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Forensic Chemistry Study: Analysis of Phenylpropanolamine Hydrochloride in Urine by UV-Vis Spectroscopy

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***ABSTRACT**: Phenylpropanolamine hydrochloride (PPA) is a stimulant commonly found in some over-the-counter decongestants and appetite suppressants, but its misuse and potential health risks necessitate reliable forensic detection methods. This study aims to develop and validate a UV-Vis spectrophotometric method for the quantitative analysis of PPAin human urine samples. The method involves determining the maximum absorption wavelength (λmax) of PPAstandard and measuring the absorbance of urine samples at this wavelength. Calibration curves were constructed using standard solutions of PPA, resulting in a strong linear relationship with a correlation coefficient (r) of 0.9989 and a regression equation of y = 0,0528x + 0,0145. Urine samples from consumers were analyzed, revealing a PPAconcentration of 10.59 µg/mL. The presence of chromophore groups in the benzene ring of PPAfacilitates ultraviolet light absorption, allowing sensitive detection by UV spectroscopy. The proposed method demonstrated high accuracy, precision, and rapid analysis capability, making it suitable for forensic toxicology applications. The quantification of PPAin urine provides valuable information for monitoring drug intake, supporting legal investigations, and ensuring public safety. In conclusion, UV-Vis spectrophotometry offers a reliable, cost-effective, and non-destructive technique for the forensic analysis of PPAin biological fluids, contributing significantly to the field of forensic chemistry.*
***KEYWORDS:** Urine Analysis, PPA, UV-Vis Spectroscopy, Forensic, Drug.*

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I. INTRODUCTION

Forensic chemistry is a branch of chemistry that focuses on the application of chemical techniques in legal investigations. One of the key applications of forensic chemistry is the analysis of chemical compounds found in biological samples such as urine, blood, and other body tissues[1]. Phenylpropanolamine hydrochloride (PPA) is a compound widely used in medications as a decongestant or weight loss agent[2][3]. The chemical structure of phenylpropanolamine is illustrated in Figure 1 below:

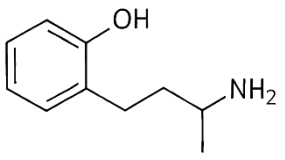

Figure 1. Structure of Phenipropanolamine (PPA)

Phenylpropanolamine hydrochloride (PPA) can also be misused as an illicit drug and may be detected in urine as forensic evidence[2][4]. PPA possesses chemical properties that allow its identification through appropriate analytical techniques[5]. One of the most effective methods for analyzing this compound is UV-Vis spectroscopy, which measures the absorption of light by the compound at specific wavelengths[6][7]. In recent years, UV-Vis spectroscopy has been widely applied in forensic chemistry due to its accuracy and high

sensitivity in detecting various chemical substances[8].The objective of this study is to apply UV-Vis spectroscopic techniques for the analysis of phenylpropanolamine hydrochloride in urine. This research aims to develop an efficient and accurate method for detecting and identifying PPAin urine samples, which can serve as a supportive tool in forensic investigations. Using this method is expected to provide a faster and more cost-effective alternative compared to conventional analytical methods such as chromatography or mass spectrometry[9].

S. Hegstad et al. (2008) reviewed the use of LCMS for detecting illicit drugs in urine, demonstrating that this technique can yield rapid and efficient identification results[10]. Ardiyanti (2014) utilized UV spectroscopy to detect drug compounds in urine; however, their study was limited to single compounds, whereas our research focuses on the more complex PPApresent in urine[2]. Although UV spectroscopy has been extensively applied in forensic analysis, there is limited research specifically employing this method to detect phenylpropanolamine hydrochloride in urine. Most previous studies have concentrated on more common compounds such as cocaine, morphine, or methamphetamine[11][12][13].Therefore, this study aims to fill this gap by providing a more specific method for detecting PPAin urine samples. The research strives to develop a faster and more affordable method compared to other analytical techniques like liquid chromatography or mass spectrometry, which require more expensive equipment and longer procedures.

II. MATERIALS AND METHOD

Materials and Equipments

Phenylpropanolamine $HCl_{(l)}$, $Chloroform_{(l)}$, $Ethanol_{(l)}$, $K_2HPO_{4(l)}$, $Methyl\ Chloride_{(l)}$, $FeCl_3$ $5\%_{(l)}$, $KI_{(s)}$ and $I_{2(s)}$. All other materials and chemicals used were of either analytical grade.Urine samples containing PPA, The UV-Vis spectrophotometer used in this study has a wavelength range of 190 nm to 1100 nm, with a resolution of 0.1 nm for accurate measurements. Equipped with a deuterium lamp for UV analysis (190-400 nm) and a tungsten lamp for analysis in the Vis range (320-1100 nm).

Preparation of PPA Standard Solution

Preparation of 1000 µg/mL phenylpropanolamine hydrochloride (PPA) standard solution then made a 100 µg/mL standard solution and a series of phenylpropanolamine hydrochloride standard solutions with concentration variations of 3 µg/mL, 6 µg/mL, 9 µg/mL, 12 µg/mL, 15 µg/mL, 18 µg/mL

Preparation of Urine of PPA Consumers

The urine sample of phenylpropanolamine hydrochloride consumers was measured as much as 50 ml, added with 50 ml of chloroform solvent, put into a separating funnel, then shaken and left for a moment. There are 2 layers (the upper layer of residual urine and the lower layer of phenylpropanolamine hydrochloride)

Urine Extraction

The results of urine preparation are then put into a beaker glass. Then the urine sample is added with 100 mL of 0.5 M K_2HPO_4 solution pH 11. Then shaken for 5 minutes and 5 ml of Methylene chloride is added to the beaker glass. Then mixed with a stirrer mixer for 5 minutes. After stirring, the sample is put into a test tube. Then centrifuged for 15 minutes. The clear layer is transferred into a beaker glass

Spot test and TLC Analysis.

Spot test analysis was used using FeCl3 which produced a purplish blue precipitateand analysis using a TLC plate.

UV Analysis.

As much as 4.5 mL of phenylpropanolamine hydrochloride standard solution was put into a 25 mL measuring flask and diluted with distilled water to the boundary line with a solution concentration of 18 µg/mL with a variation of wavelengths of 252, 254, 256, 258, 260. The highest absorbance value obtained at the optimum wavelength was observed.The extracted samples were placed into a cuvette to measure the absorbance and concentration of each sample at the optimum wavelength[14][15].

Spot Test and TLC Analysis Result

Spot test analysis produces a purplish blue precipitate. This can be seen in Table 1 below:

Table 1.Spot test result

No.	Sample	Color	Results
1	Phenylpropanolamine HCl Standard	Purplish blue precipitate	++ PPA
2	Urine	Purplish blue precipitate	++ PPA

The mechanism of color formation can be seen in Figure 2 below::

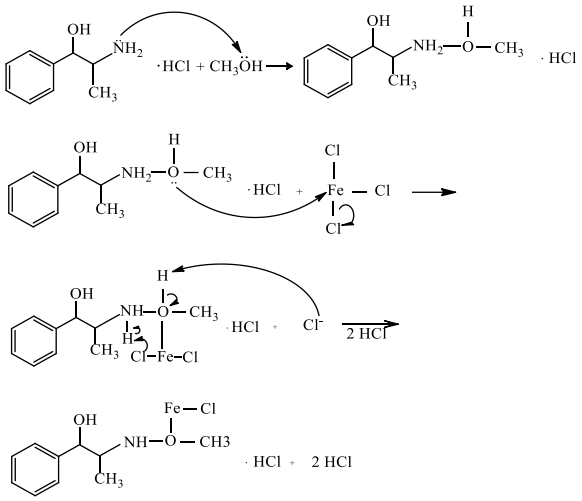

Figure 2. Reaction Mechanism of Phenylpropanolamine Hydrochloride with Iron (III) Chloride[16][2]

Figure 2 shows that a nucleophilic substitution reaction occurs, caused by phenylpropanolamine hydrochloride reacting with methanol where the NH_2 group (N as a nucleophile) attacks OH (O as an electrophile) resulting in an OH group (O as a nucleophile) attacking the Fe group (Fe as an electrophile) then Cl is released which acts as a Leavung Group. As a result of the entry of Fe into the amino acid, it produces a purplish blue precipitate[16][2].

The Rf value from the thin layer chromatography results can be seen in Table 2 below:

Table 2. Rf Values from Thin Layer Chromatography Results

No	Sample	Mobile Phase	
		Spot Distance (cm)	Rf (cm)
1	Phenylpropanolamine HCl Standard	3	0,66
2	Urine	2,9	0,64
	Mean		0,65

The Rf value is calculated based on the distance of the spot with the distance of the solvent, observed with ultraviolet light, spots that can be seen on the surface of the plate either directly or with the help of chemical reagents. In this study, the mobile phase used was chloroform: methanol with a ratio of (8:2), the solvent used in the mobile phase is a solvent that can dissolve urine samples in centrifugation. The adsorbent used is silica gel G which is polar.

UV Result

The results of the optimum wavelength determination test were obtained at a wavelength of 256 nm with an absorbance value of 0.952 (a) and Linear Regression of Standard PPA Solution (b) can be seen in Figure 3 below:

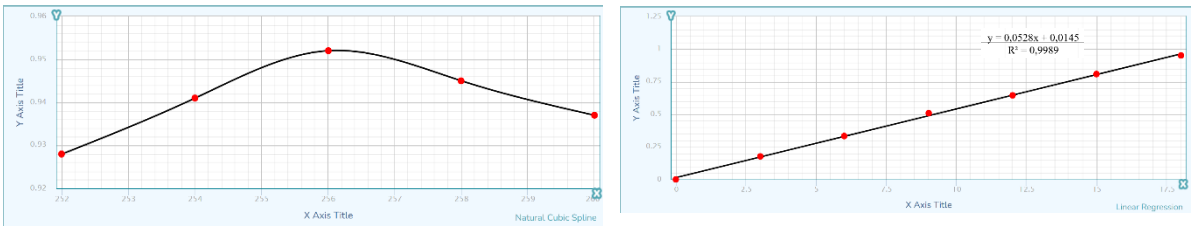

Figure 3. Determination of Optimum Wavelength (a) Linear Regression of Standard PPASolution (b)

The measurement of urine samples from consumers of phenylpropanolamine hydrochloride aims to determine the concentration of phenylpropanolamine hydrochloride in the urine by observing the maximum absorption wavelength of the phenylpropanolamine hydrochloride standard. Monitoring the maximum absorbance value optimizes the quantification of phenylpropanolamine hydrochloride levels using a detector capable of reading compounds that absorb visible and ultraviolet light.Phenylpropanolamine hydrochloride absorbs ultraviolet light due to the presence of chromophore groups within its benzene ring, which are responsible for ultraviolet radiation absorption. The determination of the optimal wavelength was performed using a UV spectrophotometer with ultraviolet light wavelengths.The results yielded a linear calibration curve with a correlation coefficient (r) of 0.9989 and a regression equation of y = 0.0528x + 0.0145, demonstrating a strong linear relationship between the concentration of phenylpropanolamine hydrochloride in the samples and the UV spectrophotometric response.Based on the Linear equation, PPA Concentration in Urine Sample is 10.59 µg/mL.

The detected concentration of phenylpropanolamine hydrochloride (PPA) in the urine sample was measured at 10.59 µg/mL using UV-Vis spectrophotometry. This concentration falls within the expected range for individuals who have recently ingested medications containing PPA, indicating effective absorption and renal excretion of the compound. In the context of forensic chemistry, accurately quantifying PPA levels in biological fluids such as urine is crucial for toxicological investigations, drug compliance monitoring, and potential abuse detection. The ability to determine PPA concentration at this level enables forensic experts to assess exposure, establish timelines of drug intake, and support legal or clinical decisions. Moreover, the non-destructive and rapid nature of UV spectroscopic analysis provides a practical and reliable approach for routine forensic screening, complementing more complex chromatographic techniques. This study reinforces the importance of spectrophotometric methods as valuable tools in forensic toxicology for detecting and quantifying stimulant compounds like PPA in biological matrices.

III. CONCLUSION

This study successfully demonstrates the application of UV-Vis spectroscopy for the quantitative analysis of phenylpropanolamine hydrochloride (PPA) in urine samples (10.59 µg/mL). The method exhibits high sensitivity and specificity, with a strong linear correlation (r = 0.9989) between PPA concentration and absorbance, confirming its suitability for forensic purposes. The detection of PPA at its characteristic maximum absorption wavelength enables rapid, reliable, and non-destructive analysis, making this approach a valuable tool in forensic toxicology for monitoring PPAconsumption. Future work may focus on method validation across diverse biological matrices and enhancing detection limits for trace analysis.

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