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A Simple Extraction Method for the Determination of Nicotine from Saliva

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A simple method is proposed for the determination of nicotine in the saliva of active smokers. Nicotine was extracted from saliva at pH 10 with chloroform and *n*-hexane (60:40). The organic layer was separated and put it in the centrifuge at 3000 rpm for 3 min and then evaporate at 35 ºC in bath water with sonication. Water molecules were bound using silica gel GF 254 through cartridge solid phase extraction (SPE). Spot test analysis was carried out using cyanogen bromide reagent produces an orange colour which indicates nicotine positive. TLC analysis was carried out to produce qualitative data with an R_f value in general = 0.75. The UV spectroscopic analysis at 260 nm produces the equation = 0.0058*x* + 0.00342 and R^2 = 0.9923. The concentration of nicotine obtained were 2.95, 2.96, 3.08, 3.22 and 2.79 µg/mL respectively.

Keywords: Nicotine, Sonication, Extraction, Saliva, UV spectroscopy.

INTRODUCTION

Cigarettes have a negative impact on the respiratory system and specifically will cause the emergence of heart disease, hypotension, cancer, liver, impotence, pregnancy and fetal disorders that can threaten the life of smokers [1]. Every single cigarette contains at least 10 mg of nicotine [2]. Nicotine is an alkaloid compound that has molecular formula $C_{10}H_{14}N_2$, which is widely contained in plants surnamed Solanaceae. One of them is the type of tobacco (Nicotiana) [3]. Nicotine and its metabolites are harmful to the body [4], nicotine can cause cancer because it contains strong carcinogenic compounds [5].

Smoker's saliva can be used as an indicator of how much a person is exposed to cigarette smoke or actively consumes cigarettes [2]. Smoker's saliva contains a certain amount of nicotine which causes damage to enzymes, allergies and other oral diseases [6]. The average human produces 700 mL of saliva every day [7]. The analysis of nicotine in cigarettes is still little carried out in an integrated manner. Nicotine can be extracted by maceration using ammonia solvent and followed by 5 mL of chloroform each in alkaline conditions [8]. The extraction results were analyzed using TLC. The data obtained is not optimal because the data generated is in the form of the qualitative data. Extraction by maceration has a disadvantage in that the nicotine is slightly extracted into the solvent [9]. One alternative that is used to overcome this is the use of sonication at 42 KHz so that nicotine can be completely distributed into the solvent [10].

The sonication method is a method by utilizing ultrasonic waves that has a very strong effect called the cavitation effect on the solution which causes the molecules of the solution to break. Some of the advantages of the sonication method are that it has a very small particle size so as to prevent the occurrence of creaming or sedimentation during storage [11], produces a large surface area so that it can accelerate the penetration of the active ingredient and facilitate its spread and is transparent in colour [12].

EXPERIMENTAL

All chemicals, analytical standards, reagents and solvents used throughout this study were of analytical grade and highly pure. Nicotine was purchased with purity of assay ≥ 99%. Methanol (Spectro) was purchased from (Sigma-Aldrich) with purity 99.9%. Other chemicals and solvents were used including

chloroform (Sigma-Aldrich) with purity 99.9% inhibitor free; NaOH from (Riedel-Dehaen AG Seelze Hannover); KH_2PO_4 (Merck), hydrochloric acid (Merck).

UV-visible spectrophotometer system: UV-VIS spectrophotometer (Beckman Coulter Du 800) was used.

Collection of saliva samples: On obtaining authorization from the ethical committee, the samples were collected at the Analytical Laboratory of the Mathematic and Natural Science, Universitas Sumatera Utara. Five saliva samples were obtained from male volunteers with active smoking status aged 17-40 years, active smokers were active and continuously smoked at least 1 (one) cigarette per day. Samples were placed in pots and stored at T = -5 ºC.

Preparation of standard nicotine solution: Nicotine (100 mg) in 100 mL (1 mg/mL) solution was prepared. After that the desired standards solution were prepared by appropriate dilution of the stock. (0,5, 1, 1,5, 2 and 2,5 µg/mL).

Extraction: The saliva sample was added to 10 mL of a mixture of chloroform and methanol (60:40). The extraction procedure was carried out at room temperature and pH 10. The organic layer was separated and centrifuged at 3000 rpm for 3 min, and then evaporated at 35 ºC in bath water with sonication. The evaporated fraction was dissolved in 2 mL of solution containing 0.2973 g of KH_2PO_4, 180 mL of methanol and 820 mL of distilled water. Water molecules were bound using silica gel GF_{254} through cartridge solid phase extraction (SPE). Spot test analysis was carried out using cyanogen bromide reagent characterized by the presence of a yellow colour and followed by ultra-violet spectroscopy.

RESULTS AND DISCUSSION

Nicotine ($C_{10}H_{14}N_2$) is an organic alkaloid compound, has a strong and stimulant effect on the human body [13,14]. For active smokers, the smoker can feel the stimulant effect of caffeine when smoking actively or passively.

The extraction process was developed using chloroform and methanol (60:40) were added with a ratio of 60:40. The separation process using room temperature and pH 10 was used to obtain the optimal extraction value. However, the nicotine extract was precipitated using the centrifugation method for 3000 rpm for 3 min, then the evaporation process was carried out at 35 ºC in bath water with sonication (42 KHz). This sonication was developed to accelerate the presence of nicotine by destroying the three-dimensional structure and speeding up the release of nicotine in the sample. An advanced process is developed by dissolving 2 mL of solution containing 0.2973 g of KH_2PO_4, 180 mL of methanol and 820 mL of distilled water. Solid phase extraction (SPE) method was developed to separate water molecules using silica gel GF_{254}.

Spot test analysis: The spot test was conducted using chloroform and methanol as solvents at a ratio of 60:40. The results of several reagents showed that cyanogen bromide reagent was the most satisfactory reagent with selectivity and sensitivity of the reagent in nicotine. Fig. 1 shows that the saliva sample of active smokers tested using a spot test with cyanogen bromide reagent produces an orange colour which indicates nicotine positive and the saliva of non-smokers does not change colour.

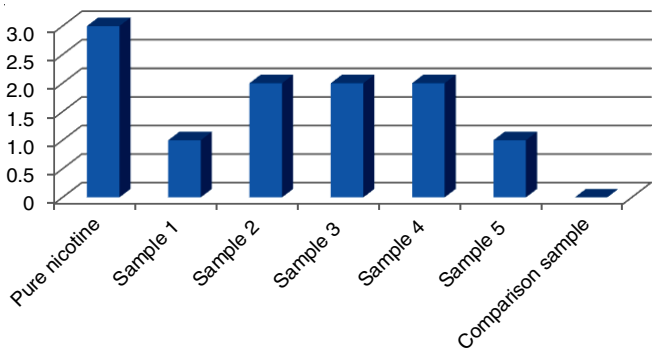

Fig. 1. Spot test analysis data

The reaction mechanism of cyanogen bromide with nicotine (Fig. 2) shows that there has been electron delocalization in

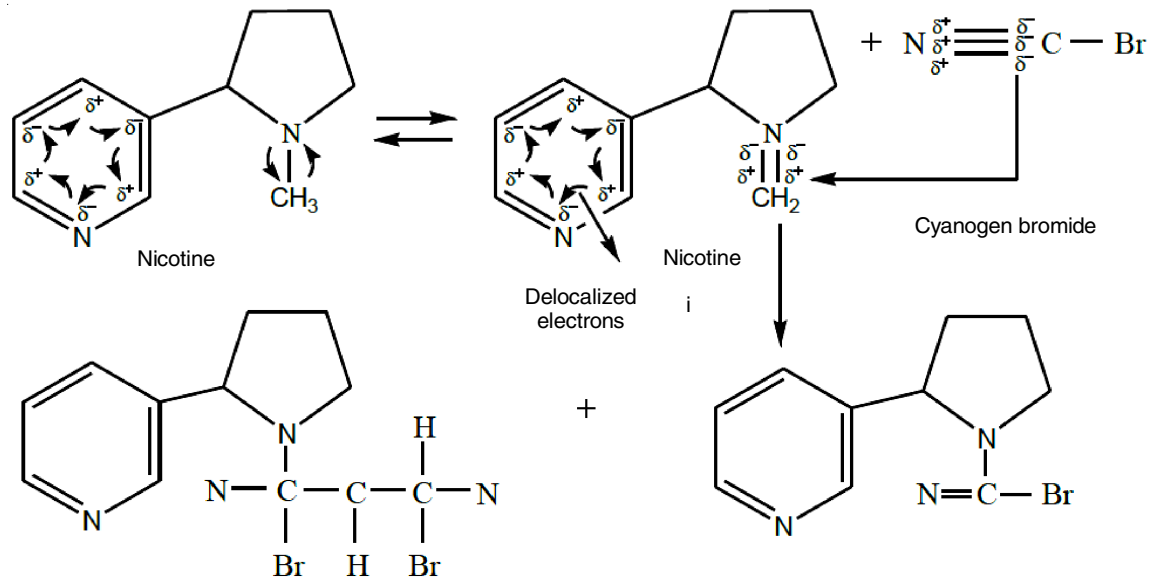

Fig. 2. Reaction of cyanogen bromide with nicotine

nicotine group due to the entry of CN in cyanogen bromide [15]. The negatively charged electrons in cyanogen bromide group will attack the positively charged electrons in nicotine, resulting in an orange precipitate [16]. In this study, the colour occurs is observed and compared with the standard and is divided into 1 (slight), 2 (moderate) and 3 (abundant).

TLC studies: Fig. 3 shows the comparison of the standard nicotine standard solution with urine samples of active smokers, which produced qualitatively known nicotine compounds from the R_f value compared to standard nicotine. The distance travelled by the stain on the TLC plate with an average value of R_f is 0.75.

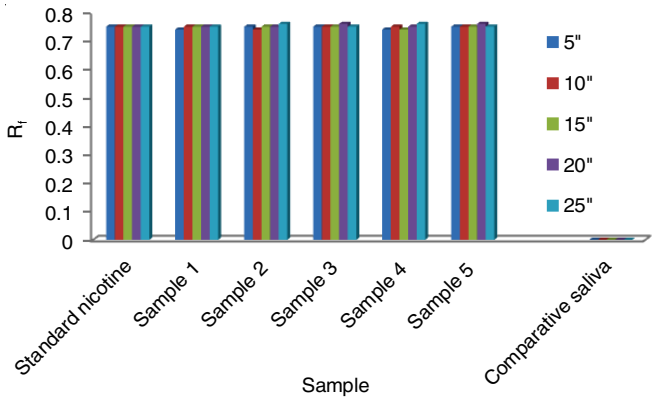

Fig. 3. TLC data

UV spectroscopy studies: In this work, nicotine standard 2.0 µg/mL was used to determine the optimum wavelength at 258, 259, 260, 261, 262 nm and observed the optimal absorbance value in order to obtain the optimal. Fig. 4 shows a peak at a wavelength of 260 nm, which is the optimum peak in UV spectroscopy with an absorbance at 0.048.

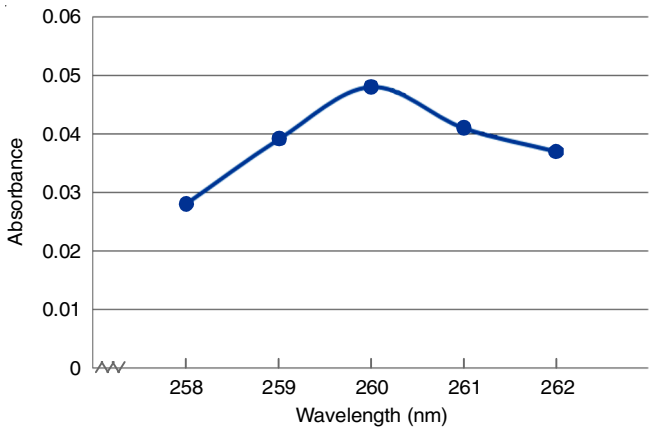

Fig. 4. Determination of the optimum wavelength in UV spectroscopy

Fig. 5 shows the concentration of standard solutions of 0.5, 1, 1.5, 2 and 2.5 µg/mL resulting in the absorptton values being 0.037, 0.0040, 0.0043, 0.0045 and 0.0049, respectively. Fig. 5 shows the equation of a straight line, namely r = 0.995 with a value of $y = 0.0058x + 0.00342$. It shows that the relationship between the concentration of nicotine samples and the response of obserbance is linear. The sample concentration has been determined based on the equation.

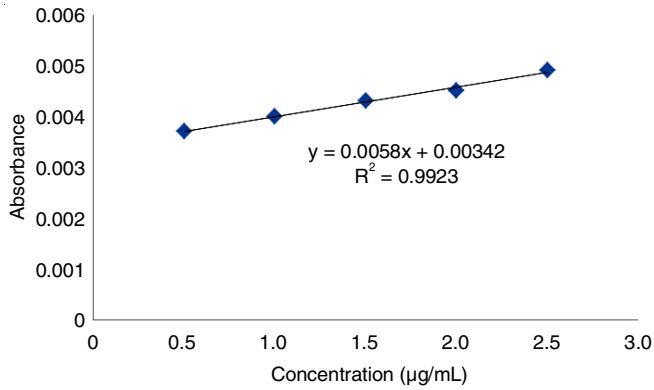

Fig. 5. Calibration curve

Fig. 6 shows the concentration of nicotine contained in the saliva obtained from five samples as follows 2.95, 2.96, 3.08, 3.22 and 2.79 µg/mL, respectively. Nicotine after being absorbed in the body's metabolic system will produce cotinine and nicotine. In this work, the concentration of nicotine that is absorbed in the saliva of active smokers . How to analyze nicotine using this simple method is a procedure that is easy to carry out in an examination laboratory in order to find out a person's history of using nicotine cigarettes.

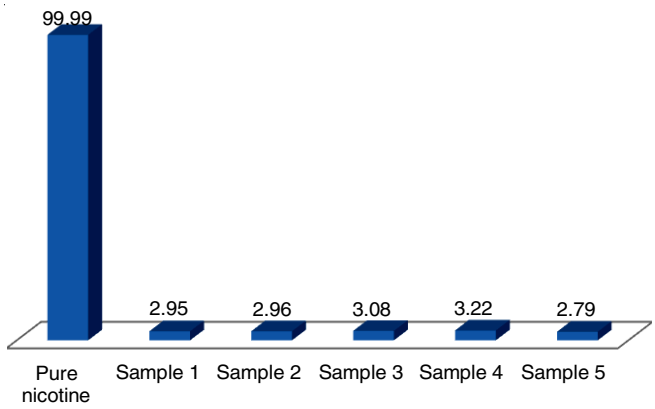

Fig. 6. Samples concentration

Conclusion

Nicotine contained in saliva can be extracted by a simple method by sonication and solid phase extraction (SPE) methods. The sonication method was carried out at 42 KHz and SPE using silica gel 254 as the stationary phase. In the extraction process pH 10 with chloroform and *n*-hexane (60:40) as mobile phase. Qualitative analysis using cyanogen bromide reagent, TLC analysis. The nicotine concentrations obtained were 2.95, 2.96, 3.08, 3.22 and 2.79 µg/mL, respectively. However, analysis of nicotine from saliva by this method contributes to the analysis of metabolites in the field of analytical chemistry.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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