Extraction and Analysis of Nicotine from the Saliva of Active Smokers using UV Spectroscopy

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Abstract: An accurate and simple method of extraction and analysis needs to be developed in the context of investigating nicotine in the saliva of active smokers. This study aims to prepare, extract and analyse nicotine from the saliva of active smokers. The preparation process is carried out from the location where the samples were taken. Extraction was carried out using sonication coupling maceration for 15 minutes. Qualitative analysis used spot test with Cyanogen bromide reagent and quantitative analysis used UV spectrophotometer. Ultra Violet (UV) spectrophotometer analysis at the optimum wavelength of 260 nm resulted in a sample concentration of Saliva A = 1.4 ppm and Saliva B = 1.6 ppm.

1 INTRODUCTION

Nicotine (*Nicotiana tobacum*) is the most important ingredient in tobacco leaves. Nicotine has the molecular formula $C_{10}H_{14}N_2$ (Fidrianny 2004). Nicotine is a clear, slightly yellow liquid that has an oil-like appearance, dissolves in water and is also soluble in organic solvents in general, such as ethanol, petroleum ether, and chloroform (Taufik et al. 2017). Nicotine is an alkaloid compound that is widely contained in plants with the genus Solanaceae (Rahmat Nur Hidayat, Adam M. Ramadhan 2016). One of them is the type of tobacco (Nicotiana). Nicotine with the chemical name 1-Methyl-2- (3-pyridyl) pyrrolidine; β-pyridyl-α- N-methylpyrolidine or with the molecular formula $C_{10}H_{14}N_2$ or $C_5H_4NC_4H_7NCH_3$ (Clayton et al. 2013).

Identification for nicotine can be done in urine, hair and including saliva (Taufik, Susilawati, et al. 2021). Saliva is the first biological fluid to be exposed to cigarette smoke in the oral cavity (Kunutsor et al. 2018). Cigarette smoke contains a variety of chemicals that can cause functional and structural changes in saliva which can reduce the flow of saliva, causing dry mouth and halitosis (Fidrianny 2004) (Kunutsor et al. 2018).

Saliva has 99% water and 1% organic and inorganic components (Jahed, Hamidi, and Galehassadi 2020). Inorganic components of saliva include: Sodium, Calcium, Potassium, Magnesium, Bicarbonate, Chloride, Rodanide and Thiocynate (CNS), Phosphate, Potassium and Nitrate. While the organic components in saliva include proteins in the form of the enzyme amylase, maltase, serum albumin, uric acid, cretinin, mucin, vitamin C, several amino acids, lysosime, lactate, and several hormones such as testosterone and cortisol (Jahed, Hamidi, and Galehassadi 2020).

The preparation of smoker's saliva which is the initial stage of work that must be carried out in various analyzes for sample preparation (Kunutsor et al. 2018). The process of separating the material from the mixture is carried out using the appropriate solvent (Alfian et al. 2018). Sample preparation is carried out in sample conditions where there are specific techniques for sampling in order to obtain a representative sample (Sisco, Najarro, and Burns 2018). This preparation aims to eliminate various annoyances (Kondeti, Mulpuri, and Meruga 2014).

Sonication maceration is a liquid-liquid extraction method that utilizes ultrasonic waves with a frequency of 42 kHz which can accelerate the contact time between the sample and the solvent

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even at room temperature (Taufik 2016). Sonication relies on wave energy that causes the cavitation process, which is the process of forming small bubbles due to the transmission of ultrasonic waves to assist solvent diffusion into the sample (Alfian et al. 2018). The sonication extraction method is also efficient and shortens the extraction time (Delmifiana and Astuti 2013).

Nicotine analysis is needed to determine the nicotine content of human metabolites (Rahmat Nur Hidayat, Adam M. Ramadhan 2016). The qualitative analysis of nicotine was carried out in several ways, such as the spot test using the Cyanogens bromide test reagent until an orange color was obtained which indicated positive nicotine (Paci et al. 2018). Where the analysis of the Cyanogen bromide test is carried out with the extraction results dropping 2 drops into the spot test, then dropping 2-3 drops of Cyanogen bromide until it dissolves in the spot test, and observing the orange color that occurs then compared with the standard and differentiated into + (slightly), + + (moderate), +++ (abundant) (Taufik, Cahyady, et al. 2021).

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The quantitative analysis of nicotine was carried out through UV spectroscopy based on the interaction of the sample with UV light. UV light has a wavelength of 190 - 380 nm as a light source, deuterium lamps can be used. Deuterium, also known as heavy hydrogen, is a stable isotope of hydrogen that is abundant in the ocean and land. The nucleus of deuterium has one proton and one neutron, while hydrogen has only one proton and no neutrons (Clayton et al. 2013). This study aims to extract by maceration the sonication coupling of nicotine contained in saliva samples of active smokers and to analyze it using UV spectroscopy.

2 METHODS

2.1 Materials

The materials used in this study were saliva, pure nicotine (sigma Aldrich), methanol, chloroform (p a merck), Cyanogen bromide reagent (Sigma Aldrich), and aquadest.

2.2 Nicotine Standard Solution Preparation

Nicotine standard solution (sigma aldrich) was prepared by varying the concentration of nicotine, respectively, 0.5 ppm, 1 ppm, 1.5 ppm, 2 ppm, 2.5 ppm.

2.3 Preparation

The saliva sample of active smokers was measured 10 ml each added 10 ml of chloroform solvent, then put it into each separating funnel, then shaken it, and let it stand for a moment, there were 2 layers (the top layer of the remaining saliva and the bottom layer of nicotine).

2.4 Extraction

The saliva used as the sample was sonication process at a frequency of 42 KHz for 10 minutes. The result of maceration was taken from the lower layer of nicotine and then diluted with the addition of 10 ml methanol. The sonication process was carried out again for 5 minutes at a frequency of 42 kHz in a sonication bath. Note: comparator saliva (not smokers) is carried out in the same manner

2.5 Spot Test Analysis

Spot test analysis was carried out using Cyanogens bromide reagent until an orange color was obtained which indicated a positive nicotine. The analysis procedure for the Cyanogen bromide test is carried out by:

1. The sample is added 2 drops of cyanogen bromide in the spot test.
2. Observed the orange color that occurs and differentiated into + (slightly), ++ (moderate), +++ (abundant).

2.6 Analysis using UV Spectroscopy

The analysis of the extracted nicotine was carried out using a UV spectrophotometer with the following procedure:

1. Turn on the Uv-Vis spectrophotometer on the back of the instrument, wait for 10-15 minutes, then connect it to the computer, and start the Windows 7 Short-cut application.
2. The cuvette used was a glass cuvette with a thickness of 10 mm, a square cuvette.
3. Inserted a blank into the UV spectrophotometer, measured the wavelength of the blank.
4. The maximum wavelength is determined.
5. Sample analysis is performed.

3 RESULTS

3.1 Collecting Samples

Saliva of smokers, and saliva of non-smokers from male volunteers obtained at Jl. Arca Gang Jawa Medan Building. All saliva samples were collected between 08:00 and 11:00 WIB. The saliva was collected in the morning by instructing the volunteers not to eat and drink at the time of collection, to let the saliva go down, to let the foam on the saliva be left for a while so that the foam would go down. The sample that has been collected is put into a beaker.

3.2 Preparation

Smoker's saliva preparation was carried out in the laboratory of the University of North Sumatra, Medan. The samples used were 10 ml of active smoker's saliva with the addition of chloroform solvent, in the smoker's saliva there is a nicotine compound that comes from cigarette consumption, which is directly exposed to cigarettes and cigarette smoke through the mouth where saliva is contained. Smoker's saliva has 2 layers perfectly where the bottom layer of nicotine and the top layer of the saliva remains. Non-smoker's saliva does not have a perfect 2-layer separation so it takes 5 minutes to let the saliva and solvent split into two layers.

3.3 Extraction

Saliva samples of active smokers that have been added with chloroform and macerated sonication, the nicotine and the remaining saliva are separated. Obtained cloudy white nicotine extract. The lower layer of the sonication maceration process was taken, then left for a while and added methanol, then re-macerated sonication with a frequency of 42 kHz and put into a vial bottle. The same was true for the comparator saliva (sample of non smokers).

3.4 Spot Test

The results of the spot test for saliva from active smokers and saliva from non-smokers can be seen in Table 4.1 below:

Table 3.1 Spot Test Result Data

Sample	abundance	Colour	Information
Nicotine standard	+++	Orange	Positive Nicotine
Saliva A	+++	Orange	Positive Nicotine
Saliva B	+++	Orange	Positive Nicotine
Comparison sample	-	-	Negative Nicotine

Table 3.1 shows that the saliva samples of active smokers from the spot test results with the cyanogen bromide test reagent produce an orange color which indicates positive for nicotine, and in the saliva of non-smokers there is no color change (negative).

3.5 UV Spectroscopy Analysis

3.5.1 Determination of the Optimum Wavelength

The results of determining the optimum wavelength of nicotine can be seen in **Figure 3.1.** the following :

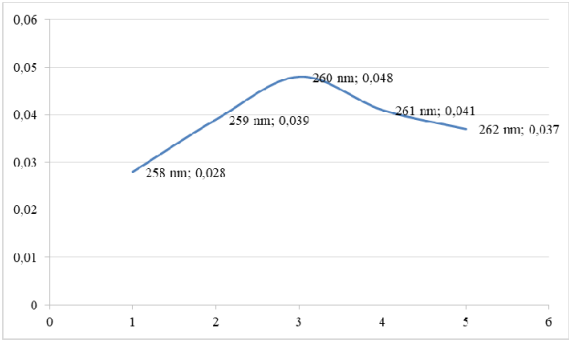

Figure 3.1. Optimum wavelength of nicotine

Figure 3.1 shows the highest peak at a wavelength of 260 nm with an absorbance value of 0.048. At a wavelength of 258 nm, an absorbance value of 0.028

was obtained, a wavelength of 259 with an absorbance value of 0.039, a wavelength of 261 with an absorbance value of 0.041 and a wavelength of 262 with an absorbance value of 0.037. So that the optimum wavelength of nicotine is at a wavelength of 260 nm.

3.5.2 Nicotine Standard Curve

The standard nicotine curve can be seen in Figure 3.2. the following :

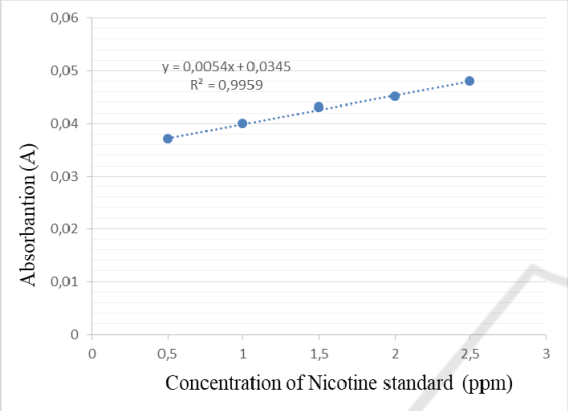

Figure 3.2. Nicotine standard curve

Figure 3.2 shows a straight line equation, namely r = 0.995 with a value of y = 0.0054x + 0.0345. Based on the figure, it shows that the relationship between the concentration of nicotine samples and the response to ultraviolet spectroscopy is proven to be linear.

3.5.3 Concentration of Nicotine

Saliva samples that have been extracted are inserted into the cuvette to measure the absorption and concentration of each sample at a wavelength (λ) of 260 nm. It can be seen in **Table 3.2**. below:

Table 3.2. Saliva Sample Concentration

No.	Sampel	Concentration (ppm)
1.	Saliva A	1,4
2.	Saliva B	1,6

Table 3.2 shows that the concentration of saliva samples is saliva A = 1.4 ppm) and saliva B = 1.6 ppm.

4 CONCLUSION

Extraction of saliva from active smokers by sonication maceration for 10 minutes with the addition of chloroform solvent at 42 KHz. Qualitative analysis of the saliva of active smokers was compared with nicotine standards, which resulted in an orange color. Ultra Violet (UV) spectrophotometer analysis at the optimum wavelength of 260 nm resulted in a sample concentration of Saliva A = 1.4 ppm and Saliva B = 1.6 ppm.

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