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A simplified approach for nicotine quantification in electronic cigarette liquids using GC-Orbitrap mass spectrometry

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Keywords

Accurate mass, electronic cigarettes, e-liquids, gas chromatography, GC-MS, quantification, GC, high-resolution, Orbitrap mass spectrometry, nicotine

Goal

To demonstrate the performance of Thermo Scientific™ Exactive™ GC Orbitrap™ GC-MS mass spectrometer for quantitative analysis of e-liquids components.

Introduction

Since the introduction of electronic cigarettes in 2007, their use has increased worldwide as an alternative to conventional tobacco cigarettes. Electronic cigarette liquids (e-liquids) can contain nicotine, a highly addictive drug that is toxic in high doses, may increase heart attack risk, and can affect reproductive health.[1] Studies have shown that nicotine can also interfere with memory and attention processing especially in adolescents.[2]

To protect human health, and to meet the obligations of the European Union under the WHO Framework Convention on Tobacco Control,[3] the Tobacco Products Directive 2014/14/EU (TPD)[4] in Article 20, contains rules for nicotine containing electronic cigarettes and refill containers. The Medicines and Healthcare products Regulatory Agency (MHRA),[5] is responsible for implementing the majority of the provisions under Article 20, including the restrictions on the nicotine strength of no more than 20 mg/mL.

Quantitative assessments of current analytical technologies for e-liquids include LC and GC coupled to both analog detectors (GC-FID, LC-UV) as well as MS.[6-11] But many of these methods can lack the sensitivity and selectivity required. High mass resolution performance and exceptional mass accuracy make GC Orbitrap a powerful solution for both qualitative and quantitative analysis of e-liquids.

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The aim of this work was to demonstrate the quantitative performance of the Exactive GC Orbitrap system for the analysis of nicotine in e-liquids samples.

Experimental

Sample and standard preparation

Flavored and flavorless e-liquid samples with specified nicotine levels of 0, 6, or 12 mg/mL were purchased locally and analyzed. Shortfill samples were also analyzed, which are supplied at 0 mg/mL specified nicotine level. Shortfills are e-liquids that can be purchased in bottles larger than the regulated limit of 10 mL, into which the user can add a nicotine shot prior to use. They are not regulated under TPD within the UK as they contain 0% nicotine upon purchase.

For target quantitative analysis of nicotine in e-liquid samples, liquid split/splitless sample injection was used. Each e-liquid sample was first diluted 50 µL to 5 mL with acetonitrile (LC/MS grade), mixed, then further diluted taking 100 µL of the diluted sample and 50 µL of the internal standard (0.1 mg/mL, 8-hydroxyquinoline), then made up to 1 mL with acetonitrile in a GC vial ready for analysis.

Calibration standards (ranging from 46 to 13,792 ng/mL nicotine) were prepared in acetonitrile, diluting from a certified e-liquid standard acquired from LGC (Teddington, UK). In addition, the calibration standards contained an internal standard (8-hydroxyquinoline) to a final concentration of 5000 ng/mL.

Instrument and method setup

An Exactive GC Orbitrap mass spectrometer coupled with a Thermo Scientific™ TRACE™ 1310 gas chromatograph, configured with a Thermo Scientific™ TriPlus™ RSH™ autosampler and a Thermo Scientific™ Instant Connect split/splitless (SSL) injector, was used in all experiments.

Compound separation was achieved on a Thermo Scientific™ TG-WaxMS 30 m x 0.25 mm i.d. x 0.25 µm film capillary column (P/N 26088-1420).

The mass spectrometer was tuned and calibrated in <1.5 min using FC43 (CAS 311-89-7) to achieve mass accuracy of <0.5 ppm. The system was operated using electron ionization (EI) mode, using full-scan and 60,000 mass resolution (full width at half maxima, measured at *m/z* 200). Additional details of the instrument parameters are shown in Tables 1 and 2.

Table 1. GC and injector conditions

TRACE 1310 GC system parameters				
Injection volume (µL):	1.0			
Liner:	Thermo Scientific™ LinerGOLD™ 4 mm i.d. 78.5 mm length (P/N 453A1255-UI)			
Inlet (˚C):	260			
Carrier gas (mL/min):	He, 1.2			
Inlet module and mode:	SSL, split mode			
Split ratio:	10:1			
Purge flow (mL/min):	5.0			
Column:	TG-WaxMS 30 m x 0.25 mm i.d. x 0.25 µm film capillary column (Thermo Scientific™ TraceGOLD™ GC Column) (P/N 26088-1420)			
Oven temperature program:	**RT (min)**	**Rate (˚C/min)**	**Target temperature (˚C)**	**Hold time (min)**
Initial	0	-	40	3.00
Final	3.00	13	250	6.00
Run time	25	-	-	-

Table 2. Mass spectrometer conditions

Exactive GC Orbitrap mass spectrometer parameters	
Transfer line (˚C):	250
Ionization type:	EI
Ion source (˚C):	230
Electron energy (eV):	70
Acquisition mode:	Full-scan
Mass range (Da):	75–500
Mass resolution:	60,000 FWHM at *m/z* 200

Data processing

Data were acquired and processed using Thermo Scientific™ TraceFinder™ software. TraceFinder single platform software integrates instrument control, method development functionality, and qualitative and quantitation data processing.

Results and discussion

The objective of this study was to evaluate the utility of Orbitrap-based GC-MS technology for quantitative analysis of e-liquids for nicotine, using direct liquid injection, accessing various analytical parameters including chromatographic resolution, instrument sensitivity, and linearity.

Chromatography

Good chromatographic separation was obtained using the GC conditions described in Table 1. The total ion chromatogram (TIC) for nicotine and 8-hydroxyquinoline (internal standard) are shown in Figure 1.

In order to develop a quantitative workflow for nicotine analysis using TraceFinder software, a compound database (CDB) to store compound information was produced (see Figure 2). The overlaid extracted ion chromatograms (EICs) achieved for target and confirming ions for nicotine over the calibration range are shown in Figure 3.

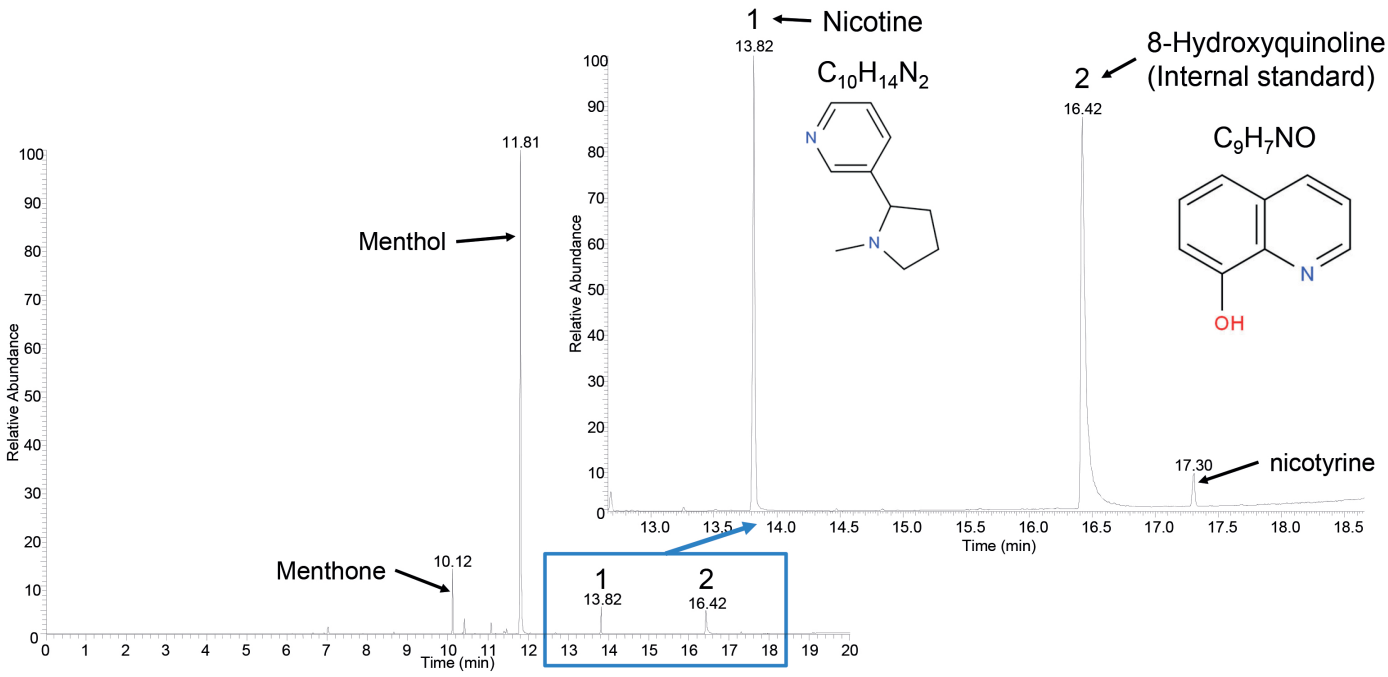
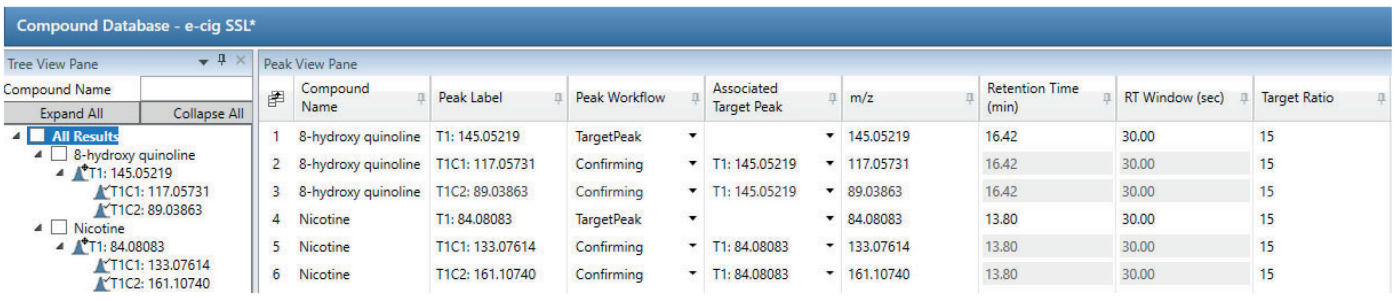


Figure 1. Total ion chromatogram (TIC) for [1] nicotine and [2] 8-hydroxyquinoline (internal standard) in a standard at 13,792 ng/mL for nicotine and 5000 ng/mL for 8-hydroxyquinoline. For peaks at retentions times 12.12, 11.81, and 17.30 minutes, additional compound names were identified, based on the top NIST library search results achieved for the background subtracted mass spectra for each peak.



	Compound Name	Peak Label	Peak Workflow	Associated Target Peak	m/z	Retention Time (min)	RT Window (sec)	Target Ratio
1	8-hydroxy quinoline	T1: 145.05219	TargetPeak		145.05219	16.42	30.00	15
2	8-hydroxy quinoline	T1C1: 117.05731	Confirming	T1: 145.05219	117.05731	16.42	30.00	15
3	8-hydroxy quinoline	T1C2: 89.03863	Confirming	T1: 145.05219	89.03863	16.42	30.00	15
4	Nicotine	T1: 84.08083	TargetPeak		84.08083	13.80	30.00	15
5	Nicotine	T1C1: 133.07614	Confirming	T1: 84.08083	133.07614	13.80	30.00	15
6	Nicotine	T1C2: 161.10740	Confirming	T1: 84.08083	161.10740	13.80	30.00	15

Figure 2. CDB for nicotine and 8-hydroxyquinolone, detailing the target and confirming ions (*m/z*), retention time (RT), RT window, and the target ion ratio for each compound

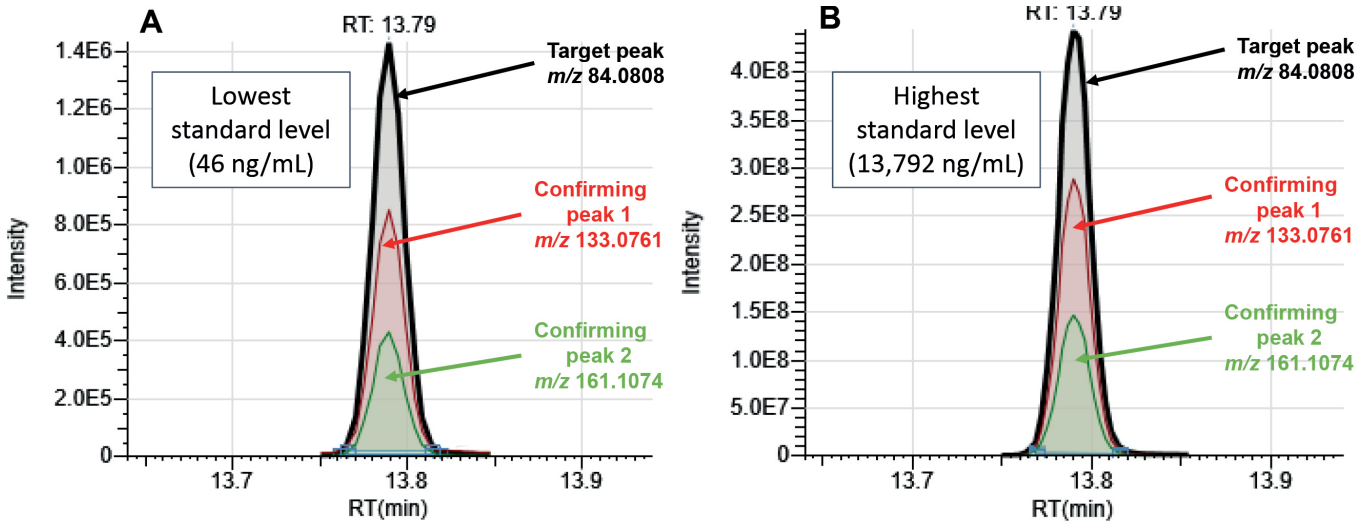


Figure 3. Overlaid EICs for target and confirming ions for [A] the lowest (46 ng/mL) and [B] the highest (13,792 ng/mL) calibration standards in the developed quantification method

Quantification and sensitivity

The quantitative performance of the Exactive GC Orbitrap GC-MS system was tested for nicotine. System sensitivity, linearity, and peak area repeatability were evaluated. Additionally, mass accuracy of the target compounds was assessed across the concentration ranges. Linearity was assessed using 11 calibration levels ranging from 46 to 13,792 ng/mL (equivalent to 0.046 to 13.79 mg/mL in the prepared e-liquid samples). Data was acquired using full scan, with compound detection based on retention time (±0.5 min window), accurate mass (±5 ppm window), and ion ratio of quantification vs. confirming ion (±15% window). Collecting full scan data enables the retrospective qualitative targeted or non-targeted screening of the collected data if required at a later date.[12] Nicotine was easily detected in the lowest calibration standard, 46 ng/mL (equivalent to 0.046 mg/mL in the prepared e-liquid samples).

Mass accuracy

Maintaining mass accuracy and spectral fidelity is critical for correct compound identification in potentially complex e-liquid samples. Figure 4 illustrates the mass accuracy achieved over the calibration range for nicotine; a mass accuracy of <1 ppm was achieved for each ion in the spectra.

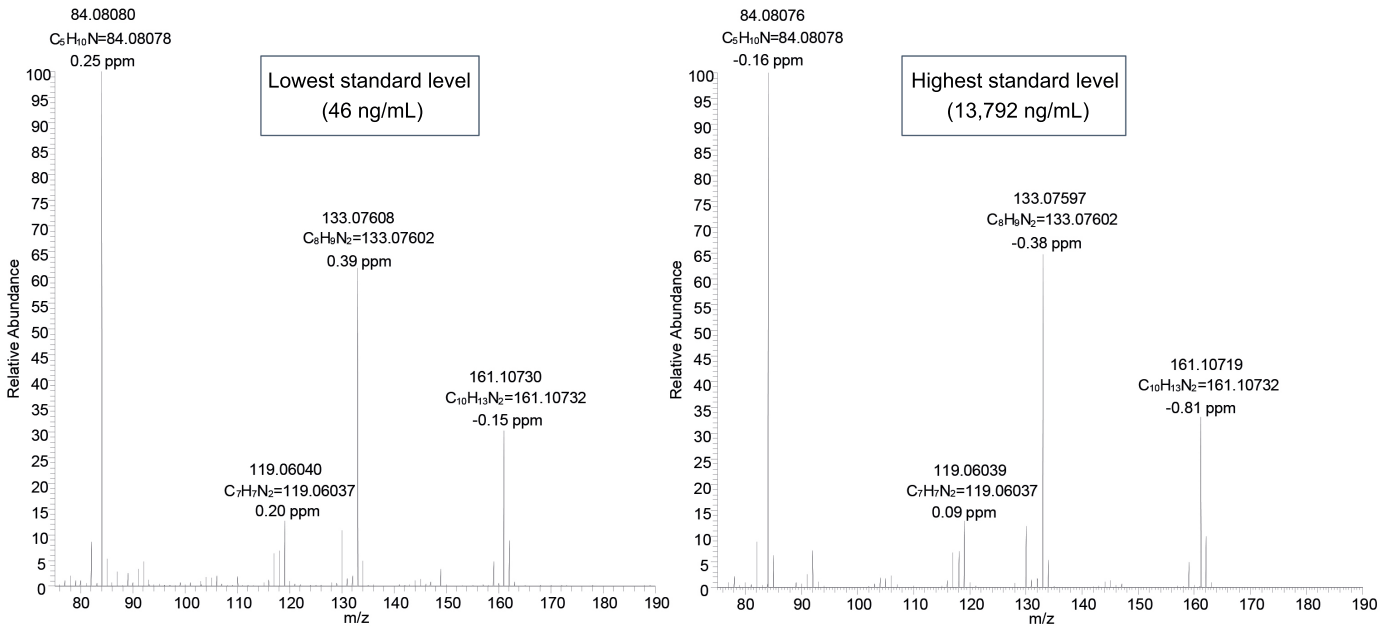


Figure 4. Mass spectra for the lowest (46 ng/mL) and highest calibration (13,792 ng/mL) standards for nicotine. Consistent <1 ppm mass accuracy was obtained for each ion in the spectra. Annotated are the measured mass, the elemental composition, and the theoretical mass as well as the mass accuracy (ppm).

Peak area repeatability in matrix

In order to have confidence in the routine nicotine quantitation results achieved, the stability of responses in the matrix is critical. Repeatability of nicotine responses in matrix were assessed by carrying out n=3 repeat injections of two e-liquids with stated nicotine concentrations of 6 mg/mL. Excellent repeatability was obtained as shown in Table 3, with %RSD for quantification ions peak area counts between 3.7% and 3.9%.

Linearity of response

To assess compound linearity, 11 calibration levels (46 to 13,792 ng/mL) were quantified using an internal standard calibration, using a 1/x weighting factor. Excellent peak area repeatability for the internal standard (8-hydroquiniline) was acheived, as shown in Figure 5A, with a %RSD of 5.3. Excellent linearity was demonstrated for nicotine, with an R^2 value of 0.9991, an average residual %RSD of 4.4, and an amount deviation tolerance at each point of <10%. An example calibration curve for nicotine is shown in Figure 5B where both the coefficient of determination (R^2) and the residual %RSD are annotated.

Table 3. Nicotine results summary for replicate injections (n=3) for two e-liquid samples with stated nicotine concentration of 6 mg/mL, f = branded flavored, and g = flavorless. The concentration results (mg/mL) are internal standard corrected.

Nicotine results summary						
Sample:	**f**			**g**		
Replicate number	**Peak area**	**RT (min)**	**Conc. (mg/mL)**	**Peak area**	**RT (min)**	**Conc. (mg/mL)**
1	227988757	13.78	5.523	274002489	13.78	7.053
2	234018241	13.77	5.619	290141336	13.77	7.422
3	245967546	13.77	5.463	29386353	13.77	7.297
Average	235991515	13.77	5.535	285993392	13.77	7.257
Std dev	9150386	0.006	0.079	10547493	0.006	0.1877
%RSD	**3.88**	**0.042**	**1.422**	**3.69**	**0.042**	**2.59**
Claimed (mg/mL)			6.00			6.00
% of claimed			92.25			120.96

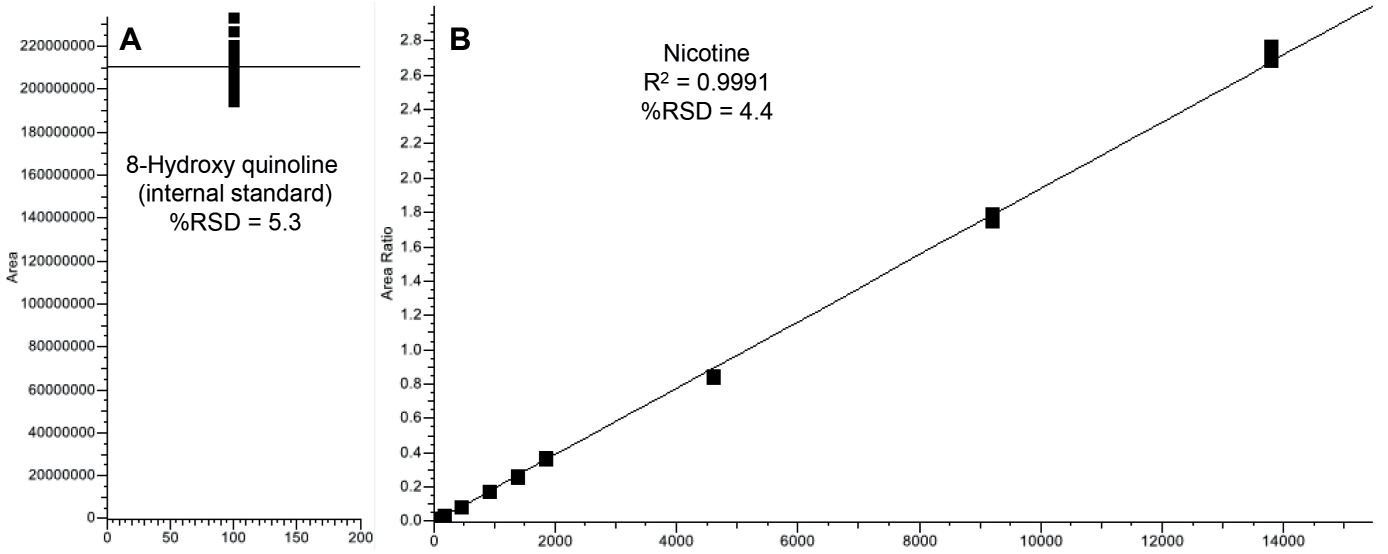


Figure 5. (A) Average peak area for 8-hydroxyquinline (internal standard), and (B) example calibration curve for nicotine, illustrating the linearity obtained, over 11 calibration levels ranging from 46 to 13,792 ng/mL (two injections per calibration level)

Quantitation of nicotine in e-liquid samples

Ten e-liquid samples were prepared and analyzed as detailed; concentrations of the nicotine identified are illustrated in Figure 6A. The samples analyzed were quantified using an internal standard calibration, with the internal standard added to all samples and standards to the same concentration. For the three e-liquids with specified nicotine concentrations, the results versus the specified nicotine concentrations are shown in Figure 6B. Many studies have shown that nicotine content in many e-liquid samples is highly variable when compared to the label claims,[13,14] which is illustrated here with the deviations of the results from the claimed concentrations between -8% and +21%.

A

Sample	Description	Declared nicotine concentration (mg/mL)	Nicotine concentration (mg/mL)
a	Flavorless	0	<0.046
b	Flavored (branded)	0	<0.046
c	Flavored (branded)	0	<0.046
d	Flavored (vanilla)	0	<0.046
e	Flavored (mint)	0	<0.046
f	Flavored (branded)	6	5.5
g	Flavorless	6	7.3
h	Flavored (lemon)	12	12.2
i	Flavored (strawberry)	0	<0.046
j	Flavored (lemon)	0	<0.046

B

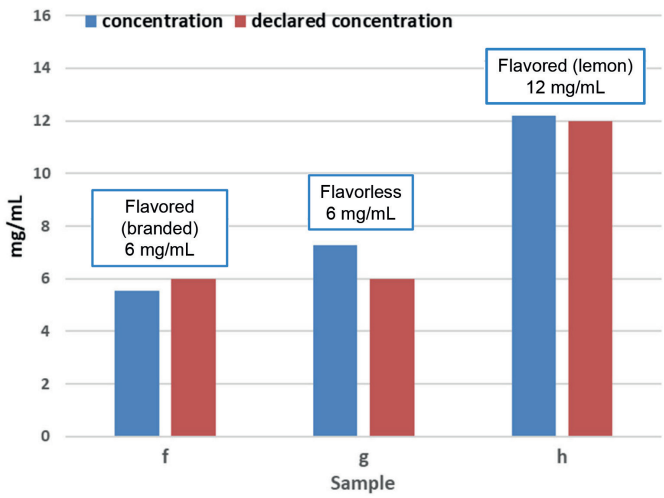


Figure 6. (A) Calculated concentration of nicotine detected in the analyzed e-liquid samples, and (B) detected nicotine results versus declared value, for the three e-liquids with specified nicotine concentration

Conclusions

- The results of this study demonstrate that using Orbitrap-based GC-MS technology, with high mass resolution performance, and exceptional mass accuracy, provides excellent solutions for the quantitative analysis of e-liquids.
- In the targeted analysis of nicotine using liquid injection: linearity was demonstrated with R^2 = 0.999 and residual values RSD% = 4.4%, over 11 calibration levels ranging from 46 to 13,792 ng/mL (equivalent to 0.046 to 13.79 mg/mL in the prepared e-liquid samples); mass accuracy of <1 ppm was obtained for all ions in the nicotine spectra (from the lowest to the highest standard).
- Quantitative targeted analysis for nicotine in ten e-liquid samples, including flavored and flavorless, with declared nicotine levels of 0, 6, or 12 mg/mL, and two shortfill flavored e-liquid samples was performed. Replicate measurements for nicotine containing samples indicated excellent precision with %RSD < 3 achieved.

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