J Forensic Sci, January 2008, Vol. 53, No. 1
doi: 10.1111/j.1556-4029.2007.00601.x
Available online at: www.blackwell-synergy.com

CASE REPORT

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A Fatal Case of Suspected Anaphylaxis with Cefoperazone and Sulbactam: LC-MS Analysis

ABSTRACT: Cefoperazone and sublactam are prescribed in combination and used in the treatment of moderate to severe bacterial infections. Serious anaphylaxis is a rare side effect. This report describes a fatal case of suspected anaphylaxis after intravenous administration of a combination of the two drugs. Heart blood was analyzed for cefoperazone by protein precipitation with acetonitrile and by liquid-liquid precipitation for sublactam after protein precipitation with aqueous acetonitrile, followed by tandem mass spectrometry in the product ion scan mode for identification and by liquid chromatography mass spectrometry in the selected ion monitoring mode for quantitation. Calibration curves for cefoperazone and sublactam were linear over the range 0.07 to 1.93 and 0.046 to 0.914 μg/ml respectively. The decedent's blood concentrations of cefoperazone and sublactam were 0.368 and 0.143 μg/ml respectively. As these concentrations were below concentrations reported after single dosing studies and below those considered to be minimally inhibitory, death was presumed to have been caused by hypersensitivity and not an overdose. In conclusion, this procedure is useful for detecting and quantitating cefoperazone and sublactam in postmortem blood and may be useful in the evaluation of anaphylaxis.

KEYWORDS: forensic science, forensic toxicology, cefoperazone, sulbactam, liquid chromatography-mass spectrometry

Cefoperazone is a third-generation cephalosporin antibiotic with a broad spectrum of activity against most gram-positive and gram-negative bacteria. Some organisms, however, are resistant to cefoperazone by producing β-lactamase (1). Sulbactam, a β-lactamase inhibitor, lacks significant antibacterial activity to most organisms as a single agent (2). *In vitro*, the combination of cefoperazone and sulbactam shows a marked degree of synergy against some cefoperazone-resistant organisms (3). For this reason, the intravenous co-administration of cefoperazone and sulbactam is effective for the treatment of moderate to severe bacterial infections caused mainly by β-lactamase-producing organisms. Moreover, in Japan, these antibiotics have been conventionally used for treatment of a cold.

The third-generation cephalosporins have common serious side effects, such as bleeding, benign diarrhea, pseudomembranous colitis, and hypersensitive reactions (4,5). Especially, anaphylaxis is a rare (frequency, 0.0001–0.1%) (6) but serious and life-threatening hypersensitive reaction. This reaction occurs even at doses or at blood concentrations lower than clinical or intoxication levels. Therefore, the determination of the blood level of the drug may indicate suspected intoxication or a hypersensitive reaction.

The aim of our work was to identify and quantify cefoperazone and sulbactam in the heart blood after their injection in a case of suspected hypersensitive reaction. Methods for quantifying cefoperazone and/or sulbactam in serum or plasma using high-performance liquid chromatography (HPLC) with UV detection have been reported (7–12). However, UV detection does not give the information necessary to identify a drug and may not be sensitive enough to determine cefoperazone/sulbactam below the clinical level. We therefore designed specific and sensitive methods using liquid chromatography-mass spectrometry (LC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS).

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Received 1 April 2006; and in revised form 1 Sept. 2006; accepted 23 June 2007.

Case History

A 33-year-old male went to a clinic for treatment of a cold. His physician diagnosed him with a cold and a nurse administered sulbactam sodium/cefoperazone sodium (500 mg each) by intravenous drip after dissolving in a total of 200 mL of electrolyte infusion solution. A few minutes after the infusion started, the patient went into respiratory distress and lost consciousness rapidly. After the patient was transferred emergently to the local hospital, he received emergency resuscitation including administration of methylprednisolone sodium succinate and epinephrine intravenously, but nonetheless expired in the emergency department. Postmortem blood was obtained from the heart for toxicological analysis.

Materials and Methods

Chemicals and Reagents

Cefoperazone sodium was obtained from Sigma (St. Louis, MO). Sulbactam sodium was obtained from Wako Pure Chemical (Osaka, Japan). Blank human blood was supplied from Tokyo Metropolitan Police Hospital (Tokyo, Japan). All the other chemicals used in the experiments were of analytical grade.

Standard stock solutions containing 1 mg/mL of cefoperazone and sulbactam (as sodium salt) were prepared in distilled water and stored at −20°C. Working standards were prepared from standard stock solutions by appropriate sequential dilutions with distilled water and stored at 4°C. Calibrators and quality control (QC) samples for quantitative analysis were prepared by spiking these working standards in blank blood as the referred to hereinafter.

Extraction Procedure

The blank blood, which was previously hemolyzed by freezing and thawing, and decedent's blood were centrifuged (4000 × *g*,

5 min, 4°C) and these supernatants were used for the following analysis.

Cefoperazone— For quantitative analysis, 0.8 mL of acetonitrile was added to 200 μL of the blood samples, in which 10 μL of working standard solutions (for calibrators and QC samples) or distilled water (for decedent's blood) were previously spiked. After blood coagulum was broken into small pieces using a spatula, the mixture was shaken for 1 min with a vortex mixer and was centrifuged (15,000 × *g*, 10 min, 4°C). The supernatant was transferred

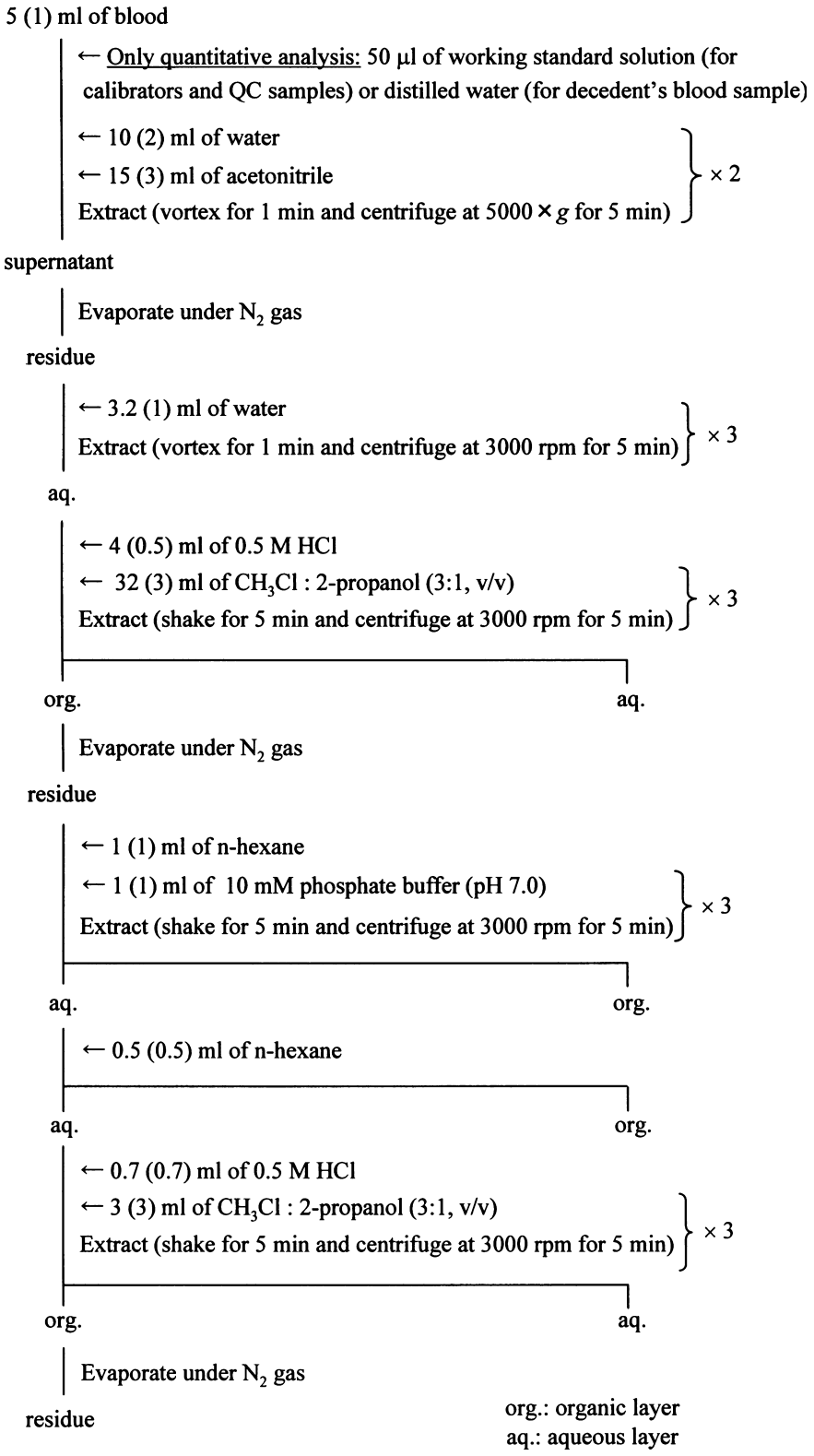

FIG. 1—*Extraction procedure for sulbactam. The numbers before and within parentheses indicate the volume of the solvents for qualification and quantification procedures, respectively.*

to another microtube and evaporated to dryness under a stream of nitrogen. The residue was reconstituted with 100 μL of 0.1% formic acid–methanol (90:10, v/v). A 10-μL aliquot was used for the analysis.

For qualitative analysis, 4 mL of acetonitrile was added to 1 mL of the blood samples. After extraction and evaporation of solvent as previously described, the residue was reconstituted with 200 μL of 0.1% formic acid–methanol (90:10, v/v). A 10-μL aliquot was used for the analysis.

Sulbactam— The extraction procedure is summarized in Fig. 1. The residue was reconstituted with 100 μL of mobile phase. A 20-μL (for qualification) or 10-μL (for quantification) aliquot was used for the analysis.

Method Validation— The linearity of the method was evaluated using calibrators with five different concentrations of cefoperazone (1.93, 0.967, 0.484, 0.242, and 0.097 μg/mL) or sulbactam (0.914, 0.457, 0.183, 0.091, and 0.046 μg/mL). The calibration curves were constructed using the peak areas versus the nominal concentrations of the analytes by an external standard method. The regression parameters for the slope, intercept, and correlation coefficient were calculated by weighted ($1/x$) linear regression using Correlation2-2 freeware (http://homepage3.nifty.com/m_nw/j-frame.htm). The accuracy of the calibration curves was tested by a comparison of the back-calculated concentrations and the nominal concentrations for all calibration levels.

Limit of detection (LOD) and limit of quantitation (LOQ) were defined as the lowest concentration with a signal-to-noise ratio of at least 3:1 and 10:1, respectively.

Test to determine the recovery, precision, and accuracy of the method were performed by using QC samples. The concentrations of QC samples were as follows: 1.74, 0.774, and 0.145 μg/mL for cefoperazone and 0.731, 0.229, and 0.073 μg/mL for sulbactam. The recoveries were calculated by comparing the peak areas for each analyte after sample extraction to those of standards prepared in a blank blood matrix. Intra- and inter-day accuracy was tested by a comparison of the mean back-calculated concentrations and the nominal concentrations. Intra- and inter-day precision was expressed as the coefficient of variation (CV) of the experimental values at each concentration.

Chromatographic Separation— Chromatographic separation of cefoperazone was performed with a Mightysil RP-18 column (150 × 2.0 mm i.d., 5 μm, Kanto Chemical, Tokyo, Japan) maintained at 40°C. The mobile phase was a gradient of methanol in 0.1% formic acid with a flow rate of 0.2 mL/min. The percentage of methanol was set at 10% for 5 min, then raised to 55% in 20 min and held at 55% for 5 min.

Chromatographic separation of sulbactam was performed with a Shodex MSpak GS-320 2D (150 × 2.0 mm i.d., 6 μm, Showa Denko K.K., Tokyo, Japan) maintained at 40°C. The mobile phase was 0.1% formic acid–methanol (90:10, v/v) and was pumped at a flow rate of 0.2 mL/min.

TABLE 1—*Mass spectrometric conditions of quantitative analysis.*

	Cefoperazone	Sulbactam
Ionization polarity	Positive	Negative
Monitoring ion	m/z 530	m/z 232
Cone voltage	45 V	25 V
Capillary voltage	5 kV	4 kV
Cone gas flow	50 L/h	50 L/h
Desolvation gas flow	350 L/h	350 L/h
Source temperature	100°C	100°C
Desolvation temperature	300°C	300°C

TABLE 2—*Mass spectrometric conditions of qualitative analysis.*

	Cefoperazone	Sulbactam
Ionization polarity	Positive	Negative
Precursor ion	m/z 646	m/z 232
Scan range	m/z 300–700	m/z 60–250
Spray voltage	4 kV	4 kV
Capillary voltage	43 V	27 V
Capillary temperature	250°C	200°C
Sheath gas flow rate	72 L/h	72 L/h

Apparatus and Mass Spectrometric Conditions— The quantitative analysis was carried out using a Waters LC-MS system (Milford, MA) consisting of a 2690 series high-performance liquid chromatograph (solvent degasser, pump, autosampler, and column oven) and a ZQ single-quadrupole mass spectrometer equipped with an electrospray ionization (ESI) interface. MS data were collected in selected ion monitoring (SIM) mode. The main MS conditions of the mass spectrometer are summarized in Table 1.

The qualitative analysis was carried out using a Shimadzu LC-10ADvp series high-performance liquid chromatograph (solvent degasser, pump, autosampler, and column oven) and a Thermo Finnigan LCQ ion trap mass spectrometer equipped with an ESI interface. MS data were collected in product ion scan mode. The main MS conditions of the mass spectrometer are summarized in Table 2.

Results

The calibration curves were linear in the range of 0.097–1.93 μg/mL for cefoperazone and 0.046–0.914 μg/mL for sulbactam, with correlation coefficients that were routinely greater than 0.995 in all cases. The accuracy of the calibration curves was between 81.6 and 114.4%. LOD and LOQ were 0.048 and 0.097 μg/mL for cefoperazone and 0.011 and 0.046 μg/ml for

TABLE 3—*Recovery of the analytes (cefoperazone: n = 5, sulbactam: n = 4).*

Analyte	Spiked (μg/mL)	Recovery (%)
Cefoperazone	1.74	72.6 ± 4.1
	0.774	65.5 ± 5.8
	0.145	73.1 ± 2.3
Sulbactam	0.731	74.7 ± 7.4
	0.229	62.0 ± 7.3
	0.073	68.1 ± 4.8

TABLE 4—*Validation of intra-day assay (cefoperazone: n = 5, sulbactam: n = 4).*

Analyte	Nominal (μg/mL)	Measured (μg/mL)	Accuracy, %	CV, %
Cefoperazone	1.74	1.63 ± 0.09	93.9	5.7
	0.774	0.690 ± 0.062	89.1	9.0
	0.145	0.134 ± 0.003	92.3	2.1
Sulbactam	0.731	0.733 ± 0.069	100.2	9.4
	0.229	0.235 ± 0.028	102.7	11.7
	0.073	0.072 ± 0.005	98.1	6.7

TABLE 5—*Validation of inter-day assay (n = 3).*

Analyte	Nominal (μg/mL)	Measured (μg/mL)	Accuracy, %	CV, %
Cefoperazone	1.74	1.57 ± 0.15	90.2	9.8
	0.774	0.736 ± 0.029	95.1	3.9
	0.145	0.142 ± 0.008	98.2	5.6
Sulbactam	0.731	0.700 ± 0.087	95.8	12.5
	0.229	0.225 ± 0.023	98.0	10.0
	0.0731	0.070 ± 0.003	96.0	3.5

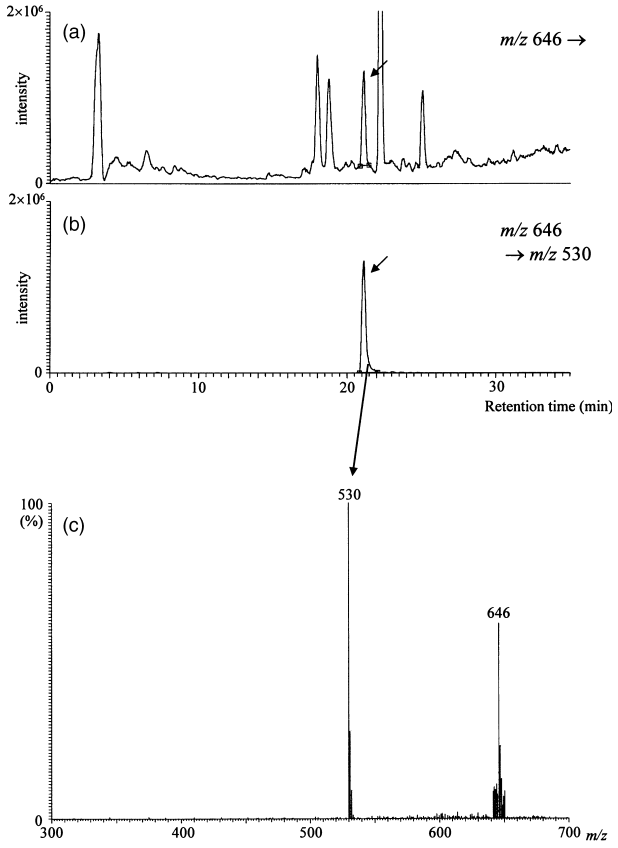

FIG. 2—*LC-MS analysis of the patient's blood sample. Analytical conditions for cefoperazone. Total ion chromatogram (a) and mass chromatogram (b) of m/z 530 in the product ion scan mode using m/z 646. (c) Product ion spectrum at m/z 646 for the 21.2-min peak.*

sulbactam, respectively. These LOD and LOQ values were considered adequate for the purposes of this study.

As shown in Table 3, the recovery percentages of cefoperazone and sulbactam ranged between 65.5 and 73.1% and between 62.0 and 74.7%, respectively. Accuracy and precision data were shown in Table 4 (intra-day) and Table 5 (inter-day). The intra- and inter-day accuracy was between 89.1 and 102.7%. The intra- and inter-day precision was within 12.5% at three concentrations for the two analytes.

The LC-MS/MS chromatograms and MS/MS spectra obtained from the extract of the deceased's blood, that of blank blood, and pure standard (for cefoperazone) or the extract of standard-spiked blank blood (for sulbactam) under each analytical condition are shown in Figs. 2–4 (cefoperazone) and Figs. 5–7 (sulbactam). In qualifying sulbactam, we used the standard-spiked blank blood instead of the pure standard, because the retention time of the sulbactam tended to shift by the effect of blood matrix. The peaks of both drugs were separated from interfering peaks. Judging from the

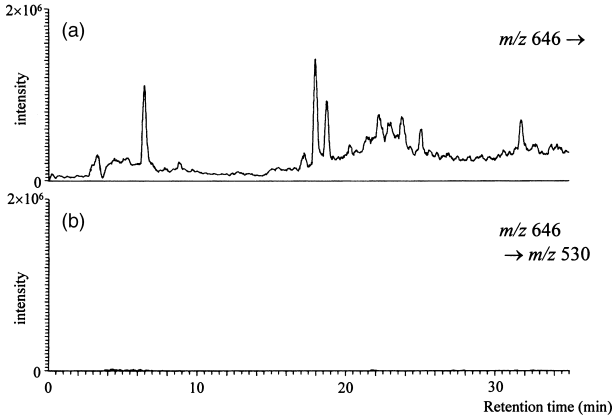

FIG. 3—*LC-MS analysis of the blank blood. Analytical conditions for cefoperazone. Total ion chromatogram (a) and mass chromatogram (b) of m/z 530 in the product ion scan mode using m/z 646.*

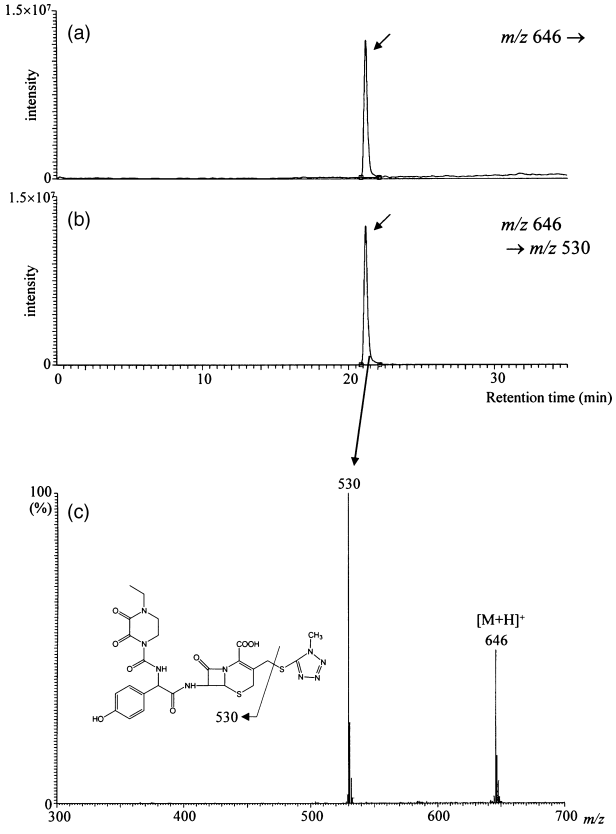

FIG. 4—*LC-MS analysis of the cefoperazone standard (48.4 ng on column). Analytical conditions for cefoperazone. Total ion chromatogram (a) and mass chromatogram (b) of m/z 530 in the product ion scan mode using m/z 646. (c) Product ion spectrum at m/z 646 for the 21.2-min peak.*

mutual correspondences of retention time and MS/MS spectra with the sample and standard (or standard-spiked blank blood), the sample contained cefoperazone and sulbactam. The concentrations of cefoperazone and sulbactam quantified by LC-MS in SIM mode were 0.368 and 0.143 μg/mL, respectively.

Discussion

There are several reports on analytical methods for cefoperazone and sulbactam in serum and plasma. However, these were not directly applicable to this case, for the following reasons:

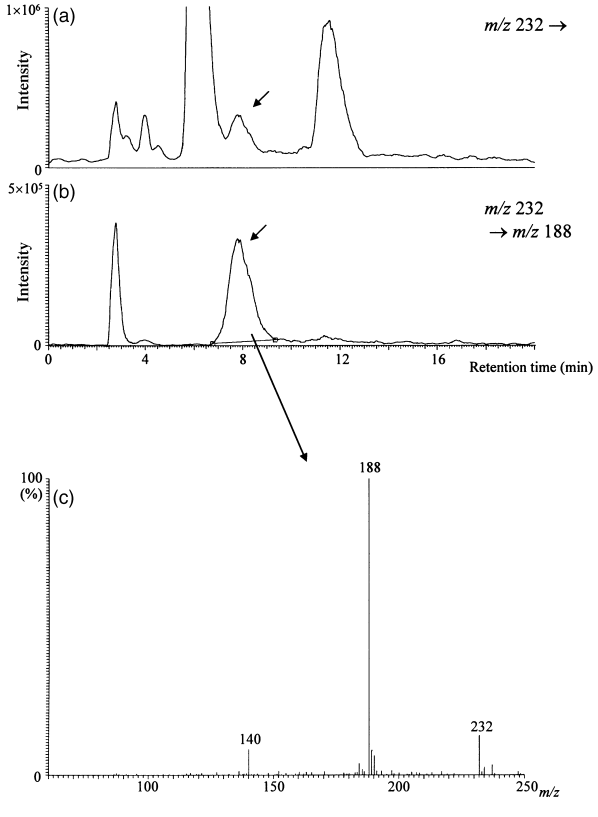

FIG. 5—*LC-MS analysis of the patient's blood sample. Analytical conditions for sulbactam. Total ion chromatogram (a) and mass chromatogram (b) of m/z 188 in the product ion scan mode using m/z 232. (c) Product ion spectrum at m/z 232 for the 7.8-min peak.*

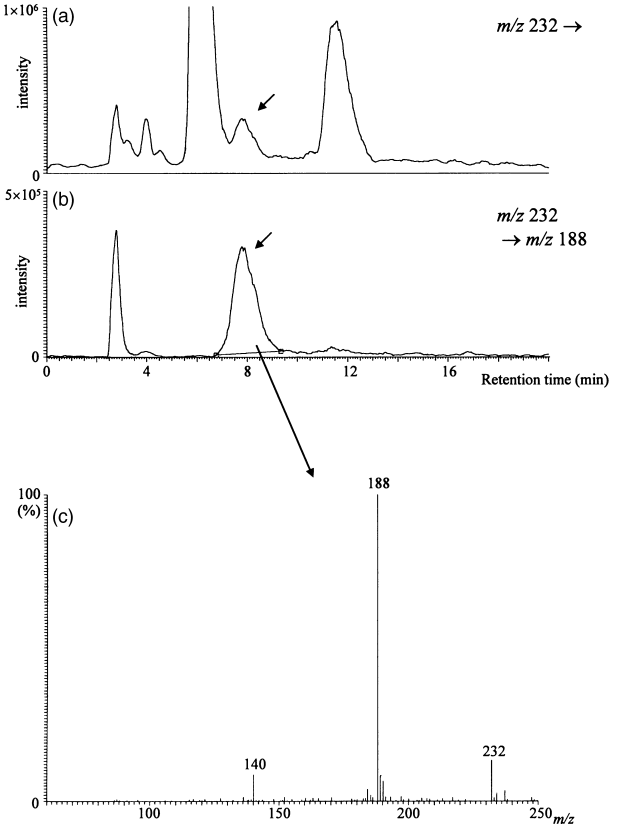

FIG. 6—*LC-MS analysis of the blank blood. Analytical conditions for sulbactam. Total ion chromatogram (a) and mass chromatogram (b) of m/z 188 in the product ion scan mode using m/z 232.*

1 Most of the studies were performed by HPLC with UV detection. It was impossible to apply those conditions to LC-MS because of the nonvolatility of the mobile phase.
2 The matrices targeted in the previous studies were serum or plasma. However, in this case we used whole blood, which is encountered commonly in forensic laboratories.

Cefoperazone was able to be recovered by simple deproteination using acetonitrile (recovery: >60%). This recovery was lower than that from serum samples reported by Kalman et al. (11). However, it was enough to determine cefoperazone in the blood in this case.

In the case of sulbactam, acetonitrile gave a poor recovery (31.1%, $n = 2$) in the preliminary study. Therefore, we selected aqueous acetonitrile as the deproteination condition. Because aqueous acetonitrile left a residue that was too dirty to allow direct application to HPLC, the deproteinized residue was extracted by a mixture of chloroform/2-propanol (3:1, v/v) under acidic conditions and was cleaned up by *n*-hexane under neutral conditions, as shown in Fig. 1. This procedure was effective for removing heme in whole blood, which was the major hindrance to the extraction of whole blood using organic solvent under acidic conditions (13).

The blood concentrations of cefoperazone and sulbactam were 0.368 and 0.143 μg/mL, respectively. Pharmacokinetic data obtained by bioassay showed that after intravenous infusion of cefoperazone sodium (500 mg) and sulbactam sodium (500 mg) over 60 min to healthy volunteers, mean serum levels of cefoperazone/sulbactam were 58.38/23.56 μg/mL at 1 h and 18.32/3.00 μg/mL at 3 h (14). Minimum inhibitory concentration (MIC) was dependent on species and strains of bacteria. For example, that against *Staphylococcus aureus*, which was one of the major targets, was 0.78–3.13 μg/mL as the total β-lactam concentration of cefoperazone sodium/sulbactam sodium (1:1) (15). The levels of the antibiotics in the decedent's sample were lower than the pharmacokinetic data and the MIC against *S. aureus*. This might be caused by not receiving full dosage, because he went into respiratory distress and lost consciousness rapidly a few minutes after the infusion started.

Toxic concentrations of cefoperazone and sulbactam have not been reported. Most of the severe side effects of cefoperazone and sulbactam are not dependent on blood concentration or dosage. One exception is that cefoperazone tends to cause bleeding when used in doses >4 g per day (5). Judging from the blood concentrations of the two drugs in this case, and the absence of other symptoms the patient suffered anaphylaxis caused by cefoperazone and/or sulbactam. However, it was difficult to make a diagnosis of anaphylaxis for the following reasons:

1 There was no information about the patient's possible history of hypersensitivity to these drugs or to other cephalosporins.
2 There was no information about serum tryptase and IgE levels, which would be elevated in the case of anaphylaxis (16,17).
3 There was no information about autopsy findings related to anaphylaxis (e.g., laryngeal edema).

In conclusion, it was possible to exclude acute toxicity by overdose based on the blood concentrations of cefoperazone/sulbactam. The determination of the two drugs in the blood will facilitate the judgment of anaphylaxis.

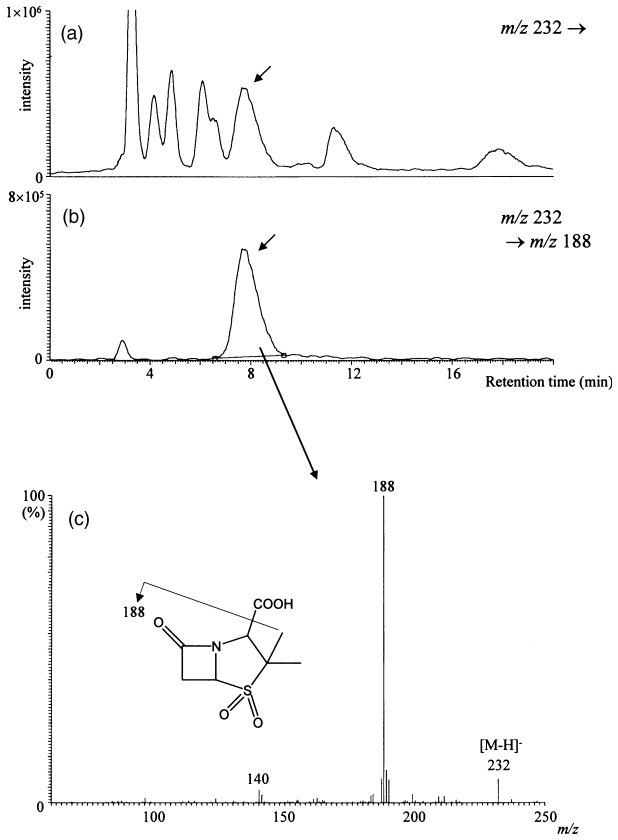

FIG. 7—*LC-MS analysis of the sulbactam-spiked blood sample (at 0.183 μg/mL). Analytical conditions for sulbactam. Total ion chromatogram (a) and mass chromatogram (b) of m/z 188 in the product ion scan mode using m/z 232. (c) Product ion spectrum at m/z 232 for the 7.7-min peak.*

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