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Is surface-enhanced Raman spectroscopy (SERS) a good alternative to separation techniques for nicotine dosage in e-liquid boosters?

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ABSTRACT

Since 2014, electronic cigarettes must follow the European Directive on tobacco products. In Belgium, the transposition of this directive requires that nicotine-containing e-liquid boosters cannot exceed a concentration of 20 mg mL^{-1} to ensure consumers safety. Nowadays, accurate analytical methods available to measure nicotine levels in e-liquid products involve chromatography. The development of alternative analytical tools being faster, greener and adaptable to in-field analyses are therefore required. Surface-enhanced Raman scattering is a spectroscopic technique that significantly enhances inherent Raman scattering signals, improving detection limits, when analytes are adsorbed onto metallic nanostructures such as gold nanoparticles (AuNPs). This study introduces new SERS methods for quantifying nicotine in e-liquid boosters using two different Raman spectrophotometers based on a transmission (SETRS) and a backscattering detection mode. The transmission Raman spectrophotometer has a better sample representativity, which is very interesting to perform SERS on liquids samples, and an autosampler offering facilities for routine analyses as a benchtop equipment while the second spectrophotometer was a handheld Raman device allowing to expand the use of the developed SERS method to in-field analyses. These SERS analyses were performed using lab-synthetized AuNps and by adding an isotope-edited internal standards (IEISs) being nicotine-d4 to mitigate some repeatability issues. These methods were finally validated according to the ICH Q2 (R2) guidelines for a working range from 100 to 300 µg L^{-1} of nicotine concentrations using a total error risk-based approach considering the acceptance limits fixed at 15 % and a risk level of 5 %.

1. Introduction

During the last decade, the use of electronic cigarettes grew in popularity. They became an alternative for smokers, especially for young people, due to their attractive various flavours [1,2]. Since 2014, electronic cigarettes must follow the European Directive on tobacco products [3]. In Belgium, the transposition of this directive requires that nicotine-containing e-liquid boosters cannot exceed a concentration of 20 mg mL^{-1} to ensure consumers safety. Moreover, the composition of these e-liquid boosters is also under this new legislation, as several additives (e.g. colourants, vitamins, etc.) are henceforth forbidden in the interest of public health. This new legislation called for the development of new analytical tools to control the conformity of these products and more especially to determine the nicotine dosage in the e-liquid refill flasks (nicotine booster). Many laboratories employ separation techniques such as liquid chromatography (LC) and gas chromatography (GC) coupled with detectors like DAD detector or mass spectrometer for the specific detection of the different compounds of interest [4–7]. Even if these separation techniques are commonly used in this context, the development of alternative techniques being faster, more environmentally friendly and adaptable to in-field analyses are required. Surface-enhanced Raman scattering (SERS) is a powerful emerging technique that significantly enhances the detection limit of Raman scattering when the target analyte is adsorbed onto metallic nanostructures acting as antennas amplifying the signal while keeping all the benefits of this vibrational spectroscopy [8]. Various substrates permit this enhancement, but colloidal gold and silver nanoparticles remain commonly used by the SERS community since these SERS substrates are

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economic and easy to manipulate while having a high enhancement factor. Gold nanoparticles (AuNPs) present the advantages of being less impacted by oxidation phenomenon and more monodisperse in terms of size and shape than silver nanoparticles when synthetized via chemical reduction [9,10]. These nanoparticles, being in suspension, can form high-field structures, such as aggregates known as hot spots, which play crucial role in SERS applications by significantly contributing to signal enhancement. The aggregation step enables to get the nanoparticles close to each other, allowing the combination of the signal exaltation, but also close the analyte of interest leading to the SERS effect [11]. The challenges that need to be considered using metallic nanoparticles in suspension as SERS substrate to permit the use of SERS in the routine analysis are (i) the metallic nanoparticles synthesis repeatability issues since few protocols were robustly optimized [12] (ii) the poor repeatability of the SERS analyses linked to a lack of uniformity in the response due to nanoparticles dispersion and (iii) the lack of control of the aggregation process and hot spot disparity throughout the samples [13,14]. Several researchers highlighted that the improvement of the analytical performance of SERS is possible through the addition of an internal standard (ISTD) in the sample preparation. Among ISTDs, the use of isotope-edited internal standards (IEISs) has been proposed due to their identical chemical properties to the analyte while offering distinct spectral features for accurate quantification [15–17]. This strategy allows to mitigate the issues coming from the SERS substrate and the poor repeatability of its synthesis, the aggregation state and variations in excitation or collection of the Raman system [18]. Furthermore, the distinct spectral features of the analyte and the IEIS enable the use of univariate data analysis. In this study, two different Raman systems were also investigated, first, to assess their impact on the method analytical performances, and second, to explore two analysis scenarios. In a first way, the SERS spectral acquisitions were performed on a transmission Raman spectrophotometer (SETRS). This transmission mode implies the detection of Raman photons on the opposite side of the incident photons unlike the classical backscattering mode which detects scattered photons on the same side of the incident photons. The transmission mode is interesting as it enhances sample representativeness by increasing the sampling volume [19–21]. This property is attractive with colloidal suspension of nanoparticles where hot spots may be unevenly distributed. The benefits of performing SETRS acquisitions were recently demonstrated by Horne et al. [22]. Therefore, the interest was also focused on this equipment since it is combined with a XY autosampler which can offer analysis facilities in routine in a benchtop format. Additionally, SERS spectral acquisition was performed using a handheld Raman device to extend the application of the developed SERS method to in-field analyses.

The aim of this study was therefore to develop SERS methods allowing the quantitative determination of nicotine in e-liquid boosters (range μg L^{-1}) by acquiring spectral data on both equipment. While SERS has previously been employed for the identification [23,24] and quantitative determination of nicotine in water [25] and e-liquid samples [26,27] using various SERS substrates, the originality of this study lies in its comprehensive approach. For the first time, the robustness of a simple developed SERS method was demonstrated by evaluating its analytical performance under varying acquisition modes, using different equipments (to simulate various analysis scenarios), across several batches of SERS substrate and involving multiple operators over different days. These factors are critical for addressing the repeatability challenges commonly reported in the literature and establishing the applicability of SERS as a routine analytical tool. To ensure reliability, the method was validated in accordance with ICH Q2 (R2) guidelines. The SERS samples were prepared using lab-synthetized gold nanoparticles in suspension (AuNps) as the SERS substrate. Diluted reconstituted e-liquid booster spiked with various nicotine concentrations and nicotine-d4 (used as an IEIS), were prepared as calibration and validation standards. The dilution step of the reconstituted booster was necessary to minimize matrix interferences, resulting in nicotine concentration range within the μg L^{-1} which supports the suitability of SERS/SETRS analysis. Finally, the validated method was tested on a real e-liquid booster sample in routine conditions to demonstrate its practical applicability.

2. Materials and methods

2.1. Chemicals and reagents

(+/-) Nicotine-d4 solution (certified reference materials) and gold (III) chloride hydrate (trace metals basis) were purchased from Merck KGaA (Darmstadt, Germany). Potassium chloride (analysis grade) was obtained from VWR (Radnor, PA, USA). Trisodium citrate 98 (98 %) and L-nicotine 99 + % were bought from Acros Organics (Geel, Belgium). Glycerol and propylene glycol (Ph. Eur.) were purchased from Fagron (Nazareth, Belgium). All solutions were prepared using Milli-Q water 103 (18.2 MΩ.cm, Milli-Q Plus 185, Millipore, Burlington, USA).

2.2. Preparation of gold nanoparticles (AuNps)

All steps were carried out in the dark. The AuNps synthesis follows the standard protocol of Lee-Meisel [28]. For the chemical reduction, 48 mg of gold (III) chloride ($HAuCl_4$) were solubilised in 100.0 mL of Milli-Q water in a volumetric flask. This $HAuCl_4$ solution was then carefully transferred into a 3-neck round bottom flask placed in a Drysyn heating mantle (Asynt, UK) with the temperature set to 150 °C. Once the solution reached boiling, a 1 % sodium citrate solution was added at a precise rate of 5 mL min^{-1} using a Dosimat dosing device (Metrohm AG, Switzerland) through one of the necks of the flask. The reaction was allowed to proceed for 1 h. Then, the resulting AuNPs suspension was cooled down at room temperature and kept away from the light.

2.3. Preparation of calibration and validation samples

A reconstituted nicotine booster was prepared by weighing 200 μL of nicotine in a 10.0 mL volumetric flask and filled up to the mark with a mixture of glycerol/propylene glycol 50/50 (V/V) mimicking the matrix. This solution was diluted 20,000 times with Milli-Q water to prepare a nicotine stock solution at 1 mg L^{-1}. Then, the calibration and validation samples were prepared using qualified automatic pipets in glass vials (VWR, Pennsylvania, USA) by mixing the correct amount of the stock solution of nicotine with freshly defrosted nicotine-d4 solution at 1.5 mg L^{-1} and Milli-Q water as described in Table 1. These resulting mixtures are referred as *analyte* in the rest of the manuscript.

2.4. Preparation of the SERS samples

The SERS samples were prepared by mixing 400 μL of colloidal AuNps with 400 μL of *analyte* in a glass vial (VWR). The resulting mixture was vortexed for 10 s (Reax top, Heidolph, Schwabach, Germany). Then 200 μL of KCl 0.3 M were added, and the glass vial was vortexed for 10 supplementary seconds. A blank was made with 400 μL of AuNps and 400 μL of Mili-Q water vortexed for 10 s. Then, 200 μL of KCl 0.3 M were added before vortexing the final mixture for 10 s. The 2/2/1 ratio of the AuNps, *analyte* and aggregation promoter was optimized based on a previous work [29]. A 10-s vortex blend time was implemented to ensure the suspension's homogeneity and promote AuNPs-analyte aggregation.

2.5. Stability measurements

Given that the aggregation of AuNPS is a dynamic process, experiments were conducted to monitor the evolution of the SERS signal over time. Samples with a target concentration of 300 ppb were placed in the Raman transmission spectrophotometer (TRS 100, Agilent Technologies, Santa Clara, California, United States) and exposed to the laser

Table 1
Preparation of validation and calibration standards referred as analyte in the rest of the manuscript.

For each series of validation	Concentration levels	Nicotine stock solution (1 mg L^{-1}) in µL	Nicotine-d4 stock solution (1.5 mg L^{-1}) in µL	Milli-Q water in µL	Concentration of nicotine in *analyte* (µg L^{-1})	Concentration of nicotine-d4 in *analyte* (µg L^{-1})
Calibration set (n = 1)	1	200	200	1600	100	150
	3	300	200	1500	150	150
	5	600	200	1600	300	150
Validation set (n = 3)	1	200	200	1600	100	150
	2	250	200	1550	125	150
	3	300	200	1500	150	150
	4	400	200	1400	200	150
	5	600	200	1200	300	150

every 4 s for 30 min with a laser power of 350 mW. An exposition of 0.65 s and three accumulations were fixed with a laser spot size of 4 mm and medium collection lens optics. Three spectra were acquired at each time point. These experiments were conducted exclusively using the transmission system only as the backscattering system does not support programming multiple acquisitions over a fixed period.

2.6. Validation design

Six series of validation were done. One validation series consists in the analysis of at least one calibration set and three validation sets. One calibration set comprised three nicotine concentrations in the *analyte* at 100, 150 and 300 µg L^{-1} while one validation set was composed of five concentrations of nicotine in the *analyte* at 100, 125, 150, 200 and 300 µg L^{-1} as illustrated in Table 1. To obtain the intermediate fidelity, the validation was carried out on six days with two operators. Four syntheses of AuNps were produced by two operators to perform this validation. These AuNps syntheses were pooled in pairs to have sufficient volume for each validation series. Each pair of colloidal AuNps was used for 3 days of the validation process.

Three spectra were acquired from each sample during the validation process on both two equipment. Regarding the Raman transmission system (TRS 100, Agilent Technologies, Santa Clara, California, United States), a laser power of 650 mW combined with an exposition time of 1.5 s and three accumulations were used as fixed parameters. For the handheld Raman backscattering system (TruScan™ RM handheld Raman analyser with the TruTools™ package, Thermo Fisher Scientific, Waltham, MA, USA), a laser power of 250 mW was settled with 2 seconds of exposition time and 3 accumulations.

2.7. Data processing

The most intense band of nicotine (1030 cm^{-1}) and of nicotine-d4 band (995 cm^{-1}) were measured from the baseline corrected spectra (Whittaker filter, lambda = 100,000, p = 0.001) after the relevant spectral range between 350 and 1800 cm^{-1} was selected using MatLab® R2020b (The MathWorks, Natick, MA, USA) coupled with PLS_Toolbox 8.9.2 (Eigenvector Research, Inc., Wenatchee, WA, USA). The ratios 1030 cm^{-1}/995 cm^{-1} were determined using Microsoft® Excel® 2016 (Redmond, Washington, USA) and were used to compute the β-expectation tolerance limits with β = 95 % in the Enoval 4.1d software (Cencora Pharmalex Belgium, Mont St-Guibert, Belgium). A linear regression model was selected.

2.8. Real e-liquid booster analysis

The developed SERS method was applied on a real e-liquid booster sample at a concentration of 20 mg mL^{-1}purchased in a Belgian vape shop using the protocol described in the Section 2.3. This sample was diluted 20,000 times with Milli-Q water. The final sample was prepared by mixing 300 µL of the diluted solution of the e-liquid booster with 200 µL of the nicotine d-4 stock solution at a concentration of 1.5 mg mL^{-1} with 1500 µL of Milli-Q water.

3. Results and discussion

3.1. Signal stabilisation study

The aggregation step consists commonly in the addition of a salt in the SERS sample preparation, in this case potassium chloride, to modify the ionic force in the medium of the colloidal suspension and decreasing the electrostatic barrier which allows to get the nanoparticles close to each other but also close to the analyte of interest. In this context, an important exaltation of signal can be observed thanks to the hot spots formation [30]. However, the SERS signal may vary over time since the aggregation is a dynamic process requiring a sample incubation time to reach a SERS signal stabilisation [11]. Therefore, a signal stability study was therefore performed over the time. The experiments were done using the transmission system since it allows the user to program multiple sample spectral acquisition over time and is equipped with an autosampler facilitating the analyses. The mean intensity ratios 1030 cm^{-1} (specific band intensity related to nicotine)/995 cm^{-1} (specific band related to nicotine-d4) determined from the SERS spectra acquired were reported versus time as it is illustrated in the Fig. 1. Bell et al. described that signal degradation may be linked to photochemical damage caused by constant laser intensity aimed towards the sample [9]. However, using a transmission system, the laser power per unit area is lower than in conventional backscattering mode since the laser is not focused on a specific point of the sample but rather a volume of the sample. In addition, no visual degradation of the samples was noticed, and the bands position in the SETRS spectra were unchanged over the time. The decrease in the signal that can be observed in the Fig. 1 is most likely linked to the stability of the colloidal suspension. It was observed that the signal tends to stabilize around 15 min. As the objective of this work was to develop a SERS quantitative method competing with separation techniques, the priority was set on the decrease of the signal variability rather than the sensitivity. An incubation time of 15 min for the SERS samples was fixed for the validation process.

3.2. Specificity study

The Fig. 2 shows the SERS signal of nicotine, nicotine-d4 and a blank acquired using the transmission spectrophotometer. These SERS spectra were acquired using a concentration of 100 µg L^{-1} of nicotine representing the lowest concentration of the calibration set while the nicotine-d4 was at a concentration of 150 µg L^{-1} being the concentration used during the validation process. This figure demonstrated the specificity of the method since distinct bands were highlighted for the nicotine and nicotine-d4 from the blank despite of their similar structures. Also, the diluted matrix, being a mixture of glycerol/propylene glycol 50/50 mimicking the e-liquid booster composition, did not interfere with the detection of nicotine and nicotine-d4 as illustrated by the blank SERS spectrum (Fig. 2).

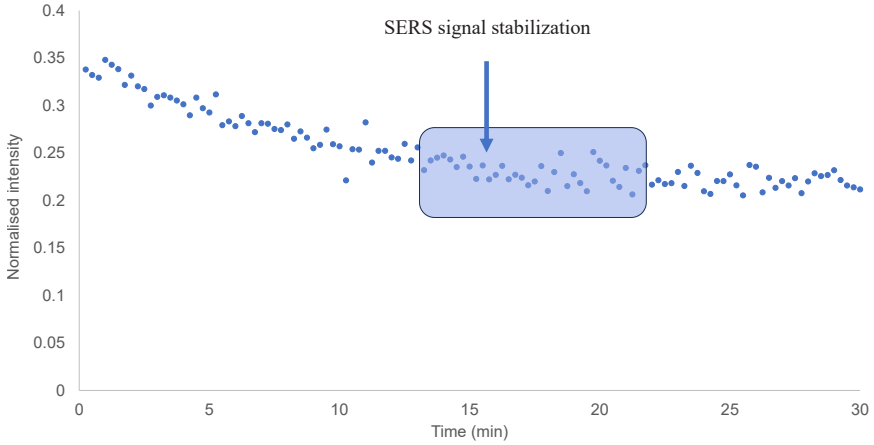

Fig. 1. Representation of the mean ratio nicotine$^{-1}_{1030\ cm}$/nicotine-d4$^{-1}_{995\ cm}$ reported versus time during the signal stabilization study. The area of interest is highlighted by the arrow.

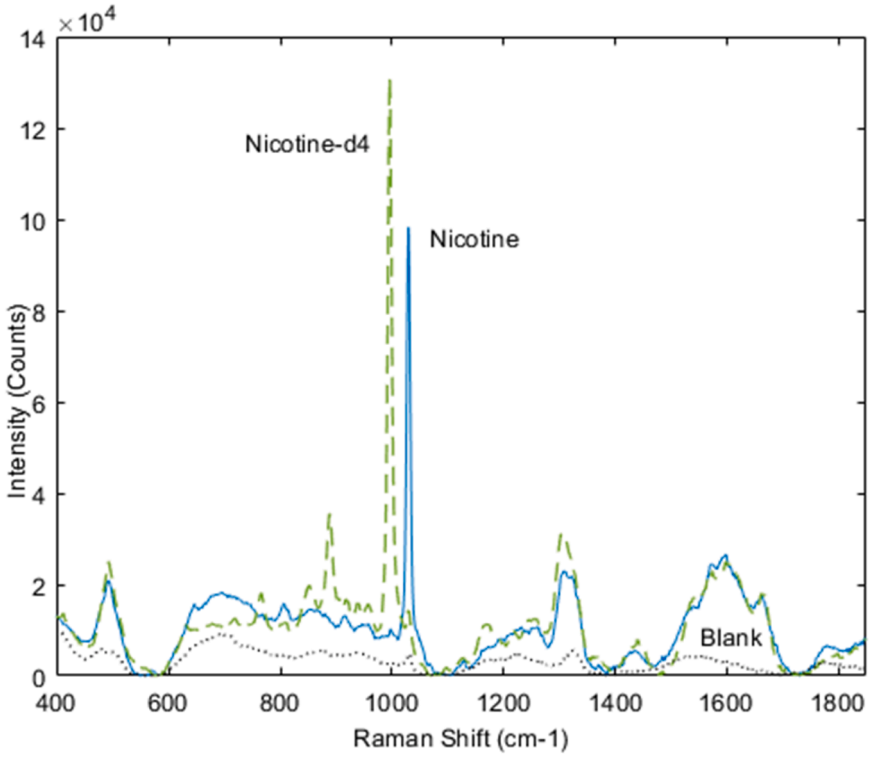

Fig. 2. SETRS spectra of nicotine at 100 μg L^{-1} (solid line), nicotine-d4 at 150 μg L^{-1} (dashed line) and a blank (dotted line).

3.3. Feasibility study of performing a quantitative determination of nicotine in reconstituted e-liquid booster

The next step consists in investigating how the nicotine concentration influences the SETRS and SERS signals. A feasibility study was implemented involving SERS samples containing AuNps, nicotine at increasing concentration, a fixed concentration of the IEIS at 150 μg L^{-1} and the aggregating agent prepared as described in the Section 2.3. The range of nicotine concentrations was set from 100 to 300 μg L^{-1}. This range was considered since a dilution step of the samples is required to minimize interferences from the matrix being a mixture of propylene glycol and glycerine. A Raman spectrum of this matrix is illustrated in the Fig. S1 in the Supplementary data. A Raman spectrum acquired from a real e-liquid booster at a concentration of 20 mg mL^{-1} is also showed to highlight the technical limitations of performing a quantitative determination of nicotine using classical Raman spectroscopy due to the intense signal scattered from the matrix. The reconstituted diluted samples were analysed using both Raman spectrophotometers. The Figs. 3(a) and 3(b) represents the normalized SETRS and SERS spectra acquired from these analyses. As described in the literature, the use of a IEISs in the sample preparation allowed to reduce the variability coming from the aggregation state and from the excitation or collection in the Raman system. The interest of using nicotine-d4 in the sample preparation is illustrated in Figs. 3(c) and 3(d). The normalization of the band intensity situated at 1030 cm^{-1} (belonging to nicotine) with the band intensity situated at 995 cm^{-1} (belonging to nicotine-d4) enabled to obtain a good linearity of the response function. However, it is important to note that a competition for the metallic nanoparticles surface has been described as a major limitation of using isotope as an internal standard since the intensities ratio analyte to IS may not be linear with

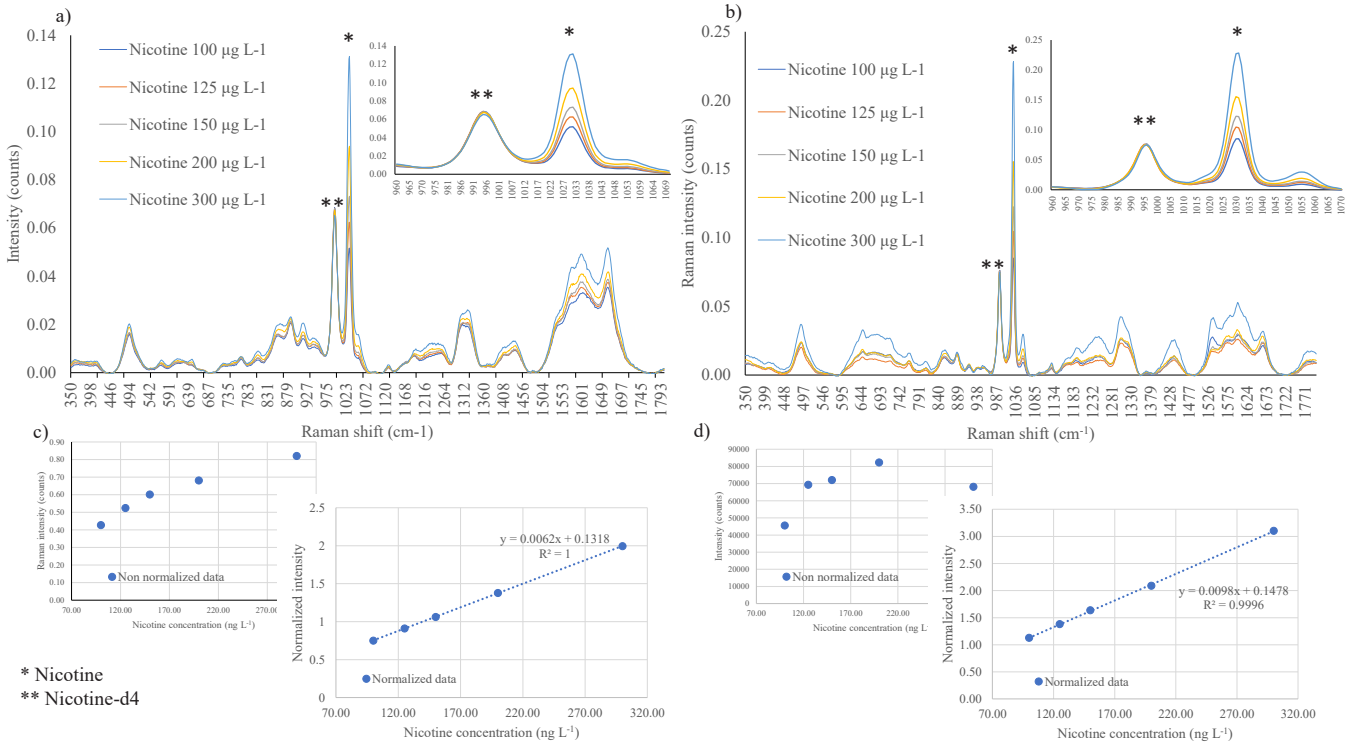

Fig. 3. SERS (a) and SETRS (b) spectra acquired from samples with increasing nicotine concentrations from 100 et 300 µg L^{-1}. The specific bands related to nicotine (*) and nicotine-4d (**) are highlighted. The interest of using a IEIS is demonstrated for the SERS (c) and for the SETRS (d) analyses according to the response function.

the analyte concentration if a saturation of the enhancement area is reached [31]. But as illustrated in this work, this limitation was circumvented by working with a short range at very low concentrations.

3.4. Methods validation according to the ICH Q2 (R2)

A univariate data analysis was selected to develop the quantitative method due to its simplicity in terms of samples preparation and spectral analysis. Moreover, the need for multivariate model maintenance is eliminated as the calibration process accounts for the variability in SERS response due to different batches of metallic nanoparticles [32,33]. This approach was feasible because nicotine and nicotine-d4 exhibit specific bands free from matrix interferences as shown in Fig. 2 and Fig. 3(a) and (b). The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) Q2(R2) guidelines recommend evaluating the specificity, range, accuracy and precision (repeatability, intermediate precision) of an analytical method used to quantify the content of a product. Even if the nicotine content in an e-liquid is not classified as a pharmaceutical product despite its current used as a nicotine substitute, the ICH Q2 (R2) guidelines were followed to ensure adherence to rigorous analytical standards. The specificity of the methods was demonstrated in Section 3.2. A total error risk-based approach combining systematic and random errors was used to assess the analytical performances of the two methods [34,35]. The calibration model retained was a linear regression with coefficient of determination greater than 0.98. For both SETRS and SERS, the lower limit of quantification (LLOQ) was set at 99.96 µg L^{-1} and the upper limit of quantitation (ULOQ) at 299.9 µg mL^{-1}. The methods precision was estimated through the repeatability (intra-series variability) determined from the analyses of three independent replicates for each concentration level and expressed as RSD ranging from 3.5 % to 4.6 % for the SETRS and from 1.8 % to 3.5 % for the SERS as detailed in Table 2. The intermediate precision (accounting intra + inter-series variability) is ranging from 3.5 % to 4.6 % for the SETRS and from 1.8 % to 3.5 % for the SERS. The relative bias presented a maximum value of 2.6 % for SETRS and 1.4 % for the SERS. These analytical performances were obtained from 6 series of validation with several batches of AuNps and two operators to capture a maximum of variability sources. These results are highly compelling and underscore the value of adding IEIS in the SERS sample preparation highlighting its effectiveness in enhancing analytical performances.

The accuracy profiles of the univariate spectral analysis for both SETRS and SERS methods is presented in Fig. 4. The acceptance limits were fixed at 15 % with a risk level of 5 % (which means that the model

Table 2
Repeatability, intermediate precision, bias and β-expectations tolerance interval for each concentration level for the developed quantitative SERS and SETRS methods using a univariate data analysis of the normalized intensities of the specific band related to nicotine at 1030 cm^{-1}.

Theoretical concentration (µg/L)	Repeatability (RSD%)		Intermediate precision (RSD%)		Bias (%)		β-expectations tolerance interval (%)	
	SETRS	SERS	SETRS	SERS	SETRS	SERS	SETRS	SERS
100.0	4.1	2.8	4.1	2.8	− 0.1	− 0.6	[− 8.9, 8.8]	[− 6.6, 5.5]
125.0	4.6	2.7	4.6	2.7	0.7	0.0	[− 9.4, 10.9]	[− 5.9, 5.8]
150.0	3.5	1.8	3.5	1.8	0.5	0.4	[− 7.0, 8.1]	[− 3.6, 4.4]
200.0	4.1	3.5	4.1	3.5	2.6	1.4	[− 6.4, 11.7]	[− 6.2, 9.1]
300.0	3.7	2.8	3.7	2.8	0.1	− 0.6	[− 7.9, 8.2]	[− 6.6, 5.5]

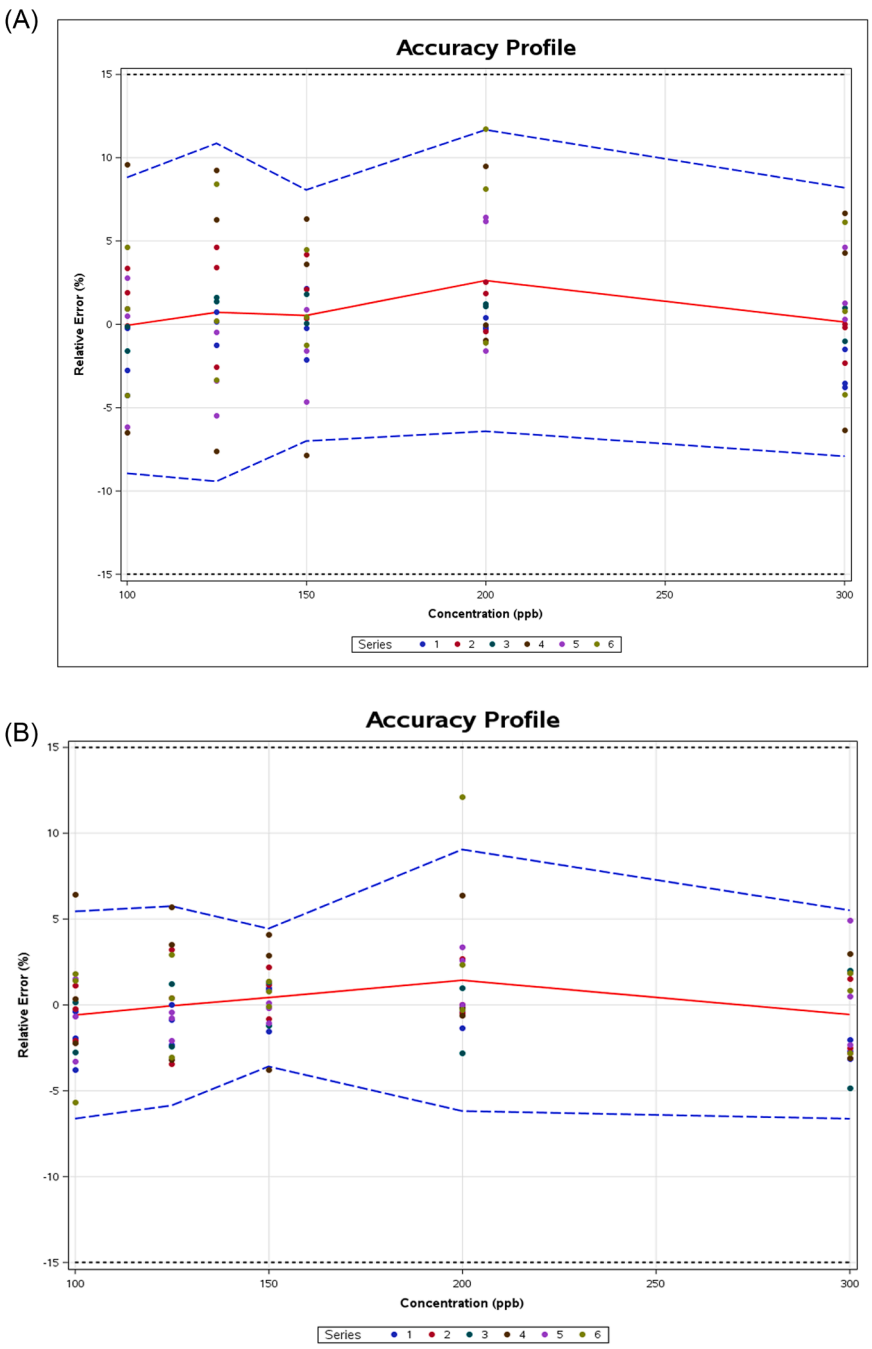

Fig. 4. Accuracy profiles obtained for SETRS (4a) and SERS (4b) quantification of nicotine in diluted reconstituted e-liquid booster based on a linear regression model. The plain red line represents the relative bias, the dashed blue lines are the 95 % β-expectation tolerance interval, dotted black lines are the acceptance limits and the colored dots represent the back-calculated results from the validation series.

tolerates 5 % of the samples to fall outside these limits). These values were adopted in the absence of specific regulatory guidelines for nicotine quantification in e-liquid beyond the general requirement that nicotine content must not exceed 20 mg mL^{-1}. The developed methods have been demonstrated fit for purpose as they were successfully validated. The β-expectations tolerance interval falls within the acceptance limits across the entire concentration range. These methods are the first one being validated according to the ICH Q2 (R2) guidelines in the context of nicotine determination using SERS. They present the advantage of being compatible with two different analysis scenarios: SETRS combined with a XY autosampler which offers analysis facilities in routine benchtop setup while SERS method developed on a handheld spectrophotometer enables in-field-analyses which can be a valuable feature in the context of customs inspection.

The analytical performances of both methods are comparable indicating that one equipment could be used to perform in-field analyses while the second as a confirmatory test in the laboratory if a sample is stated as a non-compliant sample. Although separation methods cited in the literature for nicotine quantification in e-liquids are considered as the gold standard due to their robustness and superior analytical performances, the SERS/SETRS techniques developed in this study offer significant advantages. These include rapid acquisition of the results and a reduced environmental impact as they eliminate the need for mobile phases during analysis. Furthermore, the robustness of the SERS methods demonstrated in this work is sufficient to support its implementation as a reliable analytical tool for the quality control of e-liquid

boosters.

3.5. Real e-liquid booster analysis in routine conditions

To demonstrate the robustness of the developed methods, the analysis of a real e-liquid booster sample was analysed using a new batch of AuNps prepared by a different operator. Calibration standards were prepared as described in Table 1 at a concentration of 100, 150 and 300 µg mL^{-1} with nicotine-d4 added at a concentration of 150 µg mL^{-1}. The SERS samples were prepared following the steps in the Section 2.4 and were analysed using both Raman spectrometers under the same parameters as those employed during the validation process. The mean SERS spectra obtained from the calibration standards and the real sample are illustrated in the Fig. S2 and S3 in the Supplementary data. These SERS spectra confirm that the dilution step effectively resolved issues related to matrix interferences. The nicotine concentration in the e-liquid booster was determined from the calibration data. The real sample was analysed in independent triplicates and the results are presented in Table 3. The nicotine concentration in the e-liquid booster was found to be within ± 5 % of the declared concentration (20 mg mL^{-1}) demonstrating the practical applicability of the SETRS/SERS methods.

Table 3
Nicotine concentration determination in a real sample of e-liquid booster (20 mg mL^{-1}) using the SETRS/SERS methods.

	Concentration of nicotine using SETRS method (mg mL^{-1})	Concentration of nicotine using SERS method (mg mL^{-1})
e-liquid booster sample (n = 3)	19.48 ± 0.08	19.77 ± 0.29

4. Conclusion and perspectives

The initial question posed was "Is SERS a good alternative to separation techniques for the nicotine dosage in e-liquid boosters? Based on the results of this study, the answer appears to be yes. While it is true that the analytical performances of separation techniques remain superior, SERS can offer significant advantages including rapid result acquisition and a reduced environmental impact by eliminating the need for mobile phases. Moreover, the robustness of the SETRS/SERS methods demonstrated in this study supports their implementation in the context of quality control of e-liquid boosters. These quantitative methods were validated in compliance with ICH Q2 (R2) guidelines for nicotine concentrations working range from 100 to 300 µg mL^{-1}. The analytical performances of both methods were promising as the validation process accounted for several sources of variability including two equipments with distinct acquisition modes, operator differences, AuNps batch variations and day-to-day effect. These good performances can be attributed to the addition of a IEIS in the sample preparation which effectively mitigates the variability stemming from SERS substrate batch-to-batch effect, aggregation states and equipment fluctuations.

It is relevant to note that the developed methods allow for two analytical scenarios. The SETRS setup equipped with a XY autosampler is ideal for routine benchtop analysis providing reliable results in a laboratory setting. Meanwhile a handheld device broadens the method's applicability to in field-analysis which could be particularly beneficial for customs inspection and other on-site analyses.

Regarding the perspective, it would be interesting to conduct an in-depth study of the transmission mode system to further enhance the analytical performances of the SETRS method. Factors such as the influence of geometry and position of the vial, laser beam size and the configuration of collection lens optics were not examined in this study and could provide valuable insights for optimizing transmission-based SERS measurements.

CRediT authorship contribution statement

Kenza Lahrichi: Formal analysis. **Julie Horne:** Writing – review & editing. **Kevser Kemik:** Formal analysis. **Pierre Beckers:** Writing – original draft, Methodology, Formal analysis. **Charlotte De Bleye:** Writing – original draft, Project administration, Methodology, Conceptualization. **Eric Ziemons:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Philippe Hubert:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Pierre-Yves Sacré:** Validation, Software, Methodology.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Kevser Kemik reports financial support was provided by Fund for Scientific Research. She is a FRIA grantee. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jpbao.2025.100054.

Data availability

Data will be made available on request.

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