Batch and Reverse Flow Injection Spectrophotometric Determination of Nicotine

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Abstract

Simple and rapid methods for the determination of nicotine using batch and reverse flow injection spectrophotometric system were developed. Nicotine was extracted from different brand of cigarettes. The methods were based on the reaction between nicotine and excess of sodium hypochlorite (NaClO) to form nicotinic acid followed by reaction of NaClO with methyl orange dye (MO). The absorbance of remaining MO in acidic medium at 505 nm was measured. In the batch method, Beer's law was obeyed in linear range (0.3 – 15 µg/mL) of nicotine with a detection limit of 0.13 µg/mL and correlation coefficient of 0.999. While in reverse flow injection, the calibration graph was linear under the optimum conditions in the range (0.5 – 23.0 µg/mL) of nicotine with a detection limit of 0.13 µg/mL and correlation coefficient of 0.9991. The accuracy and precision of both methods were checked by calculating relative error (E%) and relative standard deviation (RSD%) respectively.

Both methods were applied successfully for the determination of nicotine in multiple brands of cigarette products which collected in Erbil City market. The results were compared with standard HPLC method showing no significance differences between two methods.
Keywords: *Reverse flow injection analysis, Nicotine, Spectrophotometric, Cigarette.*

Introduction

Nicotine is a natural ingredient acting as a botanical insecticide in tobacco leaves (Benowitz et al., 2009). Nicotine is 3-(1-methyl-2-pyrrolidinyl) pyridine, it is a colourless, pale yellow, hygroscopic oily liquid present in the leaves of Nicotiana tabacum. It is one of the highly toxic chemicals belonging to the tobacco alkaloids (Basher et al., 2009).

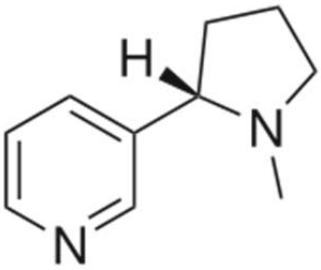

Fig. (1): Structure of Nicotine

Several analytical methods have been reported for the determination of Nicotine by spectrophotometric methods in cigarettes environmental samples (Asthana *et al*., 2004; Al-Tamarh *et al*., 1999; Figueiredo *et al*., 2009) , and gum tablets (Petal *et al*., 2014), It was also determined by LC-MS/MS (Abdullah *et al*., 2016), gas chromatography (Magni *et al*., 2016), flow injection (FI) (Lin *et al*., 2008), and HPLC (Patel et al., 2013). Some of these methods are expensive and time consuming, in addition they are poor in terms of sensitivity and specificity.

Flow injection analysis (FIA) has wide applications mainly due to reduction of the analysis time and reagent consumption compared with conventional manual procedures.

The aim of this work is to develop analytical methods with high accuracy and precision to determine extracted nicotine from different brands of cigarette consumed in Erbil City – Kurdistan Region - IRAQ. The methods were based upon the reaction of nicotine with hypochlorite in the presence of methyl orange (MO) in acidic medium. The hypochlorite was used as an oxidizing agent for nicotine (Morris *et al*., 1999; , Afkhami *et al*., 2005; Fifield *et al*., 2000). The excess of hypochlorite oxidize the methyl orange which leads to bleaching the color of the indicator (MO). Consequently, a proportional increase in the absorbance at the respective λmax is observed with increasing concentrations of nicotine, Fig. (2) explains how nicotine was affected on the color formed.

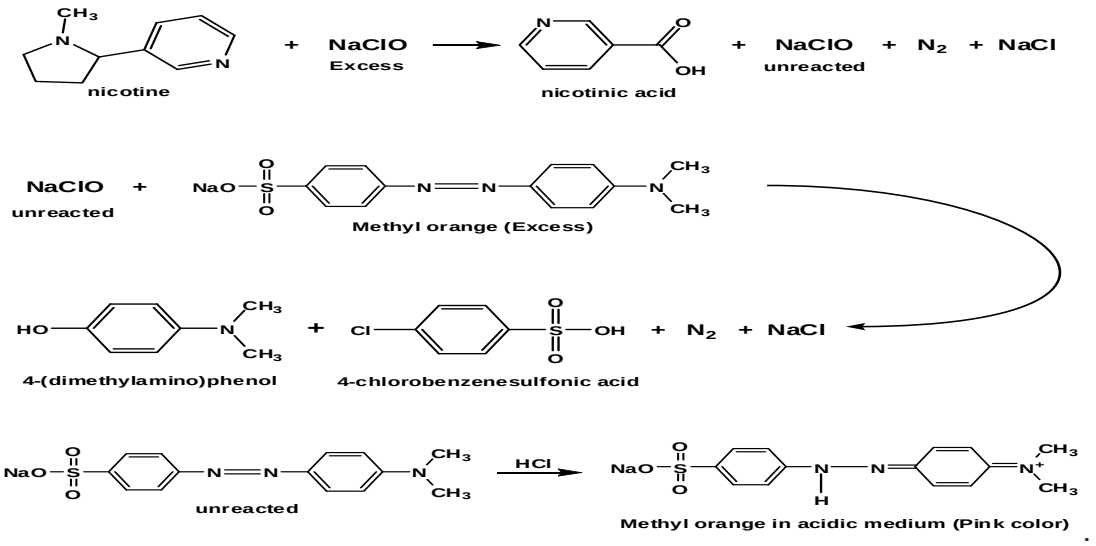

Fig. (2): Proposed Mechanism of nicotine determination

Experimental

Apparatus

The spectral measurements were carried out on a (CECIL CE 3021) UV-Vis spectrophotometer. Absorbance measurements of the batch method were carried out on a JENWAY 6300 spectrophotometer using 1 cm glass cell. A magnetic stirrer (Lab. Companion MS-HP-3000) and water bath (Lab. Companion shaking BS-11, Korea) were used when the reaction requires stirring and heat control respectively. For reverse flow injection analysis part, the schematic design of FI-system Fig. (3) is a multichannel peristaltic pump (DESAGA Heidelberg-England) with silicone pump tubes (0.8-mm i.d.) used to deliver the flow streams. A six-way injection valve (Rheodyne-USA, with variable loop volumes) and PTFE tubes with home-made T-pieces and mixing coil (0.8-mm i.d., different length) were used to connect and mixing of different flow streams. The colored product were monitored spectrophotometrically using JENWAY 6300 spectrophotometer connected to a recorder (PM 8251 A PHILIPS), through a flow-cell (Sterna-micro-flow cell, 100 µL and 1.0 cm path length).

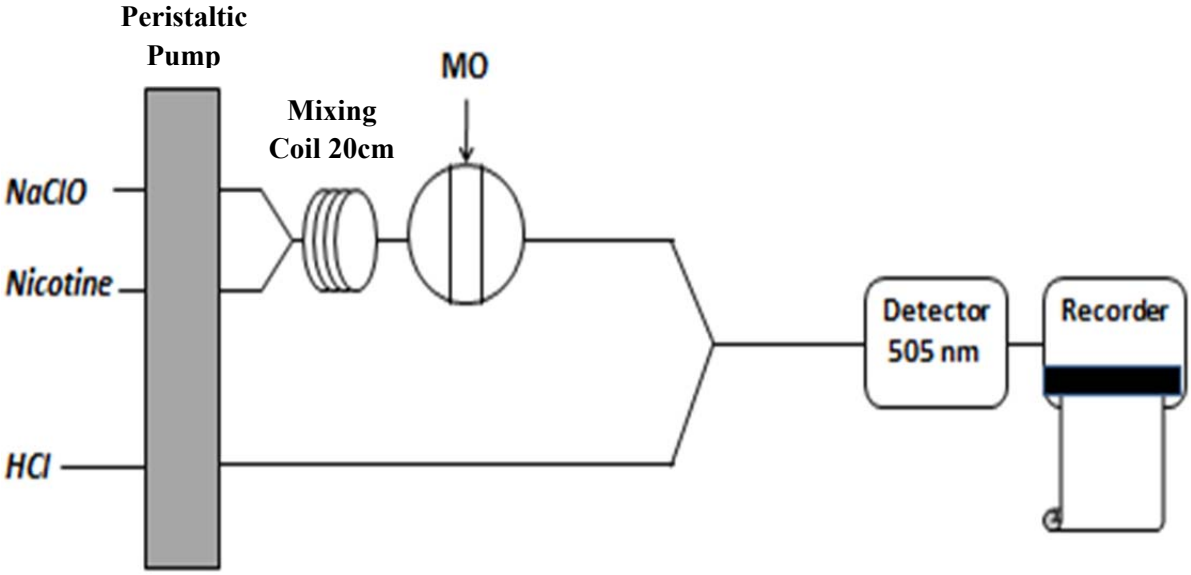

Fig. (3): Schematic diagram of reversed-FIA design proposed for the determination of nicotine.

Reagents

All chemicals were used of analytical grade reagents.

Sodium hypochlorite (NaClO): 0.1 M sodium hypochlorite was prepared daily by diluting 11.57 mL sodium hypochlorite (13%, 1.23 g/mL - BDH) in distilled water and diluting to the mark with distilled water in a 250 mL volumetric flask. It was stored in a dark bottle, and protected from light. Other solutions were prepared by serial dilutions.

Methyl Orange: $0.1x10^{-2}$ M methyl orange was prepared by dissolving 0.163 gm methyl orange (HOPKIN & WILLIAMS) in distilled water and diluting to the mark with distilled water in a 500 mL volumetric flask.

Hydrochloric acid: 1.0 M hydrochloric acid was prepared daily by diluting 20.71 mL hydrochloric acid (37%, 1.19 g/mL- BDH) in distilled water and diluting to the mark with distilled water in a 250 mL volumetric flask.

Sodium hydroxide solution: 5% of sodium hydroxide solution was prepared by dissolving 5 gm of sodium hydroxide (BDH) in approximately 90 mL of distilled water, once dissolved the solution made up to 100 mL with distilled water. This solution was standardized with standard hydrochloride solution.

Standard nicotine solution: 1000 µg/mL of stock solution of nicotine (98%, 1.01 g/mL-162.23 g/mol - BDH) was prepared by dissolving 1.01 mL in a small portion of distilled water then made up it to 1 L in a volumetric flask. It was stored in a dark bottle, and protected from light. Desired concentrations were obtained by diluting the stock solution.

Sample preparation: Table (1) illustrates nicotine contained in a common brands of cigarette purchased from different markets in Erbil city.

Table (1): The trade name and nicotine content in different brands of cigarette.

Trade name	Country of manufacturing	Nicotine content in (mg/cigarette)
Marlboro	Switzerland	0.6
Kent	Germany	0.7
Prestige	Bulgaria	0.7
Pine	Korea	0.7
Pleasure	Korea	0.9
Gitance	European Union	0.6
Gauloises	European Union	0.4
Milano	United Arab Emirates	0.4
ESSE	Korea	0.4

The nicotine content in cigarettes which were subjected to analysis, by the following procedure:

An accurately weighed amount of 10 cigarettes from mixed content of 20 cigarettes (without filter and paper). The content was dissolved in 350 mL of 5% sodium hydroxide solution with stirring for 10 min and left 24 hr for macerating sample, then the solution was filtered by using buchner funnel with filter paper (Schleicher & Schüll- number 589), the filtrate is collected in separation funnel.

Papers of the sample were collected in the beaker, 50 mL distilled water was added and stirring for 10 min. All filtrate collected in the same separation funnel which used in the previous step. The separation process is done by using diethyl ether with repeating three times, all remaining ether collected and vaporized. The Nicotine extract is dissolved in 100 mL methanol and collected in dark bottles at -4°C (Aljamaan *et al*., 2013).

General Batch Procedure

A 1.0 mL of $3x10^{-3}$ M of NaClO and 3.0 mL of 100 µg/mL nicotine (real concentration for nicotine 12 µg/mL) were added to the 25 mL volumetric flask and mixed well for 1.0 min (Time 1) followed by the addition of 1.0mL of $3x10^{-4}$ M methyl orange and mixed again for 1.0 min (Time 2). Finally, 1.0 mL of 0.25 M HCl was added and mixed for 1.0 min (Time 3) and the solution was diluted to the mark with distilled water then, the absorbance measured at 505 nm against a reagent blank.

General Reverse Flow Injection Analysis (rFIA) Procedure

The reverse flow injection analysis (rFIA) manifold shown in Fig.(2) was used for determination of nicotine. The system consists of three stream lines; the first one to deliver $1.2x10^{-3}$ M NaClO merged with the second one; 20

µg/mL standard nicotine solution using 20 cm mixing coil at a flow rates 2.0 mL/min using multi-channel peristaltic pump. $1.2x10^{-5}$ M methyl orange was injected with reagent volume 100 µL and merged with the combined line. Eventually, the product of these three components were coupled with the third one; 0.01M HCl and directed towards flow cell in the detector. The Δpeak height (absorbance of the analyte – absorbance of the blank) of the product formed was continuously recorded at a wave length 505 nm.

Results and Discussion

Absorption and Spectra

The absorption spectra were taken by (A) mixing 1.0 mL of 0.3 $x10^{-3}$ M methyl orange with 1.0 mL of 0.25 M HCl, shaking for 1.0 min and the solution was diluted to the mark with distilled water in 25 mL volumetric flask. The pink color absorbs strongly at 505 nm Fig (4A),

Mixing 1.0 mL of $3x10^{-3}$ M of NaClO and 3.0 mL of 100 µg/mL nicotine (12 µg/mL) were added to the 25 mL volumetric flask and mixed well for 1.0 min followed by the addition of 1.0mL of $3x10^{-4}$ M methyl orange and mixed again for 1.0 min then 1.0 mL of 0.25 M HCl was added and the solution was diluted to the mark with distilled water. The pink color showed a maximum absorbance at 505 nm Fig. (4B).

Mixing 1.0 mL of $3x10^{-3}$ M of NaClO and 3.0 mL of distilled water in 25 mL volumetric flask for 1.0 min followed by the addition of 1.0 mL of $3x10^{-4}$ M methyl orange and mixed again for 1.0 min. Finally, 1.0 mL of 0.25 M HCl was added and the solution was diluted to the mark with distilled water. The pink color showed a maximum absorbance at 505 nm Fig.(4C).

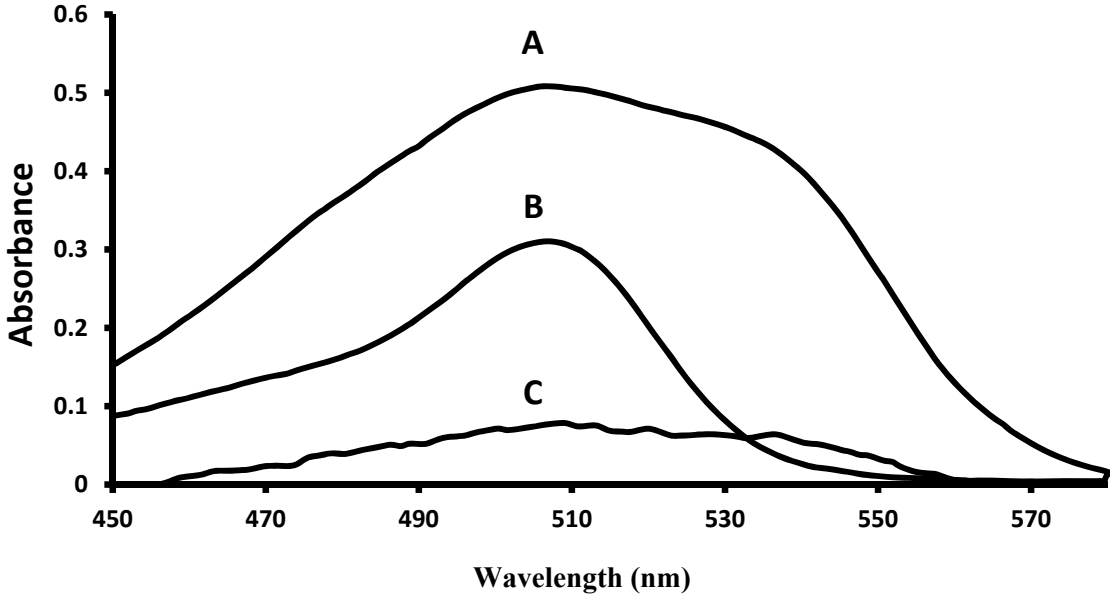

Fig. (3): The spectra of (A) MO solution, (B) Nicotine sample solution, (C) Blank solution.

Optimizations

Effect of order of addition

The effect of addition of the reactants is very important. The effect of order of additions on the oxidation process of nicotine was studied by measuring the absorbance of solutions prepared by different sequence of additions against a blank solution prepared in the same manner. Experiments showed that (Oxidant + Nicotine + MO + Acid), gave the best results.

Selecting the best acid

The effect of using different acids (HNO_3, HCl, CH_3COOH, H_2SO_4, H_3PO_4) on the intensity of the color were studied. According to the results obtained, HCl was found to be the more suitable acid to acidify the reaction medium. Table (2) shows the results obtained.

Table (2): Effect of difference type of acids on intensity of color

Type of acids	HNO_3	HCl	CH_3COOH	H_2SO_4	H_3PO_4
Absorbance					
ΔA	0.162	0.236	0.132	0.174	0.196

Effect of sodium hypochlorite concentration

The effect of sodium hypochlorite (3x10-3 M) in the range of 0.4 - 2.8 mL for batch and 2 x 10-4 – 14 x 10-3 M for rFIA on the sensitivity of the reaction were studied as shown in Fig.(5) and Fig. (6). It was found that ΔA (difference between higher absorbance and lower absorbance) 1.2 mL for batch, and ΔPeak height of 0.6 x 10-3 M for rFIA were the optimum for this work.

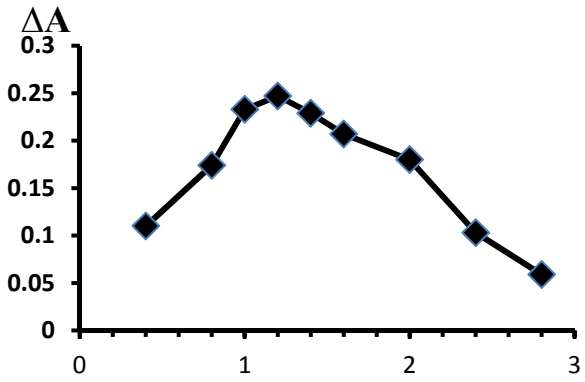

Fig. (5): Effect of 0.3 x 10^{-2} M NaClO in batch

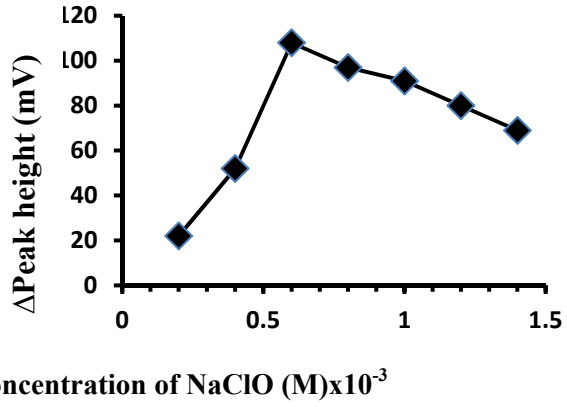

Fig. (6): Effect of NaClO (rFIA)

Effect of methyl orange concentration

The study was performed to predict the effect of the methyl orange (3 x10-4 M) volume on the color intensity by measuring the ΔA at different methyl orange volumes in the range 0.2 - 1.6 mL. The same study was examined at 1.2x10-5 – 9.6x10-5 M concentration for rFIA part. The optimum ΔA was obtained at 1mL methyl orange and 3.6x10-5 M was found enough to develop the color to its full intensity which gave a maximum Δpeak height for rFIA part as shown in Fig. (7) and (8).

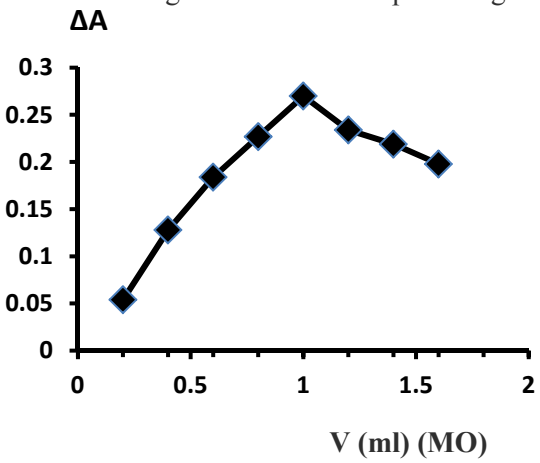

Fig. (7): Effect of MO concentration **(Batch)**

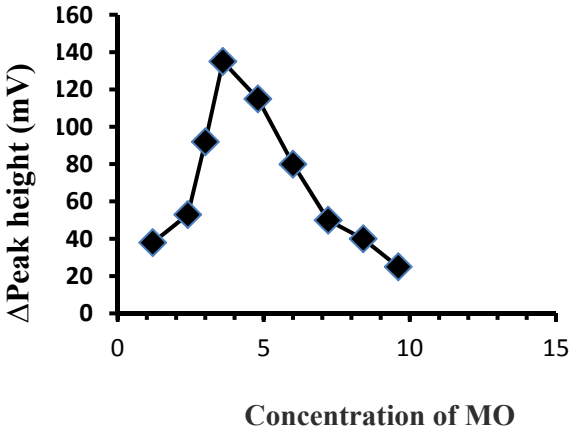

Fig. (8): Effect of MO concentration **(rFIA)**

Effect of hydrochloric acid concentration

Various volumes (0.2 - 1.6mL) of 0.25 M HCl solution were added to solution, It was found that 1.0 mL enough to develop the color of its full intensity which gave a maximum value of ΔA (Fig. (9)). Different concentrations of HCl were also studied in the range of 0.01 – 0.07 M for rFIA. As shown in Fig. (10), the concentration of 0.05 M was found to be the optimum.

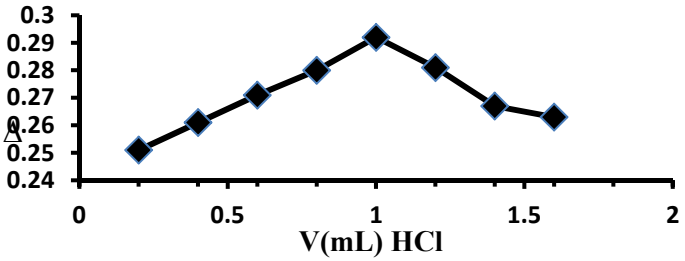

Fig. (9): Effect of HCl concentration (Batch)

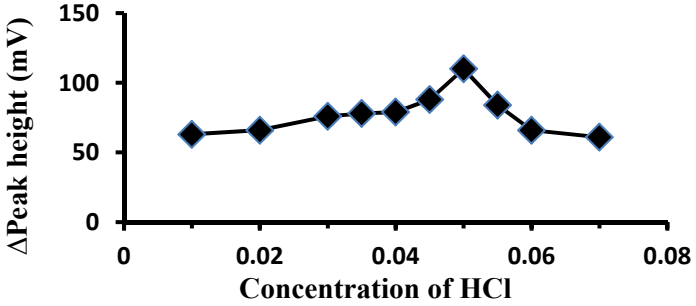

Fig. (10): Effect of HCl concentration (rFIA)

Optimizations of Physical Parameters

In order to produce the best Δpeak height, Table (3) shows the best results for both batch and rFIA methods physical parameters.

Table (3): The physical parameters for both techniques

Parameter	Batch	rFIA
Coil length (Time 1)	1 min.	1 min
Coil length (Time 2)	1 min.	1 min
Coil length (Time 3)	1 min.	1 min
Temperature	25˚C	25˚C
Flow Rate	--	2 mL/min.

(--) not measured

Calibration Graph for Batch Method

The calibration graph was constructed between the ΔA and the concentration of nicotine (µg/mL) using optimum experimental conditions, Table (4) shows a good linearity over the range of concentration of nicotine (0.3-15 µg/mL), with correlation coefficient R=0.999.

Table (4): Data from the calibration graph in batch method.

Linear range (µg/mL)	Detection limit (µg/mL)	Molar absorptivity (L/mol.cm)	Correlation coefficient (R)
0.3 -15	0.13	2887	0.999

The accuracy (relative error percent (%E)) and precision (relative standard deviation (%RSD)) of the method under optimum experimental conditions was evaluated, Table (5) shows good results for accuracy and precision of batch method.

Table (5): Accuracy and precision determination of batch method.

Nicotine concentration (µg/mL)		E %	RSD %	Recovery%
Standard solution	Found from proposed method			
5.0	5.14	2.8	1.42	102.8
10.0	9.86	-1.4	0.47	98.6

n=5

Calibration Graph for rFIA Method

Under optimum conditions, the linear calibration graph over the range 0.5 – 23 µg/mL of nicotine was constructed. Table (6) shows the statistical variables.

Table (6): Data from the calibration graph of nicotine determination in rFIA method.

Linear range (µg/mL)	Detection limit (µg/mL)	Molar absorptivity (L/mol.cm)	Correlation coefficient (R)
0.5 -23	0.13	26082	0.9991

The precision and accuracy of this method was summarized in Table (7).

Table (7): Accuracy and precision for the rFIA determination of Nicotine.

Nicotine concentration (µg/mL)		E %	RSD %	Recovery%
Standard Solution	Found from proposed method			
8.0	8.12	1.56	1.15	101.5
16.0	16.21	1.31	0.45	101.3

n= 5

Application

Both methods were applied for the determination of nicotine content in tobacco of different cigarette brands in Erbil city/ Kurdistan of IRAQ. The results were compared with HPLC analysis for the same formulations (Alali et al., 2003) as standard method by calculating the percentage error as shown in Tables (8) and (9). From both tables we concluded there are no significant difference between accuracy and precision of the two methods.

Table (8): Comparison of batch method with standard method for determination of 5 µg/mL nicotine using t-test and F-test.

Brands of cigarettes	Amount found (µg/mL)		Difference between two methods	E%
	Present method	Standard method		
Marlboro	9.984	9.814	0.17	1.73
Kent	8.342	8.418	-0.076	-0.90
Prestige	7.864	7.951	-0.087	-1.09
Pine	5.151	5.094	0.057	1.11
Pleasure	8.632	8.541	0.091	1.06
Gitance	6.143	6.225	-0.082	-1.31
Gauloises	7.196	7.115	0.081	1.13
Milano	6.451	6.558	-0.107	-1.63
ESSE	9.884	9.943	-0.059	-0.59
Mean	7.738	7.739	-0.001	
SD	1.658	1.634	0.101	
$t_{calculated}$=0.039	t_{table}= 3.18			
$F_{calculated}$=1.029	F_{table}= 3.69			

Table (9): Comparison of rFIA method with standard method for determination of 5 µg/mL nicotine using t-test and F-test.

Brands of cigarettes	Amount found (µg/mL)		Difference between two methods	E%
	Present method	Standard method		
Marlboro	9.872	9.814	0.058	0.59
Kent	8.381	8.418	-0.037	-0.86
Prestige	7.989	7.951	0.038	0.47
Pine	5.134	5.094	0.04	0.78
Pleasure	8.585	8.541	0.044	0.51
Gitance	6.282	6.225	0.057	0.91
Gauloises	7.169	7.115	0.054	0.75
Milano	6.513	6.558	-0.045	-0.68
ESSE	9.904	9.943	-0.039	-0.39
Mean	7.758	7.739	0.018	
SD	1.626	1.634	0.045	
$t_{calculated}$=1.258	t_{table}= 3.18			
$F_{calculated}$=0.989	F_{table}= 3.69			

Conclusion

A simple and efficient reverse-flow injection method with spectrophotometric detection were proposed for the determination of nicotine in different cigarette brands. The reagent injection method overcomes manifold blockage problems. The time required for sample preparation in the present methods are short and reagent consumption is low, hence the method are highly economic and can be used on routine basis for the determination. Based on the results obtained, the proposed methods are accurate, precise, reproducible and economical.

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ریگای کلاسیك وتەکنیکەکانی دەرزی لیدانی رۆیشتوو بۆ خەملاندنی نیکۆتین

کورتیا لێکولینێ:

ئەم تۆژینەوەیە بریتیە لە هەر یەك لە ریگای کلاسیك وریگای پیچەوانەییدەرزی لیدانی رۆیشتوو بۆ خەمەلاندنی نیکۆتین لە جۆری جیاوازی جگەرە. وئەم دوو ریگایە بەندە لە سەر کارلیکی نیوان نیکۆتین وبریکی زیاد لە سۆدیۆم هایپوکلورایت وە دروستبوونی نیکوتینك ئەسید، هەروەها کارلیکی زیادەی سۆدیۆم هایپوکلۆرایت کە لە گەل بریکی زیاد لە میسایل ئۆرەنج، زیادەی میسایل ئۆرنجەکە لە ناوەندیکی ترش بەرزترین تیشکە مژینمان دەداتی لە 505 نانۆمەتر. لە ریگایە کلاسیکە کە هیلی پیوەری خەملاندن دروست کرا لە مەودای (0.3 -15) مایکرۆکام/ ملیلیتر بە هاوکۆلکەی بەستنەوە 0.999 وە سنووری ناسینەوە یەکسان بوو بە 0.13 مایکرۆگرام/ملیلیتر.

سیستەمی پیچەوانەی دەرزی لیدانی بەکارهینرا بۆ خەملاندنی نیکۆتین،ئەنجامەکان دەریانخست کە پەیوەندیەکی هیلی هەیە لە باری گونجاودا لە مەودای (0.5-23)مایکرۆگرام/ملیلیتر وە سنووری ناسینەوە (0.13) مایکرۆگرام/ملیلیتر وە بە هاوکۆلکەی بەستنەوە 0.9991. وردبینی ریك کاری ریباوەکە بۆ هەردوو ریگاکە ئەنجام درا بە هۆی لادانی پیۆەری ریژەیی (RSD%) وە هەلەی ریژەیی (E%) بە بەکارهینانی دوو پەیتی جیاواز.

الطريقة الكلاسيكية وطريقة الحقن الجرياني لتقدير النيكوتين

الخلاصة:

يتضمن هذا البحث طريقتين تحليلتين لتقدير النيكوتين في أنواع مختلفة من السجائر ومن مختلف المناشئ: الطريقة الأولى تقدير النيكوتين طيفياً بالأعتماد على التفاعل بين النيكوتين وزيادة محسوبة من هايبوكلوريت الصوديوم لتكوين حامض النيكوتينيك، يعقبها تفاعل الزيادة من هايبوكلوريت الصوديوم مع كمية ثابتة من المثيل البرتقالي. وتعطى الكمية المتبقية من المثيل البرتقالي أقصى امتصاصية في 505 نانومتر في الوسط الحامضي. يمكن تطبيق قانون بير في مديات التركيز (0.3– 15 ميكروغرام/مل) مع حد الكشف بمقدار (0.13 ميكروغرام/مل) مع معامل الأرتباط بمقدار 0.999. بينما تتضمن الطريقة الثانية طريقة تحليل الحقن الجرياني المعكوس لتقدير النيكوتين، تم الحصول على علاقة خطية بين التراكيز المختلفة للنيكوتين والأمتصاصية تحت الظروف المثلى للتحليل (0.5 – 23 ميكروغرام/مل) مع حد الكشف (0.13 ميكروغرام/مل) ومعامل الأرتباط 0.9991.

تم أختبار الدقة والتوافق للطريقتين بحساب الأنحراف المعياري النسبي (RSD%) والخطأ النسبي(E%) لمستويين مختلفين من التراكيز.