



Sensitive Chromogenic Spectrophotometric Method for the Determination of Nicotine Using 1 [(Bromomethyl(phenyl) methyl]-2-(2,4-dinitrophenyl) hydrazine

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طريقة طيفية كروموجينية حساسة لتقدير النيكوتين باستخدام 1- (بروموميثيل(فينيل)ميثيل)-2-(2,4-دينيتروفينيل) هيدرازين

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Abstract:

This study aimed to establish an efficient analytical method for the detection and quantification of nicotine using 1-[(bromomethyl)(phenyl)methyl]-2-(2,4-dinitrophenyl) hydrazine (BPMDNPH) as derivatizing reagent. The proposed method is based on a simple, rapid, and highly sensitive spectrophotometric approach. The work included the determination of the optimum absorption wavelength as well as the evaluation of the complex stability time to ensure accurate measurements. Nicotine was quantified spectrophotometrically at the maximum absorption wavelength ($\lambda_{max} = 455$ nm). The measured nicotine concentrations ranged from 0.16 to 0.97 ppm, with a detection limit (LOD) of 0.13577 $\mu\text{g mL}^{-1}$. Additional analytical performance parameters, including the limit of quantification (LOQ) and method sensitivity, were also determined. The calibration curve showed excellent linearity, with a correlation coefficient (R^2) of 0.99105 and a molar absorptivity of $4.9 \times 10^4 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$. The stability of the BPMDNPH–nicotine complex was assessed under the optimum experimental conditions. The findings indicated that the absorbance of the BPMDNPH complex remained stable throughout the monitoring period. The developed method was successfully applied to determine nicotine in tobacco products marketed locally in Libya. The obtained results revealed deviations ranging from 0.06 to 0.21 (SD) from the labeled values, with an overall relative standard deviation (RSD%) of 1.8%.

Keywords: Nicotine, Hydrazone, 1-Bromoacetophenone, Cigarette, KMnO₄.

المخلص:

هدفت هذه الدراسة إلى تطوير طريقة تحليلية فعّالة للكشف عن النيكوتين وتحديد كميته باستخدام كاشف مركب الهيدرازون 1-[(بروموميثيل(فينيل)ميثيل)-2-(2,4-ثنائي نيتروفينيل)]. تعتمد الطريقة المقترحة على نهج طيفي ضوئي بسيط وسريع وحساس للغاية. شمل العمل تحديد الطول الموجي الأمثل للامتصاص، بالإضافة إلى تقييم زمن استقرار المركب لضمان دقة القياسات. تم قياس كمية النيكوتين طيفياً عند أقصى طول موجي للامتصاص ($\lambda_{max} = 455$ نانومتر). تراوحت تراكيزات النيكوتين المقاسة بين 0.16 و 0.97 جزء في المليون، مع حد كشف (LOD) قدره 0.13577 ميكروغرام/مل. كما تم تحديد معايير أداء تحليلية إضافية، بما في ذلك الحد الكمي (LOQ) وحساسية الطريقة. أظهر

منحنى المعايرة خطية ممتازة، بمعامل ارتباط (R^2) قدره 0.99105 ومعامل امتصاص مولي قدره 4.9×10^4 لتر•مول⁻¹•سم⁻¹. تم تقييم استقرار المعقد الناتج (BPMDNPH-نيكوتين) في ظل الظروف المثلى، حيث أشارت النتائج إلى أن امتصاصية المعقد الناتج ظلت ثابتة، مما يدل على الاستقرار الفائق للمعقد. تم تطبيق الطريقة المطورة بنجاح لتحديد النيكوتين في منتجات التبغ المسوّقة محليًا في ليبيا. وكشفت النتائج عن انحرافات تتراوح من 0.06 إلى 0.21 (الانحراف المعياري) عن القيم المحددة، مع انحراف معياري نسبي إجمالي قدره 1.8%.

الكلمات المفتاحية: النيكوتين، الهيدرازون، 1-برومواسيتوفينون، سيجارة، برمنجنات البوتاسيوم.

Introduction:

Nicotine (Nic) is a naturally occurring alkaloid found in the nightshade family of plants, known as the Solanaceae family [1]. This toxic organic compound triggers various pharmacological effects throughout the body, particularly in the nervous system. Nicotine is present in several plants, including tobacco, tomatoes, potatoes, green peppers, eggplants, and coca leaves. To ensure their survival and reproduction, plants have evolved mechanisms to protect themselves from various threats, such as microbes and herbivorous animals. As a defense strategy, many plants, most notably tobacco, produce nicotine to deter insects and other potential predators [2].

Nic is one of the primary active components in tobacco, functioning as both a relaxant and a stimulant depending on the depth of inhalation by smokers. However, excessive exposure to nicotine can be harmful to health [3]. There is a general consensus that nicotine alters the acute effects of alcohol [4]. The reported threshold limit value for nicotine is 0.05 mg/m³ [5].

Numerous recent studies have provided substantial evidence suggesting that nicotine may increase the risk of developing various diseases, including Alzheimer's disease [3], lung cancer, bladder cancer, and cancers of the larynx and esophagus [5], Parkinson's disease, and cardiovascular diseases [6,7]. Several methods have been used to analyze nicotine in cigarettes, including HPLC [8], radioimmunoassay [9], GC-MS [10,11], CE [12,13], TLC [14], and ASS [5]. However, many of these methods involve lengthy extraction procedures prior to nicotine analysis and the use of highly toxic compounds. However, spectrophotometric methods still the most useful and easily applicable method for the determination of nicotine. The majority of these methods rely on the cleavage of the pyridine ring [15,16].

An early report on the spectrophotometric determination of Nic was published by Asthana et al. [15]. Their method involves the bromination of Nic to form a dibromonicotine complex, which then reacts with potassium iodide to liberate iodine. The released iodine subsequently oxidizes leuco crystal violet ($\lambda = 592$ nm). Omara and Younis [14] also reported on the spectrophotometric determination of Nic in tobacco, which involves the reduction of Fe³⁺ to Fe²⁺ in the presence of potassium ferricyanide in an acidic medium. This reaction produces a Prussian blue complex (KFe[Fe(CN)₆]) with a wavelength of $\lambda = 736$ nm. The first method is complicated, time-consuming, and has a low detection range of 0.2 – 2.2 µg/mL. The second method can detect nicotine within a narrow concentration range of 0.1 – 4.4 µg/mL, but it relies on toxic cyanide reagents, raising safety and health concerns. Therefore, it is essential to develop an efficient method for determining nicotine content in tobacco.

Hydrazone compounds have potential applications as chromogenic ligands. They can serve as chromogenic reagents for the determination of free fatty acids [17], ions such as Fe³⁺ [18], Cu²⁺ [19], Ag⁺ [17], La³⁺ [20], and the acetate anion [21]. This paper will focus on the environmental application of 1-[(bromomethyl)(phenyl)methyl]-2-(2,4-dinitrophenyl) hydrazone (BPMDNPH) [17] (Figure 1) in the removal of nicotine from aqueous solutions. One notable biological application of this ligand is its role as an apoptotic inducer in cancer cells, particularly tongue cancer cells, making it a promising candidate for cancer chemoprevention studies [22]. The compound exhibited significant growth-inhibitory effects against tongue cancer cells, with a low IC₅₀ value of 0.01 mg/ml, and acted in a dose- and time-dependent manner. Additional studies of the same ligand also demonstrated its high selectivity and efficiency in removing Cu²⁺.

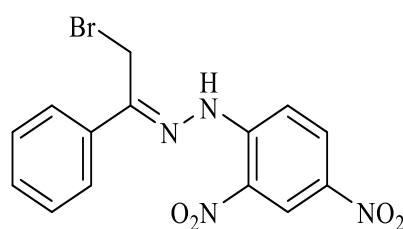


Figure (1): The molecular structure of BPMDNPH

Material and methods:

Experimental:

Apparatus:

UV/VIS Spectrophotometer (Analytikjene, Germany), Rotatory evaporator (Büchi, Switzerland), FT-IR Spectrometer (Perkin Elmer, USA).

Materials:

Nicotine, 2,4-Dinitrophenylhydrazine, 1-Bromoacetophenone, potassium permanganate, KMnO_4 , H_2SO_4 , and ethanol.

Synthesis of the ligand, BPMDNPH:

The hydrazone ligand (BPMDNPH) (Figure 1) was synthesized and characterized following reported method by our research group, and during the investigation of a silica-functionalized hydrazone for the extraction of metal ions [23,24].

General procedure:

The procedure was based on the redox method of Nic with KMnO_4 in aqueous that previously reported by Omara and S. Attaf [5] and Yesgat [25]. In this work, a series of 10 ml standard flasks were prepared by adding 2 ml of 0.8 M sodium hydroxide, followed by varying volumes of a standard nicotine solution ranging from 0.16 to 0.97 ppm. Next, 2 ml of an acidified KMnO_4 solution (4×10^{-4} M) was added, followed by 3.5 ml of BPMDNPH ligand solution (7×10^{-5} M in ethanol). The final volume was adjusted to the mark using ethanol. The absorbance of each solution was measured at a wavelength of $\lambda_{\text{max}} = 455$ nm, using a reference solution for comparison. Under the optimized conditions for forming the Nic- KMnO_4 -BPMDNPH complex, a calibration curve was constructed.

Extraction and determination of nicotine in cigarette tobacco [5]:

Weigh 10 g of cigarette into a 250 ml beaker. Add 100 ml of 0.8 M NaOH solution and stir well for 15 minutes. Filter the mixture using a Buchner funnel, pressing down firmly on the residue. Add 30 ml of distilled water to the filtrate and stir again. Transfer the solution to a separating funnel and extract with 10 ml of chloroform. Repeat the extraction process three times. Combine all extracts in a conical flask and evaporate the chloroform using a rotary evaporator, avoiding excessive heat as nicotine hydrolyzes upon intense heating. Once evaporation is complete, transfer the resulting nicotine to a 100 ml flask and dilute with distilled water. Finally, measure the absorbance of the product using a UV/VIS spectrophotometer.

Results and discussion:

FT-IR Analysis:

The FT-IR spectrum (Figure 2) of the BPMDNPH ligand displayed a distinct N–H stretching vibration at 3281.31 cm^{-1} . The C–H stretching vibrations were predominantly observed near 3102.59 cm^{-1} . Notably, the disappearance of the characteristic C=O stretching band, typically present in acetophenone, along with the appearance of a strong absorption band at 1614 cm^{-1} attributed to C=N stretching provides clear evidence for the successful synthesis of the BPMDNPH ligand [24,26]. The presence of nitro (NO_2) groups was confirmed by the appearance of asymmetric and symmetric stretching vibrations at 1514 cm^{-1} and 1361 cm^{-1} , respectively. Additionally, C–N stretching vibrations were identified within the $1267\text{--}1327 \text{ cm}^{-1}$ range. Vibrations corresponding to the aromatic ring were observed between 1581.19 and 1594 cm^{-1} .

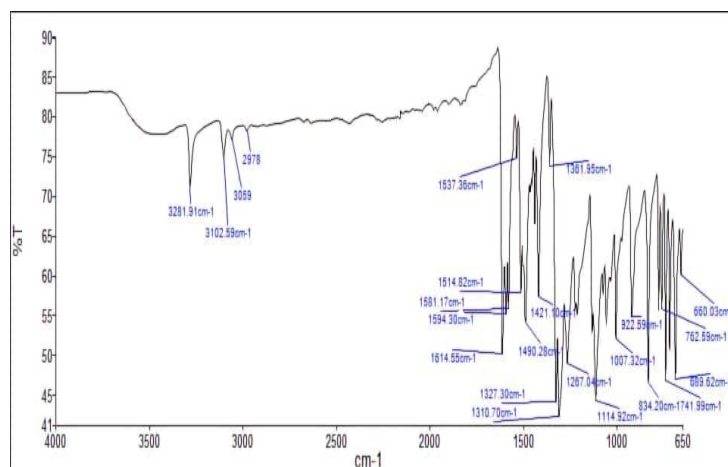


Figure (2): FT-IR spectrum of BPMDNPH.

Complexation formation:

Nicotine with KMnO_4 :

Effect of nicotine concentration:

The effect of varying Nic concentrations (ranging from 0.2 to 10×10^{-4} M) on the formation of a complex with KMnO_4 ions in an acidic medium, prior to the addition of the hydrazone ligand, was investigated by measuring absorbance at 610 nm. Results (Figure 3) showed a significant increase in absorbance with rising Nic concentrations, indicating the formation of a Nic- KMnO_4 complex. At higher concentrations, the absorbance plateaued, suggesting saturation of the reaction. The optimal concentration for complex formation was determined to be 4×10^{-4} M, which yielded the highest absorbance under the experimental conditions.

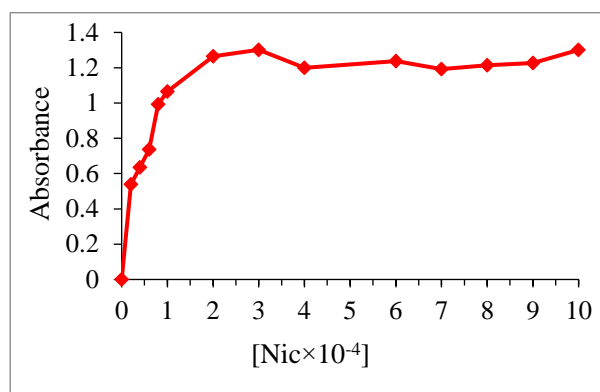


Figure (3): Effect of Nic concentration on the of Nic- KMnO_4 complex formation ($\lambda=610$ nm).

Effect of KMnO_4 concentration:

The effect of KMnO_4 concentration on the formation of the Nic- KMnO_4 complex was also examined in the absence of the hydrazone ligand. As shown in (Figure 4), absorbance increased with KMnO_4 concentration up to 4×10^{-4} M, indicating the optimal concentration for complex formation. Beyond this point, further increases had little effect, suggesting saturation of nicotine and a stable stoichiometric ratio between the reactants.

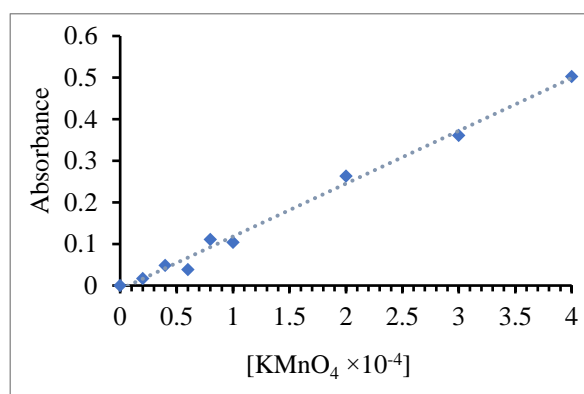


Figure (4): Effect of KMnO_4 concentration on Nic- KMnO_4 complex formation ($\lambda=610$ nm).

Nic- KMnO_4 -BPMDNPH complex:

Effect of solvent:

Due to the ligand's low solubility in aqueous solutions, several organic solvents, including methanol, ethanol, and DMSO, were utilized. The effect of these solvents on the absorption properties of the Nic- KMnO_4 -BPMDNPH complex was examined. Results showed that solvent type significantly affected both absorbance intensity and the maximum wavelength (λ_{max}) (Figure 5). DMSO exhibited higher absorbance at 610 nm, indicating greater complex stability, while ethanol caused a notable shift in (λ_{max}) to 455 nm, suggesting a structural change in the complex likely due to differences in polarity or hydrogen bonding. Thus, ethanol was most effective in altering the complex's nature, whereas DMSO enhanced absorbance intensity.

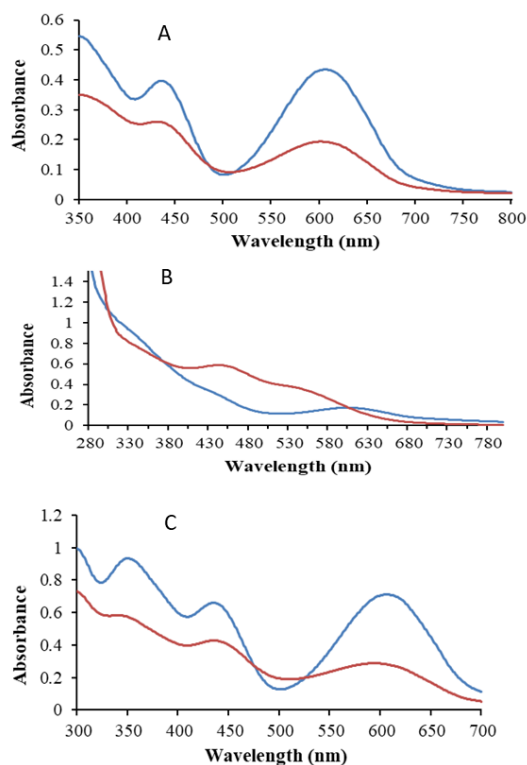


Figure (5): Nic-KMnO₄-BPMDNPH complex formation in; (A) Methanol, (B) Ethanol and (C) DMSO.

Effect of ligand concentration:

The effect of ligand (BPMDNPH) concentration on the complex formation in ethanol was studied over a range of concentrations 1.0 to 10×10⁻⁵M. The absorbance increases gradually with the increase in ligand concentration until it reaches a maximum value at 7×10⁻⁵ (Figure 6), indicating the optimum effect of the ligand concentration of the ligand. After this concentration, no more increase in the absorbance was observed, indicating that further increase in ligand concentration does not significantly enhance complex [(Nic-KMnO₄)-BPMDNPH] formation.

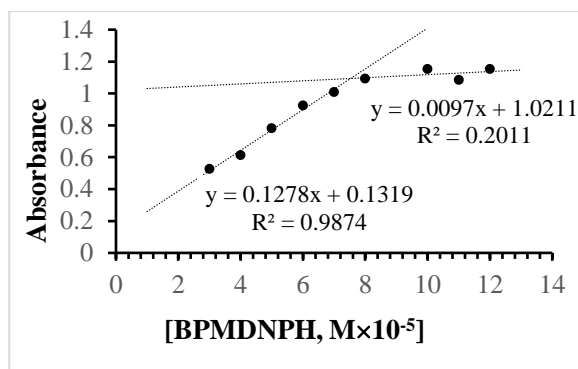


Figure (6): The optimum ligand (BPMDNPH) concentration in the (Nic-KMnO₄)-BPMDNPH complex. (λ=455nm)

Effect of nicotine concentration:

The effect of nicotine concentration on complex formation was also investigated. Very low concentrations of nicotine, ranging from 0.16 to 1.62 ppm, were used (Figure 7). The figure shows a gradual increase in absorbance with increasing nicotine concentration, indicating effective complex formation. Notably, nicotine could be detected even at extremely low concentrations, reflecting the high sensitivity of the method. This method is capable of detecting and quantifying nicotine at very low concentrations (ppm level), making it suitable for sensitive analytical determinations.

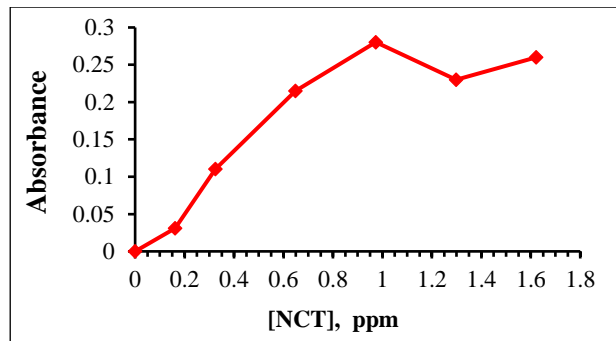


Figure (7): Effect of Nic concentration on the (Nic-KMnO₄)-BPMDNPH complex formation.

Effect of equilibrium time:

In the absence of (BPMDNPH), the stability of the Nic-KMnO₄ complex formed between nicotine (4×10⁻⁴ M) in a basic medium (NaOH, 0.8 M) and KMnO₄ (4×10⁻⁴ M) was investigated over one hour. The absorbance was measured at regular intervals (every 10 minutes) using a UV-Vis spectrophotometer to monitor any changes in complex concentration over time (Figure 8b). Additionally, the stability of the Nic-KMnO₄-BPMDNPH complex was examined over the same duration, with absorbance measurements taken every 10 minutes for a total of 60 minutes. (Figure 8a) shows that the absorbance remained nearly constant throughout this period. The results indicate that the complex is stable over time, with no significant degradation or change in absorbance.

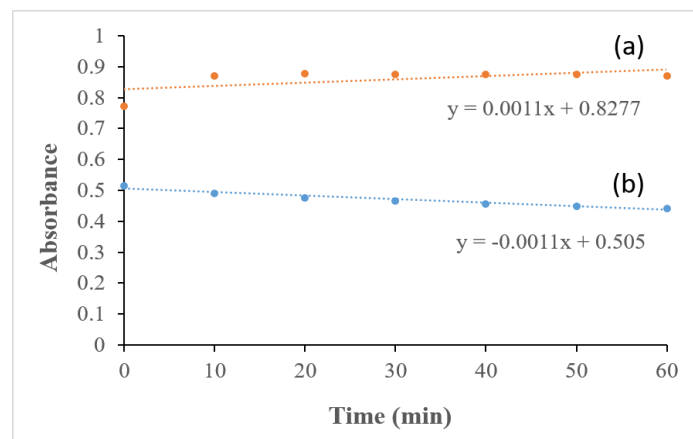


Figure (8): Effect of time on (a) the (Nic-KMnO₄)-BPMDNPH complex formation and (b) on the Nic-KMnO₄ complex formation.

The calibration curve:

After ensuring the optimum conditions for obtaining the maximum. The calibration curve was (Figure 9). These values were then plotted against concentrations that conformed to Beer's law (linear range). The molar absorption coefficient, linear regression equation, correlation coefficient, and other relevant parameters were calculated. The point of intersection of the regression line was also determined. Results are summarized in Table 1.

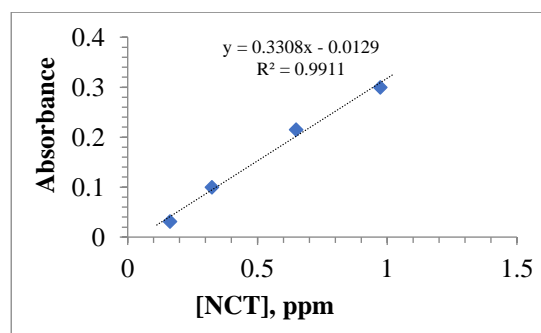


Figure (9): Effect of Nic concentration of (Nic-KMnO₄)-BPMDNPH complex formation (λ_{max} = 455nm).

Table (1): Regression statistics and the optimum condition for the method.

Multiple R	0.99552
Correlation coefficient (R ²)	0.99105
Standard Error	0.01385
Coefficient	0.33076
Standard Error	0.01361
Absorption maximum (λ_{max})	455 nm
Beer's Law Limit ($\mu\text{g/mL}$)	0.16-1.0 ppm
Sensitivity	0.3308
Limit of detection (LOD)	0.13577 $\mu\text{g ml}^{-1}$
Limit of quantitative (LOQ)	0.41144 $\mu\text{g ml}^{-1}$
Limit of linearity	0.97 $\mu\text{g ml}^{-1}$
Standard Deviation (SD)	0.31593
Molar absorptivity	$4.9 \times 10^4 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$
Regression Equation ($y = mx+c$)	$Y = 0.3308x - 0.0129$
Intercept	0.0129

Determination of nicotine in cigarette tobacco:

The current method was applied to real cigarette samples, revealing clear discrepancies between the labeled and experimentally determined nicotine contents (Figure 10). For example, "Wing" contained 0.8 mg versus the claimed 0.2 mg, and "Bon" had 0.5 mg compared to 0.1 mg. These differences may result from labeling inaccuracies, manufacturing variations, or additives. Conversely, "Napoli" showed lower nicotine than labeled. Standard deviations ranged from ± 0.06 to ± 0.21 , indicating variable reproducibility. These findings highlight the importance of direct chemical analysis for accurate nicotine assessment, crucial for public health, regulation, and consumer transparency.

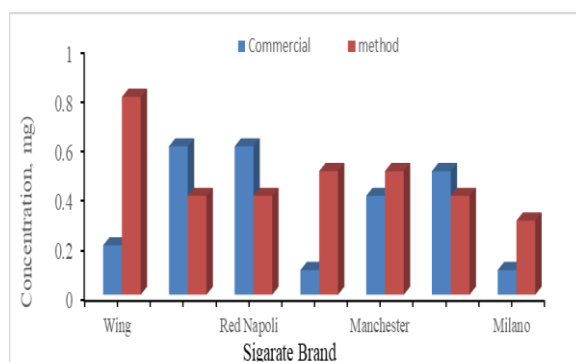


Figure (10): Comparison of manufactured versus experimentally calculated concentrations of tobacco products.

Comparison with Previous Studied:

The present method for nicotine determination was compared with two previously reported methods described above [14,15]. Table 2 summarizes the main differences among these methods. Results indicated that the present method exhibited higher efficiency by detecting nicotine over a broader range (0.16 – 6.0 $\mu\text{g/mL}$) and utilizing a stable complex that can be measured after one hour, without the need for hazardous reagents. This makes the method safer, more practical, and well-suited for the routine analysis of nicotine in tobacco products.

Table (2): Comparison between the present method and previously reported methods for Nic determination.

Method	1	2	3
λ_{max} (nm)	736	592	455
Beer's Law Limit ($\mu\text{g/mL}$)	0.1-4.4	0.2-2.2	0.16-6.0
RSD%	-	-	0.31593
LOD ($\mu\text{g/mL}$)	-	-	0.13577
LOQ ($\mu\text{g/mL}$)	-	-	0.41144
Molar absorptivity ($\text{Lmol}^{-1}\text{cm}^{-1}$)	3.05×10^4	1.4×10^6	4.9×10^4
Reference	[14]	[15]	Present work

Conclusion:

The findings of this research demonstrate the efficiency of the organic reagent used to develop a suitable spectrophotometric method for detecting and quantifying nicotine in various cigarette brands available in the Libyan market. This method shows high responsiveness to low nicotine concentrations and is characterized by its sensitivity, speed, and simplicity. The influence of factors such as KMnO_4 concentration, BPMDNPH concentration, nicotine concentration, and reaction time to determine the optimal conditions for forming a stable complex with maximum absorption. The linear regression results, along with the calculated standard deviation and detection limit, confirm the method's high sensitivity and precision. The presence of the hydrazone ligand enhances the stability of the complex and facilitates the extraction of nicotine from real samples. Overall, the developed method demonstrates strong potential for application in analytical laboratories and can serve as a reliable tool for assessing nicotine levels, whether for regulatory monitoring or future research purposes.

References:

1. S. Mahmood, S. Jameel, and Z. Ahmad, "Isolation, purification and complex formation of nicotine alkaloid," *Engineering and Applied Science Letters*, vol. 1, no. 2, pp. 30–36, 2018, <http://dx.doi.org/10.30538/psrp-easl2018.0009>.
2. A. Steppuhn, K. Gase, B. Krock, R. Halitschke, and I. Baldwin, "Nicotine's defensive function in nature," *Public Library Of Science Biology (PLOS Biology)*, vol. 2, no. 8, p. e217, 2004, <https://doi.org/10.1371/journal.pbio.0020217>.
3. T. Durazzo, N. Mattsson, and M. Weiner, "Smoking and increased Alzheimer's disease risk: a review of potential mechanisms.," *Alzheimer's & Dementia*, vol.10, pp. 122–145, 2014, View at: Google Scholar
4. A. Hossain and S. Salehuddin, "Analytical determination of nicotine in tobacco leaves by gas chromatography–mass spectrometry," *Arabian Journal of Chemistry*, vol. 6, no. 3, pp. 275–278, 2013, <https://www.sciencedirect.com/science/article/pii/S187853521000211X>.
5. H. Omara and S. Attaf, "Spectrophotometric determination of nicotine in cigarette tobacco and biological samples of smokers," *World Journal of Pharmacy and Pharmaceutical Sciences*, vol. 3, no. 8, pp. 1327–1340, 2014,
file:///C:/Users/pc2023/Desktop/nicotine/nicotine2_241125_090916.pdf.
6. D. Haack, R. Baumann, H. McKean, H. Jameson, and J. Turbek, "Nicotine exposure and Parkinson disease," *American Journal of Epidemiology*, vol. 114, no. 2, pp. 191–200, 1981, View at: Google Scholar.
7. N. Benowitz, "The role of nicotine in smoking-related cardiovascular disease," *Preventive Medicine*, vol. 26, no. 4, pp. 412–417, 1997, View at: Publisher Site | Google Scholar.
8. C. Oddeze, A. Pauli, and J. Pastor, "Rapid and sensitive high-performance liquid chromatographic determination of nicotine and cotinine in nonsmoker human and rat urines," *Journal of Chromatography B: Biomedical Sciences and Applications*, vol. 708, no. 1–2, pp. 95–101, 1998, View at: Publisher Site | Google Scholar
9. H. Van Vunakis, H. Gijka, and J. Langone, "Radioimmunoassay for nicotine and cotinine," in *Environmental Carcinogens—Methods of Analysis and Exposure Measurements*, vol. 12, pp. 293–299, 1993, View at: Google Scholar
10. B. Jung, B. Chung, S. Chung, M. Lee, and C. Shim, "Simultaneous GC-MS determination of nicotine and cotinine in plasma for the pharmacokinetic characterization of nicotine in rats," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 20, no. 1–2, pp. 195–202, 1999, View at: Publisher Site | Google Scholar
11. D. M. Bordin, M. Alves, O. Cabrices, E. de Campos, and B. de Martinis, "A rapid assay for the simultaneous determination of nicotine, cocaine and metabolites in meconium using disposable pipette extraction and gas chromatography–mass spectrometry (GC-MS)," *Journal of Analytical Toxicology*, vol. 38, no. 1, pp. 31–38, 2014, View at: Publisher Site | Google Scholar
12. J. Sun, H. Du, and T. You, "Determination of nicotine and its metabolite cotinine in urine and cigarette samples by capillary electrophoresis coupled with electrochemiluminescence," *Electrophoresis*, vol. 32, no. 16, pp. 2148–2154, 2011, View at: Publisher Site | Google Scholar
13. N. Nuchtavorn, M. Ryvolova, F. Bek, M. Macka, C. Phechkrajang, and L. Suntornsuk, "Potential of capillary electrophoresis (CE) and chip-CE with dual detection (C^{4}D and fluorescence detection) for monitoring of nicotine and cotinine derivatization," *Analytical Sciences*, vol. 29, no. 3, pp. 339–344, 2013, View at: Publisher Site | Google Scholar
14. H. Omara and H. Younis, "Spectrophotometric determination of nicotine in cigarette tobacco in Libyan market using iron (III) and potassium ferricyanide," *Sirte University Scientific Journal*, vol. 5, no. 2, pp. 65–74, 2015, <https://journal.su.edu.ly/index.php/susj/en/article/view/1106>.

15. A. Asthana, R. Rastogi, G. Sunita, and V. Gupta, "A simple spectrophotometric method for the determination of nicotine in environmental samples," *Journal of the Chinese Chemical Society*, vol. 51, pp. 949–953, 2004, [https:// DOI:10.1002/jccs.200400141](https://doi.org/10.1002/jccs.200400141).
16. A. Ali, N. Razak, and I. Rahman, "1-[(Bromomethyl)(phenyl)methylene]-2-(2,4-dinitrophenyl) hydrazine," *Acta Crystallographica Section E*, vol. 65, no. 6, pp. o1221–o1222, 2009, DOI:10.1107/S1600536809016225.
17. A. S. M. Ali, A. M. Abdurrrhman. "Determination of free fatty acids in palm oil samples by non-aqueous flow injection using salicylaldehyde-2,4-dinitrophenylhydrazone as colorimetric reagent". *Chemical and Materials Engineering* 1(3): pp. 96-103, 2013. DOI: 10.13189/cme.2013.010306.
18. A. S. Mohamed, A. M. Abusenaina and H. M. Younis, "Spectrophotometric Study of Fe(III) complexes with the newly synthesized Schiff base compound derived from 4,4'-diaminodiphenylmethane". *Libyan Journal of Ecological & Environmental Sciences and Technology*. Vol. 1 No. 1, 68 -78. 2019.
19. Y. C. Chan, A. S. Mohamed, B. Salleh, H. Rosli, C. K. Quah. "Cu (II) complexes of 2-(diphenylmethylene) hydrazinecarboxamidederivatives: Synthesis, characterization and antifungal activity against *Fusarium oxysporum* sp. *Cubense* tropical race 4". *Arabian Journal of Chemistry* 10, pp. S3493–S3500, 2017.
20. A. Salhin, A. K. M. Alhony, A. M. Abusniena and H. M. Younis. "A new lanthanum (III) sensor based on the immobilization of acenaphthenequinone-[N [(2,4-dinitrophenyl)]hydrazone on a triacetylcelluloseMembrane". *International Journal of Engineering, Applied and Management Sciences Paradigms (IJEAM)*, Vol. 54 Issue 10, pp. 1-9. 2020. ISSN 2320-6608.
21. A. S. Mohamed, S. A. S. Krmous, and R. M. Ragwan. "Spectroscopic study of hydrazone-based ligands as calorimetric reagents for acetate anion". *Sirte University Scientific Journal* Vol. 14, No. 1, pp. 53-59, 2024. DOI: 10.37375/susj.v14i1.2802.
22. N. Zulkepli, K. Rou, W. Wan Sulaiman, A. Salhin, B. Saad, and A. Seenii, "Synthetic hydrazone derivative acts as an apoptotic inducer with chemopreventive activity on a tongue cancer cell line," *Asian Pacific Journal of Cancer Prevention*, vol. 12, pp. 259–263. 2011.
23. A. Salhin, N. Abdul Razak and I. A. Rahman. 1-[(Bromomethyl)(phenyl)methylene]-2-(2,4-dinitrophenyl) hydrazine. *Acta Cryst. E*65, pp. o1221–o1222. 2009. DOI:10.1107/S1600536809016225.
24. A. Ali, N. Abdul Razak, and I. Ab Rahman, "Batch adsorption study for the extraction of silver ions by hydrazone compounds from aqueous solution," *The Scientific World Journal*, vol. 1, pp. 10, 2012, ID 351967, 2012, <https://doi.org/10.1100/2012/351967>.
25. D. Yesgat. "Determination of level of nicotine in some commercial cigarettes available in ethiopia using UV-Vis spectrometer." *Journal of Biomolecules and Biochemistry* 6. 2, pp. 1–5. 2022. doi:10.37532/puljbb.22.6(2).06-10.
26. A. Salhin and S. Alshaqsi, "Preparation, characterization and utilization of immobilized 1-((bromomethyl)(phenyl)ethyl)-2-(2,4-dinitrophenyl) hydrazine for solid phase extraction of copper (II) ion," M.S. thesis, School of Chemical Sciences, Universiti Sains Malaysia, 2011.