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UV SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF PURE NICOTINE

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Abstract

Ultraviolet (UV) spectrophotometry is a simple, precise, and economical analytical technique widely used for quantitative estimation of organic and pharmaceutical compounds. The present study focuses on the development and validation of a UV spectrophotometric method for the estimation of pure nicotine. Nicotine, a tertiary amine alkaloid primarily found in tobacco leaves, possesses characteristic UV absorbance due to its conjugated pyridine and pyrrolidine rings, exhibiting a maximum absorption wavelength (λmax) around 260 nm. Methanol was selected as the optimal solvent because of its high transparency in the UV region and excellent solubility for nicotine. The proposed method was validated according to the International Council for Harmonisation (ICH) Q2(R2) guidelines by assessing parameters such as linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), robustness, and ruggedness. The calibration curve demonstrated good linearity within the range of 2–12 µg/mL with a correlation coefficient (r^2) greater than 0.998. Recovery studies confirmed the accuracy of the method, while %RSD values below 2% indicated excellent precision. The LOD and LOQ were found to be within acceptable limits, establishing the method's sensitivity and reliability. The results confirm that the developed UV spectrophotometric method is simple, accurate, reproducible, and suitable for routine quality control analysis of nicotine in pure and pharmaceutical formulations.

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Introduction

Ultraviolet (UV) spectrophotometry remains a widely used analytical approach for rapid, cost-effective quantification of small organic molecules because it requires relatively simple instrumentation, minimal sample preparation and—when applicable-provides acceptable sensitivity and precision for routine assays [1].

Nicotine is the principal alkaloid found in tobacco leaves and is largely responsible for the pharmacological and addictive properties of tobacco products [2]. Structurally, nicotine is a tertiary amine composed of a pyridine and a pyrrolidine ring, giving rise to its characteristic UV absorbance bands. Because of its toxic and psychoactive nature, accurate quantitative determination of nicotine is essential in the analysis of tobacco, pharmaceutical formulations, biological fluids, and nicotine-replacement products [3, 4].

Among several analytical methods, UV spectrophotometry has gained importance as a simple, economical, and rapid technique for the estimation of nicotine in pure and formulated forms [5]. The method is based on the absorption of ultraviolet radiation (200–400 nm) by nicotine molecules, where the absorbance follows Beer–Lambert's law, relating absorbance to concentration [6]. The wavelength of maximum absorbance (λmax) for nicotine generally lies between 259–262 nm, depending on solvent and pH conditions [7]. This characteristic absorption makes UV spectrophotometry suitable for routine estimation and quality control analysis.

Recent review studies have highlighted the role of spectrophotometric methods as valuable alternatives to chromatographic techniques (like HPLC and GC) when rapid screening and cost-effectiveness are desired [8, 9]. Although chromatographic methods provide higher specificity and sensitivity, UV spectrophotometry remains practical for laboratories with limited resources and is frequently used in

developing countries for preliminary quantification of nicotine content in raw materials or formulations [10].

The development and validation of UV spectrophotometric methods for nicotine estimation are carried out following International Council for Harmonisation (ICH) guidelines, assessing parameters such as linearity, accuracy, precision, and limit of detection [11].

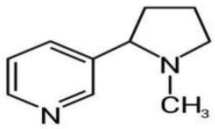

Fig 1: Chemical Structure of Nicotine

Chemical name	:	Nicotine
Molecular formula	:	$C_{10}H_{14}N_2$
Molecular weight (molar mass)	:	162.23 g/mol
Colour	:	Colourless to pale yellow (darkens on exposure to light Or air due to oxidation)
Physical state	:	Oily liquid (alkaloid)
Taste	:	Burning, acrid, bitter
Odour	:	Characteristic, tobacco-like, Pungent.
Structure	:	Contains a pyridine ring and Pyrrolidine ring- both Nitrogen-containing Heterocycles.

Structural Description

Nicotine is composed of two linked rings: A pyridine ring (aromatic nitrogen-containing ring).

Aim and Objectives

To develop and validate a simple, accurate, precise, and cost-effective UV spectrophotometric method for the estimation of pure nicotine in accordance with ICH Q2 (R2) guidelines.

Objectives

1. To study the UV absorption characteristics of pure nicotine and determine its λmax in selected solvents.
2. To optimize solvent conditions and prepare calibration standards for quantitative analysis.
3. To validate the developed method by evaluating linearity, accuracy, precision, LOD, LOQ, robustness, and ruggedness.
4. To assess the applicability of the method for routine estimation and quality control of nicotine in pharmaceutical and analytical laboratories.
5. To evaluate robustness and ruggedness of the method by introducing deliberate variations in analytical parameters such as wavelength and solvent composition.
6. To assess repeatability and intermediate precision to ensure the method's reproducibility within and between analytical runs.

Role of UV spectrophotometric method in UV analysis

The UV spectrophotometric method plays a crucial role in analytical chemistry, particularly for the qualitative and quantitative determination of various pharmaceutical compounds. It is one of the most widely used analytical techniques due to its simplicity, sensitivity, accuracy, and cost-effectiveness [12, 13].

In UV analysis, this method is based on the absorption of ultraviolet light (200–400 nm) by molecules containing π-electrons or non-bonding electrons, which undergo electronic transitions when exposed to UV radiation [14]. The amount of light absorbed at a particular wavelength is directly proportional to the concentration of the analyte, following Beer–Lambert's law [15].

Furthermore, the method serves as an essential preliminary analytical tool before employing advanced chromatographic or spectroscopic methods, as it allows quick screening of samples and determination of λmax, which is vital for method development and validation [16].

Principle of UV Spetrophotometric Method

The basic principle of ultraviolet (UV) absorption spectroscopy is based on the interaction between ultraviolet radiation (200–400 nm) and matter, where certain molecules absorb energy and undergo electronic transitions between molecular orbitals [17]. When UV light passes through a sample, part of the radiation is absorbed by electrons, causing promotion from a ground state (π or n orbital) to an excited state (π*, σ*, or n*) 18].

The amount of light absorbed depends on the nature of the molecule, particularly the presence of chromophores (light-absorbing groups) and auxochromes (groups that modify absorption intensity or wavelength) [19]. Typical chromophores include double bonds, carbonyl groups, and aromatic rings, which contain delocalized π electrons that can easily absorb UV light [20].

The quantitative relationship between absorbance and concentration is described by the Beer–Lambert law, which states that absorbance (A) is directly proportional to the concentration (C) of the absorbing species and the path length (l) of the cell:

$$A = \varepsilon cl,$$

Where ε is the molar absorptivity [21].

This principle forms the foundation of UV-visible spectrophotometry, enabling the identification and quantification of compounds based on their characteristic absorption maxima (λmax) [22].

Beer-Lamberts Law

Beer–Lambert's Law, also known as Beer's Law, describes the linear relationship between the absorbance (A) of light by a substance and its concentration (c) in solution as well as the path length (l) of the sample cell. It is mathematically expressed as:

$$A = \varepsilon c l$$

where A is absorbance (unitless), ε is the molar absorptivity ($L \bullet mol^{-1} \bullet cm^{-1}$), c is the concentration ($mol \bullet L^{-1}$), and l is the path length (cm).

This law forms the fundamental basis of UV–Visible spectrophotometry, as it allows the determination of unknown concentrations of analytes by measuring their absorbance at specific wavelengths. The principle assumes that the system follows linearity-that is, absorbance is directly proportional to both concentration and path length under ideal conditions [23, 24].

However, deviations from Beer–Lambert's Law may occur due to instrumental limitations, chemical interactions, high concentrations, or stray light [25]. Proper calibration and wavelength selection are therefore crucial in UV–Vis spectrophotometric analysis.

Absorption Maxima (λmax) for Nicotine

Nicotine, a tertiary amine alkaloid, exhibits characteristic ultraviolet (UV) absorption due to its conjugated aromatic structure. The molecule contains a pyridine ring and a pyrrolidine ring, both of which contribute to its electronic transitions. These chromophoric groups undergo π→π* and n→π* electronic transitions when exposed to UV light, giving rise to measurable absorbance in the UV region.

The absorption maximum (λmax) of nicotine is generally found between 259–262 nm, depending on the solvent and pH conditions (26). The π→π* transitions are mainly responsible for the strong absorption observed near 260 nm, attributed to the aromatic pyridine moiety in the nicotine structure [27, 28].

In methanol and ethanol solutions, the λmax is typically observed around 259–260 nm, while in aqueous and phosphate buffer solutions, it may shift slightly to 261–262 nm due to solvent polarity and hydrogen bonding effects(29)(30) .

The λmax value is of analytical importance because it represents the wavelength at which nicotine shows maximum absorbance, ensuring maximum sensitivity and accuracy in quantitative UV spectrophotometric determination. Therefore, most analytical and pharmaceutical studies use 260 nm as the selected analytical wavelength for the estimation of pure nicotine and its formulations [29-31].

Materials and Reagents

Chemicals used

1. Nicotine used:

Pure nicotine (≥99% purity) is used as the reference standard. It is usually obtained from certified suppliers such as Sigma-Aldrich or Merck. The pure compound is weighed accurately and dissolved in a suitable solvent to prepare stock and working solutions [32, 33].

2. Solvents

Methanol (UV grade): Most frequently used solvent due to its high transparency in the UV region and excellent solubility for nicotine [33, 34] .

Ethanol (AR grade): Serves as an alternative solvent in several analytical methods [35].

Phosphate Buffer (pH 7.4): Employed to study the behavior of nicotine at physiological

3. Reagents

Hydrochloric Acid (HCl, 0.1 N): Used for pH adjustment or to aid solubility of nicotine during preparation [34].

4. Other Laboratory Materials

Volumetric flasks, pipettes, and beakers for solution preparation Whatman filter paper or 0.45 µm membrane filters to remove impurities. Amber glassware is often used to prevent degradation of nicotine, which is light-sensitive [34, 35].

Solvent selection

In UV spectrophotometric analysis of pure nicotine, the choice of solvent significantly influences the accuracy and reliability of the estimation. Methanol is commonly employed due to its ability to dissolve nicotine effectively and its minimal interference in the UV region. For instance, a study by Navapariya et al. (2025) utilized methanol as the solvent and reported a maximum absorbance wavelength (λmax) for nicotine at 261 nm, with a linear concentration range of 2–12 µg/mL and a regression coefficient of 0.998 [3].

Additionally, the solvent's polarity can affect the UV absorption characteristics of nicotine. Research by Sah et al. (2025) demonstrated that nicotine exhibits different UV absorption spectra in various solvents. In carbon tetrachloride (CCl_4), nicotine showed a maximum absorbance at approximately 259 nm, whereas in dimethyl sulfoxide (DMSO), the λmax shifted to around 251 nm.

Therefore, selecting an appropriate solvent is crucial for accurate nicotine quantification. Methanol is a preferred choice [37], for its effectiveness and minimal interference, but alternative solvents like CCl_4 and DMSO may be considered depending on specific analytical requirements.

Preparation of Reagents

- All chemicals used were of analytical-reagent grade unless stated otherwise. Doubly distilled water was used throughout.
- Nicotine and total alkaloids. These were extracted from tobacco leaves and standardized spectroscopically.
- Bromine water. A saturated solution of bromine in water was prepared. This solution was prepared daily.
- Formic acid, 90% solution.
- Potassium iodide, 1 % solution.
- Starch solution. A 100 mg amount of soluble starch was made into a paste with a few drops of hot water and diluted to 100 ml using nearly boiling water.
- Zinc acetate solution. A mixture of 21.6 of zinc acetate and 3 ml of acetic acid was diluted to 100 ml with water.
- Potassium hexacyanoferrate (rI), 10.6% mlv. A 1.06 g amount of potassium hexacyanoferrate (rr) was dissolved in 10 ml of water [38].

UV- Visible Spectrophotometer Details

Introduction

UV–Visible spectrophotometry is one of the most widely used analytical techniques for qualitative and quantitative determination of chemical substances that absorb light in the ultraviolet (200–400 nm) and visible (400–800 nm) regions of the electromagnetic spectrum. The method is based on the measurement of the intensity of light absorbed by a sample solution at a specific wavelength compared to a reference or blank solution [39, 40].

Principle

Where ε is the molar absorptivity coefficient [41].

When monochromatic light passes through a homogeneous medium, part of the light is absorbed, and the remaining is transmitted. The amount of light absorbed depends on the molecular structure and concentration of the analyte [42].

Instrumentation

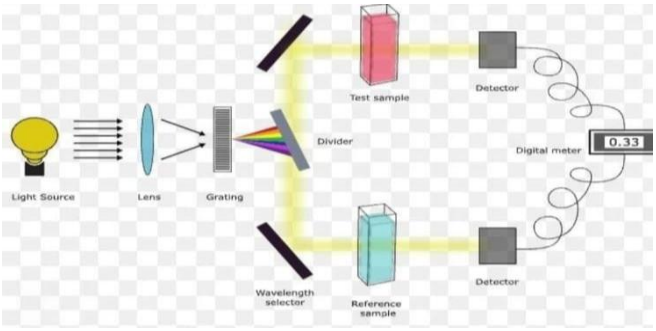

Fig 2: Image of UV–Visible spectrophotometer.

A UV–Visible spectrophotometer consists of the following main components:

Light source: Deuterium lamp for UV region and tungsten/halogen lamp for visible region.

Monochromator: To isolate a specific wavelength of light using prisms or diffraction gratings.

Sample holder/cuvette: Usually made of quartz for UV and glass for visible region.

Detector: Converts transmitted light into electrical signals (photodiode or photomultiplier tube).

Readout device: Displays absorbance or transmittance digitally [43, 44].

Instrumental parameters and specifications:

1. Wavelength Range:

A UV–Visible spectrophotometer typically operates in the range of 190–1100 nm.

The UV region covers 190–400 nm, while the visible region spans 400–800 nm.

The deuterium lamp is used for UV radiation, and the tungsten/halogen lamp for visible light [44, 45].

2. Spectral Bandwidth (Slit Width):

The spectral bandwidth determines the resolution of the spectrophotometer.

Typical bandwidths are 0.5–5.0 nm.

A narrower slit width provides higher resolution but reduces light intensity, while a wider slit increases intensity but decreases resolution [45].

3. Photometric Range and Accuracy:

Absorbance range: –0.3 to 3.0 Abs (depending on model).

Photometric accuracy: ±0.002 Abs or better.

Photometric repeatability: ±0.001 Abs.

4. Wavelength Accuracy and Repeatability:

Wavelength accuracy ensures correct wavelength calibration; it typically ranges within ±0.3 nm.

Wavelength repeatability is within ±0.1 nm, providing reliable reproducibility in repeated measurements [46].

Analytical Method Validation Parameters

Accuracy

The accuracy of method was determined using the standard addition method at three various levels: 50%, 100%, and 150%. Standard addition may be done through the standard amount addition to the sample for each level. Starting concentration is the standard stock of 100 µg/ml; 0.4 ml was taken then diluted to 10 ml to produce 4µg/ml solution.(47) From this solution, 5ml was taken and from prepared 8 µg /ml solution 5ml was taken and added to the first resulting in a concentration of 6µg/ml, which is equivalent to 50% addition of the original 4µg/ml solution. 100% addition was performed by combining it with the original 4 µg/ml solution to result in a 12µg/ml solution. For the addition of 150%, a 16µg/ml solution was added to the 4µg/ml solution.

Precision

Precision is the degree of agreement among individual test results when a method is applied repeatedly to multiple samplings of a homogeneous sample under prescribed conditions (48). It reflects the closeness of repeated measurements and is usually expressed as the standard deviation (SD) or percent relative standard deviation (%RSD) of a series of observations.

According to ICH Q2 (R2) guidelines, precision is evaluated at three levels: repeatability, intermediate precision, and reproducibility.

1. Repeatability (Intra-day Precision)

Repeatability refers to the precision under the same operating conditions over a short time interval by the same analyst using the same equipment (49). It indicates the method's stability during routine use. For UV spectrophotometric analysis, repeatability is assessed by preparing and analyzing six replicates of a single concentration of nicotine solution. The %RSD is then calculated using the formula:

$$\text{\%RSD} = \frac{\text{Standard Deviation}}{\text{Mean}} \times 100$$

A %RSD value of ≤2% is generally considered acceptable for assay-level concentrations.

2. Intermediate Precision (Inter-day Precision)

Intermediate precision evaluates variations within laboratories, such as differences between days, analysts, or instruments (50). This ensures that the analytical method remains consistent under varying but controlled conditions. In nicotine analysis, similar samples are analyzed by two different analysts or on different days, and %RSD values are compared. Typically, %RSD ≤2% demonstrates satisfactory intermediate precision.

3. Reproducibility (Between-Laboratory Precision):

Reproducibility represents precision between laboratories (inter-laboratory studies) and is essential for methods intended for transfer or regulatory us [51]. This parameter verifies that the method yields comparable results under variable laboratory environments.

Linearity

Linearity is an essential parameter in the validation of an analytical method. It evaluates the ability of the method to elicit test results that are directly proportional to the concentration of analyte within a specified range. In UV spectrophotometry, linearity is assessed by analyzing standard solutions of the analyte at different concentrations and plotting the absorbance against concentration to obtain a calibration curve. The relationship should ideally follow Beer–Lambert's law, which states that absorbance is directly proportional to concentration within a certain range [52].

The correlation coefficient (r^2) obtained from the calibration curve is used to determine the degree of linearity; an r^2 value close to 1.000 indicates good linearity. Typically, a linear range is chosen where the absorbance values fall between 0.2 and 1.0 to ensure accurate measurements.

A linear calibration curve ensures that the analytical method can reliably quantify the analyte concentration in unknown samples, which is critical for both qualitative and quantitative analysis [53]. The assessment of linearity is carried out according to ICH Q2 (R1) guidelines for method validation [54].

Limit of detection (lod) and limit of quantitation (loq)

Limit of Detection (LOD)

The Limit of Detection (LOD) is the lowest amount of analyte in a sample that can be detected but not necessarily quantified under the stated experimental conditions. It is an important validation parameter used to assess the sensitivity of an analytical method(55). LOD is generally determined based on the signal-to-noise ratio (S/N), often considered as 3:1, or using statistical methods derived from the standard deviation of the response and the slope of the calibration curve (LOD = 3.3 × σ/S), where σ is the standard deviation of the response and S is the slope of the calibration curve .

LOD ensures that the analytical method can identify even trace amounts of the analyte, which is particularly crucial in pharmaceutical and environmental analysis.

Limit of Quantitation (LOQ)

The Limit of Quantitation (LOQ) is defined as the lowest concentration of analyte that can be determined with acceptable precision and accuracy under the stated conditions. It indicates the method's quantitative capability and is typically calculated using the equation LOQ = 10 × σ/S, corresponding to a signal-to-noise ratio of 10:1 [56].

LOQ is essential to ensure that the analytical method provides reproducible and reliable results for low analyte concentrations, especially in drug assays and trace impurity determination [57].

Robustness and Ruggedness

Robustness

Different concentrations of nicotine solution like (4 µg/ml, 6 µg/ml 8 µg/ml) was analysed at different wave length that includes 259nm, 260nm,261nm 262nm, 263nm to determine robustness of the method. Robustness is a measure of an analytical method's capacity to remain unaffected by small but deliberate variations in method parameters, indicating its reliability during normal usage. It assesses how method performance is influenced by minor changes such as pH, temperature, wavelength, or mobile phase composition. A robust method ensures consistent analytical results under varied conditions, which is crucial for reproducibility and quality assurance in analytical laboratories [58].

In UV spectrophotometric analysis, robustness can be evaluated by altering the wavelength slightly (±1–2 nm), changing solvent composition, or modifying sample preparation conditions to verify that absorbance and calculated concentrations remain consistent.

Ruggedness

Ruggedness refers to the degree of reproducibility of test results obtained under varied conditions, such as different analysts, instruments, laboratories, or days. It demonstrates the method's reliability across different environments and operators. Ruggedness testing is essential to confirm the method's suitability for routine use in various laboratory settings [59].

For example, in UV spectrophotometric estimation of nicotine, ruggedness may be tested by analyzing samples using two different spectrophotometers or by two different analysts to ensure the results remain comparable.

The reproducibility of a result under typical expected operating conditions varies from instrument to instrument and analyst to analyst, and this is measured by ruggedness. Experiment [60], was carried out employing several approaches to ascertain the method's robustness.

Discussion

Absorption Spectra of Nicotine

Nicotine, an alkaloid found predominantly in tobacco plants, exhibits characteristic absorption in the ultraviolet (UV) region due to the presence of conjugated double bonds and nitrogen-containing heterocyclic rings in its structure.

In UV–visible spectrophotometric studies, nicotine shows a prominent absorption maximum (λmax) in the range of 259–262 nm when dissolved in ethanol or aqueous solvents. This absorption is attributed mainly to the $\pi \rightarrow \pi^*$ electronic transitions in the pyridine ring [61]

The intensity and position of the absorption peak can vary slightly depending on solvent polarity, pH, and sample concentration. The linearity of absorbance with concentration in this region forms the basis for the quantitative estimation of nicotine using UV spectrophotometry.

The absorption spectra typically show a sharp peak around 260 nm, confirming the electronic transitions associated with aromatic chromophores present in nicotine. This spectral behavior is consistent across pure samples and tobacco extracts analyzed under controlled laboratory conditions.

The absorption spectra typically show a sharp peak around 260 nm, confirming the electronic transitions associated with aromatic chromophores present in nicotine. This spectral behavior is consistent across pure samples and tobacco extracts analyzed under controlled laboratory conditions [62].

Calibration Curve Data

A calibration curve is a graphical representation that shows the relationship between the concentration of an analyte and its

corresponding absorbance (or response). It is used to determine unknown concentrations based on the principle that absorbance is directly proportional to concentration as per Beer–Lambert's law.

In UV spectrophotometric analysis, the calibration curve is constructed by:

1. Preparing a series of standard solutions of known concentrations.
2. Measuring the absorbance of each solution at the analyte's λmax (wavelength of maximum absorbance).
3. Plotting absorbance vs. concentration to obtain a straight line.
4. Determining the correlation coefficient (R^2), slope, and intercept from the regression equation [63].

A correlation coefficient (R^2) value close to 1 indicates excellent linearity and reliability of the method.

The calibration curve data are essential for quantitative analysis, method validation, and determination of unknown samples.

Table 01: Estimation of Nicotine in Pharmaceutical Formulations.

Method	Principle/technique	Sample types/Matrices	Key validation parameters/advantages
Micellar liquid chromatography with electrochemical detection.	Use of a C18 column, mobile phase with sodium dodecyl sulphate + pentanol etc., detection via electrochemical cell.	Pharmaceuticals such as chewing gum, dermal patches; also biological fluids.	Short analysis time (<8 min), linearity >0.999, LOD ~4 ng/mL, LOQ ~12 ng/mL, precision/repeatability <1.8 %.
RP-HPLC (Reversed Phase High-Performance Liquid Chromatography	Standard chromatographic separation (C18) + UV detection, etc.	Bulk nicotine, immediate-release and [64], extended-release dosage forms (e.g. gums)	Very good specificity vs excipients, linearity (r ~0.9993), accuracy ~100 %, acceptable retention time, etc.
UV-Visible Spectrophotometry	Nicotine absorbs at a specific λ (e.g. 261 nm), simple dilution, often in methanol	Pure nicotine; simple formulations / pure drug form	Linearity over µg/mL (e.g. 2-12 µg/mL), R^2 ~0.998; precision & accuracy within acceptable limits; LOD/LOQ ~0.4 / ~2.5 µg/mL
Spectrophotometric Reaction (Redox / Colorimetric)	Reaction of nicotine with e.g. potassium permanganate + base → colored product measured at certain λ	Nicotine in solution; possibly pharmaceutical formulations with minimal interfering substances	Beer's law obeyed over ~0.1-7.5 µg/mL; LOD ~0.08 µg/mL; RSD ~2.2 % for replicate measurements
TLC- / HPTLC-Densitometry	Thin layer chromatography (silica plates) + densitometric detection (absorbance/spot size)	Tobacco products or possibly formulated samples; nicotine and/or cotinine	Good separation, R_f reproducible, correlation >0.99; useful in simpler laboratories without expensive equipment

Applications

1. Estimation in tobacco products or e-liquids

Introduction Estimation of nicotine in tobacco products and e-liquids is essential for quality control, safety assessment, and regulatory compliance. Analytical techniques such as UV spectrophotometry, gas chromatography (GC), and high-performance liquid chromatography (HPLC) are widely employed for quantitative determination of nicotine due to their accuracy and sensitivity.

2. Analytical Techniques

1. **UV Spectrophotometry**: A simple and cost-effective method based on measuring absorbance of nicotine at a specific wavelength (usually around 260 nm). It is suitable for rapid screening and routine analysis of tobacco extracts and e-liquid samples.
2. **High-Performance Liquid Chromatography (HPLC)**: A highly accurate and precise technique for estimating nicotine concentration in complex matrices like e-liquids and cigarette tobacco.

Gas Chromatography (GC): Used when nicotine needs [65], to be separated from other volatile components in tobacco smoke or vapor samples.

Mass Spectrometry (MS): Often coupled with GC or HPLC for confirmation and quantification of nicotine at trace levels.

3. Applications

- Determination of nicotine concentration in cigarettes, cigars, chewing tobacco, and e-liquids.
- Assessment of nicotine delivery in vaping products for public health research.
- Quality control during manufacturing of nicotine replacement therapies and e-liquid formulations.
- Evaluation of nicotine degradation during storage or heating in electronic cigarettes.

Advantage and limitaations of uv spectrophotometry

Benefits of the method

Ultraviolet (UV) spectrophotometry is one of the most widely used analytical techniques in pharmaceutical, biochemical, and environmental laboratories due to its simplicity, sensitivity, and cost-effectiveness. The following are the major benefits:

1. Simplicity and Ease of Use

UV spectrophotometry requires minimal sample preparation and straightforward instrumentation. It allows rapid analysis without the need for complex separation steps or expensive reagents, making it ideal for routine quality control and preliminary screening of compounds [66].

2. Cost-Effectiveness

Compared with chromatographic techniques such as HPLC and GC, UV spectrophotometry offers a more economical alternative, both in terms of equipment cost and operational expenses. This makes it particularly suitable for laboratories with limited resources or for large-scale routine analysis.

3. Rapid and Non-Destructive Measurement

The method provides quick results, often within minutes, and can be performed without destroying the sample, which is beneficial when limited quantities of the analyte are available.

4. Good Sensitivity and Accuracy

Modern UV spectrophotometers offer high precision and sensitivity in the microgram per milliliter range, which is adequate for most pharmaceutical formulations and biological samples.

5. Versatility

UV spectrophotometry can be applied to a wide range of compounds that absorb UV radiation, including drugs, natural products, and biomolecules. It is also used for kinetic studies, impurity profiling, and stability testing.

6. Compliance with Validation Standards

The technique is easily validated under ICH guidelines (Q2R1/Q2R2) for parameters such as accuracy, precision, linearity, robustness, and detection limits, ensuring regulatory acceptance for pharmaceutical applications [67].

7. Environmental Friendliness

The method uses small volumes of relatively safe solvents, reducing chemical waste and making it an environmentally sustainable option compared to chromatographic methods.

Possible Interferences and Drawbacks

1. Interference from Co-absorbing Species

Other compounds present in the sample that absorb at or near the analytical wavelength (λmax) can interfere with measurements, leading to overestimation or underestimation of the analyte. This is a common limitation when analyzing complex mixtures such as herbal extracts, biological fluids, or multi-component formulations in.

2. Solvent Effects

The choice of solvent can significantly affect the absorption spectrum of the analyte. Solvent polarity, hydrogen bonding, and pH can cause shifts in λmax or alter molar absorptivity, potentially leading to inaccurate quantification if not properly controlled [68].

3. Low Specificity

UV spectrophotometry cannot differentiate between compounds with similar chromophores. Unlike chromatographic methods (HPLC or GC), it lacks the separation step, which limits its use in samples containing multiple UV-absorbing substances.

4. Deviations from Beer–Lambert Law

At high concentrations, UV absorbance may deviate from linearity due to molecular interactions, aggregation, or stray light, affecting the reliability of quantitative analysis [69-71].

5. Sample Degradation

Some analytes are light-sensitive or chemically unstable in solution. Exposure to UV light or air during analysis can cause degradation, leading to inaccurate results.

6. Limited Sensitivity

Although suitable for most routine assays, UV spectrophotometry generally has lower sensitivity compared to chromatographic or mass spectrometric techniques, making it less suitable for trace-level detection.

7. Instrumental Limitations

Factors such as wavelength accuracy, slit width, stray light, and photometric noise can introduce errors if the instrument is not properly calibrated and maintained.

Conclusion

A simple, accurate, and precise UV spectrophotometric method was successfully developed and validated for the estimation of pure nicotine. The method exhibited good linearity (2–12 µg/mL), high accuracy, and %RSD values below 2%, confirming excellent precision. LOD and LOQ values demonstrated adequate sensitivity, and robustness studies showed reliability under minor variations. This method is rapid, cost-effective, and suitable for routine quality control of nicotine in pure and pharmaceutical formulations.

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Conflicts of Interest

The authors declare no conflicts of interest.

Author Contribution

Both contribute equally

Financial Support

None

Ethical Considerations and Informed Consent

Not Applicable

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