Simple HPLC method for rapid quantification of nicotine content in e-cigarettes liquids

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ABSTRACT

Electronic nicotine delivery systems (ENDs) are gaining popularity in Jordan as alternatives to tobacco cigarettes with an estimation of 10% of tobacco smokers switching to ENDS. Since nicotine is toxic and highly addictive substance, it is important to develop and validate an easy and rapid analytical method to accurately measure nicotine level in e-liquids. A simple high performance liquid chromatography–photodiode array detection (HPLC–PDA) method was developed and validated for rapid determination of the actual nicotine content in 11 of the most popular e-liquids brands available in the Jordanian market and compared to the nicotine levels appeared in the labeled packaging. The new method of analysis showed an excellent linearity with correlation factor equal to 0.9994 with analytical range between 100 and 1,000 μg/mL, and Limit of detection (LOD) and Limit of quantification (LOQ) of 32.6 μg/mL and 98.9 μg/mL, respectively. The results showed that the actual measured nicotine concentrations ranged from 0 to 25.81 mg/mL with percent deviation ranged from 63.1% less than to 3.24% more than the labeled concentration on packaging. And more than 10% deviation difference in actual nicotine concentrations versus labeled were found in 9 of the 11 e-liquid products (82%). In conclusion, nicotine labelling among e-liquids products have not accurately reflect the actual content which may have potential negative impact on users.

KEYWORDS

nicotine, e-cigarettes, e-liquid, HPLC, labeling, ENDs, health hazard

INTRODUCTION

Tobacco smoke contains numerous hazardous components and contributes to serious adverse outcomes [1]. In Jordan, tobacco smoking is widely popular among the general population. In 2015, the World Health Organization (WHO) announced that Jordan has ranked 2nd in the world with smokers percentage of 70.2% among males older than 15 years and ranked 6th with percentage of 40.5% for both genders among countries with the highest smoking rates [2]. Electronic nicotine delivery systems (ENDs) use is increasing worldwide and most adults’ users perceptions and reasons for ENDs are for tobacco smoking cessation [3]. Moreover, recent randomized controlled trials have revealed that ENDs are more effective for smoking cessation than nicotine replacement [4] and nicotine patches [5]. Therefore, tobacco smokers in Jordan have started switching to use ENDs instead of regular tobacco smoking. Till now, no data is available regarding the prevalence of ENDs use. Yet, an estimation of 1 from 10 regular tobacco smokers have either attempted or switched to ENDs. Several studies have revealed that e-liquids containing nicotine used in ENDs are greatly variable than what are stated on labels [6–15]. Hence, Jordan Food and Drug Administration (JFDA) has recently issued specific legislations for tobacco and nicotine delivery systems including ENDs licensing and regulation in July 2019, which necessitate the utilization of reliable analytical methods to control these products and their contents for quality and safety.

Nicotine, an alkaloid composed of pyridine and pyrrolidine rings, affects large variety of biological functions including gene expression, regulation of hormone secretion and enzyme activities [16]. In addition to being highly addictive, nicotine adversely affects many organs...
including heart, reproductive system, lungs, kidneys, in addition to its carcinogenic potential [17]. Nicotine is a toxic poison and has rapid action on organs especially peripheral and central nervous systems. In severe poisoning, tremors, prostration, cyanosis, dyspnea, convulsion, progression to collapse and coma and even death may arise from paralysis of respiratory muscles and/or central respiratory failure with the fatal dose of 30–40 mg/m³ for 30 min, assuming a breathing rate of 50 L per minute and 100% absorption [18].

Nicotine concentration has been determined by several analytical techniques including gas chromatography with flame ionization detector (GC–FID) [9], and gas chromatography-mass spectrometry (GC–MS) [8, 10]. Since LC is a workhorse technique used for efficient and tedious analytical procedures [19–21]. It has been employed successfully for nicotine quantification in e-liquids such as liquid chromatography-mass spectrometry (LC–MS) [6, 7], in addition to HPLC with photodiode array detection (HPLC–PDA) [13, 14].

As the aim of our research group is to seek pharmaceutical products safety [22], the aim of this study was to develop and validate an easy and straightforward HPLC method for rapid determination of nicotine content in 11 of the most common e-liquids brands available in the Jordanian market and to compare the levels of actual nicotine with the labeled packaging to investigate both safety and quality.

### MATERIALS AND METHODS

#### E-liquids and chemicals

All reagents were analytical grade reagents obtained from Sigma–Aldrich unless otherwise stated. Eleven samples from the most popular brands of e-liquids were obtained locally from Jordanian market. A reference e-liquid was prepared in the lab comprising propylene glycol, glycerol and pure nicotine (Alfa Aesar, UK). Table 1 shows the detailed description of each of e-liquid brands and are indicated as e-liquid 1–11. Nicotine reference standard for calibration was purchased from Sigma–Aldrich (St. Louis, MO, USA).

#### Instrumentation and HPLC–PDA analytical conditions

Waters 2690 Alliance HPLC system equipped with a Waters 996 photodiode array detector (Milford, MA, USA) was employed for method development, validation and samples analysis. The analytical column used was C₁₈-Thermo (4.6 × 250 mm, 5 µm). The Mobile phase consisted of 0.1% triethylamine in water buffer and acetonitrile (70%;30%) at pH = 7.0 in isocratic conditions and ambient temperature and was delivered at a flow rate of 1 mL/min. Nicotine was identified at UV wavelengths between 210 and 400 nm and quantifications was carried out at 254 nm.

#### Calibration standards, quality control (QC) and samples preparations

Calibration curve (n = 3) was constructed for nicotine measurement from six standard solutions namely: 100, 200, 400, 600, 800, and 1,000 µg/mL. The standard solutions were prepared by a dilution of proper amount from stock standard solution (1 mg/mL) with methanol and were stored at 4 °C. The QC’s samples were prepared at 3 levels as QCL low (300 µg/mL), QC medium (700 µg/mL), and QC high (950 µg/mL), all were triplicate. Each of the diluted solutions was filtered using 0.22 µm syringe filter then 10 µL aliquot were injected. A 100 µL of each e-liquid samples (triplicate) was added to 1.9 mL methanol and sonicated for 5 min then vortexed for 2 min. Each sample was filtered using 0.22 µm syringe filter then 10 µL sample was injected. The calibration curve covering the range 100–1,000 µg/mL for the analytes was prepared to validate the method linearity.

#### Method validation

The analytical method was validated as follow:

### Table 1. The description of brands of e-liquids used for analysis (obtained from Jordan)

| Brand of e-liquid | Date of purchase | Date of expiration | Labeled level of nicotine (mg/mL) | Flavor |
|------------------|------------------|--------------------|----------------------------------|--------|
| e-liquid 1       | September, 2019  | October, 2021      | 25                               | Tobacco|
| e-liquid 2       | September, 2019  | May, 2021          | 20                               | Fruit  |
| e-liquid 3       | September, 2019  | July, 2021         | 10                               | Watermelon|
| e-liquid 4       | September, 2019  | September, 2021    | 20                               | Strawberry|
| e-liquid 5       | September, 2019  | June, 2021         | 25                               | Lemon-mint|
| e-liquid 6       | September, 2019  | May, 2021          | 25                               | Apple  |
| e-liquid 7       | September, 2019  | January, 2021      | 25                               | Tobacco|
| e-liquid 8       | September, 2019  | September, 2021    | 25                               | Tobacco|
| e-liquid 9       | September, 2019  | February, 2021     | 3                                | Tropical fruit|
| e-liquid 10      | September, 2019  | May, 2021          | 3                                | Milk vanilla|
| e-liquid 11      | October, 2019    | July, 2021         | 0                                | Grape  |
| Ref. e-liquid    | October, 2019    | NA                 | 15                               | No flavor|
Selectivity. Method selectivity is important to distinguish the target analyte from endogenous substances and other compounds in e-liquid samples. The selectivity of the method was evaluated using a prepared e-liquid with zero nicotine concentration by comparing the peak signal at the target analyte retention time in blank samples with the peak signal at the target analyte retention time at limit of quantitation (LOQ) sample.

Precision and accuracy. Within-run and between-run accuracy and precision were evaluated at three replicates of three QCs levels in one analytical run and three consecutive days respectively.

Limit of detection (LOD) and Limit of quantification (LOQ). The calculation of both LOD and LOQ were based on the Standard Deviation (SD) of Intercepts of the calibration curves (σ) and the slope of the calibration curves (S) for nicotine standards (n = 3). The LOD and LOQ were expressed according to the following equations (standards and blanks injected 3 times consecutively):

\[
\text{LOD} = \frac{(3.3 \times \sigma)}{S}
\]

\[
\text{LOQ} = \frac{(10 \times \sigma)}{S}
\]

The LOQ is the lowest reliable concentration in the calibration curve that could be quantified by the analytical method. In order to further validate the LOQ of the method experimentally, the analyte signal at the analyte retention time of a blank matrix was compared to the analyte signal at the same retention time of an LOQ sample prepared from the same matrix.

RESULTS AND DISCUSSION

Method development and validation

The development and validation of an analytical method for quantification of nicotine in e-liquid samples has met the acceptance criteria of FDA guidelines [23]. In which the sample processing and preparation involved only a simple and effective dilution procedure and also no carry over was reported of the analyte. Moreover, the method was selective where no interfering peaks at the retention time of nicotine were observed. Likewise, the method showed excellent linearity with correlation factor equal to 0.9994 over the analytical range of 100–1,000 μg/mL with calculated LOD and LOQ of 32.6 and 98.9 μg/mL, respectively. Moreover, within-run and between-run accuracy and precision were 99.2, 1.82% and 101.4, 4.1% respectively. This indicates that the developed method is reliable, accurate and reproducible.

Table 2 summarizes the parameters of the calibration curves.

| Parameter                        | Value                                      |
|----------------------------------|--------------------------------------------|
| Nicotine standards t_R average (min) ± SD | 4.790 ± 0.046                              |
| Calibration curve equation       | \( y = 9951.6x - 198370 \)                 |
| Determination coefficient \( (R^2) \) | 0.9994                                     |
| LOD                              | 32.6 μg/mL                                 |
| LOQ                              | 98.9 μg/mL                                 |
| Within-run accuracy              | 99.2%                                      |
| Within-run precision             | 1.82%                                      |
| Between-run accuracy             | 101.4%                                     |
| Between-run precision            | 4.1%                                       |

Table 2. Parameters of the calibration curve for the employed nicotine standards of the chromatographic conditions for HPLC/ PDA detector

Fig. 1. Chromatogram of an e-liquid sample (nicotine t_R = 4.853 min) with injection volume: 10 μL; column: C_{18}-Thermo (4.6 × 250 mm, 5 μm); detector: UV wavelengths between 210 and 400 nm, quantifications was carried out at 254 nm
levels in packaging. Table 3 lists the comparison of the measured concentration of nicotine with the labeled packaging.

The percent deviation of nicotine concentration from labeled concentration was determined using following equation (1):

\[
\text{Deviation from label} = \frac{(\text{measured value} - \text{labelled value}) \times 100}{\text{labelled value}}.
\]

The printed labeling of nicotine concentrations from 11 brands of e-liquids ranged from 0 to 25 mg/mL. However, variations existed in actual nicotine concentrations between bottles of e-liquids. The results showed that the measured concentration ranged from 0 to 25.81 mg/mL, with maximum percent deviation equal to 63.1% less than the labelled concentration in one sample and 3.24% more than the labelled concentration of nicotine in another sample.

The differences between actual and labeled nicotine content have been determined in several studies and presented as percent deviation of actual nicotine concentration from labeled concentration and are summarized in Table 4. The percentages of e-liquids in which the actual quantified nicotine concentrations that deviated by more than ±10% from the manufacturer labels were 10% [24], 18% [15], 52% [12], 56% [25], 63% [26], 65% [27], and 67% [9]. In this study, more than ±10% deviations were detected in 9 out of 11 e-liquids (82%) which are more than what stated in previous similar studies. Since Jordan is relatively new market for ENDS, most manufacturers and sellers obviously lack adequate knowledge of quality and safety of e-liquids. However, the new regulations issued by JFDA handle ENDS as medication in terms of quality and safety. Yet, special attention should be made in controlling all products available in the market. And urgent requirement for countrywide labeling standards for such products and need of measurement of concentration levels of nicotine in all products in the market.

### Table 3. The comparison of the measured concentration of nicotine with the labeled packaging in terms of % Deviation from label (n = 3)

| Brand of e-liquid | Labeled level of nicotine | Measured nicotine concentration (Triplicate average ± SD) | Deviation from label* (%) |
|------------------|--------------------------|----------------------------------------------------------|---------------------------|
| e-liquid 1       | 25                       | 19.11 ± 1.28                                             | -23.6                     |
| e-liquid 2       | 20                       | 14.19 ± 0.72                                             | -29.1                     |
| e-liquid 3       | 10                       | 6.38 ± 0.91                                              | -36.2                     |
| e-liquid 4       | 20                       | 16.86 ± 1.08                                             | -15.7                     |
| e-liquid 5       | 25                       | 18.27 ± 1.63                                             | -26.9                     |
| e-liquid 6       | 25                       | 22.58 ± 1.45                                             | -9.7                      |
| e-liquid 7       | 25                       | 25.81 ± 2.24                                             | +3.24                     |
| e-liquid 8       | 25                       | 22.42 ± 1.67                                             | -10.3                     |
| e-liquid 9       | 10                       | 3.69 ± 0.41                                              | -63.1                     |
| e-liquid 10      | 3                        | 1.75 ± 0.10                                              | -41.6                     |
| e-liquid 11      | 0                        | Not detected                                             | 0                         |
| Ref. e-liquid 20 | 20                       | 19.85 ± 0.83                                             | -0.75                     |

* Deviation from label = (measured value – labelled value) × 100/labelled value.

for the employed nicotine standards and validation results. Fig. 1 illustrates the chromatogram of one of the analyzed e-liquids showing nicotine peak around 4.9 min.

### Table 4. Summary of literature findings of actual nicotine levels in e-liquids compared to labeling

| literature       | Matrix                | Analysis technique | e-liquid samples number | Nicotine level labeled (mg/mL) | >10% difference between actual and labeled nicotine conc. | Percent deviation | Country |
|------------------|-----------------------|--------------------|-------------------------|--------------------------------|----------------------------------------------------------|------------------|---------|
| Etter et al. [24]| e-cigarette liquid    | UHPLC-DAD          | 20                      | 6–30                           | 2                                                        | -15 to 21%       | Sweden |
| Davis et al. [27]| e-cigarette liquid    | HPLC-DAD           | 54                      | 0–18                           | 35                                                       | -1.3 to 89.7%    | USA    |
| Kim et al. [15]  | e-cigarette liquid    | GC-TSD             | 32                      | 0–18                           | 6                                                        | -32.2 to 3.3%    | Korea  |
| Farsalinos et al. [12] | e-cigarette liquid | GC-FID             | 21                      | 12–18                          | 11                                                       | -21 to 22.1%     | Greece |
| Peace et al. [9] | e-cigarette liquid    | HPLC-MS            | 27                      | 6–22                           | 18                                                       | -36 to 31%       | USA    |
| Raymond et al. [26]| e-cigarette liquid | HPLC-DAD           | 35                      | 18                             | 22                                                       | -35 to 52%       | USA    |
| Bennani et al. [25]| e-cigarette liquid | HPLC-DAD           | 32                      | 3–24                           | 18                                                       | -100 to 3.3%     | Morocco |

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CONCLUSION

The developed HPLC–PDA method was successfully applied for rapid determination of nicotine content, in less than 5 min, in 11 widespread e-liquids with LOD and LOQ of 32.6 and 98.9 μg/mL, respectively. The validated method was straightforward, accurate and precise over a wide range of nicotine concentrations of e-liquids in the market. A variation in nicotine concentrations exist between the labeled and actual content. And more than 10% difference in actual nicotine concentrations versus labeled was found in 9 of the 11 e-liquid products (82%) which necessitate the urgent need for labeling standards for these products in terms of nicotine content.

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