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Abstract

Most of the common organic solvents are excreted into urine as metabolites. A correlation exists for several organic solvents between the amount taken in and the amount of metabolites excreted. Many methods have been developed for the measurement of these urinary metabolites. The methods were classified into three group: colorimetry, gas chromatography and high performance liquid chromatography. The characteristics and availability of these methods in a laboratory for routine work were reviewed. The correction equation for the amounts of metabolites in spot urine is discussed.

KEYWORDS: organic solvents, urinary metabolites, colorimetry, gaschromatography, high performance liquid chromatography.

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— REVIEW —

**QUANTITATIVE DETERMINATION OF URINARY
METABOLITES IN SUBJECTS EXPOSED TO
ORGANIC SOLVENTS**

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Abstract. Most of the common organic solvents are excreted into urine as metabolites. A correlation exists for several organic solvents between the amount taken in and the amount of metabolites excreted. Many methods have been developed for the measurement of these urinary metabolites. The methods were classified into three groups: colorimetry, gas chromatography and high performance liquid chromatography. The characteristics and availability of these methods in a laboratory for routine work were reviewed. The correction equation for the amounts of metabolites in spot urine is discussed.

Key words : organic solvents, urinary metabolites, colorimetry, gas chromatography, high performance liquid chromatography.

Organic solvents are widely used in industry. It is difficult to estimate the average concentrations of organic solvents in air since the concentrations vary considerably, especially in places where they are used only intermittently. In order to solve this problem, a personal sampler was devised at first, but in the case of masked workers, estimation of amounts inhaled through the mask by leakage was difficult by the personal sampler. The amount of organic solvent inhaled depends on the volume of inspiration, and the amounts present in the body is related to the toxicity of the solvents. It is more practicable, therefore, to calculate the average concentration of organic solvents inhaled from the metabolites excreted in urine, since according to Roach's theory (1) metabolites are excreted in urine in proportion to the amounts of organic solvents present in the body.

Since Elkins (2) proposed a "biological threshold limit" of environmental pollutants, many investigators have confirmed a validity of the proposal. Some have studied methods for estimating the amount of organic solvents to which workers were exposed from the amount of their urinary metabolites. In a previous report (1962), the authors described the determination of urinary hippuric acid in workers exposed to toluene (3). According to Pagnotte, *et al.*(4), the only report (3) available in the literature on hippuric acid excretion studies performed

TABLE I. ASSAY OF URINARY METABOLITES BY COLORIMETRIC METHODS, WITH OR WITHOUT PC AND TLC

Solvents	Metabolites	Pretreatment	Color developments	Wave length*	Reference
Toluene Xylenes ¹⁾	HA, MHAs ²⁾ (Sum)	PC Butanol/Acetic acid/ Water (4:1:1)	p-Dimethylaminobenzal- dehyde(DAB) Acetic anhydride 135°C	460	(3) (5)
Toluene Xylenes ¹⁾	HA, MHAs ²⁾ (Sep)	Apply extraction with ethyl acetate PC or TLC Toluene/Acetic acid/ Water(100:50:2.3)	DAB Acetic anhydride 135°C	460	(6)
Toluene Xylenes ¹⁾	HA, MHAs ²⁾ (Sep)	TLC Chloroform/Acetone/ Acetic acid/ (40:10:5)	DAB Acetic anhydride Pyridine	460	(7)
Toluene Xylenes ¹⁾	HA, MHAs ²⁾ (Sum)	Dried ethyl acetate extract ^{a)} or urine itself ^{b)}	Benzenesulfonyl chloride (BSCl)	380	a) (6) b) (5)
Toluene Xylenes ¹⁾	HA, MHAs ²⁾ (Sum)	Urine added phosphate buffer, superimposed BSCl sol.	Pyridine-BSCl	380	(8)
Toluene Xylenes ¹⁾	HA, MHAs ²⁾ (Sum)	Ethyl acetate extract dried ethyl acetate extract ^{a)} or urine itself ^{b)}	DAB Pyridine- Acetic anhydrid	460	a) (9) b) (10)
Phenol	Phenol sulfate Phenol glucuronide	Hydrolysate	Gibb's reagents ³⁾	610	(11)

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Trichloro ethylenes	Total trichloro compounds. TCE-OH ⁽¹⁾ TCA	Chromium-trioxide 100°C, 10min=total trichloro compounds (TTCs) Subtract TCA from TTCs No treatment	Pyridine Potassium hydroxide Pyridine-pot. hydroxide ⁽³⁾ Same as above added p-toluidine in formic acid	530 500	(12) (13)
Trichloro ethylene	TCE-OH TCA	Same as above			
Methyl- acetate Methanol	Methanol Methanol**	Addition of sulfuric acid, potassium permanganate and activated charcoal		575	(14)
Carbon disulfide	2-Thiothiazolidine-4-carboxylic acid (15)		Chromotropic acid Iodo-azide	[***] (16)	

PC : paper chromatography TLC : Thin layer chromatography 1) o-, m- and p-xylenes 2) o-, m- and p-MHAs 3) Gibb's reagents; 2,6-dichloroquinone-chloro-imide. 4) TCE-OH= Total trichloro compounds-TCA 5) pot. hydroxide (Potassium hydroxide). (*).nm. (**).not metabolite. (***)time iodine colour disappeared.

TABLE 2. ASSAY OF URINARY METABOLITES BY GASCHROMATOGRAPHY

Organic solvents	Metabolites	Specimen after pretreatment	Column	Temperature	Detectors	Reference
Toluene m-Xylene	HA, and MHAs ⁽¹⁾	Ethyl acetate(EA) extract added diazometan(DM) (added IS : HDA) ⁽¹⁾	Chromosorb W(AW-DMCS) coated with 3% SE30	160°C	FID	(17)

TABLE 2. Continued

Toluene, o-and m-or-p-xylenes	HA, o-and m-or-p-xylenes	EA added DM (added IS ; HDA) ²⁾	Chromosorb W-AW coated with 3% or 10% SE-30	190°C or 200°C	FID	(18)
Benzene or phenol	Phenol sulfite Phenol glucuronide	Acid hydrolysate	Tenax-GC 80-100 mesh	150°C	FID	(19)
Styrene	PGOA, MA	TMS or Me with DM (added IS ; MYA) ³⁾	OV-25 NPGE ⁴⁾ or	150°C or 170°C	FID	(20) (21)
Chlorinated phenol	Chlorinated phenol conjugates	Chloroform-di- ethylene ether extract from acid hydro- lysates	SE-glass Capillary column	90°C -180°C	ECID	(22)
Trichloro ethylene	TCE-OH β -glyceroidase hydrolysate TCA	Extracted DM	Chromosorb W-AW coated with Reoplex 400+H ₃ PO ₄ (15+2%)	95°C	FID	(23)
DMSO DMSO ₂	Dimethyl- sulfoxide Dimethyl- sulfone	Methyl-disulfite added IS	Shimalite W coated with 5% polyethylene glycole	160°C	FPD	(24)
Methyl chloride	5-methyl cystein	Hydlysate headspace	Chromosorb Q coated with 10% OV-101	100°C	FID	(25)

DEGs-Diethylene glycol succinate, IS ; internal standard, PGOA= phenylglyoxalic acid. MA= Mandelic acid, 1) Methyl-hippuric acids. 2) Hepta decanoic acid. 3) myristic acid. 4) NPGE= neopentylglycol succinate on chromosorb w (AW-DMCs) 60-80 mesh.

TABLE 3. ASSAY OF URINARY METABOLITES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Organic solvent	Metabolites	Specimen after pretreatment	Column	Mobile phase	Reference
Toluene, o-and m-or p-xylenes	HA, o-and m-or p-MHAs	Urine or ethyl-acetate extract added IS (o-MHA)	Lichrosorb RP ₁₈ , Nucleosil 5C ₁₈	Methanol/aq/acetic acid (20/80/0.2)	(26, 27, 28) *
Toluene o-, p-, m-xylene	HA, MHAs ¹⁾	MEK extract phenocyl estrification (added IS, MA)	Lichrosorb 5I-100	n-hexane/chloroform (25/175) Lichralorb SI-100	(29) *
Styrene	PG, MA (IS, MHA)	Methanol ethyl alcohol (added IS, MHA)	Nucleosil 5C ₁₈	Aq/acetonitrile/acetic acid (95/5/0.02)	(30) *
Benzene and phenol	Phen, -sulfate			Methanol/aq/acetic acid (50/50/0.2)	
			Nucleosil 5C ₁₈	containing 0.05M tetra-n-butylammonium bromide (TBAB)	(31) *
	Phen, -glucoronide	Urine		Methanol/aq/acetic acid (20/80/0.2) containing TBAB	
Chlorobenzene	p-Chlorophenyl mercapturic acid	Ether extraction	Nucleosil 5C ₁₈	Ethanol/aq/phosphoric acid (50/50/0.26)	(32) *
	Conjugates of 4-chloro catechol	Ether extraction hydrolysate		Methanol/aq (50/50)	**
Trichloroethylene	Trichloro-acetic acid	None	Hitachi gel 2618 (H form)	Phosphoric acid (1%)	(33) ***

Note ; Optical density measured at 245 nm except trichloroacetic acid at 225 nm/1. 1) MHAs o-, m- and p-xylenes, m-, p-MHA can be separated. IS ; Internal standard, MEK ; Methyl-ethyl-ketone, PG ; Phenyl-glyoxilic-acid. MA ; Maledic acid, Phen ; Phenyl. * detected at 254 nm. **detected at 285 nm. ***detected at 220 nm.

on industrial workers is that by Ogata, *et al.*

Up to date, industrial investigators have developed a method for detection and quantitation of urinary metabolites in workers as indices of exposure to organic solvents. An advantage of this method is that the urine specimen can be taken without giving pain to the subject. In estimating solvents in the body from metabolites, however, investigators have to consider errors introduced by difference in metabolic rate by factors such as age and sex.

In this report, the authors summarize assay methods for urinary metabolites of most organic solvents for the convenience of industrial investigators. The assay methods for the metabolites of most of the widely used organic solvents are classified into three kinds and summarized in Tables 1, 2, and 3.

Colorimetric determination (Table 1) (5-16) is widely used and needs the simple equipment available in any laboratory. The metabolites of toluene and xylene are measured together in urine as hippuric acid (HA) and methyl hippuric acid (MHA). For the separation of HA and MHA, paper chromatography (PC) and thin-layer chromatography (TLC) are necessary as pretreatment of samples. The addition of pyridine, p-dimethylaminobenzaldehyde (DAB) and acetic anhydride to glycine conjugates gives the most stable color development. The trichloroethylene (TCE) metabolites were detected by hydrolysis of the glucuronide of trichloroethanol (TCE-OH) in urine, these oxidization to trichloroacetic acid (TCA) by chromium trioxide. The TCA thus produced is allowed to react with pyridine and potassium hydroxide in a boiling water bath, and the product is reacted with p-toluidine hydrochloride in formic acid. In urine containing TCE-OH glucuronide and TCA, total trichloro-compounds are obtained after their oxidation to TCA. The assay of TCA is done as described above except that the digestion step is omitted. The difference between the two results is taken as an estimate of the TCE-OH content. The sensitivity of this reaction is about four times that of the pyridine potassium hydroxide reaction and the color which develops is stable (13).

Excellent analytical sensitivity and specificity ought to be possible with gas-chromatographic methods (GC); however, pretreatment with carcinogenic diazomethane for methylesterification of HA, MHA and TCA is necessary. TCE-OH glucuronides are hydrolyzed by β -glucuronidase to TCE-OH (Table 2) (17-25).

High performance liquid chromatography (HPLC) has been widely used recently for analysis of organic solvents. Non-volatile metabolite in urine can be assayed by this method without special pretreatment. However, metabolites of aliphatic hydrocarbons are less sensitive compared to those of aromatic hydrocarbons, because detection in HPLC is based on the absorption of ultraviolet light and aliphatic hydrocarbons have no maximum absorption peak at ultraviolet wave-lengths (Table 3) (26-33).

The values obtained by three methods were compared, and analyzed statistically. Based on the results, conversion equations were derived for calculation

as in the case of HA (34).

Some correction for the amount of urinary metabolite from a sample of spot urine should be made when calculating. The author has found that the excretion rate of hippuric acid from human subjects following inhalation of toluene is a more consistent measurement than normalization by the use of specific gravity. The distribution of the normalized "rate" of a minute amount of urinary hippuric acid and that normalized by specific gravity were compared to that of "unnormalized" (Table 4). The standard deviation and relative standard deviation became less in general in the descending order of "rate", urinary concentration corrected for specific gravity and urinary concentration, uncorrected. The results in Table 4 also indicate a parallel relationship between toluene density and urinary hippuric acid in all of the parameters (35).

TABLE 4. AMOUNT OF HIPPURIC ACID IN THE URINE FOLLOWING TOLUENE EXPOSURE

Exposure period (hours)	0-3		4-8	
Toluene exposure (ppm)	100	200	100	200
HA conc, uncorrected (mg/liter)				
Mean	2.95	3.74	3.09	8.19
S.D. ¹⁾	0.83	0.59	0.70	2.62
Relative S.D. ²⁾	0.28	0.15	0.23	0.32
Specific gravity, corrected (mg/liter) ³⁾				
Mean	2.58	3.71	2.81	5.85
S.D.	0.40	0.67	0.66	1.24
Relative S.D.	0.16	0.18	0.23	0.21
Rate (mg/min)				
Mean	2.09	3.13	3.10	4.61
S.D.	0.35	0.36	0.84	0.80
Relative S.D.	0.17	0.12	0.27	0.17

Urine taken from subjects during 0-3 and 4-8 h exposure with one break of an hour.

1) S.D.=standard deviation. 2) Relative S.D.=S.D./Mean 3) Specific gravity, corrected=1.024.

$$\text{Corrected value} = \text{Observed value} \times \frac{24}{(\text{Specific gravity}-1) \times 10^3}$$

Conc; concentration.

Measurement of each metabolite in considering exposure to mixed vapors is necessary. When the effect of each solvent is considered to be additive, for example, toluene and xylene, other have reported estimation of the combined MCA (35). The MAC (maximum allowable concentrations) for toluene and xylene are different in various countries so it is necessary to estimate hippuric and methyl hippuric acids separately. It is likely that the toxicities of toluene and xylene are additive, and the estimated total exposure should be calculated similarly.

The fraction of combined MAC (maximum allowable concentration) likely to have been present can be calculated from the following equation.

$$\frac{\text{Concentration of hippuric acid}}{\text{Mean concentration of hippuric acid for maximum allowable concentration of toluene}} + \frac{\text{Concentration of methyl hippuric acid}}{\text{Mean concentration of methyl hippuric acid for maximum allowable concentration of xylene}} = \text{Fraction of combined maximum allowable concentration.}$$

Values of the fraction greater than 1 would indicate that the subject had probably been exposed to a total concentration greater than the combined MAC (35).

Recently mixed solvent has come to be widely used in workshops. Medical supervisors should be aware of the effects of mixed solvents. The measurement of each metabolite is necessary.

The assay of urinary metabolites of organic solvents can serve as a diagnostic procedure. Quantitation of metabolites is important to estimate the body burden, as an "inhalation index" or "body burden index" rather than an "exposure index", since the amount of urinary metabolites parallels the amount of organic solvent in the body. Therefore, appropriate method should be used at the discretion of medical investigators and supervisors. The estimation of urinary metabolites, therefore, provides valuable information which serve to verify organic solvent exposure and inhalation in the individual workers, as in the case of toluene (36).

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