Journal of Pharmaceutical and Biomedical Analysis 85 (2013) 33–39

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Rapid and sensitive determination of propofol glucuronide in hair by liquid chromatography and tandem mass spectrometry

Hee Seung Kim*, Jae Chul Cheong, Jae Il Lee, Moon Kyo In

Forensic Chemistry Laboratory, Forensic Science Division, Supreme Prosecutors' Office, Seoul, Republic of Korea

ARTICLE INFO

Article history:
Received 9 May 2013
Received in revised form 12 June 2013
Accepted 18 June 2013
Available online 3 July 2013

Keywords:
Propofol glucuronide
Human hair
LC–MS/MS
Micro pulverization
Methanol extraction

ABSTRACT

A fast, sensitive and selective liquid chromatography–tandem mass spectrometry (LC–MS/MS) method for the detection and quantitation of propofol glucuronide in human hair has been developed and validated. Propofol glucuronide was extracted from 10 mg of hair using a simple methanol extraction method, with recovery greater than 91% at 3 quality control samples (15, 100, 4000 pg/mg). A reversed phase column (C_8) was used to analyze and the mobile phase was composed of ammonium formate and acetonitrile gradient at a flow rate of 0.2 mL/min. The lower limit of quantitation (LLOQ) was 5 pg/mg and the assay was linear to 5000 pg/mg. The intra- and inter-day precision (% CV, coefficient of variation) ranged from 1.26 to 4.50% while the accuracy (% RE, relative error) were -4.24 to 4.4%. The matrix effects were monitored at 3 different concentrations and the %CV of the results for these concentrations was less than 10.6%. Propofol glucuronide was stable during processing and analysis in human hair. The procedure was validated and applied to the analysis of hair samples in human subjects previously administered in propofol.

1. Introduction

Propofol, 2,6-diisopropylphenol, is an intravenous short-acting sedative hypnotic agent used for the induction and maintenance of general anesthesia. It is given as a single bolus injection or continuous infusion to induce anesthesia as well as intermittent bolus injection to achieve a desired level of sedation. Propofol is one of the most widely used anesthetic agent in the world and it was the preferred anesthetic induction agent in 96.5% of ambulatory urologic and orthopedic surgical cases in the United Kingdom in 2000 and 50% of patients undergoing cardiac surgery involved propofol as an anesthetic agent in French survey [1].

The abuse potential of propofol gained worldwide attention after the tragic death of a high profile popular singer Michael Jackson during the summer of 2009. Since then, numerous accidental death and abuse cases involving propofol were reported throughout the world. A group potentially abusing propofol is medical professionals. A survey of propofol abuse in academic anesthesia programs conducted by the Journal, Anesthesia & Analgesia suggests that one or more incidents of propofol abuse or diversion in the past 10 years were reported by 18% of department participated in the survey and propofol abuse among doctors and nurses increased 500% over 10 years [2]. Furthermore, of the 25 reported individuals abusing propofol, 7 died as a result of the propofol abuse, 6 of whom were residents [2].

Despite the significant potential for abuse and dependency as well as the recent recognition in recreational purpose, propofol is not scheduled as a controlled substance by the United States Drug Enforcement Administration (USDEA). On the other hand, Korean Food and Drug Administration (KFDA) has classified propofol as a controlled substance in 2011 after numerous cases of accidental death and overdose involving propofol were reported [3,4]. There have been numerous recommendations among medical professionals that the USDEA and other international agencies should consider regulating propofol as a controlled substance [5].

Propofol is rapidly metabolized to glucuronide and sulfate adducts in the liver and excreted as inactive by the kidneys. The structure of propofol glucuronide is illustrated in Fig. 1. The plasma half-life of propofol is between 2 and 4 min in initial distribution phase while the slow redistribution half-life is between 30 and 60 min [6]. The terminal elimination half-life is between 3 and 12 h and may increase with prolonged use [6]. This is due to the slow return of propofol from the deep compartment but does not contribute significantly to the clinical effect [1]. The active parent drug found in the urine is less than 1% while approximately 2% remained as unchanged form in feces [6].

The quantitation of propofol and its main metabolite propofol glucuronide in biological matrices such as blood, plasma or hair has been reported numerous cases and commonly employs gas

* Corresponding author at: Banpo Dae Ro 157, SeoCho Gu, Seoul, Republic of Korea. Tel.: +822 535 4173.
E-mail addresses: hskjay@spo.go.kr, hskjay@hotmail.com (H.S. Kim).

0731-7085/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jpba.2013.06.027

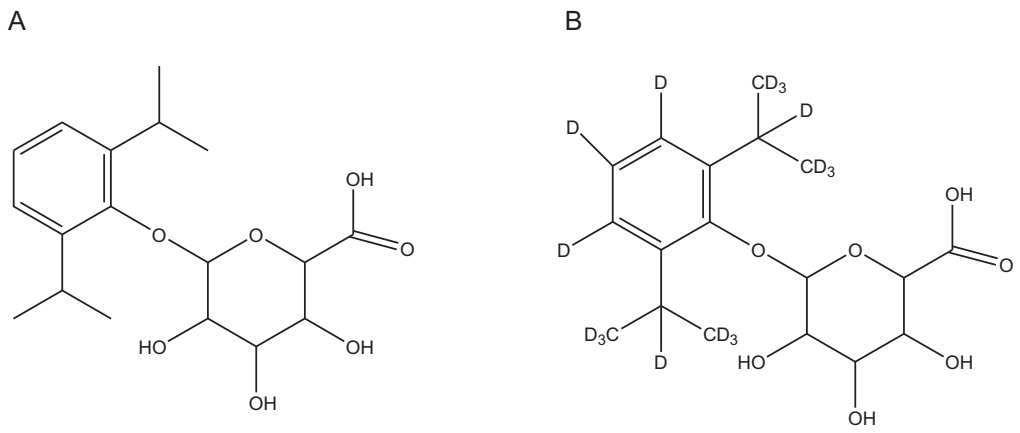

Fig. 1. Structures of propofol glucuronide (A) and deuterated (d_{17}) propofol glucuronide.

chromatography coupled with mass spectrometry (GC–MS) and liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) [7–10]. Although these methods were successfully used for determining propofol and propofol glucuronide from biological matrices, the lowest amounts determined in these reports are not suitable for high sensitive forensic applications. Furthermore these methods employed time consuming derivatization steps or intensive sample preparation steps involving liquid or solid phase extractions [7–10].

In this study, a highly sensitive and rugged LC–MS/MS method for determination of propofol glucuronide in human hair is developed and validated for the first time. The applicability of this method in assessing propofol in human hair from suspicious illegal propofol users is described.

2. Materials and methods

2.1. Chemicals and reagents

Propofol glucuronide (99.5% purity in lithium salt form) was purchased from Synfine Research (Ontario, Canada) while propofol glucuronide d_{17} was from Toronto Research Chemical (Toronto, Canada). Ammonium formate and HPLC grade methanol were obtained from Sigma Aldrich (St. Louis, MO). Nylon syringe filters (13 mm in I.D, and 0.2 μm in pore size) are purchased from Whatman (Piscataway, NJ). All other reagents were HPLC or better grade and purchased from Fisher Scientific (Pittsburgh, PA).

2.2. Instrumentation

Analysis was performed on an API 4000 triple-quadrupole mass spectrometer (Applied Biosystems/MDS Sciex, Foster City, CA) equipped with a Turbo Ion Spray ionization source operated at 550 °C. The HPLC system consisted of an Agilent 1290 binary pump, an Agilent 1290 autosampler and an Agilent 1290 column oven (Agilent, Wilmington, DE). The chromatographic separation was performed on an XTerra C_8 column (150 mm × 2.0 mm, 3.0 μm) (Waters Corp, Milford, MA). The injection volume was 10 μL and the oven temperature was 25 °C. Before each injection, the needle was washed with methanol:water (50:50, v/v). Mobile phase (0.2 mL/min) was (A) 10 mM ammonium formate and (B) acetonitrile using the following gradient program: 5% B for 1.5 min, linear gradient to 90% B between 1.5 and 8.0 min, then re-equilibrated to initial conditions between 8.01 and 12 min. Under these conditions, retention times for propofol glucuronide and propofol glucuronide-d_{17} were 6.58 and 6.54 min, respectively (Table 1). Both Q1 and Q3 quadrupoles were optimized to unit mass resolution, and the mass spectrometer conditions were optimized for each analyte. The instrument was operated in negative-ion mode with an ion spray voltage of −4500 V. The curtain gas was set at 25, ion source gas 1 at 40, ion source gas 2 at 50 and the collision gas was set at 9. Multiple reaction monitoring (MRM) transitions monitored for each analyte, along with the analyte-specific parameters, are listed in Table 1.

2.3. Standards and quality control samples

Stock solutions at 0.1 mg/mL (free base) were prepared in methanol for each analyte, propofol glucuronide and propofol glucuronide-d_{17}. Dilutions from these stock standards were prepared and used to make standard and quality control (QC) samples in hair. The concentrations of working standards and QC samples are followed, 0.5, 1, 5, 20, 100, 400 and 500 ng/mL in methanol for working standard solutions and 1.5, 10 and 400 ng/mL for working QC solutions. Each standard (100 μL) was spiked onto an Eppendorf tube containing 10 mg of pulverized hair and these gave hair standards containing 5, 10, 50, 200, 1000, 4000 and 5000 pg/mg of propofol glucuronide. Similarly, hair QC samples were prepared at 15, 100 and 4000 pg/mg. Internal standard, propofol glucuronide-d_{17}, solution was prepared at 10 ng/mL in methanol.

2.4. Hair analysis

Drug-free hair was obtained from a 41-year-old male volunteer and used as the blank matrix. Hair samples from suspicious propofol users were obtained from the Narcotics Department at Seoul District Prosecution Service and these samples were generally pulled out or cut as close as possible to the skin from the posterior vertex.

Sample extraction was performed with methanol. Briefly, hair samples, approximately 10 mg, were vortex washed twice with ultra pure water and acetone. After air drying, hair samples were transferred to 2.0 mL Eppendorf tube. Hair samples were micropulverized with 4 steel beads using a Tissuelyzer II (Qiagen, Valencia, CA) at the frequency setting of 30 for 5 min. Methanol (1.0 mL) and internal standard (50 μL) were added to pulverized hair samples and vortexed for 5 s. Then, the mixture was sonicated for 60 min at 50 °C using a Branson 8510 ultrasonicator (Danbury, CT) for extraction. Samples were then filtered through nylon syringe filters and dried under air stream at 40 °C using a TurboVap LV (Biotage, Uppsala, Sweden). Dried samples were then reconstituted with 100 μL of methanol and kept at 4 °C until analysis.