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PRECONCENTRATION AND DETERMINATION OF NICOTINE IN TOBACCO SAMPLES BY DISPERSIVE MICRO-SOLID PHASE EXTRACTION AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY

(Pemekatan dan Penentuan Nikotin dalam Sampel Tembakau oleh Pengekstrakan Fasa Pepejal-Mikro Dispersif dan Gas Kromatografi-Spektrometri Jisim)

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Abstract

In this study, the dispersive micro-solid phase extraction (D-µ-SPE) method was optimised and validated for preconcentration and determination of nicotine in tobacco samples, with the use of C_{18} as sorbent, coupled with gas chromatography-mass spectrometry (GC-MS). The optimisation of three extraction parameters, namely extraction time (min), mass of sorbent (mg), and desorption volume (mL), were investigated to provide better insight into its reliability as a low-consumption sorbent and rapid operation. The obtained parameters suitable for nicotine extraction were 10 min of extraction time, 50 mg of C_{18} sorbent, and 1.0 mL of desorption solvent. Nicotine was successfully extracted from tobacco samples with good linearity in the range of 0.1 – 500 mg/L, with a coefficient of determination (R^2) of 0.9997. The calculated LOD resulted in a value of 0.0983 mg/L, with the calculated LOQ being 0.2978 mg/L. The precision of the method revealed a percent relative standard deviation (% RSD) of 2.6%. In addition, good relative recovery (%) of nicotine in real samples was also achieved in the 75.5 – 96.4% range, with % RSD ranging from 3.1 - 5.7%. Hence, this study proposed an improvement to the green method towards nicotine analysis with low consumption of organic solvent, good sorbent absorptivity, and rapid and high selectivity for separation.

Keywords: Dispersive micro-solid-phase extraction, nicotine, tobacco samples, green method, C_{18} sorbent

Abstrak

Dalam kajian ini, kaedah pengekstrakan fasa pepejal-mikro dispersif (D-µ-SPE) telah dioptimumkan dan disahkan untuk pemekatan dan penentuan nikotin dalam sampel tembakau, dengan menggunakan C_{18} sebagai penjerap serta dicantumkan dengan kromatografi gas-spektrometri jisim (GC-MS). Pengoptimuman tiga parameter pengekstrakan, iaitu masa pengekstrakan (min), jisim penjerap (mg), dan isipadu penyaherapan (mL) turut dijalankan untuk memberikan gambaran yang lebih baik tentang kebolehpercayaannya sebagai penjerap yang dapat digunakan dalam kuantiti yang rendah serta operasi pengekstrakan yang pantas. Parameter yang diperoleh yang sesuai untuk pengekstrakan nikotin daripada sampel tembakau ialah 10 min masa pengekstrakan, dengan penggunaan sebanyak 50 mg penjerap C_{18}, dan isipadu penyaherapan sebanyak 1.0 mL. Nikotin berjaya diekstrak daripada sampel tembakau dengan kelinearan yang baik dalam julat 0.1 – 500 mg/L, dengan pekali penentuan (R^2) sebanyak 0.9997. Had pengesanan (LOD) yang dikira memberikan nilai 0.0983 mg/L, dengan had kuantitatif (LOQ) sebanyak 0.2978 mg/L. Ketepatan kaedah tersebut menghasilkan peratus sisihan piawai relatif (%RSD) sebanyak 2.6%. Di samping itu, pemulihan relatif (%) yang baik terhadap nikotin dalam sampel sebenar juga dicapai dalam julat 75.5 – 96.4%, dengan % RSD berjumlah dalam julat 3.1 - 5.7%. Oleh itu, kajian ini dapat mencadangkan penambahbaikan kepada kaedah hijau untuk analisis nikotin dengan penggunaan pelarut organik yang rendah, dapat memberikan penyerapan penjerap yang baik, kaedah yang pantas, dan kebolehasingan yang tinggi.

Kata kunci: Pengekstrakan fasa pepejal-mikro dispersif, nikotin, sampel tembakau, kaedah hijau, penjerap C_{18}

Introduction

Nicotine is a type of alkaloid mainly found naturally in plants from the Solanaceous family, including tobacco, tomato, potato, and green pepper [1]. Chemically known as 3-(1-methyl-2-pyrrolidinyl) pyridine, its content can reach 30-60 mg/kg, making it an immensely toxic alkaloid [2]. The human body absorbs approximately 1 mg of nicotine from smoking a cigarette, which typically contains between 8 and 20 mg [3].

The use of nicotine in tobacco products is undoubtedly a worldwide concern as it is known to cause various adverse health effects to consumers and people inhaling nicotine from second-hand smoke. It is highly addictive to its consumers, has severe systemic effects, and can also harm kidneys, lungs, reproductive systems, heart, and other vital organs [4]. Nicotine consumption also has the potential to be cancerous. It can impair the genome, interfere with cellular metabolic processes, and elevate the growth and spreading of altered cells, allowing nicotine to possess mutagenic and stimulate tumor-related activities in the body [5]. Nicotine, when acting on nicotinic cholinergic receptors, can endanger the body's systems, leading to its highly addictive nature [6]. Therefore, determining the content of nicotine in tobacco products is crucial as different brands of tobacco products, such as cigarettes, may vary in the amount of nicotine present in them [3]. Figure 1 shows the chemical structure of nicotine.

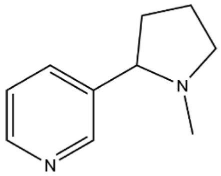


Figure 1. Chemical structure of nicotine

Sample preparation is a critical step in sample analysis. Previous studies have recorded solvent extraction as an extraction method for various samples, including nicotine in tobacco samples. A recent study utilized a solvent extraction method for the extraction of nicotine with ether, kerosene, and sulfuric acid as the extraction solvents [7]. Solvent extraction, or its other name which is liquid-liquid extraction (LLE), is an extraction technique that involves separation of compounds from two unidentical, immiscible liquids, about the liquids' respective relative solubility, typically with an organic solvent and water [8]. This extraction technique requires an extraction solvent, which is immiscible in the aqueous mixtures, to allow efficient separation of two differing liquids. In addition, the targeted analytes must be compatibly soluble and display a high partition coefficient in the extraction solvent [9]. This extraction technique involves shaking and inverting the mixture of two differing, immiscible liquids regularly for a few minutes to ensure the two liquid phases are well-mixed [9]. Expelling the solvent from the liquid phases can be done with a handheld pipette for less dense solvents than water, while denser solvents can be withdrawn from the bottom of a separatory funnel [9]. However, using organic solvents in this method may be toxic to the environment, time-consuming, and unable to separate many compounds with different polarity [8].

In recent years, dispersive micro-solid phase extraction (D-µ-SPE) has increased in recent years. D-µ-SPE is a method of extraction displayed in a miniaturized version of dispersive solid phase extraction (dSPE), extracting targeted analytes from samples rather than clean-up [11]. Sonication, stirring, vortex, or shaking are used to assist sorbent dispersion in the sample solution [11]. This is then followed by centrifugation, filtration, or applying an external magnetic field to separate the solid sorbent [10]. The last step includes analysis of the analytes obtained in small volumes after being isolated from the sorbent [11]. D-µ-SPE absorbs fewer solvents, has a more straightforward procedure, and requires less time for operation in addition to minimizing solvent wastage, as opposed to the traditional solid phase extraction (SPE) method, which has some drawbacks involving solvent wastage, significant generation of secondary waste, apart from being lengthy and involving tedious procedures [10]. C_{18} silica, a micromaterial-type sorbent, is used for clean-up, in addition to primary-secondary amine (PSA), for the isolation of interference matrix from liquid extracts for the analysis of pesticides, specifically in vegetables [12]. As for the use of C_{18} in nicotine extraction, it has been reported in a study [13] in which nicotine was extracted from the hair of smokers and non-smokers with the use of SB-C_{18} column for SPE, assisted by molecularly imprinted polymer (MIP) as sorbent. Additionally, a study has also applied C_{18} as a sorbent through matrix solid-phase dispersion extraction method for the analysis of polyphenols in tobacco [14]. However, when it comes to the separation and preconcentration of nicotine from tobacco plants or products, there are still limited studies on the D-µ-SPE method and the use of C_{18} as a sorbent. The employment of D-µ-SPE for preconcentration of nicotine has been reported by a study [2] that applied graphene oxide as sorbent for separation and preconcentration of nicotine from environmental and biological water samples, which was then analyzed by gas chromatography with flame ionization detection (GC-FID). Other reported works did apply the D-µ-SPE method with C_{18} as a sorbent, even though it does not involve preconcentration and extraction of nicotine, such as the extraction and determination of ofloxacin and sparfloxacin from human plasma [15] and for determination of polycyclic aromatic hydrocarbon (PAHs) in water samples [16]. Considering the limitations of some sample preparation or extraction methods mentioned earlier, as well as the limited research on the efficiency of D-µ-SPE on the separation and preconcentration of nicotine in tobacco samples, it calls for an improved and alternative method to overcome these limitations.

The present study proposes an efficient technique using D-µ-SPE with C_{18} as the sorbent for preconcentration and determination of nicotine in tobacco samples, coupled with gas chromatography-mass spectrometry (GC-MS). C_{18} sorbent, commonly used in SPE [12], was studied for its potential as a quick and simple method for preconcentration and determination of nicotine in tobacco samples, utilizing D-µ-SPE. The method offers the possibility of an efficient clean-up procedure, utilizing C_{18} sorbent for a fast, easy, and cost-effective approach to preconcentration and determination of nicotine.

Materials and Methods

Chemicals and materials

Nicotine (≥ 99%), methanol (MeOH) of HPLC grade and DSC-18 (C_{18} sorbent, 50 µm particle size) were purchased from Sigma-Aldrich. Tobacco samples of different brands (labelled as ‘M’, ‘G’, and ‘D’) were obtained from the tobacco market available in Malaysia**.**

Preparation of standard and spiked solutions

1000 mg/L of nicotine stock solution was prepared in HPLC-grade methanol. The stock solution was stored in amber glass bottles in the refrigerator at 4 °C when not in use. A series of working standard solutions were prepared in methanol by dilution before analysis to prevent the decomposition of analytes. All spiked solutions were diluted with methanol and stored at 4 °C until further use.

Sample preparation and pre-treatment

Sample preparation was based on the methods described in the literature with some modifications [15, 17]. 2 g of tobacco samples were weighed and placed into 50 mL polyethene centrifuge tubes. 15 mL of methanol was then added to each sample and sonicated for 15 min. The resulting supernatant of each sample was then filtered through a 20-25 µm filter paper.

Optimization of parameters using one-factor-at-a-time (OFAT)

The optimization parameters used to determine the efficiency of C_{18} sorbent for the extraction of nicotine using the D-µ-SPE method were extraction time (min), mass of sorbent (mg), and desorption volume (mL). The parameters were optimized using the One-factor-at-a-time (OFAT) approach. Each sample was spiked with 10 mg/L nicotine standard solution for the optimization study. The optimum results obtained from each parameter were used to implement subsequent parameters. The optimal conditions were then utilized for real sample analysis. For real sample analysis, the samples were spiked with 50 mg/L and 100 mg/L nicotine standard solutions. The concentration of nicotine in the filtrate and percent (%) relative recovery was calculated using the following equations:

$$\text{Concentration of nicotine} = \frac{\text{Peak area of spiked nicotine filtrate samples}}{\text{Retention factor}} \tag{1}$$

$$\%\ \text{Relative recovery} = \frac{\text{Amount recovered}}{\text{Actual amount of spiking}} \times 100 \tag{2}$$

Dispersive-micro solid phase extraction (D-µ-SPE) procedure

This part of the procedure was based on a method from the literature [15] with some modifications. 1 mL of 10 mg/L nicotine standard solution was spiked in a 50 mL centrifuge tube containing 4 mL of prepared tobacco sample filtrate and 50 mg of C_{18} sorbent. The samples were then sonicated for 10 min and centrifuged at 5000 rpm for 4 min. The sorbent was carefully collected and transferred into a microcentrifuge tube. To desorb the analytes, 1 mL of MeOH was added to the centrifuge tube containing the sorbent and vortexed for 5 min. Subsequently, to allow separation and settling of the sorbent, it was centrifuged again for another 5 min. The resulting supernatant was then filtered through a 0.22 µm nylon membrane filter before the analysis on GC-MS. Figure 2 shows the D-µ-SPE procedure by using C_{18} as a sorbent.

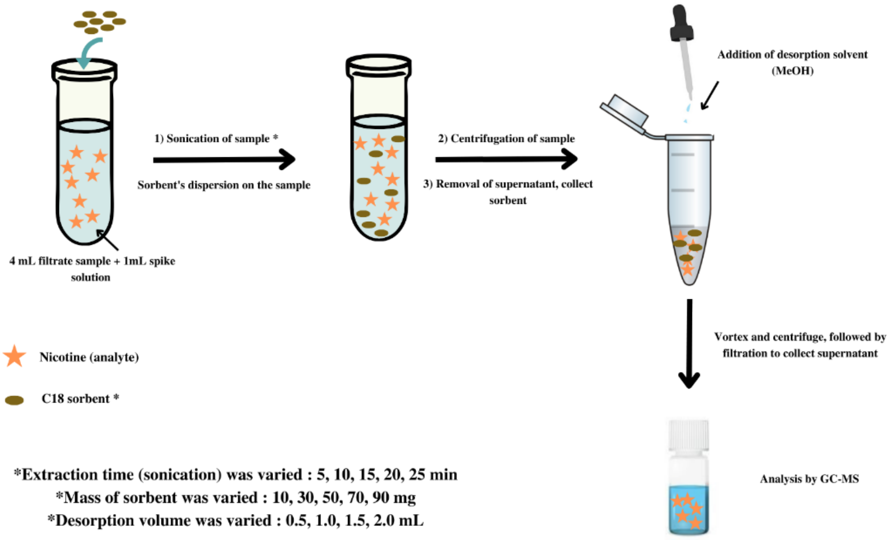


Figure 2. Dispersive micro-solid-phase extraction (D-µ-SPE) procedure by using C_{18} as sorbent

Chromatographic conditions

Chromatographic conditions were based on a method from the literature [18]. An Agilent 6890 gas chromatograph with 5973 inert mass spectrometers (USA) was used for analyzing nicotine in tobacco samples. The chromatographic separation was carried out on an InertCap 5MS/Sil column, with specifications of 0.25 mm I.D. x 30 m and df = 0.25 µm. Detection was done by MS scan (45 – 500 m/z) with an interface temperature of 280 °C. The carrier gas employed was helium, with a 36.5 cm/sec flow rate. An injection temperature of 250 °C was used. 1.0 µL of the sample was injected into the GC-MS using splitless mode for 1 min, with a vent flow of 50 mL/min. The analysis of the samples was carried out under the following column temperature program; the initial temperature was set to 60 °C and held for 1 min, then ramped up at 10 °C/min, followed by raising the temperature to 280 °C and held for 7 min.

Validation of the analytical method

For the D-µ-SPE method, the efficiency of the analytical performance was assessed through linearity, the limit of detection (LOD), the limit of quantification (LOQ), precision, and accuracy. To assess the linearity of the analytical method, an eight-point calibration curve for nicotine was plotted by varying the concentrations of the spiked standards to achieve 0.1, 0.5, 1, 10, 50, 100, 200, and 500 mg/L in the filtrate solution (methanol obtained from the filtration of cigarette samples). Limit of detection (LOD) and limit of quantification (LOQ) were established through linear regression analysis. The precision of the method was calculated in terms of repeatability by determining the relative standard deviation (RSD). The RSD was then presented and expressed as a percentage (%RSD). The accuracy of the method was expressed in terms of percent relative recovery. Real samples were spiked with 50 mg/L and 100 mg/L nicotine standards, and the accuracy of the method was determined through percent relative recovery.

Results and Discussion

Optimization of dispersive-micro solid phase extraction (D-µ-SPE)

Optimization of D-µ-SPE parameters for the extraction of nicotine from tobacco samples was performed by varying the parameters, including extraction time, mass of sorbent, and desorption volume. These parameters were optimized based on the one-factor-at-a-time method (OFAT), where the other parameters were kept constant during the optimization of one parameter. The extraction of the samples was done in triplicates for each parameter optimized. Samples were also spiked with 50 mg/L and 100 mg/L nicotine standard solutions for real sample analysis using the optimized conditions and parameters obtained from the OFAT method.

Effect of extraction time

The effect of extraction time is vital in determining the extraction efficiency of the sample to extract the analyte selectively. For this research, the extraction efficiency was carried out by allowing the C_{18} sorbent to come into contact with nicotine (analyte) in the tobacco samples. The interaction between the sorbent and analyte in the samples was individually exposed for 5 min, 10 min, 15 min, 20 min, and 25 min. After each subsequent analysis, a graph of peak area (m/z) versus extraction time (min) was plotted to determine the optimized condition for the extraction time of nicotine. The effect of the extraction time, or the duration when the sorbent is in contact with nicotine in the samples, is shown in Figure 3.

From the results obtained for the extraction time of nicotine in tobacco samples, it was observed that the extraction efficiency of nicotine increased substantially from 5 min to 10 min. The highest extraction efficiency was acquired at the 10th minute, as shown in Figure 3(a). From 10 to 20 min, there was a gradual drop in the peak area of the analyte. The peak area remained constant from 20 min to 25 min. This implied that there was no significant improvement in the extraction efficiency of nicotine from tobacco samples, even when the extraction time was lengthened. The decline in extraction performance or efficiency in relation to its extraction time can be attributed to the back extraction of the analyte [11]. Hence, 10 min extraction time was chosen as the optimum extraction time to be used for further optimization of another parameter.

Effect of mass of sorbent

In the D-µ-SPE approach, it is necessary to consider the mass of sorbent as a crucial parameter since the amount of sorbent can significantly affect the efficiency of analyte extraction from the sample matrix through the number of adsorption sites [19]. The optimization of the mass of C_{18} sorbent involved varying the amount to 10, 30, 50, 70, and 90 mg. After each subsequent analysis, a graph of peak area (m/z) versus mass of C_{18} sorbent (mg) was plotted to determine the optimized condition for the mass of C_{18} sorbent used to adsorb nicotine from tobacco samples efficiently. The interaction mechanism between C_{18} sorbent and nicotine involves hydrophobic interactions [13], π-π stacking, and Van der Waals forces. The hydrophobic alkyl chains of the C_{18} sorbent interact with the nonpolar regions of nicotine, while the aromatic rings in both the C_{18} sorbent and nicotine engage in π-π stacking interactions. Van der Waals forces further contribute to their attraction. The functional groups involved are the hydrophobic alkyl chains of C_{18}, the aromatic rings in both the sorbent and nicotine and the secondary amine group in nicotine. These interactions collectively facilitate nicotine's retention and separation on the C_{18} sorbent. Based on Figure 3(b), it can be observed that with an increasing mass of C_{18} sorbent up to 50 mg, the extraction efficiency of nicotine from tobacco samples increased significantly, with the highest extraction efficiency attained at 50 mg mass of C_{18} sorbent. This effect may be attributed to the increased likelihood of adsorbing analytes onto the sorbent due to the higher number of adsorption sites [19]. However, with an increasing mass of C_{18} sorbent beyond 50 mg (70 mg to 90 mg), the extraction efficiency started to decrease slowly. This condition may be due to the agglomeration of C_{18} sorbent that suppressed the action of the active sites of the sorbent, thereby lowering the extraction efficiency [15]. Furthermore, it is worth noting that samples may have trouble progressing through the system if the amount of sorbent increases [20]. Thus, to efficiently extract nicotine from tobacco samples, the mass of C_{18} sorbent of 50 mg was utilized as the second parameter for further optimization of another parameter and applied for subsequent use in real-sample analysis of tobacco samples.

Effect of desorption volume

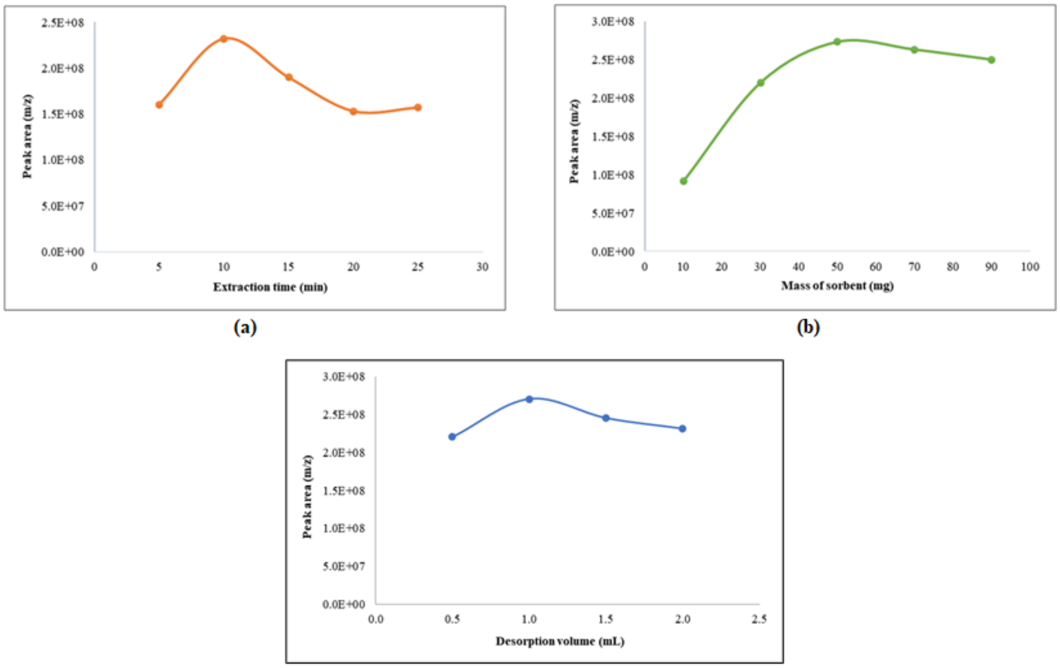
The effect of desorption volume was also taken into consideration for efficiency studies in the extraction of nicotine in tobacco samples. This is because the efficiency of desorption capability is affected by the volume of desorption solvent used [21]. This study used methanol as the desorption solvent, and the desorption volume varied to 0.5 mL, 1.0 mL, 1.5 mL, and 2.0 mL. After each subsequent analysis, a graph of peak area (m/z) versus desorption volume (mL) was plotted to determine the optimized condition for the volume of desorption solvent used to adsorb nicotine from tobacco samples efficiently. Based on Figure 3(c), it can be observed that with an increasing desorption volume from 0.5 mL to 1.0 mL, the extraction efficiency of nicotine from tobacco samples also increased substantially. However, increasing the desorption solvent volume from 1.5 mL to 2.0 mL only lowered the extraction efficiency of nicotine from tobacco samples. This condition may be attributed to the maximum achievement of analyte enrichment when applying a lower volume of desorption solvent. Additionally, only a low volume of desorption solvent was necessary for achieving higher sensitivity of analyte extraction [22]. Hence, a desorption volume of 1.0 mL of methanol was utilized as the third parameter and applied for subsequent use in the real sample analysis of tobacco samples.

Optimum extraction conditions

The final applied C_{18}- D-µ-SPE conditions were as follows: extraction time, 10 min; mass of sorbent, 50 mg; and desorption volume, 1.0 mL. Thus, these optimum extraction conditions were applied for subsequent analysis on real samples.

Method validation and analytical performance of D-µ-SPE

Method validation was then carried out based on the optimum conditions obtained from the D-µ-SPE method. Relative recovery (%), linearity, precision, limit of detection (LOD), and limit of quantification (LOQ) were obtained for validation of this study. Good linearity in the range of 0.1 – 500 mg/L was obtained for the analyte of nicotine, with the coefficients of determination (R^2) of 0.9997. The calculated LOD was 0.0983 mg/L, while the LOQ was calculated to be 0.2978 mg/L. The precision of the method was measured through percent relative standard deviation (% RSD), which gave a value of 2.6%. Table 1 shows the validation data for D-µ-SPE of nicotine from tobacco samples using sample brand M.



(a) (b) (c)

Figure 3. Optimization for the extraction performance of C_{18} using D-µ-SPE. (a) Effect of extraction time, (b) mass of sorbent and (c) desorption volume on C_{18}-D-µ-SPE of nicotine in tobacco samples.

Table 1. Quantitative results of D-µ-SPE for nicotine from tobacco samples

Sample	Analyte	Linear Range (mg/L)	Coefficient of Determination (R^2)	LOD (mg/L)	LOQ (mg/L)	RSD (%, *n* = 3)
Brand M	Nicotine	0.1 - 500	0.9997	0.0983	0.2978	2.6

Application of D-µ-SPE on real samples

The developed D-µ-SPE-GC-MS method was successfully applied to the extraction of nicotine from tobacco samples. The optimized parameters performed previously were used to obtain the actual concentration (mg/L) of nicotine in the tobacco samples. Relative recoveries (%) were also calculated on the real samples by spiking each real sample with 50 mg/L and 100 mg/L standard nicotine. Relative recoveries (%) of real samples showed good recoveries ranging from 75.5% to 96.4%. The analysis on real samples also displayed good repeatability with relative standard deviation (% RSD) ranging from 3.1% to 5.7%. Table 2 shows relative recoveries (%) of nicotine in tobacco real samples and % RSD (*n* = 3) of D-µ-SPE.

Table 2. Relative recoveries (%) of nicotine in tobacco real samples and %RSD (*n* = 3) of D-µ-SPE

Real samples	Spike Added (mg/L)	Actual Concentration (mg/L)	Relative Recoveries (%)	RSD (%) (*n* = 3)
Brand D	50	41.594	83.2	3.5
	100	84.793	84.8	4.5
Brand G	50	48.189	96.4	3.1
	100	75.489	75.5	5.7
Brand M	10	8.574	85.7	4.3

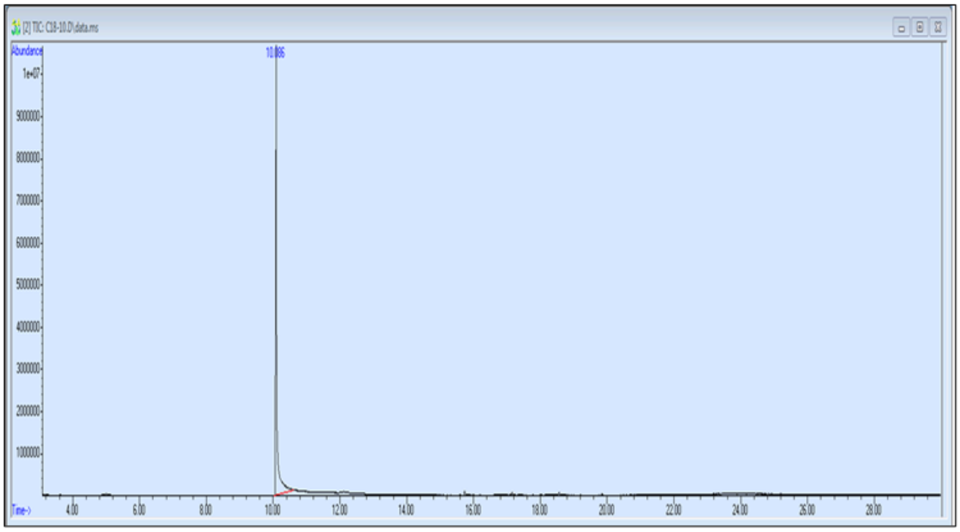


Figure 4. GC-MS chromatogram obtained for nicotine from the optimization on the effect of extraction time at 10th min

Comparison with other reported methods

Previous studies have applied various extraction methods for nicotine from different samples. A comparison study is outlined in Table 3, displaying the developed method from existing/published studies alongside the method proposed by this research for preconcentration and determination of nicotine in tobacco samples. As shown in Table 3, most of the extraction methods for nicotine from tobacco samples involve longer extraction time. For instance, a reported method [23] took 120 min to extract nicotine from waste tobacco samples using an aqueous two-phase system and solvent reverse extraction method. Extraction of nicotine by the HS-SPME method [24] and the electro-membrane extraction (EME) method [25] reported extraction time of 30 min and 20 min, respectively. In contrast, the proposed D-µ-SPE method exhibited a low extraction time of 10 min, which can significantly reduce tedious laboratory work. However, a method reported earlier using surface-enhanced Raman scattering (SERS) [26] showed the fastest extraction time, with nicotine extracted between 3 and 5 min. However, the present D-µ-SPE method demonstrated better % recovery of nicotine ranging from 75.5% to 96.4% from tobacco samples, compared to the previously developed method [26], which reported 51.47% nicotine recovery. Furthermore, the limit of detection (LOD) for the proposed D-µ-SPE method also showed a good detection limit.

Conclusion

In conclusion, the optimization and validation of the dispersive micro-solid phase extraction method using C_{18} as the sorbent have been successfully employed for the extraction and determination of nicotine in tobacco samples, coupled with gas chromatography-mass spectrometry (GC-MS) for chromatographic analysis. Three parameters were studied for optimization purposes: the effect of extraction time of the D-µ-SPE method, the effect of the mass of C_{18} sorbent, and the effect of desorption volume to extract and pre-concentrate nicotine from tobacco samples. An extraction time of 10 min, mass of C_{18} sorbent of 50 mg, and desorption volume of 1.0 mL were found to be the optimized conditions for extraction. Nicotine was successfully extracted using the above optimum conditions with the D-µ-SPE method. Good linearity in the range of 0.1 - 500 mg/L was also achieved for the analyte of nicotine, with a coefficient of determination (R^2) of 0.9997. The limit of detection (LOD) was calculated to be 0.0983 mg/L, and the limit of quantification (LOQ) was calculated to be 0.2978 mg/L. The precision of the method was measured through the percent relative standard deviation (% RSD), which gave a value of 2.6%. Additionally, good relative recovery (%) of nicotine in real samples was achieved in the 75.5 - 96.4% range, with % RSD ranging from 3.1 - 5.7%. Hence, the D-µ-SPE method proves to be green, reducing excessive solvent consumption while being selective and rapid in preconcentration and determination of nicotine from tobacco samples. It shows promise as an alternative method for future analytical laboratory work in nicotine analysis. Compared to the previously reported methods, the D-µ-SPE method used in the present study demonstrated one of the fastest extraction times and one of the highest recoveries of nicotine from tobacco samples. A future extension to this research may be to explore more optimization conditions and study other parameters and factors that may contribute to improving the efficiency of C_{18} as a sorbent for nicotine extraction from tobacco samples using the D-µ-SPE method.

Table 3. Comparison of extraction performance between previous reports and the present work for extraction and determination of nicotine from tobacco samples.

Extraction methods	Extraction Time (min)	LOD (mg/L)	% Recovery	% RSD	References
Aqueous two-phase system and solvent reverse extraction	120	-	96.1	-	[23]
Headspace solid-phase micro-extraction (HS-SPME)	30	-	99.56 -99.80	3.35	[24]
Electromembrane extraction (EME)	20	0.02	81.0 – 112.8	0.2 – 7.7	[25]
Fabrication of gold nano bipyramids (Au NBPs) – AAO substrates to burn cigarettes	3 – 5	2.2	51.47	10.18	[26]
Solvent extraction	Not stated	-	0.6	-	[27]
Ultrasound-assisted extraction of nicotine-related alkaloids from tobacco samples using 1:3 CH_3OH-CH_2Cl_2	>20	1.3	95.7	1.5	[28]
Supercritical fluid CO_2 extraction	Not stated	-	47.40	-	[29]
Dispersive micro-solid phase extraction	10	0.0983	75.5 – 96.4	3.1 – 5.7	This work

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