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Research Article

# UV Spectrophotometric Method for Estimation of Pure Nicotine

Vatsal Navapariya *, Milind M Thosar, Madhav Zinzuvadiya, Dharmesh Rathava, Dharmil Pathak

Faculty of Pharmacy, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India

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*Address for Correspondence:

Vatsal Navapariya, Faculty of Pharmacy, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India

Abstract

**Background**: Nicotine is an alkaloid that has high addictive and toxic properties. It can be found in all parts of tobacco plants (Nicotiana tobacum).

**Objective**: The aim of investigation was to develop a simple UV-visible Spectrophotometric method for the determination of Nicotine in its pure form further to validate the developed method.

**Material and Methods**: Nicotine was estimated using UV Visible double beam spectrophotometer at the wavelength of maximum absorption (261 nm) in Methanol. The drug was characterized by boiling point, and Infra-Red (IR) techniques. The analysis of the drug was carried out by novel UV-Visible method which was validated analytical parameters like linearity, precision, accuracy, robustness, ruggedness per guidelines laid down by International Conference on Harmonization (ICH).

**Result:** By the interpretation of spectra the drug was confirmed. The linear response for concentration range of 2–12 µg/ml of Nicotine was recorded with regression coefficient 0.998. The accuracy was found between 98–101 %. Precision for intraday and interday was found to be 0.886 and 1.06 respectively, which are within the limits. To establish the sensitivity of the method, limit of detection (LOD) and limit of quantification (LOQ) were determined which were found to be 0.422 µg/ml and 2.513 µg/ml respectively.

**Conclusion**: The UV method developed and validated for nicotine drug was found to be linear, accurate, precise and economical which can be used for the testing of its pharmaceutical formulations.

**Keywords:** Nicotine Estimation, Spectrophotometric Method, Analytical Method Development, Validation Parameters, Pharmaceutical Analysis, Linearity and Precision, LOD and LOQ Determination

## INTRODUCTION:

Nicotine (1-methyl-2-(3-pyridyl)pyrrolidine) is a drug obtained from the plant Nicotiana tabacum.

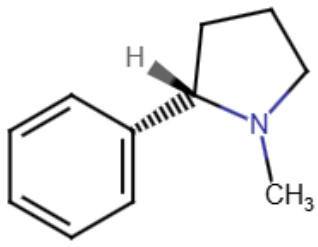


Figure 1: Structure of Nicotine

It is a colourless to pale yellow oily liquid with a tobacco-like odour. It is a highly addictive alkaloid found in all parts of the tobacco plant.[1] Specifically, Nicotine acts on nicotinic cholinergic brain receptors as well as other parts of the nervous system primarily by releasing or facilitating the production of a variety of neurotransmitters including dopamine, norepinephrine, serotonin, acetylcholine, vasopressin and beta endorphin.[2] The presence of both a pyrrolidine and a pyridine nitrogen means that nicotine is dibasic with p*K*a of 7.84 and 3.04 at 25 °C.[3]There are Very few spectrophotometric methods are available for the determination of nicotine. Smith and Cooke [4] They used di-ethyl-thio-barbituric acid as a spectrophotometric reagent for tobacco alkaloids. The absorption At 536 nm was measured and the method applied to the determination of nicotine in urine samples.[5] In this paper, a simple, UV spectrophotometric method is described for the determination of pure nicotine.

### Mechanism of Action:

Nicotinic acetylcholine receptors are ligand-gated cation channels that are widely distributed throughout the nervous system and body.[6] but this section will focus on neuronal nAChRs.[7] Nicotinic acetylcholine receptors are pentameric ligand-gated cation channels consisting of five different subunits that form a central aqueous pore which has an entry for cation inflow when activated by the receptor.[7,9] Each subunit has an extracellular N-

terminal end, the part which takes part in ligand binding; three hydrophobic transmembrane domains termed M1 to M3, an intracellular loop, a fourth hydrophobic transmembrane domain termed M4 and an extracellular C-terminus. The activation of nAChRs is achieved by the binding of the endogenous neurotransmitter acetylcholine or exogenous ligands like nicotine.[10,11]

## MATERIAL AND METHOD

### Material

Nicotine API was supplied by Nico Orgo Privet Ltd, Dakor, Gujrat, India as a gift sample. For standard plot Ultra Violet(UV) spectroscopy was used of double beam UV-1900i: SHIMADZU spectrophotometer. Finnpipette™ F3 1(0-100µL), UV chamber(ohmetron), TLC plate (Silica gel 60 $F_{254}$)

### Method

### Characterization of drug:

We have received COA from the supplier for our Nicotine API that includes all the confirmatory data related to API

### TLC (thin layer chromatography):

A thin-layer chromatography (TLC) method was employed for the alkaloid test using Dragendorff's reagent. The nicotine sample was applied to a TLC plate, and chloroform:methanol (19:1) was used as the mobile phase. After development, the plate was dried and sprayed with Dragendorff's reagent. The presence of nicotine was confirmed by the appearance of an orange or brown spot, indicating a positive result for alkaloids.

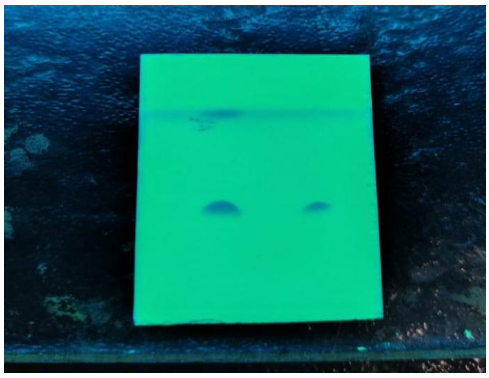

Figure 2: UV active image of spots of nicotine

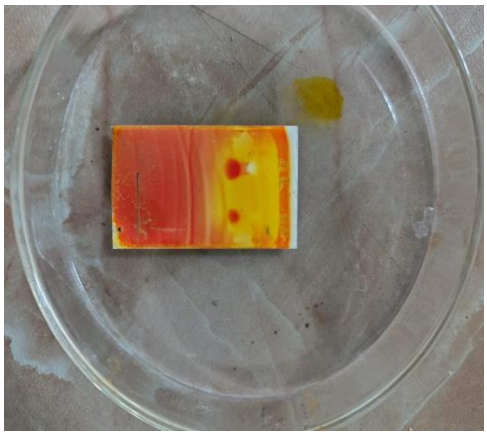

Figure 3: Dragendorff's active spots of nicotine

### Infrared Spectroscopy

The IR technique is used to measure the absorption of various infrared radiations by the target material, to produce an IR spectrum that can be used to identify functional groups and molecular structure in the sample. Then the FTIR spectra were recorded between 4000 and 400 $cm^{-1}$. The resolution was 2 $cm^{-1}$.

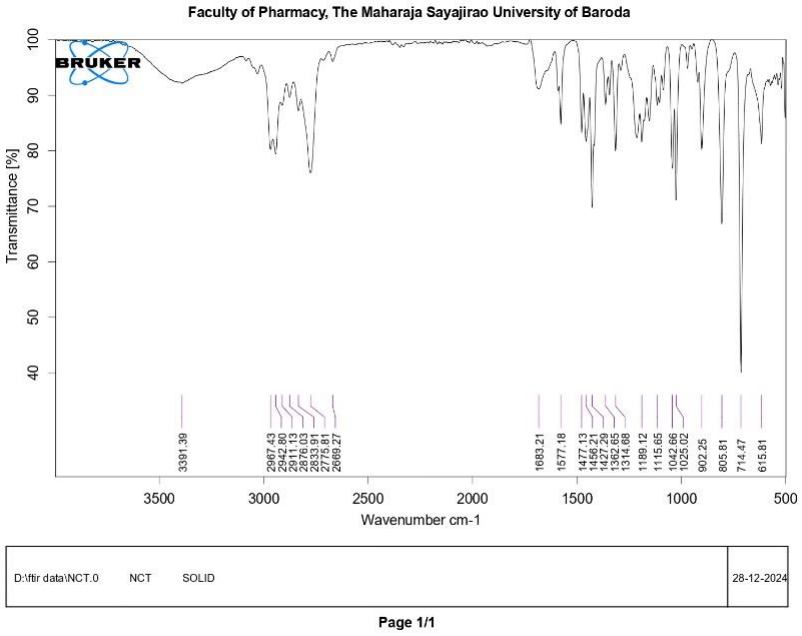


Figure 4: Infrared Spectra of Nicotine

### Method

### Stock solution

10 microliters pure (equivalent to 10 mg nicotine) Nicotine was accurately weighed and transferred into a 100 mL volumetric flask this will form 100 ppm stock solution.

### Determination of maximum wavelength

Nicotine 50 µg/ml solution was scanned in UV spectrophotometer within the wavelength range of 200–400 nm. Methanol solvent was used as blank.

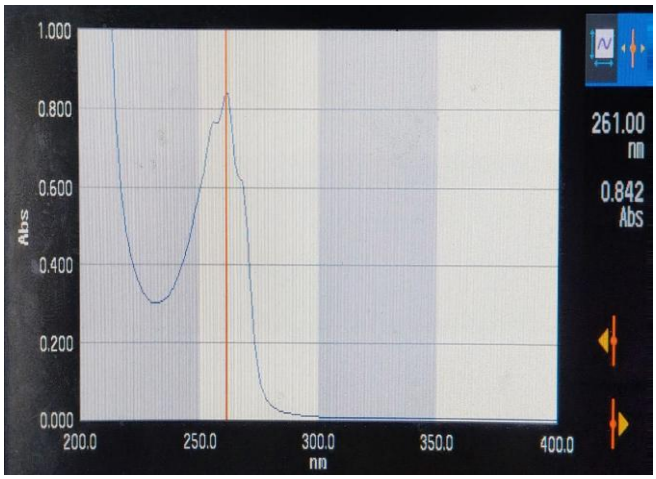


Figure 5: Maximum wavelength at 261nm.

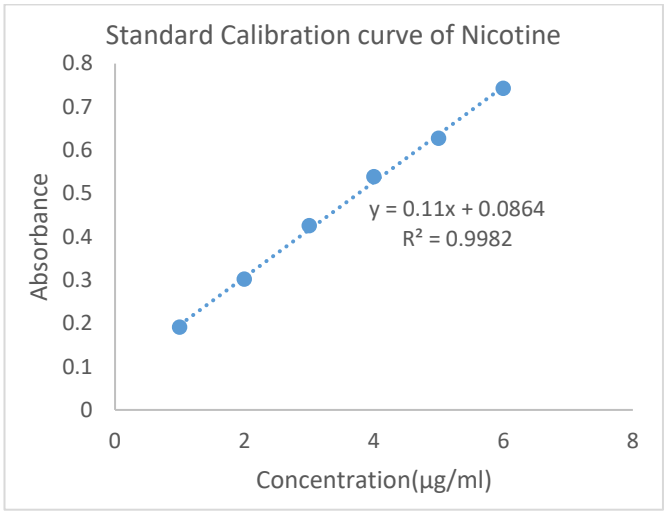


Figure 6: Standard calibration curve of Nicotine

### Preparation of standard plot/Calibration curve

- 0.4 mL of the stock solution was transferred to another volumetric Flask and volume made up to 10 ml with methanol in Achieve the outcome solution that equals 4 µg/ml.
- Dilutions were then prepared from the stock solution to Get 2 µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml, 10 µg/ml 12 µg/ml and solution concentration.
- methanol was taken as blank and absorbance of various dilutions these were measured at 261 nm.

### Analytical method validation

Validation can be said to be (ICH) setting documented proof, that gives a high assurance that a selected activity can systematically produce the desired result or product which meets its pre-set specifications and quality characteristics. The following parameters were evaluated for method validation.[12]

### Linearity and range

Using such an analytical methodology that offers linearity enabled it examine data that are directly proportional to the concentration of an analyte in simples placed within a given range towards determining the predicted methodology's linearity, 2-12 µg/ml of the Standard solution of Nicotine was prepared by using stock solution and examined. Every measurement was carried out three times.[13]

**Table 1:** Standard Calibration Curve of Nicotine

| Sr no. | Conc.( µg/ml) | Absorbance |
|---|---|---|
| 1 | 2 | 0.191 |
| 2 | 4 | 0.302 |
| 3 | 6 | 0.426 |
| 4 | 8 | 0.539 |
| 5 | 10 | 0.628 |
| 6 | 12 | 0.743 |

### Precision

The system precision is a measure of the Differences in method. It was estimated by performs three replicate analysis of the same working solutions, precision method was Demonstrated by intraday and interday variation studies. The intra-precision of the developed UV The method was determined by preparing the sample of Same batch in a decision with three Concentrations: 2,4,6 µg /ml and three replicates (n=3) each on same day at 1hours, 3hours, 6hours. The percent RSD of the results was used to evaluate the method precision. The interday was Determined by assaying the sample in triplicate (n=3) a day for three consecutive days.

**Table 2** Repeatability results in precision test for 4 µg/ml solution.

| Conc.( µg/ml) | Absorbance | Statistical Analysis |
|---|---|---|
| 4 | 0.355 | Mean = 0.3546 |
| 4 | 0.353 | SD = 0.00258 |
| 4 | 0.354 | %RSD = 0.73% |
| 4 | 0.357 | |
| 4 | 0.351 | |
| 4 | 0.358 | |

**Table 3** Repeatability results in precision test for 6 µg/ml solution.

| Conc.( µg/ml) | Absorbance | Statistical Analysis |
|---|---|---|
| 6 | 0.526 | Mean = 0.5296 |
| 6 | 0.524 | SD =0.003933 |
| 6 | 0.531 | %RSD = 0.743% |
| 6 | 0.530 | |
| 6 | 0.533 | |
| 6 | 0.534 | |

**Table 4** Repeatability results in precision test for 8 µg/ml solution.

| Conc.( µg/ml) | Absorbance | Statistical Analysis |
|---|---|---|
| 8 | 0.701 | Mean = 0.64016 |
| 8 | 0.705 | SD = 0.003933 |
| 8 | 0.708 | %RSD = 0.74% |
| 8 | 0.510 | |
| 8 | 0.509 | |
| 8 | 0.708 | |

**Table 5:** Intra-day Precision study

| Conc.( µg/ml) | Absorbance (10:00am) | Absorbance (12:00pm) | Absorbance (2:00pm) | Statistical Analysis |
|---|---|---|---|---|
| 4 | 0.261 | 0.262 | 0.258 | Mean = 0.3834 |
| 6 | 0.408 | 0.382 | 0.382 | Mean SD = 0.003388 |
| 8 | 0.510 | 0.505 | 0.502 | Mean %RSD =0.882% |

**Table 6:** Inter-day Precision study

| Conc.( µg/ml) | Absorbance (Day 1) | Absorbance (Day 2) | Absorbance (Day 3) | Statistical Analysis |
|---|---|---|---|---|
| 4 | 0.337 | 0.342 | 0.345 | Mean = 0.41306 |
| 6 | 0.385 | 0.388 | 0.381 | Mean SD = 0.005298 |
| 8 | 0.522 | 0.511 | 0.510 | Mean %RSD = 1.06% |

**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. From this solution, 5ml was taken and from prepared 8 µg/ml solution 5ml was taken and added to the first 5ml of 4µg/ml thus 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.

**Table 7** Accuracy study results

| Conc.(µg/ml) Initial | Initial Absorbance for 4 µg/ml | Level addition(in%) | After addition Absorbance | Altered concentration (µg/ml) | Average % recovery |
|---|---|---|---|---|---|
| 4 | 0.256 | 50 | 0.380 | 5.937 | 98.95 |
| 4 | 0.256 | 100 | 0.508 | 7.953 | 99.41 |
| 4 | 0.256 | 150 | 0.656 | 10.09 | 100.9 |

**LOD and LOQ:**

The smallest amount of an analyte that exists in the sample, and can be detected by analysis, is termed the limit of detection (LOD). The smallest amount of an analyte present that could be quantitated with acceptable precision and accuracy is termed the limit of quantification (LOQ). The LOQ and LOD are calculated as shown below using the following equations:

LOQ = 10 s/m

LOD = 3 s/m

In these equations, "s" is the standard deviation of the response, and "m" is the slope of the corresponding calibration curve.

**Ruggedness:**

**Table 8** Ruggedness study

| Conc.( µg/ml) | Analyst 1 | Analyst 2 | Statistical Analysis |
|---|---|---|---|
| 4 | 0.264 | 0.268 | Mean = 0.3923 |
| 6 | 0.403 | 0.398 | SD = 0.00354 |
| 8 | 0.513 | 0.507 | %RSD = 0.926% |

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

**Specificity:**

Specificity is the ability to accurately and precisely quantify the analyses of interest in the laboratory in the presence of other foreseen components within the sample matrix. It determines the size of the population. Among the other active drugs are confounding variables which include degradation, contaminants, and additives. Here we have used DMSO (Dimethyl Sulfoxide) as a confounding variable for specificity study in which 5ml volume of 4ppm nicotine sample with another 5ml with 2% DMSO and then after we haven't observed any major changes in absorbance reading as that taken earlier.

**Table 9** Robustness study results

| Conc.(µg/ml) | Absorbance | Absorbance with (2% DMSO) |
|---|---|---|
| 4 | 0.221 | 0.216 |
| 6 | 0.342 | 0.340 |
| 8 | 0.495 | 0.483 |

**Robustness:**

Different concentrations of Nicotine solution like (4 µg/ml,6 µg/ml,8 µg/ml) was analysed at different wavelength that includes 259nm, 260nm, 261nm, 262nm, 263nm to determine robustness of the method.

**Table 10:** Robustness study results

| Conc.(µg/ml) | Absorbance at 259nm | Absorbance at 260nm | Absorbance At 262nm | Absorbance at 263nm | Statistical Analysis |
|---|---|---|---|---|---|
| 4 | 0.207 | 0.212 | 0.210 | 0.208 | Mean = 0.3406 |
| 6 | 0.335 | 0.341 | 0.338 | 0.334 | SD = 0.003091 |
| 8 | 0.472 | 0.481 | 0.480 | 0.478 | %RSD = 0.93% |

## RESULTS AND DISCUSSION:

From dissolution point of view, attempt was made to dissolve the Nicotine in methanol. The calibration curve of Nicotine plotted at 261 nm (Figure no-1) a linear relationship was obtained between 2-12 µg/ml. The accuracy of the method was determined by calculating mean percentage recovery it was found to be 98–101 % (Table no-7). Further precision was calculated as repeat ability, inter and intraday variations and %RSD was less than one (Table-4). The LOD value was found to be 0.422 µg/ml and LOQ value was found to be 2.513 µg/ml.

## CONCLUSION:

In this paper Accurate, economical, and convenient methodology for the nicotine in pure API form using UV spectrophotometric method was established. The % RSD values for Intraday and Interday was found to be less than 2 which is very significant. the limit of detection and limit of quantification of the developed strategy ware found to be 0.422 µg/ml and 0.513 µg/ml this indicating that

developed method is sensitive. Developed method was also robust and rugged as the %RSD for both the tests was found to be less than 2%.

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