Extraction and Analysis of Nicotine in the Urine of Active Smokers after given by Vitamin C

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Abstract: Nicotine is a class of addictive compounds that can cause dependence. Vitamin C contains antioxidants that can ward off free radicals in the body. Nicotine extraction and analysis are a series of processes that are indispensable in order to produce nicotine in optimal concentrations. This study aims to extract and analyze nicotine in the urine of active smokers after giving vitamin C. The descriptive method was developed for the extraction of nicotine in urine. Preliminary test using Cyanogen bromide showed the presence of nicotine in the urine of smokers for 7 days. The same thing is evidenced by the thin layer chromatography data resulting in an average Rf value of 0.6. The concentration of nicotine in urine decreased from the first day of vitamin C administration to day 7. This indicates that there is an effect of vitamin C on the urine of active smokers.

1 INTRODUCTION

Cigarettes are one of the most dangerous deadly objects in the world (Rahmat Nur Hidayat, Adam M. Ramadhan 2016). Consuming cigarettes continuously can cause various health problems such as cancer, respiratory problems, and heart problems. Cigarettes are one of the biggest contributors to death today. The death rate caused by smoking in Indonesia has reached 57,000 people every year (Sari, Zulaikhah, and PH 2019).

Cigarette consumption in Indonesia reaches 215 billion sticks per year. In Indonesia there are 60% smokers, 59% of whom are men and 37% are women. In Indonesia, tobacco plus cloves and other ingredients are mixed to make kretek cigarettes. In addition to kretek, tobacco can also be used as rolled cigarettes, white cigarettes, cigars, pipe cigarettes, and smokeless tobacco, cylinders of paper measuring 70-120 mm in length and 10 mm in diameter containing chopped tobacco leaves. The basic ingredient of cigarettes is tobacco.

Tobacco contains a variety of chemicals that can be addictive to a person, even if they don't want to try again (Solarino et al. 2009).These health problems are related to the content of free radicals in cigarette smoke. in one suction, an estimated 1014 free radical molecules enter the body (Rahmat Nur Hidayat, Adam M. Ramadhan 2016). Cigarette smoke contains ± 4000 chemical compounds with 60 chemical compounds that have been identified as cancer-causing and genotoxic. These chemical compounds circulating in the testicular blood vessels damage the spermatozoa with their cytotoxic effect. These chemical compounds consist of nicotine, tar, carbon monoxide, acetone, arsenic, ammonia, hydrogen cyanide and so on (Clayton et al. 2013).

Vitamin C is one of the most widely taken nutritional supplements (Pacier and Martirosyan 2015). Health professionals as well as a number of health benefits for vitamin c such as boosting immunity or preventing common diseases and cancer (Lorensia et al. 2018). If free radicals are too high, natural anti-oxidants alone are not enough to ward off free radicals. Therefore, additional antioxidants are needed to inhibit oxidation

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reactions, neutralize and scavenge free radicals. Water-soluble vitamins that can function well as antioxidants are commonly known as vitamin C or ascorbic acid. This vitamin C plays an important role in protecting cell damage caused by free radicals. Because structural vitamin C is similar to glucose and can replace glucose in various chemical reactions (Pacier and Martirosyan 2015).

Nicotine extraction from smoker's urine can be done in several ways, namely extraction, maceration, reflux, and distillation. Extraction by means of maceration gives an advantage, so that it is not damaged and so that more tends to be obtained. Extraction by maceration was identified by sonication and electrosynthesis methods (Taufik et al. 2017). Fidrianny (2004) has carried out the preparation and analysis of nicotine contained in smoker's urine where chloroform is used as a solvent in sample preparation The electrolysis coupling maceration method is the best extraction method compared to the results of the maceration method (Muhammad Taufik, Rid Wanto, Athaillah, Anny Sartika Daulay, Lilis Karlina Siahaan, Desi Ardilla, Mariany Razali 2017). However, this study has a weakness where the solvent used has not been optimized. In this work, the use of UV spectroscopy was also developed at a wavelength of 260 nm. However, this study is a basic study in order to search for nicotine in the urine of smokers simply in the laboratory.

2 MATERIALS AND METHOD

2.1 Sample Collection

The sample was collected purposively, namely based on the criteria determined by the researcher. The sample obtained was the urine of volunteers who consumed cigarettes that had been given vitamin C and not given vitamin C. The samples were taken within 24 hours after the volunteers consumed cigarettes. The urine sample used was from Garu II Medan Amplas. The dose of with Vitamin C = 50 mg x 2. Samples were taken until day 7.

2.2 Administration of Vitamin C

Filling in the questionnaire sheet including name, age. Followed by giving vitamin C 50 mg / tab x 2 for 2 times a day morning and evening to volunteers for 7 days.

2.3 Preparation and Extraction

Each 25 ml active smoker's urine sample was added with 25 ml of chloroform inserted into a separating funnel. The lower layer was put into a 50 ml beaker glass and followed by maceration of the electrosynthetic coupling for 15 minutes. The result of maceration is evaporated until the solvent evaporates. The nicotine evaporated was diluted at pH 9 then continued with qualitative and quantitative analysis.

2.4 Preliminary Test using Cyanogen Bromide

The extracted sample was dripped with Cyanogen bromide reagent until a yellow color was obtained which indicated positive nicotine, observed the yellow color that occurred and compared it with the standard differentiated into + (slightly), ++ (moderate), +++ (abundant).

2.5 TLC Analysis

Thin layer chromatography (TLC) analysis was performed using the mobile phase of Methanol: Chloroform (50:50). The stain appearance solution used was dragendorff reagent. Physically, the stain analysis uses UV light.

2.6 UV Analysis

The results of the smoker's urine sample which was macerated with electrosynthetic coupling were analyzed quantitatively using MDA 50 UV-Vis spectrophotometry. This instrument was switched on for 10-15 minutes and used a wavelength of 200-800 nm. The cuvette used was a glass cuvette with a thickness of 10 mm, a square shape. In this work, the blank used is methanol.

3 RESULTS AND DISCUSSION

3.1 Preparation and Extraction

The sample was taken from 5 respondents who were active smokers. The preparation has a very important role in determining the success of the extraction and analysis process. The extraction process uses chloroform as a solvent. This sample extraction aims to separate nicotine from the urine of active smokers. The electro synthetic method was

developed to shorten the extraction time. This technique is a way to synthesize which is based on electrochemical techniques. In this study, the electro synthetic coupling maceration was developed for 15 minutes with a voltage of 20 V.

3.2 Preliminary Test using Cyanogen Bromide

Preliminary test results using Cyanogen bromide can be seen in Table 1 in the following:

Table 1: Preliminary test results

Sample	Day						
	1	2	3	4	5	6	7
A	++	++	++	+	+	+	+
B	+	++	++	++	+	+	+
C	++	++	++	+	+	+	+
D	++	++	++	+	+	+	+
E	++	++	+	+	+	+	+

Table 1 showed that the urine of active smokers in the analysis showed positive results for nicotine compounds. This is indicated by the appearance of orange deposits. In this study, the greatest nicotine was obtained on the third day then decreased until the 7th day.

3.3 TLC Analysis

The results of TLC analysis is presented in Table 2 as follows:

Table 2: Sample Rf value

Sample	Day	Stain distance	Rf
A	I	6.5	0.92
	II	6.6	0.94
	III	6.6	0.94
	IV	6.5	0.92
	V	6.6	0.94
	VI	6.6	0.94
	VII	6.6	0.94
	VIII	6.6	0.94
B	I	6.4	0.91
	II	6.3	0.90
	III	6.2	0.88
	IV	6.0	0.85
	V	5.9	0.84
	VI	6.0	0.94
	VII	6.0	0.94
C	I	6.6	0.94
	II	6.6	0.94
	III	6.6	0.94
	IV	6.6	0.94
	V	6.6	0.94
	VI	6.6	0.94
	VII	6.6	0.94
	VIII	6.1	0.87
D	I	6.1	0.87
	II	6.2	0.88
	III	6.6	0.94
	IV	6.6	0.94
	V	6.6	0.94
	VI	6.6	0.94
	VII	6.6	0.94
	VIII	6.6	0.94
E	I	6.6	0.94
	II	6.6	0.94
	III	6.6	0.94
	IV	6.7	0.95
	V	6.5	0.92
	VI	6.5	0.92
	VII	6.6	0.94
	VIII	6.6	0.94
Nicotine standard		6.6	0.94

Table 2 shows that the Rf values of samples A, B, C, D, and E from Day 1 to 7 are almost the same as for standard nicotine (Rf = 6.6).

3.4 Quantitative Analysis using UV

UV-Vis spectrophotometry was developed to generate quantitative data. Initially, the standard solution was made from nicotine standard solutions with concentrations of 0.5, 1.0, 1.5, 2.0, and 2.5 ppm. The blank used is methanol. To determine the nicotine concentration, first the maximum wavelength must be determined at 260 nm. Determination of wavelength used a concentration of 2 ppm. The results of the analysis of nicotine concentration for the 5 samples used can be seen in Figure 1.

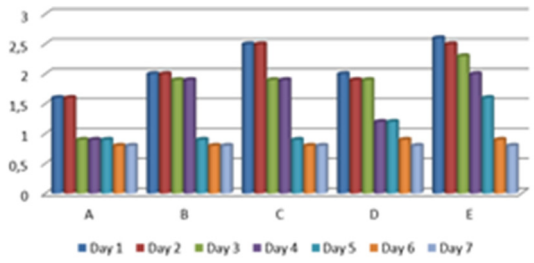

Figure 1: The results of the analysis of nicotine levels

Figure 1 showed data from five urine samples of active smokers that had been extracted using

chloroform. The nicotine concentration table in the sample can be seen in Table 3.

Table 3: Nicotine concentration

No	Sampel urin perokok									
	A		B		C		D		E	
	λ(A)	Concentration (ppm)	λ(A)	Concentration (ppm)	λ(A)	Concentration (ppm)	λ(A)	Concentration (ppm)	λ(A)	Concentration (ppm)
1	0,910	1.6	1,071	2.0	1,260	2.5	1,071	2.0	1,301	2.6
2	0,914	1.6	1,071	2.0	1,260	2.5	1,051	1.9	1,260	2.5
3	0,635	0.9	1,036	1.9	1,036	1.9	1,051	1.9	1,201	2.3
4	0,638	0.9	1,056	1.9	1,046	1.9	0,762	1.2	1,076	2.0
5	0,633	0.9	0,636	0.9	0,631	0.9	0,762	1.2	0,910	1.6
6	0,623	0.8	0,620	0.8	0,613	0.8	0,646	0.9	0,633	0.9
7	0,616	0.8	0,627	0.8	0,613	0.8	0,627	0.9	0,629	0.8

Figure 1 and Table 3 shows the nicotine concentration generated from the five analyzed samples. The concentration of urine generated from day 1 to day seven is reduced. This shows that Vitamin C given to active smokers has an effect on urine levels.

4 CONCLUSION

The extraction of nicotine in the urine of active smokers can be developed using electro synthetic coupling maceration. The extraction results were analyzed using a preliminary test and thin layer chromatography indicated the presence of nicotine in the smoker's urine. The urine concentration decreased until the 7th day after administration of Vitamin C by testing using UV spectroscopy at a wavelength of 260 nm.

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