International Journal of Chemical and Biomedical Science
2015; 1(1): 1-5
Published online March 10, 2015 (http://www.aascit.org/journal/ijcbs)

Keywords
Glue,
Hippuric Acid,
Color Test,
Ultra Violet –Visible

Received: December 15, 2014
Revised: February 13, 2015
Accepted: February 14, 2015

Qualitative and Quantitative Analysis of Hippuric Acid in Glue Sniffer's Urine Using G.S Kit and UV-Visible Method

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Citation
Abdul Rahim Yacob, Mohamad Raizul Zinalibdin. Qualitative and Quantitative Analysis of Hippuric Acid in Glue Sniffer's Urine Using G.S Kit and UV-Visible Method. *International Journal of Chemical and Biomedical Science.* Vol. 1, No. 1, 2015, pp. 1-5.

Abstract
Inhalant abuse is defined as inhalation of solvents or volatile organic substances for pleasure or short term enjoyment. The most common d material is glue due to its low price and easy access. Glue solvent sniffing produces fast and pleasurable experience to the abuser. Glue normally contains toluene will then metabolite to hippuric acid and excreted in urine for glue sniffer's. A mobile G.S. Kit developed by this group was employed of pyridine, benzenesulphonyl chloride and distilled water and use for screening or qualitative analysis. Survey shows that positive samples of hippuric acid in urines would change the color from yellow to red. All positive samples were brought back to lab for quantitative analysis. In this study, quantitative analysis of hippuric acid using UV-Vis at wavelength 417 nm was determined. The plotted results gave a regression coefficient of 0.994, while the recovery, accuracy and coefficient variance were 96.57%, 2.94% and 0.50% respectively. This method was successful in screening urine samples of suspect toluene abusers or glue sniffers among secondary school children at Johor Bahru and suspect abuser from Hospital Sultanah Aminah with the collaboration of the National Anti Drug Agency.

1. Introduction

Toluene also known as methyl benzene or phenyl methane is a clear insoluble liquid with typical sweet smell of benzene compound [1]. This organic solvent is widely used in printing, painting, automotive, shoemaking, adhesive material and in pharmaceutical industries [2]. In paint and contact adhesives some grades of toluene contain traces of xylene and also benzene [1]. Toluene has a low boiling point, flammable chemical and easy to evaporate. It is commonly sniffed by drug abusers.

In Malaysia, the organic volatile solvent abused has been observed since early 1980s. The problem of solvent abuse is however predominant in East Malaysia (i.e., Sabah and Sarawak) and in Johore, the southern part of West Malaysia which is nearby to Singapore as reported by Navaratnam (1988) [3]. The trend of inhalant abuse in Malaysia however has remained stable during the last 5 years, which is in contrast to other countries like Thailand and Singapore. The types of substances contain solvent of abused include paint thinner, nail polish remover, gasoline and glue. Preventive education on various aspects of inhalant abuse and its effects has been part of the preventive education measure on drug abuse in Malaysia. In the smaller affected states, pamphlets on inhalant abuse have

been promoted and campaigned by the departments of education for use in schools, professionals and teachers.

At presently in Malaysia, no legislation relates specifically to the abuse of inhalants or glue sniffers. Nonetheless, existing legislation (i.e., the Juvenile Courts Act of 1947) could be used to a limited extent to help children or adolescents younger than 16 years old who are inhalant abusers. They can be placed in an approved home by the juvenile court. The police also can charge the glue sniffer under the Minor Offense Ordinance of 1955 if they also disturb the peace once they use high on abuse.

Reported by newspaper three teenagers aged 18 to 20 were found dead nearby school in Cheras, Kuala Lumpur and the Police had found three empty glues can nearby [5]. Report from these newspapers and by previous none published data it is feared that this problem might lead to "time bomb" disasters for the future generation in Malaysia.

Reported by Yacob *et al* (2009), solvent in glue is toluene. Toluene can be absorbed into the blood stream from the lung and the gastrointestinal tract. Brain and liver will serve as then reservoir for toluene [6]. Following inhalation or oral exposure to toluene, approximately 60 – 75% of absorbed toluene is metabolite to benzoic acid [7, 8]. Refer to figure 1, the initial step begin with the side chain oxidation of toluene to benzyl alcohol by cytochrome P450 enzymes. The benzyl alcohol is then oxidized to benzoic acid by alcohol and aldehyde dehydrogenases. Benzoic acid is subsequently conjugated with glycine to form hippuric acid [7] as shown by the reaction in figure 2. Benzoic acid however may also be conjugated with glucuronic acid to form benzoyl glucuronide and excreted in urine. Less than 1% of the absorbed toluene undergoes ring hydroxylation to form o-, and p-cresol, which are also excreted in urine as glucuronide or sulphate conjugates [9, 10,11].

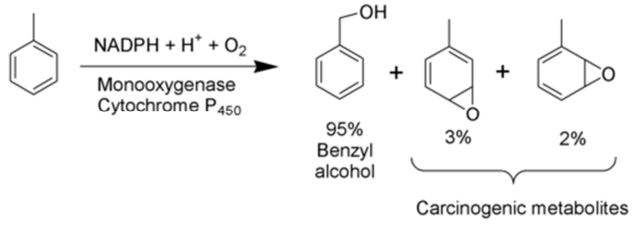

Figure 1. *First step of toluene metabolite*

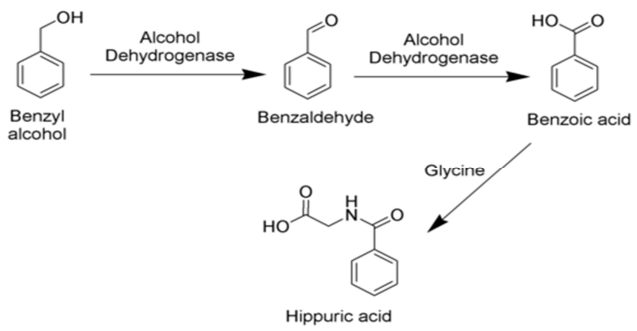

Figure 2. *Second step of toluene metabolite*

Novel color reaction for hippuric acid was first developed by Gaffney *et al* (1954) based on azlactone formation from the reaction of aldehyde with hippuric acid. Here the hippuric acid will convert to a deep orange colored azlactone, 2-phenyl-4-(p-dimethylamino) benzal-5-oxazolone by the reaction of hippuric acid with acetic anhydride and p-dimethylaminobenzaldehyde [12]. Following in 1960, Charles J.U discover new method using pyridine and benzenesulfonyl chloride to develop deep red color in the present of hippuric acid [13].

In this study, the novel color reaction will be applied to determine qualitative and quantitative determination of toluene metabolites in glue sniffer urine using Ultra Violet Visible (UV-Vis) spectroscopy. The method of color test will use pyridine and benzenesulphonyl chloride which will turn yellow to red by addition of distilled water reported by Manabu Yoshida et al (2005). This research will adapt this color reaction which is use as a screening test for glue abuser among the secondary school in Malaysia and might be help the National Anti Drug Agency and Royal Police of Malaysia to prevent the glue abuser among student and teenagers.

2. Experimental

2.1. Reagents

Hippuric Acid 98%, Benzenesulphonyl chloride (BSC) 98%, Toluene 99.3%, and pyridine 98% purchased from Sigma Aldrich (USA).

2.2. Instrument

Ultra Violet Visible (UV-Vis) Perkin Elmer (Germany).

2.3. Determination of Hippuric Acid Using UV-Vis

2.3.1. Sample Selection

Sample urine obtained from secondary school around Johore Bahru, West Malaysia.

2.3.2. Sample Preparation

(i) Urine Sample

0.1 ml of urine sample of will be added to 0.25 ml pyridine and 0.1 ml BSC with 1.0 ml distilled water. Colour obtained then recorded.

(ii) Preparation of Standard Hippuric Acid

12.5 mg, 25.0 mg, 50.0 mg, 100 mg, 150 mg and 200 mg of hippuric acid respectively will be weighed into 100 ml volumetric flask. The different volumetric flask will be label as standard HA 0.125, HA 0.25, HA 0.5, HA 1.0, HA 1.5 and HA 2.0 accordingly. The solution then will be marked up with distilled water. All standards then sonicate and read using UV-Vis Perkin Elmer at wavelength 417nm. The calibrated curve of hippuric acid will then developed and the significance and accuracy of concentration hippuric acid with absorbance obtain.

3. Results and Discussion

3.1. Qualitatively Analysis of screening Urine Sample Using Colour Test Method

The colour test using G.S Kit for screening urine sample was shown by Figure 3. In negative result of hippuric acid, the mixture urine became colourless and transparent. The urine sample A and B however illustrated light red colour which is similar to the hippuric acid calibrated standard. Thus prove the present of hippuric acid in the urine samples. This figure also shows a standard hippuric acid of 2.0 mg/ml has the same colour development.

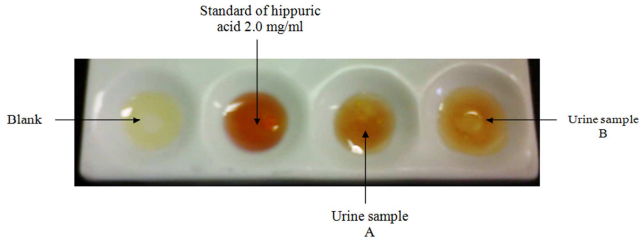

Figure 3. *Colour test for screening urine sample.*

Figure 4 shows the proposed chemical equation between benzenesulphonychloride, pyridine and hippuric acid that took part during in the reaction. In that equation, the chloride ion will attacked the amine group or hydroxyl group to produce hydrochloric acid. Pyridine is thus one of the indicators in this reaction which is similar to phenolphthalein. When the acidic reacted with the pyridine, the red colouration will develop. The equations show that hydrochloric acid that reacts with pyridine will form the red colour. This is an indicative reaction for the presence of hippuric acid in urine glue solvent abuser.

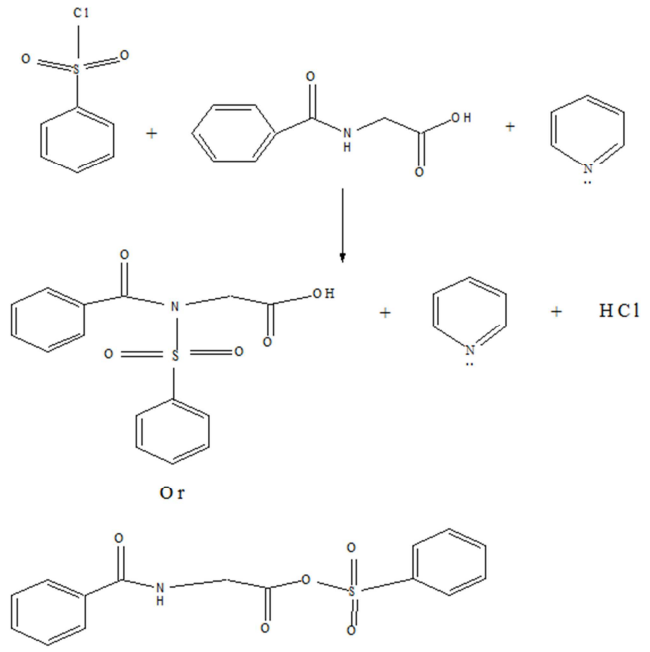

Figure 4. *Chemical equation of colour test method*

3.2. Quantitatively Analysis Calibration of Standard

A color chart was prepared for the semiquantitation of hippuric acid concentration ranging from 0.125 mg/ml to 6.00 mg/ml respectively. The UV-Vis of the mixtures was scanned from 300 to 700 nm using Perkin Elmer Ultra Violet Visible (UV-Vis) spectrometry hippuric acid taken at wavelength 417 nm. Based on the table 1, the color test method was successful to determine the presence of hippuric acid at various concentrations. These results thus proved that this method is suitable to be applied to detect hippuric acid.

Table 1. *Concentration using color test method of hippuric acid at 417 nm wavelength.*

Concentration of Hippuric Acid	Absorbance
0.000	0.000
0.125	0.149
0.250	0.284
0.500	0.337
1.000	0.654
1.500	0.797
2.000	0.993
3.000	1.500
6.000	2.667

From the calibration curve of hippuric acid standard using the colour test method, the R^2=0.9933 and the amount hippuric acid will be calculated using the equation y=0.4324x + 0.1293.

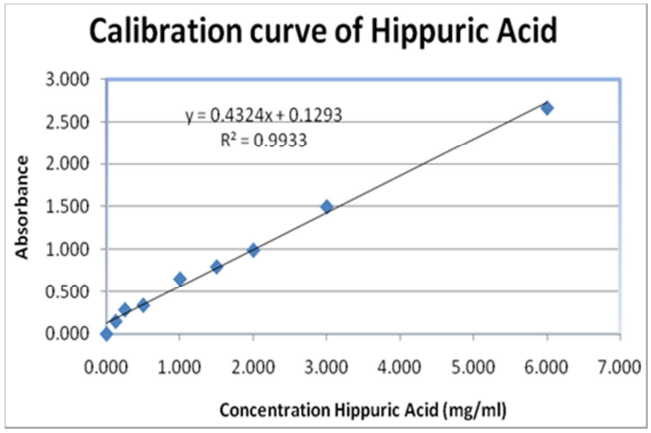

Figure 5. *Calibration curve of hippuric acid using colour test method*

3.3. Result of Screening Sample at Secondary School

22 students were involved in the screening urine test at Seri Rahmat Secondary School, Johore Bahru. A number of 4 students gave positive results for hippuric acid in urine sample while the others gave negative results. At the same time, the National Anti Drug Agency was screening 5 drugs using dip strip kit to the same samples and none of the students are tested positive. The positive hippuric acid sample will be further analysing quantitatively using Ultra Violet Visible (UV-Vis) available in the lab. The colour reaction is shown in Figure 6. Even though, the colour test

method is quite useful for qualitative analysis, the reddish colour of the reaction mixture faded gradually, so that semi quantification should be performed as soon as possible.

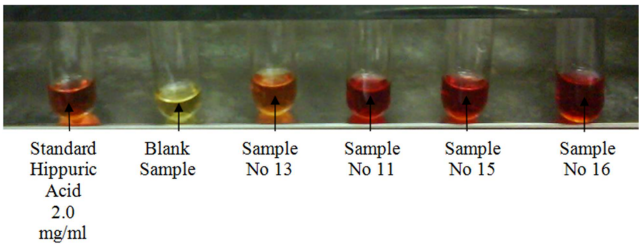

Figure 6. *Colour test for screening urine sample at secondary school*

3.4. Result of Quantitatively of Hippuric Acid Using Colour Test by Ultra Violet Visible

Based from the screening urine test using color test method the result show four students gave the positive of existence of hippuric acid. From the calibration curve of hippuric acid using color test method the equation of y=0.4324x + 0.1293 the amount hippuric acid will be calculate with the R^2=0.9933. Calculation amount of hippuric acid in urine has been show at Table 2.

Table 2. *Result of amount of hippuric acid using novel color reaction screening urine sample at secondary school.*

Sample number	Amount of hippuric acid (mg/ml)
11	3.35
13	1.40
15	2.54
16	3.00

Based from the Manabu *et al.,* (2005) stated that the amount of hippuric acid level more than 2.0 mg/ml indicates the sniffering of toluene with high probability and that a level from 1.0 to 2.0 mg/ml suggests the possibility of toluene abuse. Based from the article, the normal human body will produce at least 0.10 mg/ml hippuric acid per day [14]. The result shows in the Table 3.1.6 that three of the students have high probability of toluene abuse while others have possibility of toluene abuse.

4. Conclusion

For screening of glue abuser urines, in this study, G.S Kit color test has been used successfully. The quantitative analysis of hippuric acid using Ultra Violet Visible (UV-Vis) at wavelength 417 nm was also successful. It is calculated and by the intensity of red coloration, a graph of calibration was plotted. The coefficient value (R^2) for this method was determined at 0.9933 which indicates linearity and precision.

By this study, the secondary schools screened, four of them gave positive results for hippuric acid. Three of them have high probability of toluene abuse while one gave possibility of toluene abuser.

This novel color test thus useful for screening of glue sniffer and toluene abuse qualitatively and quantitatively in a way to help the National Anti-Drug Agency, Department of Education and Royal Police of Malaysia to prevent and stop this problem before become pandemic.

Acknowledgment

Special thanks to National Anti Drugs Agency (AADK) for collaboration in this research, financial support from Ministry of Science Technology and Innovation (MOSTI) and Department of Chemistry, Johore branch.

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