Comparison of Methods for the Extraction of Nicotine from an E-Cigarette Liquid Product Using Attenuated Total Reflectance Fourier-Transform Infrared Spectroscopy

Iffa Zaahiera Mohd Hizam Fakhruddin[1], Yong Gong Yu[1,2] and Muhammad Jefri Mohd Yusof[1,3]*

[1]Department of Diagnostic and Allied Health Science, Faculty Health Life and Sciences, Management and Science University, 40100 Shah Alam, Selangor, Malaysia

[2]School of Graduate Studies, Postgraduate Centre, Management and Science University, 40100 Shah Alam, Selangor, Malaysia

[3]Forensic Science Programme, Centre of Diagnostic, Therapeutics & Investigation (CODTIS), Faculty of Health Sciences, Basement 1, Perpustakaan Tun Seri Lanang, Universiti Kebangsaan Malaysia, Bangi 43600, Malaysia

*Corresponding author (e-mail: jefri@ukm.edu.my)

The increasing use of electronic cigarettes has intensified the need for reliable quantification of nicotine in e-liquids, particularly due to concerns regarding product mislabelling and the associated health risks. This study compares three nicotine extraction techniques, namely salting out, acid protonation and basic digestion, prior to quantitative analysis using Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR). A calibration curve constructed from serially diluted nicotine standards in the range of 0.5 to 36 mg/mL produced a strong linear correlation with an R^2 value of 0.985, confirming its suitability for quantification. A commercial e-liquid was subjected to each extraction method and subsequently analysed using ATR-FTIR. Only the acid protonation method enabled successful quantification, yielding a nicotine concentration of 15.953 mg/mL with a recovery of 88.63 %, while the salting out and basic digestion methods produced no measurable amounts. These findings demonstrate that ATR-FTIR, when combined with an appropriate extraction strategy, provides a rapid and practical approach for the quantification of nicotine in e-liquids, with potential applications in regulatory monitoring and forensic assessment.

Keywords: Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR), nicotine quantification, smoking, forensic chemistry, electronic cigarettes (vape)

Received: August 2025; Accepted: December 2025

E-liquids generally contain propylene glycol (PG), vegetable glycerine (VG), and nicotine, while flavourings, sweeteners, and colourants are added to enhance the vaping experience [1]. Nicotine in e-liquids exists in two primary forms: free-base nicotine and protonated, or salt-based, nicotine. Free-base nicotine produces a stronger throat sensation, whereas nicotine salts are created by combining nicotine with acids to reduce harshness and improve absorption efficiency in the lungs [2]. This smoother mode of delivery often encourages greater inhalation volumes, which raises concerns about increased addiction risk, particularly among non-smokers and young people. These concerns are further intensified by the mislabelling of nicotine concentrations in commercial products. Several studies have reported detectable amounts of nicotine in e-liquids marketed as nicotine-free, highlighting significant ethical, legal, and public health issues [3].

Several analytical techniques have been used to detect nicotine, including high-performance liquid chromatography (HPLC), ultraviolet-visible (UV-Vis) spectroscopy, gas chromatography and electrochemical sensors [4,5]. However, these methods often require extensive sample preparation and sophisticated instruments, and in some cases involve the use of hazardous solvents [6]. Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy provides a rapid, non-destructive, and reagent-free alternative that is suitable for both qualitative and quantitative analysis [7]. The discrimination between nicotine-containing and non-nicotine-containing e-liquids has previously been demonstrated using mid infrared and near infrared spectroscopy combined with chemometric analysis, where trained models were able to distinguish samples without any form of pretreatment [8]. In a separate study, FTIR spectroscopy was used to quantify nicotine content in e-liquids by comparing sample spectra with those of standard nicotine solutions to obtain correlation coefficients. The nicotine levels determined by FTIR were then verified through GCMS analysis to assess the accuracy of the FTIR technique. Although a strong correlation was reported, the authors concluded that FTIR was unsuitable as a standalone method for nicotine quantification [9].

A major limitation of the previous work was the absence of a selective extraction step. The e-liquids were analysed directly, resulting in substantial matrix interference from PG, VG, and other additives, which obscured the nicotine signal in the spectra. This highlights a clear research gap, namely the need for selective extraction procedures that remove interfering components prior to spectroscopic analysis. The present study addresses this gap by evaluating several nicotine extraction approaches designed to minimise matrix effects before FTIR measurement.

To date, several extraction approaches for quantifying nicotine in e-liquids have been reported. For example, nicotine content has been measured using Fourier Transform Ion Cyclotron Mass Spectrometry (FT-ICR-MS) following a series of acidic sample preparation steps. The results indicated that nicotine concentrations deviated from manufacturers' claims by values ranging from -2.94 % to +25.20 % [10]. Salting-out assisted liquid-liquid extraction using sodium hydroxide and acetonitrile in combination with Ultra Performance Liquid Chromatography coupled with Tandem Mass Spectrometry (UPLC-MS/MS) has also been applied, and this method produced acceptable recovery values [11]. In addition, basic digestion has been used to extract nicotine from hair samples, allowing successful detection using ATR-FTIR spectroscopy [12]. Methods involving liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) and gas chromatography coupled with mass spectrometry (GC-MS) have also been developed to quantify various forms of nicotine salts in e-liquids, yielding highly satisfactory recoveries [13]. A rapid GC-MS method has likewise been introduced to determine the levels of PG, VG, and nicotine in e-liquids, incorporating derivatisation during sample preparation. However, this technique produced a wide range of standard errors, from -41.70 % to +27.80 % when compared with the declared values [14].

The main objective of this study is to develop selective nicotine extraction methods, namely salting-out, acid protonation and basic digestion, and to evaluate their compatibility with ATR-FTIR detection. ATR-FTIR offers several advantages over other analytical techniques, including high throughput and the ability to generate results within a short period of time. A calibration curve for nicotine standards was constructed, and the extraction efficiencies of each method were assessed to identify the most effective approach for reliable nicotine quantification in selected e-liquids. The findings of this study are expected to support more accurate forensic preliminary screening and to strengthen regulatory oversight within the vaping industry.

EXPERIMENTAL

Chemicals and Materials

A single Malaysian e-liquid product (brand *Meledak*, flavour *Japanese Fluffy Cake*) with a declared nicotine concentration of 18 mg/mL was used. The formulation consisted of PG (40 %), VG (60 %), and flavourings. The sample was stored in its original sealed container and refrigerated at 4-8 °C prior to analysis.

Characterization Methods

Nicotine Standard Preparation

A standard nicotine solution (CAS No. 54-11-5, 25 mL, 1000 mg/mL) was prepared by serial dilution of the stock solution using ethanol as the diluent. Eight standard solutions containing 36.00, 24.00, 18.00, 12.00, 6.00, 3.00, 1.50 and 0.50 mg/mL of nicotine were produced to construct a calibration curve for ATR-FTIR analysis.

Salting-Out Technique

Adapted from Aldeek et al. [11], nicotine extraction was performed using a salting-out liquid-liquid extraction technique. In brief, 500 µl of e-liquid was transferred into a labelled 50 mL centrifuge tube and fortified with 100 µl of a nicotine standard solution at 18 mg/ml prepared in ethanol. The pH of the aqueous phase was adjusted to a value of 10 or greater using 15 mL of 2 M NaOH, which converted nicotine salts into free-base nicotine to facilitate extraction into the organic phase. The mixture was vortexed vigorously for ten minutes, after which 10.00 ml of acetonitrile was added as the extraction solvent together with 1.00 g of NaCl to promote phase separation. The tube was securely capped and vortexed for a further period of 10 - 15 min, and then centrifuged at 4000 rpm for 10 min. The lower organic layer, containing nicotine dissolved in acetonitrile, was carefully withdrawn using a pipette while avoiding contamination from the aqueous phase. The extracts were analysed directly using ATR-FTIR spectroscopy.

Acid Protonation

Following the method described by Ogunwale et al. [10], 20 µL of e-liquid was added to a solution containing 37 % HCl (10.0 µL, 49.2 µmol) in 915.0 µL methanol at room temperature. The mixture was vortexed for at least 30 min to ensure protonation of nicotine. Then, 20 µL of the reaction mixture was withdrawn and analysed directly by FTIR spectroscopy in triplicate.

Basic Digestion

Adapted from Marcus et al. [12], 2.0 mL of e-liquid was transferred into a dry 50 mL centrifuge tube, after which 10 mL of 2 M NaOH was immediately added. The mixture was vortexed for 30 min to initiate alkaline digestion and convert nicotine salt into free-base nicotine. After mixing, 8 mL of deionised water was added to bring the total volume to approximately

20 mL, followed by vortexing for 1 min. The solution was then transferred to a separatory funnel and extracted with 20 mL dichloromethane (DCM) in a 1:1 ratio. The funnel was shaken vigorously for 2 min with intermittent venting, and then allowed to separate for 5 min. The upper organic layer was collected and dried over 1 g of anhydrous sodium sulphate. The extract was evaporated at 30 to 40 °C for 30 min until approximately 1.0 mL remained, after which it was subjected to ATR-FTIR analysis.

Data Analysis

Nicotine was quantified using a calibration curve generated using the standard solutions. Spectral data were processed with LabSolutions IR software (version 2.31, Shimadzu, Japan). A linear regression model was applied to correlate absorbance intensity with the known concentrations, and an R^2 value greater than 0.9 was considered acceptable for quantitative analysis. All samples were analysed in triplicate, and the mean values obtained were used for subsequent evaluation.

RESULTS AND DISCUSSION

FTIR Spectrum of Nicotine

The FTIR spectrum of pure nicotine was obtained using an ATR-FTIR spectrometer and served as the reference for identifying nicotine-specific absorption peaks in both the standard solutions and the e-liquid extracts. Nicotine contains several functional groups, including an aromatic ring, C–H stretching vibrations and a tertiary amine, each producing distinctive absorption bands. As shown in Figure 1, the ATR-FTIR spectrum was annotated with alphabetical markers (a to h) to highlight the wavenumber regions associated with these characteristic absorptions. These annotated regions were subsequently used for quantitative analysis by evaluating either peak height or peak area in order to construct the calibration curves.

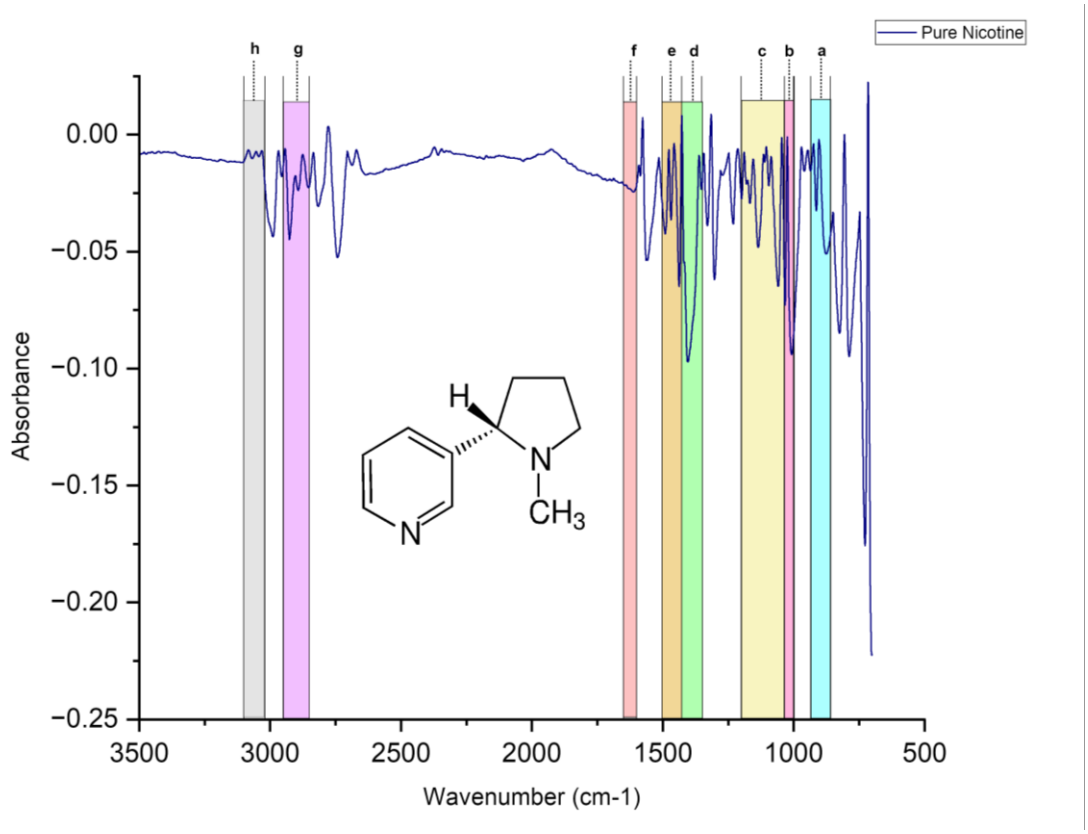

Figure 1. FTIR spectrum of pure nicotine. **a**. 860–934 cm^{-1} (C–H out-of-plane bending), **b**. 998–1034 cm^{-1} (C–N stretching vibration) **c.** 1000–1200 cm^{-1} (C–C and C–N stretching) **d**. 1350–1450 cm^{-1} C–H bending (CH_2/CH_3 groups), **e**. 1403–1503 cm^{-1} (CH_2 scissoring and aromatic C=C stretching)**, f**. 1600–1650 cm^{-1} (aromatic C=C stretching)**, g**. 2850–2950 cm^{-1} (C–H stretching)**, h**. 3020–3100 cm^{-1} (=C–H stretching).

Table 1. FTIR calibration parameters for nicotine quantification using peak heights.

Peak No.	Wavenumber Range (cm^{-1})	Regression Equation	R^2 Value
1	860 – 934	Height = 0.001925.Conc + 0.1762	0.940
2	998 – 1034	Height = 0.003587.Conc + 0.1939	0.985
3	1000 – 1200	Height = 0.005025.Conc + 0.4045	0.958
4	1350 – 1450	Height = 0.001740.Conc + 0.07371	0.941
5	1403 – 1503	Height = 0.002254.Conc + 0.05221	0.950
6	1600 – 1650	Height = 0.001954.Conc + 0.01041	0.883
7	2850 – 2950	Height = 0.08408.Conc + 0.06400	0.666
8	3020 – 3100	Height = 0.003693.Conc + 0.02664	0.549

Calibration Curve

For quantifying nicotine concentration using ATR-FTIR, a calibration curve was constructed based on peak height measurements, following the approach described by Fekhar et al. [15]. Eight wavenumber regions were evaluated, and the band at 998 to 1034 cm^{-1} was identified as the most suitable for calibration, yielding the highest R^2 value of 0.985. This indicated a strong linear relationship between nicotine concentration and absorbance height (Table 1). The corresponding regression equation was:

$$Height = 0.003587.\text{Conc} + 0.1939 \quad (1)$$

Method Validation: Accuracy, Repeatability and Extraction Precision

Method validation was carried out to assess the accuracy and precision of the ATR-FTIR calibration model and the extraction procedure. Accuracy was evaluated using a spiked standard solution with a known nicotine concentration of 9.00 mg/mL, which yielded a value of 8.95 mg/mL. This corresponded to a recovery of 99.44 %, falling within the accepted analytical range of 95-105 %. Repeatability was assessed through triplicate measurements of the same standard, resulting in minimal variation and an RSD of 0.11 %, indicating excellent precision. Validation of the extraction process using an e-liquid sample with a declared concentration of 18 mg/mL produced a mean detected concentration of 15.62 mg/mL, corresponding to a recovery of 86.76 % and an RSD of 0.19 %. Table 2 summarises these validation results, including the accuracy of the calibration curve, repeatability of the peak measurements, and precision of the extraction procedure. These findings demonstrate that both the calibration model and the extraction method provided reliable quantitative results suitable for determining nicotine content in e-liquids.

Quantification of Nicotine Content

After establishing the calibration curve, nicotine concentrations obtained from the three extraction methods were calculated. Table 3 presents the final nicotine concentrations (mg/mL) and corresponding percentage recoveries for each method, calculated using LabSolutions IR software.

Table 2. Validation results for the ATR-FTIR calibration and extraction method.

Parameter	Expected Value	Detected Value	% Recovery	% Error	RSD (%)	Interpretation
Accuracy (spiked standard)	9.00 mg/mL	8.95 mg/mL	99.44	0.56	–	Within 95–105% acceptance
Repeatability (standard, n=3)	–	8.94–8.96 mg/mL	–	–	0.11	Excellent precision (RSD < 2 %)
Extraction accuracy (e-liquid, n=3)	18.00 mg/mL	15.59–15.65 mg/mL	86.76 (mean)	–	0.19	Acceptable for complex matrices
Extraction precision (n=3)	–	15.59–15.65 mg/mL	–	–	0.19	Good precision (RSD < 5 %)

Table 3. Final nicotine concentrations (mg/mL) in e-liquid samples extracted by salting-out, acid protonation, and basic digestion methods.

Method	Detected Concentration (mg/mL)	SD (mg/mL)	n	Expected Concentration (mg/mL)	Yield (%)
Salting-out	N/A	–	3	18.000	N/A
Acid protonation	15.953	0.030	3	18.000	88.63
Basic digestion	N/A	–	3	18.000	N/A

Among the three approaches, only the acid protonation method yielded a quantifiable nicotine concentration, with a detected value of 15.953 mg/mL. This corresponded to a recovery of 88.63 % relative to the reference concentration of 18 mg/mL, with a deviation of 11.37 %. The success of this method can be attributed to the action of HCl, which protonates nicotine and converts it from its free-base form into a more stable protonated form ($NicH^+$). This result is in agreement with previous findings in which acidic digestion enabled quantification of nicotine using FT-ICR-MS, although substantial variation was reported, with deviations of -2.94 % to +25.20 % from labelled values [10]. The present results therefore demonstrate the feasibility of ATR-FTIR for nicotine quantification when appropriate sample preparation procedures are applied. This aligns with earlier reports showing that FTIR performed without prior extraction was insufficient for quantitative purposes and should be limited to qualitative identification only [8,9].

In contrast, both the salting-out and basic digestion methods produced nicotine concentrations below the detection limit, recorded as N/A in the "Detected Concentration" and "Yield" columns. The poor performance of the salting-out method may be attributed to incomplete or unstable phase separation, which may arise from inadequate salt concentration, suboptimal salt choice, or incompatibility between nicotine and the extraction solvent. The complex matrix of e-liquids, consisting of PG, VG, and flavouring agents, may further contribute to matrix suppression, where co-extracted compounds interfere with nicotine's spectral signals or hinder its transfer into the organic phase [2].

Similarly, the failure of the basic digestion method reflects inherent limitations in its design. Free-base nicotine is highly volatile, making it unsuitable for extraction procedures that involve heating or solvent evaporation, as losses are likely to occur. Furthermore, strong alkaline conditions may reduce nicotine stability, leading to lower recovery. Additional experimental repetitions were conducted to evaluate the reproducibility of both methods, and the outcomes consistently showed that nicotine could not be quantified. Potential improvements may include the use of sealed low-temperature systems to minimise volatilisation losses, shortening digestion times, or incorporating a neutralisation step before drying [16]. However, our results show that both the salting-out and basic digestion methods were unsuitable for nicotine quantification using ATR-FTIR.

The current study examined only a single e-liquid product, and the form of nicotine present in the sample was not known. Future work should involve analysing a broader range of e-liquids to investigate how different nicotine forms influence extraction efficiency and recovery under various experimental conditions. Determination of nicotine type may be conducted through label inspection, pH measurement, liquid–liquid extraction behaviour or Nuclear Magnetic Resonance (NMR) spectroscopy [2,17]. Understanding whether nicotine exists in free-base or protonated form is crucial, as free-base nicotine is lipophilic and readily partitions into organic solvents, whereas protonated nicotine is hydrophilic and preferentially dissolves in aqueous or acidic media [14]. This knowledge would enable more informed selection of extraction strategies for accurate ATR-FTIR quantification.

CONCLUSION

This study evaluated three extraction approaches for the quantification of nicotine in an e-liquid using ATR-FTIR spectroscopy. Only the acid protonation method produced clear and quantifiable nicotine signals, allowing reliable determination of nicotine concentration from the calibration model. The salting out and basic digestion methods did not yield detectable nicotine levels and were therefore unsuitable under the conditions applied. These findings demonstrate that ATR-FTIR, when supported by a compatible extraction strategy, provides a rapid and practical approach for quantitative analysis of nicotine in e-liquids. This work highlights the importance of selecting an appropriate extraction method to ensure accurate spectroscopic measurement and offers a foundation for further development in regulatory and forensic settings.

ACKNOWLEDGEMENTS

We would like to express our gratitude to the Management and Science University for providing financial support through the MSU Seed Grant, project code SG-006-022022-FHLS.

REFERENCES

1. Etter, J. F., Zäther, E. & Svensson, S. (2013) Analysis of refill liquids for electronic cigarettes. *Addiction*, **108(9)**, 1671–1679. https://doi.org/10.1111/add.12235.

2. Gholap, V. V., Kosmider, L., Golshahi, L. and Halquist, M. S. (2020) Nicotine forms: Why and how do they matter in nicotine delivery from electronic cigarettes? *Expert Opinion on Drug Delivery*, **17(12)**, 1–10. https://doi.org/10.1080/17425247.2020.1814736.

3. Bennani, I., Alami Chentoufi, M., El Karbane, M., Cheikh, A. and Bouatia, M. (2020) E-cigarette quality control: Impurity and nicotine level analysis in electronic cigarette refill liquids. *The Scientific World Journal*, **2020**, 1–7. https://doi.org/10.1155/2020/3050189.

4. Jerome, R. and Sundramoorthy, A. K. (2020) Preparation of hexagonal boron nitride doped graphene film modified sensor for selective electrochemical detection of nicotine in tobacco sample. *Analytica Chimica Acta*, **1132**, 110–120. https://doi.org/10.1016/j.aca.2020.07.060.

5. Chien, J. -Y., Gu, Y. -C., Tsai, H. -M., Liu, C. -H., Yen, C. -Y., Wang, Y. -L., Wang, J. -K. and Lin, C. -H. (2020) Rapid identification of nicotine in electronic cigarette liquids based on surface-enhanced Raman scattering. *Journal of Food and Drug Analysis*, **28(2)**, 302–308. https://doi.org/10.38212/2224-6614.1064.

6. Yong, G. Y. and Yusof, M. J. M. (2025) Detection of nicotine and cotinine in keratinized samples: A review. *Malaysian Journal of Analytical Sciences*, **29(2)**, 1349.

7. Liu, G. -L. and Kazarian, S. (2022) Recent advances in studies of cultural heritage using ATR-FTIR spectroscopy and ATR-FTIR spectroscopic imaging. *The Analyst*, **147**, 1230–1245. https://doi.org/10.1039/d2an00005a.

8. Deconinck, E., Bothy, J. L., Barhdadi, S. & Courselle, P. (2016) Discriminating nicotine and non-nicotine containing e-liquids using infrared spectroscopy. *Journal of Pharmaceutical and Biomedical Analysis*, **120**, 333–341. https://doi.org/10.1016/j.jpba.2015.12.054.

9. Hamzah, N. H., Mohd Aris, F. N., Mukhni, N. H., Abdul Manap, M. R. The detection of nicotine in e-liquids using rapid Fourier-transform infrared (FTIR) spectroscopy spectral comparison method. http://dx.doi.org/10.2139/ssrn.5554305.

10. Ogunwale, M. A., Chen, Y., Theis, W. S., Nantz, M. H., Conklin, D. J. and Fu, X. -A. (2017) A novel method of nicotine quantification in electronic cigarette liquids and aerosols. *Analytical Methods*, **9(29)**, 4261–4266. https://doi.org/10.1039/c7ay00501f.

11. Aldeek, F., Lopez, V. and Miller, J. H. (2023) Salting-out-assisted liquid-liquid extraction method for the determination of nicotine from oral traditional and innovative tobacco products using UPLC-MS/MS. *ACS Omega*, **8(34)**, 31256–31264. https://doi.org/10.1021/acsomega.3c03474.

12. Marcus, N. X. Y., Yong, G. Y., Nashvinder, K. J. S. and Yusof, M. J. M. (2024) Attenuated total reflectance Fourier transform infrared spectroscopy analysis of cotinine as biomarker in hair of e-cigarette smokers. *Journal of Management and Science*, **22(2)**, 41–48.

13. Han, S., Chen, H., Su, Y., Cui, L., Feng, P., Fu, Y., Tian, Y., Liu, T., Hou, H. & Hu, Q. (2022) Simultaneous quantification of nicotine salts in e-liquids by LC-MS/MS and GC-MS. *Analytical Methods: Advancing Methods and Applications*, **14(42)**, 4185–4192. https://doi.org/10.1039/d2ay00799a.

14. Dagla, I., Gikas, E. & Tsarbopoulos, A. (2023) Two Fast GC-MS Methods for the Measurement of Nicotine, Propylene Glycol, Vegetable Glycol, Ethylmaltol, Diacetyl, and Acetylpropionyl in Refill Liquids for E-Cigarettes. *Molecules*, **28(4)**, 1902. https://doi.org/10.3390/molecules28041902.

15. Fekhar, M., Daghbouche, Y., Bouzidi, N. and El Hattab, M. (2023) ATR-FTIR spectroscopy combined with chemometrics for quantification of total nicotine in Algerian smokeless tobacco products. *Microchemical Journal*, **193**, 109127. https://doi.org/10.1016/j.microc.2023.109127.

16. Kheawfu, K., Kaewpinta, A., Chanmahasathien, W., Rachtanapun, P. and Jantrawut, P. (2021) Extraction of nicotine from tobacco leaves and development of fast dissolving nicotine extract film. *Membranes*, **11(6)**, 403. https://doi.org/10.3390/membranes11060403.

17. El-Hellani, A., El-Hage, R., Baalbaki, R., Salman, R., Talih, S., Shihadeh, A. & Saliba, N. A. (2015). Free-Base and Protonated Nicotine in Electronic Cigarette Liquids and Aerosols. *Chemical Research in Toxicology*, **28(8)**, 1532–1537. https://doi.org/10.1021/acs.chemrestox.5b00107.