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Rapid detection of dihydrocodeine by thermospray mass spectrometry

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Abstract

Rapid assay of dihydrocodeine (DHC) by thermospray mass spectrometry is explored. Liquid–liquid extractions of blood, urine and gastric contents were injected into a thermospray mass spectrometer, to which there was no column connected, and DHC was assayed by the flow injection method. The mass spectra of DHC under thermospray ionization and filament-on ionization modes consist of the MH^+ ion of m/z 302 alone, which was clearly detected in the samples. Although DHC should be quantitated by gas chromatography–mass spectrometry, this method is applicable for rapid identification of DHC in biological materials.

Keywords: Dihydrocodeine

1. Introduction

In cases of drug poisoning, rapid identification of the drugs in biological materials such as blood, urine and gastric contents is often required, especially in emergency medicine. Dihydrocodeine (DHC), a narcotic analgesic usually used as an antitussive, has been analyzed by gas chromatography [1], high-performance liquid chromatography [2,3], or gas chromatography–mass spectrometry (GC–MS) [4–6]. GC–MS analysis may be the most precise method regarding both identification and quantitation, but the procedures, such as extraction and derivation of the drugs, preliminary studies to determine the appropriate experimental conditions, and pattern analysis of fragment ions in the mass spectra, are troublesome and time-consuming. Rapid immunoassay kits such as Triage (Biosite Diagnostics, San Diego, CA, USA) are very simple and rapid in screening of abused drugs and their metabolites in urine, but Triage detects DHC only as a kind of opiate. To identify the detected opiate, the sample must be analyzed further by mass spectrometry. We investigated thermospray mass spectrometry (TSP-MS) to explore a more rapid and identifiable assay for DHC in biological materials.

2. Materials and methods

Left and right ventricular blood, urine and gastric contents were extracted from a fire victim, who was suspected of abusing drugs before her death by carbon monoxide poisoning. Her body surface was

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wholly charred, but her organs, heart blood, urine and gastric contents were only slightly affected by heat. The specimens were stored at −20°C until drug analysis was performed. Standard DHC was purchased from Dainippon Pharmaceutical. As a negative control, peripheral blood, urine and gastric contents were obtained from healthy volunteers and other autopsied cadavers who had no history of using narcotic analgesics. Detection limits of DHC in TSP-MS were determined using specimens treated with appropriate amounts of standard DHC.

For the Triage test, non-pretreated urine and the liquid–liquid extraction of urine (described below) were used according to the manufacturer's instruction.

For TSP-MS, 2 ml of blood, urine or gastric contents were alkalized to pH 10 by adding appropriate amounts of 28% ammonia water, then 5 ml of ethyl acetate were added and shaken vigorously for 5 min. Following centrifugation (1200×*g*, 10 min), the organic phase was dried under a stream of nitrogen. The residue was dissolved in 100 μl of 20% methanol containing 0.1 *M* ammonium acetate. After centrifugation (1200×*g*, 10 min) to remove lipids in the sample, 20 μl of supernatant were injected into a Shimadzu LC–MS QP1000EX liquid chromatograph–quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) with a Vestec thermospray interface (Vestec, Houston, TX, USA), and a Rheodyne Model 7125 injector (Rheodyne, Cotati, CA, USA) fitted with a 100 μl loop or Shimadzu SIL-7A autoinjector. No column was connected to the apparatus, and the assay was performed using the flow injection method. The mobile phase consisted of 20% methanol containing 0.1 *M* ammonium acetate, and the flow phase used a Shimadzu LC-9A pump at a rate of 1.0 ml/min. The scanning mass range was *m*/*z* 140–640 with a scanning interval of 1 s. The exit temperatures of the vaporizer and block and the tip heater temperature of the ion source were 160, 305 and 310°C, respectively. The repeller was set at 0 V. Positive ion TSP-mass spectra were obtained by TSP ionization mode (filament-off mode) or TSP on-filament ionization mode (filament-on mode).

For GC–MS, 200 μl of 10 μg/ml diazepam were added as an internal standard into 5 ml of the specimen. Following alkalization, liquid–liquid extraction using 15 ml chloroform was performed twice. The organic phase was dehydrated with an appropriate volume of anhydrous sodium sulfate, and evaporated under stream nitrogen. The residue was dissolved in 10 μl of ethyl acetate, and 0.5 μl of solution were assayed using a QP2000A GC–MS (Shimadzu) equipped with a CBP1-M25 column (25 m×0.2 mm I.D., 0.25 μm film thickness, Shimadzu). The conditions were: carrier gas, helium at a flow-rate of 0.56 ml/min; column temperature, 60°C (maintained for 3 min) to 240°C programmed at 10°C/min; injection temperature, 200°C; injection, split method (1:85); interface temperature, 250°C; mode, electron-impact ionization; ion source temperature, 250°C; electron energy, 70 eV; total emission current, 400 mA; scanning mass range, 40–600 *m*/*z*; and scanning interval, 1.0 s.

3. Results

When the Triage test was performed using urine from the fire victim, a red bar appeared at the 'opiate' detection zone on the device membrane. Triage detects more than 300 ng/ml of opiates such as morphine, codeine, heroin and DHC. To identify the opiate, further analysis by mass spectrometry was required.

A TSP-mass spectrum of standard DHC is shown in Fig. 1. Under both filament-off and -on modes, simple mass spectra were obtained. An MH^+ ion of *m*/*z* 302 was detected as the base ion, but no fragment ion was observed. The spectra of specimens (blood, urine and gastric contents) extracted from healthy volunteers and autopsied cadavers, who had no history of using DHC, did not include the *m*/*z* 302 ion but ions less than *m*/*z* 200. However, the spectra of the specimens containing standard DHC showed that impure ions corresponding to biological substances and contents in the specimens reduced the relative intensity of the base ion of DHC. Therefore, under both the filament-off and -on modes, detection limits of DHC in blood, urine and gastric contents were 60, 150 and 200 ng/ml, respectively, while the limit of DHC in aqueous solution was 2.5 ng/ml.

In the TSP-mass spectra of the liquid–liquid extractions of blood, urine and gastric contents from the fire victim, the *m*/*z* 302 ion was clearly detected (Fig. 2). The MH^+ ions of other opiates, 6-acetylmorphine, codeine, heroin, ethylmorphine,

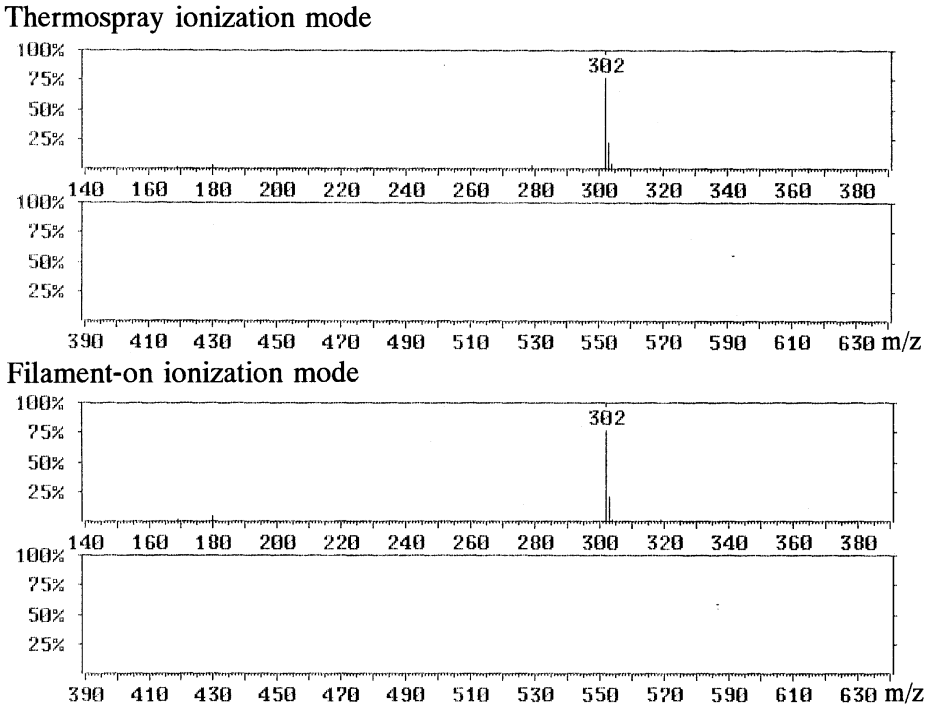

Fig. 1. Mass spectra of DHC by TSP-MS.

morphine, morphine-*N*-oxide and oxymorphone are *m*/*z* 328, 300, 370, 314, 286, 302 and 302, respectively. By TSP-MS, the opiate detected by the Triage test was focused to DHC, oxymorphone or morphine-*N*-oxide.

Then, further qualitative and quantitative analyses were performed by GC–MS. DHC is characterized by *m*/*z* 301, 164, 70 and 244 ions (Fig. 3), while morphine-*N*-oxide by *m*/*z* 285, 162, 215 and 124 ions, and oxymorphone by *m*/*z* 301, 115, 70 and 216 ions, so that DHC in the specimen extracts was easily monitored by GC–MS chromatograms of the ions (Fig. 4). DHC was not detected in control samples, but was found in all materials from the victim at a retention time of about 27.53 min (Table 1). The concentrations quantitated by GC–MS suggested that the victim had taken usual doses of DHC as an antitussive.

4. Discussion

To explore the usefulness of rapid mass-spectrometric assay to identify unknown drugs in biological materials, TSP-MS was investigated in this study. In the assay, no separation column was connected to the apparatus for rapid identification. Therefore, ions corresponding to both drugs and biological substances (impurities) contained in the specimens were detected at the same time in TSP-mass spectra. Generally, MH^+ ions of drugs are detected as base ions, because little fragmentation of the molecules occurs. Drugs were thus identified only by differences in their molecular weights. Since ions corresponding to the impurities were less than *m*/*z* 200, drugs with molecular weights greater than 200 were identified with this method. Relative intensities of MH^+ ions of the drugs may be reduced by the impure ions, but not by fragmentation. Contamination of the mass spectrometer in the TSP-MS was the same as that in GC–MS, because liquid–liquid extractions of the specimens were used.

In the fire death case, opiate was detected in the urine with the Triage test. The *m*/*z* 302 ion detected by TSP-MS assay may have been derived from DHC, morphine-*N*-oxide or oxymorphone, but their spectra could not be identified due to codetection of the impure ions. This fact, however, did not indicate

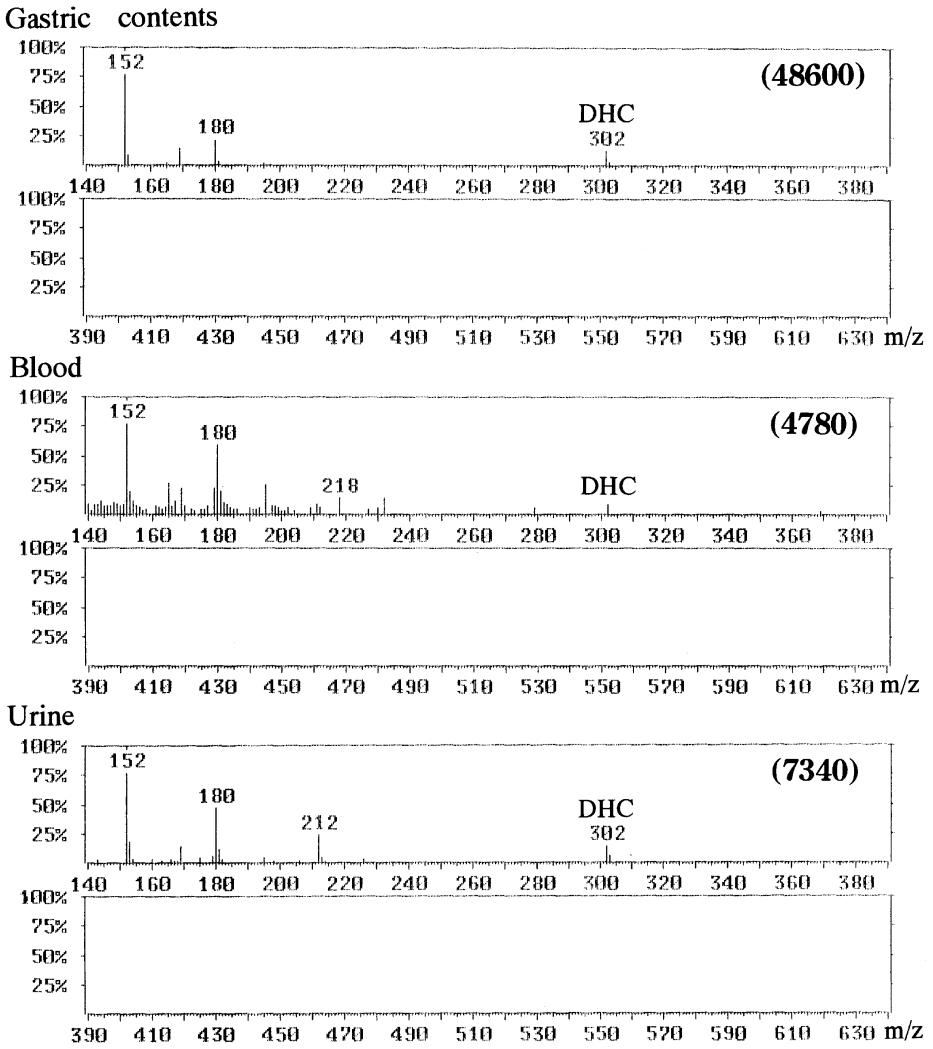

Fig. 2. Mass spectra of extracted samples by TSP-MS using the flow injection method. Intensity of the base ion is presented in the parenthesis.

low applicability of the TSP-MS assay, because base ions of other opiates, 6-acetylmorphine, codeine, heroin, ethylmorphine and morphine, could be identified easily (data not shown). DHC, morphine-*N*-oxide and oxymorphone should have thus been identified based on their metabolic features or by GC–MS analysis, as described below.

Morphine-*N*-oxide is produced during the metabolism of morphine in the presence of tacrine or amiphenazole in humans, and may be reversely metabolized to morphine in their absence [7–9]. Therefore, morphine is always detected in biological specimens containing morphine-*N*-oxide, whereas the MH^+ ion of morphine was not detected in the samples. Furthermore, morphine-*N*-oxide can be obtained neither commercially nor illegally, at least in Japan. These facts suggested that the *m*/*z* 302 ion was not the MH^+ ion of morphine-*N*-oxide. On the other hand, oxymorphone (Numorphan), a semisynthetic narcotic analgesic derived from thebaine, is

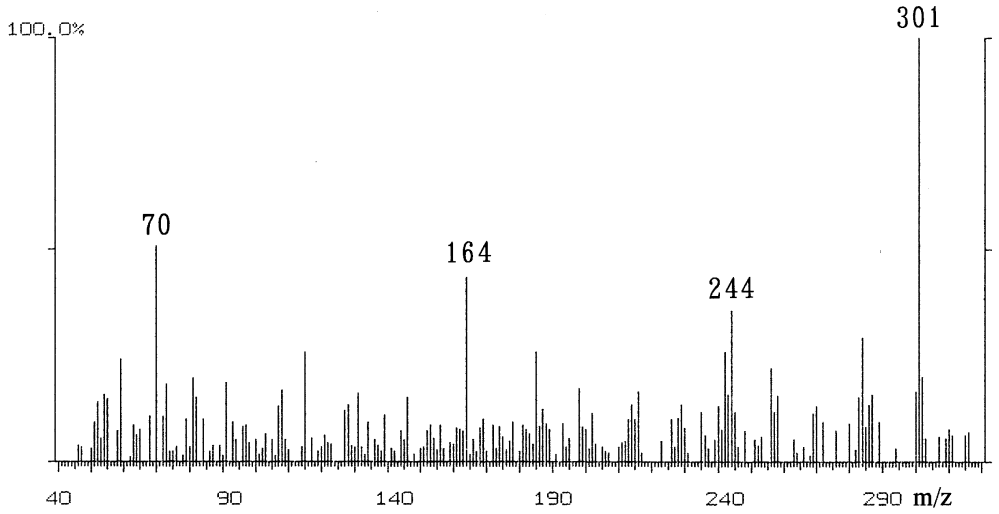

Fig. 3. Mass spectrum of DHC in blood by GC–MS.

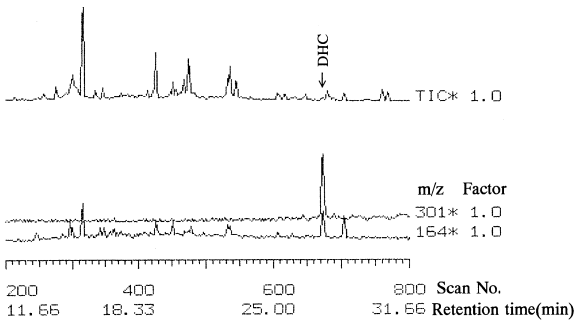

Fig. 4. Mass chromatogram of blood extract by GC–MS.

prescribed primarily as a postoperative analgesics as it is approximately ten times more potent than morphine and produces mild sedation and little depression of the cough reflex [10]. Due to oxymorphone's limited availability, it is not commonly found in toxicological investigations. Furthermore, oxymorphone is extensively metabolized in humans to 6α- and 6β-oxymorphol following conjugation. Therefore, when biological specimens are analyzed by TSP-MS following administration of oxymorphone, MH^+ ions of the oxymorphols (m/z 304) are detected more intensely than that of oxymorphone. Morphine-*N*-oxide and oxymorphone are usually administered parenterally, so that these opiates are rarely found in gastric contents.

Table 1
Concentrations and retention times of DHC in biological specimens

	Concentration (μg/ml)	Retention time (min)
Blood	0.15	27.46
Urine	0.37	27.46
Gastric contents	11.4	27.38
Standard	–	27.53

In conclusion, the m/z 302 ion detected in the samples was considered to be the MH^+ ion of DHC, which was confirmed and quantitated by GC–MS. Thermospray liquid chromatography–mass spectrometry (TSP–LC–MS) was not performed for quantitation of DHC, due to low reliability and precision of the assay. For accurate TSP–LC–MS analysis of DHC, deuterium-labelled DHC, rather than diazepam, should be used as an internal standard [11], to compensate for signal instability commonly observed in TSP–LC–MS analyses [12]. DHC may be analyzed with sufficiently-high precision by GC–MS using diazepam as an internal standard, while a deuterium-labelled internal standard is also suitable for GC–MS analysis.

Although drugs in biological materials should be identified by GC–MS, TSP-MS is more rapid and

simpler, and can detect one drug, or at least several drugs, among the suspected substances. TSP-MS may thus be useful in many situations such as emergency medicine in which administered drugs must be identified as soon as possible.

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