CHUGOKUGAKUEN J. 2005
Vol. 4, pp. 23-26

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

Original Article

Preparation of Standard for Biological Monitoring of Organic Solvent

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Artificial urines containing major components of human urine, as ingredients, were prepared as specimens for the survey of quality control. The hippuric acid, m-methylhippuric acid and mandelic acid of various concentrations were spiked into the artificial urines. Thereafter, the specimens were kept at 30 °C, 1 °C and −20 °C for 1, 2 and 4 weeks, and change in concentrations during storage was examined by high performance liquid chromatography. The ratio of the concentrations of the three acids after storage to those before storage was about 99 per cent or above under the storage condition at 1 °C for 2 weeks and −20 °C for 4 weeks. Crcatinine was more unstable than the three acids under these storing condition at 30 °C and 1 °C. The metabolites were more stable when they were kept in lower temperatures.

Key Words: artificial urine, quality control, hippuric acid, m-methylhippuric acid, mandelic acid, creatinine

Introduction

In Japan, industrial health control is accomplished by control of the working environment, work practice management and health care. Periodical biological monitoring of workers exposed to lead and eight popular organic solvents, including mixed solvents, became mandatory as of October l, 1989, in accordance with an ordinance issued by the Ministry of Labor.

A quality-control programme 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, quality-control surveys in occupational health, organized by national federations and by international societies as well as on a commercial basis, have been carried out. [2, 3, 4, 5, 6].

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 this study I tried to prepare new artificial urine containig ingredients of urea and creatinine. Moreover, Na-K phosphate buffer was added instead of sulfur (pH

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4.0) for neutralization. The stability of the three solvent metabolites prepared by this artificial urine was examined for the survey of quality control.

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.

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 2.5 g/l, 1.73 g/l and 0.96 g/l of final concentration, and m-MHA was dissolved in artificial urine to obtain 1.50 g/l, 0.95 g/l and 0.48 g/l of final concentration, and MA was dissolved in artificial urine to obtain 1.00 g/l, 0.64 g/l and 0.29 g/l of final concentration. The concentrations of creatinine were 0.94, 0.98 and 0.97 g/l at 30 °C, 1 °C and − 20 °C, respectively.

4. Storage condition of sample

The samples were preserved for one, two and four weeks at 30 °C, 1 °C and − 20 °C.

5. High performance liquid chromatography

Analysis of urinary metabolites: Urine specimens were diluted 100-fold with distilled water. The diluted samples were centrifuged at 2000 rpm × 5 min and 10 μl of the supernatant thus obtained were used for HPLC.

Table I Components of aritificial 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 hydogenphosphate 12-water	5.3 g
total	1 *l*

An analysis of urinary metabolites by automated HPLC components (Toyo Soda Co, Tokyo) has been described previously [7]. A stainless-steel column (ϕ 4.6 mm × 150 mm) packed with octadecyl-silanized silica gel (TSK gel, ODS-80 TM, 5 μm, Toyo Soda Co.) and with a jacket attached for temperature control was used throughout the investigation. The flow rate was 0.7 ml/min, producing a pressure of 100 kg/cm^2 in the separation procedure. The column temperature was 25 °C. The effluents of urinary metabolites were monitored at a wave length of 225 nm. To separate urinary creatinine and organic acids, a mixed solution of [20 mM KH_2PO_4 (pH 3.3) containing 3 mM sodium 1-decanesulfonate]/CH_3CN (85/15) was used as a mobile phase.

Results

Artificial urine containing HA, m-MHA, MA and creatinine was preserved for a certain period under various conditions. Changes in the concentrations of three metabolites and creatinine in artificial urine are shown in the Table 2, 3 and 4.

In the case of storage condition at 30 °C, the ratio of the concentrations of HA, m-MHA and MA after storage to those before storage were former 98% until one week in a high concentration, an inside concentration and a low concentration. HA and m-MHA decreased up to 94% in two weeks. Creatinine decreased from 83% to 92%. In the case of storage condition at 1 °C, HA, m-MHA and MA were former 97% until four weeks in a high concentration, an inside concentration and a low concentration. The change of creatinine showed from 93% to 99%. In the case of storage condition at − 20 °C, HA, m-MHA and MA kept former 97% until four weeks in high concentration and an inside concentration and a low concentration. The concentration of creatinine kept 97% or more.

Discussion

The artificial urine in this study is the one that the uric acid and the creatinine of a density almost near human urine were added to past artificial urine and sulfuric acid was changed into Na-K phosphate buffer. The advantages of using this 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. On the other

Table 2 Changes of the hippuric acid (HA), m-methylhippuric acid (m-MHA), mandelic acid (MA) and creatinine (Cr) in the case of strage condition at 30 °C.

	0	7 days	14 days	28 days
HA (g/l)	2.50	2.45 ± 0.04	2.44 ± 0.01	2.47 ± 0.04
	100%	98%	98%	99%
m-MHA (g/l)	1.50	1.47 ± 0.03	1.47 ± 0.01	1.47 ± 0.02
	100%	98%	98%	98%
MA (g/l)	1.00	0.98 ± 0.02	0.98 ± 0.01	0.96 ± 0.02
	100%	98%	98%	96%
Cr (g/l)	0.94	0.78 ± 0.01	0.71 ± 0.01	0.65 ± 0.09
	100%	83%	76%	69%

	0	7 days	14 days	28 days
HA (g/l)	1.73	1.70 ± 0.05	1.68 ± 0.04	1.70 ± 0.02
	100%	98%	97%	98%
m-MHA (g/l)	0.95	0.93 ± 0.03	0.92 ± 0.02	0.95 ± 0.01
	100%	98%	97%	98%
MA (g/l)	0.64	0.63 ± 0.01	0.62 ± 0.01	0.62 ± 0.01
	100%	98%	97%	97%
Cr (g/l)	0.98	0.89 ± 0.02	0.81 ± 0.02	0.71 ± 0.01
	100%	91%	83%	73%

	0	7 days	14 days	28 days
HA (g/l)	0.96	0.93 ± 0.03	0.91 ± 0.05	0.92 ± 0.02
	100%	97%	94%	96%
m-MHA (g/l)	0.48	0.46 ± 0.01	0.45 ± 0.03	0.46 ± 0.01
	100%	97%	94%	97%
MA (g/l)	0.29	0.28 ± 0.01	0.28 ± 0.02	0.28 ± 0.01
	100%	97%	97%	97%
Cr (g/l)	0.97	0.90 ± 0.02	0.80 ± 0.04	0.73 ± 0.02
	100%	92%	83%	75%

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 the case of strage condition at 1 °C.

	0	7 days	14 days	28 days
HA (g/l)	2.50	2.48 ± 0.06	2.50 ± 0.05	2.50 ± 0.08
	100%	99%	100%	100%
m-MHA (g/l)	1.50	1.49 ± 0.04	1.50 ± 0.03	1.49 ± 0.05
	100%	99%	100%	99%
MA (g/l)	1.00	0.99 ± 0.03	1.00 ± 0.02	0.97 ± 0.03
	100%	99%	100%	97%
Cr (g/l)	0.94	0.91 ± 0.02	0.90 ± 0.02	0.87 ± 0.03
	100%	97%	96%	93%

	0	7 days	14 days	28 days
HA (g/l)	1.73	1.71 ± 0.03	1.71 ± 0.06	1.71 ± 0.03
	100%	99%	99%	99%
m-MHA (g/l)	0.95	0.94 ± 0.02	0.94 ± 0.03	0.95 ± 0.02
	100%	99%	99%	100%
MA (g/l)	0.64	0.63 ± 0.01	0.63 ± 0.02	0.63 ± 0.01
	100%	99%	99%	99%
Cr (g/l)	0.98	0.94 ± 0.02	0.96 ± 0.04	0.97 ± 0.01
	100%	96%	98%	99%

	0	7 days	14 days	28 days
HA (g/l)	0.96	0.95 ± 0.04	0.95 ± 0.06	0.94 ± 0.04
	100%	99%	99%	98%
m-MHA (g/l)	0.48	0.47 ± 0.02	0.47 ± 0.03	0.47 ± 0.02
	100%	99%	99%	99%
MA (g/l)	0.29	0.29 ± 0.01	0.29 ± 0.02	0.29 ± 0.01
	100%	100%	100%	100%
Cr (g/l)	0.97	0.95 ± 0.04	0.96 ± 0.07	0.96 ± 0.04
	100%	98%	99%	99%

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

Table 4 Changes of the hippuric acid (HA), m-methylhippuric acid (m-MHA), mandelic acid (MA) and creatinine (Cr) in the case of strage condition at − 20 °C.

	0	7 days	14 days	28 days
HA (g/l)	2.50	2.48 ± 0.15	2.48 ± 0.07	2.48 ± 0.05
	100%	99%	99%	99%
m-MHA (g/l)	1.50	1.49 ± 0.09	1.49 ± 0.04	1.49 ± 0.02
	100%	99%	99%	99%
MA (g/l)	1.00	1.00 ± 0.06	1.00 ± 0.03	0.98 ± 0.02
	100%	100%	100%	98%
Cr (g/l)	0.94	0.92 ± 0.06	0.92 ± 0.02	0.91 ± 0.02
	100%	98%	98%	97%

	0	7 days	14 days	28 days
HA (g/l)	1.73	1.71 ± 0.05	1.71 ± 0.06	1.73 ± 0.03
	100%	99%	99%	100%
m-MHA (g/l)	0.95	0.94 ± 0.03	0.94 ± 0.03	0.96 ± 0.02
	100%	99%	99%	101%
MA (g/l)	0.64	0.64 ± 0.02	0.63 ± 0.02	0.63 ± 0.01
	100%	99%	99%	99%
Cr (g/l)	0.98	0.95 ± 0.03	0.97 ± 0.03	0.98 ± 0.02
	100%	97%	99%	100%

	0	7 days	14 days	28 days
HA (g/l)	0.96	0.95 ± 0.04	0.95 ± 0.01	0.94 ± 0.07
	100%	99%	99%	98%
m-MHA (g/l)	0.48	0.47 ± 0.02	0.47 ± 0.01	0.47 ± 0.04
	100%	99%	99%	99%
MA (g/l)	0.29	0.29 ± 0.01	0.29 ± 0.01	0.29 ± 0.02
	100%	100%	100%	100%
Cr (g/l)	0.97	0.96 ± 0.04	0.96 ± 0.01	0.97 ± 0.07
	100%	99%	98%	100

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

hand, the demerit is that the authorization of the accuracy of the method of measurement is insufficient because the urine element is fewer than human urine.

Three metabolites can be preserved until one week at the storage condition at 30 °C and until two weeks at the storage condition at 1 °C, but creatinine is unstable at 30 °C. Consequently, the storage condition at 1 °C is safe.

Three metabolites can be preserved for four weeks at the storage condition at − 20 °C. However, it is necessary to do the process of the decompression and freezing carefully.

Creatinine adjustment has been thought to be an effective measure in case of the spot urine specimen, which is very concentrated or diluted. In this study, it turned out that creatinine was more unstable than the metabolites. Therefore, the correction with the creatinine is possible only by − 20 °C.

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Accepted March 31, 2005.