Al-Mukhtar Journal of Sciences 34 (3): 216-224, 2019

Determination of Nicotine in Libyan Smokers' Urine Compared with that of Nonsmokers using Reversed Phase – High Performance Liquid Chromatography (RP-HPLC)

Galal Elmanfe[1*], Suad K. Omar [1], Noreldin S.Y. Abdolla[2] and Amna M. Hassan[1]
[1]*Chemistry Department, Faculty of Science, Omar Al-Mukhtar University, Al-Bayda- Libya*
[2]*Chemistry Department, Faculty of Science, Tobrouk University, Tobrouk, Libya*

Received: 15 July 2019/ Accepted: 15 November 2019
Doi: https://doi.org/10.54172/mjsc.v34i3.323

Abstract: The aim of the present study was to evaluate the levels of nicotine in twenty urine samples taken from ten smokers and ten non-smokers in Libya. Each volunteer was required to complete a questionnaire before providing the urine sample. The evaluation of the nicotine concentrations was carried out by means of a simple, rapid, cost effective but reliable, one-step extraction technique-reversed-phase high-performance liquid chromatography which was developed and validated for this purpose. The criteria and factors taken into consideration for this evaluation and validation include the linearity, precision, accuracy, limit of detection, and limit of quantitation. The urine samples from the smokers presented nicotine concentrations in the range of 0.037-1.979 µg/*ml*, with an average of 0.663 µg/*ml*. The range of the nicotine concentrations in non-smokers, on the other hand, was from 0.017-1.331 g/*ml*, where 0.273 µg/*ml* is the average value. Statistical analyses show that the nicotine concentrations were very significant in the smoker samples in contrast with the nonsmoker samples.

Keywords: Cigarettes; Smoking; Nicotine; Urine; Extraction; RP-HPLC

INTRODUCTION

Smoking, especially cigarettes, certainly has a harmful impact on the health of smokers and people around them, along with economic issues for smokers. As the recognition of such problems has become more widespread, the movement to ban smoking has gained momentum (Tollison, 1988; Watson & Witten, 2001). Cigarette smoking is the leading cause of mortality and morbidity in our society (Giovino, 1999), and the leading cause of preventable disease and death in world (Jha et al., 2008). Each year, tobacco deaths in the United States are estimated to be higher than those from motor vehicle accidents, suicide, fires, homicide, AIDS, alcohol, heroin and cocaine combined (U.S. Department of Health and Human Services, 2000). People often commence smoking at the age of 16-17 years, and adolescent males tend to continue smoking for another 16 years and females for 20 years (Pierce & Gilpin, 1996). Every day, approximately 3000 teenagers and children become regular tobacco users (Stevens, A. Marie Barron, Carol A. Ledbetter, Katie M. Foarde, & Menard., 2001). Some research studies that have focused on adolescent smoking and its relation to several individual and family backgrounds, have discovered that white people are more likely to engage in smoking in comparison to other racial groups (Allen et al., 2003; Orlando, Tucker Joan S, Ellickson Phyllis L., & J., 2004; Wills & Cleary, 1997). Furthermore, it is more common for non-smokers to come from two-parent families or families in which the parents have higher levels of education. (Orlando et al., 2004). Smoking is considered to be a high risk factor for chronic obstructive pulmonary disease, cancer and atherosclerosis, etc., (Gupta, Prakash, Gupta,

Corresponding Author: [1]Galal Elmanfe, galal.elmanfe@omu.edu.ly, Chemistry Department, Faculty of Science, Omar Al-Mukhtar University, Al-Bayda- Libya

& Gupta, 1997; Padmavati, 2002). The World Health Organisation estimates that deaths resulting from cigarette smoking in India might exceed 1.5 million yearly by 2020 (Pasupathi, Bakthavathsalam, Rao, & Farook, 2009; Rani, Bonu, Jha, Nguyen, & Jamjoum, 2003). Nicotine (3-(1-methyl-2-pyrrolidinyl)pyridine), which exists in the leaves of nicotine tobacco, is considered to be the most toxic chemical in tobacco alkaloids (Rodricks, 1992; Wu, Siems, Hill, & Hannan, 1998). It is one of many thousands of substances that make up tobacco contributing to its flavor, aroma, and physiological effects. Nicotine is a tracer for environmental tobacco smoke (ETS) due to the fact that it specifies the tobacco (Jones, 1994). In addition, it is a chemical that is commonly used as a natural insecticide, as well as being a highly addictive drug (Hamm, 1982).

Certain modifications were made for the purpose of the isolation and determination of nicotine in urine in smokers, constituting liquid–liquid extraction with binary solvents (Davoli, Stramare, Fanelli, Diomede, & Salmona, 1998; Elmanfe & Abdulla, 2014; Massadeh, Gharaibeh, & Omari, 2009) to get better detection limit, linearity over high range, recovery, and no interference peaks. The one-step extraction method used in this study was more rapid and simpler than other extraction methods, but still reliable. It was developed specifically for this purpose, with consideration given to methods recommended by previous researchers (Ceppa, El Jahiri, Mayaudon, Dupuy, & Burnat, 2000). The utilization of a single extraction step with 5-10 *ml* of a solvent mixture is another advantage of the method developed. The analyses of the method were all developed and validated using HPLC. This study was aimed to estimate the concentration of nicotine in smokers and nonsmokers' urine samples using RP-HPLC.

MATERIALS AND METHODS

Chemicals and reagents: All chemicals, analytical standards, reagents, and solvents used throughout this study were of analytical grade and high quality (≥ 99.9%) without any further purification and were obtained from Sigma-Aldrich, Fluka, Riedel-Dehaen AG Seelze Hannover and Merck) unless mentioned otherwise.

Preparation of standard solutions: Standard nicotine solution: 100 mg in 100 *ml* (1 mg/*ml*) solution was prepared. After that, the desired standards solution were prepared by appropriate dilution of the stock. (5, 10, 15, 20 and 25 µg/*ml*). The solution of Potassium dihydrogen phosphate (KH_2PO_4): (0.2973 g) of salt was dissolved in one litre or (0.5946 g) of KH_2PO_4 in two litres. This standard solution was used as the mobile phase for HPLC, with its pH modified by means of dropwise addition of orthophosphoric acid (pH ≈ 3.2). Sodium hydroxide (5M) solution is prepared by dissolving 20 g of NaOH in 100 *ml* of H_2O to make 5M of the solution. Also, 0.25M of hydrochloric acid was prepared.

Instrumentation: The HPLC system (Thermo Series P2000 Pump) Autosampler, Series 200 UV/Vis (from 190 to 1000 nm, Series 200 Autosampler, Series 200 Analytical Pump, Series 200 Column Oven, and 20 *µl* loop injector. The stationary phase represents the analytical column which was a Brownlee Bio C18 column of 250×4.6 mm and 5 µm particle size. The HPLC operating conditions are shown in Table 1.

Table (1). HPLC operating conditions

Mobile Phase	**a:** 82% phosphate buffer (KH_2PO_4); pH≈3.2
	b: 18% MeOH
Flow rate	1 *ml*/min
Injection	10 *µl*

Standard Solutions (HPLC Calibration): Calibration standards in the range (5-25 µg/*ml*) were prepared by serial dilution from the stock solution of nicotine and the calibration curve for it as shown in Figure 2. Figure 3 displays the chromatograms of different concentrations of nicotine (5-25 µg/*ml*) at 258 nm.

ISSN: online 2617-2186 print 2617-2178

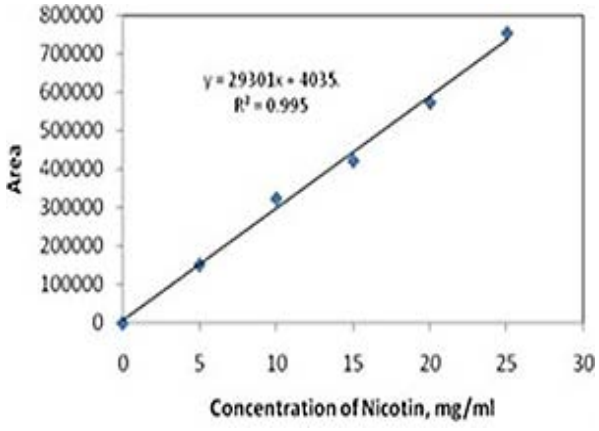

Figure (1). Calibration curve for standard solutions of nicotine, expressed on a linear scale.

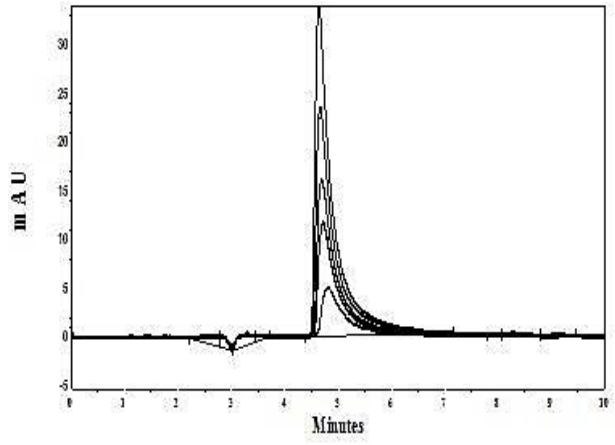

Figure (2). Chromatograms of different concentrations of nicotine by HPLC.

Sample collection: The samples were collected at the Medical Laboratory of the Clinic of Omar Al-Mukhtar University, El-Beida in Libya. A total of 20 samples were taken, 10 from male smokers, eight from male non-smokers and two from female non-smokers. All samples were taken at the same time. The detailed content of each volunteer was tailored according to the answers in their individual questionnaires. The collected data were classified on the basis of smokers' urine (male) and nonsmokers' urine (male/female) and the data are shown in Tables 2, 3, respectively. Note: all the urine samples were collected and transported immediately to the laboratory and kept at ˍ 80 °C until analysis.

Table (2). Samples collection from male smokers' urine.

S. No.	Age/Year	Smoking Peri-od/year	cigarette brands	Amount smoked/Daily	Time/ min
1	40	20	Milano	20	5
2	50	30	Milano	20	5
3	22	15	Eagle	20	15
4	42	20	Onis White	20	10
5	18	8	Platinum	30	15
6	36	17	Oris Blue	20	10
7	48	25	Oris Blue	20	3
8	48	25	D.G	20	3
9	50	30	Onis White	8	60
10	21	14	Oris Blue	2	5

S. No. = Sample Number; Time/min = After Smoking

Table (3). Samples collection from male and female nonsmokers' urine.

S. No.	Volunteer Age /Year	Volunteer Gender
11	32	Female(pregnant)
12	33	Male
13	60	Male
14	22	Male
15	18	Male
16	45	Female
17	13	Male
18	9	Male
19	16	Male
20	21	Male

Extraction of Nicotine: The extraction procedure was carried out according to those described in the literature, with minor modifications (Davoli et al., 1998; Elmanfe & Abdulla, 2014; Massadeh et al., 2009) at room temperature and neutral pH. A 0.5 *ml* urine sample was placed in a glass test tube. Each sample was alkalinized with 200 *µl* of 5 M NaOH, then vortex mixed at 2800 rpm for 2 minutes. An aliquot (6 *ml*) of the dichloromethane/diethylether (1:1 v/v) was used for one-step single extraction, then vortex mixed again at 2800 rpm for 2 minutes. When the or-

ISSN: online 2617-2186 print 2617-2178

ganic layers were centrifuged (3000 rpm for 3 minutes) to break the emulsion formed, they were transferred to a new glass tube containing 40 μl of 0.25M HCl. After that, the organic phase was then evaporated at 35 ºC in a water bath until dry and reconstituted to (2 *ml*) with a mobile phase consisting of a mixture of 0.2973 g of KH_2PO_4, (820 *ml*) of distilled water, and 180 *ml* of methanol (HPLC-grade). The samples (aliquot 10 μl) were injected automatically onto the HPLC and analyzed.

RESULTS

This section explains the results obtained in this study, as well as highlighting the efficiency of the methods used, together with the instrumentation. The results indicate that the nicotine in the smokers' urine was in the range of 0.037-1.979 µg/*ml* with an average of 0.663 µg/*ml* (see Table 4), while the nicotine in the non-smokers' urine was in the range of 0.017-1.331 µg/*ml* with an average of 0.2735 µg/*ml* (see Table 5). According to the detailed results shown in Tables 4 and 5, there was a significant difference in nicotine concentrations between smokers and non-smokers at a 95% confidence level, but the average concentration of nicotine in smokers' urine was greater than that in non-smokers urine.

Table (4). Concentrations of nicotine in male smokers' urine by HPLC,(n=3)

Sample No.	Age/year	Gender	Area	Concentration of nicotine/ppm (µg/*ml*) in 0.5 *ml* of Urine
1	40	5	9787	1.9793
2	50	5	3284	0.2587
3	22	15	1576	0.8464
4	42	10	3927	0.0374
5	18	15	144	1.339
6	36	10	26489.33	0.7727
7	48	3	12153	0.2793
8	48	3	6076.5	0.7023
9	50	60	0	0.000
10	21	5	5251.333	0.4184
		Average		0.663
		ST		0.618

Table (5). Concentrations of nicotine in male and female nonsmokers' urine by HPLC

Sample No.	Age /year	Gender	Area	Concentration of nicotine/ppm (µg/*ml*) in 0.5 *ml* of Urine
11	32	Female (pregnant)	129	0.0172
12	33	Male	18971.67	0.5140
13	60	Male	42723.67	1.3314
14	22	Male	11552.67	0.2587
15	18	Male	4535.333	0.1178
16	45	Female	444	0.0629
17	13	Male	1029.5	0.1035
18	9	Male	612	0.0714
19	16	Male	6109	0.1344
20	21	Male	5862.333	0.1236
		Average		0.2735
		ST		0.397

ISSN: online 2617-2186 print 2617-2178

DISCUSSION

Linearity of the technique was appreciated by successive dilutions of high concentration nicotine samples. Limit of detection and quantitation were determined, as well as precision and confidence limit for the mean.

Study Statistic:

Linearity: Examination of calibration curves was conducted by computing a linear least-squares regression analysis on the plot of the peak area ratio of nicotine versus concentration over the range of 5-25 µg/*ml.* In the RP-HPLC (utilizing five concentration levels) and in the equation of the regression curve was y=29301x+4035 with correlation coefficients (R^2), it was always higher than 0.995. It should be noted that the extractions were carried out at a neutral pH and room temperature, as shown previously.

Limit of detection (LOD): Limit of detection (LOD) is defined as the concentration of analyte required to give a signal equal to three times the standard deviation of the blank. The LOD was calculated using the following equation:

$$LOD = 3\ S_{y/x}/b$$

Where *S* is the average of the standard deviation $S_{y/x}$ of the peak area /concentration ratio (peak area of standard solution/its concentration), and *b* is the average of the slope of a calibration curve. In the present study, the limit of detection (LOD) value for nicotine in urine samples using HPLC was 2.26 µg/*ml.* Compared with other studies, the LOD value for nicotine using HPLC was 0.15 ng/*ml* (0.00015 µg/*ml*) in a urine sample (Massadeh et al., 2009) whereas LOD value reported for nicotine in urine using GCMS was 0.2 ng/*ml*

$$X_t = \overline{X} \pm (ts/\sqrt{n})$$

(0.0002 µg/*ml*) (Shin, Kim, Shin, & Jee, 2002). In both studies, the LOD values were much smaller than our result (LOD = 2.26 µg/*ml*). Knowing that the concentration range in these studies was much smaller than that in our study.

Limit of quantitation (LOQ): Limit of quantitation (LOQ) is defined as the concentration of analyte required to give a signal equal to ten times the standard deviation of the blank. The LOQ was calculated using the following equation:

$$LOQ = 10\ S_{y/x}/b$$

The LOQ of nicotine in the present study was determined by HPLC to be 6.85 µg/*ml.* in urine. The LOQ of nicotine using RP-HPLC in urine sample has been previously reported to be 0.5 ng/*ml* (0.0005 µg/ml), whereas the LOQ of nicotine in urine was determined by HPLC to be 0.66 ng/ml (0.00066 µg/*ml*) (Massadeh et al., 2009). Compared with previous assays, LOQs achieved in this study were less sensitive than those obtained in a previous study (Massadeh et al., 2009). Taking into account the difference in the concentration range of the standard solutions between these studies and our study.

Accuracy and precision: Accuracy is expressed as a relative error percent (% R.E.). Precision is expressed as a relative standard deviation percent (% RSD). For the accuracy, a standard working solution of nicotine was prepared. The prepared standards were injected 3 times as a test sample. From the respective area counts, the concentrations of the nicotine were calculated using the detector responses. The accuracy (% R.E.) = 6.18 %. Precision of the proposed method was also determined by running calibration series solutions at 5-25 µg/*ml* and then was evaluated in terms of repeatability and expressed as the relative standard deviation (RSD,%). The results of precision ranged between 0.182 and 2.603 %, indicating a good repeatability.

Confidence Limit (or Interval) for the Mean: This is the limit (above and below)

ISSN: online 2617-2186 print 2617-2178

around x that μ must lie, with a given degree of certainty (probability or confidence level). In our study, the confidence limit for the mean of nicotine in smokers' urine samples using RP-HPLC was xt = 0.663 ± 0.0442; whereas its value for nicotine in non-smokers' urine samples was xt = 0.273 ± 0.0259.

CONCLUSION

The concentrations of nicotine in the urine were mostly less than expected, but the average concentrations of nicotine in the male smokers' urine samples were higher than those in the male and female non-smokers' urine samples. This study employed a highly efficient extraction method and the modified methods used were applicable and reliable in determining nicotine in urine using HPLC. It also provided good results in terms of LOD, LOQ, correlation coefficient, percentage of R.E. and percentage of RSD. We recommend further research to be carried out to examine nicotine concentrations and their metabolisms in urine and serum samples. In addition, further techniques such as GC–MS, HPLC-ESI-MS, HPLC-UV or/and HPLC-FL and statistical analysis should be carried out to provide further information and confirm these results.

ACKNOWLEDGEMENT

The authors would like to thank all the volunteers who participated in the collection of samples. Many thanks are extended to the staff of the Medical Laboratory of the Clinic of Omar Al-Mukhtar University for their collaboration. Very special thanks are due to Dr. Osama Khreit, Omukalthum Abduljalil, Othman Eznien, and Ahmed B. Eznien for providing laboratory facilities and their technical assistance.

REFERENCES

Allen, J. A. V., D. Haviland, M. L. Healton, C. Davis, K. C. Farrelly, M. C. Husten, & C. G. Pechacek, T. (2003). Tobacco use among middle and high school students, United States, 2002. *Morbidity and Mortality Weekly Report, 52*(1096), 1096. doi: http://link.galegroup.com/apps/doc/A111400677/EAIM?u=leicester&sid=EAIM&xid=f900bc67

Ceppa, F., El Jahiri, Y., Mayaudon, H., Dupuy, O., & Burnat, P. (2000). High performance liquid chromatographic determination of cotinine in urine in isocratic mode. *Journal of Chromatography B: Biomedical Sciences and Applications, 746*(2), 115-122. doi: https://doi.org/10.1016/S0378-4347(00)00306-6

Davoli, E., Stramare, L., Fanelli, R., Diomede, L., & Salmona, M. (1998). Rapid solid-phase extraction method for automated gas chromatographic–mass spectrometric determination of nicotine in plasma. *Journal of Chromatography B: Biomedical Sciences and Applications, 707*(1), 312-316. doi: https://doi.org/10.1016/S0378-4347(97)00579-3

Elmanfe, G., & Abdulla, A. (2014). *Spectrophotometric Determination of Nicotine in Some Libyan Smokers' Blood and Compared with Nonsmokers*. Paper presented at the 1st Conference on Chemical Sciences and its Applications, 21-23 April 2014, Elbaida-Libya

Giovino, G. A. (1999). Epidemiology of tobacco use among US adolescents. *Nicotine and Tobacco Research 1, (Suppl 1):*, S31-40. doi: 10.1080/14622299050011571

Gupta, R., Prakash, H., Gupta, V. P., & Gupta, K. D. (1997). Prevalence and determinants of coronary heart disease in a rural population of India. *Journal*

ISSN: online 2617-2186 print 2617-2178

of Clinical Epidemiology, 50(2), 203-209. doi: 10.1016/S0895-4356(96)00281-8

Hamm, J. G. (1982). *The Handbook of Pest Control* (1st ed.). Frederick Fell Publishers Inc.: New York.

Jha, P., Jacob, B., Gajalakshmi, V., Gupta, P., Dhingra, N., Kumar, R., . . . Richard, P. (2008). A Nationally Representative Case Control Study of Smoking and Death in India. *New England Journal of Medicine, 358*(11), 1137-1147. doi: 10.1056/NEJMsa0707719

Jones, F. E. (1994). *Toxic Organic Vapors in the Workplace*. Lewis Publishers: London, CRC Press Inc.: New York. 224.

Massadeh, A. M., Gharaibeh, A. A., & Omari, K. W. (2009). A Single-Step Extraction Method for the Determination of Nicotine and Cotinine in Jordanian Smokers' Blood and Urine Samples by RP-HPLC and GC-MS. *Journal of Chromatographic Science, 47*(2), 170-177. doi: 10.1093/chromsci/47.2.170

Orlando, M., Tucker Joan S, Ellickson Phyllis L., & J., K. D. (2004). Developmental Trajectories of Cigarette Smoking and Their Correlates From Early Adolescence to Young Adulthood. *Journal of Consulting and Clinical Psychology, 72*(3), 400-410. doi: 10.1037/0022-006X.72.3.400

Padmavati, S. (2002). Prevention of heart disease in India in the 21st century: need for a concerted effort. *Indian Heart J, 54*(1), 99-102.

Pasupathi, P., Bakthavathsalam, G., Rao, Y. Y., & Farook, J. (2009). Cigarette smoking effect of metabolic health risk: A review. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews, 3*(2), 120-127. doi: https://doi.org/10.1016/j.dsx.2009.02.006

Pierce, P., & Gilpin, E. (1996). How long will today's new adolescent smoker be addicted to cigarettes? *American journal of public health, 86*, 253 - 256.

Rani, M., Bonu, S., Jha, P., Nguyen, S. N., & Jamjoum, L. (2003). Tobacco use in India: prevalence and predictors of smoking and chewing in a national cross sectional household survey. *Tobacco Control, 12*(4), 1-8. doi: 10.1136/tc.12.4.e4

Rodricks, J. W. (1992). *Calculated Risks* (1st ed.). Cambridge University Press. pp. 251- 256.

Shin, H. S., Kim, J. G., Shin, Y. J., & Jee, S. H. (2002). Sensitive and simple method for the determination of nicotine and cotinine in human urine, plasma and saliva by gas chromatography mass spectrometry. *Journal of Chromatography B, 769*(1), 177-183. doi: https://doi.org/10.1016/S1570-0232(02)00007-7

Stevens, K. R., A. Marie Barron, Carol A. Ledbetter, Katie M. Foarde, & Menard., S. W. (2001). Legislation, Policy, and Tobacco Use Among Youth: Implications for Health Care Providers. *Journal of School Health, 71*(3), 89-95. doi: 10.1111/j.1746-1561.2001.tb07298.x

Tollison, D. R. (1988). *Clearing the air: perspectives on environmental tobacco smoke*: Lexington, Mass.: Lexington Books, MA, U.S.A.

ISSN: online 2617-2186 print 2617-2178

U.S. Department of Health and Human Services. (2000). *Healthy People 2010: Understanding and Improving Health* (2nd ed.): Washington, DC: U.S. Government Printing Office.

Watson, R. R., & Witten, M. (2001). *Environmental Tobacco Smoke*. Boca Raton London New York Washington, D.C.: CRC Press.

Wills, T. A., & Cleary, S. D. (1997). The validity of self-reports of smoking: analyses by race/ethnicity in a school sample of urban adolescents. *American journal of public health, 87*(1), 56-61. doi: 10.2105/ajph.87.1.56

Wu, C., Siems, W. F., Hill, J. H. H., & Hannan, R. M. (1998). Analytical determination of nicotine in tobacco by supercritical fluid chromatography ion mobility detection. *Journal of Chromatography A, 811*(1), 157-161. doi: https://doi.org/10.1016/S0021-9673(98)00223-4

ISSN: online 2617-2186 print 2617-2178

تقدير النيكوتين في عينات بول مدخنين ليبيين ومقارنة مع غير المدخنين باستخدام جهاز كروماتوجرافيا السائل عالي الكفاءة – طور معكوس(RP-HPLC)

جلال محمد المنفي[1]*، سعاد خليفة عمر[1]، نور الدين أسويكر يحي عبد الله [2]، آمنة منصور حسن[1]

[1] *قسم الكيمياء، كلية العلوم، جامعة عمر المختار، البيضاء، ليبيا*

[2] *قسم الكيمياء، كلية العلوم، جامعة طبرق، طبرق، ليبيا*

تاريخ الاستلام: 27 يونيو 2019 / تاريخ القبول: 00 يوليو 2019

Doi : https://doi.org/10.54172/mjsc.v34i3.323

المستخلص: تهدف هذه الدراسة لتقدير النيكوتين في 20 عينة من البول مأخوذة من عشرة مدخنين وعشرة غير مدخنين في ليبيا. كان على كل متطوع استكمال استبيان قبل أخذ عينة البول. تم تقدير النيكوتين من خلال عملية استخلاص بمرحلة واحدة سريعة، رخيصة، عالية الأداء وموثوق بها، وذلك باستخدام تقنية التحليل الكروماتوجرافي السائل عالي الأداء–معكوس الطور، وقد تم تطويرها والتحقق منها لهذا الغرض. تشمل المعايير والعوامل التي أخذت في الاعتبار لهذا التقييم والتحقق: الخطية والدقة والحساسية وحدود الكشف وحدود الكميات. تراوحت تركيزات النيكوتين في عينات البول من المدخنين من 0.037 إلى 1.979 ميكروجرام / مل، وبمتوسط 0.663 ميكروجرام / مل. من ناحية أخرى، تراوحت تركيزات النيكوتين لدى غير المدخنين من 0.017 إلى 1.331 ميكروجرام / مل، وبمتوسط 0.273 ميكروغرام / مل. تبين التحاليل الإحصائية أن تركيزات النيكوتين كانت كبيرة في عينات المدخنين على عكس عينات غير المدخنين.

الكلمات المفتاحية: السجائر؛ التدخين؛ النيكوتين. البول. الاستخلاص؛ RP-HPLC.

*[1]جلال محمد المنفي galal.elmanfe@omu.edu.ly قسم الكيمياء، كلية العلوم، جامعة عمر المختار، البيضاء - ليبيا.