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ORIGINAL ARTICLE

Postmortem distribution of PV9, a new cathinone derivative, in human solid tissues in a fatal poisoning case

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Abstract In our previous study, we identified PV9 [1-phenyl-2-(pyrrolidin-1-yl)octan-1-one] in human blood and urine in a fatal poisoning case. The victim was an 18-year-old woman. After ingesting "aroma liquid" solution, the victim showed various symptoms including low levels of consciousness, and was taken to a hospital emergency department. Although the victim received intensive medical treatment including an intravenous drip infusion of a large volume of transfusion solution, she was pronounced dead about 20 h after admission. In this study, we carefully examined the postmortem distribution of PV9 in nine solid tissues of the victim collected at forensic autopsy. The extraction of PV9 and internal standard (IS) PV8 [1-phenyl-2-(1-pyrrolidinyl)-1-heptanone] was performed by acetonitrile deproteinization, followed by modified QuEChERS dispersive solid-phase extraction and filtration through Captiva ND Lipids cartridges. Anaysis was performed by liquid chromatography–tandem mass spectrometry. Because this study dealt with various kinds of human matrices, we used the standard addition method to overcome matrix effects. After thorough validations, such as checking the product ion mass spectra, selected reaction monitoring chromatograms, linearity of the standard addition calibration curves, the intraday and interday repeatability, matrix effects, and recovery rates for the method, the concentrations of PV9 in nine solid tissue specimens were measured using PV8 as IS. The highest level of PV9 was found in the kidney at 907 ± 19.5 ng/g followed by the skeletal muscle, pancreas, adipose tissue, liver, lung, spleen, heart muscle, and brain. The lowest level of PV9 in the brain was 212 ± 11.9 ng/g. The high level of PV9 in the kidney suggests that this drug tends to be rapidly excreted into urine via the kidney as was the case for α-pyrrolidinovalerophenone. The low concentration of PV9 in the brain was unexpected, because this drug is a psychotropic drug with a long hydrophobic side chain, and is considered to cross the blood–brain barrier very easily. To our knowledge, this is the first demonstration of the distribution of the new pyrrolidinophenone derivative PV9 in human solid tissues in a poisoning case.

Keywords PV9 · α-POP · 1-Phenyl-2-(pyrrolidin-1-yl)octan-1-one · Cathinone derivative · Distribution in human organs · LC–MS–MS

Introduction

Cathinone derivatives and synthetic cannabinoids are now the most widely distributed drugs of abuse in the world [1–6]. Recently, we encountered a fatal case of drug poisoning, in which PV9 [α-POP; 1-phenyl-2-(pyrrolidin-1-yl)octan-1-one] was judged as the cause of death. We described identification and quantitation of PV9 in an "aroma liquid" product, antemortem body fluid specimens, and postmortem whole blood with a newly modified QuEChERS extraction procedure followed by liquid chromatography–tandem mass spectrometry (LC–MS–MS) [7]. In the present study, we carefully examined the postmortem distribution of PV9 in nine solid tissues of the victim using a remodified QuEChERS dispersive solid-phase extraction plus filtration through Captiva ND Lipids cartridges. To date, data on the distribution of PV9 in

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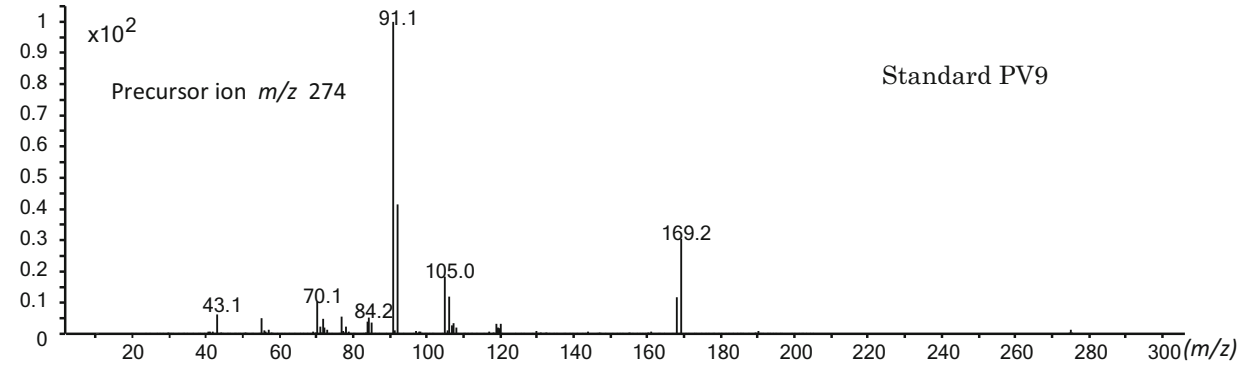
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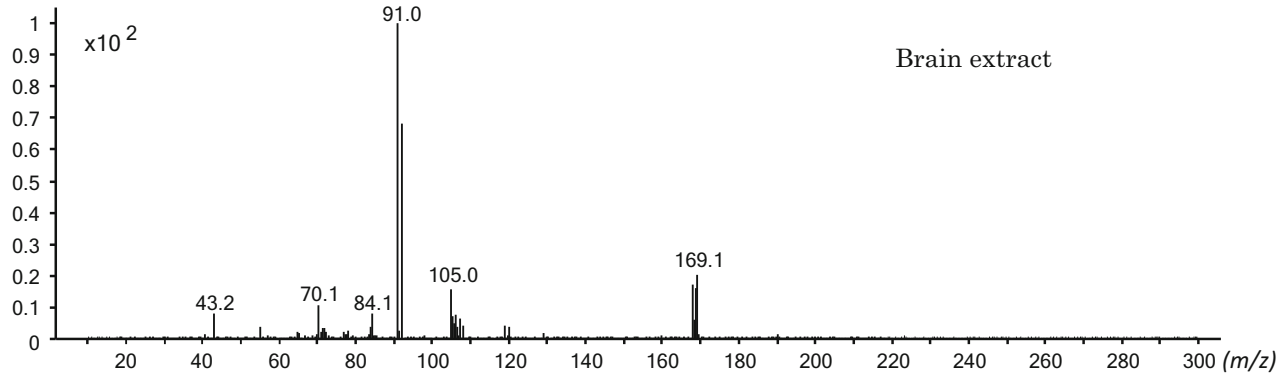
70.1
84.2
(m/z)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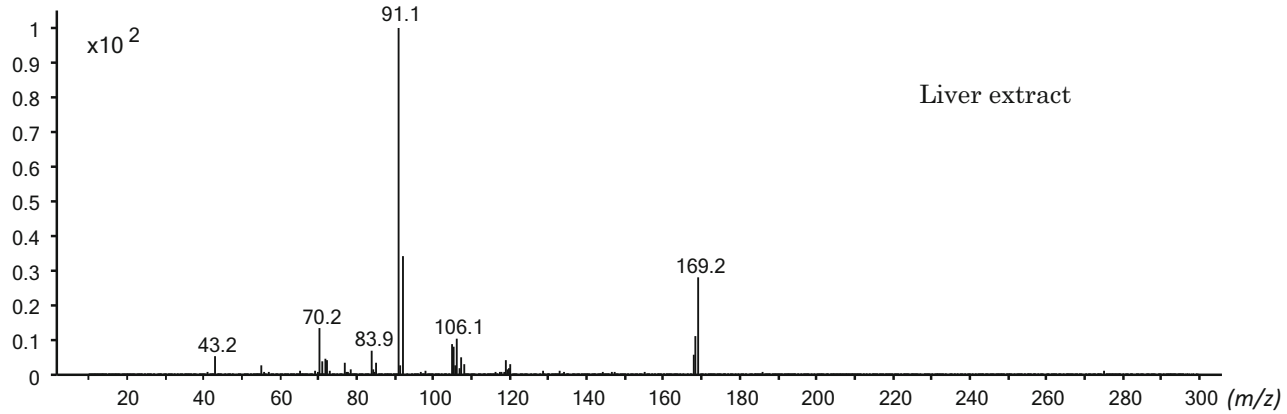
(m/z)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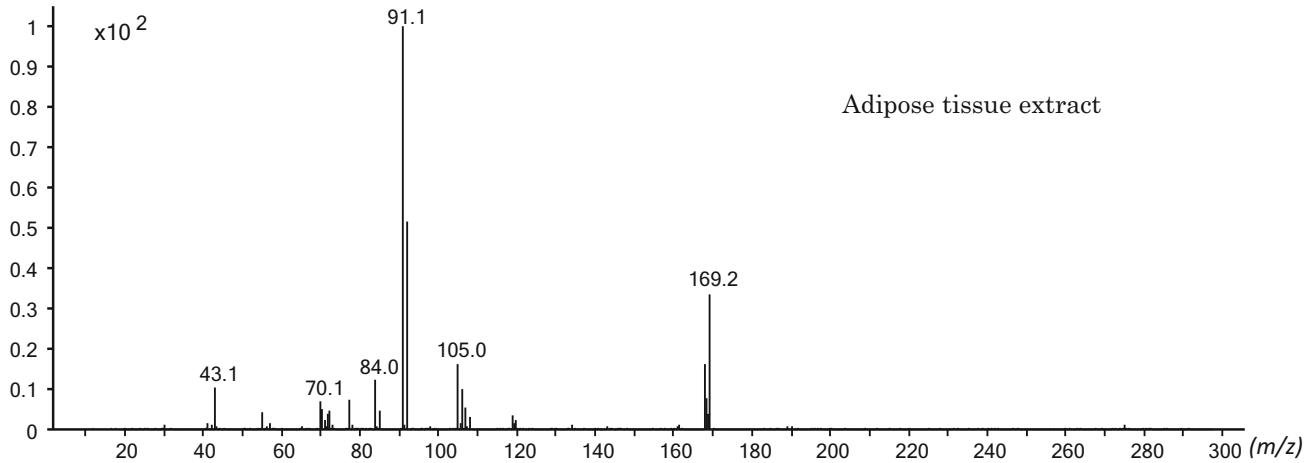

x10 2
Adipose tissue extract
91.1
169.2
105.0
84.0
43.1
70.1
(m/z)

◀**Fig. 1** Product ion mass spectra obtained from reference standard PV9 and the extracts of the postmortem brain, liver, and adipose tissue of the deceased, recorded by liquid chromatography–tandem mass spectrometry (LC–MS–MS)

human solid tissues in a fatal poisoning case have not been reported.

Case history

The details of the case history before autopsy are given in the previous report [7]. An 18-year-old woman ingested "aroma liquid" solution and soon after showed various symptoms that included shivering, convulsion, and low levels of consciousness. She was taken to a hospital emergency department, where she received intensive medical treatment including an intravenous drip infusion of a large volume of transfusion solution and gastrolavage. Despite the efforts of the medical team, the woman was pronounced dead about 20 h after admission. The deceased was taken to our department for forensic autopsy. Although the postmortem interval was 6 days at the beginning of the autopsy, the cadaver was relatively fresh with almost no putrefaction because of its storage in a morgue refrigerated at 4 °C until the day of the autopsy. She was 154 cm tall and weighed 56.4 kg. By macroscopic observation, there were many abrasions, injection marks, and scars on various parts of her skin, but these were not considered serious or life-threatening. Internally, the brain was edematous, and both lungs were congestive. Inside the heart, a large amount of dark-red fluidal blood was observed. The liver showed fatty degeneration. No obvious disorders related to her death could be found. There was no urine in the urinary bladder. About 10 g each of the brain, lung, heart muscle, liver, spleen, kidney, pancreas, skeletal muscle, and adipose tissue just inside the abdominal skin were collected, and frozen at −80 °C until analysis.

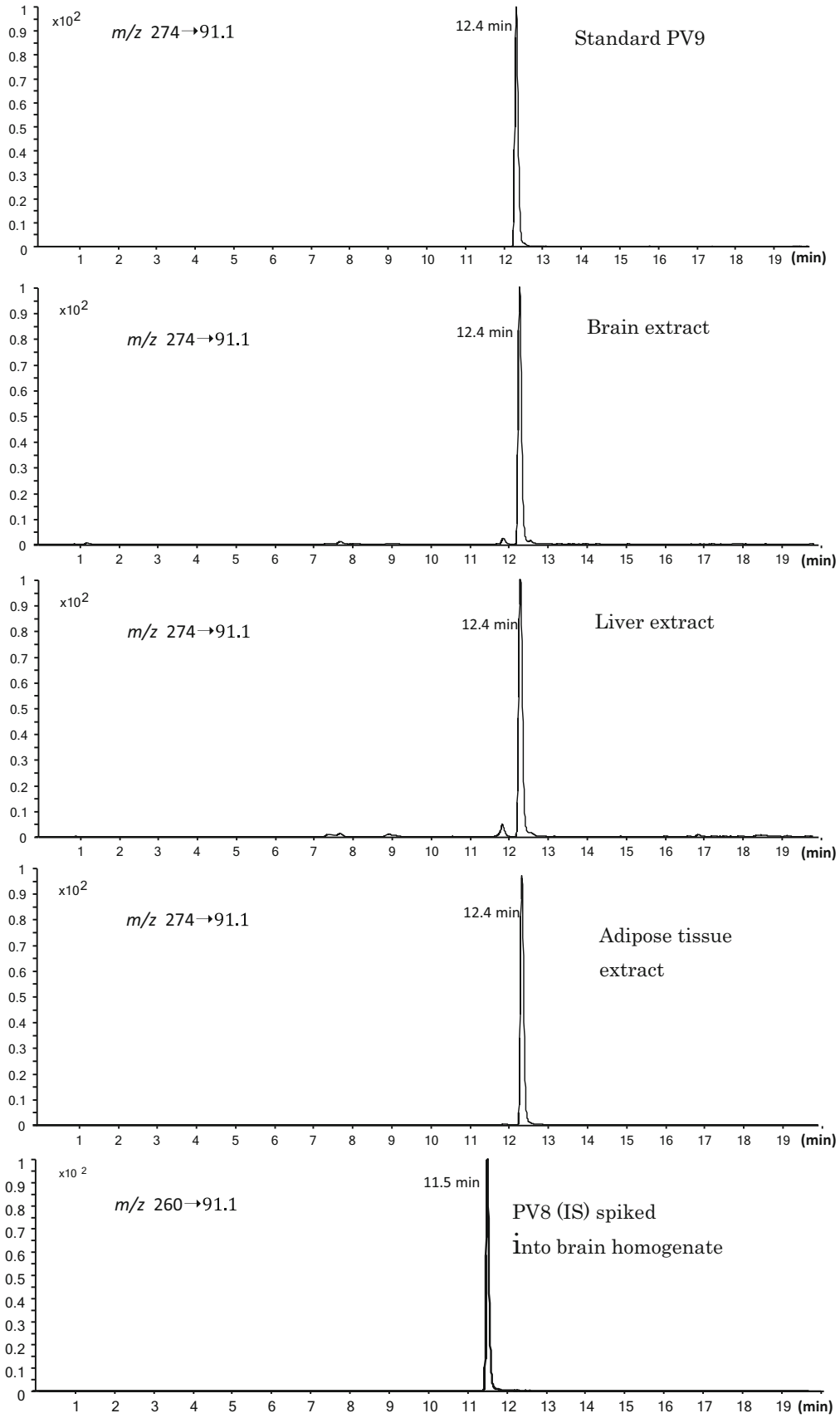
The alcohol analysis for blood by gas chromatography was negative. The immunochemical drug screening using a Triage DOA kit for urine specimens (Alere, Waltham, MA, USA) collected at the hospital was also negative; the urine specimens obtained at the hospital and kept at −80 °C were probably markedly diluted by the intensive intravenous drip infusion. This was also the case for blood specimens obtained at the hospital. The concentrations of PV9 in the antemortem whole blood, antemortem urine, and postmortem femoral vein blood collected before the autopsy were 45.7, 20.3, and 180 ng/ml, respectively [7]. The NAGINATA screening for the conventional drug and toxic substances in whole blood using gas chromatography–mass spectrometry (GC–MS) [8] showed a low level of caffeine. Because our in-house MS screening for drugs of abuse suggested no drugs, we consulted the Cayman Spectral Library [9], which strongly suggested the presence of PV9 in the solution of aroma liquid.

Materials and methods

PV9-HCl [α-POP-HCl, 1-phenyl-2-(pyrrolidin-1-yl)octan-1-one monohydrochloride] and PV8-HCl [α-PHPP-HCl, 1-phenyl-2-(1-pyrrolidinyl)-1-heptanone monohydrochloride] [7] were purchased from Cayman Chemical (Ann Arbor, MI, USA). Other common chemicals 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. Captiva ND Lipids cartridges (3-ml capacity) and 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_{18EC}), and 150 mg of magnesium sulfate, were obtained from Agilent (Santa Clara, CA, USA).

Extraction procedure

A 100-mg portion of each solid tissue was placed in a 5-ml plastic centrifuge tube containing 4.9 ml of acetonitrile and 100 ng of PV8-HCl (internal standard, IS) dissolved in 10 µl of acetonitrile. The tissue was minced with clean surgical scissors. Five stainless beads were added to the mixture, and the tube was capped, held to a bead beater-type shaking machine (Beads Crusher µT-12; TAITEC), and vigorously shaken at 3,200 rpm for 5 min. For the adipose tissue, the mixture was heated at 80 °C for 10 min before the bead beater crushing; for other tissues, heating was unnecessary. After tissue crushing, the suspension mixture without the beads was transferred to a large test tube, 5 ml of acetonitrile was added, and it was shaken gently. Six 1-ml portions were taken from the 10-ml suspension mixture; to each 1-ml portion was added (or not added) an appropriate amount of PV9-HCl dissolved in 10 µl of acetonitrile to construct a standard addition calibration curve in a capped 1.5-ml plastic centrifuge tube. The tube was vortexed for 30 s, and centrifuged at 10,000 rpm for 2 min. The supernatant was decanted into the QuEChERS dispersive-SPE centrifuge tube (2-ml capacity) containing PSA, C_{18EC}, and magnesium sulfate, vortexed for 30 s, and centrifuged at 10,000 rpm for 2 min. The upper acetonitrile layer was filtered through a Captiva ND Lipids cartridge. A 3.5-µl aliquot of the eluate was analyzed by LC–MS–MS.


x10^2
m/z 274→91.1
12.4 min
Standard PV9
x10^2
m/z 274→91.1
12.4 min
Brain extract
x10^2
m/z 274→91.1
12.4 min
Liver extract
x10^2
m/z 274→91.1
12.4 min
Adipose tissue extract
x10^2
m/z 260→91.1
11.5 min
PV8 (IS) spiked into brain homogenate
(min)

◀**Fig. 2** Selected reaction monitoring chromatograms for the reference standard PV9 and the extracts of the postmortem brain, liver, and adipose tissue and for PV8 (internal standard) spiked into the brain tissue, recorded by LC–MS–MS

Table 1 Standard addition calibration equations for α-PV9 in solid tissues of the victim

Specimen	Equation[a]	Correlation coefficient (r)
Brain	$y = 0.000100x + 0.0213$	0.998
Lung	$y = 0.000102x + 0.0343$	0.999
Heart muscle	$y = 0.000112x + 0.0288$	0.998
Liver	$y = 0.0000982x + 0.0370$	0.998
Spleen	$y = 0.000104x + 0.0279$	0.997
Kidney	$y = 0.0000989x + 0.0897$	0.998
Pancreas	$y = 0.000102x + 0.0641$	0.997
Skeletal muscle	$y = 0.000107x + 0.0732$	0.996
Adipose tissue	$y = 0.000104x + 0.0544$	0.998

[a] If y equals 0, the preexisting concentration (x) can be calculated as a minus value

LC–MS–MS conditions

LC–MS–MS was conducted on an Agilent 1200 LC-SL system connected to a 6460 Triple Quad LC/MS tandem MS instrument (Agilent). The LC-SL system contained a microdegasser and a high-performance autosampler. LC conditions were: separation column, ZORBAX Eclipse Plus C18 column (100 × 2.1 mm i.d., particle size 1.8 μm, Agilent); injection volume, 3.5 μl; flow rate, 0.25 ml/min; elution mode, gradient with 10 mM ammonium formate/0.1 % formic acid in distilled water (A) and acetonitrile (B) from 90 % A/10 % B to 100 % B in 20 min followed by isocratic elution with 100 % B for 10 min. Column and autosampler were operated at room temperature.

Tandem MS conditions were: interface, electrospray ionization (ESI) mode; polarity, positive; ion source temperature, 320 °C; ion source voltage, 500 V; quantitation, selected reaction monitoring (SRM) mode using peak area; ion transitions, m/z 274 → 91.1 for PV9 and m/z 260 → 91.1 for PV8 (IS); collision and fragment energies, 21 and 100 V, respectively, for both compounds.

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

Standard addition method

The standard addition method [10] was employed to quantitate PV9 in human solid tissue specimens. This method can overcome the matrix effects and recovery rate differences. In addition, the method requires no blank human specimens that are negative for a target compound; generally, the collection of nonessential human tissue sample should be avoided because of ethical reasons. The principle and the calculation method of the standard addition method are described in our previous report [3, 7, 11].

Matrix effects and recovery rates

Although the standard addition method can overcome matrix effects and negates the influence of variable recovery rates, it is useful to consider the matrix effects, especially when LC–MS–MS is used and lipid-rich matrices are analyzed. To determine the matrix effects and recovery rates for PV9 in the solid tissue specimens, we first measured all concentrations of PV9 in the matrices by the standard addition method. According to the concentration of PV9 in each matrix, we prepared reference standard PV9 solutions dissolved in acetonitrile at two concentrations; one with a concentration equal to that in each matrix, and the other with a concentration 100 times lower than that in the matrix. Then a 0.1-g portion of a matrix was processed again following the procedure described before only with addition of IS at the initial step; although six 1-ml portions of the suspension mixture were usually taken from the 10-ml suspension, we took four 1-ml portions from it in this experiment. They were purified through the QuEChERS dispersive-SPE centrifuge tubes and the Captiva ND Lipids cartridges without any addition of PV9 according to the method described before.

Table 2 Examples of intraday and interday repeatability for determination of PV9 in three solid tissues of the victim

Specimen	Intraday ($n = 5$)		Interday ($n = 5$)	
	Concentration found[a] (ng/g)	Repeatability (%RSD)	Concentration found[a] (ng/g)	Repeatability (%RSD)
Brain	212 ± 11.9	5.60	219 ± 15.7	7.18
Liver	377 ± 13.9	3.70	375 ± 28.9	7.71
Adipose tissue	526 ± 20.0	3.80	657 ± 70.3	10.7

[a] Data given as mean ± standard deviation (SD) obtained by intraday or interday determinations ($n = 5$ each)

RSD relative standard deviation

The acetonitrile eluates from the three final Captiva ND Lipids cartridges were combined. To 1 ml of the combined eluate, 10 µl of acetonitrile PV9 solution at the concentration equal to that in the corresponding solid tissue was added and shaken gently; a 3.5-µl aliquot of it was injected into the LC–MS–MS system to obtain a peak area designated as *A*. A 3.5-µl aliquot of another eluate from a Captiva ND Lipids cartridge without any addition was also injected into the LC–MS–MS system to obtain a peak area designated as *B*. A 3.5-µl aliquot of the PV9 acetonitrile solution without any extraction, the concentration of which was 100 times lower than that in the corresponding matrix, was finally injected into the LC–MS–MS system to obtain a peak area designated as *C*. The matrix effect and recovery rate was calculated as follows. Matrix effect $(\%) = [(A - B)/C] \times 100$. Recovery rate $(\%) = [B/(A - B)] \times 100$.

Results and discussion

Identification of PV9 by LC–MS–MS

In our previous report [7], we first identified PV9 in whole blood of this victim by LC–MS–MS. Figure 1 shows the product ion mass spectra of the extracts of the brain, liver, and adipose tissue in comparison with the spectrum of the reference standard PV9. The spectra obtained from the three organs agreed well with that of the standard, confirming the presence of PV9 in the organs.

Validation for quantitative analysis of PV9

Figure 2 shows the SRM chromatograms of reference standard PV9, PV9 extracted from the brain, liver, and adipose tissue, and PV8 spiked into the brain as IS. Sharp peaks appeared at 12.4 min for PV9 and 11.5 min for PV8 (IS). All chromatograms showed almost no impurity peaks and very low backgrounds.

In our previous report describing the identification of PV9 in whole blood and urine of this victim [7], we confirmed that PV8 did not coexist with PV9 in this deceased.

Table 1 shows the standard addition calibration equations and their correlation coefficients for PV9 in the nine solid tissues of the deceased. All of them showed satisfactory linearity with correlation coefficients greater than 0.995. The detection limit (signal-to-noise ratio = 3) of PV9 by this method was estimated to be around 0.05 ng/g.

Because we employed the standard addition method for quantitation without the use of blank specimens, it was impossible to present the usual accuracy and precision data. Instead, as shown in Table 2, we repeated intraday and interday determinations of PV9 in the brain, liver, and adipose tissue as an example. The repeatability expressed as relative standard deviation was not greater than 10.7 %.

Table 3 Matrix effects and recovery rates for determination of PV9 in three tissue specimens obtained from the victim

Specimen	Matrix effect (%)	Recovery (%)
Brain	110 ± 3.30	83.6 ± 1.11
Liver	89.7 ± 3.02	99.8 ± 2.48
Adipose tissue	94.8 ± 2.73	71.4 ± 2.33

Data given as mean ± SD obtained from triplicate determinations

Table 3 shows the matrix effects and recovery rates for quantitation of PV9 in the brain, liver, and adipose tissue calculated as described before. Satisfactory values for matrix effects not lower than 89.7 %, and recovery rates not lower than 71.4 % were obtained after the three-step purification: deproteinization with acetonitrile, QuEChERS dispersive-SPE, and filtration through Captiva ND Lipids cartridges.

Concentration of PV9 in nine solid tissues of the victim

Table 4 shows the distribution of PV9 in nine solid tissues. The highest concentration of PV9 was observed for the kidney, followed by the skeletal muscle, pancreas, adipose tissue, liver, lung, spleen, heart muscle, and brain. In our previous reports [3, 11], we reported the postmortem distribution of α-pyrrolidinovalerophenone (α-PVP) and α-pyrrolidinobutiophenone (α-PBP) in body fluids and solid tissues of human cadavers. PV9, α-PVP, and α-PBP are all α-pyrrolidinophenone derivative analogs. The concentrations of α-PVP and α-PBP were also highest in the kidney among solid tissues, and much higher concentrations were detected in urine samples. Therefore, the α-pyrrolidinophenone derivatives including PV9 tend to be rapidly excreted into urine via the kidney.

The concentration of PV9 in the brain was lowest among the solid tissues tested in this study (Table 4); a relatively low level was also found for α-PVP in the brain [3]. This was unexpected, because Zaitsu et al. [2] reported that α-pyrrolidinophenone derivatives have high lipophilicity due to the pyrrolidine ring substituion at the nitrogen atom, resulting in higher blood–brain barrier permeability.

In this case, the victim received intensive medical treatment including an intravenous drip infusion of a large amount of transfusion solution while still alive, which probably lowered the concentrations of PV9 in body fluids and the solid tissues of the victim. In a fatal case of α-PVP poisoning in which the victim received no treatment, the levels of α-PVP in various solid tissues [3] were higher than those of PV9 in the present case where intensive drip infusion treatment was administered (Table 4).

Table 4 Concentrations of PV9 in nine solid tissues of the victim

Specimen	Concentration (ng/g)
Brain	212 ± 11.9
Lung	337 ± 16.0
Heart muscle	258 ± 5.78
Liver	377 ± 10.1
Spleen	269 ± 5.57
Kidney	907 ± 19.5
Pancreas	628 ± 28.2
Skeletal muscle	686 ± 14.0
Adipose tissue	526 ± 20.0

Data given as mean ± SD obtained from triplicate determinations, expressed as concentrations of the free base form of PV9

Conclusions

We analyzed the postmortem distribution of PV9 in nine solid tissues collected from the body of a woman, who was judged to have died of PV9 poisoning. After acetonitrile deproteinization, QuEChERS dispersive-SPE, and filtration through Captiva ND Lipids cartridge for solid tissue specimens, almost no matrix effects were found, even when the analysis was performed by LC–MS–MS. Although the victim received intensive medical treatment including an intravenous drip infusion of a large amount of transfusion solution, appreciable concentrations of PV9 were detected in nine solid tissues of the victim. All of the concentrations in solid tissues were higher than that of postmortem whole blood in the femoral vein [7]. Our reported values of PV9 in human solid tissues will be informative for judgement on whether PV9 can be considered the cause of death when autopsy is performed. To our knowledge, this is the first report describing the distribution of PV9 in human solid tissues in a fatal poisoning case.

Conflict of interest There are no financial or other relations that could lead to a conflict of interest.

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