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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.