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ORIGINAL ARTICLE

Detection of hippuric acid: A glue solvent metabolite, using a mobile test kit

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Abstract Many testing kits for screening common drugs are widely used in Malaysia, such as the dip strip urine drug testing kit which is specified for detecting drug in abuser. However, currently there is no specific testing kit for screening urine samples to detect glue abusers. This research is dedicated to develop a specific color test method to emphasize the screening for glue abusers, known as G.S. Kit. It contains benzenesulphonyl chloride, pyridine and distilled water. The urine sample is mixed with pyridine and benzenesulphonyl chloride and followed by distilled water. A positive result will be in the form of a color reaction from yellow to red. This novel G.S. Kit would be quite useful for the screening and detection of glue sniffing and toluene abusing qualitatively, as a way of helping National Anti Drug Agency, Department of Education and Royal Police of Malaysia in preventing glue sniffing from becoming a social pandemic.

1. Introduction

Toluene is also known as methyl benzene or phenyl methane. It is a clear and water insoluble liquid with a distinguished sweet smell of the existing benzene compound (Ikeda et al., 1990). This chemical is widely used as an organic solvent in printing, painting, automotive, shoemaking, adhesive material and pharmaceutical industries (Serap et al., 2001). Normally, toluene is found in many products including paint and contact adhesives as a solvent. Besides that, some grades of toluene contain traces of xylene and benzene (Ikeda et al., 1990). It is also a common substance in glue and paint thinner that are sniffed by drug abusers.

Toluene abuse via glue sniffing is a growing problem and is considered nowadays as drug abusing in Malaysia. Even though it is an alternative to drugs, the national registry of abusers in Malaysia does not require the reporting of glue sniffers thus, there is no national statistics on the extent and nature of the problem (Sharifah, 1990). Based on newspaper articles, three teenagers aged 18–20 were found dead near a school in Cheras, Kuala Lumpur and Police found three cans of glue nearby (Jasmine, 2005). By the information from the newspapers and electronic media it is feared that glue sniffing problem

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might lead to bigger disasters for future generations in Malaysia.

Yacob and Zinalibdin (2009) have described that toluene is a major compound that gets absorbed in the body of glue sniffers when they sniff glue through the nose (Yacob and Zinalibdin, 2009). Due to this reason, this research will focus on the metabolites of toluene which are hippuric acid and benzoic acid. The pharmacokinetics of toluene is explained below.

Toluene can be absorbed into blood directly from the lung, the gastrointestinal tract, mucosa and via physical penetration through the skin. The brain and liver serve as reservoirs for toluene (Ameno et al., 1989). Yamazaki et al. (1992) in their research explained that more than 80% of the absorbed toluene is metabolized by mixed-oxidase enzyme system to benzoic acid and hippuric acid in the liver and kidney before being excreted as part of urine (Yamazaki et al., 1992). Some absorbed toluene (0.4–1.1%) is hydroxylated and excreted as a mixture of ortho-, para-, meta-cresol Anderson and Loomis, 2003.

Among the metabolites, hippuric acid is a traditional biomarker in the biological monitoring of toluene exposure but *p*-cresol and hippuric acid are used by the endogenous and dietary sources (Zuppi et al., 2003).

In order to aid the enforcement agencies to prevent glue abuse a new color test method to test the presence of toluene metabolites is employed by using pyridine and benzenesulphonyl chloride with distilled water. In the presence of hippuric acid, the reaction develops a deep red color as described by Yoshida et al. (2001). This test was first developed by Umberger and Fiorese (1960) and then modified by Yoshida by adding distilled water to observe the color change using naked eyes Umberger and Fiorese, 1960.

Yoshida et al. (2001) in his research did not illustrate with pictures the color test method. It focussed on samples of people exposed to toluene but did not mention the equation and reaction of the color test method. Besides that, they did not discuss clearly the analytical method for qualitative analysis using a spectrophotometer. Yacob and Zinalibdin (2010) have illustrated good and comprehensive results for the color test method compared to Yoshida and they have done quantitative analysis using UV–Vis but did not deliver their research about G.S. Kit Yacob and Zinalibdin, 2010.

However, this research was expanded to include the equation and reaction of the color test method, focussing on urine sample of glue abusers and explained deeper into the quantitative analysis of hippuric acid using UV–Vis. In addition, this research developed a simple mobile test kit for screening and detecting glue sniffers based on the color test method. The result obtained using the G.S. Kit is similar to UV–Vis result but is faster with the result obtained within only 5 min and is also comparable to the dip strip urine drug testing kits. Besides this, this kit can be a fast and in situ tool to help the National Anti Drug agency for screening and detecting glue abusers among school students and teenagers.

2. Experimental

2.1. Reagents

Hippuric acid 98%, benzenesulphonyl chloride (BSC) 98%, and pyridine 98% purchased from Sigma Aldrich.

2.2. Equipment

Sample bottle, rubber teat, micro pipette, pipette tips and pasture pipette are part of the G.S. Kit and Ultra Violet Visible (UV–Vis) Perkin Elmer.

2.3. Development and validation of standard series color chart of G.S. Kit

2.3.1. Preparation of hippuric acid standard

An amount of 12.5, 25.0, 50.0, 100, 150, 200 and 300 mg of hippuric acid was weighed into seven different 100 mL volumetric flasks. The volumetric flasks were labelled as hippuric acid (HA) 0.125, HA 0.25, HA 0.5, HA 1.0, HA 1.5, HA 2.0 and HA 3.0. The solution was filled to the mark with distilled water and then all standards were sonicated in a waterbath.

2.3.2. Preparation of hippuric acid standard for color test method

A quantity of 0.1 mL hippuric acid standard for HA 0.125, HA 0.25, HA 0.5, HA 1.0, HA 1.5, HA 2.0 and HA 3.0 were

Figure 1 G.S. Kit picture.

added to 0.25 mL pyridine and 0.1 mL BSC with 1.0 mL distilled water.

2.4. Determination of hippuric acid in urine using G.S. Kit

2.3.4. Sample selection

The samples were received from screening urine samples from students in secondary schools around Johor Bahru, Malaysia.

2.3.5. Manual instructions of G.S. Kit

(a) 0.1 mL urine samples and 2.0 mg/mL of hippuric acid standard were pipetted into the sample bottles.
(b) 0.25 mL pyridine 98% was added to the sample bottles containing urine and hippuric acid standard.
(c) 0.1 mL benzenesulphonyl chloride 98% and 1 mL of distilled water were added to the mixtures.
(d) Lastly, the mixture color of the mixture changes from yellow to red if hippuric acid is present in the urine samples. The color of the original urine samples was compared to the hippuric acid standard to detect glue abuse.

2.3.6. Safety precautions

(a) The G.S. Kit is to be stored at room temperature.
(b) The person conducting the analysis should wear a safety mask along with safety glove as a safety precaution from chemical exposure.
(c) The analysis must be conducted at the lab or open space with a good ventilation system.
(d) The mobile kit must be stored at an appropriate place away from the children.

3. Results and discussion

3.1. G.S. Kit-mobile test kit

3.1.1. G.S. Kit

The G.S. Kit-mobile test kit contains chemicals such as benzenesulphonyl chloride, pyridine and distilled water in 10 mL amber bottles, 20 sets of 5 mL sample bottles, a micro pipette, a pasteur pipette, rubber teat and pipette tips along with the standard series color chart of hippuric acid concentrations. Other than that, a manual instruction and safety precaution booklet on how to use the kit, mask and rubber glove were also provided. Fig. 1 illustrates a picture of the G.S. Kit.

3.1.2. Standard series color chart of hippuric acid concentration

A light yellow color appears if the sample contains a low concentration of hippuric acid. The yellow color turns red as the concentration of hippuric acid increases. If there is no hippuric acid in the sample, the mixture will become colorless and transparent. The color change between 0.125 and 3.0 mg/mL hippuric acid standards is shown in Fig. 2. The result of the urine samples was compared to the series of the color chart. Limit of detection (LOD) hippuric acid using color test method is 0.125 mg/mL. There is no color change in the mixture even though the analysis is repeated several times.

3.2. Result of screening urine samples

3.2.1. Screening urine sample using color test method

The color test for screening urine sample is shown in Fig. 3 by using the G.S. Kit. If there is no presence of hippuric acid in urine sample, the mixture becomes colorless and transparent. The urine samples A and B illustrated light red color similar to the color of the standard hippuric acid of 2.0 mg/mL. Thus proving that hippuric acid is present in these samples. The figure shows the color change without adding distilled water and is useful only to differentiate the positive and negative results but not the concentration of hippuric acid present. Because of this, Yoshida et al. (2001) added distilled water to differentiate the red color development if high concentrations of hippuric acid are present and it is shown clearly in Fig. 5.

The reaction of the color test method gives the red color development. Fig. 4 shows the chemical equation between benzenesulphonylchloride, pyridine and hippuric acid. In the equation, the chloride of benzenesulphonylchloride attacks the amine group or hydroxyl group of hippuric acid and forms an ester or ether group. Then, the chloride atom forms hydrochloric acid. Pyridine is an acid indicator for this reaction as well as phenolphthalein which develops the red color. that indicates the presence of hippuric acid in the urine samples.

Although the red color formed was from reaction of pyridine with hydrochloric acid it cannot distinguish the level of hippuric acid concentration. Thus, Yoshida et al. (2001) came up with a new solution of adding distilled water to the mixture. Distilled water is slightly acidic and it has hydrogen ion (H^+), hydroxide ion (OH^-) and some metals. By addition of distilled

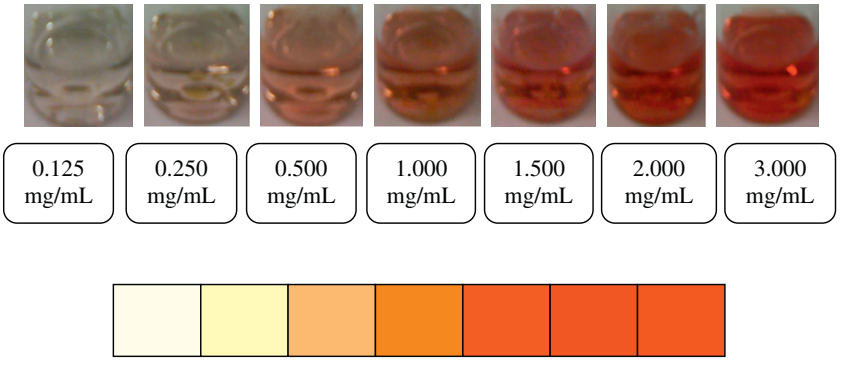

Figure 2 Standard series color chart of hippuric acid concentrations.

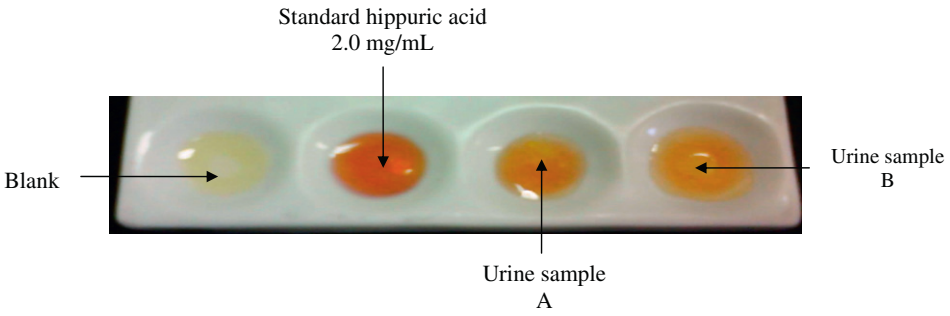

Figure 3 Color test for screening urine sample.

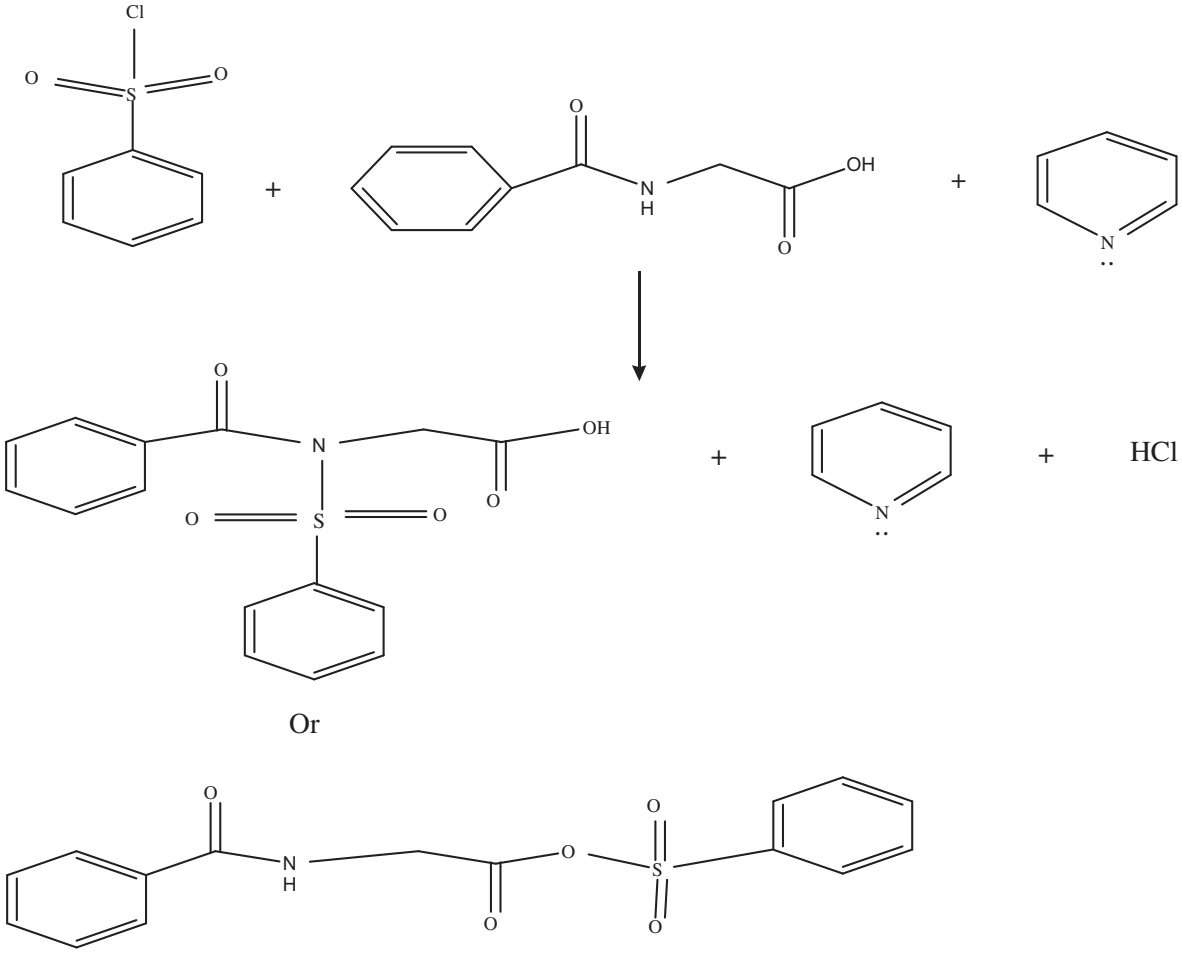

Figure 4 Chemical equation of color test method.

water, acidic value of the mixture will increase and the red color formed is based on the hippuric acid concentration.

3.3. Result of screening sample at secondary school

Forty four students were involved in the screening urine test using G.S. Kit at a secondary school in Johor Bahru. Four students gave positive results for hippuric acid in the urine samples while others gave negative results. At the same time, the National Anti Drug Agency was conducting a screening test for 5 drugs using the dip strip kit on the same samples and none of the students tested positive. The positive hippuric acid sample was analyzed quantitatively using Ultra Violet Visible (UV–Vis) spectrometer. The color test reaction is shown in Fig. 5. Even though, the color reaction is quite useful for qualitative analysis, the color of the reaction mixture will fad gradually. So, semi quantification should be performed as soon as possible. The G.S. Kit is a fast tool for detecting glue abuse with the results obtained within 5 min.

3.3.1. Color test for hippuric acid using UV–Vis

The UV–Vis spectra of the mixtures were recorded at 300–700 nm using Perkin Elmer Ultra Violet Visible (UV–Vis). A color chart for the semiquantitation of hippuric acid was pre-

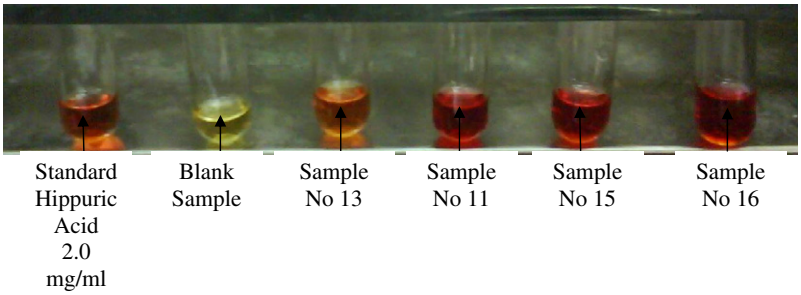

Figure 5 Color test for screening urine sample at the secondary school.

Table 1 Concentration of hippuric acid standard using color test method 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

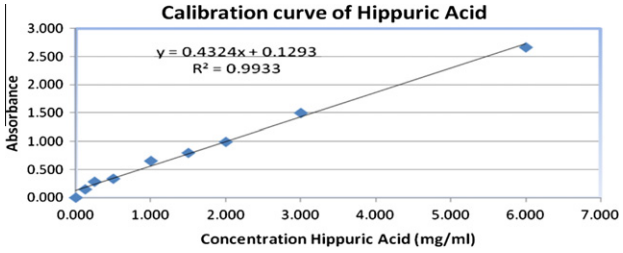

Figure 6 Calibration curve of hippuric acid using color test method.

pared using standard samples of 0.125, 0.25, 0.50, 1.0, 1.50, 2.0, 3.0 and 6.0 mg/mL hippuric acid. Table 1 shows the concentration of hippuric acid using color test method at wavelength 417 nm. From the table, it is obvious that the color test method has successfully determined the presence of hippuric acid at different concentrations relative to the intensity of red color developed by the mixture.

Fig. 6 demonstrates the calibration curve of hippuric acid using color test method with correlation value of hippuric acid concentration and absorbance. It is useful to determine hippuric acid concentration qualitatively.

Based onthe screening urine test using G.S. Kit the result shows that four students gave the positive presence of hippuric acid that can be determined by calculation based on calibration curve equation. Calculated amount of hippuric acid in urine is shown in Table 2.

A few articles have defined the detected amount of hippuric acid to be considered as glue abuse. Yoshida et al. (2001) suggested that 2.0 mg/mL hippuric acid indicated high probability of being a glue sniffer while 1.0–1.5 mg/mL suggested the possibility of being a glue sniffer. Normal level of hippuric acid produced by the human body is 0.1 mg/mL per day.

Table 2 Result of amount of hippuric acid using color test method by UV–Vis for screening urine sample at secondary school.

Sample number	Sample amount, mg/mL ± RSD*
	Hippuric acid
11	3.35 ± 0.03
13	1.40 ± 0.009
15	2.54 ± 0.01
16	3.00 ± 0.03

* Based on triplicate samples.

Based on the stated definition, the results in Table 2 show the test for the positively tested students. Three of the students have high probability of being glue sniffers while one has the possibility of being a glue sniffer. From the qualitative and quantitative analysis of hippuric acid, it can be concluded that these 3 students may be positive glue sniffers and from this, it was proved that the G.S. Kit provides significant and accurate result, good linearity and detection limit comparable to UV–Vis instrument.

4. Conclusion

The color test method produced by the G.S. -mobile test kit is very useful, and fast with good linearity and accuracy. This kit is useful for detecting and screening glue sniffer's urine. Other than that, this kit has been patented by KASS SDN BHD as a patent product and the ID number is PI 20101467. The G.S. Kit might be used by National Anti Drug Agency and Royal Police of Malaysia forpreventing glue abuse among students and teenagers.

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