Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 854 (2007) 204–210
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

# SPE–GC/FTD determination of *N*-methyl-2-pyrrolidone and its metabolites in urine

Ryuichi Kubota [a], Yoko Endo [a,*], Akito Takeuchi [b], Yoshinori Inoue [c], Hiroko Ogata [a], Masanori Ogawa [a], Tomoo Nakagawa [d], Nobuhiko Onda [a], Ginji Endo [c]

[a] *Research Center for Occupational Poisoning, Tokyo Rosai Hospital, Japan Labour Health Organization, 4-13-21 Ohmori-Minami, Ota-ku, Tokyo 143-0013, Japan*
[b] *Chugoku-Shikoku Safety and Health Center, Japan Industrial Safety and Health Association, Japan*
[c] *Department of Industrial Medicine, Graduate School of Medicine, Osaka City University, Japan*
[d] *Yokohama City Institute of Health, Japan*

Received 14 November 2006; accepted 16 April 2007
Available online 25 April 2007

**Abstract**

An analytical method using a combination of solid-phase extraction (SPE) and gas chromatography with a flame thermionic detector (GC/FTD) was developed for determination of *N*-methyl-2-pyrrolidone (NMP), *N*-methylsuccinimide (MSI), and 2-hydroxy-*N*-methylsuccinimide (2-HMSI) in human urine. The SPE cartridge of poly(divinylbenzene/hydroxymethacrylate) used was directly loaded with urine sample, followed by elution with methyl isobutyl ketone (MIBK) and subsequent centrifugation, and the supernatant was injected into the capillary GC using a DB1701. This method allowed efficient separation of NMP, MSI, and 2-HMSI, which were nearly free of interference by other GC peaks arising from urine. Recoveries of NMP, MSI, and 2-HMSI from the SPE cartridge were about 98, 101, and 67%, respectively, with limits of detection of 0.04, 0.02, and 0.06 mg/L, respectively, which met the regulatory requirements. The present method was used for assay in biological monitoring of workers exposed to NMP in their occupational environment.


*Keywords:* NMP; *N*-Methyl-2-pyrrolidone; *N*-Methylsuccinimide; 2-Hydroxy-*N*-methylsuccinimide; Solid-phase extraction; Flame thermionic detector; Gas chromatography

## 1. Introduction

*N*-Methyl-2-pyrrolidone (NMP) has been used in many industries as an organic solvent for dissolution of various resins [1–3]. NMP has also been reported to be a potential skin penetration enhancer in humans [4]. The recent annual volume of production of NMP is estimated to be 9000 tons in Japan [5] and 38,000 tons in the European Union [6]. Those workers are at high risk of exposure to NMP who work in places where NMP is manufactured or used. The Maximale Arbeitsplatz Konzentration (MAK) value for NMP was set at 20 ppm (82 mg/m$^3$) based on reproductive toxicity observed in animal experiments by Deutsche Forschungsgemeinschaft (DFG) in Germany [7,8], while the Japan Society for Occupational Health has recently recommended an occupational exposure limit (OEL) for NMP of 1 ppm because of a human case of stillbirth and feasible exposure levels [9].

NMP is rapidly absorbed via inhalation, ingestion, and dermal penetration [10,11]. In human urine, NMP and three metabolites, 5-hydroxy-NMP (5-HNMP), *N*-methylsuccinimide (MSI), and 2-hydroxy-MSI (2-HMSI), have been identified following exposure to NMP [12,13]. The proportions detected were 0.5–2% as NMP, 44–58% as 5–HNMP, 0.1–2% as MSI, and 20–37% as 2–HMSI [11–15]. The biological half-lives of NMP and these metabolites in urine are 3.2–4.5, 4–7.3, 8, and 17 h, respectively [11–15]. All three metabolites have been suggested to be suitable for use as biomarkers of NMP exposure [11,13–15]. As the metabolite with highest concentration, 5-

* Corresponding author. Tel.: +81 3 3742 7301; fax: +81 3 5735 9977.
*E-mail address:* yokoendo@tokyoh.rofuku.go.jp (Y. Endo).

1570-0232/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2007.04.022

HNMP was proposed for use as a Biological Exposure Index for exposure to NMP vapor by ACGIH in 2006 [16]. However, Jönsson, and Åkesson [17] reviewed the biomarkers to NMP exposure and reported that 2-HMSI in plasma and urine is appropriate as a biomarker from the toxicokinetic perspective. Concentrations of 2-HMSI were found to strongly correlate with exposed NMP concentration. In addition, there exist the following advantages of 2-HMSI for use in biological monitoring: (1) it has the longest half-life, which is useful for repeated exposure in the workplace; (2) it is less sensitive to sampling timing; and (3) it has the highest concentration next to 5-HNMP, enabling low risk of contamination in analytical work.

Analytical methods of NMP and its metabolites have been developed using combined separation and detection [13,18,19]. NMP and its metabolites range from lipophilic NMP and MSI to hydrophilic 2-HMSI and 5-HNMP, and since their total analysis requires elaborate extraction from a biological fluid matrix, analysis has been performed with two separate runs, with organic and aqueous phases [13]. NMP in organic phase was determined by capillary gas chromatography with FTD [12,17], MSI and 2-HMSI in aqueous phase were determined by capillary GC/MS in negative-ion chemical ionization mode, and 5-HNMP required trimethylsilyl derivatization of its hydroxyl group prior to GC/MS measurement because it is thermolabile [17]. Subsequently, as an alternative method, LC/MS/MS incorporating atmospheric pressure ionization (API) and collision-induced dissociation (CID) was introduced for analysis of 5-HNMP and 2-HMSI directly without derivatization [19,20]. Since all of these mass spectrometric methods require the use of deuterium-labeled synthetic 5-HNMP and 2-HMSI substances as an internal standard, the availability of which is very limited, use of them in biomonitoring laboratories is still limited. Although HPLC–UV detection is preferred because it is very commonly performed, the present authors' experience suggested that the sensitivity and specificity of UV detection are not high enough for determination of the TLV level of NMP exposure. At present, the use of LC/MS or LC/MS/MS is required for the higher sensitivity and specificity of mass spectrometries [19,21]. On the other hand, GC equipped with a flame thermionic detector (GC/FTD) has high sensitivity and selectivity in detecting organic nitrogen compounds such as NMP and carbamate pesticides [22,23], though thermolabile metabolites such as 5-HNMP cannot be directly applied to it [18]. GC/FTD has been used for NMP vapor detection with public methods [24].

Recently, high-performance solid-phase extraction (SPE) based on copolymer resin packing has become available for hydrophilic and lipophilic mixtures, and the clean-up procedure using it enables rapid selective extraction of urinary metabolites [25].

This study aimed to develop an easy-to-use and rugged analytical method by applying recent progress in SPE and capillary GC/FTD to the determination of urinary levels of NMP and its metabolites for workers exposed to NMP. The analytical method developed is designed for biological monitoring of exposure to NMP at levels below the Japanese OEL value for NMP. Because of its thermolability, as noted above, measurement of 5-HNMP was excluded from the present study [18].

## 2. Experimental

### 2.1. Chemicals

NMP, MSI, 2-HMSI, and *N*-ethylsuccinimide (ESI) used as an internal standard were purchased from Wako Pure Chemicals (Osaka, Japan), Avocado Research Chemicals (Heysham, Lancashire, England), Synthelec (Lund, Sweden), and Tokyo Kasei Kogyo (Tokyo, Japan), respectively. Isobutyl acetate and ethyl acetate of reagent grade, acetonitrile and methanol of HPLC grade, and methyl isobutyl ketone (MIBK) of atomic absorption spectrometric grade were purchased from Wako Pure Chemicals. High-purity water was prepared with a Milli-Q water system (Millipore, Bedford, MA, USA).

### 2.2. Solid-phase extraction

A cartridge packed with poly (divinylbenzene/hydroxymethacrylate) solid phase (Nobias PR-SG1, Hitachi High-Technologies, Tokyo, Japan) was used. It contained 240 mg of the solid phase in a 12 mm diameter, 6 mm long cartridge, and was fitted with a vacuum manifold for passage of the conditioning eluent. The SPE cartridge was pre-conditioned by initial passage of 5 mL of acetonitrile, followed 5 min later by a second 5 mL of acetonitrile, and then 10 mL of Milli-Q water, as recommended by the manufacturer. Unless otherwise stated, 2.0 mL urine sample and 0.2 mL of ESI (200 mg/L) were loaded, and the cartridge was dried before elution with organic solvent. To determine optimal conditions for SPE operation, the followings were examined for recoveries of NMP, MSI, and 2-HMSI:

#### 2.2.1. Effect of pH

The cartridge was loaded with 2.0 mL of standard aqueous solutions containing NMP, MSI, and 2-HMSI at 2 mg/L each. The pH of the sample solution was adjusted with oxalic acid or sodium carbonate solution.

#### 2.2.2. Drying time

The urine sample-loaded cartridge was dried with dry nitrogen gas flow at 10 L/min (pressure at 100 kPa) for up to 15 min. Dryness was evaluated by measuring cartridge weight.

#### 2.2.3. Effects of solvents used for elution

The cartridge was spiked with urine sample containing the above three compounds at 1.0 mg/L each. The eluent solvents tested were methanol, ethyl acetate, isobutyl acetate, and MIBK at 2.0 mL.

### 2.3. Gas chromatography

A gas chromatograph equipped with an FTD, a liquid autosampler, and a data processor (GC-17A/FTD-17, AOC-1400, and C-R7A plus, respectively, Shimadzu, Kyoto, Japan) and a DB1701 capillary column of 30 m × 0.53 mm i.d. and 1 μm thickness (J & W Scientific, CA, USA) were used. The operating temperature of the GC injector was 250 °C. The column temperature was programmed to be held at 100 °C for 1 min,

increased to 240 °C at 10 °C/min, and then held for 8 min at 240 °C. Helium was used as carrier gas at 16 mL/min. Hydrogen and air were set for the FTD at flow rates of 4 and 150 mL/min, respectively. The split–splitless injector was operated in 1:10 split mode. Retention times of NMP, MSI, ESI (internal standard), and 2-HMSI were 3.2, 4.1, 4.4, and 5.8 min, respectively. Peak areas of NMP, MSI, and 2-HMSI were corrected by the peak area of ESI.

The effluent from the SPE cartridge was centrifuged at 3000 rpm for 10 min and then 1 μL of the supernatant was injected into GC.

### 2.4. Calibration, limits of detection, and variations

The GC/FTD linear range was examined using MIBK standard solution of concentration ranging from 0.5 to 60 mg/L. For quantitative GC analysis, calibration curves, limits of detection, and variability were examined as follows:

(1) Calibration curves were prepared by injecting into the GC/FTD 1 μL of standard aqueous solutions that contained NMP, MSI, and 2-HMSI of known concentrations of 0.5, 1.0, 5.0, and 10.0 mg/L and subjecting them to the SPE procedure. The GC peak area ratio of each compound relative to that of ESI was plotted against concentration of NMP, MSI, or 2-HMSI. In addition, linearity was evaluated, using urine samples spiked with these three compounds at known concentrations of 0.5, 1.0, 5.0, and 10.0 mg/L.
(2) Limits of detection for NMP, MSI, and 2-HMSI were calculated according to the definition given by Gibbons [26], as 3.14 times the standard deviations of the concentrations corresponding to the peak area ratio obtained for 1 μL injection of standard solution containing these compounds at 1.0 mg/L each. In addition, the limits of detection of NMP, MSI, and 2-HMSI were evaluated for urine samples spiked with these three compounds at 1.0 mg/L each.
(3) The within-day variation on GC analysis was determined by performing GC analysis of five samples prepared from the same urine spiked with NMP, MSI, and 2-HMSI at 1 and 10 mg/L within 1 day. The between-day variation was determined by repetition of experiments on 5 days (five replicates per sample) using the same urine, which was spiked with the compounds of concern, followed by SPE–GC/MS measurement. Measurements were performed for two different urine samples at two levels of concentration, 1 mg/L and 10 mg/L. These two types of variations were measured as standard deviations for NMP, MSI, and 2-HMSI.

### 2.5. Urine samples obtained with or without exposure to NMP

The urine samples used as those without exposure to NMP were from 20 male volunteers who had not been exposed to NMP. The urine samples from five NMP-exposed workers who used NMP as a solvent in a factory were collected at the end of a work-shift on a Friday evening. The workers' urine was stored in a freezer at −20 °C until analysis.

Worker NMP exposure was monitored with an Organic Vapor Monitor 3500 passive sampling device (3M, MN, USA) for 8 h. NMP was determined with GC/MS (6890N/5973inert, Agilent Technologies, CA, USA) using a DB-624 capillary column (60 m × 0.25 mm i.d., 1.4 μm film thickness; J & W Scientific, CA, USA), with $m/z = 99$ and 44. The vapor concentration of NMP was calculated according to the manufacturer's Technical Data Bulletin [27], using a sampling rate of NMP of 28.8 mL/min, and with a recovery of NMP from the charcoal adsorbent pad by dichloromethane of 81%.

## 3. Results and discussion

### 3.1. Optimization of SPE

Recovery was calculated using the chromatographic peak area ratio for the SPE-processed sample against standard MIBK solution containing known amounts of the compounds of concern. Table 1 shows effects of eluting solvents on recoveries of NMP, MSI, and 2-HMSI from the SPE cartridge. MIBK was the best choice for extraction of NMP and MSI among the three solvents tested. Methanol was also examined as an eluting solvent, though unidentified GC peaks from the methanol effluent were found to interfere with GC peaks of NMP, MSI, and 2-HMSI, as described in Section 3.2. The compounds of concern ranged from the hydrophilic 2-HMSI to hydrophobic NMP and MSI. The hydrophobic compounds interact with the solid-phase moiety of divinylbenzene, while the hydrophilic ones bind to the polar moiety of hydroxymethacrylate or distribute in the water trapped in the pores of the solid phase [28,29]. This may account for the lower recoveries of 2-HMSI. These interactions can be governed by the nature of the eluent used. Based on the results of evaluation noted above, MIBK was selected as eluting solvent and used throughout this study.

Recovery is, as noted above, thought to be influenced by the residual urinary water in the solid phase, and, as shown in Fig. 1, recoveries dramatically increased with time of drying of the cartridge. The dotted line indicates the relative weight change of the cartridge. Amount of water in the cartridge gradually decreased and then leveled off after 10 min of passage of dry nitrogen gas. The recoveries of NMP, MSI, and 2-HMSI were 28, 73, and 36% before passage of dry nitrogen, increased to 102, 107, and 68% at 10 min, respectively, and leveled off thereafter. Drying of the loaded SPE cartridge with nitrogen gas flow was the most important factor in quantitative extraction of urinary NMP and its metabolites.

Table 1
Effects of eluting solvent on recoveries of NMP, MSI, and 2-HMSI from SPE cartridge loaded with the spiked urine sample of these compounds of 1.0 mg/L each

| Eluent | Recovery (%) | | |
|---|---|---|---|
| | NMP | MSI | 2-HMSI |
| Ethyl acetate | 81 ± 1 | 93 ± 1 | 62 ± 2 |
| Isobutyl acetate | 88 ± 1 | 79 ± 1 | 53 ± 1 |
| Methyl isobutyl ketone | 93 ± 2 | 99 ± 1 | 61 ± 1 |

The values are expressed as means ± SD ($n = 3$).

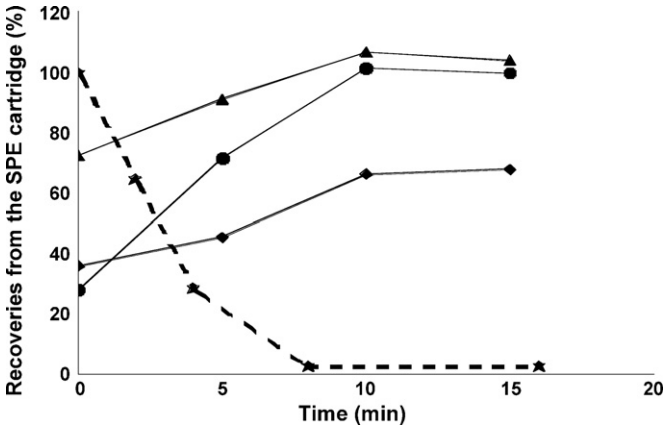


Fig. 1. Effects of drying of loaded SPE cartridge on recoveries of NMP, MSI, and 2-HMSI. The SPE cartridge was loaded with urine sample spiked with three compounds at 1.0 mg/L each. Dry nitrogen was passed through the SPE cartridge at a flow rate of 10 L/min. The dotted line shows cartridge weight. Circles, triangles, and squares indicate NMP, MSI, and 2-HMSI, respectively.

The recoveries of NMP and MSI were found to be independent of pH over the entire range of pH examined, as shown in Fig. 2. The recovery of 2-HMSI was maintained constant at about 60–65% in the acidic and neutral range, but was decreased to 50% at pH 8 and down to 0% at pH 9. In general, the normal pH of human urine is weakly acidic, though it varies physiologically from 5.0 to 7.5 [30]. The pH of the urine samples used in this study ranged from 5.5 to 6.7.

Therefore, the present findings of SPE optimization indicated that 2.0 mL of MIBK as an eluting solvent and 10-min drying of the cartridge under a nitrogen gas flow of 10 L/min, without adjustment of pH of urine samples, enables quantitative extraction of NMP, MSI, and 2-HMSI with the selected SPE cartridge. Table 2 shows recoveries for spiked urine from 20 different individuals who were not exposed to NMP, with CV values less than 10%. Recoveries of NMP, MSI, and 2-HMSI are 98, 99–103, and 65–69%, respectively.

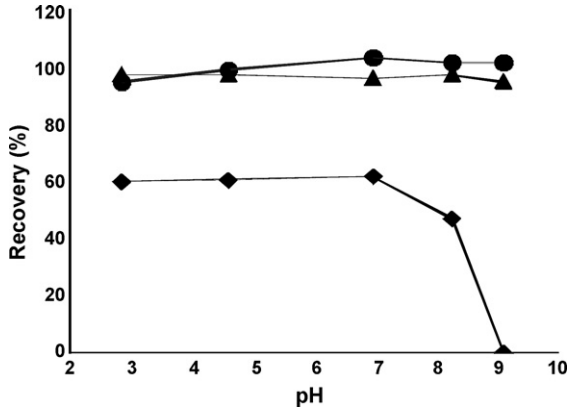


Fig. 2. Effects of pH of sample solution on recoveries of NMP, MSI, and 2-HMSI. The SPE cartridge was loaded with standard aqueous solution containing these compounds at 2 mg/L each. Circles, triangles, and squares indicate NMP, MSI, and 2-HMSI, respectively.

### 3.2. Interference-free GC Separation

Fig. 3 shows gas chromatograms for the following three substances: (a) SPE–MIBK effluent for the standard aqueous solution containing NMP, MSI, 2-HMSI, and ESI (internal standard), (b) a blank urine sample, spiked only with the same amount of ESI, from a volunteer not exposed to NMP, and (c) the same urine sample spiked with the same amounts of these four compounds. As seen in the insert in Fig. 3, an unidentified peak minor to the peak of 2-HMSI was found, depending on the individual urine sample, at a retention time near that of 2-HMSI, and was chromatographically subtracted. The authors noted the optimal flow rate of carrier gas should be less than a half of 16 mL/min and observed improvement in separation of this minor peak and 2-HMSI as the valley point between those peaks lowered to about a half with 8 mL/min of the carrier gas flow. Nevertheless, these chromatograms indicated relatively little interference with GC peaks corresponding to NMP, MSI, or 2-HMSI for human urine, as well as efficient clean-up with the SPE used, in addition to the benefits of chromatographic separation with the DB1701 capillary column and highly selective detection of the compounds concerned with use of FTD. As seen in the example of analysis of a worker's urine in Fig. 4, use of methanol as the eluent for SPE yielded a chromatogram including many interfering peaks and a poor baseline due to contaminants, indicating the need for improved separation and a more selective system. The chromatograms suggest that the DB1701 capillary column effectively elutes the abundant polar interfering compounds in urine nearly at void volume.

Within-day variation, expressed as the CV, was 0.5–2.1% for NMP, 0.7–2.4% for MSI, and 0.8–2.6% for 2-HMSI. Between-day variation was examined using spiked urine samples which were stored in a refrigerator at 4 °C. The variation over 8 days was 0.6–2.1% for NMP, 0.8–2.0% for MSI, and 2.0–4.9% for 2-HMSI for 5 days repetition of experiments, as shown in Table 3. Both within-day and between-day variations were sufficiently low for NMP and those two metabolites. The results of between-day variation test supported the fact that storage in a refrigerator for at least 1 week did not affect these concentrations in urine.

These findings indicate that this analytical method is sufficient for practical urine analysis with the rugged and easy-to-use features of GC/FTD and aids biomonitoring work when dedicated GC/MS or LC/MS/MS instrumentation is not available. The internal standard used in this study, *N*-ethylsuccinimide, enabled inexpensive analysis compared with the deuterated synthetic substances required for mass spectrometry. LC/MS and LC/MS/MS are prone to changes in sensitivity or marked change in ionization efficiency for coexisting compounds, and use of an adequate internal standard is thus always preferred with them [31].

### 3.3. Quantitative analysis

#### 3.3.1. Calibration and limits of detection

GC/FTD linearity was checked with MIBK standard solution over the range of 0.5–60 mg/L and confirmed between 0.5 and

Table 2
Individual differences in recoveries (%) of NMP, MSI, and 2-HMSI from the SPE cartridge loaded with the urine samples of 20 non-exposed volunteers spiked with these three compounds of 1 or 10 mg/L each

| Sample | pH | Urine spiked with 1 mg/L | | | Urine spiked with 10 mg/L | | |
|---|---|---|---|---|---|---|---|
| | | NMP | MSI | 2-HMSI | NMP | MSI | 2-HMSI |
| Recoveries (%) | | | | | | | |
| A | 6.0 | 98 | 104 | 64 | 95 | 96 | 68 |
| B | 6.0 | 96 | 106 | 69 | 100 | 101 | 71 |
| C | 6.5 | 95 | 104 | 71 | 96 | 96 | 70 |
| D | 6.5 | 95 | 101 | 70 | 103 | 102 | 71 |
| E | 6.0 | 99 | 103 | 62 | 99 | 99 | 68 |
| F | 6.0 | 96 | 106 | 52 | 101 | 102 | 62 |
| G | 6.0 | 95 | 105 | 59 | 98 | 99 | 68 |
| H | 6.0 | 95 | 102 | 71 | 103 | 100 | 70 |
| I | 6.0 | 94 | 104 | 61 | 100 | 98 | 64 |
| J | 6.0 | 97 | 102 | 62 | 100 | 97 | 67 |
| K | 5.6 | 102 | 104 | 71 | 96 | 103 | 72 |
| L | 6.0 | 94 | 93 | 73 | 91 | 98 | 65 |
| M | 6.5 | 97 | 108 | 62 | 95 | 94 | 64 |
| N | 6.7 | 99 | 106 | 65 | 95 | 102 | 71 |
| O | 6.1 | 99 | 105 | 73 | 95 | 94 | 70 |
| P | 6.3 | 100 | 98 | 61 | 95 | 102 | 71 |
| Q | 6.0 | 102 | 96 | 70 | 97 | 102 | 69 |
| R | 6.1 | 100 | 95 | 66 | 96 | 102 | 69 |
| S | 5.5 | 98 | 103 | 67 | 97 | 104 | 72 |
| T | 6.1 | 103 | 107 | 54 | 98 | 97 | 70 |
| Mean (%) | | 97.7 | 102.6 | 65.2 | 97.5 | 99.4 | 68.6 |
| SD | | 2.8 | 4.1 | 6.0 | 3.0 | 3.0 | 2.9 |
| CV (%) | | 2.8 | 4.0 | 9.3 | 3.1 | 3.1 | 4.2 |

20 mg/L for NMP and all three metabolites. However, above 40 mg/L, peak area deviated from the regression line obtained for 0–20 mg/L, i.e., 9% below at 40 mg/L and 13% below at 60 mg/L. Use of a higher split ratio for injection into GC/FTD enables extension of the linear response range. This study was therefore subsequently limited to 20 mg/L, and, for reasons of safety, linear calibration curves for NMP, MSI, and 2-HMSI were set between 0.5 and 10.0 mg/L for the SPE processed standard aqueous solutions and the spiked urine samples, which is a workable range for practical applications. The results obtained, such as appropriate correlation coefficients greater than 0.999, small intercepts, and different, compound-dependent slopes, are indicated in Table 4. The slopes, intercepts, and correlation coefficients of the calibration equations obtained with the spiked urine samples are quite similar to those obtained with standard aqueous solutions.

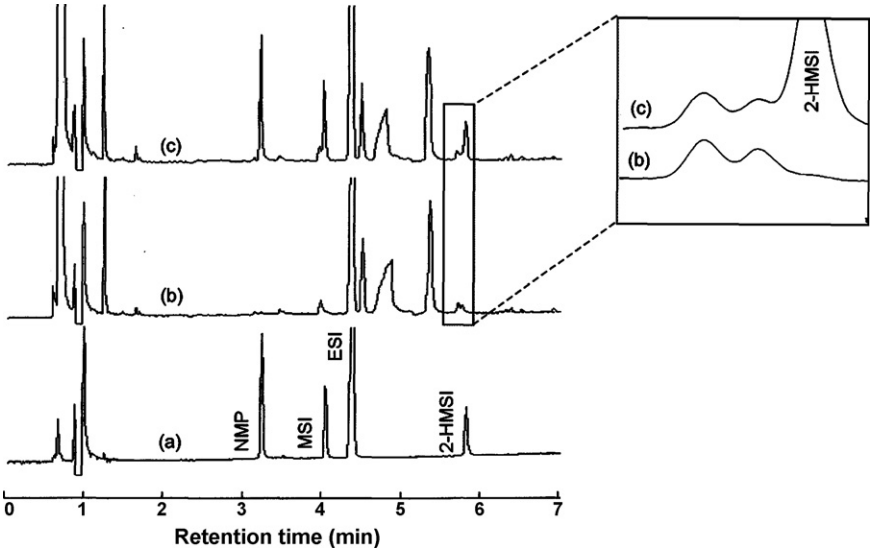


Fig. 3. Chromatograms of NMP and its metabolites. (a) Standard aqueous solution containing NMP, MSI, and 2-HMSI at 1 mg/L each and ESI as an internal standard, (b) a blank urine sample spiked with ESI, and (c) the same urine sample spiked with the same amounts of these compounds and ESI as in (a).

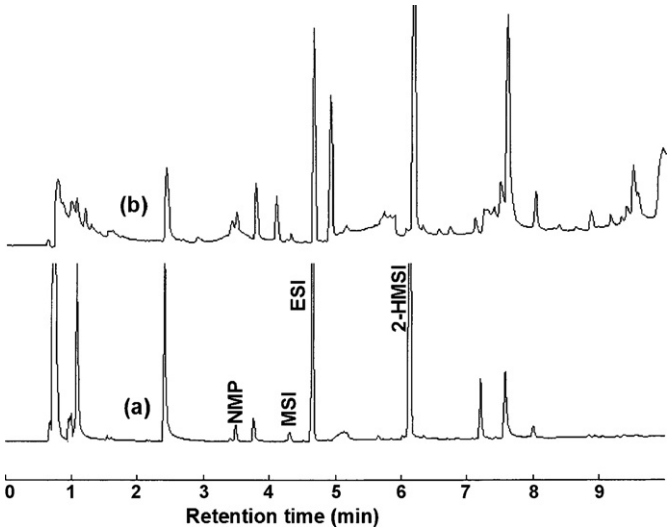


Fig. 4. Chromatograms of a worker's urine sample eluted from SPE with MIBK (a) and methanol (b). The values obtained were 0.7 mg/L for NMP, 0.7 mg/L for MSI, and 39.9 mg/L for 2-HMSI. *Note*. Retention times are slightly shifted compared with Fig. 3 due to slight differences in GC operation.

These findings indicate that this method appropriately removes the urine matrix, enabling accurate determination of the quantities of NMP, MSI, and 2-HMSI in urine.

The limits of detection were calculated as 0.04 mg/L for NMP, 0.02 mg/L for MSI, and 0.06 mg/L for 2-HMSI using the procedures described in Section 2.4. No differences in the limit of detection for NMP, MSI, or 2-HMSI were found between the standard aqueous solution and the spiked urine samples. These limits of detection could be assessed using urine samples, as follows.

### 3.3.2. Application to biological monitoring

From workers exposed to 3.2 ± 1.1 ppm NMP in the atmosphere for 8 h, urine samples were collected at the end of a work-shift (Friday evening) and NMP and its metabolites were determined as shown in Table 5, in which mean values of the concentrations of NMP and its metabolites are shown. The quantity of 2-HMSI, which exceeded the upper range of 10 mg/L, was diluted before analysis. Mean concentrations of NMP, MSI, and 2-HMSI were 1.7, 0.8, and 27.8 mg/g creatinine, respectively. Bader et al. [6] reported that at 1.01 mg/m$^3$ (0.25 ppm) of NMP, exposure resulted in a mean NMP in urine of 206 μg/g creatinine, and 2-HMSI in urine ranged from below the limit of detection to 14.7 mg/g creatinine. The exposure levels of their workers were about one-tenth those of our workers, and urinary concentrations of NMP and 2-HMSI of their workers were about one-tenth those of our workers. Since the biological half-life of 2-HMSI in urine has been reported to be about 17 h [17], and it is a better indicator of percutaneous absorption [11], 2-HMSI in urine appears to be a good indicator of exposure for exposed workers.

Table 3
Within-day and day-to-day variations of recoveries obtained from 5 days repeated experiments

| Compounds | Day | Recovery (%) | |
|---|---|---|---|
| | | Urine X spiked as 1 mg/L[a] | Urine Y spiked as 10 mg/L[a] |
| NMP | First | 99.4 ± 0.6 | 95.2 ± 0.7 |
| | Second | 99.5 ± 1.5 | 96.1 ± 0.9 |
| | Third | 97.6 ± 2.1 | 96.4 ± 1.4 |
| | Fourth | 97.4 ± 1.3 | 96.2 ± 2.0 |
| | Fifth | 94.4 ± 0.5 | 96.7 ± 1.1 |
| Mean | | 97.7 | 96.1 |
| SD | | 2.1 | 0.6 |
| MSI | First | 105.1 ± 2.4 | 100.3 ± 1.0 |
| | Second | 105.9 ± 2.1 | 100.9 ± 0.7 |
| | Third | 104.3 ± 1.9 | 101.3 ± 1.0 |
| | Fourth | 104.7 ± 0.9 | 101.1 ± 1.9 |
| | Fifth | 103.9 ± 1.6 | 96.5 ± 1.0 |
| Mean | | 104.8 | 100.0 |
| SD | | 0.8 | 2.0 |
| 2-HMSI | First | 70.7 ± 1.4 | 73.3 ± 1.1 |
| | Second | 71.8 ± 1.8 | 69.5 ± 0.9 |
| | Third | 70.6 ± 0.6 | 72.6 ± 1.6 |
| | Fourth | 69.3 ± 0.8 | 70.6 ± 1.3 |
| | Fifth | 68.1 ± 1.6 | 64.6 ± 0.8 |
| Mean | | 70.1 | 70.1 |
| SD | | 1.4 | 3.4 |

The values are expressed as means ± SD ($n$ = 5).
[a] Urines X and Y were from NMP non-exposed volunteers.

Table 4
Calibration equations for NMP, MSI, and 2-HMSI with the standard aqueous solutions and the spiked urine samples containing these compounds

| Compounds | Concentration range (mg/L) | Slope (L/mg) | Intercept | Correlation coefficient |
|---|---|---|---|---|
| Standard aqueous solution | | | | |
| NMP | 0.5–10 | 0.0997 | +0.0012 | 0.999 |
| MSI | 0.5–10 | 0.0662 | +0.0010 | 0.999 |
| 2-HMSI | 0.5–10 | 0.0400 | −0.0065 | 0.999 |
| Spiked urine | | | | |
| NMP | 0.5–10 | 0.1034 | +0.0030 | 0.999 |
| MSI | 0.5–10 | 0.0697 | +0.0056 | 0.999 |
| 2-HMSI | 0.5–10 | 0.0412 | +0.0060 | 0.999 |

Table 5
Urinary levels of NMP, MSI, and 2-HMSI for workers exposed to NMP in the workplace and concentration of NMP in workplace air

| NMP in air (ppm) | Urine (mg/L) | | |
|---|---|---|---|
| | NMP | MSI | 2-HMSI |
| 3.2 ± 1.1 | 1.3 ± 0.7 (1.7 ± 2.1)[a] | 0.8 ± 0.2 (0.8 ± 0.4)[a] | 40.3 ± 32.4 (27.8 ± 12.5)[a] |

The values are expressed as means ± SD ($n$ = 5).
[a] Corrected by creatinine unit: mg/g creatinine.

Since the limits of detection of NMP, MSI, and 2-HMSI were, as noted above, found to be 0.04, 0.02, and 0.06 mg/L, respectively, this analytical method is applicable down to one-tenth level, i.e., exposure to 0.1 ppm NMP in the workplace in routine biological monitoring, and meets the requirements of the Japanese OEL occupational health value of 1 ppm for NMP in workplace air.

## 4. Conclusion

The analytical method we developed, which combines the most recent developments in SPE and gas chromatography with FTD and a DB1701 capillary column, is useful for biological monitoring by urinary assay of NMP exposure in the workplace atmosphere to levels at or below those required by regulatory agencies throughout the world. As a next step, complete evaluation of this method should be performed in parallel with established GC/MS or LC/MS/MS methodologies and extended to workplace surveillance.

## Acknowledgements

This study was a part of the "Research and Development Project on the 13 Fields of Occupational Injuries and Illness of the Japanese Labour, Health, and Welfare Organization", and was supported by a Grant-in-Aid for Scientific Research (17659178) from the Japan Society for the Promotion of Science.

## References

[1] Health & Safety Executive, *N*-Methyl-2-Pyrrolidone Risk Assessment Document No. EH72/10, HSE Books, Norwich NR3 IBQ, UK, 1997.
[2] International Programme on Chemical Safety, Concise International Chemical Assessment Document No 35, World Health Organization, Geneva, 2001.
[3] H.J. Beaulieu, K.R. Schmerber, Appl. Occup. Environ. Hyg. 6 (1991) 874.
[4] S.A. Akhter, B.W. Barry, J. Pharm. Pharmacol. 37 (1985) 27.
[5] The Chemical Daily Co. Ltd., Handbook of Chemical Products-14303, The Chemical Daily Co. Ltd., Tokyo, 2003, p. 843 (in Japanese).
[6] M. Bader, W. Rosenberger, T. Rebe, S.A. Keener, T.H. Brock, H.J. Hemmerling, R. Wrbitzky, Int. Arch. Occup. Environ. Health 79 (2006) 357.
[7] Deutsche Forschungsgemeinschaft, List of MAK and BAT Values 2006, Wiley-VCH, Weinheim, 2006.
[8] Deutsche Forschungsgemeinschaft, in: H. Greim (Ed.), Occupational Toxicants. Critical Data Evaluation for MAK Values and Classification of Carcinogens, Wiley-VCH, Weinheim, 1994, p. 147.
[9] Japan Society for Occupational Health, J. Occup. Health 44 (2002) 170.
[10] C. Ursin, C.M. Hansen, J.W. Van Dyk, P.O. Jensen, I.J. Christensen, J. Ebbehoej, Am. Ind. Hyg. Assoc. J. 56 (1995) 651.
[11] B. Åkesson, M.A. Carnerup, B.A. Jönsson, Scand. J. Work Environ. Health 30 (2004) 306.
[12] B. Åkesson, B.A. Jönsson, Drug Metab. Dispos. 25 (1997) 267.
[13] B. Åkesson, K. Paulsson, Occup. Environ. Med. 54 (1997) 236.
[14] B. Åkesson, B.A. Jönsson, Scand. J. Work Environ. Health 26 (2000) 213.
[15] B.A. Jönsson, B. Åkesson, Int. Arch. Occup. Environ. Health 74 (2001) 289.
[16] ACGIH, TLVs and BEIs 2006, ACGIH, Cincinnati, OH, 2006.
[17] B.A. Jönsson, B. Åkesson, Int. Arch. Occup. Environ. Health 76 (2003) 267.
[18] B.A. Jönsson, B. Åkesson, J. Chromatogr. B 694 (1997) 351.
[19] M.A. Carnerup, B. Åkesson, B.A. Jönsson, J. Chromatogr. B 761 (2001) 107.
[20] M.A. Carnerup, M. Spanne, B.A. Jönsson, Toxicol. Lett. 162 (2006) 139.
[21] M.A. Carnerup, A.M. Saillenfait, B.A. Jönsson, Food Chem. Toxicol. 43 (2005) 1441.
[22] B. Kolb, M. Auer, P. Pospisil, J. Chromatogr. Sci 15 (1977) 53.
[23] W.M. Drapper, J. Agric. Food Chem. 43 (1995) 2077.
[24] NIOSH Method 1302, in: M.E. Cassinelli (Ed.), NIOSH Manual of Analytical Methods, Center for Disease Control and Prevention, Cincinnati, OH, 1998.
[25] J.J. Pesek, M.T. Matyska, in: N.J.K. Simpson (Ed.), SPE Sorbent and Formats, Marcel Dekker, New York, 2000, p. 19.
[26] R.D. Gibbons, in: R.A. Meyers (Ed.), Encyclopedia of Analytical Chemistry, Wiley, Chicheter, UK, 2000, p. 2343.
[27] 3M Occupational Health and Environmental Safety Division, Technical Data Bulletin No. 1028, 3M, St. Paul, 2000.
[28] M. Shibukawa, N. Ohta, N. Onda, Bull. Chem. Soc. Jpn. 63 (1990) 3490.
[29] M. Shibukawa, Bunseki Kagaku 55 (2006) 149 (in Japanese).
[30] H. Kikuchi, in: K. Nakahara (Ed.), Comprehensive Manual for Biological & Immunological Aspects of Clinical Pathology, Nippon Rinsho, Osaka, 2004, p. 98 (in Japanese).
[31] K. Georgi, K.S. Boos, Chromatographia 63 (2006) 523.