Detection and identification of morphine in blood of male white rats \textit{(rattus norvegicus)} by ultraviolet-visible spectrophotometry

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Abstract. The blood of male white rats has been experimentally characterized by Ultraviolet-visible (UV-Vis) Spectrophotometry. Ultraviolet-visible (UV-Vis) spectrophotometry appears as an affordable option to monitor morphine composition in blood of male white rats \textit{(Rattus norvegicus)}. Morphine compounds were identified following two different methods, namely qualitative and quantitative methods. Qualitative analysis of this research by the marquis test and quantitative analysis with spectrophotometry method. The male white rats \textit{(Rattus norvegicus)} received morphine and it was maintained drug free for 10 days. After that, the blood was taken from the male white rats \textit{(Rattus norvegicus)} and were extracted the blood using organic solvents, namely methanol and chloroform (1: 1). In the extraction process was used sonication (42 KHz) at temperature 500° C. The qualitative test proves the presence of morphine content with respective concentrations of 1%, 2%, 3%, 4% and 5%. Furthermore, identification of ultraviolet-visible (UV-Vis) spectrophotometry showed that morphine levels in each subcutaneous, that is 0%; 0.6%; 1.0%; 1.6% and 3.5%. The percent transmittance on each samples are 99.9%; 76.9%; 68.8%; 55.9% and 31.0%. The successful application of this simple methodology to a blood of male white rats (Rattus norvegicus) suggests that this approach has practical utility for confirming the identity of abused drugs like morphine detected by ultraviolet-visible (UV-Vis) spectrophotometry.

1. Introduction

In 2016, Indonesia declared a State of emergency drugs (narcotics and dangerous drugs). Almost all community groups ranging from workers, students, street children and even now more worried again used among children and circulation can not be stopped so easily. Illegal use of narcotics is the impact of global developments that affect the behavior of nations in the world, especially teenagers. Narcotics abuse not only affects human quality, but also can increase the number and quality of crime, economic vulnerability, politics and social culture \cite{1}.

Based on data from the national narcotics agency (BNN) in 2015 obtained that in January 2015 narcotics users of shabu-shabu reached of 2,320,000. In North Sumatra recorded 115,290 users of shabu-shabu. Among the students, there were 87,800 users of shabu-shabu \cite{2}.

Identification of narcotics (especially morphine type) in the laboratory requires highly tested methods with optimal results \cite{3}. Marti and Okid (2009) \cite{4} have used white male rat \textit{(Rattus norvegicus)} strains of 2-3 months old male wistar strains as research samples because they have
similarities with humans in terms of physiology, anatomy, nutrition, pathology, and metabolism. In this study, will be given treatment of mouse cells through subcutaneous injection [5].

Morphine is a natural alkaloid derived from the opium poppy *Papaver somniferum*, and is the most widely used opioid to treat moderate to severe pain. Morphine is a phenanthrene derivative and a weak base (pKₐ 7.9) that is highly soluble in water under acidic conditions and poorly soluble in lipids at physiological pH (7.4). It is a full agonist at the μ opioid receptor. In humans, the binding affinity (Ki) for the μ opioid receptor has been reported to be 14 nM [6]. The effects of morphine are mainly associated with μ opioid receptor activation and include analgesia, respiratory depression, reduced gastrointestinal motility, nausea, and sedation [7].

Morphine is a powerful opioid analgesic widely used for relieving severe pain, such as the pain associated with cancer and surgery. Morphine is rapidly absorbed in subcutaneous administration by achieving peak analgesia at 30-60 min and 50-90 min after injection. The half-life of subcutaneous morphine is 3-4 hours. Morphine is distributed throughout the body, especially in parenchymal tissues such as the kidneys, lungs, liver, and spleen. Low concentrations are found in striated and brain tissue. Morphine diffuses through the placental barrier and is also found in breast milk. Approximately 35% bind to proteins, especially to albumin serum levels begin to decline about 1.5-2 hours, and 90% are found in the urine within 24 hours. Approximately 7-10% of the doses encountered in stool, mostly after conjugation and excretion through the gland [8].

![Figure 1: Structure of Morphine](image)

The identification of morphine compounds in human metabolic compounds has been carried out by several researchers. Suaniti (2007) [9] has conducted quantitative determination of morphine in urine by spectrophotometry. In the study, the initial identification was performed using thin layer chromatography (TLC) with a suitable solvent for morphine was toluene: acetone: ethanol: ammonia in the ratio of 45: 45: 7: 3. At the maximum wavelength obtained was 287 nm obtained morphine about 20 - 120 ng / ml. The Thin Layer Chromatography (TLC) method has also been developed by Harborne (1987) [10] and the optimum conditions obtained using an ethyl acetate solvent: methanol, and 80 ml, 13 ml, and 4 ml ammonia solutions were obtained morphine with an Rf value of 0.30 gray bluish. Spectrophotometry method is a simple and easy method in analyzing morphine in samples of metabolite compounds including blood.

2. **Materials and Methods**

2.1. **Materials**

The materials used in this study were standard solutions of morphine, sulfuric acid, glacial acetic acid, formaldehyde, chloroform, ethyl acetate, n-hexane, aquadest, methanol, and sodium hydroxide.
2.2. Instrumentation

The instrument of this research are, beaker glass, test tube, watch glass, dropper dropper, syringe, funnel, mouse cage, oven, sonication, chamber, Ultraviolet-visible (UV-Vis) spectrophotometry device.

2.3. Animals

Male white rats (Rattus norvegicus) (4-6 months) were purchased from pharmacy laboratory at University of Sumatera Utara. The weight of rats is 150 gram. The sample size is 50 tails and has not physical damage, and has been maintained for 2 weeks in a cage to adjust the environment.

2.4. Blood Sampling Male Rats (Rattus norvegicus) And Sampling Extraction

Blood samples of male white rats (Rattus norvegicus) were taken at the tail after three days injected as much as 5 cc. The extraction process was carried out as follows:

Blood samples of 5 cc male white rat were extracted first using chloroform and methanol (1:1) (5% ethyl acetate and n-hexane) (total volume is 10 cc). In the extraction process used sonication for 42 KHz at a temperature of 500°C. At the bottom piped and placed on the beaker glass, left to evaporate. Then add as much as 5cc methanol. pH was made of 9 by using a basic sodium hydroxide solution.

3. Results and Discussion

3.1. Marquist Test

In this experiment, in vivo testing was performed which usually showed large deviation results compared with in vitro experiments, due to biological variation. For such variations to be minimal, animals with the same species or same strain, same age, and same sex, are kept in the same condition[10]. Animal experiments commonly used in scientific research are mice. Male white rat (Rattus norvegicus) have been known to be perfect, easy to maintain, and were relatively healthy animals suitable for various studies.

In general the first stage in investigating a case of a drug is used color test techniques. Mixing the two substances will cause a color change in the reaction material. In the marquis test the reaction material will also change color to purple when mixed with substances containing opium, especially morphine or heroin. But for morphine-type drugs, the reaction material will turn purple.

The color change in the marquis test can be seen in the picture below:

Figure 2 The marquis color test
The marquist test in blood of male white rats was used add 8-10 drops (± 0.25 ml) of 37% formaldehyd solution into glacial acetic acid and concentrated sulfuric acid as much as 90 drops. And then the solution is dropped into the extracted solution. Observed purple deposits that occur and are compared with the standard and differentiated into + (slightly), ++ (medium), and +++ (overflow).

### Table 1 Marquist Test Results

| Sample | Methanol : Chloroform | 1 : 1 | 1 : 2 | 2 : 1 |
|--------|----------------------|------|------|------|
| 0%     | -                    | -    | -    | -    |
| 1%     | +                    | -    | +    |   +  |
| 2%     | ++                   | +    |   +  |   +  |
| 3%     | ++                   | +    |   +  |   +  |
| 4%     | ++                   | -    |   +  |   +  |
| 5%     | ++                   | +    |   +  |   +  |

The table above shows the obtained qualitative test on each morphine concentration, that is at the average morphine concentration obtained a positive result on the content of morphine in the blood of male white rat.

Based on the results of research on the analysis of morphine in male white rats obtained qualitative test results conducted by Marquist Test method. The result showed that the optimal solvent ratio was obtained using solvet of chloroform: methanol is 1:1.

### 3.2. Ultraviolet-visible (UV-Vis) Analysis

Determination of morphine levels in the blood of male white rats is using Ultraviolet-visible (UV-Vis) spectrophotometry method was carried out at 300 nm wavelength based on the group between absorbance and wavelength. The following can be seen absorbance data from morphine concentration respectively, that is 1%, 2%, 3%, 4% and 5%.

In the table below can be seen the amount of morphine content in the blood of male white rat (Rattus norvegicus). There is a calibration curve for standard solution of morphine 1%, 2%, 3%, 4% and 5%

### Table 2 The results on Absorbance and % Transmittance of Morphine Standard Solution by Using Ultraviolet-Visible (UV-Vis) Spectrophotometry

| No. | Concentration of Morphine Standard Solution | Absorbance(A) | % Transmittance |
|-----|-------------------------------------------|----------------|-----------------|
| 1.  | 1                                         | 0.206          | 62.3            |
| 2.  | 2                                         | 0.321          | 47.8            |
| 3.  | 3                                         | 0.418          | 38.2            |
| 4.  | 4                                         | 0.493          | 32.1            |
| 5.  | 5                                         | 0.762          | 17.3            |
Figure 3 Spectrum of Morphine on UV-Visible Spectrophotometry

The graph above can be seen measuring morphine is done at 300 nm wavelength with the highest absorbance, is 0.762 and has a transmittance of 17.3%. On the calibration curve obtained also the correlation coefficient of 0.9331 and the correlation is linear. The ultraviolet-visible (UV-Vis) spectrophotometry method has the ability as an analytical method that responds directly or with the help of good mathematical transformation, proportional to the concentration of the analyte in the sample. The positive correlation coefficient indicates that the relationship is unidirectional and this indicates the measurement of morphine in the blood of male white rats are accurate.

Determination of morphine level in male white rats blood was done by using ultraviolet-visible spectrophotometer and standard solution of morphine with various concentrations of 1%, 2%, 3%, 4% and 5%. In the table below can be seen making a standard solution of morphine for male white blood rats. The analysis of morphine content using Ultraviolet-visible (UV-Vis) spectrophotometry using form aquabides. The spectrum of morphine showed an absorbance of 0.509 and a concentration of 3.5%. Based on the results of this study, an optimum dosage is found at 5% subcutaneous and Ultraviolet-visible (UV-Vis) spectrophotometry method can be an effective method in determination of morphine on blood sample of male white rats. Then it can be concluded, with the increase of each morphine concentration to the ratio of solvent then also increase the concentration for morphine in the blood of male white rats.

4. Conclusion
The results of qualitative tests with the method of marquist test is showed positive on morphine content in male white blood rats show that the ratio of 1%, 2%, 3%, 4% and 5% and solvents of methanol : chloroform (1:1). Determination of morphine level was done by using Ultraviolet-visible (UV-Vis) spectrophotometry with correlation coefficient 0.9331, and concentration of each morphine shows that the ratio of 1%, 2%, 3%, 4% and 5%, the concentration is 0%; 0.6%; 1.0%; 1.6% and 3.5%. The transmittance (%) in a row are 99.9%; 76.9%; 68.8%; 55.9% and 31.0%, respectively. So it can be concluded Ultraviolet-visible (UV-Vis) Spectrophotometry method can be used as an effective and accurate method in the determination of morphine in the blood of male white rat.
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