry is primarily a quantitative analytical technique concerned with the absorption of near-UV (180-390 nm) or visible (390-780 nm) radiation by chemical species in solution. Spectroscopy in the ultraviolet (UV), visible (Vis) and near-infrared (NIR) region of the electromagnetic spectrum.

Spectroscopy: Spectroscopy is a branch of science that deals with study of interaction of UV radiation with matter. Spectroscopy is a most useful tool available for study of atomic & Molecular structure.

a) Atomic Spectroscopy: The atomic spectroscopy deals with study of interaction of UV radiation with atoms.

b) Molecular spectroscopy: The molecular spectroscopy deals with study of interaction of UV radiation with molecules.

Spectrophotometer is a device which are design to determine spectrum of a compound. UV spectroscopy = 200 – 400 nm.

UV visible spectroscopy = 400 – 800 nm⁽¹⁸⁾

Principle:

The principle of UV visible spectroscopy is based on absorption of ultraviolet light or visible light by a chemical compound which gives spectra. 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 discrete-dimensional 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 describes the excitation of an electron from its ground state to its excited state.



Fig. No. 03: UV-1900 Shimadzu UV- Visible spectroscopy

Beer Lambert law:

Beer Lambert law states that, "The absorbance (A) of monochromatic beam is directly proportional to concentration (C) & Path length (l).

$$A = \epsilon C L$$

Where,

A = Absorbance

ϵ = Molar absorption coefficient

C = Molar concentration L = Path length.

Beer-law Lambert's states:

It can be stated that when the thickness and concentration of absorbing media rises arithmetically, the intensity of a monochromatic light beam passing through transparent material drops exponentially.

$$A = \log_{10} \frac{I_0}{I_t}$$

Where,

A = Solution absorbance at a specific wavelength of the light beam I_0 denotes the intensity of the incident light beam.

I_t stands for the intensity of the transmitted light beam.

a = Molecule absorption at the wavelength of the beam

b = Cell path length in cm.

c = is the mole/lit concentration of the solution.

Beer's law is said to be obeyed over a concentration range if a plot of concentration against absorbance is a straight line that passes through the origin.

Limitations of Beer Lambert law:

1. The light source used must be monochromatic.
2. This is not suitable for concentrated solutions.⁽¹⁹⁾

UV-Visible Spectroscopy in Action:

The detection of functional groups (chromophores), the extent of conjugation, the detection of polynuclear compounds by comparison, and the detection and study of chromophores are all examples of UV-Visible Spectroscopy. The colorimetry method can be used to determine highly colored solutions that aren't visible to the naked eye. Titrations of precipitation can also be done using the photometric approach. The radiant power scattering is reduced by the suspended particles. As a result, the titrations are carried out to a constant turbidity condition.

UV spectroscopy is most commonly used to investigate the extent of configuration, distinguish between conjugated and non-conjugated compounds, analyze geometrical isomerism, tautomerism, structural properties in different solvents, and distinguish between equatorial and axial conformations. It is used to determine molecular weight, detect contaminants, and identify unknown chemicals, among other things.

Instrumentations:

The Essential components of UV-VIS Spectrophotometer are as follows:

1. Light Sources (UV and visible)
2. Monochromator
3. Sample containers (Cuvette)
4. Detector
5. Amplifier and recorder.

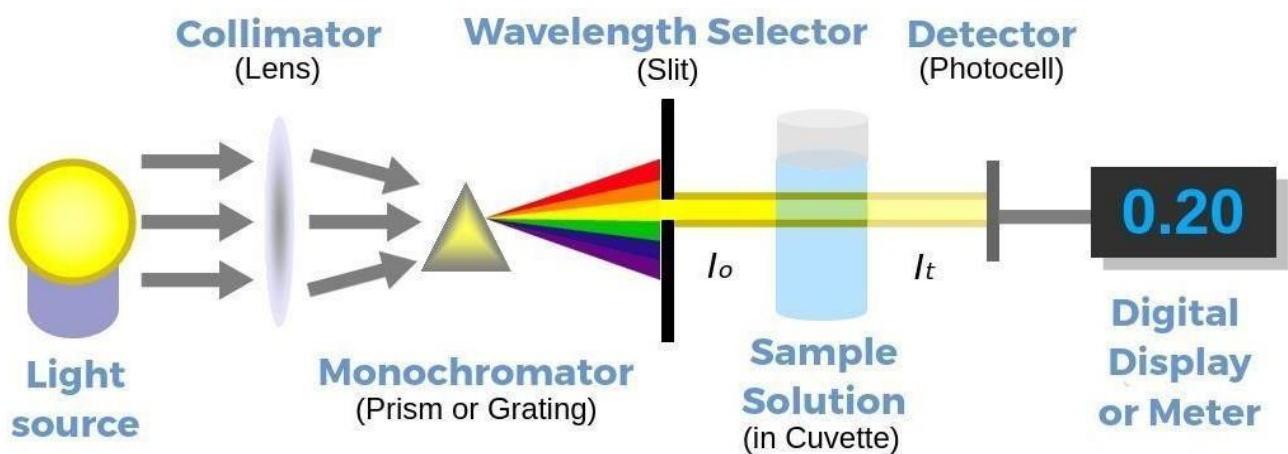


Fig. No. 04: UV Instrumentation.

1. Light Source :

The light source used must provide consistent & stable light. A continuous source, or one that produces radiation at a variety of wavelengths, is necessary for UV-Vis Spectroscopy. Assorted UV radiation sources include the following:

- a) Hydrogen & deuterium lamps
- b) Tungsten filaments lamps
- c) Xenon arc lamps.

2. Monochromator :

It separates polychromatic light into single spectral line. A monochromator is an optical device that is used to select a narrow band of a wavelength of light.

- A) Slit
- B) Mirror
- C) Lens
- D) Prism
- E) Grafting

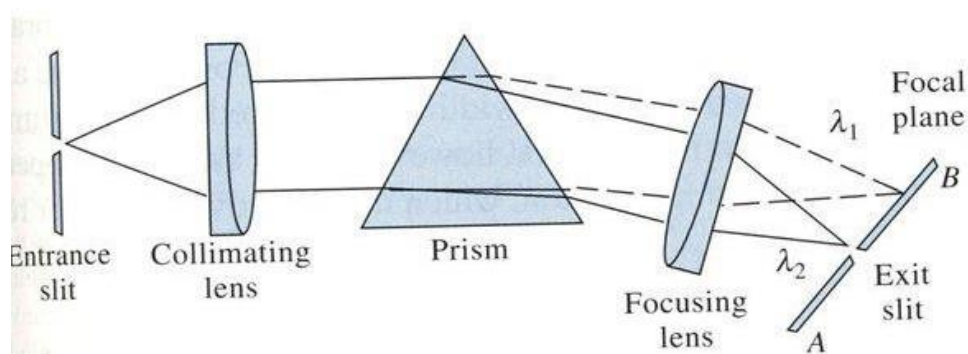


Fig. No. 05: Monochromator

A monochromator is an optical instrument which measures the light spectrum. Light is focused in the input slit and diffracted by a grating. In this way, only one color is transmitted through the output slit at a given time. Spectra are then recorded wavelength by wavelength, rotating the grating.

There are modified types of monochromator, for example the Fastie-Ebert monochromator with a common collimator/refocusing mirror, and devices with two gratings for better resolution. The quality of the diffraction grating can be important for the performance: Its diffraction efficiency determines the power losses. Monochromator is a mechanism that emits monochromatic light from a light source. A dispersive element, generally a prism or diffraction grating, is used to create the monochromatic light. The most common materials for laboratory X-ray monochromator are pyrolytic graphite for broad band use and either silicon, germanium, or quartz for narrow band use.⁽²⁰⁾

There are two types of monochromator:

- 1) Prisms
- 2) Grating systems.

3. Sample & reference cells :

The cuvette are generally made up of quartz & borosilicate. One beam passthrough sample solution & second beam pass through reference solution. The cuvette are generally transparent.

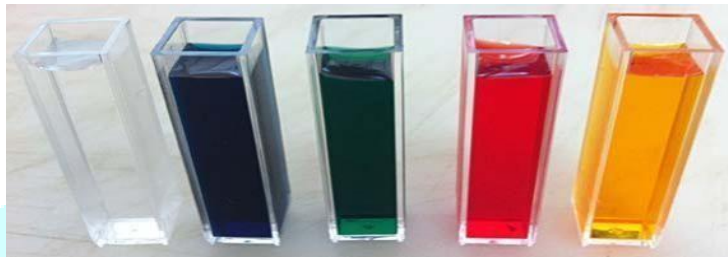


Fig. No. 06: Sample Solution in Cuvette

A cuvette is a type of sample holder for liquid samples. Often, they are made of plastic, borosilicate glass, or quartz. Stellar Net offers glass cuvettes for experiments in the visible or NIR ranges and quartz cuvettes for experiments in the UV range. Cuvettes also come with two or four polished sides. When you rinse the cuvettes with water, the water dilutes the sample (concentration changes). Therefore, you must rinse the cuvettes with the sample (rather than water) so you can avoid changing the concentration of your sample.

5. Detector :

The Detector is responsible for detection of radiation. The intensity of radiation from reference cell is stronger than beam of sample cell. A photo detector is a semiconductor device which converts light energy to electrical energy. It consists of a sample P-N junction diode and is designed to work in reverse biased condition. The photons approaching the diode are absorbed by the photodiode and current is generated.

Photodetectors, also called photo sensors, are sensors of light or other electromagnetic radiation. There is a wide variety of photodetectors which may be classified by mechanism of detection, such as photoelectric or photochemical effects, or by various performance metrics, such as spectral response.

TYPES OF DETECTOR:

1. Barrier Layer Cell Detector
2. Photo Tube Detector
3. Photo Multiplier Tube Detector
4. Silicon Photodiode Detector

BARRIER LAYER CELL DETECTOR :-

Barrier layer cell detector is also called as photo volatile cell. It consists of semiconductor (selenium) which is deposited on strong base ion. A very thin layer of silver or gold is placed over the surface of semiconductor. To act as collector electrode radiation when falls on the surface electron on produce. Electron are produce and their it is converted into electric current. To dose not required power supply.

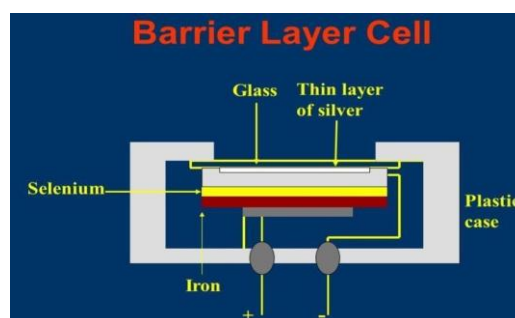


Fig. No. 07: Barrier layer cell detector

A photoelectric detector which is made of iron coated with a semiconductor film when light from 250-750nm hits this cell, you get a current; this is a cell which is mainly good for intense light sources, because there is not a huge signal enhancement. Also known as a self-generating barrier layer cell. A photoelectric detector that converts radiant flux directly into electrical current.

5. Recorder :

The recorder detect & record the data of the experiment. It also stores the data in computer when it connected to computer. The signal from detector is received by recording system. Recording is done by recording pen. The earliest instruments were simple and directly connected the amplifier detector signal to a chart recorder. Nowadays, all experimental settings are controlled by a computer and detector signal are digitized processed and stored. The device used to accept the signal transmitted from the analyzer and display it for use in process operation or other decision making. Displayed as measured property or concentration in the accepted units of that property or concentration.

Advantage's of UV visible spectroscopy :

1. UV visible spectroscopy gives accurate results.
2. Easy to handle
3. Cost effective instrument.

Disadvantage's of UV visible spectroscopy :

1. The results can be affected by Temperature, PH, impurities etc.
2. Only liquid samples are possible to analyses.
3. Require proper handling of cuvette.

Applications of 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.
6. It is used to check nucleic acid purity
7. It is used in detection of impurities.
8. Structural elucidation of organic compounds.

❖ Various Spectrophotometric methods:

Various spectrophotometric methods are available for assay of substances in multicomponent samples.

1. Simultaneous equation method (vierodt's method)
2. Absorbance ratio method
3. Geometric correction method
4. Orthogonal polynomial method
5. Difference spectrophotometry
6. Derivative spectrophotometry
7. Two wavelength method
8. Absorption factor method (absorption correction method).⁽²¹⁾

❖ Method development :

Analytical method development and validation are keys elements of any pharmaceutical's development program. The analysis method UV is developed for identifying, quantifying, or purifying compounds of interest. This technical brief will emphasis on development and validation activities useful to drug products.

Method development is a process ensures that all the laboratory resources are effectively utilized while methods meet the specification needed at each step of drug development activity. Method validation requires by regulatory agencies at certain stages of the drug approvals process, is defined as the "process of signifying that analytical procedure is useful for their intended use." Understanding of the physical and chemicals characteristic of drug allows one to select the most appropriate UV method development from the available vast literature. Information concerning the sample for example molecular weight, structure and functional group presents, pKa values and Ultra Violet spectra, compound's solubility should be compiled. The requirement of removal of insoluble impurities by filtration, centrifuge, dilution, or concentration to control the concentration, extraction, derivatization for detection etc. should be checked. For pure compounds, the sample solubility should be identified whether it is organic solvents soluble or water soluble as this helps to select the method development.

Analytical method development and method validation is an important process in the drug discovery. Without validated analytical method a drug cannot enter into the market although the drug shows good potency. This is to ensure the quality and safety of the drug.

UV-Visible spectroscopy is an analytical technique that measures the amount of discrete wavelengths of UV or visible light that are absorbed by or transmitted through a sample in comparison to a reference or blank sample.

This is done by measuring the intensity of light that passes through a sample with respect to the intensity of light through a reference sample or blank.⁽²²⁾

The developed methods will be validated by using various validation parameters:

After the development of UV spectrophotometric method for the estimation of Colchicine (antigout drug) validation of developed methods will be carried as per ICH guidelines.

Validation is same as verification; Validation is the process “where the specific requirement is acceptable for an intended usage” and Verification means “provision of assurance that a particular provided items fulfils particular requirement.” The scope and importance of an analytical process may be defined in advanced of validation process. It includes explaining the analytical range description of apparatus and procedure, level of validation and necessary criteria required. Validation range is explained as concentration of analyte within the method might be considered as validated. This range is neither highest nor lowest promising amount of the analyte that could be find out by the method. The method could be validated for various purposes like screening and for quantitative method. It could be used on various machine available in company, equipment, or instrument present in the laboratory or in various laboratory on different climatic conditions.

- Accuracy
- Precision (Repeatability and Reproducibility)
- Linearity And Range
- Limit Of Detection (LOD)
- Limit Of Quantitation (LOQ)
- Selectivity
- Specificity
- Robustness
- Ruggedness
- Stability And System Suitability

1.Accuracy:

Accuracy can be defined as the —closeness of the measured value to the true value.

If a method has to be highly accurate the measured value of analyzed method should be identical to true value. Accuracy is determined by recoveries studies.

Accuracy can be determined by three ways.

- 1.Comparison to a reference standard
- 2.Standard addition of the analyte
- 3.Recovery of the analyte spiked into blank matrix.

Recovery studies give the information of whether tablet excipients interfere with the drug spectra.

2.Precision:

Precision can be defined as —the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogenous sample.

According to ICH guidelines there are three types of precision.

- 1.Repeatability
- 2.Intermediate precision
- 3.Reproducibility

Repeatability is the —precision of a method under the same operation conditions over a short period of time. Intermediate precision is the —agreement of complete measurements when the same method is applied many times within the same laboratory. Reproducibility examines the precision between laboratories and is often determined in collaborative studies or method transfer experiments.

A method may found to be precise if the %RSD<2.

3.Linearity and Range:

Linearity of a method can be defined as a —measure of how well a calibration plot of response vs. concentration approximates a straight line. Measurements are performed at several analyte concentrations. Using the values obtained from linearity data a calibration curve can be plotted and slope, intercept and correlation coefficient of the calibration curve provides the desired information on linearity. The range of the method can be defined as the lower and upper concentrations for which the analytical method has adequate accuracy, precision and linearity.

4.Limit of detection and Limit of quantification:

The limit of detection can be defined as —the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The LOD is often based on a certain signal-to-noise ratio, typically 2 or 3. The quantitation limit of an individual analytical procedure can be defined as —the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is particularly used for the determination of impurities and degradation products. The LOD and LOQ may be expressed as $LOD = 3.3 \times \sigma/S$ and $LOQ = 10 \times \sigma/S$.

5.Specificity:

The specificity of any test method is found out by comparing test results from testing of samples containing some impurities, degradation products or placebo ingredients with those found from a testing of samples without any impurities, degradation products or placebo ingredients. Specificity could be best explained by resolution between the analyte peak and the other closely eluting peak.

6. Selectivity:

Selectivity and specificity are sometimes used interchangeably to describe the same concept in method validation. Selectivity of an analytical method is defined by the ISO as property of a measuring system used with specified measurement procedure whereby it provides measured are independent of other measured or other quantities in the phenomenon body or substance being investigated.

7. Robustness:

It is a measure of its ability to be unaffected by small but intentional changes to the technique parameters and gives evidence as to how reliable it will be under typical conditions. For the determination of method robustness, several chromatographic parameters.

8. Ruggedness:

The ruggedness of an analytical method refers to how consistent the test results are when the same samples are analyzed under a variety of normal test conditions. These conditions can include different laboratories, analysts, and operational and environmental conditions that may vary but still fall within the specified parameters of the assay. To determine the ruggedness of an analytical method, the reproducibility of test results is measured as a function of the assay variable. This reproducibility can then be compared to the precision of the assay under normal conditions to obtain a measure of the method's ruggedness.⁽²³⁾

❖ Plan Of Work :**Literature Review**

Review existing literature on analytical methods for antitumor drugs. Understand the UV absorption characteristics and chromatographic behavior of the drugs. Study stability- indicating methods and related analytical techniques.

Method Selection and Design

Colchicine drug have been Chosen to be analyzed simultaneously. Decide on the dosage forms (e.g., tablets) to be analyzed. Select appropriate UV wavelengths and chromatographic conditions to minimize interference.

Sample Preparation

Develop sample preparation methods to extract drugs from the dosage forms. Optimize the extraction process to ensure complete recovery and minimal matrix effects.

UV Spectrophotometric Method

Prepare standard solutions of individual antitumor drug. Determine the suitable UV wavelengths for each drug. Construct calibration curves for each drug using UV absorption measurements. Validate the method for linearity, accuracy, precision, and specificity.

Method Validation

Perform validation studies for UV method. Validate parameters including linearity, accuracy, precision, robustness, and sensitivity. For stability-indicating method, assess selectivity, accuracy, and precision in the presence of degradation products.

Data Analysis and Reporting

Analyze the data obtained from method validation and application studies. Prepare a comprehensive report detailing the methods, validation results, and applications.⁽²⁴⁾

❖ Chapterization :

Certainly, breaking down the topic into chapters will help you organize your study and research more effectively. Here is a chapterization for the topic "Development and Validation of Estimation of Colchicine Drug in Bulk and Pharmaceutical Dosage Form by UV Spectrophotometric method.

Chapter 1: Introduction

- Drug profiles
- UV Instrumentation
- Analytical method introduction

Chapter 2: Aim & Objectives

- Title of developed method
- Development method and validate the methods.

Chapter 3: Need for the study

- Discussed sampling in the subjected in the study
- For the Development method and validate the method.

Chapter 4: Review of literature

- Review of analytical methods for Colchicine drug analysis
- UV spectrophotometric methods in pharmaceutical analysis

Chapter 5: Research Methodology

- UV Development Method
- UV Validation of the Methods

Chapter 6: Results and Discussion

- Include the Results of developed methods
- Discussion of the findings in the context of objectives

Chapter 7: Summary and Conclusion

- Summary of key findings and contributions.
- Conclusion of the study's success in achieving objectives

Chapter 8: References

- Listing of all sources, literature, and references used in the study.

Scope of the present study:

According to a literature review, UV Spectrophotometry technique were used to estimate the quality and quantity of drugs and their formulations qualitatively and quantitatively. Many of these procedures necessitated the use of complex and expensive analytical tools, which many rural laboratories cannot afford for routine and quick examination. Many laboratories find it difficult to obtain these devices and utilize them for regular analysis on a daily basis. The UV Visible Spectrophotometric method is the most cost-effective, quick, and low- cost way of measurement. To achieve this, specialized, sensitive, quick, and highly reliable analytical processes must be created, which require simple methodology and inexpensive instruments that are readily available in all laboratories.

However, the use of modern equipment eliminates the challenges associated with determining minute amounts of breakdown products or analyzing drug metabolites in physiological fluids. The measurement of chosen medicines in technical grade, formulations, and biological samples was done using UV Visible spectrophotometry techniques in this study.⁽²⁵⁾

II. Objectives –

- 1) To develop a simple, rapid and reliable UV Spectrophotometric method for estimation of selected phytochemical in its pharmaceutical dosage form.
- 2) To validate the developed UV-Spectrophotometric methods.
- 3) To carry out the assay on pharmaceutical dosage form.

III. Need for the study:

When no authoritative methods are available, new methods are being developed for analysis of phytochemical or products.

When there is no official drug or drug combinations available in the pharmacopeias.

When there is no decorous analytical process for the existing drug in the literature due to interference caused by the formulation excipient. Analytical methods for the quantitation of the analyte in biological fluid are found to be unavailable. The existing analytical procedures may need costly reagents and solvents. Analytical methods for a drug in a combination with other drug may not be available. Identification of sources and Quantitation of Potential errors. Determination if Method is Acceptable for intended use. It is cost effectiveness method development. When there is do not follow ICH Guidelines for the existing drug in the literature. According to Literature, when compared to other ways, these methods take less time and are simpler to implement. According to ICH recommendations, these procedures will be tested and validated for a variety of factors.⁽²⁶⁾

IV. Review of Literature:

1) *Abdulbaqi et al., (2017)* have developed and validated a simple yet rapid method under the title “A Simple HPLC-UV Method For The Quantification Of *Colchicines* In Bulk And Formulation And Its Validation.”

The main aim of the present study was A simple, rapid, specific and stability indicating HPLC- UV method for the determination of *Colchicines* in pure and formulation was successfully developed. The method was statistically confirmed to be accurate, precise and reproducible. *Colchicine* is commonly used to treat gout. Other than its involvement in several anti-inflammatory pathways, colchicine also interrupts inflammation some stimulation through blocking microtubule assembly. Moreover, it is used to treat familial Mediterranean fever and incident cancer in male gout patients.. The developed method was validated in accordance with the ICH Guidelines. Also Preparation of the sample solution and the absorbance will be 246 nm wavelength.⁽²⁷⁾

2) *Sravya et al., (2019)* have developed and validated a UV- spectrophotometric method under the title “Development And Validation of UV Spectrophotometric Method For Estimation Of *Colchicine* In Bulk And Pharmaceutical Dosage Forms.”

The main aim of the present study was describe a method to analyze *Colchicine* using UV Spectrophotometry. The method was validated as per ICH guidelines and the parameter validated were linearity, range, accuracy. *Colchicine* has FDA approval for gout prophylaxis and treatment of acute gout n flares. *Colchicine* has been used off-label to treat several other conditions, including hepatic cirrhosis, primary biliary cirrhosis, and pseudo gout. The aim of the present study was the developed method were validated according to ICH guidelines and successfully applied for the determination of colchicine in tablet with the UV spectra of 246 nm.⁽²⁸⁾

3) *Omar et al., (2019)* have developed and validated a simple yet rapid and precise UV method under the title “Spectrophotometric Method For The Determination Of *Colchicine* In Pure And Pharmaceutical Forms.”

The main aim of the present study was simple, rapid, spectrophotometric method for the determination of *Colchicine* in pure as well as in dosage form is described. The method was used for to determination of colchicines in its pharmaceutical tablet with high quality accuracy and precision. *Gloriosa* is a climbing herb, is widely distributed throughout the tropical areas of Africa and Asia. Other than its use as an ornament, it is also a source of medicinal agents. The essential well-known compound is *Colchicine*, our goal was to develop a method that could accurately measure. The aim of the present study was describe a method to analyze *Colchicine* using UV Spectrophotometry of the wavelength 246 nm.⁽²⁹⁾

4) *Mohammed et al., (2020)* have developed and validated a UV method under the title “Stability Indicating Chromatographic And UV Spectrophotometric Methods For Determinations Of *Colchicine*.”

The main aim of the present study was the developed method were validated according to ICH guidelines and successfully applied for the determination of *Colchicine* in tablet. *Colchicine* is an alkaloid that used in the relief of the acute gout. Three simple, sensitive, selective and rapid methods have been developed for determination of *Colchicine* in the presence of alkaline degradation product with good applicability on its pharmaceutical formulations with UV detection of 246nm.⁽³⁰⁾

5) *Tantaway et al., (2021)* have developed and validated a simple yet rapid method under the title “Method Development And Optimization For *Colchicines* Estimations In Toxicological Analysis Using Dispersive Liquid-Liquid Micro-Extraction And HPLC-UV.”

The main aim of the present study was the sample was analyzed was Zycolchin 0.5 mg. The developed method was validated in accordance with the ICH Guidelines. Also Preparation of the sample solution. The preliminary analysis was performed with UV-VIS Spectrophotometer. The analysis was performed using a Shimadzu LC-10AT with SPD-10A UV-VIS Detector. The sample was analyzed as Zycolchin 0.5 mg.⁽³¹⁾

6) *Bhumika parmar et al., (2022)* have developed and validated a simple yet rapid method under the title “UV Visible Spectroscopy Method Development And Validation For Estimation of Molnupiravir In Solid Dosage Form.”

The main aim of the present study was these method was validated as per ICH guidelines for various parameters such as accuracy, linearity, precision, robustness, limit of detection and limit of quantification. The main intent of this investigation was to develop and validate uncomplicated, forehanded, time saving and efficient for the resolution of capsule in the solid dosage form. Standard and sample solution of Molnupiravir prepared in distilled water.⁽³²⁾

V. MATERIAL & METHODS

Instruments & Appratus :

The absorbance of the drug were carried out by using Shimadzu company model 1900 UV visible double beam spectrophotometer with 1 cm matched quartz cell, spectral band width is 1 nm, supported by UV win 5.0 software. All weight are taken on analytical balance. Sonicator was used for dissolving *Colchicine* in distilled water. Volumetric flask & Pipette of borosilicate glass were used for the development and validation of proposed analytical method.

Reagents & Chemicals :

Colchicine was purchased from Raheja Chemicals & Surgicals Pvt. Ltd Pune Maharashtra. Zycolchin 0.5 mg tablet were purchased from local market. All the reagents are used as analytical grade.

Sr. No.	Instruments & Chemicals	Company Name
01	UV-1900 Spectrophotometer	Shimadzu
02	Analytical balance	Mettler Toledo India Pvt. Ltd.
03	Sonicator	Labline India Pvt. Ltd.
04	Volumetric Flask	Medilab India Pvt. Ltd. Mumbai
05	Colchicine	Raheja Chemicals Pune
06	Zycolchine 0.5 mg tablet	Zydus Healthcare India Pvt. Ltd

Table. No. 03: Used instruments & Chemicals

Experimental Work –

A) UV Spectrophotometric development method –

Selection of solvent :

It depends on the type of material using for study and its absorption maximum wavelength. Different types of UV solvents are available like ethanol, methanol, chloroform, hexane, benzene, acetone Generally organic compounds specifically those with a high degree of conjugation also absorb light in the UV or visible regions of the electromagnetic spectrum. For these determination water could be used for water soluble compounds and ethanol for polar organic compounds. Solubility of drugs was performed in various polar and non-polar solvents. Distilled Water was selected for UV Spectrophotometry as solvent it is because of easy availability, cheap and the solubility of sample for determination of λ max.

Selection of wavelength :

The wavelength equivalent to the maximum absorbance of target compound is chosen for UV analysis. This selection shows maximum sensitivity because major response is found for a specific analyst concentration. The best wavelength is the one which

shows highest molar absorptivity λ_{\max} without interfering those substance which absorb at the similar wavelength. For the selection of wavelength for the estimation of *Colchicine* first scanning of absorbance from 200nm to 400nm was done to find out the λ_{\max} with help of standard stock solution of 10 $\mu\text{g/ml}$ placed at 1cm path length quartz cuvette.⁽³³⁾

Solubility profile test :

Solubility test for the drug *Colchicine* was performed by using various solvents, which includes distilled water, methanol, ethanol, orthophosphoric acid, DMSO and phosphate buffer saline pH 6.4. Hence the present work is performed to develop UV Spectrophotometric analysis of *Colchicine* using more economic solvents i.e. Distilled water.⁽³⁴⁾

Preparation of stock solution :

The stock solution of *Colchicine* was prepared at concentration of 1mg/ml in distilled water. Weigh accurately 100 mg of *Colchicine* and transfer to 100 ml volumetric flask. Then dissolve the drug by adding small amount of solvent with vigorous shaking for 3 to 5 minutes. Then the final volumes was made up to the volume with solvent.

Preparation of Working Standard Solution:

Fresh stock solution 1 ml was further diluted to 100 ml with distilled water to get the solution having the concentration 10 $\mu\text{g/ml}$.

Determination of λ_{\max} :

From the above working standard solution, 5ml was pipette out into a 10 ml volumetric flask and the volume was made up to the mark with distilled water to prepare a concentration 50 $\mu\text{g/ml}$. Then the sample was scanned in UV-VIS Spectrophotometer in the range 200-400 using distilled water as blank and the wavelength corresponding of maximum absorbance λ_{\max} was found to be 246 nm.

The drug was showing good solubility in water, and hence was selected as the solvent. When the working standard solution of colchicine was scanned in UV range between 200 and 400 nm, the drug was showing maximum absorbance at 246 nm, which was selected as λ_{\max} .⁽³⁵⁾

Preparation of Calibration Curve :

From the working standard solution, pipette out 2 ml, 4 ml, 8ml, 12 ml, 16 ml, 20 ml and diluted to 20 ml using solvent distilled water. The solution were scanned at 246 nm using solvent distilled water as a blank. Then the calibration curve was plotted by taking concentrations on X- axis and absorbance on Y-axis. The curve shows linearity in the concentration range 2-20 $\mu\text{g/ml}$. Linearity was performed by taking eight non-zero concentrations of colchicine in the range of 2- 20 $\mu\text{g/ml}$. Correlation coefficient (r^2) obtained was 0.995, proving the method to be linear in the range of 2-20 $\mu\text{g/ml}$.⁽³⁶⁾

B) UV Spectrophotometric Validation method –

Analytical method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from methods validation can be used to judge the quality, reliability and consistency of the analytical results; it is an integral part of any good analytical practice. Analytical methods used to be validated or revalidated.

- Before their introduction into routine use
- Whenever the condition changes or which the method has been validated

The USP has published specific guidelines for method validation for compound evaluation. USP defines right steps for validation.

- Accuracy
- Precision
- Specificity
- Limit of detection
- Limit of quantitation
- Ruggedness
- Robustness

The FDA has also published guidelines for the validation of bio-analytical methods. Wegscheider has published procedure for method validation with a special focus on calibration, recovery experiments, method comparison and investigation of ruggedness.

1) Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy means test output match with true value. To study the accuracy of proposed method, recovery studies were carried out by standard addition method at three different levels (50%, 100% and 150%). Here to a pre-analyzed sample solution, standard drug solution was added and then percentage drug content were calculated. The Percentage recovery of the added pure drug was calculated as follows

% recovery = $[(C_t - C_s) / C_a] \times 100$ Where,

C_t = Total drug concentration measured after standard addition C_s = Drug concentration in the formulation sample

C_a = Drug concentration added to formulation

2) Precision:

Inter-day and Intra-day precision

The repeatability of the method was confirmed by the formulation analysis, repeated for six times with the same concentration. The percentage RSD was calculated. The intermediate precision of the method was confirmed by intra-day and inter-day analysis i.e. the analysis of the formulation was repeated three times in the same day at an interval of one hour and on three successive days, respectively. Repeatability is also termed intra- assay precision. Intermediate precision expresses within laboratory variations different days, different analysis, different equipment etc. Reproducibility expresses the precision between laboratories.⁽³⁷⁾

3) Linearity and Range :

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration of analyte in the sample. The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. For both drugs dilutions of standard stock solutions were assayed as per the developed methods. The Beer-Lambert's concentration range for colchicine was found to be 2- 20 µg/ml respectively. Linearity is the ability of analytical procedure to obtain a response that is directly proportional to the concentration (amount) of analyte in the sample. If the procedure is linear, the analyte concentration in the sample falls within a range that may be readily transformed mathematically into a relationship that is directly

proportionate to the test.⁽³⁸⁾

4) Ruggedness Study :

This includes different analysts, laboratories, columns, instruments, sources of reagents, chemicals, solvents. The degree of correlation between test findings analysed from the same samples under varied standard test conditions shows the analytical method's dependability. The ruggedness of the method was studied by changing the experimental condition such as, Changing to another column of similar type and different operations in the same laboratory.

It shows that the precision within laboratories variations like different analyst. Ruggedness of the method was assessed by for the standard 2 times with different analyst by using same equipment. The result was indicated as %RSD.

5) Robustness :

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal range. Robustness traditionally has not been considered as a validation parameter in the strictest sense because usually it is investigated during method development, once the method is at least partially optimized. When thought of in this context, evaluation of robustness during development makes sense as parameters that affect the method can be identified easily when manipulated for selectivity or optimization purposes.

6) Limit of Detection & Limit of Quantification :

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The LOD and LOQ were separately determined with the help of calibration curve. The residual standard deviation of a regression line or the standard deviation of y- intercepts of regression lines were used to calculate the LOD and LOQ. The LOD and LOQ were calculated by using the average of slope and standard deviation of response (Intercept). The LOD and LOQ of proposed methods were determined using calibration standards.

L LOD= $3.3\sigma/S$ and LOQ= $10\sigma/S$

Where,

S is the slope of the calibration curve and

σ is the standard deviation of response (intercept)

The smallest concentration of an analyte that could be measured but never calculated in a sample is known as the LOD. LOD is defined as a concentration at a given signal-to-noise ratio, often 3:1. The least amount of an analyte found in a specimen that is determinable using respectable precision and accuracy there within the parameters of the method's circumstances is known as LOQ.⁽³⁹⁾

7) Solution stability data for sample :

Stability in solution was evaluated by the standard solution and the test preparation. The solution was stored at 5 °C at ambient temperature without protection from light and tested after 12, 24, 36, and 48 hrs. The responses for the aged solution were evaluated by comparison with freshly prepared solutions. The stability study of the stored standard solution and test preparation were performed and solutions were found to be stable for up to 48 hrs. The assay values obtained after 36 hr. were statistically identical with the initial value without measurable loss.

8) UV Spectra of Marketed Formulation :

The developed UV method was used for analysis of marketed tablet dosage form (Brand name -Zycolchin). The sample solution was prepared at concentration of 10 µg/mL using water as solvent and its absorbance was measured at 246 nm. The concentration of sample solution was back calculated from the linear equation.

9) Assay of Marketed Formulation (0.5 mg) :

2 tablet were weighed from which a powder equivalent to 1 mg of Colchicine was taken in 100 ml volumetric flask and it was dissolved in 50 ml of solvent by intermediate shaking of the flask for about 20 min and diluted up to the mark with the solvent. Then the solution was filtered using Whatmann filter paper No. 40 and make up to the volume by using solvent. From this filtrate 5 ml is pipetted and make up to 10µg/ml and scanned for absorbance and the UV result was indicated by % Purity.^{(40) (41)}

VI. Result & Discussion:

1) Specificity and Selectivity:

Analysis wavelength selection of *Colchicine* solution was scanned in the UV range (200- 400). in spectrum *Colchicine* shows lambda max at 246 nm.

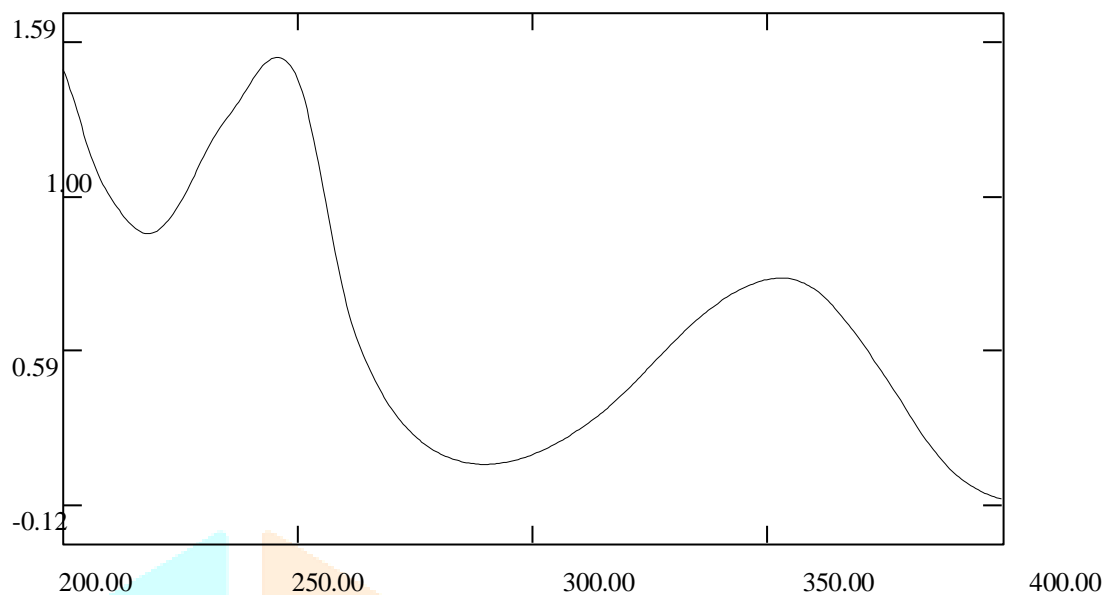


Fig. No. 08: UV Spectra of *Colchicine*

2) Linearity :

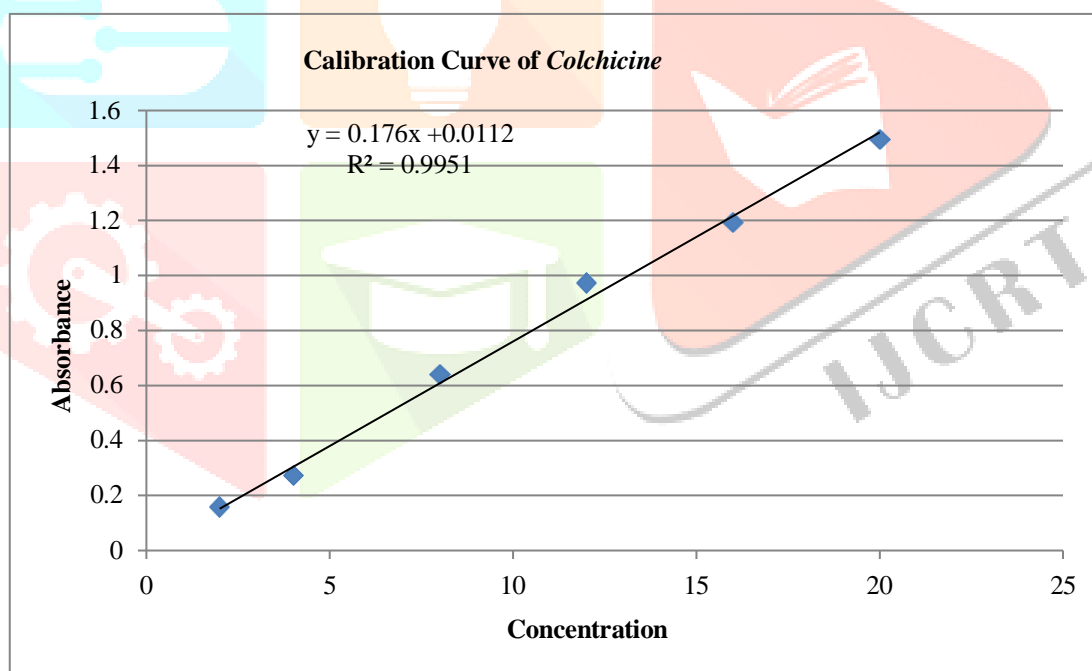


Fig. No. 09: Calibration Curve of *Colchicine*

Linearity was performed by taking eight non-zero concentrations of *colchicine* in the range of 2-20 µg/ml. The absorbance was measured at 246 nm and the graph was plotted taking concentration on X-axis and absorbance on Y-axis. Correlation coefficient (r^2) obtained was 0.995, proving the method to be linear in the range of 2-20 µg/ml.

Concentration	Absorbance
2	0.157
4	0.273
8	0.639
12	0.974
16	1.194
20	1.494

Table. No. 04: Linearity data

Regression analysis of the calibration curve for proposed method:

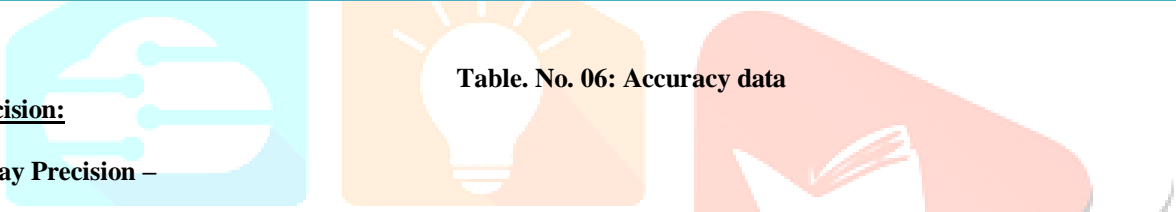
Parameters	Results
λ max	246 nm
Beer's law	2-20 $\mu\text{g/ml}$
Correlation coefficient (R^2)	0.995
Regression equation ($y = mx + c$)	$y = 0.176x + 0.0112$
Slope (m)	0.176
Intercept (c)	0.0112
LOD ($\mu\text{g/ml}$)	2.94
LOQ ($\mu\text{g/ml}$)	8.92

Table. No. 05: Regression analysis of the calibration curve of proposed method

3) Accuracy:

The accuracy of the developed method was determined at three levels. The sample solutions were spiked at 50%, 100% and 150% concentration levels and the % recovery was calculated. It was found to be in the range of 98.06 – 100.77% proving the method to be accurate.

Sr. No.	Recovery level	Amt. of sample taken (A) µg/ml	Amt. of std. add (B) µg/ml	Total amt. (A+B) µg/ml	Absorbance	% Recovered
01	50	8	4	12	0.918	100.77
02	100	8	8	16	1.192	98.41
03	150	8	12	20	1.482	98.06


Table. No. 06: Accuracy data
4) Precision:**Interday Precision –**

Sr. No.	Concentration µg/ml	Abs. Day 1	Abs. Day 2	Abs. Day 3
1	10	0.828	0.831	0.842
2	10	0.835	0.842	0.830
3	10	0.820	0.840	0.845
4	10	0.836	0.838	0.836
5	10	0.842	0.828	0.828
6	10	0.831	0.838	0.839
Average	-	0.832	0.836	0.836
SD	-	0.007	0.005	0.006
% RSD	-	0.909 %	0.652 %	0.799 %

Table. No. 07: Interday Precision data

Intraday Precision –

Sr. No.	Concentration $\mu\text{g/ml}$	Abs. Morning	Abs. Afternoon	Abs. Evening
1	10	0.848	0.840	0.847
2	10	0.846	0.843	0.849
3	10	0.843	0.847	0.843
4	10	0.846	0.848	0.846
5	10	0.847	0.846	0.846
6	10	0.846	0.846	0.848
Average	-	0.846	0.845	0.846
SD	-	0.001	0.002	0.002
% RSD	-	0.198 %	0.351 %	0.245 %

Table. No. 08: Intraday Precision data

To assess the precision, intra-day study was conducted by measuring the absorbance of the working standard solution at concentration of 10 $\mu\text{g/mL}$ at six different time points within same day. The %RSD calculated was found to be 0.264 % for intra-day precision and 0.79% for inter- day precision.

5) Robustness:

The change in concentration i.e. 20 $\mu\text{g/ml}$ and obtained results shown that there is negligible effect on results. The result of robustness was found to be in boundary i.e. the relative standard deviation is less than 2.0 %. Hence the performed parameter was found to be validated.

Sr. No.	Concentration ($\mu\text{g/ml}$)	Absorbance (A) Change in solvent (8:2) Methanol : Distilled water	Absorbance (B) Change in solvent (8:2) Methanol : Distilled water
1	20 $\mu\text{g/ml}$	1.494	1.465
2	20 $\mu\text{g/ml}$	1.355	1.750
3	20 $\mu\text{g/ml}$	1.445	1.865
4	20 $\mu\text{g/ml}$	1.505	1.505
5	20 $\mu\text{g/ml}$	1.205	1.494
6	20 $\mu\text{g/ml}$	1.490	1.355
Average	-	1.415	1.572
SD	-	0.117	0.193
% RSD	-	0.827 %	0.122 %

Table. No. 09: Robustness data

6) Ruggedness:

The change in analyst at concentration of 15 µg/ml showed that the obtained result does not affected by it. Two different analyst analyze the same concentration solution to give following results.

Sr. No.	Concentration (µg/ml)	Absorbance (Analyst 1)	Absorbance (Analyst 2)
1	15 µg/ml	1.193	1.192
2	15 µg/ml	1.192	1.190
3	15 µg/ml	1.190	1.185
4	15 µg/ml	1.192	1.193
5	15 µg/ml	1.194	1.194
6	15 µg/ml	1.183	1.183
Average	-	1.1906	1.1908
SD	-	0.003	0.005
% RSD	-	0.335 %	0.479 %

Table. No. 10: Ruggedness data

7) Solution Stability Data :

Sr. No.	Concentration (µg/ml)	Fresh stock solution	Old stock solution
1	2	0.149	0.15
2	2	0.152	0.151
3	2	0.148	0.147
4	2	0.151	0.149
5	2	0.147	0.152
6	2	0.15	0.154
Average	-	0.152	0.151
SD	-	0.005	0.002
% RSD	-	1.25 %	1.54 %

Table. No. 11: Solution Stability data

The stability study of the stored standard solution and test preparation were performed and solutions were found to be stable for up to 48 hrs. The assay values obtained after 36 hr. were statistically identical with the initial value without measurable loss.

8) Limit of Detection :

$$DL = 3.3 \sigma/S = 3.3 \times 0.157 / 0.176 = 2.94 \mu\text{g/ml.}$$

Where,

σ is the std. deviation of response S is the slope of calibration Curve DL is the limit of detection.

The limit of detection was found to be 2.94 $\mu\text{g/ml}$.

9) Limit of Quantification :

$$DL = 10 \sigma/S = 10 \times 0.157 / 0.176 = 8.92 \mu\text{g/ml.}$$

Where,

σ is the std. deviation of response S is the slope of calibration Curve DL is the limit of detection.

The limit of quantification was found to be 8.92 $\mu\text{g/ml}$.

10) Assay of Marketed formulation :

2 tablet were weighed from which a powder equivalent to 1 mg of colchicine was taken in 100 ml volumetric flask and it was dissolved in 50 ml of solvent by intermediate shaking of the flask for about 20 min and diluted up to the mark with the solvent. Then the solution was filtered using Whatmann filter paper No. 40 and make up to the volume by using solvent. From this filtrate 5 ml is pipetted and make up to 10 $\mu\text{g/ml}$ and scanned for absorbance and the UV result was indicated by % Purity.

Drug Name	Brand Name	Label Claim (mg)	Amt. Prepared (mg)	Amt. Found (mg)	% Assay
Colchicine	Zycolchin	0.5 mg	0.5 mg	0.493 mg	98.60 %

Table. No. 12: Assay of Marketed Formulation.

11) UV Spectra of Marketed formulations:

The developed UV method was used for analysis of marketed tablet dosage form (Brand name -Zycolchin). The sample solution was prepared at concentration of 10 $\mu\text{g/mL}$ using water as solvent and its absorbance was measured at 246 nm. The concentration of sample solution was back calculated from the linear equation. It was found to be 98.60%.

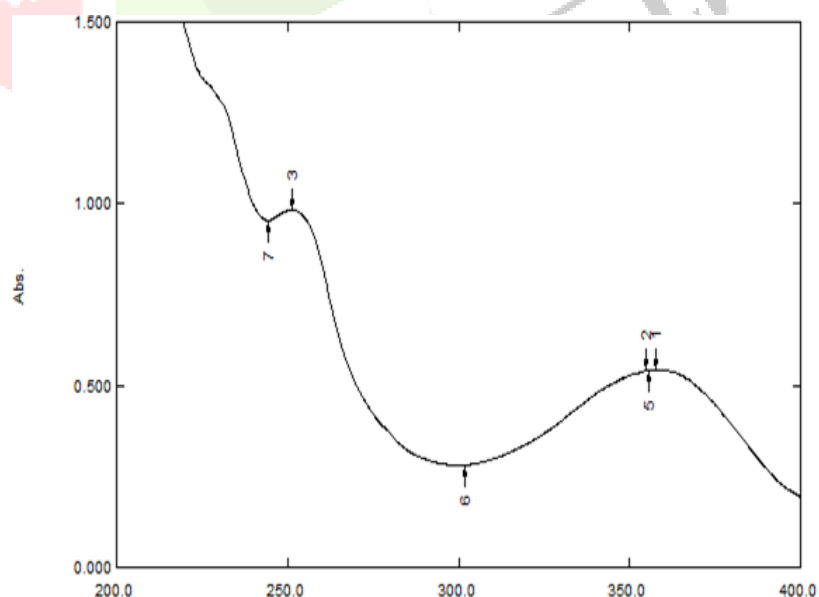


Fig. No. 10: UV Spectra of Zycolchin.

VII. Conclusion

The UV method developed for estimation of *Colchicine* in bulk and tablet dosage forms is simple, accurate, precise and sensitive. The usage of water as solvent let it to be more economic method. The proposed method was also successfully applied for quantitative estimation of marketed tablet dosage form and hence can be used for routine analysis.

The present analytical method was validated as per ICH Q2(R1) guideline and it meets to specific acceptance criteria. The analytical approach was determined to be specific, precise, linear, accurate, robust, and having stability suggesting features. The current analytical technique can be applied to the desired outcome. According to ICH guidelines, a UV-visible spectrophotometric method for evaluating *Colchicine* in a single dosage form utilising distilled water as a solvent has been developed and confirmed. The benefits of the proposed method for analytical purposes are fast determination, cost-effectiveness, easy sample preparation, good reproducibility, simple, economical, accurate and practical. Therefore, the proposed method for evaluating *Colchicine* may be recommended in routine quality assurance research in pharmaceutical industries. The developed method was simple, precise, accurate, highly sensitive, reproducible and inexpensive. The planned method was found to be appropriate for detection of *Colchicine* and it is also gives the satisfactory recovery of analyst, which can be simply applied for the analysis of *Colchicine* in pure form.

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