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Case Report

**Postmortem distribution/redistribution of buformin in body fluids and solid tissues in an autopsy case using liquid chromatography—tandem mass spectrometry with QuEChERS extraction method**

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A. Wurita and K. Hasegawa contributed equally to this work.

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## **Abstract**

An autopsy for a suicidal case of a male in his 40s, who had died of poisoning due to ingestion of a large amount of buformin, was performed at our department. Buformin is biganide class agent used for patients of diabetes mellitus, which can occasionally cause severe lactic acidosis. The autopsy was performed about 10 days after his death, and the direct cause of his death was judged as asphyxia due to the aspiration of stomach contents into the airway. The nine body fluids and eight solid tissues specimens were dealt with for investigating postmortem distribution/redistribution of buformin in a whole body; femoral vein blood, right and left heart blood, pericardial fluid, urine, bile, stomach contents, small intestine contents, cerebrospinal fluid, the brain, lung, heart muscle, liver, spleen, kidney and skeletal muscle were examined. For extracting buformin from specimens, a modified QuEChERS method including dispersive solid-phase extraction was employed, followed by the analysis by liquid chromatography tandem mass spectrometry (LC-MS/MS). Buformin in various kinds of human matrices were quantified by the standard addition method in this study, which can overcome the matrix effects and recovery rates without use of blank human matrices. All concentrations of buformin in specimens examined in this case were extremely higher than those of previously reported poisoning cases. The concentrations of buformin in left and right

heart blood and femoral vein blood specimens of this case were 399, 216 and 261  $\mu\text{g/mL}$ , respectively; although the direct cause of his death was judged as asphyxia due to occlusion of airway with stomach contents, the vomiting was thought to be provoked by buformin poisoning. In this study, marked differences of buformin concentrations between brain tissue and cerebral spinal fluids, and other specimens were observed, which suggested that its distribution was influenced also by blood-brain-barrier. Although a number of buformin poisoning cases were published so far, they gave sporadic data on its concentrations and/or distribution in some limited human specimens. This study is the first to describe detailed distribution/redistribution of buformin in a whole human body quantified by using LC-MS/MS.

**Keywords:** Buformin, Postmortem distribution/redistribution, Blood-brain barrier, QuEChERS method, Standard addition method, LC-MS/MS

## **1. Introduction**

Buformin (1-butylbiganide) was firstly synthesized in as early as 1950s [1] and is one of oral anti-hyperglycemic drugs classified as biganide class, which is used for patients of non-insulin-dependent (type 2) diabetes mellitus. Nowadays, buformin is no longer registered as anti-hyperglycemic drugs in many countries [2], because of its adverse-effects and complications such as severe lactic acidosis, hypothermia, shock and rhabdomyosis [3]; its analog phenformin has also been withdrawn from most of markets in the world including Japan since 1970s. Although a number of fatal cases due to biganide class drugs have also been reported [4-8], buformin is still being prescribed in Romania, Spain and some other countries including Japan bringing about various complications [9]. In addition, such oral anti-hyperglycemic drugs have been sometimes reported to be illegally contained in dietary supplements or oral products [10].

Recently, an autopsy case of suicide involving the ingestion of a large amount of buformin was experienced. In this report, the distribution/redistribution of buformin in nine body fluids including cerebrospinal fluid (CSF), stomach and small intestine contents, and eight solid tissues obtained from the cadaver at the autopsy is presented.

Although sporadic data on buformin in human specimens have been published in both bioanalytical and forensic area [11-13], this study is the first to demonstrate detailed distribution of buformin in a whole human body measured by liquid chromatography tandem mass spectrometry (LC-MS/MS).

## **2. Case history and autopsy findings**

On an October day, a male in his 40s was found dead in supine position, lying on a mat of his room; the victim was living by himself, and the room temperature was about 23°C during investigation by police. In the dust box of his room, empty blister packages of 308 buformin (50 mg) tablets were found, which were equivalent to 15.4 g buformin, thought to be ingested prior to his death; in his room several other prescribed medicine such as thiamazole, levothyroxine sodium hydrate, azilsaltan, amlodipine and atorvastatin, besides remaining buformin were also found. Police investigation disclosed that he had been diagnosed as diabetes mellitus, hyperlipidemia, hypertension, hypothyroidism and schizophrenia at the time of accident, and the above medicines were recently prescribed by a physician. Therefore, the police first suspected that the victim

died of buformin poisoning; neither any criminal evidences nor a suicide note was found during the investigation of his room.

A forensic autopsy for the victim was performed at our department, and the postmortem interval estimated at the beginning of the autopsy was about 10 days. The male victim was 174 cm high and weighed 72.7 kg. Postmortem rigidity was relatively weak at all joints. Prominent lividity accompanied by marked petechiae was found on his back. Upon the external findings, his face was quite congested along with small petechial hemorrhages observed at conjunctivae of the right eyelid. Inside the oral and nasal cavity, relatively large volume of dark reddish fluid was present. Although there were small abrasions and scar on the various parts of his skin, they were not serious, providing almost no contributions to his death. In addition to the above findings, no notable wounds were found on the surface of his body.

As internal findings, a large amount of dark reddish fluids including a lot of froth and stomach contents were observed inside the trachea and the bronchi. The both lungs were congested markedly; the right and left lungs weighed 772 g and 722 g, respectively. Almost all organs including the brain were also congested. A relatively large volume of liquid and clotted blood were presented inside the heart. Inside the stomach, there was 356 g contents including dark brown fluids and food debris; neither tablets nor its residue

could be found in the stomach. Except for the above findings, there were neither pathological findings nor injuries by macroscopic observation.

### **3. Materials and methods**

#### *3.1. Materials and biological samples*

Buformin hydrochloride and phenformin hydrochloride (internal standard, IS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other common chemicals used were of the highest purity commercially available. Plastic centrifuge tubes with caps (5-mL capacity, 6 × 1.5 cm external diameter) and stainless beads (5 mm external diameter) for crushing solid tissues were purchased from TAITEC, Saitama, Japan. The QuEChERS dispersive solid-phase extraction (SPE) centrifuge tubes with caps (2-mL capacity), each of which contained 25 mg of primary-secondary amine (PSA), 25 mg of end-capped octadecylsilane (C<sub>18</sub>EC) and 150 mg of magnesium sulfate, and Captiva ND Lipids cartridges (3-mL capacity) were purchased from Agilent (Santa Clara, CA, USA).

Whole blood specimens from the femoral vein and the right and left atria in heart, bile, pericardial fluid, cerebrospinal fluid, urine, stomach contents (pH was around 5-6



by test paper), small intestine contents and solid tissue specimens (brain, lung, heart muscle, liver, spleen, kidney and skeletal muscle from psoas major) were obtained from the deceased at autopsy, and kept frozen at -80°C until analysis;

### *3.2. LC-MS/MS conditions*

LC-MS/MS with electrospray ionization (ESI) was conducted on an Agilent 1200 LC-SL system connected to a 6460 Triple Quad LC/MS tandem MS instrument (Agilent). The Agilent LC-SL system included a microdegasser and a high-performance autosampler. For LC separation, a ZORBAX Eclipse Plus C18 column (100 × 2.1 mm i.d., particle size 1.8 µm; Agilent) was used. The LC conditions were: injection volume, 3.5 µL; flow rate, 0.2 mL/min; elution mode, gradient with 10 mM ammonium formate/0.1% formic acid in distilled water (A) and acetonitrile (B) from 95% A/5% B to 50% A/50% B over 5 min, followed by isocratic elution with the initial solvent composition for 10 min in post running. The column and autosampler were operated in room with air conditioning, where the temperature was controlled around 25°C, during analysis.

The tandem MS condition were: interface, ESI mode; polarity, positive ion mode; ion source temperature, 320°C; ion source voltage, 500 V; sheath gas flow 12 L/min, quantification, selected reaction monitoring (SRM) mode using the peak area; ion transitions,  $m/z$  158  $\rightarrow$  43.1 for buformin,  $m/z$  206  $\rightarrow$  60.1 for phenformin (IS); fragmentor voltage and collision energy were 120 and 40 V for bufoumin, 120 and 13 V for phenformin, respectively.

Data acquisition, peak integration, and calculation were performed with a computer workstation (Agilent Masshunter, Revision Acquisition B. 02. 01, Qualification B. 03. 01SP2 and Quantification B. 04. 00).

### *3.3. Extraction procedure for human specimens*

A 100  $\mu$ L volume each of body fluid was added to 9.9 mL of distilled water, followed by mixing with 10  $\mu$ g of phenformin (IS) dissolved in 10  $\mu$ L of acetonitrile. To the mixture, one of the various amounts 0, 5, 10, 50, 100 and 500  $\mu$ g of buformin was also added for quantitation by standard addition method; but for the cerebrospinal fluid, each of 0, 1.0, 5.0, 10 and 50  $\mu$ g of buformin was added. Then, the mixture was shaken gently and centrifuged at 10,000 rpm for 2 min in a plastic test tube. Each 0.1 mL aliquot

of the mixture was further added to 0.9 mL of acetonitrile, resulting in 1.0 mL of volume. Then, this mixture was decanted into a QuEChERS SPE centrifuge tube, vortexed for 30 s, and centrifuged at 10,000 rpm for 2 min. The 600  $\mu$ L volume of upper acetonitrile layer was passed through a Captiva ND Lipids cartridge. A 3.5  $\mu$ L aliquot of the eluate was then analyzed by LC-MS/MS; for stomach contents, 10  $\mu$ L volume was used for the extraction procedure, because of much higher concentration of buformin than in other body fluids

For the solid tissues, 1.0 g each of solid tissue specimen was placed in a 5-mL plastic tube with a cap containing 4-mL of distilled water; for a solid tissue specimen, it was carefully minced with surgical scissors. Then, five stainless beads were added to the mixture. The plastic tube was capped and held to a bead beater-type shaking machine (Beads Crusher  $\mu$ T-12; TAITEC), followed by vigorously shaking at 3,200 rpm for 5 min. Then, the homogenate except the beads was transferred to a large plastic test tube containing 5 mL of distilled water, resulting in almost 10 mL of volume. To the 1.0 mL aliquot of the homogenate, 9.0 mL of distilled water containing 10  $\mu$ g of IS with one of 0, 5, 10, 50, 100 and 500  $\mu$ g of buformin were also added, followed by shaking and centrifuge at 10,000 rpm for 2 min; for the brain tissue, each of 0, 1.0, 5.0, 10 and 50  $\mu$ g of buformin was added. Then, each 0.1-mL aliquot of the mixture was further added to

0.9 mL of acetonitrile, resulting in 1.0 mL of volume, and following procedure was exactly the same with that for liquid specimens.

In the above procedure, the authentic specimens except for stomach contents were diluted to 1,000 folds with distilled water and acetonitrile, because of extremely high concentration of target compounds in specimens tested, and also for deproteinization; for stomach contents, they were further diluted to 10,000 folds.

## 4. Results and discussion

### *4.1. Blood Alcohol and drug screening test on autopsy*

At autopsy, routine analysis of blood alcohol using gas chromatography with flame ionization detector (GC-FID) showed negative results for both heart blood and urine specimens. Immunochemical drug screening kit Triage Drugs of Abuse panel (Alere, Waltham, MA, USA) for the urine specimen also showed negative results. By the NAGINATA screening for conventional drugs and toxic compounds in human whole blood and urine specimens using gas chromatography mass spectrometry (GC-MS) [14], no targeted drugs were detected from femoral vein blood and urine specimens of the

present deceased; the screening method use neutral organic extracts and is capable of detecting wide range of acidic and basic drugs as target compounds with semi-quantitative results.

In addition to the routine screening, because buformin was strongly thought to be involved in this case, further analysis for the stomach contents was carried out on buformin. Then, by another in house screening using GC-MS, extremely large amount of buformin could be successfully detected from the stomach contents; it was confirmed that other anti-diabetic drugs such as phenformin could not be detected in all specimens of this case by LC-MS/MS.

#### *4.2. Standard addition method*

The standard addition method [15] was employed to measure concentration of buformin in different human matrices in this study. Recently, this method is frequently used for quantification of target compounds in forensic toxicological field [16-22] including various human specimens such as body fluids, solid tissues, and of constituents in mushrooms, because this method can overcome the differences in matrix effects and even recovery rates according to kinds of specimens in our studies. In addition to overcoming the matrix effects and recovery rates, the method requires no matched blank

human matrices without target compound(s); generally, the collection of blank human samples for non-purposive use should be avoided, because of ethical reasons. The detailed procedure and the calculation method for quantification were described in our previous literature [21].

#### *4.3. Matrix effects, recovery rates, and repeatability*

Although the standard addition method can overcome the matrix effects and recovery rates as described above, it should be of interest to examine the differences of matrix effect and recovery rate according to kind of specimens; the matrix effects and recovery rates of buformin in our analysis seem of use and informative for readers. Generally, the matrix effects and recovery rates for quantifications are investigated by the procedures as previously described by Matsuzewski et al. [23] using matrix-matched blank specimens. However, because the standard addition method without the use of blank human matrices was employed, it is impossible to present the values of matrix effects and recovery rates using the matrix-matched procedure in this study. The detailed procedures and the calculation method for matrix effects and recovery rates without use

of the blank human matrices in standard addition method were also described in our previous report [21].

Instead of accuracy and precision data using blank human matrices, intra-day and inter-day determinations of the target compound were repeated in each matrix, examining their relative standard deviation of the repeatability in this study.

#### *4.4. Product ion spectra and selected reaction monitoring chromatograms*

Figure 1 shows examples of product ion spectra by LC-MS/MS obtained from the reference standard buformin and phenformin, plus of extracts from femoral vein blood, urine, and liver of the deceased. The product ion spectra obtained from these specimens coincided well with that of the reference standard buformin, without additional impurity peaks. The base peak appeared at  $m/z$  43.1, which was used for quantitative analysis; for phenformin, peak at  $m/z$  60.1 was used quantitative analysis.

Figure 2 shows SRM chromatograms for the reference standard buformin, for extracts from femoral vein blood, urine and liver and for phenformin spiked into the femoral vein blood specimen of the deceased. The retention times of buformin and IS were 2.31 and 2.37 min, respectively. According to product ion spectra of buformin, 2

transitions (158.1 => 43.1 and 158.1 => 60.1) were used as quantifier and as qualifier ions in this analysis; for IS, 206.1 => 60.1 and 206.1 => 105.0 transitions were used as quantifier and qualifier ions, respectively. The qualitative confirmations of buformin and IS in samples were performed by 2 transition SRM chromatograms coupled with product ion spectra. It was confirmed that all specimens in this case did not contain phenformin, which was enable us to use it as IS. All peaks appeared sufficiently sharp and symmetric; background levels were generally very low, and there were no impurity peaks which interfered with the target and IS peaks.

#### *4.5. Validation of the method*

Table 1 shows the standard addition calibration equations for buformin in all specimens tested. The correlation coefficient values obtained from all specimens were not smaller than 0.992. By extensive dilution of some samples with distilled water, the detection limit (signal-to-noise ratio  $\geq 3$ ) was around 1 ng/mL or g, and the lower quantification limit (signal-to-noise ratio  $\geq 10$ ) was around 10 ng/mL or g, respectively.

As shown in Table 2, each intra-day and inter-day repeatability of buformin in the femoral vein blood, urine and liver specimens was examined. The repeatability,



expressed as relative standard deviations, was not greater than 12.1% for all specimens tested, showing that repeatability in this method were satisfactory.

In this study, extensive dilution, deproteinization and QuEChERS dispersive solid-phase extraction coupled with filtration through a Captiva ND Lipids cartridge prior to LC-MS/MS analysis were employed for extraction. However, moderate suppressive matrix effects were found for buformin in femoral vein blood specimens, showing depressive matrix effect of 73.3% although such effects were not marked for the liver specimen. After compensation calculation of suppressive matrix effects for buformin in the above specimens, the recovery rate values for specimens tested were not lower than 96.4%, showing that they were satisfactory (Table 3). However, it should be addressed that the use of the standard addition method can compensate the recovery rates and matrix effects.

Stabilities of the target compounds in the femoral vein blood and liver samples were also assessed for seven days under two conditions. The samples were left at room temperature (25 °C) and -80 °C (to be used as 100% control), and then extraction procedures followed by LC—MS/MS analysis were conducted for each specimen. The buformin in tested samples were found to be stable at room temperature for seven days, as shown in Table 4.

#### *4.6. Postmortem distribution/redistribution of buformin in the specimens of the cadaver*

Table 5 shows the postmortem distribution of buformin in the nine body fluids including stomach contents and eight solid tissues obtained from the victim.

Each concentration of buformin in left and right heart blood and femoral vein blood specimens of this case were 399, 216 and 261  $\mu\text{g/mL}$ , respectively. The high concentration in left heart blood is probably due to postmortem transportation of basic xenobiotic from the lung via pulmonary vein, and the low concentration in femoral vein blood is due to the loss by diffusion across the thin wall of peripheral femoral vein [24].

According to previous articles [8], plasma concentration of buformin in six intoxication patients who could recover from severe acidosis averaged 1.7  $\mu\text{g/mL}$ , the concentration of which ranged between 1.1 and 2.4  $\mu\text{g/mL}$ . As for the fatal cases, buformin concentrations in plasma specimen obtained from 4 patients were reported to average 1.9  $\mu\text{g/mL}$ , ranging from 1.0 to 3.4  $\mu\text{g/mL}$  [8]; to our best efforts, neither toxic nor fatal buformin concentration in whole blood specimens for intoxication cases could not be found, which were published in scientific articles or reports.

In our case, concentrations of buformin in blood specimens and other tissues were extremely higher than those previously reported. Concentrations of buformin determined in the left and right heart blood and the femoral vein blood specimens of this case were 399, 216 and 261  $\mu\text{g/mL}$ , respectively, showing two orders of magnitude higher than those in plasma that were said to be “intoxication” and “fatal” cases. As for distribution of buformin in solid tissue specimens in a “fatal” case, concentrations in the liver, the kidney, the heart and lung were reported (by gas chromatography; neither details nor validation were given); they were 5.2, 98, 3.0 and 2.8  $\mu\text{g/g}$ , respectively [4]. On the other hand, those in our case were 478, 834, 225 and 275  $\mu\text{g/g}$  showing also much higher values than those in the corresponding specimens as described. Almost all previous literatures and reports on intoxications due to buformin have dealt with accidental clinical cases which showed severe acidosis occurred during treatment for diabetes mellitus; normal therapeutic plasma level of buformin were between 0.2 and 0.6  $\mu\text{g/mL}$  [8] with therapeutic oral buformin dose being  $258\pm 25$  mg/day [7]. On overdose case of buformin, one previous report on non-fatal suicidal case presented that the patient had ingested 2100 mg of buformin, which was about ten-fold daily therapeutic dose, and resulted in showing 5.4  $\mu\text{g/mL}$  in plasma [5]. In our case, it was estimated that the victim ingested orally about 300 tablets, each of which contained 50 mg of buformin, resulting in as high as 15,000

mg oral ingestion at once. The estimated amount of buformin in this case was about 60-times and 7-times higher than those of therapeutic dose and non-fatal suicidal case, respectively. Therefore, the extremely high oral dose was considered to contribute to very high buformin concentrations in specimens and to his death of the present deceased. Considering the buformin concentrations in specimens in this case, it was strongly estimated that the victim suffered from severe lactic acidosis. Although the concentrations of lactic acid in blood specimens was tried to be examined, it was impossible because of influences of postmortem changes. To the best of our knowledge, buformin concentrations in authentic human specimens presented in this report were the highest among published articles to date.

In the present case, marked differences in concentrations of buformin between brain tissue, cerebrospinal fluid and other specimens were observed; each buformin concentrations of brain tissue and cerebrospinal fluid specimen were 25.8 and 11.2  $\mu\text{g/g}$  or mL, respectively; concentrations of buformin in brain tissue and cerebrospinal fluid specimen were found to be about one order of magnitude lower than those in other specimens examined, showing distinct gradient of buformin concentrations between them. In general, distributions of compounds in human whole bodies are largely influenced by polarities (lipophilic- or hydrophilic properties) of the compounds and its volume-of-

distributions ( $V_d$ ) in individuals, along with its manners of metabolism and excretions; in autopsy cases, postmortem redistribution of compounds should also be taken into consideration. Furthermore, especially in the brain, blood-brain barrier system is known to play very important role for drug distribution in living body. It is thought that hydrophilic substances are generally restricted in exchanging out of blood vessels into brain through blood-brain barrier system. On the other hand, lipophilic substances are not influenced so much as hydrophilic substances are. Buformin is belong to polar biguanide anti diabetic drug family [25], and thus this drug is also estimated to be strongly influenced in distribution between blood and brain tissues prior to death in this case. However, neither any data and literatures on  $V_d$  of buformin in human whole bodies nor its interaction with blood-brain barriers has been available, to our knowledge. Although sporadic distribution data of buformin in a fatal case were already reported [4, 13], showing its concentrations in plasma, lung, liver, bile and kidney specimens, buformin concentrations in both brain and cerebrospinal fluid were not included in the literature.

There is a possibility of postmortem collapse of the blood-brain barrier system because the interval between the death and autopsy was as long as 10 days after his death, thus the concentrations of buformin in brain and cerebrospinal fluid were thought to be influenced by its postmortem redistribution among tissues. However, our measurements

of buformin in brain tissue and cerebrospinal fluid showed that the antemortem distribution by blood-brain barrier system could also influence its postmortem distribution demonstrating remarkable differences of concentration, which is of toxicological and also clinical pharmacokinetic interests.

To our best knowledge, this report is the first to present detailed postmortem distribution of buformin in the nine body fluids including stomach contents and eight solid tissues obtained from a fatal poisoning case due to intake of extremely large amount of buformin, showing the highest concentrations among ever reported.

Although the number of published literatures dealing with concentrations of buformin in various specimens in both survival cases and its fatal cases are limited [4, 11-13]. Our results on distribution of buformin in postmortem body fluids and solid tissues are most detailed, and the method by LC-MS/M is most modernized with the highest sensitivity for buformin.

## **5. Conclusions**

Buformin is still being subscribed as anti diabetic agent in some countries including Japan. In this report, detailed postmortem distribution/redistribution of buformin in

authentic nine fluid specimens including cerebral spinal fluid and eight solid tissues obtained from a suicidal case were investigated using standard addition method, which overcome matrix effects and even recovery rates. The concentrations of buformin in specimens examined in this case were extremely higher than those of fatal or poisoning cases in previous literatures. In autopsy, a large amount of stomach contents and froth were observed inside trachea and bronchi of the victim. Therefore, the direct cause of his death was judged as asphyxia due to aspiration of stomach contents into the air way. Occlusion of the airway due to aspiration of stomach contents are usually not thought to be brought about for healthy persons in a state of clear consciousness; these findings seemed to be due to serious condition resulted in drowsiness with vomiting symptom provoked by intake of large amounts of buformin. Because many empty packages were found with the victim and buformin concentration in the stomach contents was quite high, the accidental aspiration seemed to be due to a large amount of oral buformin ingestion. Our results strongly suggested that distribution of buformin in brain and CFS were also influenced by brain-blood-barrier prior to death. Finally, it should be mentioned that this is the first report to establish the procedure for analysis of buformin in human specimens by LC-MS/MS with modified QuEChERS method including dispersive SPE.

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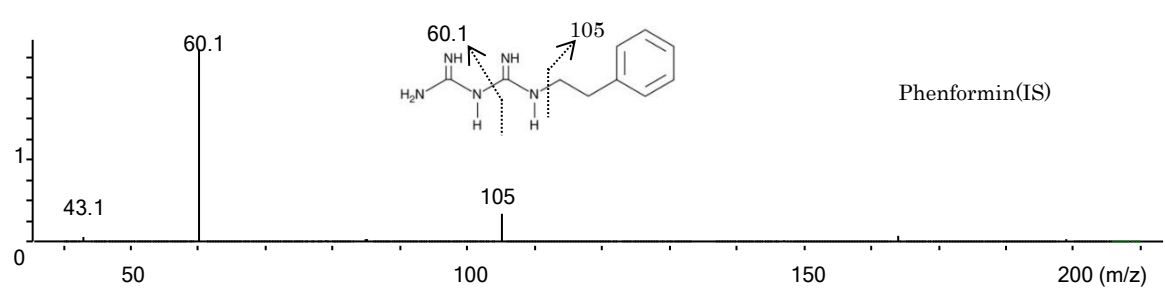
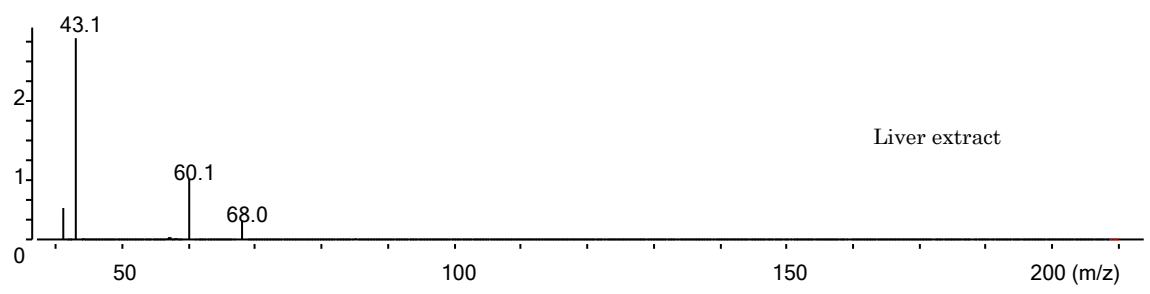
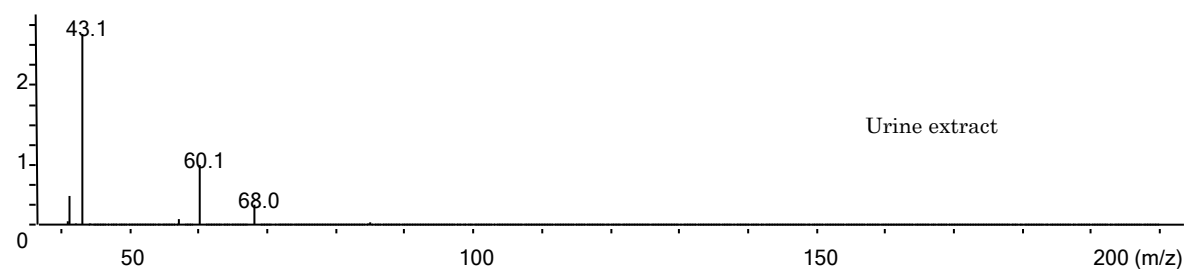
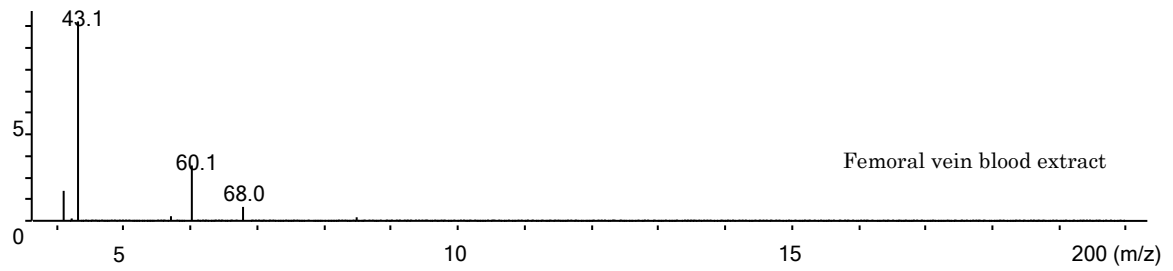
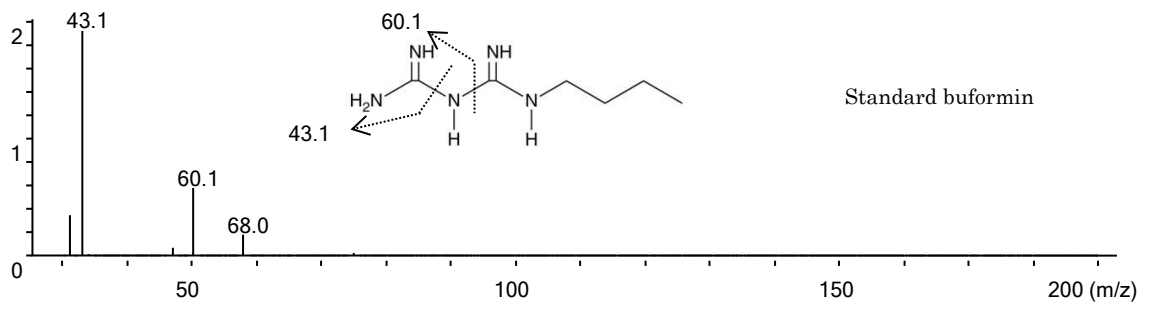


Fig 1

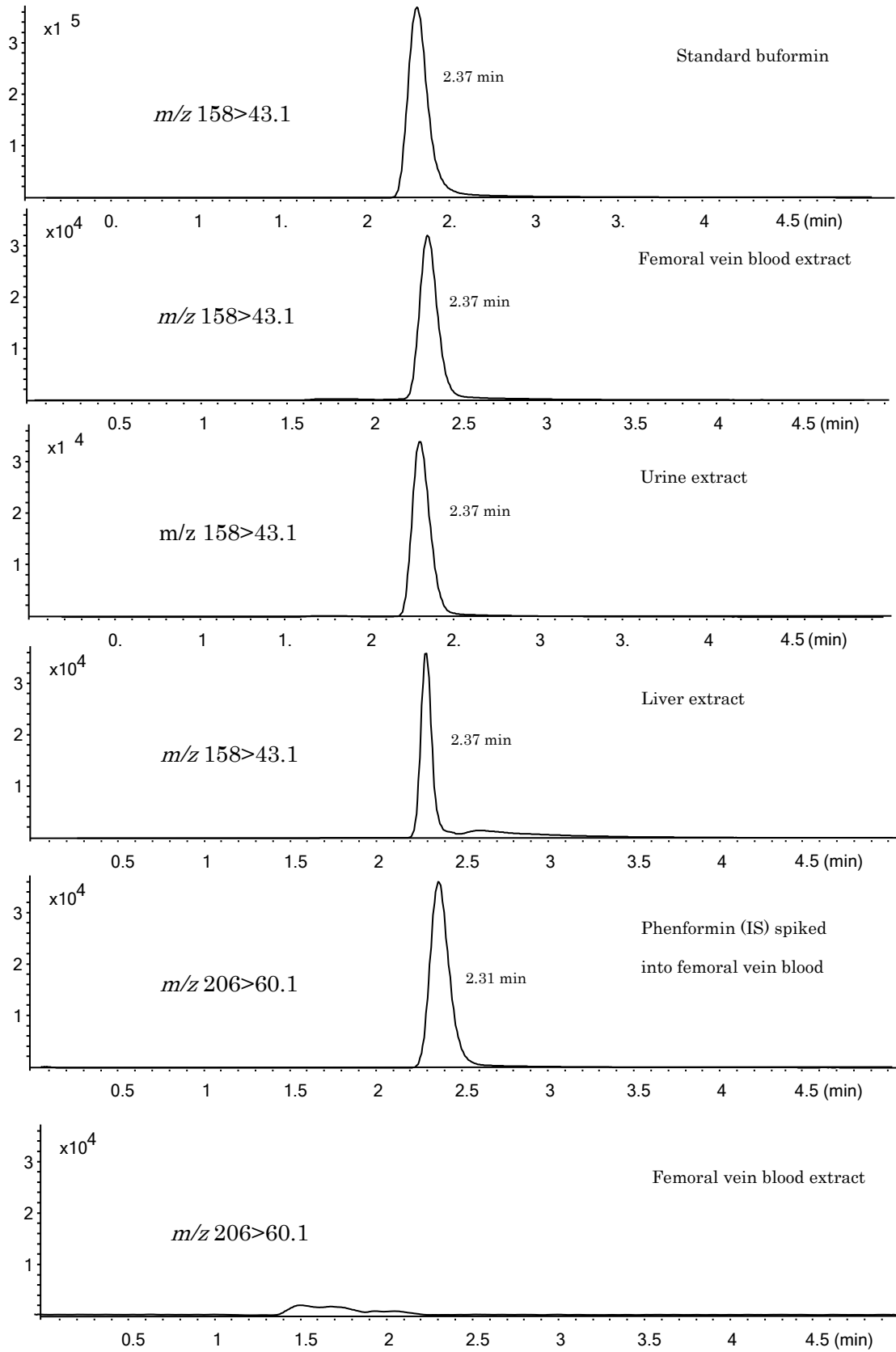


Fig 2

**Table 1** Standard addition calibration equations for buformin in body fluids and solid tissues of the deceased.

Specimen	Equation <sup>a</sup>	Correlation coefficient(r)
Femoral vein blood	$y=0.00222x + 0.579$	0.999
Right heart blood	$y=0.00237x + 0.512$	0.996
Left heart blood	$y=0.0022x + 0.877$	0.999
Cerebrospinal fluid	$y=0.0212x + 0.237$	0.999
Pericardial fluid	$y=0.00175x + 1.29$	0.994
Bile	$y=0.00269x + 2.54$	0.998
Small intestine contents	$y=0.000735x + 0.395$	0.992
Stomach contents	$y=0.000876x + 2.52$	0.995
Urine	$y=0.00269x + 1.46$	0.999
Brain	$y=0.00276x + 0.0713$	0.999
Lung	$y=0.00101x + 0.278$	0.999
Heart muscle	$y=0.00107x + 0.241$	0.993
Liver	$y=0.00117x + 0.559$	0.998
Spleen	$y=0.00152x + 0.257$	0.998
Kidney	$y=0.00265x + 2.21$	0.996
Pancreas	$y=0.000779x + 2.47$	0.995
Skeletal muscle	$y=0.00105x + 0.25$	0.998

<sup>a</sup> If  $y=0$ , preexisting concentration ( $x$ ) can be calculated as a minus value.

**Table 2** examples of intraday and interday repeatability for determination of buformin in the body fluids and solid tissues of the deceased.

Specimen	Intraday (n=5)		Interday (n=5)	
	Concentration ( $\mu\text{g}/\text{mL}$ or $\mu\text{g}/\text{g}$ ) <sup>a</sup>	Repeatability (%RSD) <sup>b</sup>	Concentration ( $\mu\text{g}/\text{ml}$ or $\mu\text{g}/\text{g}$ )	Repeatability (%RSD)
Femoral vein blood	261 $\pm$ 15.4	5.90	272 $\pm$ 20.3	7.46
Urine	543 $\pm$ 14.6	2.68	516 $\pm$ 60.2	11.7
Liver	478 $\pm$ 12.3	2.58	432 $\pm$ 52.4	12.1

<sup>a</sup> Data are given as mean  $\pm$  standard deviation (SD) obtained from five experiments each.

<sup>b</sup> RSD, relative standard deviation



**Table 3** Examples of matrix effects and recovery rates for determination of buformin in the body fluids and solid tissues of the deceased.

Specimen	Matrix effect (%)	Recovery rate (%)
Femoral vein blood	73.3±2.63	97.1±4.62
Urine	78.9±3.65	96.4±5.12
Liver	89.5±1.84	97.2±3.96

Data given as mean ± SD obtained from triplicate determinations.

**Table 4** Examples of stabilities of buformin in the body fluids and solid tissues of the deceased at room temperature for 24h and a week after storage.

Specimen	Stability (%) (25°C, 24h)	Stability (%) (25°C, 1 week)
Femoral vein blood	97.2±2.61	97.2±3.65
Urine	94.8±4.02	92.5±5.32
Liver	102±7.49	104±9.46

Data given as mean ± SD obtained from triplicate determinations.

**Table 5** Postmortem concentrations of buformin in body fluids and solid tissues of the deceased.

Specimen	Concentration ( $\mu\text{g/mL}$ or $\mu\text{g/g}$ )
Femoral vein blood	261 $\pm$ 15.4
Right heart blood	216 $\pm$ 8.96
Left heart blood	399 $\pm$ 35.6
Cerebrospinal fluid	11.2 $\pm$ 0.367
Pericardial fluid	737 $\pm$ 13.9
Bile	944 $\pm$ 33.7
Stomach contents	2880 $\pm$ 234
Small intestine contents	537 $\pm$ 67.6
Urine	543 $\pm$ 14.6
Brain	25.8 $\pm$ 0.717
Lung	275 $\pm$ 21.2
Heart muscle	225 $\pm$ 8.37
Liver	478 $\pm$ 12.3
Spleen	169 $\pm$ 2.04
Kidney	834 $\pm$ 17.3
Pancreas	3177 $\pm$ 189
Skeletal muscle	238 $\pm$ 3.14

Data given as mean  $\pm$  SD obtained from triplicate determinations.