DOI: 10.55522/jmpas.V14I1.6691 ISSN NO. 2320 – 7418

International peer reviewed open access journal

Journal of Medical Pharmaceutical and Allied Sciences

Journal homepage: www.jmpas.com CODEN: JMPACO

Research article

Chemico-analytical parameters and findings of nicotine from pan masala

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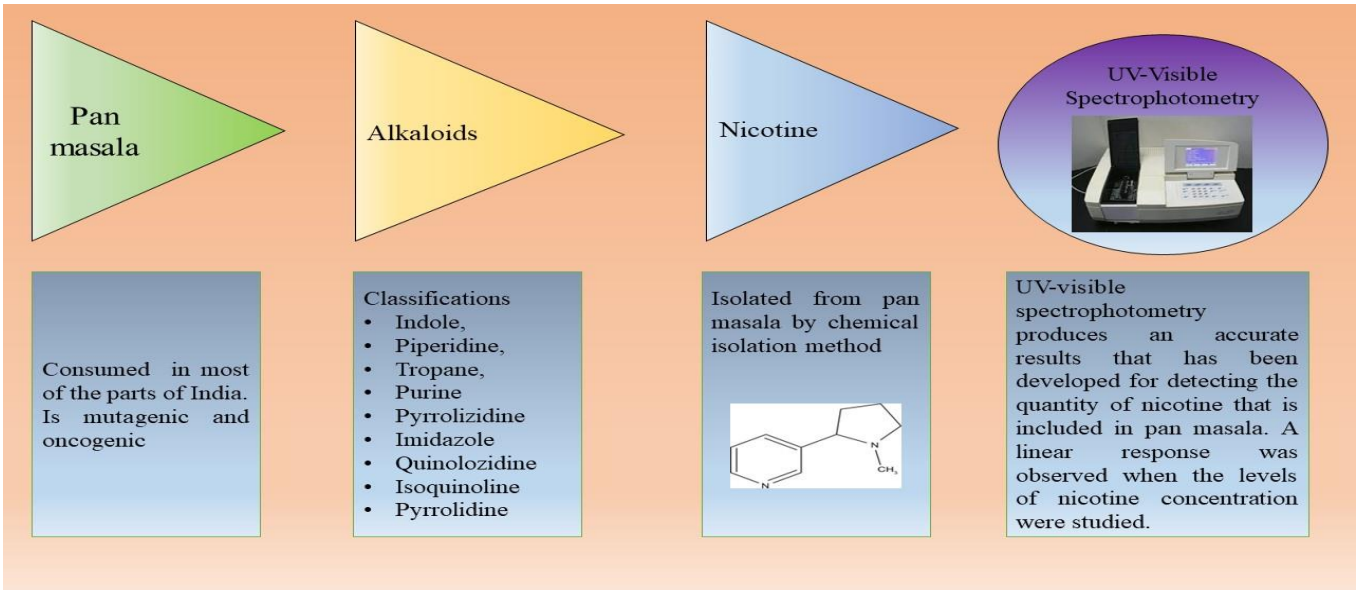
Received - 09-09-2024**, Revised** - 27-11-2024, **Accepted** - 24-01-2025 **(DD-MM-YYYY)**

Refer This Article

Vikas Kumar Pal, Priyank Yadav, Smriti Mathur, 2025. Chemico-analytical parameters and findings of nicotine from pan masala. Journal of medical pharmaceutical and allied sciences, V 14 - I 1, Pages - 6915 – 6921. Doi: https://doi.org/10.55522/jmpas.V14I1.6691.

ABSTRACT

Nicotine, is an alkaloid found in different amounts ranging from 2% to 14% in the leaves of *Nicotiana rustica, Nicotiana tobacum, Duboisia hopwoodii*, and *Asclepias syriaca*. The main perspective of this work is to extract and isolate nicotine from various brands of paan masala and perform chemical test and analytical studies on it. UV and IR parameters are applied for the purpose of standardization of it, after the isolation. Then the method development through UV is done and is compared with the standard nicotine solution. The approach is suitable for use in the regular analysis of nicotine and does not result in any influence from excipients. A straightforward method that makes use of UV-visible spectrophotometry and may produce accurate results has been developed for detecting the quantity of nicotine that is included in a nicotine gum. A linear response was observed when the levels of nicotine concentration were studied, which ranged from 0.2 to 2.4 micrograms per millilitre. The analysis of the pharmaceutical formulation that was conducted utilising the proposed method gave results that are extremely reproducible and reliable, as well as consistent with the label claims. The procedure for estimating the quantity of nicotine that was present was not hindered in any way by the substances that are commonly included in pharmaceutical preparations.

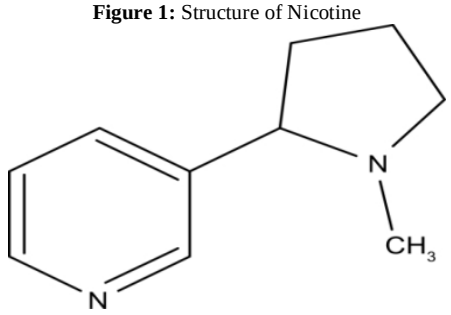


Keywords: Acetazolamide, Congestive heart failure (CHF), Comorbidities, Cardiac function, MLHFQ Questionnaire.

INTRODUCTION

The name "alkaloids" refers to a broad group of compounds that share certain structural properties, such as a ring structure and a nitrogen atom. These elements are present in compounds that belong to the group known as "alkaloids." In most cases, the structure of a heterocyclic ring will have the atom of nitrogen embedded somewhere inside its boundaries. There are many different kinds of creatures that are capable of producing alkaloids, including bacteria, fungi, plants, and mammals [1].

Figure 1: Structure of Nicotine



Nicotine, as in figure 1, can be found in amounts ranging from 2% to 14% in the leaves of Nicotiana rustica, Nicotiana tobacum, Duboisia hopwoodii, and Asclepias syriaca. Nicotine has a powerful ability to ingrain habits [2, 3].

Nicotine's addictive features include psychotropic effects, narcotic activity, and compulsive use, relapse after abstinence, physical addiction, and resistance to the drug's pull [4, 5].

Nicotine accounts for between 0.6 and 3.0% of the dry weight of tobacco. Two to seven grammes per kilogramme are found in the Solanaceae family of plants, which includes eggplants and tomatoes. This amount is less than one millionth of the total. Nicotine is an activator at nicotinic acetylcholine receptors in particular; compared to (R)-(+)-nicotine, (S)-()-nicotine is six times more potent at muscle-type nicotinic acetylcholine receptors [6-8].

Since the beginning of recorded history, India has been a major centre for the cultivation and consumption of tobacco. Chewing tobacco leaves and smoking tobacco were the two basic ways that people consumed tobacco in its early forms. Chewing tobacco leaves was also a common practice. There are currently a significant number of products available for purchase that are derived from tobacco or contain elements of tobacco.

The population of the 11 nations that comprise the WHO South-East Asia Area accounts for approximately 25.35 percent of the total population of the entire planet. This corresponds to 1.536 billion persons in total (SEAR). Individuals in this region of the world are accustomed to smoking cigarettes as well as using smokeless tobacco (SLT), in contrast to those in other regions of the world, where smoking is the more prevalent method of tobacco consumption [9]. According to the findings of the Global Adult Tobacco Survey carried out in India, among individuals who use tobacco products, 21 percent only use smokeless tobacco, while just 5 percent also smoke [10].

Tobacco products of all kinds, including cigarettes, bidis, cigars, chewing tobacco, smokeless tobacco, and various other forms of tobacco, are examples of some of the numerous items available on the market that contain nicotine and are marketed and sold under a wide variety of brand names. In addition, Gutkha and pan masala are regarded as additional products that are included in the category of items that include tobacco or nicotine. The use of Gutkha, which contains a variety of toxic and carcinogenic substances in addition to a high concentration of carcinogenic heavy metals such as lead and arsenic, is associated with an increased risk of developing cancer as well as other illnesses of the nervous system. Gutkha also contains a variety of toxic and carcinogenic substances.

The ingredients that go into making Gutkha are catechu, lime, tobacco, and betel nut, as well as artificial flavours. Women, including those who are pregnant, teenagers, and individuals who are under the age of 40 make up the majority of its users in India. Teenagers and people who are under the age of 40 also use it [11-13]. Oral sub mucous fibrosis is being observed in an increasing number of patients under the age of 40, including an increasing proportion of youngsters as well as young men and women. These patients' medical records reveal a history of consistent Gutkha consumption. Oral cancer will likely break out in a broad manner in the near future. Trismus, burning, discomfort, blanching, and repeated ulceration are the symptoms that are associated with sub mucous fibrosis, a disorder that affects the mucosa and lasts for a long time. One of the clinical criteria that must be met in order to arrive at a diagnosis of sub mucous fibrosis is the presence of palpable fibrous bands. Very common in the mucosa of the buccal and retromolar regions, as well as the region that surrounds the aperture of the mouth. Papillae are absent from the tongue of a person who has been impacted because of the effect [14].

The International Agency for Research on Cancer, which is a component of the World Health Organization, classifies areca nut as a Category I carcinogen. This means that areca nut can cause cancer in humans. Despite the absence of tobacco, there is evidence that chewing areca nuts is linked to a number of different types of cancer in humans. These cancers include those that occur in the throat, stomach, lung, and cervical regions. Dependence on areca nuts has been linked to a variety of life-threatening diseases, such as cancer, heart attacks, arrhythmias, metabolic syndrome, and diabetes. Children who are born to moms who consume areca nuts on a consistent basis tend to be of a smaller size and display withdrawal symptoms more frequently than children who are born to mothers who do not consume areca nuts [15].

Chewing tobacco, also known as Gutkha, is a proven carcinogen that increases the risk of developing oral cancer, as well as

oesophageal, pharynx, larynx, stomach, and pancreatic malignancies. Smoking tobacco is linked to many different illnesses and disorders, including cancer. This category includes a variety of conditions, including periodontal disease, peripheral vascular disease, hypertension, peptic ulcer disease, and coronary artery disease [16, 17].

Pan masala is made with a variety of ingredients, including tobacco, betel leaf, lime, areca nut, clove, cardamom, and mint, to name a few of them. There may be a combination of this element with others. This concoction contains desiccated areca nuts, catechu (Acacia catechu), slaked lime (calcium oxide and calcium hydroxide), cardamom, as well as synthetic aromas and flavours. The mixture does not expire. Copper, which is abundant in pan masala, is one of the nutrients that is known to stimulate the production of collagen as well as an increase in the cross-linking of elastin and collagen. This is because copper helps to form hydrogen bonds between the proteins [18]. In addition to this, it comes packaged in itty-bitty sachets that are not only economical but also easily attainable and transportable [19]. Areca nuts have a grainy texture, which is responsible for the localised stress and irritation they cause to the oral mucosa. This irritation is made worse by the paan masala's granular consistency. According to the second National Family Health Survey, 21 percent of adults aged 15 and older reported having consumed paan masala or cigarettes in the previous 30 days**.**

MATERIAL AND METHODS

Aim and Objective of Study

The main aim is to extract and isolation nicotine from various brands of paanmasala and perform chemical test and analytical studies on it. For the analysis purpose column chromatography, UV and IR is used.

The products like Kesar and Tulsi are used to isolate the nicotine. After the isolation characterization like solubility, melting point, UV and IR is performed. Then the method development through UV is done and then compare with the standard nicotine solution.

Isolation of Nicotine

Take 10 gm of tobacco powder and to it 40% of 100ml NaOH is added and stirred for 15 minute. Then filter it and add 30 ml distilled water to it and again filter. To the filtrate 25 ml ether is added and transfer to the separating funnel. Three times extraction is done and collect the four filtrate of ether and filter it and then evaporate on water bath. Add 4ml methanol and 10 ml saturated picric acid and place it on the ice bath. The crystal of nicotine dipicrosine is formed.

Characterization

Solubility

The solubility of nicotine is in water, ethanol, acetone and diethyl ether.

Melting Point

The melting point of nicotine is 206 ºC.

UV

The absorbance of nicotine at different concentrations is observed.

Empirical Formula: C10H14N2

M.P: -79 ºC (-110 ºF)

B.P: 247º C (477 ºF)

Molecular weight: 162.12

Density: 1.01 gm/cm3

Chemical Test

The following chemical tests are performed on the different products of nicotine including Gutkha and paan masala, as in table 1.

A small amount of sample was projected to IR spectroscopy and functional group peaks were recorded and interpreted, as in figure 2.

It has peaks on 3300 cm-1, we can see the large peak of water (it deals with a liquid film).

Between 2917 and 2849 cm^{-1}: C-H stretching.

The peak at 1571 cm^{-1}: aromatic C=N double bond stretching.

The peaks at 717 cm^{-1} and 891 cm^{-1} correspond to the out of plane bending of the C-H bond of the monosubstituted pyridinic cycle.

Table 1: Chemical tests of Nicotine

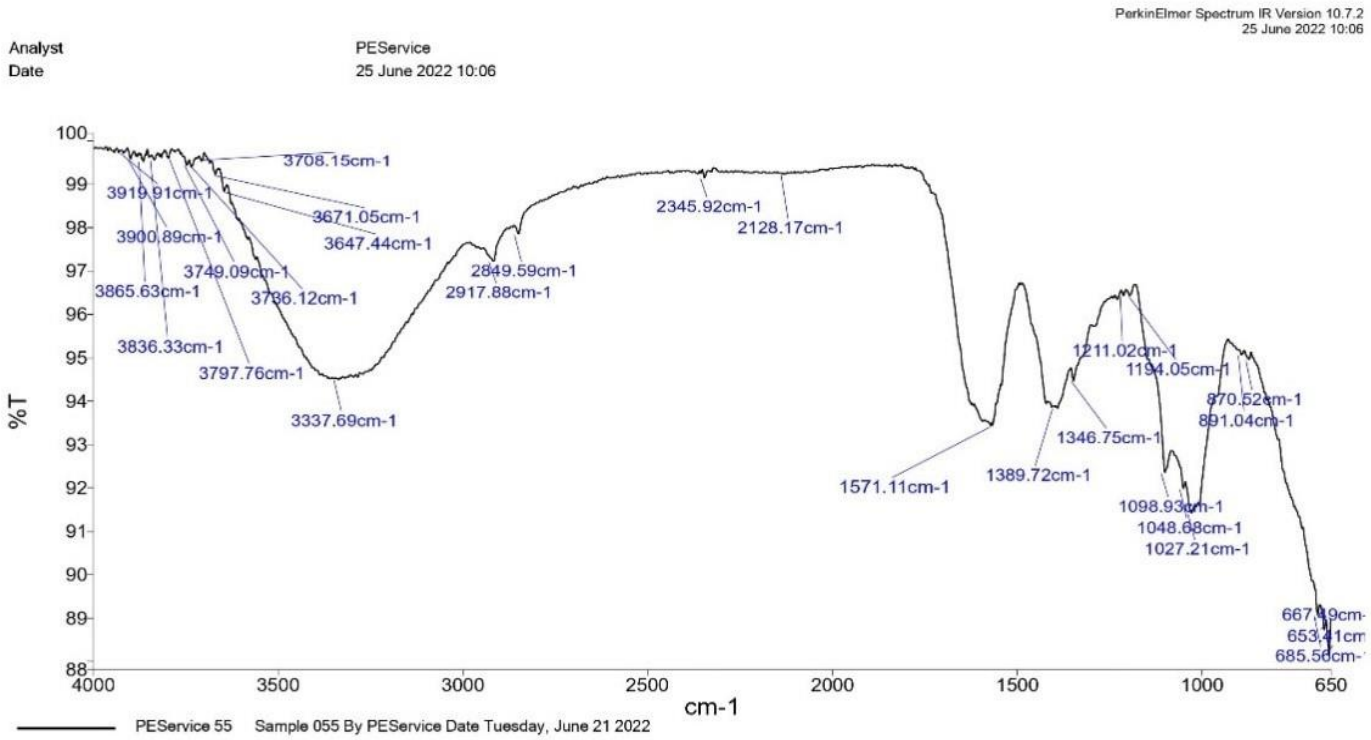
Groups	Chemical Test	Procedure	Observation	Result
Alkaloids	Wagner's Test	Small amount of sample + solution Wagner's reagent (Iodine Potassium iodide) gives brown or reddish-brown color which indicates the presence of alkaloids.	Reddish brown color was obtained	Alkaloid was present
	Hager's Test	Small amount of sample + solution of Hager's reagent (saturated solution of picric acid) gives yellow precipitate which indicates the presence of alkaloids.	Yellow precipitate was produced	Alkaloid was present
Tannins	Gelatin Test	sample solution + the aqueous solution of gelatin + sodium chloride is added gives a white buff colored precipitate which indicates the presence of tannins in the sample.	White buffed color was formed	Tannin was present
	Vanillin HCl Test	Sample solution + vanillin HCl reagent (1gm vanillin + 10 ml alcohol + 10ml concentrated HCl) gives pink color which is obtained due to the presence of phloroglucin.	Pink color was obtained	Tannin was present
Menthol	Test for Menthol	Dissolve 10mg of sample in 1ml of sulphuric acid + 1ml of 1% w/v solution of vanillin in sulphuric acid which produced an orange color then Add 1ml of water, the colour changes to violet which indicates the presence of menthol in the sample.	Orange color changes into violet colour on addition of water	Menthol was present

Validation of the Method

Linearity

Nicotine concentration-dependent calibration graphs were drawn of 0.2 to 2.4 g/ml. To do this, 10 ml volumetric flasks were filled with 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2-, and 2.4-ml dilutions of nicotine standard solutions. The amount corrected to the mark with distilled water and the absorbance of the liquids were tested versus distilled water at a certain wavelength. By charting the graph of absorbance vs concentration, a calibration curve was generated. There were three repetitions of the experiment.

Figure 2: IR Analysis of Nicotine



Precision

Method precision (Repeatability)

Accomplished by repeatedly measuring the absorbance of six distinct experimental standard concentrations of nicotine (1000g/ml) and calculating the % RSD.

Intermediate precision (Reproducibility)

The intraday and interday fluctuation of nicotine was investigated by determining absorbance three times on the same day and three times on three distinct days for 12 different calibration range concentrations(0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, 2.4 µg/ml) for 3 times. The results were expressed in terms of % RSD.

LOD and LOQ

The drug's LOD and LOQ were determined using the following equations by the International conference on Harmonization (ICH) recommendations.

$LOD = 3.3 \times \sigma/S$
$LOQ = 10 \times \sigma/S$
Where σ = the standard deviation of the response, S = Slope of calibration curve.

Accuracy (%Recovery)

Using the usual addition method at three distinct concentration ranges, a multilevel recovery study was carried out to assess the approach's precision. In the recovery investigation, previously examined sample solutions were spiked with nicotine standard solutions (50, 100, and 150%), and the combination was assessed using the suggested approach. The experiment was repeated three times, and % RSD was determined.

Method and Development

Nicotine working standard solutions were made individually in distilled water and analysed in the 200-400 nm wavelength range. Peak absorbance was seen in the spectra of nicotine standard solution at 352 nm. This wavelength was utilised for nicotine detection

RESULT AND DISCUSSION

Chemical Test

As the presence of alkaloid was tested and came positive. The tannins and menthol identification tests were undertaken because it was specified on the packaging of pan masala, thus the identification tests were performed and found to be positive.

Linearity

The developed approach was found to be linear for nicotine concentrations ranging from 0.2 to 2.4 g/ml, as in table 2. The high correlation coefficient result (R2 = 0.998) indicates that the approach is linear in the range of 0.2-2.4 g/ml. The nicotine calibration curve is given in figure 3.

Table 2: Linearity data of nicotine by UV- visible spectrophotometry

Concentration	Absorbance
0.2µg/ml	0.045
0.4µg/ml	0.064
0.6µg/ml	0.089
0.8µg/ml	0.11
1.0µg/ml	0.125
1.2µg/ml	0.138
1.4µg/ml	0.147
1.6µg/ml	0.169
1.8µg/ml	0.193
2.0µg/ml	0.204
2.2µg/ml	0.22
2.4µg/ml	0.248
R^2	0.9935
Slope	0.0175
Y intercept	0.0324
STDEV	0.063215361
LOD	11.92061086

Intermediate precision

The intraday and interday accuracy of the developed approach was evaluated by analysing the appropriate results three times on the same day and on various days over the course of one day for all ratios of nicotine standard solutions (0.-2.4g/ml). The outcome was expressed as a percentage relative standard deviation (% RSD), as in table 3, 4 and figure 4, 5.

Figure 3*:* calibration curve of Nicotine at 352 nm

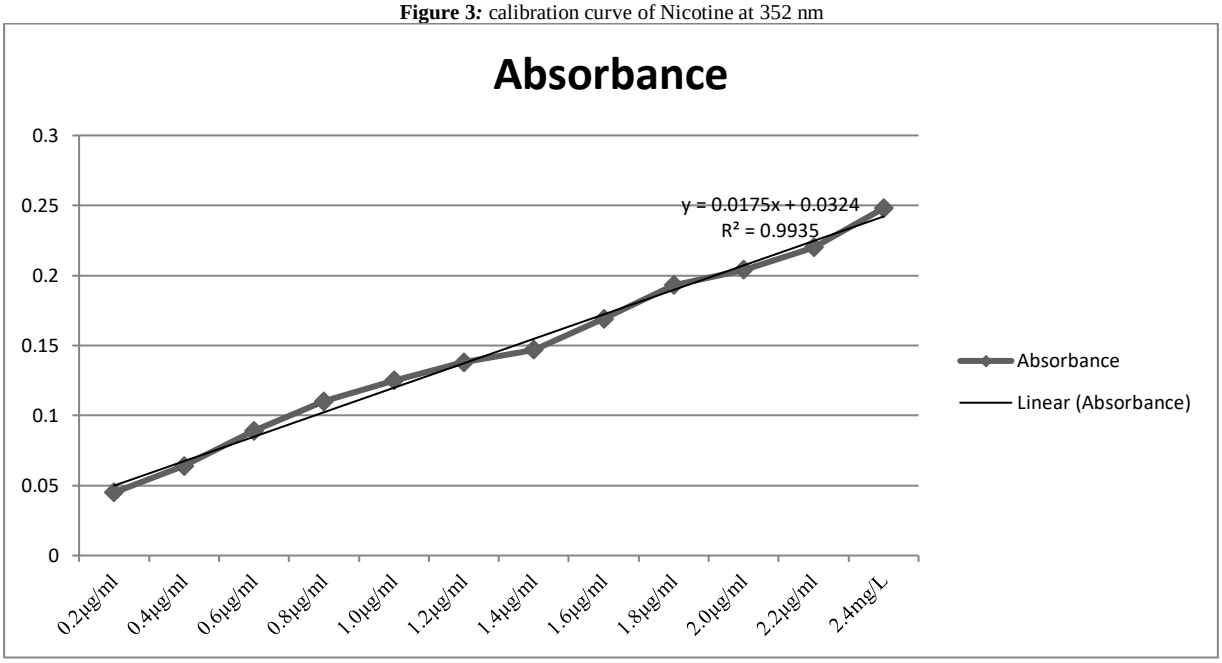


Table 3: Intraday Data

Concentration	Sample1	Sample2	Mean	Sd	CV
0.2µg/ml	0.045	0.042	0.0435	0.0015	0.034483
0.4µg/ml	0.064	0.067	0.0675	0.001893	0.028044
0.6µg/ml	0.089	0.094	0.0915	0.0025	0.027322
0.8µg/ml	0.11	0.108	0.109	0.001	0.009174
1.0µg/ml	0.125	0.122	0.1235	0.0015	0.012146
1.2µg/ml	0.138	0.134	0.136	0.002	0.012146
1.4µg/ml	0.147	0.142	0.1455	0.002566	0.017634
1.6µg/ml	0.169	0.173	0.171	0.002	0.011696
1.8µg/ml	0.193	0.194	0.1935	0.0005	0.002584
2.0µg/ml	0.204	0.202	0.201	0.001528	0.0076
2.0µg/ml	0.218	0.22	0.219	0.001	0.004566
2.4µg/ml	0.242	0.248	0.245	0.003	0.012245

Figure 4: calibration curve for Intraday Data

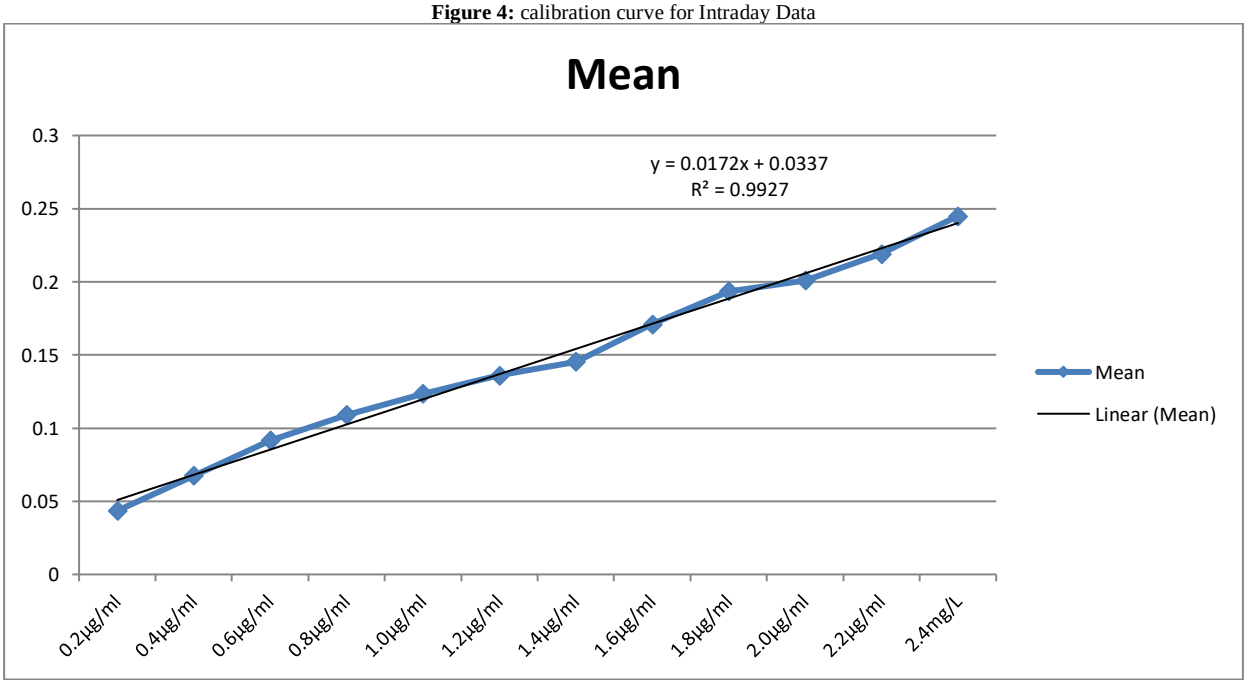


Table 4: Interday Data

Concentration	Sample1	Sample2	Mean
0.2µg/ml	0.042	0.043	0.0425
0.4µg/ml	0.061	0.062	0.0615
0.6µg/ml	0.084	0.09	0.087
0.8µg/ml	0.108	0.107	0.1075
1.0µg/ml	0.121	0.119	0.12
1.2µg/ml	0.133	0.131	0.132
1.4µg/ml	0.141	0.142	0.1415
1.6µg/ml	0.165	0.17	0.1675
1.8µg/ml	0.179	0.173	0.176
2.0µg/ml	0.202	0.193	0.1975
2.2µg/ml	0.217	0.219	0.218
2.4µg/ml	0.244	0.248	0.246

Figure 5: calibration curve for Interday Data

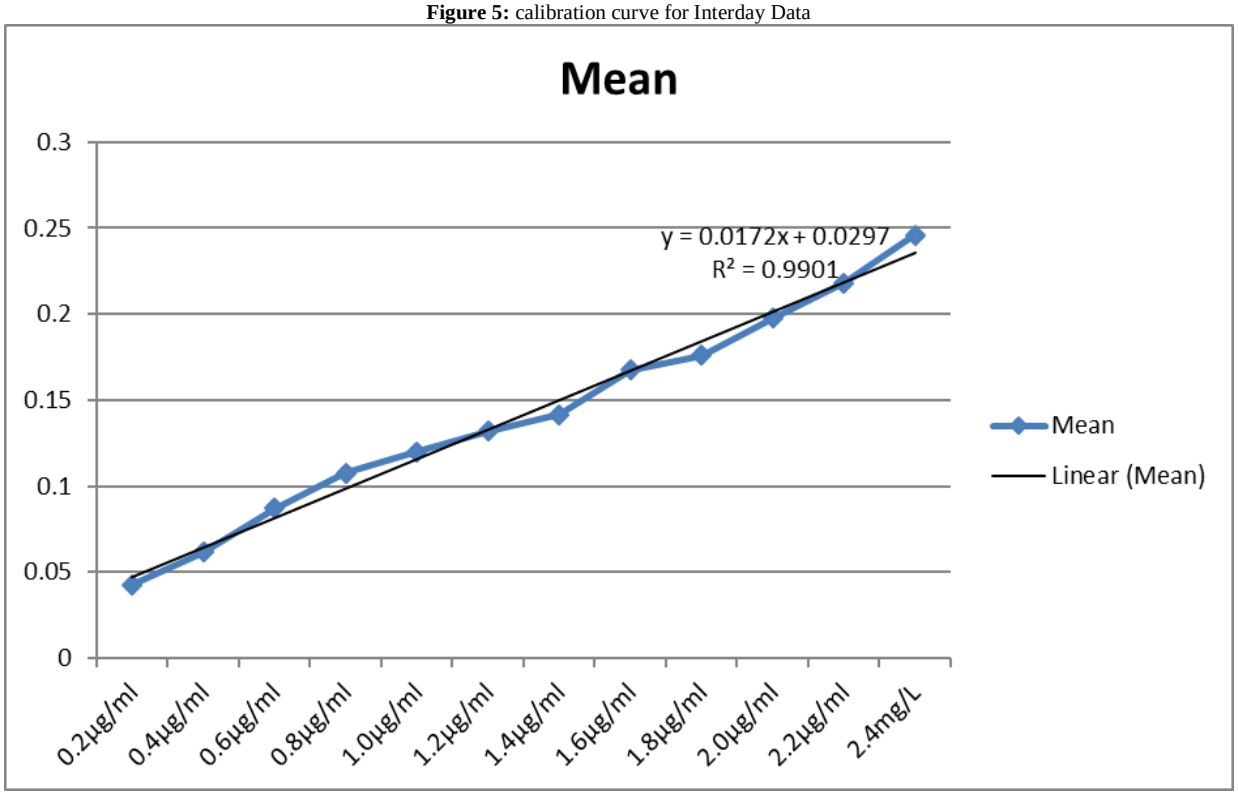


Table 5: Recovery data of proposed method

Amount of Nicotine in Masala	Amount recovered	Mean	sd	%RSD	SE	% Recovery
10µg/ml	9.86	9.86	0.074	0.0075	0.0427	98.67
	9.8					
	9.83					
20µg/ml	19.71	19.69	0.01247	0.0633	0.00719	98.45
	19.69					
	19.688					
10µg/ml	9.95	9.9	0.0454	0.0045	0.0262	99
	9.91					
	9.84					

LOD and LOQ

The nicotine LOD and LOQ values were determined to be11.92061086 µg/ml and 36.12306321µg/ml at 352 nm, respectively. Low values for LOD and LOQ indicate a delicate method.

Accuracy (% recovery)

The approach's precision was verified by estimating nicotine recovery using the usual addition method. 50, 100, and 150% of known quantities of standard solutions of nicotine were introduced to pre-quantified sample solutions of nicotine. For nicotine, the solutions were measured at 352 nm. The experiment was repeated three times, and % RSD was determined.

The recovery experiment was conducted using the normal addition procedure. The average Nicotine recovery was determined to be 98.706 0.0251. The results of recovery studies are displayed in table 5.

CONCLUSION

In many parts of the country, not only is the consumption of pan masala prevalent, but it is also simple to acquire. This is because pan masala is both common and easily accessible in these areas. It is known to cause harm to the oral cavity, liver, kidneys in addition to producing cancer, and it is carcinogenic and genotoxic as well. It is

imperative that the government move quickly in order to reduce usage and increase awareness among the general public about the dangers posed by the chemical.

A straightforward method that makes use of UV-visible spectrophotometry and may produce accurate results has been developed for detecting the amount of nicotine that is present in a nicotine gum tablet. A linear response was observed when the levels of nicotine concentration were studied, which ranged from 0.2 to 2.4 micrograms per millilitre. The evaluation of the pharmaceutical formulation that was carried out using the technique that was suggested produced results that are very reproducible and trustworthy, and they are also in good accordance with the claims that are made on the label of the drug. The procedure for estimating the amount of nicotine that was present was not hindered in any way by the substances that are commonly included in pharmaceutical formulations. As a result, the method is appropriate for use in the routine analysis of nicotine, and it does not lead to any effect from excipients.

ACKNOWLEDGEMENT

We are grateful to our mentor Dr. Yogendra Pal Sir and institute's HOD Mr. Ashwani Kumar Chaturvedi Sir for their guidance and support as well as to the Krishna Institute of Pharmacy & Sciences, Kanpur, Uttar Pradesh, India.

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