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# Liquid chromatographic–atmospheric pressure chemical ionization mass spectrometric analysis of opiates and metabolites in rat urine after inhalation of opium

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**Abstract**

To examine the urinary excretion of opiates and their metabolites following inhalation exposure of rats to opium, analytical procedures for the simultaneous determination of the compounds in opium, the vapor derived by the volatilization of opium and the urine of rats exposed to the opium vapor were developed using liquid chromatography–atmospheric pressure chemical ionization mass spectrometry (LC–APCI-MS). Seven compounds were determined in the opium, namely morphine, codeine, thebaine, noscapine, papaverine, meconic acid and meconin. All seven were extracted with 2.5% acetic acid solution and subjected to LC–APCI-MS analysis. The separation was performed on an ODS column in acetonitrile–50 m*M* ammonium formate buffer (pH 3.0) using a linear gradient program and quantitative analysis was carried out in the selected ion monitoring mode ($[M+H]^+$). For the analysis of the volatilization of opium, the opium (1 g) was added to a glass pipe, which was then heated at 300 °C for 20 min. Negative pressure (air flow-rate; 300 ml/min) was used to draw the vapor through a series of glass wool and methanol traps. The total amount of each compound in the vapor was estimated by measurement of the compounds trapped in the glass wool and methanol. Wister rats ($n=3$) were exposed to the vapor derived from the volatilization system and the urinary amounts (0–72 h) of the six opiates and metabolites including morphine-3-grucronide (M3G) and morphine-6-grucronide (M6G) were measured after solid-phase extraction. The calibration curves for those compounds in the rat urine were linear over the concentration range 10–500 ng/ml. The recoveries for each analyte from the rat urine sample spiked with standard solution were generally greater than 80%, and the relative standard deviation for the analytical procedure was less than 8% with the exception of meconin. After inhalation exposure of rats to opium, M3G (5.45–14.38 μg), morphine (2.27–4.65 μg), meconin (0.54–1.85 μg), codeine (0.54–1.85 μg), noscapine (0.34–0.40 μg) and papaverine (0.01–0.04 μg) were detected in the urine over 72 h. However, only trace levels of thebaine were observed despite it being one of the major alkaloids found in the opium. On the other hand, a relatively large amount of meconin was detected in the vapor and the urine as compared with the opium. It is suggested that the presence of meconin in biological fluids could be indicative of opium ingestion by inhalation.


*Keywords:* Opiates; Opium

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## 1. Introduction

Opium is the partly dried exudates of the poppy plant *Papaver somniferum*. It contains a number of alkaloids of considerable pharmaceutical importance, such as morphine, codeine, thebaine, papaverine and noscapine, accounting for 0.3–10% of the dry mass with the remaining alkaloids occurring in trace amounts [1,2]. Numerous analytical methods have been developed for the estimation of these compounds in opium gum by thin-layer chromatography (TLC) [3–6], gas chromatography (GC) [7,8], liquid chromatography (LC) [9–13], capillary electrophoresis [14,15] and radioimmunoassay [16]. However, so far the study of the urinary excretion profile following the consumption of opium has not been done except by Cone et al. who reported on the detection and measurement of opium alkaloids and metabolites in human urine of ''opium eaters'' by methane chemical ionization mass fragmentography [17]. They detected morphine, codeine, normorphine, norcodeine and noscapine in urine; however, no evidence was obtained for thebaine, papaverine or oripavine.

Apart from the pharmaceutical use of opium, the naturally occurring opioids contained in opium have been abused by smoking for centuries. From a forensic toxicological viewpoint, it is necessary to investigate the urinary excretion after opium ingestion by inhalation. Lichtman et al. [18] investigated the inhalation exposure of mice to volatilized opioid compounds, for example heroine, morphine, codeine and meperidine, and their pharmacological effects. However, no report has appeared on the determination of opiates in the vapor derived by volatilization of opium and the urinary excretion of opiates following the inhalation of volatilized opium.

In this study, analytical procedures for the simultaneous determination of the major opiates (Fig. 1) in opium, the vapor derived by the volatilization of opium and the urine of rats exposed to the opium vapor were developed using liquid chromatography–atmospheric pressure chemical ionization mass spectrometry (LC–APCI-MS). Furthermore, the urinary excretion of opiates and their metabolites following inhalation in rats was investigated.

## 2. Experimental

### 2.1. Chemicals and reagents

Morphine hydrochloride, codeine phosphate, thebaine, papaverine hydrochloride, noscapine and

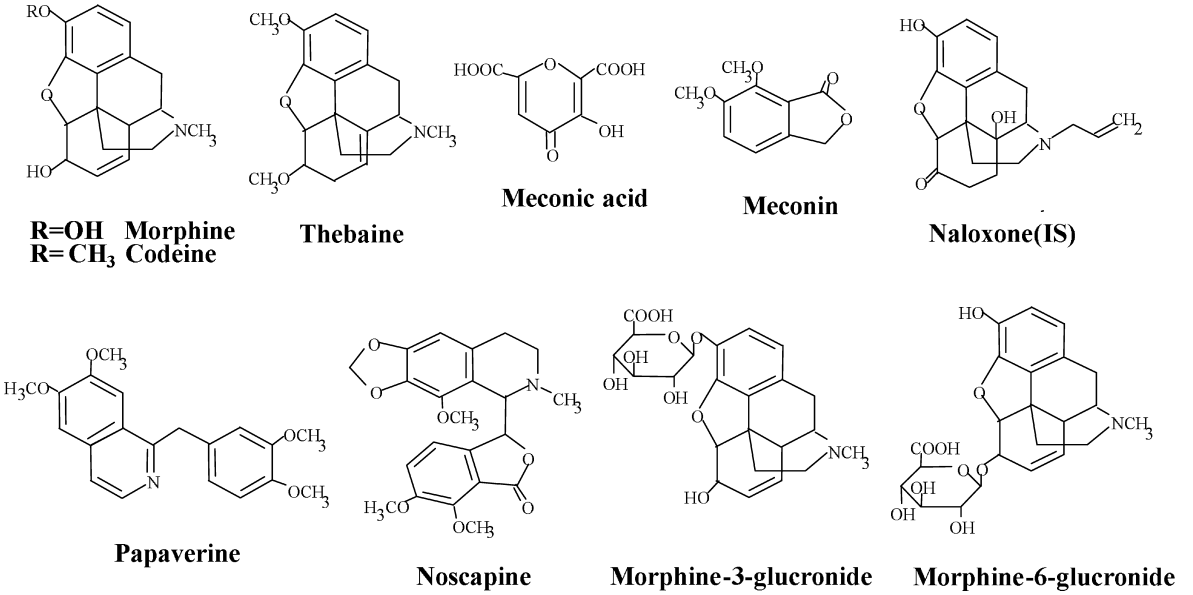


Fig. 1. Structure of opiate compounds, morphine glucronides and the internal standard (I.S.).

meconic acid were obtained from Takeda Chemical Industries (Osaka, Japan). Morphine-3-glucronide (M3G) and morphine-6-glucronide (M6G) were given from Shionogi (Osaka, Japan). Naloxone hydrochloride used as an internal standard (I.S.) was supplied by Sigma (St. Louis, MO, USA). Meconin was prepared from 2, 3-dimethoxybenzoic acid, formaldehyde solution (36–38%) and concentrated hydrochloric acid (Wako, Tokyo, Japan) according to the method previously reported [19]. Its structure and purity were confirmed by melting point (102–103 °C) [19], TLC, GC–MS [20] and $^{1}$H nuclear magnetic resonance. A solid-phase extraction column (Oasis HLB, 3 ml/60 mg, particle size 30 μm) was obtained from Waters (Milford, MA, USA) and a membrane filter (Ultrafree-CL, 0.45-μm, PTFE) was from Millipore (Bedford, MA, USA). All other chemicals and solvents were of analytical reagent grade or HPLC grade (Wako).

### 2.2. LC–APCI-MS

The LC–APCI-MS system consisted of an Agilent 1100 series HPLC system equipped with a 1100 series LC–MSD SL (Agilent Technology, Palo Alto, CA, USA). Chromatographic separation was performed in a gradient mode using an Inertsil ODS-3 column (150 mm×4.6 mm I.D., 5 μm) protected by a GL cart guard column (5 mm×4.6 mm I.D., GL Sciences, Tokyo, Japan) at 40 °C. The mobile phases were 50 m*M* ammonium formate (pH 3) and acetonitrile delivered at 0.8 ml/min. A linear gradient from 5% of acetonitrile (95% of 50 m*M* ammonium formate, pH 3) to 30% of acetonitrile over 20 min was used, and the final condition was maintained for 10 min. The mobile phase composition was then brought back to the starting point over 5 min and the column re-equilibrated over 10 min. The injection volume was 10 μl.

For the detection system, a tandem setting of a diode array detection (DAD) system and a mass detector (MSD) was adopted. Mass analysis by APCI was used in a positive mode. Nitrogen was used as the nebulization gas and delivered at a flow-rate of 13 l/min at 350 °C. The nebulizer pressure was 60 p.s.i. g (1 p.s.i.=6894.76 Pa) and the vaporizer temperature was 450 °C. The capillary voltage was 2300 V for M3G and M6G, 3000 V for meconin, papaverine and noscapine, 3200 V for codeine and naloxone (I.S.), 3400 V for thebaine, and 3600 V for morphine. The fragmentation voltage was 100 V for meconin, 120 V for thebaine, papaverine and noscapine, 130 V for morphine, M3G and M6G, and 150 V for codeine and naloxone. The corona current was 7.0 mA except codeine and naloxone (5.0 mA). The selected ions monitored as protonated molecules ($MH^+$) were as follows: $m/z$ 286 for morphine, $m/z$ 300 for codeine, $m/z$ 328 for naloxone, $m/z$ 312 for thebaine, $m/z$ 195 for meconin, $m/z$ 340 for papaverine, $m/z$ 414 for noscapine, and $m/z$ 462 (286 as the deconjugation product ion) for M3G and M6G. Only meconic acid was determined using DAD at 280 nm. Detection and integration of chromatographic peaks were performed by the Agilent CHEMSTATION data analysis system (Agilent Technology). The peak area was used as a response.

### 2.3. Standard solutions

Individual standard solutions of 1 mg/ml of each drug, morphine, codeine, thebaine, meconin, papaverine and noscapine (additionally, M3G and M6G for the urine analysis), were prepared in methanol, ethanol or distilled water, according to the solubility of the solute, and stored in the dark at −20 °C. Under these conditions, all solutions proved stable for more than 2 months at least. Mixed standard solutions, ranging from 0.1 to 5.0 μg/ml of all opiates, were prepared by mixing an aliquot of each stock solution. These solutions were prepared freshly for each analysis. Solutions of 10 μg/ml and 20 mg/ml of the I.S. (naloxone), in distilled water were also prepared.

### 2.4. Calibration curves

The drug concentrations in the samples were calculated using the peak-area ratios of the ions monitored for the target compounds versus the I.S. Calibration curves for determination of the analytes in opium gum and volatilized materials were constructed by analyzing mixed solutions of 50 m*M* ammonium formate (pH 3)–acetonitrile (4:1, v/v) spiked with the mixed standard solution. The calibration curves for the urine samples were constructed by analyzing extracted drug-free control urine spiked with the mixed standard solution. Calibration sam-

ples containing 1, 5, 10, 50, 100 and 200 μg/ml of each drug for the opium gum or 10, 25, 50, 100, 250 and 500 ng/ml for the volatilized materials and the rat urine samples were prepared just before analysis. The limit of quantitation of each drug was chosen to be the concentration of the lowest calibration standard with an acceptable limit of variance.

### 2.5. Inhalation procedure

The exposure system was according to a previously reported method [18,21,22] with a few modifications. The apparatus consisted of a U-shaped pipe constructed from glass tubing (26 cm×6 mm I.D.) as shown in Fig. 2. The pipe was heated at a constant temperature (300 °C) in a heating mantle regulated by a temperature controller (type YER, M.E.D., Tokyo, Japan), which was filled with 5-mm steel beads. One end of the glass pipe was connected to a manifold. The subject was placed in the holding tube that fitted snugly into the manifold for a nose only exposure. A glass wool filter (trap A) consisting of 1 g of silanized glass wool in a 12-cm length of Tygon tubing (6 mm I.D.) was connected to the exhaust of the manifold. This filter was successively connected to two additional traps (traps B and C) that contained 75 ml of MeOH, respectively. Vapor was drawn (300 ml/min) through the apparatus by negative pressure, which originated from a vacuum pump regulated by a switching valve and a flow regulator. Once the flow-rate and temperature of the pipe were stabilized, 1 g of small pieces of opium was rapidly inserted into the bottom of the U-glass tubing using the delivery apparatus. After exposure of rats to the volatilized opium ($n=3$, male Wister rats, 150–170 g; Japan SLC, Shizuoka, Japan) for 20 min, the urine samples were collected at 0–4, 4–10, 10–24, 24–48 and 48–72 h after inhalation, and stored at –20 °C until analysis.

### 2.6. Sample preparation

#### 2.6.1. Extraction method of the compounds from opium gum

The seized opium sample was cut into small pieces and extracted with (A) 2.5% acetic acid, (B) 2.5% acetic acid in MeOH and (C) MeOH. A 20-mg amount of opium was extracted with each solvent (1 ml×5 times) under ultrasonication for 5 min; the solution was then vortex-mixed for 1 min and centrifuged. The supernatant solutions were combined; 100 μl of naloxone (I.S.) solution (20 mg/ml) were added and it was then made up to volume (10 ml) with water. The solution was filtered by membrane filter before LC–MS analysis.

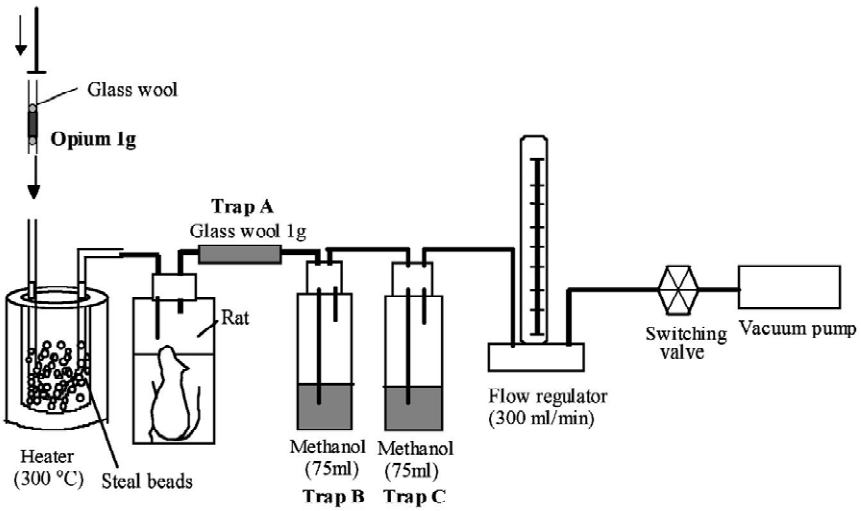


Fig. 2. Schematic for the opium volatilization and inhalation system.

*2.6.2. Recovery of volatilized opiates from the traps*

At the end of heating period, the U-glass tubing was withdrawn from the heating mantle and allowed to cool, and the vacuum pump was turned off. The glass wool (trap A) was removed from Tygon tubing, and analytes were extracted with five 10-ml portions of MeOH under ultrasonication. The Tygon tubing was rinsed with MeOH and the solution was combined with the extracts, followed by evaporation at room temperature. The residue was reconstituted in 1 ml of 50 m*M* ammonium formate (pH 3)–acetonitrile (4:1, v/v) including I.S. solution, and then membrane filtered. The MeOH solutions from traps B and C were evaporated, reconstituted and filtered as for trap A. A 10-μl volume of each solution was automatically injected into the LC–MS.

*2.6.3. Extraction of opiates from rat urine samples*

The urine sample (250 μl) was buffered with 0.5 *M* potassium carbonate solution (pH 8.0–9.0) and spiked with 25 μl of the I.S. solution (10 μg/ml). After mixing on a vortex-mixer for 10 s, the solution was extracted using a solid-phase extraction column. The column was first conditioned with 1 ml of MeOH, followed by 1 ml of water. The sample was loaded on the column and washed with 1 ml of water. The analytes were eluted with 3 ml of MeOH. The eluate was evaporated to dryness under nitrogen gas and the residue was reconstituted in 100 μl of 50 m*M* ammonium formate (pH 3)–acetonitrile (4:1, v/v). A 10-μl volume of the solution was automatically injected into the LC–MS.

## 3. Results and discussion

*3.1. Separation and determination of opiates by LC–APCI-MS*

Chromatographic separation of the five principal opium alkaloids (morphine, codeine, thebaine, papaverine and noscapine), the other opiates (meconic acid and meconin), the metabolites (M3G and M6G) and I.S. (naloxone) was studied. With DAD (UV 280 nm), a complete separation of all ten compounds was confirmed in 30 min when a linear gradient elution with 50 m*M* ammonium acetate (pH 3)–acetonitrile was adopted on the ODS analytical column. Mass detection was carried out by APCI in the positive mode. The conditions for ionization of each drug, such as the nebulizer pressure, drying gas flow-rate, drying gas temperature, vaporizer temperature, capillary voltage, fragmentation voltage and corona current were investigated using flow-injection analysis and the optimum conditions were determined as described in Experimental. The protonated molecule ions ($MH^+$) were observed as base peak ions for all the compounds investigated in this study except meconin, and the quantitative analysis was done using these ions as selected monitoring ions. No adequate fragmentation was observed for meconic acid by APCI, not only in the positive mode but also in the negative mode. Furthermore, meconic acid could not be detected by electrospray ionization mass spectrometry. It is thought that meconic acid was not stable and decomposed at high temperature. Therefore, only meconic acid was determined with DAD (UV 280 nm) tandem connected to MSD. Fig. 3 shows LC–DAD (A) and LC–APCI-MS (B) chromatograms of opiate standard mixtures (100 μg/ml).

The full scan mass spectrum of product ions of M3G and M6G had the same protonated molecular ions at $m/z$ 462 and deconjugated product ions at $m/z$ 286. In the case of high concentrations (100 μg/ml) of standard solution of M3G and M6G, the protonated molecular ions were the main ones under the analytical condition used in this study; however, the deconjugated product ions were inclined to be the major ones for analysis of lower concentrations of drugs in rat urine samples. Nishikawa et al. [23] and Bogusz et al. [24] reported that the extent of fragmentation of morphine glucuronides by APCI was affected by the concentrations of compounds or the composition of mobile phase, therefore the deconjugated product ($m/z$ 286) ion was monitored for the analysis of the urine samples. Table 1 shows the calibration functions, precisions and detection limits of standard solutions using LC–APCI-MS.

*3.2. Determination of opium alkaloids, meconic acid and meconin in gum opium*

Srivastava et al. [9] reported that five extractions with 2.5% aqueous acetic acid quantitatively ex-

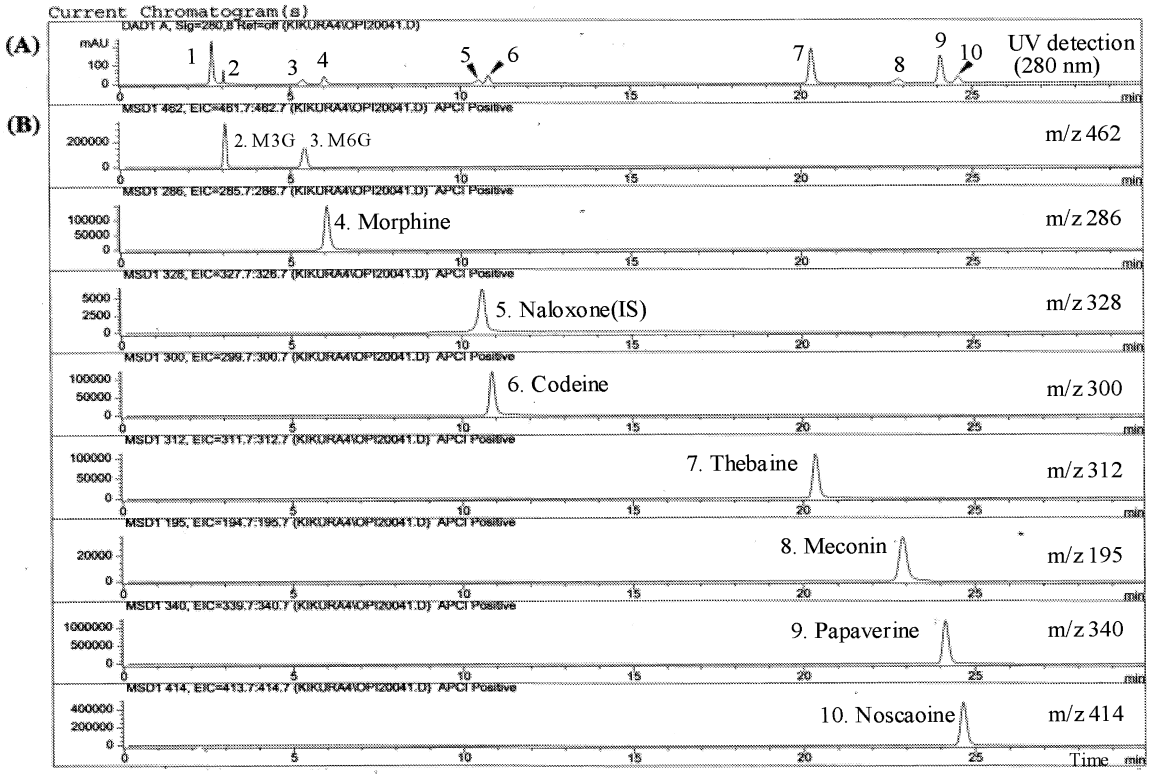


Fig. 3. HPLC–UV (A) and –MS (B) chromatograms of standard solution spiked with 100 μg/ml of opiate mixtures. 1 = meconic acid; 2 = M3G; 3 = M6G; 4 = morphine; 5 = naloxone (I.S.); 6 = codeine; 7 = thebaine; 8 = meconin; 9 = papaverine; 10 = noscapine.

Table 1
Validation results of the LC–APCI-MS analysis for standard solution

| | Detection limit (ng/ml, $S/N>3$) | Calibration curves ($r^2$) (1.0 μg/ml) | Precision (RSD, %) | |
|---|---|---|---|---|
| | | | Repeatability[a] | Reproducibility[b] |
| Meconic acid[c] | 25.0 | $y = 0.0350x - 0.0553$ ($r^2 = 0.9998$) | 0.12 | 0.70 |
| Morphine | 0.1 | $y = 0.0315x - 0.0110$ ($r^2 = 0.9995$) | 2.26 | 6.54 |
| Codeine | 0.1 | $y = 0.0386x - 0.00002$ ($r^2 = 0.9998$) | 4.38 | 9.44 |
| Thebaine | 0.1 | $y = 0.0204x - 0.0133$ ($r^2 = 0.9964$) | 1.76 | 5.31 |
| Meconin | 10.0 | $y = 0.0101x - 0.0038$ ($r^2 = 0.9975$) | 1.48 | 8.42 |
| Papaverine | 0.01 | $y = 0.1248x - 0.0274$ ($r^2 = 1.0000$) | 1.69 | 7.82 |
| Noscapine | 0.01 | $y = 0.0725x - 0.0108$ ($r^2 = 1.0000$) | 0.39 | 3.45 |

[a] Repeatability was calculated on the basis of six replicates at 50 μg/ml within a day.
[b] Reproducibility was calculated on the basis of duplicate per a day for 5 days.
[c] Determined from data measured with UV detection (280 nm).

tracted the major alkaloids from opium gum (>99% of recoveries). In this study, the acidic and neutral compounds, meconic acid and meconin, in opium gum were also analyzed in addition to the alkaloids. For sufficient extraction of these compounds from the opium, three solvents, (A) 2.5% aqueous acetic acid, (B) 2.5% acetic acid in MeOH and (C) MeOH were studied. Table 2 shows the concentrations of each compound extracted from the opium by the three extraction solvents.

The results in the observed contents were obtained using those solvents for the five extractions, in the ranges 11.0–12.0% for morphine, 7.2–8.3% for noscapine, 7.8% for meconic acid, 4.1–4.4% for codeine, 3.6–3.9% for thebaine, 1.5–2.0% for papaverine and 0.06% for meconin, although the contents with 2.5% acetic acid extraction was slightly higher as a whole. The RSD of each drug for these extraction methods was <5.5%. No difference was observed between the extraction efficiencies of the alkaloids, meconic acid and meconin using these solvents. To confirm the stability of the analytes in each solvent, the mixed standard solution was added to the solvents and treated in a similar manner as the extraction of opium. As a result, their recoveries were satisfactory—more than 97% with all solvents. Under the chromatographic conditions used, there was no interference to the target compounds or their I.S. from any endogenous materials extracted from the opium by those solvents. These results suggest that meconic acid and meconin could be sufficiently extracted from the opium with 2.5% aqueous acetic acid, similar to the other alkaloids. Therefore, 2.5% aqueous acetic acid was used as the extraction solvent.

### 3.3. Opium vaporization

An initial study was conducted to determine the U-shaped pipe temperature that produced optimal volatilization. The pipe temperatures evaluated were 200, 250 and 300 °C, which were above the melting point of the drugs (morphine, 197 °C; codeine, 154–156 °C; thebaine, 193 °C; noscapine, 176 °C; papaverine, 147 °C; meconin, 102–103 °C). The effect of temperature on volatilization was examined by maintaining a flow-rate of 300 ml/min during a 20-min heating. As a result, the maximum contents of the opiates were recovered from the traps A, B and C when the pipe was heated at 300 °C. Morphine (44.14 μg), codeine (115.13 μg), thebaine (3.15 μg), meconin (73.24 μg), papaverine (31.81 μg) and noscapine (7.6 μg) were recovered from the traps (A+B+C) under these conditions; however only a trace of meconic acid was detected (Table 3). It is known that meconic acid becomes an anhydride at a high temperature; making it difficult to detect it in the vapor. Moreover, the amounts of thebaine and noscapine recovered from the traps were only 0.01% of those in the opium. With regard to thebaine, thermal decomposition at a temperature routinely used for gas chromatographic separation (285 °C) has been reported [25]. On the other hand, a large amount of meconin was detected in the vapor as compared with the opium, and 11.63% of meconin in the opium was trapped at traps A, B and C after volatilization. The melting point of meconin is relatively low, 102–103 °C; therefore it should be easily volatilized. These results suggest that it could be possible to detect meconin in biological fluids after opium ingestion by inhalation in spite of its low concentration in the opium.

### 3.4. Selectivity, linearity and precision of the analytical method for the rat urine

Under the chromatographic conditions used, there was almost no interference with the target com-

Table 2
Comparison of three extraction methods of the opium sample

| Extraction solvent | Drug amounts (mg/g opium) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Meconic acid[a] | Morphine | Codeine | Thebaine | Meconin | Papaverine | Noscapine |
| 2.5% AcOH | 78.11±0.47 | 116.89±3.23 | 44.18±0.62 | 38.26±0.26 | 0.63±0.02 | 19.66±1.21 | 83.00±3.34 |
| 2.5% AcOH+MeOH | 77.61±1.91 | 119.83±2.83 | 43.97±1.70 | 38.93±1.11 | 0.63±0.01 | 18.93±0.99 | 79.26±4.37 |
| MeOH | 78.03±0.64 | 110.69±5.16 | 40.60±1.83 | 36.39±0.15 | 0.56±0.03 | 14.74±0.99 | 71.84±3.01 |

[a] These values were calculated using UV detection.

Table 3
Drug amounts recovered from traps A, B and C after 20-min volatilization of opium

| | Meconic acid[a] | Morphine | Codeine | Thebaine | Meconin | Papaverine | Noscapine |
|---|---|---|---|---|---|---|---|
| Trap A (μg) | Trace | 40.69±20.1 | 104.75±46.13 | 2.97±2.42 | 37.4±25.68 | 29.06±14.10 | 6.63±4.40 |
| Trap B (μg) | ND | 3.10±2.56 | 9.42±6.41 | 0.15±0.10 | 35.38±26.28 | 2.51±1.81 | 0.61±0.48 |
| Trap C (μg) | ND | 0.35±0.26 | 0.96±0.75 | 0.025±0.011 | 0.46±0.26 | 0.24±0.18 | 0.36±0.27 |
| Total (A+B+C, μg) | Trace | 44.14 | 115.13 | 3.15 | 73.24 | 31.81 | 7.6 |
| Recovery (%)[b] | – | 0.04 | 0.26 | 0.01 | 11.63 | 0.16 | 0.01 |

The U-pipe temperature was 300 °C and the flow-rate was 300 ml/min during a 20-min vaporization.
[a] These values were calculated using UV detection.
[b] Recovery means the ratio of the total amount of drug in the traps (trap A+B+C) to the amount in the opium (1 g).

pounds, M3G, M6G, morphine, codeine, thebaine, papaverine and noscapine or their I.S. from any extractable endogenous materials in the rat control urine; however, it was observed that the selected monitoring ion peak of meconin ($m/z$ 195) was partially overlapped with a small amount of endogenous material on the chromatogram. The calibration curves for these compounds were linear over the concentration range 10–500 ng/ml with good values of $r^2 \geq 0.996$. The precision and recovery data of the analytical procedure for the rat urine sample spiked with the standard solution of each compound are presented in Table 4. The precision was evaluated by RSDs of three standards analyzed within the same day. The recovery of analytes from the urine using the solid-phase extraction method was calculated on the basis of triplicated measurements at each concentration. The precision of these drugs ranged from 1.1 to 8.4% except for 50 ng/ml meconin (18.1%). The recovery values were almost more than 80% for all compounds but thebaine (71.5–72.8%) and that of meconin was almost 150%, which could be caused by the interference of endogenous material to meconin on the chromatogram. Some scatter was observed in the validation date of meconin; however, this analytical procedure could be useful for the

Table 4
Validation results of the LC–APCI-MS analysis for rat urine sample

| | Calibration curves ($r^2$) (10–500 ng/ml) | Reproducibility | | Recovery[b] (%) |
|---|---|---|---|---|
| | | Conc. (ng/ml) | RSD[a](%) | |
| Morphine-3-glucronide | $y = 0.0023x - 0.0008$ ($r^2 = 0.9999$) | 50 | 8.4 | 102.8 |
| | | 250 | 3.6 | 94.1 |
| Morphine-6-glucronide | $y = 0.0018x - 0.0013$ ($r^2 = 0.9994$) | 50 | 6.3 | 92.1 |
| | | 250 | 4.4 | 93.4 |
| Morphine | $y = 0.0107x - 0.0794$ ($r^2 = 0.9982$) | 50 | 1.1 | 95.0 |
| | | 250 | 4.0 | 109.2 |
| Codeine | $y = 0.0070x - 0.0079$ ($r^2 = 0.9997$) | 50 | 5.2 | 88.4 |
| | | 250 | 1.3 | 83.3 |
| Thebaine | $y = 0.0036x - 0.0567$ ($r^2 = 0.9954$) | 50 | 2.0 | 71.5 |
| | | 250 | 4.5 | 72.8 |
| Meconin | $y = 0.0005x - 0.0923$ ($r^2 = 0.9973$) | 50 | 18.1 | 144.2 |
| | | 250 | 5.9 | 158.5 |
| Papaverine | $y = 0.0323x - 0.0244$ ($r^2 = 0.9992$) | 50 | 2.3 | 98.1 |
| | | 250 | 4.2 | 89.9 |
| Noscapine | $y = 0.00141x - 0.0469$ ($r^2 = 0.9985$) | 50 | 3.8 | 80.5 |
| | | 250 | 4.3 | 78.1 |

[a] RSD (relative standard deviation) was calculated on the basis of triplicates at each concentration.
[b] Recovery was calculated on the basis of triplicates at each concentration.

measurement of opiates and their metabolites in the urine using LC–MS because linearity for each compound was still sufficient.

### *3.5. Excretion of opiates and their metabolites into the urine after opium inhalation by rats*

After inhalation of volatilized opium to three rats, the concentrations of opiates and the metabolites in the urine were monitored using LC–APCI-MS. The time courses of excretion of M3G, M6G, morphine, codeine, thebaine, meconin, papaverine and noscapine in the urine over 72 h are shown in Fig. 4. The LC–MS chromatogram of the extract from the urine of the rat exposed to volatilized opium is shown in Fig. 5.

The major metabolite excreted in the rat urine was M3G and its maximum excretion occurred within the first 10 h in the urine (3.6–7.1 μg/ml). Meconin (4.40–5.06 μg over 96 h), morphine (2.27–4.65 μg), codeine (0.54–1.85 μg) and noscapine (0.34–0.40 μg) were detected in the urine in relatively large amounts, and a small amount of papaverine (0.014–0.035 μg) was also detected (Table 5). Meconin was detected as almost the same amount as morphine. However, only trace levels of thebaine were observed in the urine despite it being one of the major alkaloids found in the opium, and M6G was not detected. Cone et al. reported similar results [17] with regard to a urine sample of an ''opium eater''; morphine, codeine, normorphine, norcodeine and noscapine were detected, but thebaine, papaverine and oripavine were not observed.

Fig. 6 shows the comparison of the ratios of drug amounts in the opium, vapor and urine (morphine = 100). A large amount of meconin was detected in the vapor and urine compared with the opium. Tsunoda et al. [26,27] have reported that meconin was the major metabolite of noscapine in all three species, accounting for about 3, 8 and 2% of the oral dose of

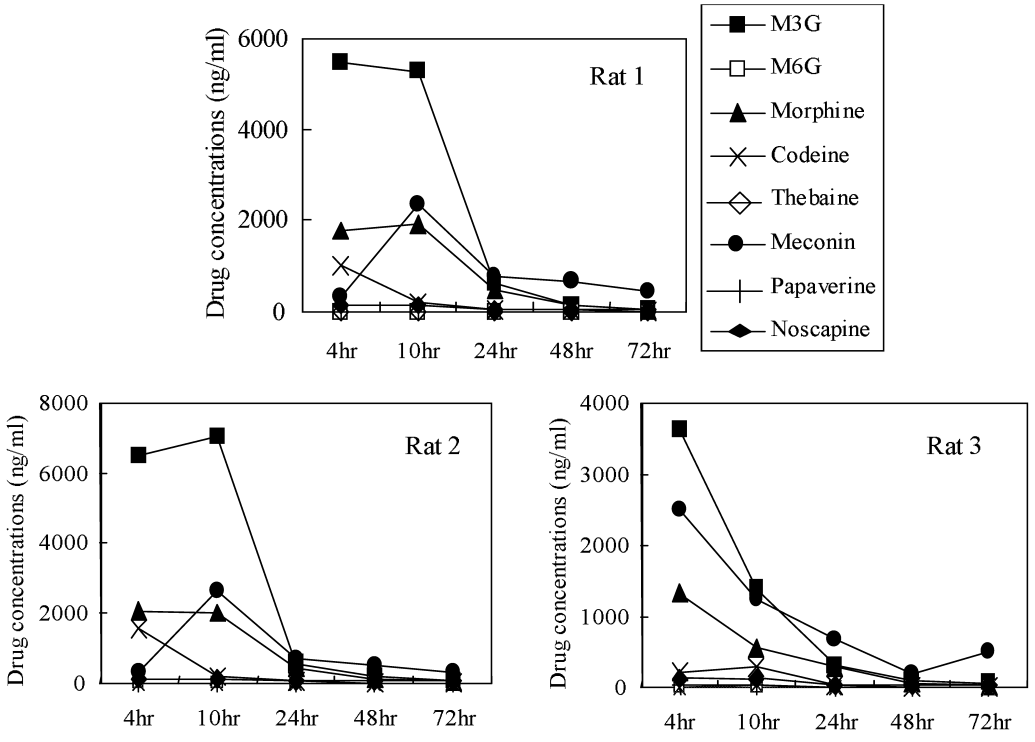


Fig. 4. Time course of excretion of opiate compounds and morphine glucronides into urine of rats 1, 2 and 3 after 20-min exposures to volatilized opium.

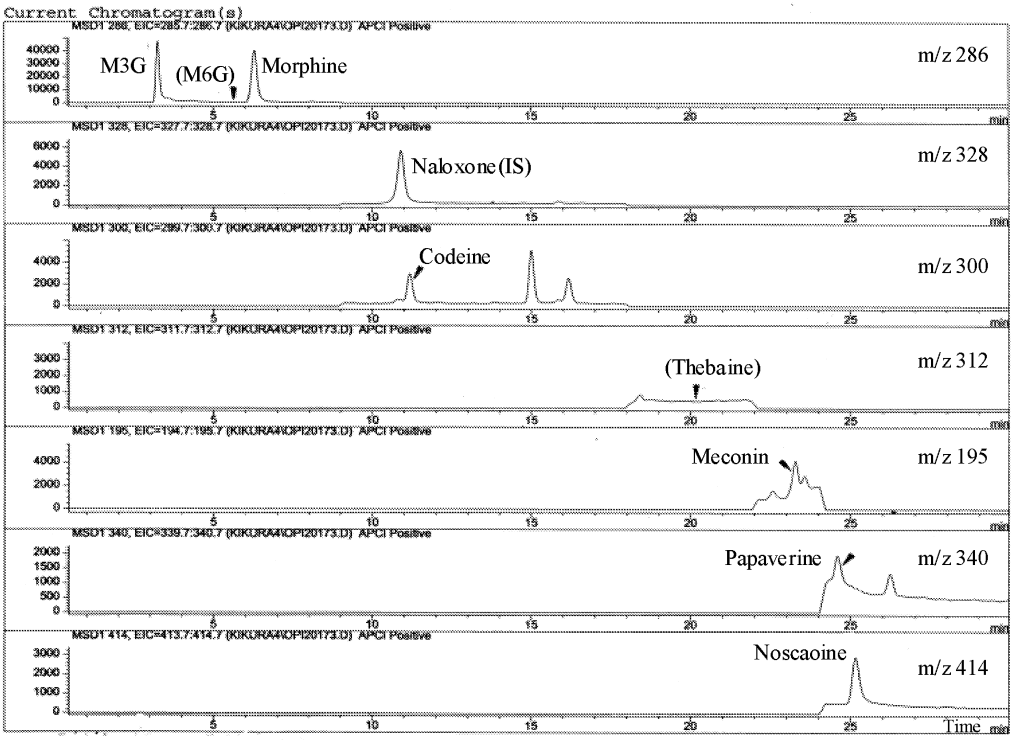


Fig. 5. LC–MS chromatogram obtained from the urine extract of rat exposed to volatilized opium (after 4–10 h).

noscapine in the first 24 h urines of rats, rabbits and humans, respectively. In our inhalation experiments, noscapine was detected only less than one fifth of meconin in the vapor, and it was thought that almost all the meconin in the urine could be derived from volatilized mecoin. These results suggest that meconin could be a noteworthy compound in biological fluids when investigating opium ingestion by inhalation.

## 4. Conclusion

Simultaneous determinations of compounds in opium, the vapor derived by the volatilization of opium and the urine of rats exposed to the opium vapor were made using LC–APCI-MS. Chromatographic separation of the five principal opium alkaloids, meconic acid, meconin, the metabolites (M3G and M6G) and the I.S. (naloxone) was com-

Table 5
Comparison of the quantitative results for the opium, vapor and urine of rats exposed to the opium vapor

| | | M3G | M6G | Morphine | Codeine | Thebaine | Meconin | Papaverine | Noscapine |
|---|---|---|---|---|---|---|---|---|---|
| Opium (mg/opium 1 g) | | ND | ND | 116.89 | 44.18 | 38.26 | 0.63 | 19.66 | 83.00 |
| Vapor (A+B+C, total μg) | Rat 1 | ND | ND | 19.96 | 72.06 | 0.60 | 22.09 | 19.50 | 2.60 |
| | Rat 2 | ND | ND | 61.44 | 174.34 | 1.90 | 57.21 | 49.91 | 9.15 |
| | Rat 3 | ND | ND | 28.82 | 98.99 | 6.96 | 41.00 | 25.99 | 11.05 |
| Urine (0–72 h, total μg) | Rat 1 | 11.59 | ND | 4.39 | 1.29 | ND | 4.60 | 0.014 | 0.40 |
| | Rat 2 | 14.38 | ND | 4.65 | 1.85 | Trace | 4.40 | 0.018 | 0.36 |
| | Rat 3 | 5.45 | ND | 2.27 | 0.54 | 0.023 | 5.06 | 0.035 | 0.34 |

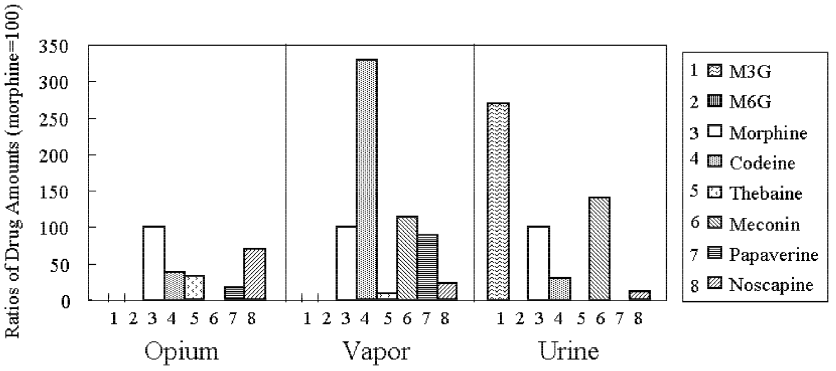


Fig. 6. Comparison between the ratios of drug amounts in the opium, vapor and urine (morphine = 100).

pleted in 30 min when a linear gradient elution with 50 m*M* ammonium acetate (pH 3)–acetonitrile was adopted on an ODS analytical column. Detection was carried out by the APCI mass analysis in positive mode and the protonated molecule ions ($MH^+$) were observed as base peak ions for all the compounds except meconin.

For the analysis of the volatilization of opium, the opium was added to the glass pipe, which was then heated at 300 °C for 20 min by maintaining the flow-rate of 300 ml/min. As a result, all compounds except meconic acid were recovered from the traps captured the vapor derived by the volatilization of opium. After inhalation exposure of opium to rats, M3G, morphine, meconin, codeine, noscapine and papaverine were detected in the urine. Only trace levels of thebaine were observed in the urine despite it being one of the major alkaloids found in the opium. A relatively large amount of meconin was detected in the vapor and the urine as compared with the opium. It is suggested that the presence of meconin in biological fluids could be indicative of opium ingestion by inhalation.

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