Simultaneous Determination of Carisoprodol and Acetaminophen in an Attempted Suicide by Liquid Chromatography–Mass Spectrometry with Positive Electrospray Ionization

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Abstract

An adult female ingested a considerable quantity of carisoprodol/acetaminophen tablets, which are not commercially available in Japan, in an attempt to commit suicide. Generally, because of lack of the appreciable ultraviolet absorbance or fluorescence, carisoprodol and its major metabolite meprobamate are determined by gas chromatography or gas chromatography–mass spectrometry. Complicated derivatization is, however, necessary to that methodology. Thus, we investigated the derivatization-free, highly sensitive, and simultaneous determination of carisoprodol, meprobamate, and acetaminophen by means of liquid chromatography–mass spectrometry (LC–MS) with positive electrospray ionization. A semi-micro ODS column was used. Ammonium acetate solution (10mM) and acetonitrile were used as mobile phase at a flow rate of 150 μL/min using gradient elution. MS parameters were as follows: capillary voltage, 3.5 kV; cone voltage, +30 V; extractor voltage, 5 kV; and ion source temperature, 100°C. Urine samples pretreated by Oasis™ HLB cartridge, or plasma samples deproleinized by adding ice-cold acetoniitrile were analyzed by LC–MS. The limits of quantitation for each compound were as follows: 0.50 ng/mL for carisoprodol; 10 ng/mL for acetaminophen; and 1.0 ng/mL for meprobamate. In the present case, carisoprodol and acetaminophen were the only drugs detected. Meprobamate was also found as the metabolite of carisoprodol in both urine and plasma. The plasma levels of carisoprodol, acetaminophen, and meprobamate on arrival were 20.5, 245, and 46.7 μg/mL, respectively. These levels were extremely high compared with therapeutic plasma concentrations. Despite the high plasma concentrations of these drugs, which correspond to fatal levels, the patient survived.

Introduction

An adult female ingested a considerable quantity of carisoprodol/acetaminophen tablets, commercially unavailable as prescription or over-the-counter (OTC) drugs in Japan, in an attempt to commit suicide. Carisoprodol (N-isopropyl-2-methyl-2-propyl-1,3-propanediol dicarbamate) is a racemic carbamate derivative used primarily as a centrally acting musculoskeletal relaxant. This compound is both structurally and pharmacologically related to meprobamate and has sedative, antipyretic, and analgesic properties. The usual adult oral dosage of carisoprodol for the musculoskeletal relaxant property is 350–700 mg per day, and 700 mg of carisoprodol administered to a normal individual produces a peak serum level of 9.3 μg/mL in 1 h (1). Carisoprodol is rapidly distributed into the central nervous system and is then metabolized by undergoing dealkylation, hydroxylation, and conjugation in the liver. Meprobamate (2-methyl-2-propyl-1,3-propanediol dicarbamate) is a major and active metabolite of carisoprodol catalyzed by cytochrome P450 (CYP) 2C19 isoform localized on human liver microsomes (2). Although its toxicity to humans and rodents is known to be mild (LD50 in mice and rats, p.o.: 2340 and 1320 mg/kg, respectively) (3), several carisoprodol-related deaths have been reported (4–7). Carisoprodol is often combined with other antipyretics or analgesics such as aspirin, acetaminophen, or morphine. Carisoprodol has also been known to be abused (8,9).

The determination of carisoprodol and meprobamate, which are aliphatic compounds without any appreciable ultraviolet absorbance or fluorescence, has been primarily carried out by using gas chromatography (5) or gas chromatography–mass spectrometry (GC–MS) (6). These methodologies are, however, not optimal because of the heat instabilities of carisoprodol and meprobamate at the injection port of a GC. In order to
avoid this problem, Stidman et al. (10) hydrolyzed carisoprodol and meprobamate with KOH, and then the hydrolysates produced were trimethylsilylated before GC–MS analysis. Although the trimethylsilylated product has a high sensitivity to a mass analyzer in comparison with the original compound, this complicated derivatization is a time-consuming process that prolongs pretreatment and analysis time. We thus investigated a derivatization-free, highly sensitive, and simultaneous determination of carisoprodol, acetaminophen, and meprobamate in urine and plasma by means of liquid chromatography–mass spectrometry (LC–MS) equipped with pneumatically assisted electrospray ionization (ESI).

Case History

A 37-year-old female was found unconscious in a hotel bed the day after she checked in. An empty package of strawberry yogurt, the leftovers of orange-colored yogurt in a bowl, and a wooden pestle were found at the scene. There was no evidence that she was physically assaulted. In view of the circumstances surrounding this case, it has been considered that she ingested the yogurt mixed with some drugs pulverized by the wooden pestle in order to commit suicide. On admission, glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) levels were 325 and 376 IU/L, respectively. Initially, the emergency measures against common drug poisoning were taken because of the lack of information concerning the causal drugs. The patient was detoxified by means of direct hemoperfusion. Although she was then treated with N-acetyl-L-cysteine because of the possibility of acetaminophen-induced hepatitis, the hepatitis deteriorated, probably because of the belated administration. After two days, the GOT and GPT levels increased to 12,190 and 9110 IU/L, respectively. Thereafter, continuous hemodiafiltration and hemodialysis were performed against renal failure, and glucagon–insulin therapy was also carried out against hepatic failure. Despite such critical condition, the patient was eventually saved.

According to the subsequent investigation, she had purchased the carisoprodol/acetaminophen tablets, round-shaped orange-colored tablets engraved with the letter "W" in a hexagonal inscription on one side, through a Web site that provides suicide-related information.

Experimental

Reagents and standards

Carisoprodol, acetaminophen (paracetamol; N-(4-hydroxyphenyl)acetamide), and meprobamate were purchased from Sigma Chemical Co. (St. Louis, MO), Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and Daichi Seiyaku Co., Ltd. (Tokyo, Japan), respectively. All other reagents and solvents used were of the highest purity commercially available, and were purchased from Wako Pure Chemical Industries, Ltd. Stock solutions of carisoprodol, acetaminophen, and meprobamate were prepared by dissolving an appropriate amount of each drug in 50 vol% acetonitrile to make a concentration of 1 mg/mL. A procaine (2-((N,N-diethylamino)ethyl 4-aminobenzoate) solution (1 mg/mL) as an internal standard (I.S.) was also prepared in the same manner.

Apparatus and analytical condition

Determination of carisoprodol, acetaminophen, and meprobamate was accomplished by means of an LC–MS system consisting of Alliance™ 2900 separations module (Waters Corp., Milford, MA) and Platform LCZ quadrupole MS equipped with an ESI interface (Micromass Ltd., Manchester, U.K.). A Symmetry™ C18 (150 mm × 2.1-mm i.d., 3.5-μm packing, Waters Corp.) reversed-phase ODS packed column was used for separating the analytes from other impurities. Ammonium acetate solution (10mM) (AcONH₄ aq) and acetoniitrile were used as mobile phases at a flow rate of 150 μL/min. The elution gradient (AcONH₄ aq/acetoniitrile ratio) was ramped as follows: 90:10 (v/v) (0–5 min), 90:10–20:80 (v/v) linearly (5–15 min), and 20:80 (v/v) (15–25 min).

Full scanning and selected ion monitoring (SIM) modes were used for data acquisition in a positive-ion mode, and the operation parameters were as follows: ESI probe capillary voltage, +3.5 kV; sampling cone voltage, +30 V; extraction cone voltage, +5 kV; ion source block temperature, 100°C; desolvation gas temperature and flow rate, 300°C and ~400 L/h, respectively; and multiplier voltage, 650 V. Both the nebulizing gas and the desolvation gas used were N₂ separated from the ambient air. In the full scanning mode, the full-scan mass spectra were acquired in the mass range of 80–500 atomic mass units (amu). Scan time and interscan delay time were 0.5 and 0.1 s, respectively. In the SIM mode, only four fragments, m/z 152 (acetaminophen), 158 (meprobamate), 176 (carisoprodol), and 237 (I.S.), were monitored. Interchannel delay time and channel span were 0.02 s and ± 0.05 amu, respectively.

Specimens and extraction procedure

Urine and plasma samples were collected in the hospital to which the patient was admitted, and stored at ~85°C until analysis. Each sample was diluted to appropriate concentration if necessary, and then was added to 100 μL of 100 ng/mL procaine as an I.S. For urine samples, Oasis HLB solid-phase extraction cartridges (Waters Corp.) were used. The cartridge was activated with 2 mL of methanol and 3 mL of H₂O, and then 1 mL of the urine sample was loaded onto the cartridge. After column washing with 2 mL of H₂O, the analytes were eluted with 2 mL of methanol. The eluate obtained was evaporated to dryness under an N₂ stream at 60°C. The residue was then reconstituted to 1 mL with 10 vol% acetoniitrile. A 20-μL aliquot of the reconstituted solution was introduced into the LC–MS system by an autoinjector. For plasma samples, deproteinization was performed by adding an equal volume of ice-cold acetoniitrile. After sonication for 30 s, the plasma/acetoniitrile mixture was left at 4°C for 15 min, followed by centrifuging at 3000 rpm for 5 min. A 20-μL aliquot of the supernatant was introduced into the LC–MS system by an autoinjector. The calibration curve for each compound was obtained by employing drug-free human urine and plasma spiked with the appropriate standards. Each analyte was quantified by the peak-area ratio.
Results and Discussion

Optimization of sampling cone voltage

To obtain higher LC–MS–ESI sensitivity to the target analytes, the sampling cone voltage was optimized. The magnitude of the cone voltage may significantly affect in-source collision-induced dissociation (CID), and careful voltage adjustment contributes to the formation of a molecular-related ion, also known as a quasi-molecular ion, or several fragment ions as required. The effect of the cone voltage on the ESI mass spectra of carisoprodol is shown in Figure 1. A standard carisoprodol solution was prepared to give the final concentration of 1 μg/mL using 10mM AcONH₄aq/acetonitrile mixture (50:50, v/v) as an infusion solvent. The infusion was carried out at a flow rate of 10 μL/min. Low voltage (< 10 V) provided only the protonated ion [M+H]+, m/z 261, and higher voltage adequate to induce in-source CID formed several fragment ions such as m/z 176 or 158 in addition to the protonated ion. Although no protonated ion was observed at high voltage (> 40 V), the Na+-adduct ion [M+Na]+, m/z 283, emerged. When the cone voltage was set at 30 V, the sensitivity of carisoprodol reached its highest value. Likewise, the cone voltage for acetaminophen and meprobamate analysis was established. Finally, in the SIM mode, we set the cone voltage at 30 V, and monitored the following fragment ions: m/z 176 for carisoprodol, m/z 158 for meprobamate, m/z 152 for acetaminophen, and m/z 237 for procaine (I.S.).

Recovery, dynamic range, and limit of quantitation (LOQ)

The calibration curve for each analyte using SIM mode with spiked urine and plasma showed high linearity from 10 to 5000 ng/mL. Each data point plotted the peak area ratio of the analyte to the I.S. The correlation coefficients were r = 0.996, 0.994, and 0.994 for carisoprodol, acetaminophen, and meprobamate, respectively. The recoveries (mean ± S.D., n = 6) from the drug-free human urine spiked with the three analytes (100 ng/mL each) were 102.0 ± 3.01% for carisoprodol, 83.8 ± 2.88% for acetaminophen, and 98.2 ± 2.79% for meprobamate. The repeatabilities represented by relative standard deviations were also 2.95, 3.44, and 2.84% (n = 6), respectively. By utilizing the Oasis HLB solid-phase extraction cartridges, both carisoprodol and meprobamate were readily and completely extracted from the matrix. Although the recovery of acetaminophen was slightly low (83.8 ± 2.88%) as compared with the other two compounds, this method was also applicable to the quantitative analysis as well as the qualitative analysis. The LOQ for each compound at the signal-to-noise ratio of 10 was as follows: 0.50 ng/mL for carisoprodol; 10 ng/mL acetaminophen; and 1.0 ng/mL meprobamate. Because of the poor retention of ODS for acetaminophen, gradient elution was adopted. As a result, acetaminophen retained poorly and carisoprodol retained tightly could be deter-
Table I. Concentrations of the Drugs Detected in Urine and Plasma at the Same Time*

<table>
<thead>
<tr>
<th>Urine</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time elapsed after admission (h)</td>
<td>Time elapsed after admission (h)</td>
</tr>
<tr>
<td></td>
<td>0  18  24  33  41  29.5  22.0  2.29  0.60  0.18  0.07</td>
</tr>
<tr>
<td>Carisoprodol 27.3</td>
<td>0  0.73  0.15  0.10</td>
</tr>
<tr>
<td>Acetaminophen 841</td>
<td>0  22.9  28.4  13.1  6.03  2.42</td>
</tr>
<tr>
<td>Meprobamate 244</td>
<td>2.29  0.73  0.15  0.10</td>
</tr>
</tbody>
</table>

* Each concentration in the table is represented in μg/mL (n = 2).

Comparison of the drug concentration between reference data previously reported by Backer et al. (6) and the present data is shown in Table II. In that report, three cases of carisoprodol-related death were described. Although the patient in the present case was eventually saved, carisoprodol concentration in the plasma was as high as the reference data. This means that carisoprodol level of the patient had reached fatal level at the time of sampling. Moreover, fatal-level acetaminophen was also detected in the plasma of the patient. Because the patient was hospitalized 16 h after the ingestion, her acetaminophen level was categorized into “high-risk group” according to the severity nomogram from Smilkstein et al. (12). Thus, it is considered that the drug levels before the hospitalization were much higher than the levels on admission. It is presumed that initial unconsciousness resulted from sedative and hypnotic properties of carisoprodol and meprobamate, and the subsequent fulminant hepatic failure and renal failure were caused by N-acetyl-p-benzoquinonimine (NAPQI), the highly reactive hepatotoxic metabolite derived from acetaminophen. Although N-acetyl-L-cysteine was treated to the patient in order to facilitate the NAPQI excretion, the treatment might have been ineffective on account of 16 h elapsed after the ingestion. Acetaminophen fortunately has no drug-drug interaction with carisoprodol or meproba-

Determination of carisoprodol, acetaminophen, and meprobamate in urine and plasma

In the present case, carisoprodol and acetaminophen were the only drugs detected. Meprobamate was also found as the N-dealkylated metabolite of carisoprodol in both urine and plasma. Changes with time in the urine and plasma concentr-
mate. Whereas acetaminophen is principally metabolized by conjugations (i.e., glucuronidation and sulfation) in the liver, carisoprodol mainly undergoes N-dealkylation catalyzed by CYP 2C19 isoform. In addition, carisoprodol and acetaminophen have diverse mechanisms of pharmacodynamic and toxicodynamic action from each other. This is believed to be the reason for the victim’s survival.

To date carisoprodol is available neither as a prescription drug nor as an OTC drug in Japan. In recent years, overdose cases using unknown drugs purchased over the Internet are increasing. For the reason that information on adverse effects of these drugs is not sufficiently provided to end-consumers, or information in foreign languages is not comprehensible to domestic consumers, both suicidal overdosing and accidental poisoning may increase further.

In conclusion, the LC-MS-ESI method we described here enabled highly sensitive, derivatization-free analysis of heat-labile drugs, such as carisoprodol or meprobamate. We also attained the simultaneous determination of carisoprodol and acetaminophen co-administered to achieve a synergistic effect. To assay the acetaminophen level simultaneously is of importance by reason of its potential toxicity. By means of the SIM, 0.5 ng/mL of carisoprodol, 10 ng/mL of acetaminophen, and 1.0 ng/mL of meprobamate could be quantitated without complicated derivatization.

References


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