

A Simple Spectrophotometric Method for the Determination of Nicotine in Environmental Samples

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A new simple and sensitive spectrophotometric method for the determination of nicotine is described. The method is based on the bromination of nicotine to form dibromonicotine which reacts with potassium iodide to liberate iodine. The iodine liberated then selectively oxidizes leuco crystal violet, and the crystal violet dye formed shows maximum absorbance at 592 nm. Beer's law is obeyed over the concentration range 0.2 µg-2.2 µg of nicotine in a final solution volume of 25 mL (0.008-0.088 ppm). The molar absorptivity of the colour system is $1.40 \times 10^6 \text{ l mol}^{-1} \text{ cm}^{-1}$. The method is free from the interference of other major toxicants. The various analytical parameters were optimized, and the method was applied for the determination of nicotine in cigarette smoke, food, and biological samples.

Keywords: Nicotine; Spectrophotometric; Leucocrystal violet.

INTRODUCTION

Nicotine (pyridine, 3-(1-methyl-2-pyrrolidinyl), (S) is one of the highly toxic tobacco alkaloids present in tobacco leaves and cigarette smoke.¹ Nicotine appears to be a promising tracer for environmental tobacco smoke (ETS) because of its specificity for tobacco.² It is also a systemic and contact insecticide and is also used as a drug and in chemical.³ The threshold limit value reported for nicotine is 0.05 mg/m³.¹ The most important symptoms of exposure to nicotine are bronchitis, emphysema, cyanosis, and exenation of the central nervous system. Excessive smoking has been implicated in lung cancer, bladder cancer, and cancer of the larynx and oesophagus.⁴

The determination of total nicotine alkaloids is of particular importance to the tobacco industry and in the area of toxicology. Various analytical methods such as GC,⁵ TLC,⁶ HPLC,⁷ ASS,⁸ capillary electrophoresis,⁹ radio immuno assay,¹⁰ GC-MS,¹¹ and circular dichroism spectropolarimetry¹² have been reported. Most of the spectrophotometric methods reported are based on cleavage of the pyridine ring with cyanogen bromide or chloride and condensation of the resulting compound with p-amino benzoic acid¹³ and diethyl thiobarbituric acid.¹⁴ Many of these methods involve a prolonged extraction procedure prior to the determination of nicotine and the use of highly toxic cyanide.^{13,14}

A new spectrophotometric method for the micro determination of total nicotine alkaloid is described. It is based on

the bromination of nicotine to form dibromo nicotine which on reaction with potassium iodide liberates iodine which selectively oxidizes leuco crystal violet, and the crystal violet dye so formed shows maximum absorbance at 592 nm. The method has been applied for the determination of nicotine in cigarette smoke, food, and biological samples. The proposed method is rapid, does not use toxic cyanide, and is highly sensitive and more selective than other reported methods. This method is also a good alternative to some of the reported costly instrumental methods.

EXPERIMENTAL

Apparatus

All spectral measurements were made with a Carl-Zeiss spectrophotometer with optically matched silica cells and a Systronics 108 UV-VIS digital spectrophotometer. A calibrated rotameter (PIMCO) was used for controlling the flow rate while sampling air. A RemiC-854/4 clinical centrifuge having a maximum centrifugal force of 1850 g with fixed swinging rotors was used for centrifugation.

A Systronics pH meter model 331 was used for all pH measurements.

Reagents

All chemicals used were of analytical reagent grade of the best available quality. Double distilled deionised water

was used throughout.

Standard nicotine solution: (Merck)

1 mg mL⁻¹ solution was prepared. Working standards were prepared by appropriate dilution of the stock.

Bromine water: (Ranbaxy Fine Chemicals Ltd.)

A saturated solution of bromine in water was prepared. This solution was prepared daily.

Formic acid (E Merck): 90% aqueous solution.

Zinc acetate (E Merck): 21.6 gm of zinc acetate and 3 mL of acetic acid to 100 mL with water.

Potassium iodide (E Merck Germany): 1 mg mL⁻¹ aqueous solution.

Leuco crystal violet (LCV) (Eastman Kodak Co. New York)

To a 1 litre volumetric flask 200 mL water, 3 mL 85% phosphoric acid and 250 mg of leuco crystal violet - i.e. 4.4'4" methyl dynetris (N-N'-dimethyl aniline) were added and shaken gently until the dye dissolved. The contents of the flask were then diluted to 1 litre with water.

Potassium hexa cyano ferrate (II): (Ranbaxy Fine Chemicals Ltd.)

10.6% w/v 1.06 gm amount of potassium hexa cyano-ferrate (II) was dissolved in 10 mL of water.

Fuller's earth (Loba Chemie): 0.5 gm

Absorbing solution: A 0.01 mol l⁻¹ sodium hydroxide solution in 10% methanol was used.

Preparation of calibration graph

To an aliquot of 0.2 µg-2.2 µg of nicotine in a 25 mL calibrated tube, 0.5 mL of bromine water was added, and the mixture was shaken gently for ~2 min. The excess of bromine was removed by the dropwise addition of formic acid after which 1 mL each of potassium iodide and leuco crystal violet (LCV) was added and shaken for a few minutes: 1 mL 2 M sodium hydroxide was added dropwise. The contents were diluted to the 25 mL mark with water, and the absorbance was measured at 592 nm against a reagent blank as reference after 25 mins.

RESULTS AND DISCUSSION**Absorption Spectrum**

The absorption spectrum of the colour system showed

maximum absorbance at 592 nm.

The reagent blank showed negligible absorbance at this wave length. Beer's law is followed over the concentration range of 0.2 µg-2.2 µg for nicotine in a final solution volume of 25 mL (0.008-0.088 ppm). The molar absorptivity and Sandell's sensitivity were found to be 1.40 × 10⁶ l mol⁻¹ cm⁻¹ and 0.0001 µg cm⁻², respectively, for nicotine.

Reproducibility of the method

The reproducibility of the method was assessed by carrying out seven replicate analyses of a solution containing 1.0 µg of nicotine in a final solution volume of 25 mL. The standard deviation and relative standard deviation of absorbance values were found to be ± 0.013 and 2.9%, respectively.

Effect of reagent concentration

The concentration range varying from 0.1 to 2 mL bromine water has been investigated, and it was found that 0.5 mL bromine water was sufficient: below this, absorbance value decreases and after 0.5 mL absorbance value remains constant, 0.2 to 2.5 mL LCV was used and it was found that 1 mL LCV was sufficient for maximum colour development: below 1 mL, absorbance value gradually decreases and after 1 mL there is no change in absorbance value. 0.2 to 2 mL potassium iodide was used: 1 mL potassium iodide was sufficient for maximum absorbance. A few drops of formic acid were sufficient for the removal of excess bromine.

Stability of colour

The crystal violet dye formed was stable for several days under optimum conditions.

Effect of time

It was found that 2 min. were sufficient for bromination, and iodine was liberated immediately on addition of potassium iodide. After adding all the reagents the final solution was kept for 25 min for complete colour development.

Effect of temperature

It was found that 35 °C-45 °C was most suitable for the colour reaction. The temperature was obtained by keeping the solution in a thermostat maintained at ~45 °C. At a higher temperature the colour gradually faded.

Effect of pH

Under optimum conditions, a pH of 5.5-6.0 was found to be the best for the formation of crystal violet from LCV. An increase of pH above 6.5 severely affected the stability and

sensitivity of the dye. Colour development did not take place below pH 5.0.

Collection efficiency

It was found that the absorption of total nicotine was complete in the first impinger. Methanolic sodium hydroxide was found to be highly efficient (99-100%) in the absorption of alkaloid at a flow rate of 1 l min⁻¹.

Effect of foreign species

The validity of the method was assessed by investigating the effect of common foreign species in the analyses of nicotine. The tolerance limit value of different foreign species in a solution containing 1.0 µg of nicotine are given in Table 1: Hg²⁺ and Mn⁴⁺ had a positive catalysing effect on the colour reaction.

The anions Cl⁻ and SO₄²⁻ did not interfere and the cations Zn²⁺, Cu²⁺ and Cd²⁺, Fe³⁺ did not interfere under the proposed optimum condition.

It was found that phenol, pyridine, and aromatic amines which interfere with other methods, did not interfere with the proposed method. However, the other components that liberate iodine from potassium iodide under the proposed experimental condition are likely to interfere.

APPLICATION

Extraction and determination of nicotine in dried tobacco leaves¹⁵

To 1 gm of crushed tobacco leaves placed in a 100 mL beaker, 10 mL of methanol was added and the mixture was allowed to stand for 30 min. To this solution, 25 mL of water and 1 mL of 2 mol l⁻¹ sodium hydroxide were added, and the mixture was kept in a boiling water bath. The solution was then boiled, cooled and then filtered through Whatman filter paper. The beaker was rinsed with water and the washings were filtered and combined with the 4 original filterates before making the final volume up to 50 mL with water. 1 mL each of zinc acetate and potassium hexa cyanoferrate (II) solutions were added, and the mixture was centrifuged at 2000 rev min⁻¹ for 5 min. The supernatant liquid was transferred into a beaker and about 0.5 mg of Fuller's earth was added. The mixture was shaken thoroughly and centrifuged. The clean supernatant liquid was discarded and the residue was treated with 10 mL of 0.01 mol l⁻¹ sodium hydroxide solution, and then filtered. To the filtrate 14 mL of 1 mol l⁻¹ HCl was added and the solution was made up to 100 mL with water. An

Table 1. Tolerance limit of foreign species in the determination of nicotine

Total Nicotine Concentration: 1.0 µg/25 mL	
Foreign Species	Tolerance limit (ppm)*
SO ₄ ²⁻ , PO ₄ ³⁻	2500
Phenol	2000
NO ₃ ⁻ , CO ₃ ²⁻	1500
Cd ²⁺ , Fe ³⁺	1250
Zn ²⁺ , Al ³⁺	850
Br ⁻ , Cl ⁻ Formaldehyde	500
Co ²⁺ , Cu ²⁺	400
Benzene, Triethanolamine	450
Sn ⁴⁺	250
Pyridine	150
Hg ²⁺ , Mn ⁴⁺	25

* The amount causing ± 2% error in absorbance values.

aliquot of this solution was taken and analysed as described above. The results are shown in Table 2.

Determination of nicotine in cigarette smoke

Different brands of cigarettes with known mass were taken and analysed as described above. For the determination of nicotine in cigarette smoke, the smoke was drawn through 10 mL of absorbing solution contained in two midjet impingers connected in series to a source of suction at a flow rate of 1 l min⁻¹. After sampling, an aliquot of the solution was taken and analysed as described above. The results of the analyses are shown in Table 3. It was found that cigarettes without filters gave more nicotine than cigarettes with filters.

Determination of nicotine in coffee

From a 0.1 gm of sample, i.e. coffee placed in a 100 mL of beaker, nicotine was extracted as described above in the procedure. The residue of the Fuller's earth after extraction was treated with 0.01 mol l⁻¹ sodium hydroxide (10 mL) and

Table 2. Determination of total alkaloids/nicotine in dried, tobacco leaves

Sample	Alkaloids found* (µg)	
	Proposed method	Reported method ¹⁷
Tobacco leaves**	28.50	28.45
	27.96	27.92
	28.79	28.80
	26.81	26.76
	28.27	28.25

* Mean of three replicate analyses.

** 0.1 gm amount was taken.

Table 3. Determination of nicotine in cigarette smoke, coffee, and tea

Sample	Nicotine found (μg)*	
	Proposed method	Reported method ¹⁷
1A. Cigarette Smoke*** (with filter)	0.99	0.98
	0.96	0.94
	1.05	1.92
	0.94	0.92
B. Cigarette Smoke*** (without filter)	1.98	1.98
	2.05	2.07
	2.20	2.16
	2.19	2.16
2. Coffee**	1.30	1.29
	1.42	1.40
3 A. Tea Leaves**	1.40	1.39
	2.12	2.05
	2.33	1.98
B. Tea (branded)**	1.86	1.74
	1.45	1.39
	1.28	1.23
	1.09	1.02

* Mean of three repetitive analyses.

** Amount of sample: 0.1 gm. After treatment diluted to 50 mL. Nicotine found in $\mu\text{g}/10$ mL of the final solution.

*** Analytical variability of the nicotine yield in four different brands of cigarettes, Nicotine found in μg per mL of the absorbing solution. Smoke analyses from five cigarettes.

filtered: to the filtrate were added 14 mL of 1 mol l^{-1} HCl and made up to 50 mL with water. An aliquot of this solution was taken and analysed as described above and by the reported method,¹⁷ (Table 3).

Determination of nicotine in tea

Tea samples of 0.1 gm (branded tea and tea leaves) were taken in a 100 mL beaker and nicotine was extracted as de-

scribed in the procedure. The residue of the Fuller's earth after extraction was treated with 0.01 mol l^{-1} sodium hydroxide (10 mL) and filtered: to the filtrate were added 14 mL of 1 mol l^{-1} HCl and made up to 50 mL with water. An aliquot of this solution was taken and analysed as described above and by the reported method,¹⁷ (Table 3).

Determination of nicotine in various food samples

Nicotine is reported to be present in apples, cabbage, and other food samples when used as an insecticide.¹⁶ Hence to further check the applicability and recovery of the proposed method, a known amount of nicotine was added to apples, cabbage, and spinach and later determined by the proposed method with excellent recoveries (> 98%) which is in agreement with the reported method¹⁷ (Table 4).

Determination of nicotine in biological samples

For assessing the applicability of the method to biological samples, blood and urine samples were taken from smokers and non-smokers. 1 mL aliquot of blood and urine samples was taken and deproteinized by adding 2 mL of 1% trichloro acetic acid.¹⁸ After 25 min, the mixture was centrifuged, and the supernatant solution was transferred into a 25 mL calibrated tube and analysed as described in the procedure. The results of the analyses are shown in Table 5.

Determination of nicotine in pan masala

Nicotine is found to be present in different brands of pan masala used in India. These pan masala are reported to cause cancer of the mouth and throat. Different brands of pan masalas were taken and after extraction nicotine was detected by the present method and found to be present in a detectable amount.

Table 4. Recovery of nicotine from food products

Sample*	Amount of nicotine added (μg)	Amount of nicotine found		Recovery%	
		Proposed method	Reported method ¹⁷	Proposed method ¹⁷	Proposed method ¹⁷
Apples**	1.0	0.98	0.96	98.00	96.00
	2.0	1.95	1.91	97.50	96.50
Spinach***	1.0	0.95	0.96	95.00	96.00
	2.0	1.90	0.96	95.00	94.00
Cabbage***	1.0	0.98	0.97	98.00	97.00
	2.0	1.97	1.97	98.50	98.50

* Mean of three repetitive analyses

** Amount of sample: 20 gms

*** Amount of sample: 5 gms

Table 5. Determination of nicotine in biological samples

Sample*		Nicotine found/ μg^{**}	
		Proposed method	Reported method ¹⁷
Blood (Zinc)	A	0.31	0.30
	B	0.19	0.17
	C	0.44	0.40
	D	ND	ND
	E	ND	ND
Urine (2 mL)	A	1.21	1.19
	B	1.09	1.06
	C	1.44	1.46
	D	0.15	0.13
	E	0.10	0.12

* Samples D and E were taken from non smokers.

** Mean of three replicate analyses. --ND-- Not detectable.

CONCLUSIONS

The present method for the determination of nicotine is rapid, simple, sensitive and more highly selective than other reported spectrophotometric methods.^{17,19,20} The method was successfully applied for the analyses of nicotine in samples of tobacco leaves, cigarette smoke, coffee, tea, food samples, and biological samples. Results (Tables 2-4) are in good agreement with those obtained by the reported method.¹⁷

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