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ORIGINAL ARTICLE

Investigating the efficiency of mechanical agitation on the quantification of nicotine in e-cigarettes, using a novel method

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Abstract

Electronic cigarettes (ECs) have gained popularity among the general public. ECs have varying nicotine levels from one e-liquid to another. Thus, a standardised method for analysing the nicotine content in e-liquids is needed. Here, a method was developed for quantitative analysis using high-performance liquid chromatography (HPLC) with mechanical agitation (MA). The same elution conditions were applied without mechanical agitation (WMA) to investigate the effect of processing on nicotine separation efficiency. The proposed method resulted in increased linearity in a concentration range of 25–500 ppm. This method also resulted in an increase in the correlation coefficient from 0.2415 to 0.9991, with a limit of detection (LOD) and limit of quantification (LOQ) of 0.09 ppm and 0.29 ppm, respectively, making it more applicable to a wide range of e-liquids in domestic and foreign markets. The analysis of seven e-liquids with concentrations of 0–50 mg/mL revealed that 67% of the samples had a lower nicotine content than that stated on the label. The opposite was true for 33% of samples. No sample matched the nicotine content stated on the label. A sample that was claimed to contain 3 mg/mL actually contained 17 mg/mL. Moreover, a sample labelled as nicotine-free contained 7 mg/mL of nicotine. Another sample was claimed to contain 50 mg/mL, but the measured value was 24 mg/mL. This value exceeds the Saudi Food and Drug Administration (SFDA) standard, which specifies that e-liquids should not contain more than 20 mg/mL of nicotine.

Keywords Electronic cigarette · Mechanical agitation · HPLC · Nicotine · E-liquid

1 Introduction

Electronic cigarettes (ECs) are devices that are powered by rechargeable batteries and convert electronic liquids (e-liquids) into aerosols (gaseous suspensions of liquid, solid, or both) [1, 2] that the user may inhale [1–4]. Based on their design, ECs may comprise one piece, two pieces (battery and cartomiser), and three pieces (where the cartridge is separated from the atomiser) (Fig. 1) [3–5].

The device allows the user to select the nicotine, flavour and strength, and increase or decrease the temperature [2, 6, 7]. The use of normal cigarettes (NCs) is known as smoking, and the user is called a smoker. The usage of ECs is known as vaping, and the user is known as a vaper. In contrast with NCs, ECs do not burn the components but rather convert the e-liquid into an aerosol, referred to as 'vapour', which is the gaseous state of the substance [1–4, 8]. The 'vapour' generated by ECs is classified into three categories: primary vapour (inhalation), secondary vapour (which unlike NCs is not formed spontaneously), and tertiary vapour (exhalation) [9]. The vaper draws air through the device and then the power button or sensor activates the battery, which in turn supplies power to the atomiser to generate aerosols resulting from the flow of e-liquid [1, 9]. Some manufacturers set the temperature of the vaporisation chamber between 40 and 50 °C, but no reliable data is available on the temperature range of the devices. The first ECs appeared on the market in 2004 [2, 4, 10], when China was the foremost manufacturer. In 2005, ECs entered the European market, where they gradually spread until they arrived in the American market in 2007. These devices help deliver nicotine without combustion and are operated using rechargeable batteries [4]. The components of the e-liquid differ from one manufacturer to another. The e-liquid is one of the most important components of an ECs as vaping would not be possible without it. The e-liquid is placed in a cartridge or cartomiser [2, 3, 8] which comes in two forms depending on the type of device

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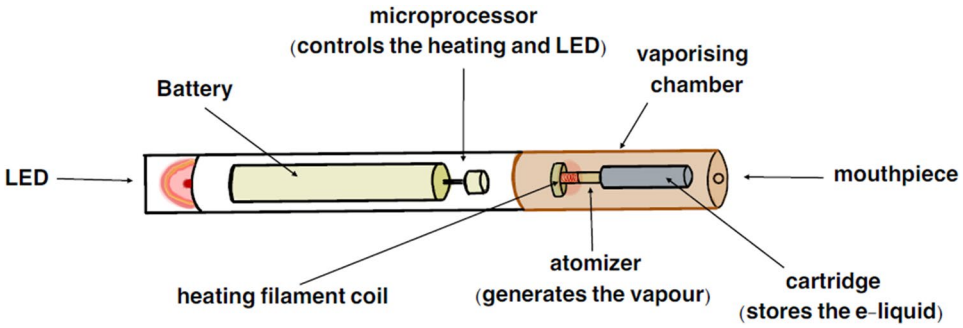

Fig. 1 Structure of an ECs (three pieces)

(Fig. 2). The first type replacement cartridge can be removed and disposed off. By contrast, the second type refillable cartridge should be refilled manually by the vaper and it cannot be removed from the device [3, 7, 11].

The replacement cartridge is considered the simplest type because it can be removed and replaced and new liquid may be put into the device immediately. On the other hand, the refillable tank requires caution when handling and care to avoid overfilling the cartridge. Furthermore, the liquid should be applied in drops, ensuring that it does not contact the skin because it contains irritating substances like propylene glycol (PG) [9]. Moreover, regular and periodic cleaning of the tank is required after the liquid has been used up and before refilling the ECs with a new one [11]. E-liquid is a highly viscous oily substance [3] that contains components such as nicotine, PG, vegetable glycerine (VG) and water in addition to flavours and fragrances [3, 12]. The excessive use of many flavourings is a concern because they can cause various health problems [6]. The level of nicotine varies from one e-liquid to another and nicotine may not be found in some e-liquids [12].

Electronic nicotine delivery systems (ENDS) are ECs where nicotine is the main component. Nicotine is an organic compound consisting of pyridine and pyrrolidine rings. Nicotine is classified as a toxic substance that causes addictiveness and has effects on the nervous system. Nicotine intake results in many symptoms such as shortness of breath and convulsions. A dose of 30–40 mg/m^3 is considered a lethal dose if exposed for 30 min [10].

The nicotine in e-liquids is extracted from tobacco [8, 13, 14] because synthetic nicotine is very expensive [9, 13]. Nicotine exists either as a salt or as a free base [3]. Nicotine salt is usually found at very high concentrations of up to 88 mg/mL [15], whereas the free base is present at lower concentrations but has a stronger 'throat hit' [16]. E-liquids are classified into two types based on whether they contain nicotine. The first category includes e-liquids with nicotine concentrations ranging from 3 to 100 mg/mL, whereas the second category includes nicotine-free liquids. The nicotine inhaled through ENDS enters the bloodstream, where it is absorbed and delivered to the brain via cholinergic receptors [6, 17], which release various neurotransmitters (internal substances that allow the transmission of signals from one nerve cell to another) [17]. This process takes 10–15 s, causing changes in feelings such as perception, anxiety, and relaxation [6, 17]. The issue of nicotine in ENDS has received increased attention because of the difference between the value recorded on the label (labelled value) and the value obtained from analysis (measured value). This difference

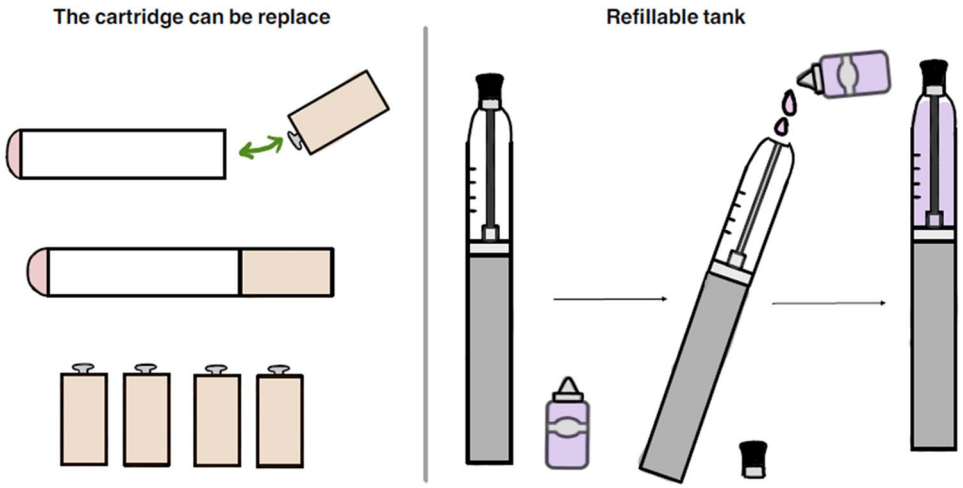

Fig. 2 Differences between the replacement and refillable cartridges

is regarded as one of the most important current discussions in the field of health. [18]

To quantify nicotine-related compounds in ECs, a study described vortexing at 1800 rpm for 30 min; 1 µL of sample was injected into a liquid chromatography-mass spectrometry (LC–MS) system [19]. Pagano and co-worker [20] performed sonication for 20 min before gas chromatography-mass spectrometry (GC–MS) analysis.

There is no reliable evidence that mechanical agitation (MA) affects nicotine quantification. Furthermore, no study has focused on the effect of processing on the difference in nicotine content. Thus, the aim of this study and its novelty are based on the development of a new, simple, and easy-to-interpret method for determining nicotine in e-liquids, using high-performance liquid chromatography with an ultraviolet detector (HPLC–UV). Also, use of MA and comparison of MA-treated samples with unprocessed samples in terms of separation efficacy. Furthermore, to increase the reliability of the data, we tested seven e-liquids purchased from Saudi stores. The nicotine concentrations on the label ranged from 0 to 50 mg/mL. The analyses were repeated three times to reduce error. Given the lack of general agreement on analysis procedures for ECs, we expect this study to produce higher estimation results, improve separation efficiency, and add a deeper understanding and perception of nicotine analysis. Thus, this study contributes to the standardisation of procedures for nicotine analysis in e-liquid samples.

2 Materials and methods

2.1 Chemicals and reagents

Nicotine hydrogen tartrate with product number 26140 (purity 95%) was obtained from BDH Chemical Ltd (Poole, England). LiChrosolv® HPLC-grade acetonitrile and methanol were obtained from Merck KGaA (Darmstadt, Germany). Ethyl acetate was procured from Chemie AG (Buchs, Switzerland). The water purification system was an Aqua Max-Ultra (YL Instrument Co, Ltd, Anyang, Korea).

2.2 E-liquid samples

Seven samples from the most popular brands of e-liquids were purchased from Saudi stores. Each e-liquid sample is described in detail in Table 1. All e-liquids were coded with numbers for analysis.

2.3 Preparation of standard nicotine and calibration solutions

A stock standard solution of nicotine hydrogen tartrate (9500 ppm) in diluent was used for analysis. Calibration solutions (n = 3) were prepared from dilutions of the stock solution in ACN: H_2O (50:50) to obtain the following concentrations: 25, 50, 75, 100, 200, 300, 400 and 500 ppm. The stock solution and calibration solutions were stored at 5 °C.

2.4 Preparation of e-liquid samples

Stock samples of e-liquid (2 g) were accurately weighed into 20 mL volumetric flasks. Samples (800, 900, 1000, and 2000 µL) of each e-liquid (n = 3) were degassed using an ultrasonic (US) homogeniser at ambient temperature, operated at 52% power, 6 s of US horn, and purged for 2 s with a mixture of ACN:H_2O (50:50) for 17 min. Then, samples were vortexed at 2500 rmp for 2 min. A 10 µL aliquot was analysed by reversed-phase (RP)-HPLC-UV.

2.5 Chromatographic conditions

A Shodex silica 5C8-4D column (100 Å, 5 µm, 4.6 mm × 150 mm) was used with a column temperature of 50 °C and a flow rate of 0.5 mL/min. The injection volume was 10 µL. UV spectra were collected over a wavelength range of 259–334 nm, and quantification was carried out at 268 nm.

Table 1 Description of e-liquids used for the analysis (purchased from Saudi stores)

Sample code	Opening date	Nicotine strength (mg/mL)	Flavour	PG:VG (%)
1	6\10\2022	30	Mello Melon	50:50
2	4\10\2022	3	Strawberry	30:70
3*	5\10\2022	35	Mango, strawberry	50:50
4*	5\10\2022	50	Watermelon	50:50
5*	23\1\2022	50	Blue Raspberry	NA
6	23\1\2022	50	Vanilla, custard, almond	29.17:36.62
7	5\6\2022	Nicotine free	2X Apple (red, green)	NA

Nicotine strength = nicotine level on the label

PG, propylene glycol; VG, vegetable glycerin; NA, not available

*Samples are listed as containing nicotine salts on the label

Eluent A consisted of 100% acetonitrile, whereas eluent B consisted of 100% water. The eluent gradient program was a series of linear gradients from an initial condition of 5% A and 95% B at 10 min, then increased to 95% A and 5% B with a total run time of 13 min (Table 2).

2.6 Method validation

The nicotine quantification in e-liquids was validated for linearity, accuracy, precision, the limit of detection (LOD) and the limit of quantification (LOQ).

2.6.1 Linearity

Nicotine standards of 25, 50, 75, 100, 200, 300, 400 and 500 ppm were prepared for plotting the calibration curve. Linearity was evaluated using a linear equation and the correlation coefficient (R^2).

2.6.2 Accuracy

The following equation was used to express accuracy as a percentage of recovery:

$$Recovery(\%) = \frac{Experimental\ results}{Theoretical\ results} \times 100 \quad (1)$$

2.6.3 Precision

Precision was expressed as a percentage of the relative standard deviation (RSD) of the analytical method, using the following equation:

$$RSD(\%) = \frac{Standard\ deviation}{Mean} \times 100 \quad (2)$$

2.6.4 LOD and LOQ

LOD and LOQ were calculated based on the standard deviation and the slope, using the following equations:

$$LOD = 3.3\frac{\sigma}{S} \quad (3)$$

Table 2 Time program for the HPLC elution

Time	Module	Command	Value (%)	Curve (min)
0.01	Pumps	Solvent B conc	95	10
10.00	Pumps	Solvent B conc	5	10
13.00	Controller	Stop		

$$LOQ = 10\frac{\sigma}{S} \quad (4)$$

$\therefore \sigma$ =standard deviation of the background response, $\therefore S$ = slope of the calibration curve.

2.7 Deviation from the label

The following equation was used to calculate the percent difference (DV) between the measured nicotine concentration and that stated on the label:

$$DV(\%) = \frac{measured\ value - labeled\ value}{labeled\ value} \times 100 \quad (5)$$

3 Results and discussion

This part presents the findings of the analyses of e-liquid samples from various brands. The optimal conditions for nicotine detection were selected and the outcomes were compared in terms of separation efficiency. In addition, the linearity, accuracy, precision, LOD and LOQ were calculated and the deviation in concentration from the label was estimated.

3.1 Preparation of standard nicotine and calibration solutions

Calibration curves were plotted for both groups MA and without mechanical agitation (WMA). The correlation coefficients were compared to determine the degree of linearity between the two variables (area and concentration). A calibration curve was drawn for standard nicotine solutions (WMA) with concentrations of 25–200 ppm (Fig. 3a). The standard solutions showed acceptable linearity, with $R^2 = 0.9974$. However, it became obvious during the analysis of the unknown samples that some of them exceeded the concentration of the standard nicotine solution (200 ppm). Thus, the concentrations were raised to 500 ppm (Fig. 3b), with $R^2 = 0.2415$. A curve with this value suggests a problem with the preparation of the sample or the instrument and may result in inaccurate results. Hence, a calibration curve was drawn for standard nicotine solutions (MA) with concentrations of 25–200 ppm (Fig. 3c). The analyses showed acceptable linearity, with $R^2 = 0.991$. The concentrations were raised to 500 ppm so that all unknown samples would fall within the concentration range of the standard nicotine solutions (Fig. 3d). The obtained $R^2 = 0.9991$ indicates that the calculated values for the unknown samples are accurate across the entire calibration range compared to the previous calibration curve (Fig. 3b).

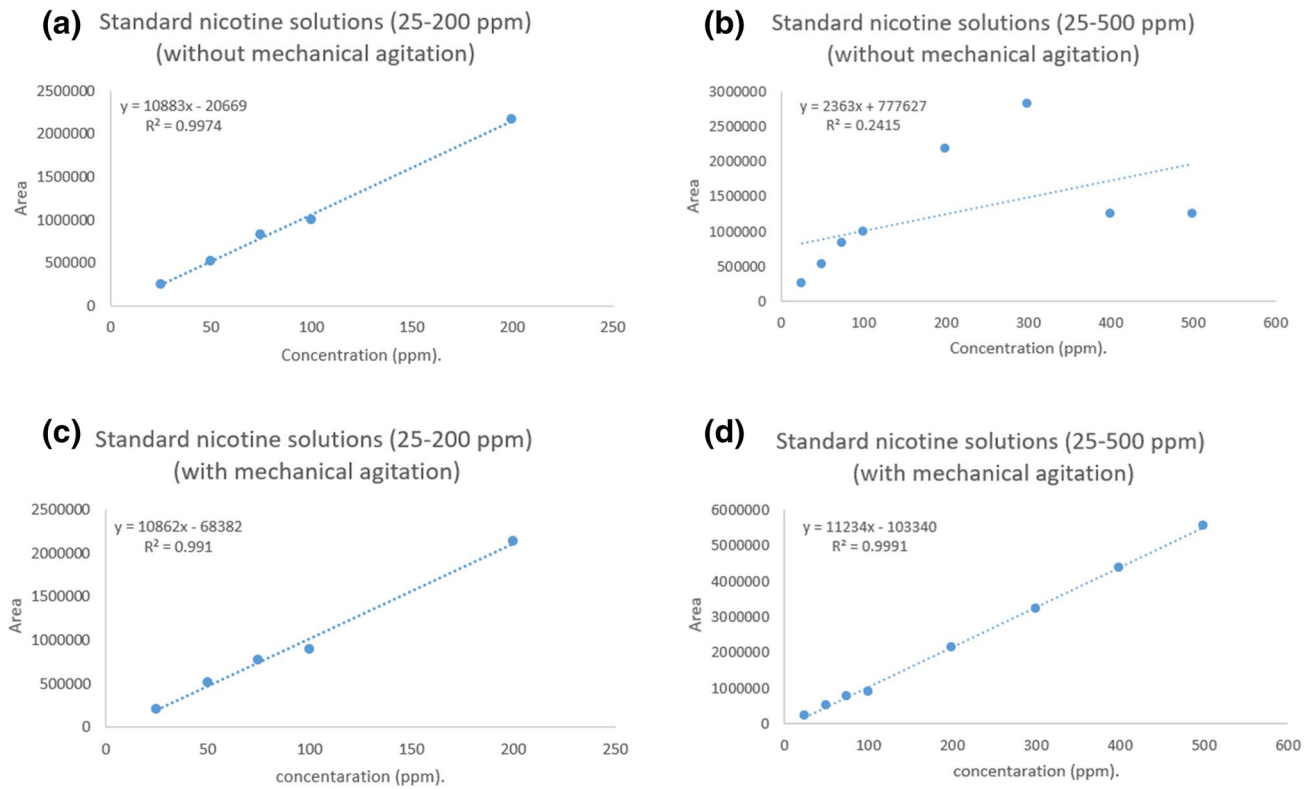

Fig. 3 Calibration curves for the standard nicotine solutions

The shapes of the peaks and their separation efficiencies were also compared. The chromatogram of the 300 ppm WMA sample shows poor separation efficiency (Fig. 4b) compared to the sample with the same concentration treated by MA (Fig. 4a). The differences in separation efficiency between the treatments became evident as the concentration of nicotine increased. For the 400 and 500 ppm WMA samples (Fig. 4d) and (Fig. 4f), no linearity was observed and the area under the peak was smaller compared to the same concentrations that were subjected to MA.

A sequential increase in the response rate was observed for standard nicotine concentrations from 25 to 500 ppm (Fig. 5), while the shapes of the peaks remained without defects. The evidence presented thus far supports the idea that MA improves the shape of the peak because vortexing enhances the mixing of the oily sample with the solvent. Moreover, sonication removes gases, which helps in reducing noise and producing a clearer peak, both of which contribute to increasing separation efficiency. To distinguish between the two groups tested, preference was assigned to samples subject to MA in terms of the correlation coefficient, linearity, peak shape and difference in the area under the peak. Thus, the same procedures were applied for the quantification of nicotine in e-liquids.

3.2 Preparation of e-liquid samples

The results of the nicotine content analysis for e-liquids based on a standard nicotine solution are summarised in Fig. 6 and Table 4. In Sample 3, nicotine indicated 10 mg/mL, whereas the concentration stated on the label was 35 mg/mL. In Sample 4, nicotine indicated 6 mg/mL, whereas, in Sample 5, it indicated 5 mg/mL. The labels of both samples stated that the nicotine concentration was 50 mg/mL.

The results obtained are consistent with what studies have mentioned regarding the possible differences in the measured values of two or more samples that, according to the label, contain the same amount of nicotine [21].

Sample 7 was claimed to be nicotine-free on the label but the analyses showed a measurable value of 7 mg/mL of nicotine. These results support the same premise [22].

A large peak appeared before the nicotine peak. This peak could be due to the interaction of humectants (VG and PG) that could turn into aldehydes, or it could be due to the addition of flavourings and fragrances that contain a large number of compounds. However, the most likely reason is the poor extraction of nicotine from tobacco leaves, which increases the possibility of impurities (secondary alkaloids) appearing. For example, this large peak appears in Samples

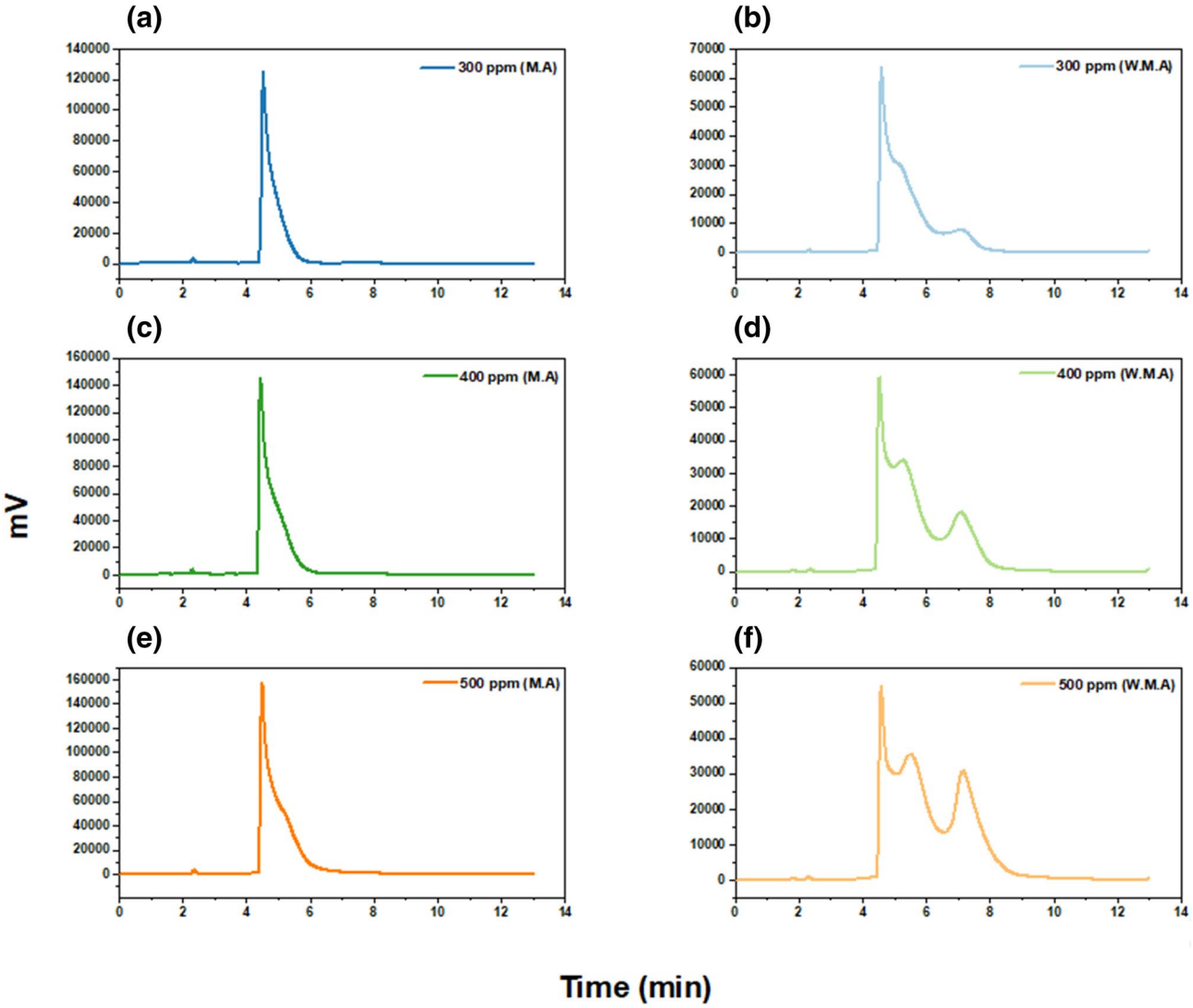

Fig. 4 Chromatograms showing the differences between standard nicotine solutions (300–500 ppm) with/without mechanical agitation

4, 5 and 7 but not in Sample 3. This is due to the degree of purity of the nicotine in e-liquid products. Sample 3 contains synthetic nicotine, whereas the other samples contain nicotine extracted from tobacco leaves, using solvents such as toluene or styrene. These solvents can extract nicotine (the primary alkaloid), as well as secondary alkaloids, resulting in a higher percentage of pollutants compared to synthetic nicotine. Most companies rely on nicotine extracted from tobacco leaves rather than synthetic nicotine, which is more expensive.

Sample 1 contained 4 mg/mL of nicotine, despite the label declaring 30 mg/mL. Sample 2 had 17 mg/mL of nicotine, which is four times the amount mentioned on the label. Overall, the sampling results revealed concentrations that are significantly lower than what is indicated on the label. No sample matched the concentration stated on the label, which suggests that manufacturers are careful during production because nicotine is classified as an addictive substance. Failing to declare the nicotine content is risky because it would expose vapers to variable doses of the alkaloid or to its unwanted presence in the case of nicotine-free e-liquids. A questionnaire was administered to 76 vapers and, as shown in Fig. 7, most of their ages ranged from 16 to 30 years; the lowest percentage corresponded to those aged 33–50 years. Most vapers knew the nicotine concentration stated on the label of the e-liquid. By contrast, 28.95% of vapers knew the e-liquid contained nicotine but did not know the concentration stated on the label. Moreover, 21.05% of vapers reported using an e-liquid that was claimed to be nicotine-free.

Furthermore, after using up the e-liquid, 37% of vapers affirmed that they would choose another e-liquid without consideration for its nicotine content, 20% would choose a

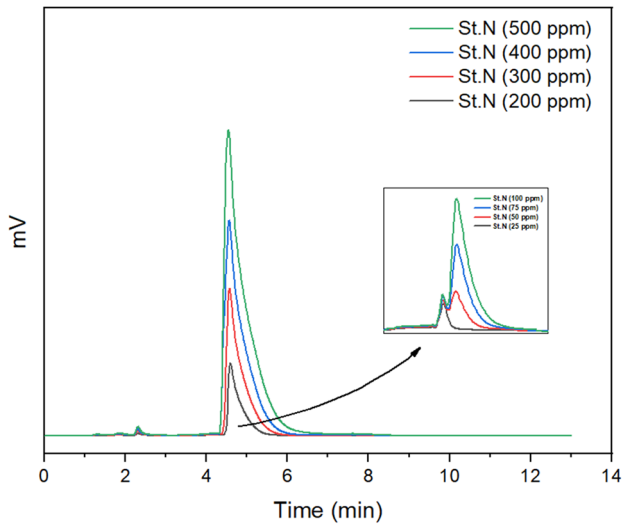

Fig. 5 Chromatogram showing all standard nicotine concentrations

product with an increased concentration of nicotine, 17% would choose a product with a reduced concentration of nicotine and 26% would always choose a product with the same level of nicotine (Fig. 8).

3.3 Method validation

3.3.1 Linearity

Linearity was measured by a calibration curve built using nicotine standard solutions. The method was found to be linear over the specified range (from 25 to 500 ppm) with linear equation $y = 11234x - 103340$ and $R^2 = 0.9991$ (Table 3).

3.3.2 Accuracy

The accuracy of an analytical method is the closeness of the test results obtained by that procedure to the theoretical yield. Accuracy ranged from 104 to 112% (Table 3).

3.3.3 Precision

The precision of the method is expressed as repeatability (%RSD). The %RSD was 1.2% (Table 3).

3.3.4 LOD and LOQ

The LOD was 0.09 ppm of nicotine. The minimum known concentration of nicotine that can be analysed quantitively (i.e., the LOQ) was 0.29 ppm (Table 3).

3.4 Deviations from the label

Seven samples of different brands available in the Saudi market were examined (Table 4). The calculated deviation from the label was used to compare the nicotine concentration on the label to the measured concentration.

The nicotine concentrations stated on the label ranged from 0 to 50 mg/mL. Differences were observed between the quantities stated on the labels and the measured quantities. The measured nicotine concentrations ranged from 4 to 24 mg/mL, with a maximum decrease of 90.6% compared to the value on the label in one sample and an increase of 451% compared to the value on the label in another sample. Five samples (67%) had nicotine contents below those stated on the labels, whereas two samples (33%) had nicotine contents above those stated on the labels.

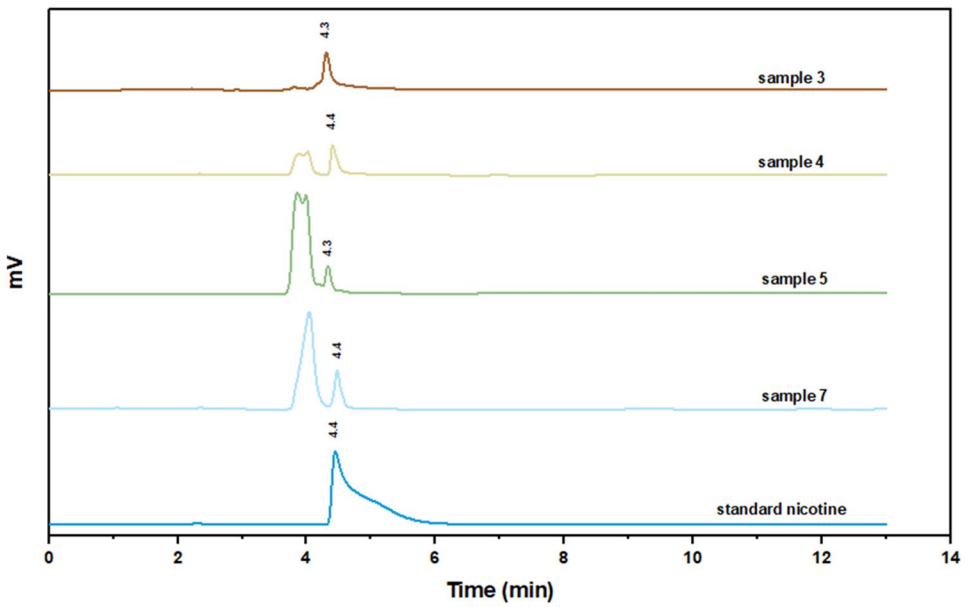

Fig. 6 Chromatograms of e-liquid samples and the standard nicotine solution

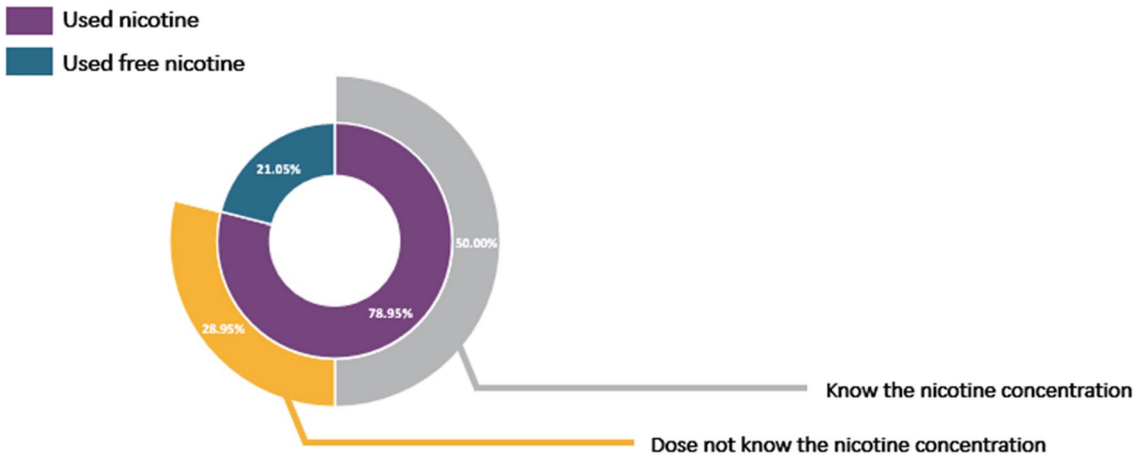

Fig. 7 A questionnaire that displays the extent to which vapers know the content of nicotine in ECs

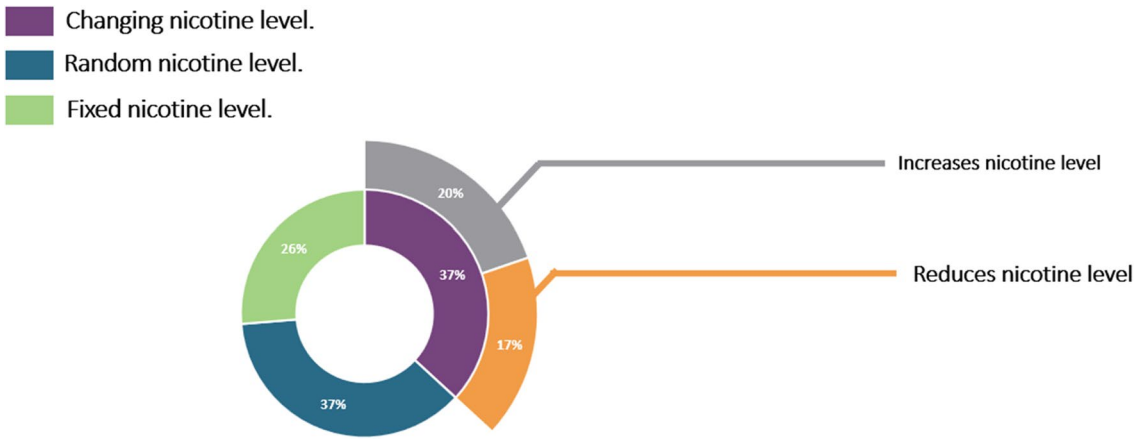

Fig. 8 A questionnaire showing the level of nicotine chosen by the vaper after using up the e-liquid

Table 3 Summary of calculations for the validation of the analytical method

Linearity					
Linear equation y = 11234x − 103340				Correlation coefficient (R^2) $\boldsymbol{R^2 = 0.9991}$	
% Recovery				% Relative standard deviation	
	Theoretical mean			Peak area (75 ppm)	
	25 ppm	50 ppm	75 ppm	Standard deviation	9482.57755
Experimental mean	28 ppm	56 ppm	78 ppm	Mean	775,514
% Rec	**112%**	**111%**	**104%**	**% RSD**	**1.2%**
LOD and LOQ					
Slope	11,234				
Standard deviation	328				
LOD	**0.09 ppm**				
LOQ	**0.29 ppm**				

% Rec: % Recovery; % RSD: % Relative standard deviation; LOD: Limit of detection; LOQ: Limit of quantification; ppm: part per million

Table 4 Comparison of nicotine concentrations measured and stated on the labels, using deviations from the labels (%)

e-Liquid sample code	Stated nicotine (Label, mg/mL)	Measured nicotine (n = 3) (Mean ± SD) (mg/mL)	[a]Deviation from the label (%)
1	30	4 ± 0.50	− 86.9
2	3	17 ± 1.54	+ 451
3	35	10 ± 0.71	− 71.3
4	50	6 ± 0.55	− 87.3
5	50	5 ± 4.11	− 90.6
6	50	24 ± 4.38	− 52.0
7	0	7 ± 0.71	Nil

[a]Deviation from the label (%) = $\frac{mesured\ value - labeled\ value}{labeled\ value} \times 100$

4 Conclusion

We developed an HPLC-based method capable of measuring the nicotine content in e-liquids subjected to MA. The method showed excellent results compared to the same experimental conditions WMA. The method was linear over a wide range of nicotine concentrations (25– 500 ppm). The investigation was carried out on seven e-liquids with LOD and LOQ values of 0.09 ppm and 0.29 ppm, respectively.

In line with previous studies, we noted that the measured nicotine concentrations differed from those stated on the labels. The results showed that 67% of samples had nicotine contents lower than those on the labels, whereas 33% had higher nicotine contents than those stated on the labels. No sample matched the nicotine content stated on the label. A sample registered as a nicotine-free e-liquid contained 7 mg/mL of nicotine and another sample claimed to contain 3 mg/mL actually had 17 mg/mL of nicotine, which is nearly four times the amount on the label. Thus, standards are required to ensure that manufacturers provide truthful information on the nicotine content of their products (i.e., that the stated amount on the label matches the measured nicotine amount). Otherwise, vapers may be exposed to unwanted levels of nicotine or use an e-liquid claimed to be nicotine-free without actually being so. A survey of 76 vapers revealed that 28.95% do not know the concentration of nicotine on the label, whereas 21% use a nicotine-free liquid because they believe it to be safer. Among vapers, 20% would buy an e-liquid with an increased nicotine content in their next purchase, whereas 17% would do the opposite and 26% would prefer the same level of nicotine. In summary, the claimed nicotine content of e-liquids is not always accurate, potentially exposing vapers to negative effects.

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Data availability My manuscript has associated data in a data repository.

Declarations

Conflict of interest The authors declare that there is no conflict of interest.

Ethical approval Not applicable.

Consent to participate All authors were participated in this work.

Consent to publish All authors agree to publish.

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