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LC–MS/MS and GC–MS methods in propofol detection: Evaluation of the two analytical procedures

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ABSTRACT

Propofol is a short-acting hypnotic agent that is commonly used to induce and maintain anesthesia. Propofol abuse and its involvement in suicide deaths have increased in recent years, especially among healthcare personnel. An example is the suicide of a 61-year-old nurse found with a propofol drip in his left arm. We describe the postmortem concentration of propofol in various tissues (femoral and cardiac blood, bile, urine, brain, and liver) and in the drip. The toxicological analyses were performed through two analytical methods, differing in derivatization reaction and in instrumentation: silylation for gas chromatograph–mass spectrometer (GC–MS), as routinely performed in our laboratory for this kind of analyses (lower limits of quantification–LLOQ–in urine and blood: 0.3 and 5 ng/ml); for liquid chromatograph–tandem mass spectrometer (LC–MS/MS) an innovative azo-coupling derivatization (LLOQ: 0.0004 and 0.1 ng/ml). This latter produces an azo-derivative (molecular composition: $C_{18}H_{22}ON_2$; molecular weight: 282 Da) highly ionizable in electro-spray ion source, both in negative and positive ionizations. These two methods were compared to evaluate the effectiveness of this new LC–MS/MS analysis. An acidic hydrolysis (HCl 6 N, 100 °C, and 1 h) was performed for the biological samples (1 ml or 1 g) irrespective of the analytical method applied. The drip content was extracted adding phosphate buffer (pH 8) and a dichloromethane/ethylacetate 8:2 (v:v) mixture. Derivatization steps were: silylation with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) + tetramethylammonium hydroxide (TMAH) for GC–MS; regarding LC–MS/MS, azo-coupling reaction with the aryl-diazonium salt (0–5 °C, and 30 min). The analyses were achieved in selected-ion monitoring for GC–MS (*m/z*, 235,250,73 propofol"; *m/z*, 252,267,27 propofol-d17) and in multiple reaction monitoring ($[M-H]^-$: *m/z* 283→241,77, azo-propofol; *m/z* 299→251,77, azo-propofol-d17) for LC–MS/MS. Autopsy showed no significant findings. Propofol concentrations were (LC–MS/MS vs GC–MS, respectively): 15.1 vs 14.5 mg/ml, drip content; 7.11 vs 6.07 μg/ml, cardiac blood; 9.50 vs 7.19 μg/ml, femoral blood; 0.64 vs 1.07 μg/ml, bile; 0.042 vs 0.051 μg/ml urine; 4.93 vs 5.89 μg/g, brain; and 7.88 vs 6.80 μg/g, liver. These values are comparable with the ones described in literature for death by acute propofol intoxication; the drip content is compatible with a diluted formulation of propofol available in Italy (20 mg/ml injectable emulsion). The comparison shows an excellent fitting of the data (R^2: 0.9362). Toxicological results proved the cause of death as acute propofol intoxication. Furthermore, the new LC–MS/MS method showed an excellent effectiveness and reliability when compared to the routinely used GC–MS method.

1. Introduction

Propofol (2,6-diisopropylphenol) is a short-acting intravenous hypnotic-amnesiac agent and is extensively used for the induction and maintenance of general anesthesia [1–4]. It is widely used in surgical procedures because of its rapid onset, low toxicity, and long duration of narcotic effects [5–7]. However, hypotension, cardiac arrhythmia, apnea, and respiratory failures are the main side effects [8–10]. Since other side effects includes euphoria, sexual hallucinations, and disinhibition [11–14], its recreational use is recently increased fueling the debate about its potential of abuse and dependence. In recent years, many cases of addiction and deaths have been reported (just as the death of the performer Michael Jackson), providing evidences about the risks of propofol

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use/abuse out of medical control [12,15–17]. Accordingly, Korea has been the first country to classify propofol as psychotropic agent in 2011. Thus, the spread of this substance for recreational or suicide purposes, and the related deaths, has been a great impulse for forensic toxicologists to validate new and more sensitive analytical procedures. Gas chromatography-mass spectrometry (GC–MS) is the most used technique for propofol detection due to its high separation capacity and detection sensitivity toward volatile compounds [18–21]. In particular, the headspace GC–MS is the simplest and fastest analysis (very low preparation time). Anyway, novel methods have been described for liquid chromatography–tandem mass spectrometry (LC–MS/MS), especially for phase II metabolites [2,22,23] as marker of propofol administration. Direct determination by means of LC–MS/MS systems is affected by the low ionization efficiency (IE) of propofol since its nonpolar nature and the lack of group is easy to ionize in electrospray ion source (ESI). Chemical derivatizations may be a useful solution to overcome this hurdle. Introduction of ionizable moieties such as charged (i.e., quaternary ammonium) or chargeable groups [24–26], may increase the IE and, moreover, may provide specific fragmentations by collision-induced dissociation (CID), making easier and more effective the analyte detection. Propofol derivatization methods are exclusively focused on the hydroxyl group [27,28]. With a previous paper [29], we were the first to shift attention to the phenolic ring taking advantage of its reactivity toward the electrophilic aromatic substitution (EAS). In that study, we presented a new derivatization procedure for LC–MS/MS detection of propofol in urine and blood by means of the EAS with aryl-diazonium salt (ArN_2^+). The reaction is known as azo-coupling (AC) reaction (Fig. 1) and the derivative (molecular composition: $C_{18}H_{22}ON_2$ and molecular weight: 282 Da) presents an azo-group (–N=N–) in *para* position. This moiety provides not only an increase in ionizability, both in negative (NIM) and positive ion modes (PIM) in ESI, but also specific fragmentation pathways (Figs. 2 and 3). The two methods were widely validated and were more sensitive than the ones described in literature (up to 200-fold than the analysis with dansyl derivatization [27] in blood and 6000-fold than detection of propofol-glucuronide in urine [20]). In this study, the new LC–MS/MS analysis was compared to the routine method in GC–MS, in order to investigate its effectiveness and reliability. Therefore, several parameters, such as time consuming, analytical procedure, quantitative findings, and their statistical processing, were evaluated. Analyses were performed on real specimens (femoral and cardiac blood, bile, urine, brain, liver, and drip content) collected from a case of suicide by propofol intoxication.

In addition, toxicological interpretation about the postmortem concentrations and the cause of death were discussed.

2. Material and methods

2.1. Chemical and reagents

Dichloromethane (DCM), diethyl ether (Et_2O), and methanol (MeOH) were purchased from Panreac Quimica S.L.U. (Castellar del Vallès, Spain). Ethyl acetate (AcOEt), sodium acetate, glacial acetic acid, hydrochloric acid (HCl), sodium nitrite ($NaNO_2$), formic acid, and n-hexane (Hex) were obtained from J.T. Baker (Deventen, Netherlands). Sodium hydroxide (NaOH) was supplied by Carlo Erba Reagenti (Milano, Italy). LC–MS CHROMASOLV® MeOH was purchased from Sigma-Aldrich (St. Louis, MO, USA). Sterile water for injection (H_2O) was obtained from B. Braun (Milano, Italy). Aniline was acquired from Riedel-de Haën (Seelze, Germany). *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and Tetramethylammonium hydroxide (TMAH) were provided by Supelco (Bellefonte, PA, USA). Propofol and propofol-d17 (internal standard, IS) methanolic standards were purchased from Chemical Research 2000 s.r.l. (Rome, Italy) and diluted to the appropriate concentrations with MeOH.

2.2. Case presentation

A 61-year-old man was found dead by his son in the house where he lived. The corpse was on the bed with an almost empty 100 ml drip (labeled "NaCl 0.9%") still inserted, with an intravenous tube, in a vessel on the elbow of the left arm. The drip content was about 5 ml of a lactescent liquid. The subject saved on his computer a farewell letter explaining the reasons for his suicide. Autopsy did not reveal any remarkable findings. Femoral and cardiac blood, bile, urine, brain, liver, and drip content samples were collected and sent to our laboratory for the toxicological analysis.

2.3. Sample preparation

2.3.1. Drip content

A total of 10 μl of drip content was added with 20 μl of phosphate buffer (pH 8), 10 μl of a 10 μg/μl solution of IS and then a liquid-liquid extraction (LLE) was performed with 100 μl of a DCM/AcOEt 8:2 (v:v) mixture.

2.3.2. Biological samples

A protein precipitation was achieved on 1 ml of blood specimens (cardiac and femoral) with 2 ml of MeOH in presence of 100 ng of IS.

Since propofol is extensively metabolized (especially to glucuronide-conjugated form) an acidic hydrolysis was performed on 1 ml or 1 g of the other biological specimens (bile, urine, brain, and liver) by adding 300 μl HCl 6 M and 10 μl of IS (50 ng/μl) at 100 °C for 1 h. After cooling at room temperature, the mixture was neutralized with 300 μl of NaOH 6 M and 20 μl of phosphate buffer (pH 8) were then added. A LLE was achieved with 3 ml of a DCM/AcOEt 8:2 (v:v) mixture.

2.4. Derivatization

2.4.1. Silylation for GC–MS

Organic layers from LLE and 100 μl of supernatant from deproteinated blood were added with 20 μl of TMAH and then dried under gentle stream of nitrogen (N_2) at 40 °C. The residue was incubated at 80 °C for 15 min with 50 μl of BSTFA. A total of 1 μl was injected in GC–MS system.

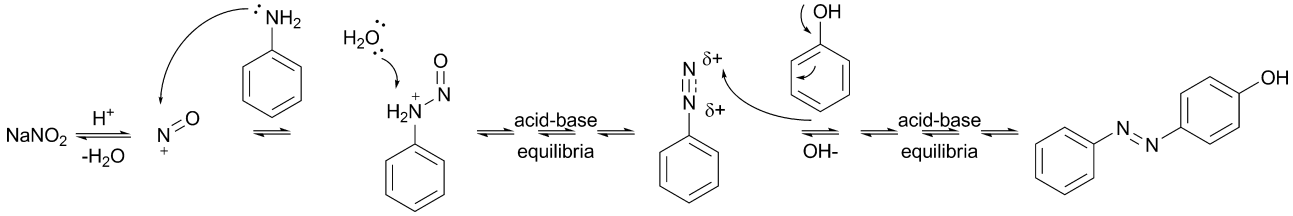

Fig. 1. Mechanism for the azo-coupling reaction.