CHUGOKUGAKUEN J. 2007
Vol. 6, pp. 1-4


CHUGOKUGAKUEN Journal
http://www.cjc.ac.jp/

*Original Article*

# Storage of Authentic Sample for Biological Monitoring of Organic Solvents

**Yoshihiro Shimada**

*Department of Human Nutrition, Faculty of Contemporary Life Science, Chugokugakuen University, Okayama 701-0197, Japan*

**An external standard added the hippuric acid, m-methylhippuric acid and mandelic acid to the artificial urine and human urine were prepared for the quality control of biological monitoring. Thereafter, the specimens were kept at 25℃ for 1, 2 and 4 weeks after freeze-drying, and change in concentrations during storage was examined by high performance liquid chromatography.**
**The ratio of the concentrations of the three acids and creatinine after freeze-drying compares to that before storage was about 99 per cent or above under the storage condition at 25℃ for 4 weeks.**

**Key Words:** artificial urine, freeze-drying, quality control, hippuric acid, m-methylhippuric acid, mandelic acid

## Introduction

A quality-control program concerned with toxicological analysis in occupational medicine is a systematic attempt to assure the precision and accuracy of the data by detecting determinate errors in analysis, preventing their recurrence, and quantitating their impact. Two concepts must be considered: accuracy and precision [1]. In several countries, national federations, international societies, as well as commercial concerns have conducted quality-control surveys in occupational health. [2, 3, 4, 5, 6].

In Japan, industrial health control legally began with monitoring of the working environment and health surveillance [7, 8]. A detailed explanation of environmental monitoring and the method of collecting air in the working environment was made by Sakabe [9]. Recently, industrial health control has involved control of the working environment, work practice management and health care [10]. The actual work situation, including the working environment, work methods, working conditions, and health risk will be evaluated from the results of biological monitoring [11].

Toluene, m-xylene, ethylbenzene and styrene are widely employed as industrial organic solvents, but it is quite difficult to estimate the average atmospheric concentrations of these solvents in the workplace since the concentrations tend to vary considerably within a working day. It is possible, however, to calculate the amounts of the solvents retained in the body after being inhaled and/or absorbed via the skin from the amounts of metabolites excreted in urine. Urinary metabolites have been used to determine concentrations of hippuric acid (HA) from toluene, m-methylhippuric acid (m-MHA) from m-xylene, and mandelic acid (MA) from ethylbenzene and styrene.

In the present report, an external standard added the hippuric acid, m-methylhippuric acid and mandelic acid to the artificial urine and human urine

Corresponding author.
Yoshihiro Shimada, PhD.
Department of Human Nutrition, Faculty of Contemporary Life Science, Chugokugakuen University, 83, Niwase, Okayama 701-0197, Japan
Tel & FAX; +1 86 293 0247

were prepared for the quality control of biological monitoring, and they were freeze-dried. The stability of these specimens was examined.

## Materials and Methods

### *1. Reagents*

All the reagents used were of reagent grade. HA, m-MHA, MA, creatinine and sodium l-decanesulfonate (for ion-pair chromatography) were obtained from the Tokyo Kasei Co., Tokyo.

### *2. Artificial urine*

The artificial urine which was used in this study contained the components shown in the table 1. Urea was first dissolved in a Na-K phosphate buffer (pH 7.4), and then the other components were dissolved in distilled water.

Table 1 Components of artificial urine

| | |
|---|---|
| Sodium chloride | 11.6 g |
| Diammonium hydrogenphosphate | 2.0 g |
| Urea | 18.0 g |
| Tartrazine | 100 mg |
| Sodium azide | 1.0 g |
| Creatinine | 1.0 g |
| Uric acid | 0.25 g |
| Potassium dihydrogenphosphate | 0.7 g |
| Sodium hydrogenphosphate 12-water | 5.3 g |
| total | 1 ℓ |

### *3. Addition concentration of metabolites*

Three kinds of solutions for HA, m-MHA and MA were made: a high concentration, an inside concentration, and a low concentration. HA was dissolved in artificial urine to obtain a final concentration of 2.42g/l and 0.97g/l and in human urine to obtain a final concentration of 2.46g/l, 1.82g/l and 1.25g/l. The m-MHA was dissolved in artificial urine to obtain a final concentration of 1.46g/l and 0.50g/l and in human urine to obtain a final concentration of 1.53g/l, 1.01g/l and 0.50g/l. MA was dissolved in artificial urine to obtain a final concentration of 0.97g/l and 0.29g/l and in human urine to obtain a final concentration of 1.02 g/l, 0.65g/l and 0.30g/l. The concentrations of creatinine were 0.88, 0.23 and 0.44g/l.

### *4. Storage condition of sample*

The samples were preserved for one, two and four weeks at 25℃ after freeze-drying.

### *5. High performance liquid chromatography*

Analysis of urinary metabolites: Urine specimens were diluted 100-fold with distilled water. The diluted samples were centrifuged at 2,000rpm × 5min and 10 $\mu$l of the supernatant thus obtained were used for HPLC.

An analysis of urinary metabolites by automated HPLC components (Toyo Soda Co, Tokyo) has been described previously [12]. A stainless steel column ($\phi$ 4.6mm × 150mm) packed with octadecyl-silanized silica gel (TSK gel, ODS-80 TM, 5 $\mu$m, Toyo Soda Co), and with a jacket attached for temperature control, was used throughout the investigation. The flow rate was 0.7ml/min, producing a pressure of 100kg/cm$^2$ in the separation procedure. The column temperature was 25℃. The effluents of urinary metabolites were monitored at a wave length of 225nm. To separate urinary creatinine and organic acids, a mixed solution of [20mM $KH_2PO_4$ (pH3.3) containing 3mM sodium 1-decanesulfonate]/$CH_3CN$ (85/15) was used as a mobile phase.

## Results

Artificial urine and human urine containing HA, m-MHA, MA and creatinine was preserved for cer-

Table 2 Changes of the hippuric acid (HA), m-methylhippuric acid (m-MHA), mandelic acid (MA) and creatinine (Cr) in the artificial urine at 25℃ after freeze-drynig

| | 0 | 7 days | 14 days | 28 days |
|---|---|---|---|---|
| HA (g/l) | 2.42 | 2.42±0.15 | 2.42±0.07 | 2.42±0.05 |
| | 100% | 100% | 100% | 100% |
| m-MHA (g/l) | 1.46 | 1.46±0.09 | 1.46±0.04 | 1.46±0.02 |
| | 100% | 100% | 100% | 100% |
| MA (g/l) | 0.97 | 0.97±0.06 | 0.97±0.03 | 0.97±0.02 |
| | 100% | 100% | 100% | 100% |
| Cr (g/l) | 0.88 | 0.87±0.06 | 0.87±0.02 | 0.87±0.02 |
| | 100% | 99% | 99% | 99% |

| | 0 | 7 days | 14 days | 28 days |
|---|---|---|---|---|
| HA (g/l) | 0.97 | 0.97±0.05 | 0.97±0.06 | 0.97±0.03 |
| | 100% | 100% | 100% | 100% |
| m-MHA (g/l) | 0.50 | 0.50±0.03 | 0.50±0.03 | 0.50±0.02 |
| | 100% | 100% | 100% | 100% |
| MA (g/l) | 0.29 | 0.29±0.02 | 0.29±0.02 | 0.29±0.01 |
| | 100% | 100% | 100% | 100% |
| Cr (g/l) | 0.88 | 0.87±0.03 | 0.88±0.03 | 0.87±0.02 |
| | 100% | 99% | 100% | 99% |

Numbers are the initial value and measured values (mean ± SD).

Table 3 Changes of the hippuric acid (HA), m-methylhippuric acid (m-MHA), mandelic acid (MA) and creatinine (Cr) in human urine at 25℃ after freeze-drying

| | 0 | 7 days | 14 days | 28 days |
|---|---|---|---|---|
| HA (g/l) | 2.46 | 2.46±0.06 | 2.46±0.05 | 2.46±0.08 |
| | 100% | 100% | 100% | 99% |
| m-MHA (g/l) | 1.53 | 1.55±0.04 | 1.53±0.03 | 1.51±0.05 |
| | 100% | 101% | 100% | 99% |
| MA (g/l) | 1.02 | 1.03±0.03 | 1.02±0.02 | 1.02±0.03 |
| | 100% | 101% | 100% | 100% |
| Cr (g/l) | 0.23 | 0.23±0.02 | 0.23±0.02 | 0.23±0.03 |
| | 100% | 100% | 100% | 100% |

| | 0 | 7 days | 14 days | 28 days |
|---|---|---|---|---|
| HA (g/l) | 1.82 | 1.82±0.03 | 1.82±0.06 | 1.80±0.03 |
| | 100% | 100% | 100% | 99% |
| m-MHA (g/l) | 1.01 | 1.01±0.02 | 1.01±0.03 | 1.00±0.02 |
| | 100% | 100% | 100% | 99% |
| MA (g/l) | 0.65 | 0.65±0.01 | 0.65±0.02 | 0.64±0.01 |
| | 100% | 100% | 100% | 99% |
| Cr (g/l) | 0.44 | 0.44±0.02 | 0.44±0.04 | 0.43±0.01 |
| | 100% | 100% | 100% | 99% |

| | 0 | 7 days | 14 days | 28 days |
|---|---|---|---|---|
| HA (g/l) | 1.25 | 1.25±0.04 | 1.24±0.06 | 1.24±0.04 |
| | 100% | 100% | 99% | 99% |
| m-MHA (g/l) | 0.50 | 0.50±0.02 | 0.49±0.03 | 0.49±0.02 |
| | 100% | 100% | 99% | 99% |
| MA (g/l) | 0.30 | 0.30±0.01 | 0.29±0.02 | 0.29±0.01 |
| | 100% | 100% | 99% | 99% |
| Cr (g/l) | 0.44 | 0.44±0.04 | 0.44±0.07 | 0.44±0.04 |
| | 100% | 100% | 100% | 100% |

Numbers are the initial value and measured values (mean ± SD).

tain periods at 25℃ after freeze-drying. Changes in the concentrations of three metabolites and creatinine are shown in the tables 2 and 3.

In the case of artificial urine, the ratio of the concentrations of HA, m-MHA and MA after storage for four weeks compared to that before storage was 100% in high, inside, and low concentrations. Creatinine rations stayed above 99% until the fourth week.

In the case of human urine, the ratio of the concentrations of HA, m-MHA and MA after storage to those before storage remained above 99% until the fourth week in high, inside, and low concentrations. Creatinine remained above 99% until the fourth week.

## Discussion

Three metabolites, HA, m-MHA and MA, can be preserved in artificial urine and human urine for four weeks at the storage condition of 25℃ after freeze-drying. The advantages of using the artificial urine are that the true values can be obtained because a known quantity of metabolites of organic solvent is added, and that preservation is easy because there is little degeneration by rot [13].

In freeze-drying of authentic samples for biological monitoring of organic solvents, there is merit in that the samples can be preserved at the normal temperatures. This enables world wide transportation of samples.

Creatinine can also be preserved for four weeks at the storage condition of 25℃ after freeze-drying. Creatinine adjustment has been thought to be an effective measure in case of the spot urine specimen, which is very concentrated or diluted. Therefore, freeze-drying is from this respect to the effective method.

## References


1. Kleinmann MT and Linch AL: Quality control laboratory licensure and accreditation. In: Kneip J. Crable V (eds) Methods for biological monitoring. A manual for assessing human exposure to hazardous substances. American Public Health Association. Washington, DC. (1988) pp 81-92.
2. Schaller KH, Angerer J, Lehnert G, Valentin H and Weltle D: External quality control programmes in the toxicological analysis of biological material in the fields of occupational medicine-experiences from three round-robins in the Federal Republic of Germany. Fresenius J. Anal. Chem. (1987) **326**, 643-646.
3. Weber JP: An interlaboratory comparison programme for several toxic substances in blood and urine. Sci. Total Environ (1988) **71**, 111-123.
4. Anglov TH, Christensen JM and Holst E: Lyophilized human whole blood for internal and external quality assurance of lead in blood assays. Fresenius J. Anal. Chem. (1990) **338**, 530-533.
5. Harada A: Quality control of analytical procedures used in biological monitoring in Japan. In: Fiserova-Bergerova V, Ogata M (eds) Biological monitoring of exposure to industrial chemicals. American Conference of Governmental Industrial Hygienists, Inc. Cincinnati, Ohio (1990).
6. Herber R, Stoeppler M and Tonks DB: Cooperative interlaboratory surveys of the determination of cadmium in whole blood. Fresenius J. Anal. Chem. (1990) **338**, 269-278.
7. Japan Industrial Safety and Health Association. Industrial Safety and Health Law and related legislation of Japan. Industrial Safety and Health Association. Tokyo (1983) pp 426-499.
8. Industrial Health Division, Industrial Safety and Health Department, Labor Standards Bureau and Ministry of Labor. Working environ-

mental measurement system in Japan. Japan Association for Working Environment Measurement Tokyo (1986) pp 1-3.
9. Sakabe H: Transactions of the forty-first annual meeting of the American Conference of Governmental Industrial Hygienists. ACGIH. Chicago, Illinois, (1979) pp 71-82.
10. Sakabe H, Ikeda M, Kosi S, Tada O, Toyama T and Hara I: Working environment measurement system in Japan. Japan Association for Working Environment Measurement. Tokyo (1991) pp 1-10.
11. Zielhuis RL and Henderson PT: Definitions of monitoring activity and their relevance for the practice of occupational health. Int. Arch. Occup. Environ. Health (1986) **57**, 249-257.
12. Ogata M and Taguchi T: Quantitation of urinary metabolites of toluene, xylene, styrene, ethylbenzene, benzene and phenol by automated high performance liquid chromatography. Int. Arch. Occup. Environ. Health (1987) **59**, 263-272.
13. Shimada Y: Preparation of standard for biological monitoring of organic solvent. Chugokugakuen J. (2005) **4**, 23-26.

**Accepted March 30, 2007.**