CASE REPORT

Determination of propofol by GC/MS and Fast GC/MS-TOF in two cases of poisoning

Paolo Procaccianti\textsuperscript{a} · Fiorenza Farè\textsuperscript{b} · Antonella Argo\textsuperscript{a} · Eleonora Casagni\textsuperscript{b} · Sebastiano Arnoldi\textsuperscript{b} · Sara Facheris\textsuperscript{b} · Giacomo Luca Visconti\textsuperscript{b} · Gabriella Roda\textsuperscript{b} · Veniero Gambaro\textsuperscript{b}

\textsuperscript{a}Department of Sciences for Health Promotion and Mother-Child Care "G. D'Alessandro"
Via del Vespro 133, 90127 Palermo, Italy

\textsuperscript{b}Dipartimento di Scienze Farmaceutiche, Università degli Studi di Milano, Via Mangiagalli 25, 20133, Milano, Italy.

CORRESPONDING AUTHOR
Gabriella Roda, Ph.D.
Tel.+39-02-50319328; Fax. +39-02-50319359
E-mail:gabriella.roda@unimi.it
Abstract

Two cases of suspected acute and lethal intoxication caused by propofol were delivered by the judicial authority to the Department of Sciences for Health Promotion and Mother-Child Care in Palermo, Sicily. In the first case a female nurse was found in a hotel room, where she lived with her mother; four 10 mg/mL vials and two 20 mg/mL vials of propofol were found near the decedent along with syringes and needles. In the second case a male nurse was found in the operating room of a hospital, along with a used syringe. In both cases a preliminary systematic and toxicological analysis (STA) indicated the presence of propofol in the blood and urine. As a result, a method for the quantitative determination of propofol in biological fluids was optimized and validated using a liquid-liquid extraction protocol followed by GC/MS and Fast GC/MS-TOF. In the first case, the concentration of propofol in blood was determined to be 8.1 μg/mL while the concentration of propofol in the second case was calculated at 1.2 μg/mL. Additionally, the tissue distribution of propofol was determined for both cases. Data emerging from the autopsy findings, histopathological exams as well as the toxicological results aided in establishing that the deaths were due to poisoning, however the manner of death in each were different: homicide in Case 1 and suicide in Case 2.

Keywords Propofol · Poisoning · Systematic and toxicological analysis · Tissue distribution · Cause of death · GC/MS-TOF
Propofol (2,6-diisopropylphenol), a sedative-hypnotic agent used for the induction of anesthesia and for sedating mechanically ventilated patients in intensive care units [1,2], is now increasingly being used for conscious sedation during endoscopic procedures. Propofol is an extremely rapid-acting intravenous anesthetic. Its advantages include less residual postoperative sedation and less psychomotor impairment compared to the barbiturates and less incidence of nausea and vomiting [3]. The blood concentration required for induction of anesthesia is generally 2-10 μg/L, while a concentration of 2-4 μg/L is sufficient to maintain it [4,5]. Propofol produces dose-dependent cardiovascular and respiratory depression with a profile similar to methohexital. Side effects include pain on injection, involuntary muscle movements, coughing, and hiccoughing [6]. It has been associated with fatal heart failure both in children [7] and in adult patients with head injuries [8]. In fact, the constellation of myocardial failure, metabolic acidosis, and rhabdomyolysis in children receiving propofol infusions for more than 48 hours has been termed the propofol infusion syndrome [9,10]. Propofol is known to induce hypertriglyceridemia, severe enough to cause pancreatitis, but only when used at a rate exceeding 100 μg kg⁻¹min⁻¹ for prolonged periods [11]. Propofol is also associated with abuse and dependency, especially among health care professionals [12-14], because of its rapid narcotic effect causing euphoria and sexual hallucinations [15].

Several fatal cases of poisoning have been reported [13-20]; in these cases a high variability in the blood concentration of propofol has been observed (from 0.08 to 8.7 μg/L) [4].

Two cases of suspected lethal intoxication caused by propofol were delivered by the judicial authority to the Department of Sciences for Health Promotion and Mother-Child Care in Palermo, Sicily in 2014. A GC/MS method previously developed and validated in our laboratory [21] was applied for the determination of volatile organic compounds (VOC) and the systematic
toxicological analysis (STA) on blood and urine collected from the two cases. In both cases STA indicated the presence of propofol in blood and urine. A method was therefore optimized and validated for the quantitative determination of propofol in the biological fluids using a liquid-liquid extraction protocol followed by GC/MS and Fast GC/MS-TOF. Blood, urine, bile and tissue concentrations were determined for both cases [22].

Case history

First case: female, nurse, 41 years old, sitting on a chair near a bed in a hotel room. Four 10 mg/mL vials and two 20 mg/mL vials of propofol were found near the decedent together with syringes and needles. Signs of acupuncture on the left elbow, forearm, hand and foot were noted. Blood, urine, bile, brain and liver were obtained at the autopsy.

Second case: male, nurse, 55 years old, found lying in an operating room with a syringe nearby. Sign of acupuncture on the right ankle. Blood, urine, brain, liver and kidney were obtained at the autopsy.

Materials and methods

Reagents, chemicals and standards

All reagents were of analytical grade and were stored as indicated by the supplier. Ethyl acetate, 2-propanol, dichloromethane, methanol, ammonia, hydrochloric acid 37%, sodium chloride, sodium bicarbonate, sodium carbonate, anhydrous sodium sulfate sodium hydroxide, O,N-bis(trimethylsilyl)trifluoroacetoamide-trimethylchlorosilane (BSTFA-1% TMCS), pH 6 buffer were purchased from Sigma-Aldrich (St. Louis, MO, USA); Thymol and sodium sulfate were obtained from Farmalabor (Canosa di Puglia, Italy). MethElute Reagent 0.2 M in methanol (TMAH) was from Thermo Scientific (Waltham, MA, USA). Propofol was purchased from Archimica S.p.a
(Origgio, Italy). Water (18.2 MΩ·cm\(^{-1}\)) was prepared by a Milli-Q System (Millipore, Darmstadt, Germany); other common chemicals were of the highest purity commercially available.

Stock solutions of propofol (0.1, 0.25, 0.50, 1, 2, 3, 10, 20, 25, 50, 100 µg/mL) and thymol (IS; 10, 100, 1000 µg/mL) were prepared in methanol and stored at 4 °C.

**Systematic and toxicological analysis (STA) [21]**

**Blood, urine and bile sample preparation**

Blood (1 mL), urine (1 mL) or bile (250 µL) was added with IS (100 µL, 10 µg/mL), saline solution (up to 2 mL), bicarbonate-carbonate buffer (50 mg, 2/1 w/w, pH 9) and extracted with ethyl acetate (4 mL). The mixture was put on a rotary shaker (20 min, 15 rpm) and then centrifuged (5 min, 5000 rpm). The organic phase was separated, sodium sulfate was added and after centrifugation (5 min, 5000 rpm) the supernatant was withdrawn and the solvent evaporated. The residue was dissolved in ethyl acetate (100 µL) before the analysis.

To evaluate specificity blood, urine or bile working standard solutions were prepared as follows: 100 µL of propofol standard solution (10 µg/mL) were placed in vial and the solvent evaporated. Blank blood (1 mL), blank urine (1 mL) or blank bile (250 µL), IS (100 µL, 10 µg/mL), saline solution (up to 2 mL), bicarbonate-carbonate buffer (50 mg, 2/1 w/w, pH 9) were added and the mixtures extracted as described before.

**Hydrolysis of propofol glucuronide and sulfate in urine and bile samples**
The sample of urine (1 mL) or bile (250 µL) was added with saline solution until a volume of 2 mL and 1 mL of 6N hydrochloric acid was added. The mixture was heated at 105 °C for 1 h. After cooling, IS (100 µL, 10 µg/mL) was added, pH was adjusted to 8 and bicarbonate-carbonate buffer (50 mg, 2/1 w/w, pH 9) was added. Then the mixtures were extracted as described before.

Hydrolyzed urine or bile working standard solutions were prepared as follows: 100 µL of propofol standard solution (10 µg/mL) were placed in vial and the solvent evaporated. Blank urine (1 mL) or blank bile (250 µL) and saline solution until a volume of 2 mL were added; the mixture was heated at 105 °C for 1 h. After cooling, IS (100 µL, 10 µg/mL) was added, pH was adjusted to 8 and bicarbonate-carbonate buffer (50 mg, 2/1 w/w, pH 9) was added. Then the mixtures were extracted as described before.

Tissue sample preparation

Each sample was homogenized with a blender or ball mill, depending on the quantity of material. The deproteinization of the biological matrix was performed by means of an ultrasonic bath: 100 mg of tissue (brain, liver or kidney) previously added with 4 mL of saline solution, bicarbonate-carbonate buffer (50 mg, 2/1 w/w, pH 9) and 100 µL of IS (10 µg/mL) were sonicated for 15 minutes at room temperature. After 5 min centrifugation, a clear supernatant was separated and extracted with ethyl acetate (4 mL). The mixture was placed on a rotary shaker (20 min, 15 rpm) and then centrifuged (5 min, 5000 rpm). The organic phase was separated, anhydrous sodium sulfate was added and after centrifugation (5 min, 5000 rpm) the
supernatant was withdrawn and the solvent evaporated. The residue was dissolved in ethyl acetate (100 µL) before the analysis.

Tissue working standard samples were prepared as follows: 100 µL of propofol standard solution (10 µg/mL) were placed in vial and the solvent evaporated. Blank tissue (100 mg), IS (100 µL, 10 µg/mL), saline solution (4 mL), bicarbonate-carbonate buffer (50 mg, 2/1 w/w, pH 9) were added and the mixtures extracted as described before.

**GC/MS**

The analyses were performed on a HP6890 Series II GC system, with a split-splitless injection system and an MSD HP5973 MS detector (Agilent Technologies, Santa Clara, CA, USA) operated in electron ionization (EI) mode (70 eV). The GC was equipped with a Rxi®-5Sil MS (5% diphenyl/95% dimethyl polysiloxane, 30 m x 0.25 mm i.d., film thickness 0.25 µm) capillary column (Restek, Bellefonte, PA, USA).

GC/MS conditions: splitless; solvent delay, 3.5 min; injector temperature, 280°C; interface transfer line, 280°C; ion source, 280°C; oven temperature program, initial 70°C, 40°C/min up to 110°C, then 15°C/min up to 300°C (3 min). Helium was used as the carrier gas at a flow rate of 1.2 mL/min. The MS detector was operated in the scan mode, acquiring ions from m/z 50 to 550. The total analysis time was 21 min.

**GC/MS-TOF**

The analyses were performed on a Dani Master GC system, with a split-splitless injection system and a Dani Master TOF Plus detector (Dani Instruments, Cologno Monzese, Italy) operated
in electron ionization (EI) mode (70 eV). The GC was equipped with a Rxi\textsuperscript{®}-5ms (Crossbond\textsuperscript{®}, 5% diphenyl/95% dimethyl polysiloxane, 10 m x 0.10 mm i.d., film thickness 0.15 μm) capillary column (Restek, Bellefonte, PA, USA).

The GC/MS conditions: split ratio 100:1; injector temperature, 250°C; interface transfer line, 280°C; ion source, 200°C; oven temperature program, initial 70°C, 20°C/min up to 200°C, then 30°C/min up to 300°C (17 s). Helium was used as the carrier gas at a flow rate of 0.5 mL/min. The MS detector was operated in the scan mode, acquiring ions from m/z 50 to 550. The total analysis time was 8 min. The selected ions were 163 and 178 for propofol and 135 and 150 for the IS.

**Method validation**

The specificity, accuracy, precision and linearity as well as the limit of detection (LOD) and limit of quantitation (LOQ) were evaluated using blood as matrix.

The specificity was assessed by extracting control (blank) blood, urine, bile, brain, liver and kidney samples. The lack of interfering peaks at the same analyte retention times conferred acceptable selectivity.

The linearity of the response of the GC/MS-TOF analysis was assessed for propofol by plotting drug/IS peak area ratios versus the total amount of drug in the standard solutions, with intervals of 25–2000 total ng of analyte (25, 50, 75, 150, 200, 500, 1250, 1500, 2000 ng\textsubscript{tot}). The calibration curve (y = 0.0007x – 0.0204) gave good correlation coefficients ($R^2 > 0.9925$) over the whole range.

Accuracy was expressed as the per cent recovery (REC) evaluated by analyzing, in triplicate, two standard propofol solutions (500 to 1250 ng\textsubscript{tot}). The averaged results were found to be satisfactory (mean %REC 86.6 at 500 and 111.1 at 1250 ng\textsubscript{tot}).

Two standard solutions (500 to 1000 ng\textsubscript{tot}) were analyzed five times in the same day and over 5 days in order to evaluate the precision of the method. The intraday and interday %CV were respectively 7.55 and 9.82% at 500 ng\textsubscript{tot}; 8.51 and 5.03% at 1000 ng\textsubscript{tot}. The obtained data demonstrated adequate reproducibility.
The LOD and LOQ were also evaluated and were found to be 10 and 25 ng evaluated as the concentration of the analyte which gives a signal to noise ratio of at least 3 and 10 respectively.

Results and discussion

STA was carried out on the biological samples of the two cases received. Blood and urine of both cases were evaluated; however bile was available only in the first case. Case 1 did not test positive for VOC; however Case 2 had a blood alcohol concentration of 0.2 g/L. Other non-volatile substances identified in the cases are reported in Table 1. As noted caffeine, cotinine and nicotine were identified in both cases and are considered toxicologically irrelevant. Of interest is the presence of a chromatographic peak whose mass spectrum correlated to silanized propofol (Fig. 1). Based on the nature of the two cases, the laboratory proceeded with developing an analytical method for the quantification of propofol in biological fluids and tissues.

Due to the low recoveries obtained with the original SPE method [21], a liquid-liquid extraction protocol was developed with ethyl acetate at pH 9 (bicarbonate/carbonate buffer) to optimize the extraction of propofol in the organic phase. Thymol was chosen as internal standard. The extracts were silanized using O,N-bis(trimethylsilyl)trifluoroacetoamide-trimethylchlorosilane (BSTFA-1% TMCS) as in the STA analysis, but due to the low reproducibility of the results by GC/MS, the determination of propofol after the liquid-liquid extraction protocol without derivatization was carried out. Unfortunately, two interfering species were detected: capric acid in blood and nicotine in urine samples (Fig. 2).

At this point the chromatographic system was completely changed, using Fast GC/TOF, with narrower and shorter capillary columns. The fast heating and cooling rate of the GC oven and the fast acquisition rate of the MS detector, allow high sensitivity and resolution and the chromatographic separation results enhanced although the shortness of the column. In these conditions, the peak of propofol was completely separated from those of capric acid and nicotine.
The method was validated using blood as matrix showing suitable selectivity, accuracy, precision, LOD, LOQ and linearity in the concentration ranges requested for propofol determination in biological specimen [5, 12-22].

The optimized method was applied for the determination of propofol in the biological specimens from the two cases. Urine and bile samples were hydrolyzed because it is known that most of propofol is conjugated with glucuronic acid [5]. A chromatogram obtained for the analysis of blood of Case 1 is depicted in Figure 3.

The results obtained analyzing the biological samples from the two cases are reported in Table 2.

The interpretation of the results should be made with particular caution. It is still widely debated whether propofol can be used to suicidal overdose. Several coroners believe that it is not possible to commit suicide with propofol because the maximum voluntarily injectable quantity of propofol before losing consciousness is not sufficient to cause death [23]. Death could be caused by a continuous intravenous infusion of the drug, with multiple organs failure mimicking propofol-related infusion syndrome. The two cases show very different propofol concentrations especially in blood and urine. In Case 2 propofol levels, found in blood and urine, were below the therapeutic range and in accordance with the literature [4-8]. Death was probably caused by the respiratory depression caused by propofol, assumed in uncontrolled conditions. The drug was probably assumed by an intravenous infusion. In fact the subject was a nurse and he was found in an operating room with a single sign of acupuncture in his arm. So suicidal hypothesis is the most likely.

Case 1 was more complicated. The very high concentration of propofol found in blood seemed incompatible with a single voluntary injection of propofol [23]. In fact propofol causes very rapid loss of consciousness. Even an intravenous infusion can hardly be responsible for a so high concentration.
Examining circumstantial data, the presence of several ampoules of “Propofol Kabi” in the room where the corpse was found, were evidenced. The corpse presented several signs of acupuncture. The police found out that the woman lived in the hotel room with her mother, also a nurse, in poor conditions; they gambled and had many debts. Probably they decided to both commit suicide, the mother injected some vials of propofol to the daughter but then changed her mind and did not kill herself. Death in the first case is then to be ascribed to an homicide rather than a suicide.

In conclusion both deaths were related to propofol poisoning though with a different manner, homicide in Case 1 and suicide in Case 2. These considerations were deduced taking into account blood and urine concentrations of propofol. To confirm the poisoning caused by this drug, also the tissues available from the autopsy were analyzed. The presence of propofol was confirmed also in all the tissues considered.

Conclusions

A liquid.liquid extraction protocol and a GC/MS and a Fast GC/MS-TOF method for the confirmation of propofol in the biological fluids was optimized and validated. The concentration of propofol was determined in blood, urine, bile, brain, liver and kidney of two suspected cases of poisoning caused by propofol. Data emerging from autopsy findings, histopathological exams and the concentrations of propofol evidenced by chemical and toxicological analysis, on the basis of literature data [4-16], allowed us to establish that both deaths were due to poisoning caused by propofol. In the first case the concentration of propofol in blood resulted to be 8.1 µg/mL while in the second one it was 1.2 µg/mL. The very different concentrations between the two cases were interpreted in two different ways: in the first case two females, mother and daughter, both nurses, decided to commit suicide with propofol, stolen by the daughter in the hospital where she worked.
The mother injected propofol in the ankle of the daughter, but then changed her mind and did not kill herself. In the second case a nurse committed suicide with an intravenous infusion of propofol.


Figure legends

**Fig. 1** SCAN analysis of case 1 blood (a); Mass spectrum of propofol-TMS (b)

**Fig. 2** Chromatograms of blood of Case 1 in GC/MS (a) and GC/TOF (b) and urine in GC/MS (c) and GC/TOF (d). A=Propofol; B=capric acid; C=nicotine

**Fig. 3** Chromatogram for the determination of propofol in blood of Case 1.
a) 

b)
**Table 1** Results of STA (n.d.= not determined)

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Case 1</th>
<th>Case 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>Cotinine</td>
<td>Cotinine</td>
</tr>
<tr>
<td></td>
<td>Caffeine</td>
<td>Caffeine</td>
</tr>
<tr>
<td>Urine</td>
<td>Nicotine</td>
<td>Nicotine</td>
</tr>
<tr>
<td></td>
<td>Cotinine</td>
<td>Caffeine</td>
</tr>
<tr>
<td>Bile</td>
<td>Nicotine</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>Cotinine</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2** Results of the quantitative determination of propofol in the biological specimens from the two cases

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Case 1 (µg/mL or µg/g)</th>
<th>Case 2 (µg/mL or µg/g)</th>
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</thead>
<tbody>
<tr>
<td>Blood</td>
<td>8.1</td>
<td>1.2</td>
</tr>
<tr>
<td>Urine</td>
<td>0.21</td>
<td>0.0073</td>
</tr>
<tr>
<td>Hydrolyzed urine</td>
<td>1276.6</td>
<td>18.3</td>
</tr>
<tr>
<td>Bile</td>
<td>3.28</td>
<td></td>
</tr>
<tr>
<td>Hydrolyzed bile</td>
<td>105.7</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>31.1</td>
<td>4.7</td>
</tr>
<tr>
<td>Liver</td>
<td>52.2</td>
<td>49.1</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td>2.3</td>
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