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Use of a Holder-vacuum Tube Device to Save On-site Hands in Preparing Urine Samples for Head-space Gas-chromatography, and Its Application to Determine the Time Allowance for Sample Sealing

Toshio KAWAI[1], Kimiaki SUMINO[1], Fumiko OHASHI[2] and Masayuki IKEDA[2]*

[1]Osaka Occupational Health Service Center, Japan Industrial Health Safety and Health Association, 2–3–8 Tosabori, Nishi-ku, Osaka 550-0001, Japan
[2]Kyoto Industrial Health Association (Head office), Kyoto 604-8472, Japan

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Abstract: To facilitate urine sample preparation prior to head-space gas-chromatographic (HS-GC) analysis. Urine samples containing one of the five solvents (acetone, methanol, methyl ethyl ketone, methyl isobutyl ketone and toluene) at the levels of biological exposure limits were aspirated into a vacuum tube via holder, a device commercially available for venous blood collection (the vacuum tube method). The urine sample, 5 ml, was quantitatively transferred to a 20-ml head-space vial prior to HS-GC analysis. The loaded tubes were stored at +4°C in dark for up to 3 d. The vacuum tube method facilitated on-site procedures of urine sample preparation for HS-GC with no significant loss of solvents in the sample and no need of skilled hands, whereas on-site sample preparation time was significantly reduced. Furthermore, no loss of solvents was detected during the 3-d storage, irrespective of hydrophilic (acetone) or lipophilic solvent (toluene). In a pilot application, high performance of the vacuum tube method in sealing a sample in an air-tight space succeeded to confirm that no solvent will be lost when sealing is completed within 5 min after urine voiding, and that the allowance time is as long as 30 min in case of toluene in urine. The use of the holder-vacuum tube device not only saves hands for transfer of the sample to air-tight space, but facilitates sample storage prior to HS-GC analysis.

Key words: Biological marker, Head-space gas-chromatography, Holder for blood collection, Vacuum tube, Urine sampling

Introduction

Head-space gas-chromatography (HS-GC) has been winning popularity typically in biological exposure monitoring for organic solvents. The use was not limited to occupational health where the method was originally developed[1–6], but also in environmental health fields[7, 8]. The method has been applied to analysis for un-metabolized mother chemicals[1, 9–12] as well as for metabolites after proper automated derivatization of metabolites to increase volatility[13–16].

Subject to physical characteristics of target analyses, the method generally needs pipetting work to take a portion of the sample (typically urine) to a head-space vial (HS-vial) immediately after sampling to avoid possible loss into or contamination from surrounding air[1]. Under practical conditions of occupational health service, however, such is not always easy to achieve quickly enough.

*To whom correspondence should be addressed.
E-mail: tkawai@jisha.or.jp

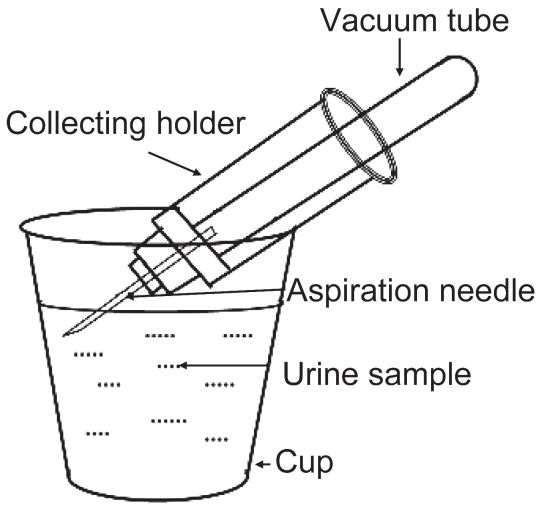

Fig. 1. Concept of taking a sample by the vacuum tube method.
Note that the picture is made transparent for better understanding.

To solve the problem, trials were made in the present study to apply a commercially available inexpensive combination of a holder and a vacuum tube (the pair which has been popularly used for venous blood sampling in clinical practice; Fig. 1) to save time and to avoid possible spoilage of hands. The successful application to 5 solvents of biological monitoring importance is presented in this report.

As discussed above, the allowance in time for transferring a urine sample aliquot into an air-tight space is a practical question for determination of solvents in urine by HS-GC. Through application of the vacuum tube method, it was possible to measure the time limit below which solvent loss remained insignificant.

Materials and Methods

Selection of test solvents and their concentrations in urine

Five organic solvents of acetone (ACET), methanol (MeOH), methyl ethyl ketone (MEK), methyl isobutyl ketone (MIBK) and toluene (TOL) were selected as they are target organic solvents of biological monitoring listed by Japan Society for Occupational Health[17], American Conference of Governmental Industrial Hygienists[18], or both. Lower values were taken as the biological exposure limits in case the values were different between the two organizations (i.e., the case of MIBK) (Table 1). An additional selection criterion was that all of them were analyzable on a single column and sensitive to flame-ionization detection as to be described later.

Table 1. Test solvents and concentrations

Solvents tested	B'OEL[a] (mg/l)	BEI[b] (mg/l)	Concentration tested (mg/l)	Limit of detection (mg/l)
Acetone	40	50	39.5	0.6
Methanol		15	15.0	1.0
Methyl ethyl ketone	5	2	1.9	0.1
Methyl isobutyl ketone	1.7	2	2.0	0.06
Toluene	0.06	—[c]	0.06	0.005

[a]Biological occupational exposure limits by Japan Society for Occupational Health (2009).
[b]Biological exposure indices by American Conference of Governmental Industrial Hygienists (2009).
[c]Not described.

Materials

Blood collecting holders (Venoject II holder) and vacuum tubes (Venoject II; aspiration volume, 5 ml) (the holder-vacuum tube device) were commercially available products of TERUMO Corporation, Tokyo, Japan. It was considered important to use the devices within the guaranteed time period. Head-space vials (HS-vial; 20 ml in capacity) were from Agilent Technologies, Santa Clara, CA, U.S.A.

Sampling procedures

Organic solvents were dissolved in urine samples at the concentrations described in Table 1. In case of least hydrophilic toluene, for example, 10 μl, e.g., of toluene was first dissolved in ca. 990 ml water in a 1,000 ml volumetric flask by repeated stirring, and the total volume was adjusted to 1,000 ml by addition of water.

A 5-ml aliquot was transferred into a HS-vial by use of a pipette (the pipette method), and the vial was sealed tightly with a septum and an aluminum cap as designed. Alternatively, the sample was first aspirated into a vacuum tube by use of a holder-vacuum tube device (the vacuum tube method) (the concept is depicted Fig. 1). The tube (containing a 5-ml aliquot of the urine sample) was stored in a refrigerator at +4°C as to be detailed below until analysis. At the time of the analysis, the sample in the tube was manually transferred to a HS-vial as quantitatively as possible, which was then sealed as designed.

Analytical instruments and conditions

The method employed was basically as previously described (Kawai *et al*. 1996). In practice, a gas-chromatogram (5890 Series II, Agilent Technologies, Santa Clara, CA, U.S.A.) with a flame-ionization detector (FID) was connected with an automated head-space air sampler (HP7694, Agilent Technologies, Santa Clara, CA, U.S.A.) and equipped with a polar column of

DBWAX (Agilent Technologies, Santa Clara CA, U.S.A.) (60 m, 0.53 mm and 1.0 μm in length, inner diameter and film thickness, respectively). H_2 was supplied at a rate of 40 ml/mim. Make-up gas was N_2 at a rate of 30 ml/min, and He gas was allowed to flow at a rate of 5.0 ml/min. The detector and the injection port were maintained at 200 and 250°C, respectively.

Under standard conditions, the sampling valve and the transfer line were kept at 100 and 120°C. Vials were equilibrated for 30 min at 60°C. Times for vial pressurization, loop fill and loop equilibration were 0.2, 0.2 and 0.005 min, respectively. A 1-ml aliquot headspace air was introduced in the split-less mode within a few seconds (He gas was allowed to flow through the sampling loop for 1 min). The column temperature was initially kept at 60°C for 10 min, was elevated at a rate of 10°C/min to reach 180°C, kept there for 1 min, elevated at a rate of 40°C/min to reach 200°C, and kept there for 1 min to be cooled down to the initial temperature. One analysis was completed in 32 min including cooling-down time.

The retention times for ACET, MeOH, MEK, MIBK and TOL were 7.6, 9.6, 9.9, 13.5 and 14.6 min, respectively. No tailing was observed in the chromatograms.

It was confirmed in a preliminary experiment that the peak area in the gas-chromatogram was proportional to the solvent concentration in the vial at least up to 1.5 times the target concentrations given in Table 1. It was also confirmed that the peak area did not differ whether the solvents were dissolved in re-distilled water or in urine (data not shown).

Reagents

The five authentic solvents, acetone (ACET, purity 99.9%), methanol (MeOH, 99.7%), methyl ethyl ketone (MEK, 99% pure), methyl isobutyl ketone (MIBK, 99.5%) and toluene (TOL, 99%) were purchased from Wako Pure Chemical Ltd., Osaka, Japan. Urine samples were obtained from non-exposed men.

Results and Discussion

Comparison of on-site time necessary for sample preparation by the vacuum tube method with that by the pipetting method

Comparison of practice by the vacuum tube method with that by the conventional pippetting method showed that about 24 s was necessary for the pipetting method per urine sample (including the time for sealing with a septum and an aluminum cap) to transfer into a HS-vial (or about 4 min for ten samples), while it was about 15 s per sample (or 2.5 min for ten samples) for the vacuum tube method to take into a vacuum tube. Further manual transfer from the vacuum tube to a HS-vial was necessary (about 2.5 min for ten samples), but it was possible to do so after transport of the sample-containing vacuum tubes to an analytical laboratory.

Whereas shortening of time for the on-site first stage urine sample preparation appeared to be rather limited, no need of skilled hands for sampling should be taken as significant contributions to facilitate the sampling procedures in a potentially congested sampling site and at the time close to the end of a shift of the day. Thus, occupational health nurses, who are familiar with the use of the holder-vacuum tube device for blood sampling, should be readily able to do the task. Even urine sample donors themselves would treat their own urine samples when properly trained. In this respect, reduced risk of spoiling hands with urine samples should be another substantial advantage of practical significance. In such cases, however, blunting of the needle in the holder must be positively recommended.

No loss of solvents by use of the holder-vacuum tube device during sample preparation

To examine if any loss would be induced when the device be employed, five urine samples each (impregnated with solvents at the concentrations given in Table 1) were prepared, and the samples (5 ml each) were transferred either directly by pipetting to each HS-vials, or first aspirated into the vacuum tubes and then manually transferred to the vials. The comparison of the results (Table 2) showed that no significant (p>0.10) loss in the solvent concentrations was induced by use of the vacuum tubes, irrespective of lipo- or hydro-philicity of the solvents (i.e., TOL vs. ACET), suggesting that the use of vacuum tube would not induce significant loss in solvent concentration.

Possible use of one holder for several urine samples

Table 2. No loss due to use of vacuum tube

Solvent	Pipetting[a]	Vacuum tube[b]	p[c]
	AM[d] ± ASD	AM[d] ± ASD	
ACET	100 ± 2.8	101.4 ± 1.7	>0.10
MeOH	100 ± 2.4	99.1 ± 2.3	>0.10
MEK	100 ± 2.5	100.3 ± 1.6	>0.10
MIBK	100 ± 4.5	96.6 ± 2.3	>0.10
TOL	100 ± 6.1	98.7 ± 3.1	>0.10

[a]By use of a pipette, a 5-ml aliquot of the solvent impregnated urine sample (n=5; for concentrations, see Table 1) was transferred to a HS-GC vial.
[b]A 5-ml portion of the solvent-impregnated urine sample (n=5) was aspirated into a vacuum tube, which then was quantitatively transferred by hand to a HS-GC vial.
[c]p for the difference between a and b.
[d]AM value after transfer by pipetting was taken as 100.

should be of practical importance, as allowance of the repeated use would speed up the sampling practice. Thus, urine samples containing solvents at the levels listed in Table 1 were aspirated into a vacuum tube, which were followed by aspiration of (solvent-free) re-distilled water without washing of the holder needle. Analysis of the water sample resulted in no detection of the solvents [i.e., below the corresponding limits of detection (LODs); for LODs, see Table 1], suggesting that there should be no significant effect of carrying over even if one holder be employed for sampling of multiple urine samples.

No significant loss of samples due to manual transfer of urine sample from a vacuum tube to a HS vial

To examine if the same amount of the sample should be aspirated into a vacuum tube and if the sample in the vacuum tube could be quantitatively transferred by hand to a HS-vial, the weights of 5 vacuum tubes were measured before and after aspiration of redistilled water (Columns A and B in Table 3). The increase in weight [i.e., C=(B–A)] was attributed to the amount of water aspirated. The AM value for C was very close to 5 (ml) and the coefficient of variation (CV) was well less than 1%, suggesting that the aspiration of 5 ml by vacuum tube method was quite quantitative.

Separately, the weights of five HS-vials (with no septum or aluminum cap) were measured before and after manual transfer of the samples to vials (Columns D and E); the increase in weight [i.e., F=(E–D)] was taken as the amount transferred. Comparison of C with F in terms of AM (%) and CV (%) indicated that the transfer was quantitative with no significant loss in the volume.

Thus, it was clear that a 5 ml aliquot was quantitatively aspirated by vacuum tube and then transferred to the HS-vial also quantitatively. The observation was in good agreement with the findings for the vacuum tube method in Table 2 that CV values (=ASD/AM in %) were <5% irrespective of the five solvents studied.

No reduction in concentration in the vacuum tube during storage in a refrigerator

It should be of practical convenience if the vacuum tube containing a urine sample could be stored for several days (e.g., over a weekend) when refrigerated. Thus, urine samples containing one of the five solvents at the concentrations listed in Table 1 were aspirated into 20 vacuum tubes and the tubes were kept in a refrigerator at +4°C. After 0 (i.e., immediately after the aspiration), 1, 2 and 3 d of storage, the samples in the tubes were transferred to HS-vials and the vials were kept refrigerated. A preliminary experiment showed that no decay in solvent concentration took place in the HS-vial when refrigerated up to 3 d at shortest. The samples were subjected to HS-GC analyses on the third day. The comparison of the results (Table 4) showed that there was no significant decay in the solvent concentrations (p>0.10) irrespective of types of solvents.

Table 4. % changes in concentration[a]

Days on storage[b]	Solvents dissolved in urine				
	Acetone	Methanol	Methyl ethyl ketone	Methyl isobutyl ketone	Toluene
0	100.0	100.0	100.0	100.0	100.0
1	103.7	101.5	104.0	105.9	106.8
2	106.1	103.3	102.8	111.7	109.1
3	102.5	98.2	103.9	107.3	103.8

[a]The values in the table are percent changes in arithmetic means (n=5) taking Day 0 values as 100%; CVs are less than 5% at largest.
[b]Stored as refrigerated.

Table 3. No significant loss of the sample during the transfer from a vacuum tube to a HS-vial

No.	A[a] (g)	B[b] (g)	C=(B–A) (g)	D[c] (g)	E[d] (g)	F=(E–D) (g)	F/C (%)
1	4.950	9.942	4.992	14.980	19.964	4.984	99.8
2	4.930	9.924	4.994	14.814	19.803	4.989	99.9
3	4.908	9.918	5.010	14.700	19.701	5.001	99.8
4	4.921	9.931	5.010	15.110	20.114	5.004	99.9
5	4.948	9.933	4.985	14.961	19.939	4.978	99.9
AM			4.998			4.991	99.9
ASD			0.011			0.011	0.03
CV(%)			0.22			0.22	0.03

[a]Weight of a vacuum tube before sampling.
[b]Weight of the vacuum tube after sampling.
[c]Weight of a HS-vial (with no septum or aluminum cap) before sample transfer.
[d]Weight of a HS-vial (with no septum or an aluminum cap) after sample transfer.

Table 5. Decay in solvent concentration in urine as a function of time after preparation

Solvents tested	Initial solvent concentration (mg/l)	Duration of time (in minutes) left in urine cup							
		0	5	10	15	20	30	40	60
ACET	39.5	100.0	98.8	96.1*	94.1*	93.4*	91.4**	86.6**	59.6**
MeOH	15.0	100.0	99.6	99.6	103.3	104.1	102.4	103.6	77.1**
MEK	1.9	100.0	98.1	93.9*	93.1*	91.9*	89.3*	84.0**	57.6**
MIBK	2.0	100.0	98.1	93.8	91.5*	89.2*	87.1**	80.5**	53.9**
TOL	0.06	100.0	101.9	106.4	103.8	102.5	98.9	88.3**	52.4**
Temperature[a]		36.0	32.0	31.0	30.0	29.3	28.3	27.5	27.0

Urine samples, spiked with the solvents at the concentrations given in the table and kept at 36°C, were divided into 8 cups (60 ml/cup) and the cups were left at a room temperature (25°C with no significan air flow) for the time periods shown in the table. At each time period, three 5-ml portions were taken by the vacuum tube method and analyzed for solvent concentrations by the HS-GC method. The values in the table are the AMs of triplicate samples, relative to the original concentrations (taken as 100). CVs were <1% in most cases and never exceeded 4%.

** and * show that the values are significantly (** for $p<0.01$, and * for $p<0.05$) lower than the corresponding value at time 0.

[a]Temperature in (°C) of samples in cups.

Measurement of the maximum time allowed for transfer of a urine sample to an air-tight space

Taking advantage of the high performance of the vacuum tube method, a pilot study as an application was conducted to measure time allowance for transferring a urine sample into an air-tight space. For this purpose, a urine sample was spiked with ACET, MeOH, MEK, MIBK and TOL at 39.5, 15.0, 1.9, 2.0 and 0.06 mg/l, respectively, warmed at 36°C (to simulate human body temperature), and divided into 60-ml portions in 8 urine sampling cups. The cups were allowed to stand at a room temperature (25°C). At 0 to 60 min, three samples (5-ml each) were taken by the vacuum tube method from one cup as scheduled, and the urine samples in the tubes (24 tubes in total) were analyzed by the HS-GC method for the five solvents. The results were summarized in Table 5, in terms of the AMs of the three determinations, taking the original solvent concentrations as 100. It is clear from the table that there was no loss of any of the five solvents at the time of 5 min, that statistically significant loss of ACET and MEK was observed at 10 min, and that the loss was significant for all of the five solvents at 60 min. Nevertheless, the time allowance was as long as 30 min for TOL and 40 min for MeOH.

Over-all evaluation of the vacuum tube method, and recommended procedure

It was possible to conclude that the vacuum tube method could sample a 5-ml aliquot of urine very quantitatively and in a hand- and time-saving manner. No spoilage of hands with urine samples should also be a practical advantage. It may be desirable for safety if the sampling needle is blunted.

Recommended procedures prior to HS-GC can be summarized as follows:

1. Collect the urine sample in a sampling cup.
2. Immediately after collection, aspirate a 5-ml portion of the urine sample into a vacuum tube by use of a holder (multiple use of one holder is acceptable). The time allowance is 5 min in general, and 30 min in case of TOL in urine.
3. Within 3 h leaving the sample-containing tube at room temperature, transfer the tube into a refrigerator (at 4°C). Samples may be stored in the tube at least for 3 d without loss when refrigerated.
4. Transfer the sample into a HS-vial manually and seal the vial for GC analysis.

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