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Determination of propofol glucuronide from hair sample by using mixed mode anion exchange cartridge and liquid chromatography tandem mass spectrometry

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ABSTRACT

The main objective of this study was to develop and validate a simpler and less time consuming analytical method for determination of propofol glucuronide from hair sample, by using mixed mode anion exchange cartridge and liquid chromatography-tandem mass spectrometry (LC–MS/MS). The study uses propofol glucuronide, a major metabolite of propofol, as a marker for propofol abuse. The hair sample was digested in sodium hydroxide solution and loaded in mixed-mode anion cartridge for solid phase extraction. Water and ethyl acetate were used as washing solvents to remove interfering substances from the hair sample. Consequently, 2% formic acid in ethyl acetate was employed to elute propofol glucuronide from the sorbent of mixed-mode anion cartridge, and analyzed by LC–MS/MS. The method validation parameters such as selectivity, specificity, LOD, LLOQ, accuracy, precision, recovery, and matrix effect were also tested. The linearity of calibration curves showed good correlation, with correlation coefficient 0.998. The LOD and LLOQ of the propofol glucuronide were 0.2 pg/mg and 0.5 pg/mg, respectively. The intra and inter-day precision and accuracy were acceptable within 15%. The mean values of recovery and matrix effect were in the range of 91.7–98.7% and 87.5–90.3%, respectively, signifying that the sample preparation, washing and extraction procedure were efficient, and there was low significant hair matrix effect for the extraction of propofol glucuronide from hair sample on the mixed mode anion cartridge. To evaluate the suitability of method, the hair of propofol administered rat was successfully analyzed with this method.

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1. Introduction

Propofol (2, 6-diisopropylphenol) is an intravenously administered hypnotic drug, widely used for both induction and maintenance of anesthesia for its short but rapid effect [1,2]. Propofol is mainly metabolized by either direct conjugation leading to propofol glucuronide, or *p*-hydroxylation with subsequent glucuronidation (or sulphation) [3]. Studies suggest, about 60% of a single dose is excreted within 5 days as the 1- and 4-glucuronide and 4-sultate conjugates of 2.6-diisopropyl-1, 4-quinol, while the remainder dose consisted of propofol glucuronide; without any traces of free propofol [3,4]. Studies report increasing abuse of propofol, and gained worldwide attention after the death of Michael Jackson, King of Pop (1958–2009) [5]. Yet, its potential has not been defined completely.

United States Drug Enforcement Administration (USDEA) has not classified propofol as a controlled substance [6]. On the other hand, Korea Food and Drug Administration (KFDA) classified the drug as a controlled substance, in 2011, after several cases of overdosing and accidental deaths were reported [2,7,8]. The medical professionals were highly potential towards the propofol abuse [9,10]. During 2000–2011, the National Forensic Service (NFS) of Korea reported 36 propofol related fatal cases in South Korea—20 cases of suicidal or accidental death of medical staffs and 16 cases of medical accident at normal therapeutic dosage. The report raises the level of precaution to be undertaken with the use of propofol [8,11]. Technically, it also alerts the need of highly sensitive analytical methods, for evaluating the metabolism of propofol and its forensic application.

Various analytical methods, such as high performance liquid chromatography (HPLC) with UV, fluorescence or electrochemical detection, gas chromatography–mass spectrometry (GC–MS) and liquid chromatography tandem mass spectrometry (LC–MS/MS), have been used for quantification of propofol and its main metabo-

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lites (propofol glucuronide) in a variety of biological samples [12], such as plasma, blood, urine or hair [5,12–14]. As propofol is rapidly metabolized and eliminated from the body, it is tricky to confirm its quantity, over time, in the biological samples. Furthermore, propofol does not get easily ionized, enough to be detected in a hair in electro-spray ionization (ESI) mass mode, resulting narrow linear-concentration range and poor reproducibility during LC–MS/MS analysis. Because of rapid metabolism and poor ionization of propofol, propofol glucuronide (a major metabolite of propofol) has been widely used as a biomarker for propofol administration. Also, studies report analysis of propofol glucuronide, instead of propofol, in the biological samples [5,12]. Hair has been preferred over other biological samples, to determine propofol glucuronide, for possessing longer preservation time. Therefore, this study aims to develop analytical method to determine propofol glucuronide, in hair.

The proteins and lipids present in hair are likely to interfere propofol glucuronide extraction processes, thus, the extraction processes need to be highly efficient. Some researchers have used water and methanol as washing and extraction solvents, respectively, with incubation time of about 16 h [12]. But, in this study, 1 M sodium hydroxide has been used for hair digestion, as sodium hydroxide can dissolve hair completely by disrupting protein disulfide bonds of proteins within an hour. During such digestion, propofol glucuronide gets dissociated and changes to negatively charged form ($pKa = 3$ to 4). On loading the sample, negatively charged propofol glucuronide binds to positive ionic sites of mixed mode exchanger. The strength of this ionic bond is strong and can withstand the washing with organic solvent. Because of these selective but powerful bindings between propofol glucuronide and cartridge sorbent, several interfering substances from the hair are removed facilitating elution of propofol glucuronide, selectively. This sensitive and improved method for determination of propofol glucuronide in hair, by using mixed mode anion exchange cartridge, has been validated by comparing with liquid-liquid extraction. To further ensure the suitability of method, authentic hair samples of rats have been applied.

2. Materials and methods

2.1. Reagents and chemicals

Propofol glucuronide, naproxen (internal standard), formic acid, sodium hydroxide and ammonium formate were purchased from Sigma (St. Louis, MO, USA). Methanol, ethyl acetate and acetonitrile were obtained from Burdick and Jackson (Muskegon, MI, USA). A 0.22 μm PVDF microporous membrane filter was purchased from Millipore (Billerica, MA). Water was purified using a Millipore (Chem-science, USA) purification system. Oasis MAX® (3 mL, 60 mg) cartridges were purchased from Waters (Milford, MA, USA).

2.2. Sample preparation

The hair sample was washed with distilled water and methanol, 2 mL each, to remove the external contamination, and then cut into small pieces measuring less than 2 mm 1 mL of 1 M sodium hydroxide solution, 50 μL of internal standard (10 ng/mL) and calibration standards (0.2, 0.4, 2, 4, 20, 40, and 200 ng/mL) were added to the 10 mL glass tube containing 20 mg of hair. The sample was then incubated at 90 °C for 1 h, so that the hair gets digested completely. The digested solution was cooled at room temperature for 10 min and transferred to 2 mL microtube, and centrifuged at 15,000*g* for 10 min. The supernatant was collected and stored at 4 °C, until extraction.

2.3. Solid phase extraction (SPE)

An Oasis MAX® extraction cartridge was preconditioned with methanol and distilled water, 2 mL each, successively. The hair sample treated with sodium hydroxide was loaded to the SPE column. The cartridge was washed with 0.01 M sodium hydroxide solution and ethyl acetate, 2 mL each, and dried for 5 min under reduced pressure. The elution was carried out with 2 mL of 2% formic acid in ethyl acetate, and evaporated to dryness under gentle stream of nitrogen. The residue was reconstituted with 100 μL of methanol and filtered through 0.22 μm PVDF microporous membrane. The filtrate was then transferred to a vial, placed in the autosampler and 10 μL was injected into the LC–MS/MS.

2.4. Liquid liquid extraction (LLE)

To evaluate the total area of LLE chromatograms, 5 blank hair samples including 20 mg hair were prepared by treating with 1 M sodium hydroxide, and extracted with 2 mL of ethyl acetate for 20 min using vortex. The extracted ethyl acetate layer was evaporated to dryness under gentle stream of nitrogen. 100 μL of methanol was added to the residue and filtered through 0.22 μm PVDF microporous membrane. The solution was transferred to a vial, and 10 μL was injected into the LC–MS/MS.

2.5. LC–MS/MS conditions

The LC–MS/MS analysis was carried out on Agilent 1290 infinity UHPLC (Agilent Technologies, CA, USA) and AB Sciex Qtrap® 4000 MS/MS (AB Sciex, MA, USA). The propofol glucuronide and internal standard naproxen were separated on Zorbax Eclipse Plus C18 (2.1 × 100 mm, 1.8 μm) column. The temperature of column and auto-sampler were maintained at 40 °C and 4 °C, respectively. The mobile phases used were 2 mM ammonium formate containing 0.2% formic acid, in water (A) and in acetonitrile (B). The gradient system used was maintained at 90% 'A' at a flow rate of 0.3 mL/min and decreased to 5% over a period of 1–6 min, where it was maintained until 8 min. The initial condition of 90% 'A' was then restored from 8.1 to 10 min.

The instrument was operated in negative-ion mode with ESI. The optimum conditions were curtain gas, 20 psi; collision activated dissociation (CAD), medium; heated nebulizer temperature, 650 °C; nebulizing gas (GS1) 50 psi; and heater gas (GS2), 50 psi. The multiple reactions monitoring (MRM) was performed on propofol glucuronide and naproxen. The optimized value (declustering potential, entrance potential, collision energy and collision cell exit potential) of each compounds, MRM transitions and retention time have been shown in Table 1.

Enhanced product ion scan mode (50–500 amu) was employed to evaluate the total peak area of LLE chromatogram and cartridge extraction method. Data were processed using the Analyst® 1.6 software (AB Sciex, USA).

2.6. Method validation

Analytical method developed in this study was validated for linearity, intra- and inter-day precision, accuracy, recovery, matrix effects and process efficiency. For calibration curves, blank hair solution treated with 1 M sodium hydroxide was spiked with standard propofol glucuronide concentrations (0.5, 1, 5, 10, 50, 100, and 500 pg/mg) and its linearity was tested by plotting calibration curve. The acceptance criteria for correlation coefficient was at least 0.990. Hair samples spiked with decreasing concentrations of the propofol glucuronide were analyzed to determine limit of detection (LOD). The lower limit of quantification (LLOQ) was established as the lowest calibration standard, with accuracy and precision less

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