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Comparison of GC/MS and LC/MS methods for the analysis of propofol and its metabolites in urine

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ABSTRACT

Gas chromatography–mass spectrometry (GC/MS) and liquid chromatography–mass spectrometry (LC/MS) were compared for their capacity to metabolite identification, sensitivity, and speed of analysis for propofol and its metabolites in urine samples. Acidic hydrolysis, liquid–liquid extraction (LLE), and trimethylsilyl (TMS) derivatization procedures were applied for GC/MS analysis. The LC/MS analysis used a simple sample pretreatment based on centrifugation and dilution. Propofol and four metabolites were successfully analyzed by GC/MS following TMS derivatization. One compound, *di*-isopropanolphenol was tentatively characterized as a new metabolite observed for the first time in human urine. The TMS derivatization greatly improved the chromatographic properties and detection sensitivity, especially for hydroxylated metabolites. The lower limits of quantitation (LLOQ) of propofol were about 325 and 0.51 ng/mL for the GC/MS scan mode and selected ion monitoring (SIM) mode, respectively. In addition, five conjugated propofol metabolites were successfully analyzed by LC–MS/MS in negative ion mode. The detection sensitivity for these conjugated metabolites could be greatly enhanced by the addition of triethylamine to the mobile phase without any loss of LC resolution capacity. The LLOQs of propofol-glucuronide (PG) were about 1.17 and 2.01 ng/mL for the LC–MS-selected ion monitoring (SIM) and multiple reaction monitoring (MRM) mode, respectively. Both GC/MS and LC/MS methods sensitively detected nine metabolites of propofol and could be used to provide complementary data for the reasonable propofol metabolism study. Urinary excretion profiles for propofol and its metabolites following administration to human were suggested based on the total ion chromatograms obtained by GC/MS and LC/MS methods, respectively.

1. Introduction

Propofol, an intravenous agent, has been widely used for the induction and maintenance of anesthesia due to its short and rapid effects [1–3]. However, owing to the high lipophilicity of propofol, infusion at high-doses can lead to hypertriglyceridemia and subsequent hypotension, bradycardia, and transient apnea [1,4]. The drug can induce cardiorespiratory depression, if used for prolonged durations, resulting in deadly consequences [4]. The risk of acute intoxication and potentially fatal human poisoning is high when propofol is overdosed [5]. Recently, this drug has entered the public domain *via* underground routes and has shown an increase in recreational use due to its strong and fast narcotic effect. Highly sensitive analytical methods therefore need to be developed for drug metabolism and forensic applications.

Propofol is excreted mainly in the urine after glucuronidation of parent drug and sulfato-and glucuro-conjugation of its hydroxylated metabolites [6]. Previous reports [7,8] have identified several metabolites in human: 4-(2,6-diisopropyl-1,4-quinol) (quinol), 4-(2,6-diisopropyl-1,4-quinol) sulfate (4-QS), 1-(2,6-diisopropyl-1,4-quinol) glucuronide (1-QG), 4-(2,6-diisopropyl-1,4-quinol) glucuronide (4-QG) and propofol-glucuronide (PG) [8–10]. Occasionally, propofol was metabolized into minor compounds as x-((2-(ω-propanol)-6-isoproyl-phenol) glucuronide) (x-2-ω-PG) and y-((2-(ω-propanol)-6-isopropyl-phenol) glucuronide) (y-2-ω-PG). Besides these metabolites, a few minor metabolites may still remain to be identified in biological samples due to their trace amounts in samples and the lack of authentic standards.

Various analytical methods have been reported for the determination of propofol and its metabolites in biological matrices such

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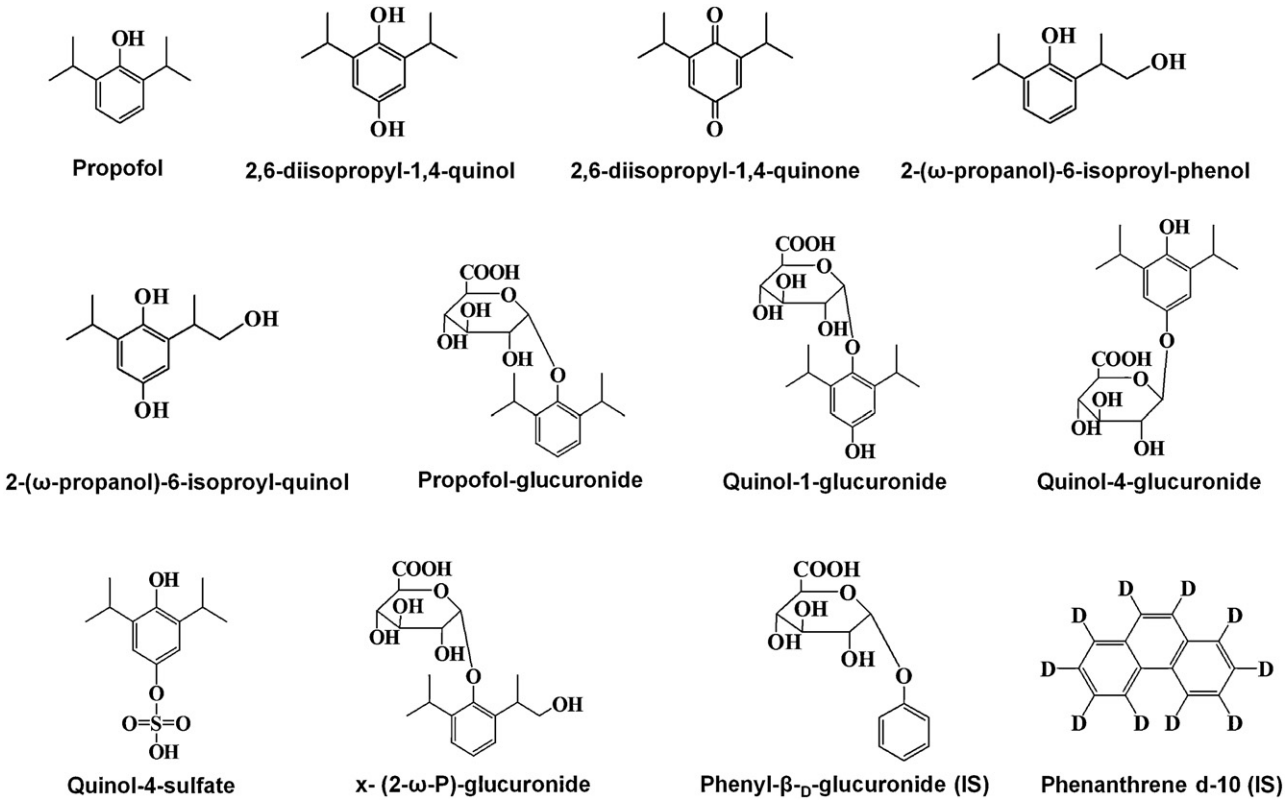


Fig. 1. Chemical structures of propofol and its metabolites present in human urine and of internal standards used in the present study.

as blood [9–11], urine [8,12–14] and hair [15] including the use of high performance liquid chromatography (HPLC) with UV detection [4,7], and gas chromatography coupled mass spectrometry (GC/MS) [14,15]. In general, GC/MS methods have been used for the determination of propofol and its metabolites on account of the high separation capacity and detection sensitivity of the volatile compounds, even though intensive sample workup such as extraction and derivatization is required [14,16]. Significant enhancements of the chromatographic properties and detection sensitivity of phenolic compounds have been reported following trimethylsilyl (TMS) derivatization [17,18]. However, GC/MS method cannot provide detailed information regarding phase II metabolites due to their incompatibility with GC/MS analysis.

The analysis of propofol metabolites in biological samples by LC/MS with either electrospray (ESI) [8,9,11,19] or atmospheric pressure chemical ionization (APCI) [20,21] has become increasingly popular in recent years. Improvement in LC/MS detection sensitivity for propofol in biological samples have also been obtained by chemical derivatization methods [11,19]. For LC/MS analysis, several sample preparation methods including centrifugation [7,21–23], liquid–liquid extraction (LLE) [12,22] and solid-phase extraction (SPE) [4,8,9] have frequently been used. At present, simple sample preparation [7,14,21] based on the centrifugation and dilution also is typically applied for convenient extraction of analytes from biological samples. Phase II propofol metabolites have been successfully determined by LC-ESI-MS/MS in negative ion mode [8,9]; however, propofol and its phase I metabolites are difficult to protonate or deprotonated which complicates their detection by LC/MS in either positive or negative ion mode. Thus, comprehensive analytical methods need to be established for the determination of propofol and its metabolites for drug metabolism and forensic applications. Although the analysis of propofol and its metabolites has been performed using both GC/MS and LC/MS [14], no report has been detailed studied on the comparative performance of both analytical methods.

The aim of this study was to optimize GC/MS and LC/MS methods for the comprehensive analysis of propofol and its metabolites in urine to give complementary result. Both analytical methods were compared in terms of metabolite identification, sensitivity, and speed of analysis. The improvement of detection sensitivities for propofol and its metabolites has been described, and their EI-mass spectra and MS/MS spectra were also obtained, which enabled reliable metabolites identification. Urinary excretion profiles of propofol and its metabolites were successfully obtained by GC/MS and LC/MS methods, respectively.

2. Materials and methods

2.1. Reagents and chemicals

Phenyl-β-D-glucuronide used as an internal standard and propofol were purchased from Sigma (St. Louis, MO, USA). A major metabolite of propofol, propofol-β-D-glucuronide, was obtained from Richmond Hill (Ontario, Canada). Phenanthrene-d10 used as an internal standard for GC/MS analysis was obtained from Supelco (Bellefonte, PA, USA). Stock solutions (1000 μg/mL) of propofol, propofol-β-D-glucuronide, phenanthrene-d10, and phenyl-β-D-glucuronide were prepared in methanol and water, respectively. Standard solutions were diluted with methanol/water (1/1, v/v) as necessary. Other metabolites that were not commercially available were identified by interpretation of their EI mass spectra and LC–MS/MS spectra. Chemical structures of propofol and its metabolites are shown in Fig. 1.

Analytical grade organic solvents (methanol, acetonitrile) were supplied by J.T. Baker (Phillipsburg, NJ, USA). Ammonium acetate,

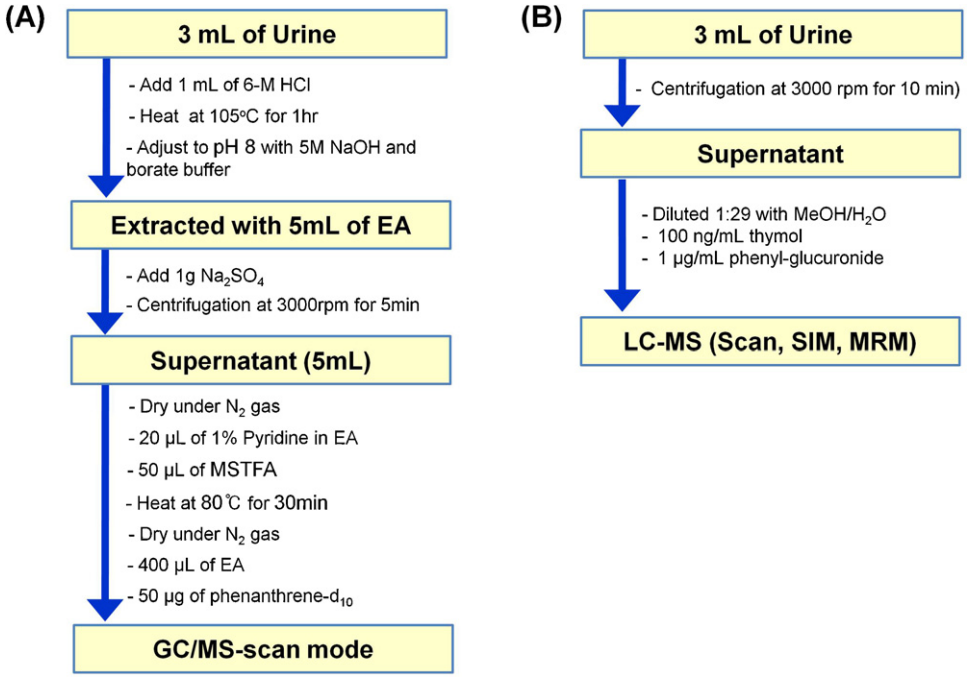


Fig. 2. Analytical GC/MS and LC/MS procedures for propofol and its metabolites in urine.

trimethylamine, and triethylamine were purchased from Sigma-Aldrich (Steinheim, Germany) and Acros Organics (Geel, Belgium). Water was purified using a Millipore (Chem-Science, USA) purification system and had a resistance greater than 18 Ω.

2.2. Sample preparation

Human urine samples obtained from three patients who were infused with propofol were provided by the Medical Center of Kyung Hee University. The dose was approximately 1 g of propofol delivered during a 4 h infusion. Urine samples were collected for the periods 0–6, 6–12, 12–18, 12–24, and 24–48 h after propofol administration and stored at −20 °C until analysis.

Urine samples (3 mL) used for GC/MS analysis were hydrolyzed with 1 mL 6M-HCl at 105 °C for 60 min. The reaction tubes were cooled to room temperature and then neutralized by addition of 5M-NaOH. The pH was then adjusted to pH 8 by addition of 0.2 mL borate buffer.

Propofol and its metabolites were extracted by adding 5 mL ethyl acetate to each hydrolyzed sample and shaking thoroughly for 5 min. The ethyl acetate layer was treated with 1 g Na_2SO_4 to remove trace water and then transferred to a glass tube and dried under a gentle nitrogen stream. The dried residue was resuspended in 20 μL 1% pyridine in ethyl acetate and derivatized with 50 μL *N*-methyl,*N*'-trimethylsilyltrifluoroacetamide (MSTFA) at 80 °C for 30 min. After cooling, the sample was diluted with 0.2 mL ethyl acetate and 30 μL (corresponding to 1.5 μg) phenanthrene-d10 used as an internal standard. One microliter of this solution was injected into the GC/MS.

Recovery was tested by spiking 3 mL of blank urine with 25 μg of propofol, adjusting the pH to 3, 6, 8, 9 and 11 with 6M-HCl or 5M-NaOH. An appropriate extraction solvent was found by testing the extraction efficiency with 5 mL of ethyl acetate, *n*-hexane, diethyl ether, and toluene. The hydrolysis test was performed by combining 1 μg of propofol-glucuronide with 3 mL of blank urine and 1 mL of 6M-HCl. The resulting solution was heated at 105 °C for 30, 45, 60, 90, and 120 min, respectively. Spiked urine samples were processed using these same procedures.

Urine samples (3 mL) for LC/MS analysis were measured into tubes by autopipette, and centrifuged at 3000 rpm for 10 min. The sample supernatant was transferred to a glass vial and diluted 1:29 with methanol/water (1:1, v/v) and then filtered through a membrane filter. Two microliters of the extracted solution were injected for analysis by LC-ESI/MS. The analytical methods for the analysis of propofol and its metabolites by GC/MS and LC/MS are depicted schematically in Fig. 2.

2.3. Gas chromatography–mass spectrometric conditions

The GC/MS analysis was performed with an Agilent 6890N gas chromatography instrument, combined with an Agilent-5973 mass spectrometer equipped with an electron ionization (EI) and quadrupole analyzer. Propofol and its metabolites were separated using a 5% phenyl dimethylpolysiloxane fused-silica capillary column (DB-5MS 30 m × 250 μm i.d., film thickness 0.25 μm, J&W Scientific, Folsom, CA, USA). The temperatures of the ion source and interface were set at 230 and 300 °C, respectively. A split injection (split ratio, 10:1) and injector temperature of 250 °C were employed and a mass scan range was from 50 to 550 amu. The electron energy was set at 70 eV. The oven temperature was programmed to hold at 80 °C for 5 min and then increase to 300 °C at a rate of 20 °C/min. Total runtime was 20 min.

2.4. Liquid chromatography tandem mass spectrometric conditions

Propofol and its metabolites were analyzed by an Agilent 1200 HPLC instrument (Agilent Technologies, Palo Alto, CA, USA) coupled directly to a triple quadrupole mass spectrometer (API 3200, MDS Sciex, Concord, ON, Canada). The chromatographic separation was performed at room temperature on Luna C18 column (Phenomenex, 150 mm length × 1.00 mm i.d.) with a particle size of 5 μm. The mobile phase consisted of 0.1 mM ammonium acetate and in water (solvent A) and methanol/acetonitrile (50/50, v/v) (solvent B), delivered at a flow rate of 50 μL/min. The gradient elution program was as follows: 9% B for 0.1 min, then 9–15% B for 4 min,

then 15–90% B for 14 min followed by an isocratic hold at 90% B for 7 min. At 25 min, B was returned to 9% over 10 min, and followed by an isocratic hold at 9% B for 25 min. The total run time for each injection was 60 min and the injection volume was 2 μL.

The mass spectrometer was operated in negative ion mode with a Turbo Ion Spray ionization source. Instrument control, data acquisition, and data analysis were performed by Analyst 1.5 software (Applied Biosystems/MDS Sciex). Nitrogen was used as the collision, desolvation, heating and nebulizing gas. The other ionization parameters were as follows: desolvation gas, 20 psi; nebulizing gas, 50 psi; heating gas, 50 psi; source temperature, 500 °; electron voltage, −4500 V; entrance potential, −10 V; collision cell exit potential, 15 V. The dwell time for each SIM transition was 150 ms.

3. Results and discussion

3.1. Sample preparation for GC/MS and LC/MS analysis

Urine samples used for GC/MS analysis required extensive sample pretreatment including acid hydrolysis, liquid–liquid extraction (LLE), and TMS derivatization. In order to hydrolyze the phase II conjugated metabolites, urine sample was applied acidic hydrolysis with 6M-HCl to cleave glucuronide and sulfate groups from the phase II conjugated metabolites. The optimizing reaction time for acid hydrolysis was studied over a time range of 30, 45, 60, 90, and 120 min for a PG spiked sample. As the hydrolysis time increased, the amount of propofol gradually increased up to 60 min, with no further increases after 90 min. Acid hydrolysis for 60 min resulted in almost complete cleavage of glucuronides based on quantitation of released propofol.

Extraction of propofol and its metabolites from urine sample was tested at various pH values (pH 3–11) and with several different organic solvents. The highest recovery yield of propofol was obtained at pH 8 where propofol ionization in aqueous solution is the lowest [24]. However, sufficient recovery of hydroxylated metabolites was obtained at pH 6.5–7.0 because these compounds had lower pK_a values than propofol. At pH 7–8, propofol and hydroxylated metabolites were successfully extracted from urine samples with about 90% recovery of propofol. The organic solvents used in this study all resulted in appropriate recovery of propofol but several impurities were also extracted in large amount. Ethyl acetate was deemed the most suitable solvent, based on its extraction yield and co-extraction of impurities.

Several extraction methods [4,8,9,14] including the SPE with various adsorbents or LLE methods have been used previously for the analysis of propofol and its metabolites by LC/MS. However, phase I and II metabolites of propofol could not be successfully extracted simultaneously by the SPE or LLE methods because of significant differences in the chemical properties between the lipophilic phase I metabolites including the parent drug and the hydrophilic phase II metabolites. Therefore, we used a previous reported sample treatment for LC/MS analysis [7,13,14] that relies on a very simple centrifugation and dilution. In the present study, we used a centrifugation and dilution in 1:29 methanol/water (1:1, v/v) which provided rapidity and convenience in the sample workup and showed the satisfactory extraction yields without any significant loss of analytes.

3.2. Analysis of propofol and its metabolites by GC/MS

Urine samples were analyzed for propofol by GC/MS using the established method shown in Fig. 3. The relative amounts of phase II conjugated metabolites were determined by comparing acid hydrolyzed and unhydrolyzed urine extracts. As shown in Fig. 3A and B, lower overall amounts of propofol and phase I metabolites were obtained in unhydrolyzed samples than in acid hydrolzed samples. The ratio of PG to propofol in urine collected for 6 hr was 1.63 based on the quantitation of propofol. Particularly, the unhydrolyzed extracts did not appear the metabolites of 2-ω-phenol and 2-ω-quinol, due to their presence at trace amount levels and poor sensitivity in GC/MS analysis. In contrast, the metabolites of 2-ω-phenol (peak 4) and 2-ω-quinol (peak 6) were observed in hydrolyzed extract, as shown in Fig. 3B. This observation indicated that most of these metabolites might be presented in urine as glucuronide or sulfate conjugated forms. A significant amount of quinone form was observed in the TIC of the hydrolyzed extract (Fig. 3B), even though this compound does not form a glucuronide conjugate, and quinone form was not detected in the TIC of unhydrolyzed extracts (Fig. 3A). In fact, quinone form is not a direct metabolite of propofol [25] but it can be formed by the tautomerization of quinol during the adjustment of neutralization after acid hydrolysis. This type of chemical conversion was inevitable during the pH adjustment with 5M-NaOH after acid hydrolysis.

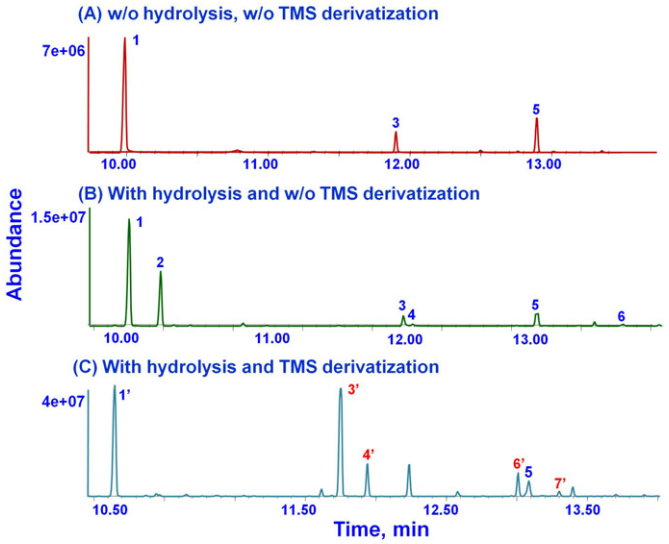


Fig. 3. Total ion chromatograms (TICs) of propofol and its metabolites extracted from urine (A) without hydrolysis, (B) with acid hydrolysis, and (C) with acid hydrolysis and trimethylsilylation obtained by GC/MS scan mode. Peak identities as follows: (1) propofol; (2) 1,4-quinone; (3) 1,4-quinol; (4) 2-ω-phenol; (5) IS (phenanthrene-d10); (6) 2-ω-quinol; (7) 2,6-*di*-isopropanol-phenol; and peak number' represents their corresponding TMS derivatives.

The detection sensitivity for propofol and its metabolites was compared with that of the TMS derivatives. The urine extracts were analyzed by dividing 2 groups; one was directly analyzed and the other was analyzed after TMS derivatization. Fig. 3B and C show TICs for underivatized and TMS derivatized extracts, respectively, obtained from a urine sample and analyzed using a DB-5MS column. The peaks for 2-ω-phenol and 2-ω-quinol, particular, showed tailing indicating their relatively strong interaction with the stationary phases (Fig. 3B). Adsorption of hydroxylated propofol metabolites to GC inlet and column surface was also noticeable and likely led to a reduction of detection sensitivity. On the other hand, all TMS derivatives of propofol and its metabolites in urine extract were successfully separated on the DB-5MS column (Fig. 3C). Their peak shapes were extremely sharp, and sensitivity was greater for the derivatized metabolites than for their free forms. The responses of TMS derivatives in GC/MS scan mode were greatly improved by approximately 1.5 times for propofol, 16.8 times for 1,4-quinol, 15.1 times for 2-ω-phenol, and 10.8 times for 2-ω-quinol. Especially, peak 7 depicted in Fig. 3C was considered to be a *di*-hydroxylated metabolites. This compound was not observed in extracts without TMS derivatization and was tentatively

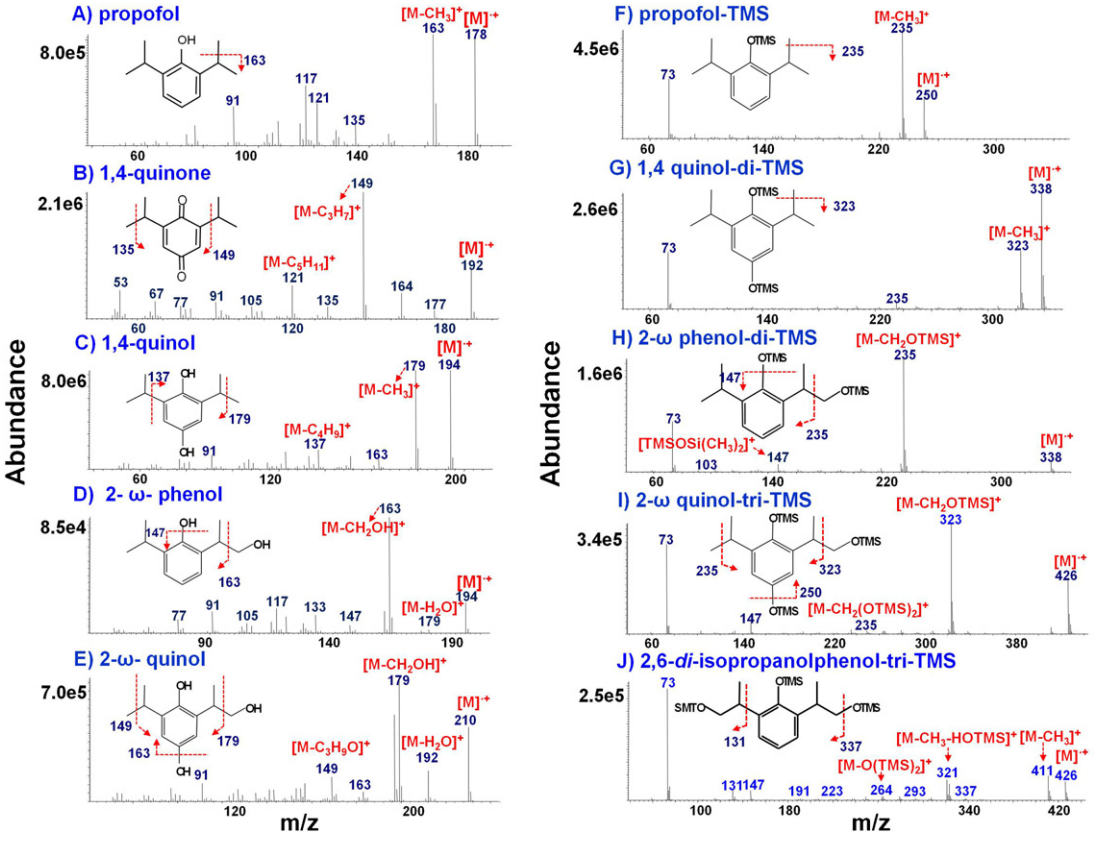


Fig. 4. EI-mass spectra of propofol and its metabolites as free forms and TMS-derivatives. (A) propofol, (B) 1,4-quinone, (C) 1,4-quinol, (D) 2-ω-phenol, (E) 2-ω-quinol, (F) propofol-TMS, (G) 1,4 quinol-di-TMS, (H) 2-ω-phenol-di-TMS, (I) 2-ω-quinol-tri-TMS, and (J) 2,6-*di*-isopropanol-phenol-tri-TMS.

characterized as 2,6-*di*-isopropanol-phenol-*tri*-TMS based on its molecular weight of 426 Da and its EI-mass spectrum. This is the first report of this particular metabolite. At the present time, the identification of this compound needs to be unambiguously confirmed.

No quinone form was seen in the TICs of TMS derivatized extracts. The reason for this might be that quinone was converted into quinol-$OTMS_2$ *via* an enolization during the TMS derivatization step. Actually, this type of conversion would be another advantage of TMS derivatization for better understanding of propofol metabolism and TMS derivatization should be a requirement for the study of propofol metabolism by GC/MS.

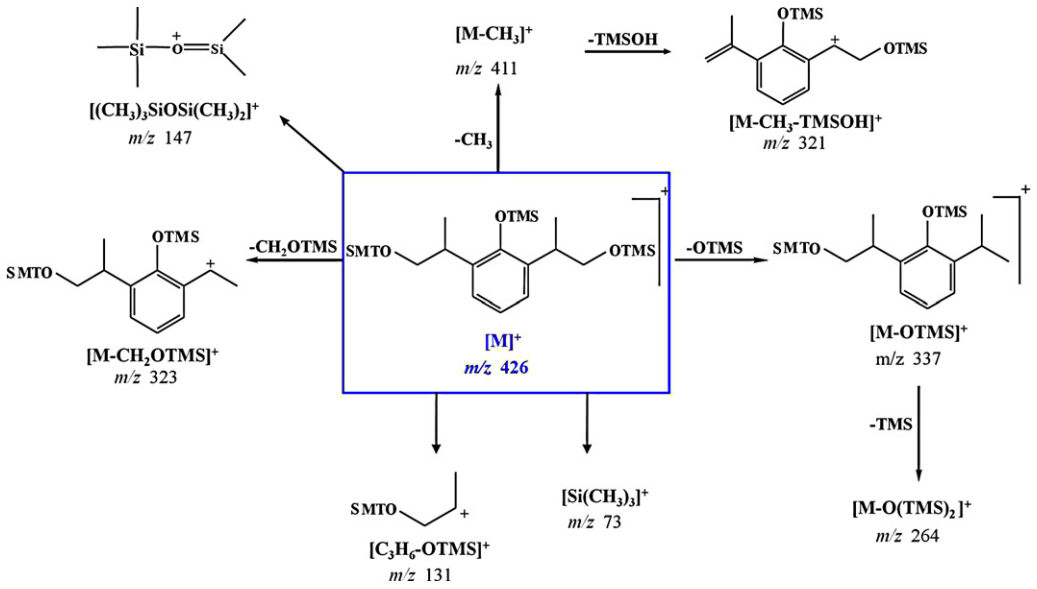
Several previous studies on various phenolic compounds showed that TMS derivatization was effective not only for mass spectral identification but also for improvement of chromatographic properties [17,18]. Derivatization was also expected to suppress adsorption of phenolic compounds to the GC inlet and column surface, because this adsorption of phenolic compounds is due to their polar hydroxyl moieties. Derivatization has been examined according to the conventional method with MSTFA [26]. However, propofol and its hydroxylated metabolites could not be successfully derivatized with MSTFA alone most likely because of steric hindrance and the lower nucleophilicity of hydroxyl groups due to the *di*-isopropyl groups at the *ortho* position. In the present study, we overcame these problems by adding pyridine as a base. Preliminary tests indicated that 1% pyridine in ethyl acetate was suitable. The TMS reaction temperature and time were also optimized to give the final derivatization procedure described in the Section 2.

The EI-mass spectra of free forms of propofol and its metabolites showed somewhat various fragment ions for their identification. As shown in Fig. 4A–E, each EI mass spectrum of the analytes was characterized by intense molecular ions and abundant fragment ions produced by cleavage of alkyl group at the benzylic position. Also, 1,4-quinone showed an intense ion at *m*/*z* 149, formed by the cleavage of a C_3H_7 radical from the molecular ion. The metabolites of 2-ω-phenol and 2-ω-quinol were identified by the presence of $[M\text{-}H_2O]^+$ and $[M\text{-}CH_2OH]^+$ ions.

As shown in Fig. 4F–J, every EI mass spectrum for the TMS derivatives showed characteristic and well-defined fragmentation patterns when compared to their free forms. The mass spectra of propofol-OTMS and quinol-OTMS derivatives were characterized by intense molecular ions and $[M\text{-}15]^+$ ions. The TMS derivatives of 2-ω-phenol and 2-ω-quinol showed characteristic molecular ions and intense $[M\text{-}CH_2OTMS]^+$ ions. The mass spectrum of the 2,6-*di*-isopropanol-phenol-*tri*-TMS derivative (Fig. 4J) showed a weak intensity molecular ion at *m*/*z* 426 and a $[M\text{-}15]^+$ ion at *m*/*z* 411, as well as several characteristic ions at *m*/*z* 323, 321, 264, and 147. These fragment ions could give the important evidence on the hydroxylation at another ω-position of 2-ω-phenol. The fragmentation pathways of this compound are suggested in Scheme 1. We did not consider this compound to be *meta*-hydroxylated metabolite such as 2-ω-hydroxylatedphenol (catechol form) because its mass spectrum was quite different from that of 2-ω-quinol-tri-TMS. This metabolite might be produced by further hydroxylation at another ω-position of 2-ω-phenol.

3.3. Analysis of propofol and its metabolites by LC/MS

The separation of propofol and its metabolites by C18-HPLC, in this study, used a mobile phase modified from previous reports [9,20,21] to improve chromatographic properties and detection sensitivity. To optimize the separation of propofol phase II metabolites, mobile phases (acetonitrile, methanol, acetonitrile–methanol) and concentration of ion-pair



Scheme 1. EI-mass fragmentation pathways of a 2,6-*di*-isopropanol-phenol-tri-TMS derivative.

agent were tested and compared. Though not shown data here, an acetonitrile–methanol mixture (1:1, v/v) as mobile phase provided reasonable separation efficiency and appropriate LC run-time. The retention times of propofol phase II metabolites increased as the portion of methanol increased. As the portion of acetonitrile increased, the LC run-time could be reduced but some of propofol phase II metabolites could not successfully separate. The concentration of ammonium acetate did not significantly affect the peak shape and separation efficiency but could alter the peak responses of propofol phase II metabolites under ESI process. As the concentration of ammonium acetate increases, the overall detection sensitivity of propofol phase II metabolites appeared to decrease due primarily to the increased ionic strength. In this study, maximum responses could be obtained at 0.1 mM of ammonium acetate. Based on the optimized LC conditions, five metabolites were successfully separated within 20 min and detected without any significant interferences in TICs (Fig. 5A).

The MS/MS spectra of phase II metabolites extracted from urine were obtained by LC–MS/MS in negative ion mode, as shown in Fig. 5B–F. Peaks 1 to 3 in TIC indicated the same deprotonated ion $[M-H]^-$ at *m/z* 369 corresponding to a glucuronide attached at three different hydroxyl positions of a hydroxyl propofol metabolite. As shown in Fig. 5B–D, the MS/MS spectra showed almost identical product ions with slight differences for the intensity of common ions. As seen in Fig. 5B–E, the characteristic ions at *m/z* 193 and 175 corresponded to $[M\text{-glucuronide}]^-$ and glucuronide ions,

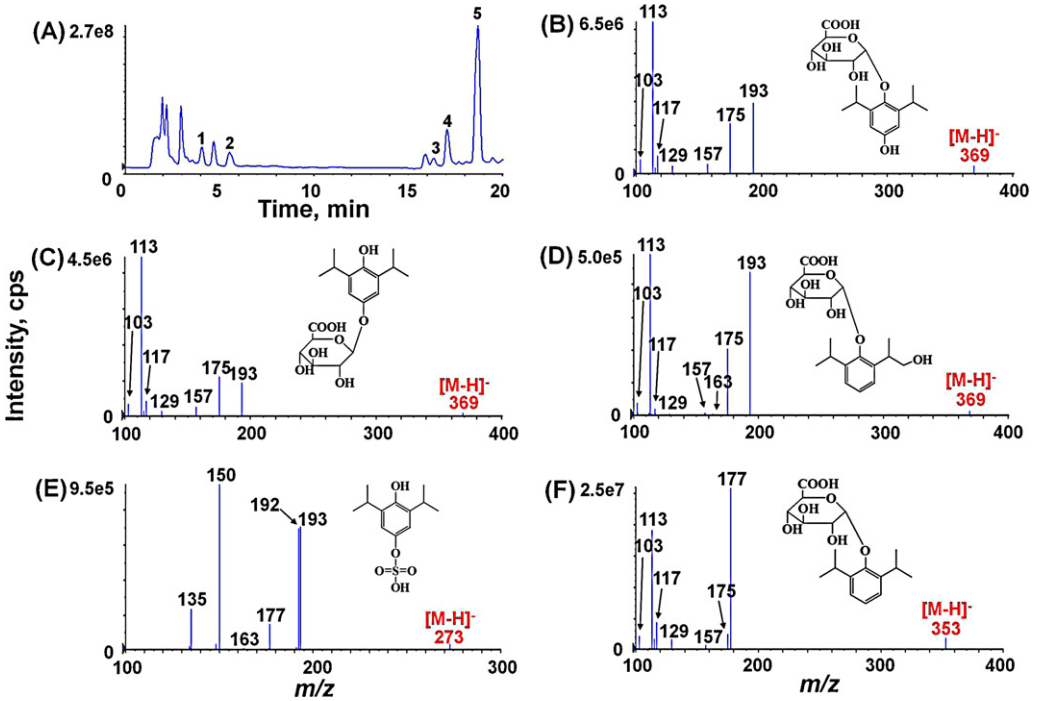
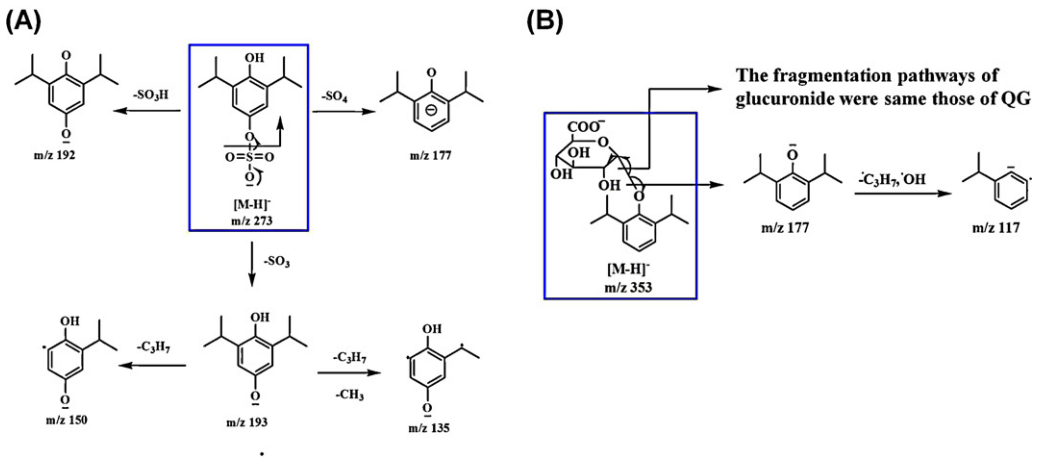


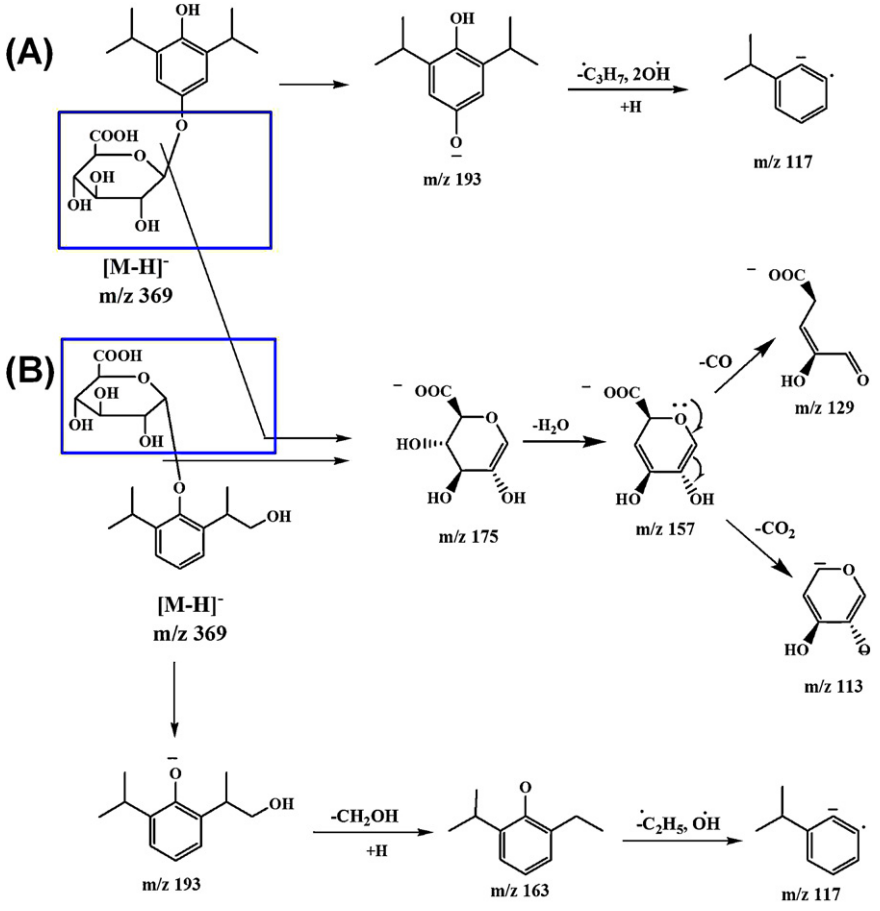
Fig. 5. (A) TICs of urine extract obtained by LC/MS-scan mode and MS/MS spectra of (B) quinol-1-glucuronide, (C) quinol-4-glucuronide, (D) x-(2-(ω-propanol)-6-isopropyl phenol) glucuronide, (E) quinol-4-sulfate, and (F) propofol-glucuronide.



Scheme 2. MS/MS fragmentation pathways of quinol-glucuronide and x-(2-(ω-propanol)-6-isopropyl phenol) glucuronide.

respectively. The fragment ions below *m*/*z* 160 were mainly formed by the cleavage of the glucuronide moiety. The fragmentation pathways of [M-H]⁻ ion for these compounds are indicated in Scheme 2. These compounds could not be easily identified and discriminated based only by their MS/MS spectra due to the lack of authentic standards. These isomers could be tentatively identified based on their elution order on a C18-HPLC column which previous studies [14,27] had shown to be: 1-QG, 4-QG, and x-2-ω-PG. This elution order was used to assign identities to peaks 1–3. The relatively small amount of peak 3 also provided evidence for the assignment of x-2-ω-PG which was consistent with previous studies [14,27]. As can be seen in Fig. 5D, the presence of an ion at *m*/*z* 163 with very weak intensity indicated that hydroxylation had occurred at the ω position. Peaks 4 and 5 were assigned as 4-QS and PG, respectively, based on their deprotonated molecules and LC peak abundance. The fragmentation pathways of [M-H]⁻ ions for PG and 4-QS are also reasonably suggested in Scheme 3.

In the present study, TMA or TEA was added to the mobile phase to test the chromatographic properties and detection sensitivity of propofol phase II metabolites in LC/MS analysis. As shown in Fig. 6A, the separation of propofol phase II metabolites was achieved within 20 min by LC–MS/MS-MRM mode. The addition of TEA to the mobile



Scheme 3. MS/MS fragmentation pathways of propofol-glucuronide and quinol-sulfate.

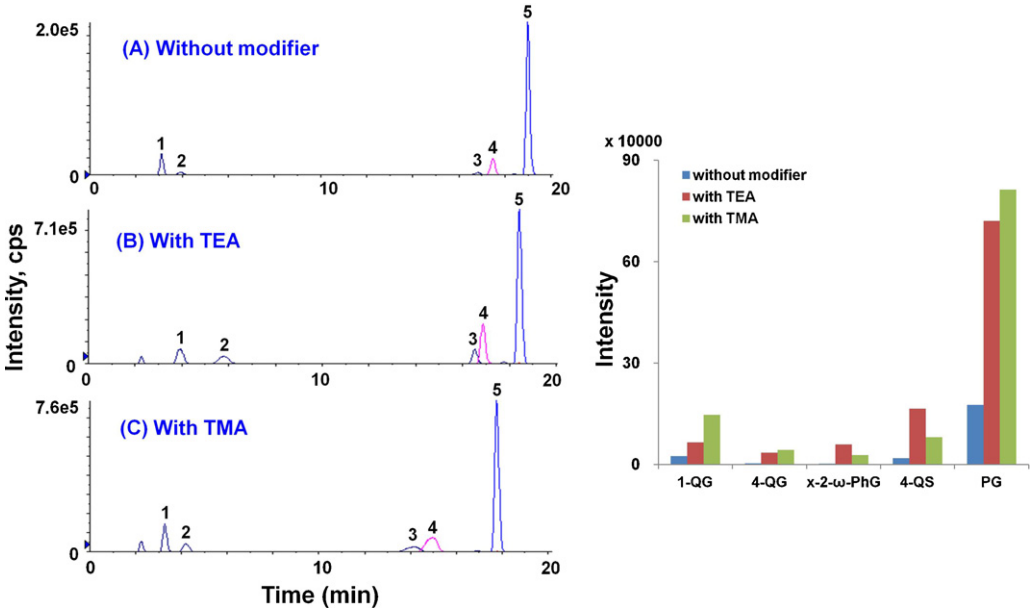


Fig. 6. TICs of phase II metabolites from human urine after propofol administration and the effects of addition of trimethylamine and triethylamine on detection sensitivity by LC/MS-MRM. Peak identities as follows: (1) quinol-1-glucuronide; (2) quinol-4-glucuronide; (3) x-(2-(ω-propanol)-6-isopropyl phenol) glucuronide; (4) quinol-4-sulfate; (5) propofol-glucuronide.

phase did not significantly change the peak resolution capacity. However, addition of TMA or TEA significantly increased the overall sensitivity for conjugated metabolites by approximately 2.68 to 19.90 fold over that achieved without additives (Fig. 6B). The reason for this is probably the ability of TMA and TEA to facilitate deprotonation of propofol phase II metabolites. The sensitivity for 4-QS was particularly improved by about 19.90 folds by the addition of TEA. The detection of 1-QG, 4-QG, x-2-ω-PG, and PG was also improved by 2.68, 10.63, 8.78, and 4.09 folds, respectively, using TEA.

The use of TMA also improved the detection sensitivity of 1- and 4-QG isomers, 4-QS, x-2-ω-PG, and PG by about 6.00, 13.39, 9.23, 4.27, and 4.61 folds, respectively. The overall LC peaks of phase II metabolites indicated slightly earlier elution than with TEA. However, x-2-ω-PG and 4-QS corresponding to peaks 3 and 4 could not be successfully separated due to peak broadening. The use of TEA gave an overall sensitivity similar to that with TMA but the phase II metabolites, especially peaks 3 and 4, were successfully separated. Therefore TEA was selected as an additive for use in the present study. The lower limit of detection (LLOD) of PG when TEA was used was about 0.48 ppb level by MRM mode and 0.38 ppb level by SIM mode. The LLOQ for PG was about 10 times lower in the present study than reported in previous studies [12,28].

Phase I metabolites and propofol were not sensitively detected even in the presence of mobile phase additives, owing to their poor ionization efficient in negative ion mode. In other words, propofol and phase I metabolites were not readily deprotonated during the ESI process because they have relatively higher pK_a values when compared with phase II metabolites.

3.4. Comparison of GC/MS and LC/MS

Fig. 7 shows the TICs and urinary excretion profiles for urine samples collected up to 48 hr after propofol administration. The GC/MS-scan mode could sensitively detect propofol and its metabolites even in urine samples collected 48 h after administration (Fig. 7A). The peaks corresponding to propofol and 1,4-quinol were still observed with high sensitivity even in the 48 h urine sample. Although other minor metabolites could be also observed in the 48 h urine sample using the GC/MS-scan mode, the propofol metabolite profile could be more sensitively followed over an even longer time period if the GC/MS-SIM mode was used. Propofol conjugated metabolites were also clearly detected in urine samples even in 48 hr samples by LC/MS-MRM mode (Fig. 7B). In particular, PG was sensitively detected in 48 h urine samples. The TICs were used as a basis for determining the urinary excretion profiles for propofol and its metabolites obtained by GC/MS and LC/MS methods, as shown in Fig. 7C and D, respectively. As shown in Fig. 7C and D, most of propofol and its metabolites were excreted during the 0–6 h collection periods. Subsequently they were gradually excreted. Urinary excretion profiles of propofol and PG were quite consistent with previous reports [7,12,13].

Table 1
Comparison of GC/MS and LC–ESI–MS methods for the analysis of propofol and its metabolites in human urine.

	GC/MS	LC-ESI-MS
Measured analytes	Propofol, quinol, 2-ω-phenol, 2-ω-quinol and their TMS derivatives	Conjugated propofol-metabolites
Time for sample preparation	300 min	20 min
Analysis time	20 min	40 min
LLOD	Scan: 79 ng/mL (P-TMS) SIM: 0.04 ng/mL(P-TMS)	SIM: 0.38 ng/mL (PG) MRM: 0.44 ng/mL (PG)
LLOQ	Scan: 325 ng/mL (P-TMS) SIM: 0.51 ng/mL (P-TMS)	SIM: 1.17 ng/mL (PG) MRM: 2.01 ng/mL (PG)
Linear range	Scan: 1–2000 μg/mL (P-TMS) SIM: 1–2000 ng/mL	SIM: 5–2000 ng/mL (PG) MRM: 5–2000 ng/mL (PG)
Correlation coefficient	Scan: 0.995 SIM: 0.997	SIM: 0.998 MRM: 0.990
Method development	Easy	Optimization of LC conditions can be challenging

P-TMS: propofol-TMS, PG: propofol-glucuronide.

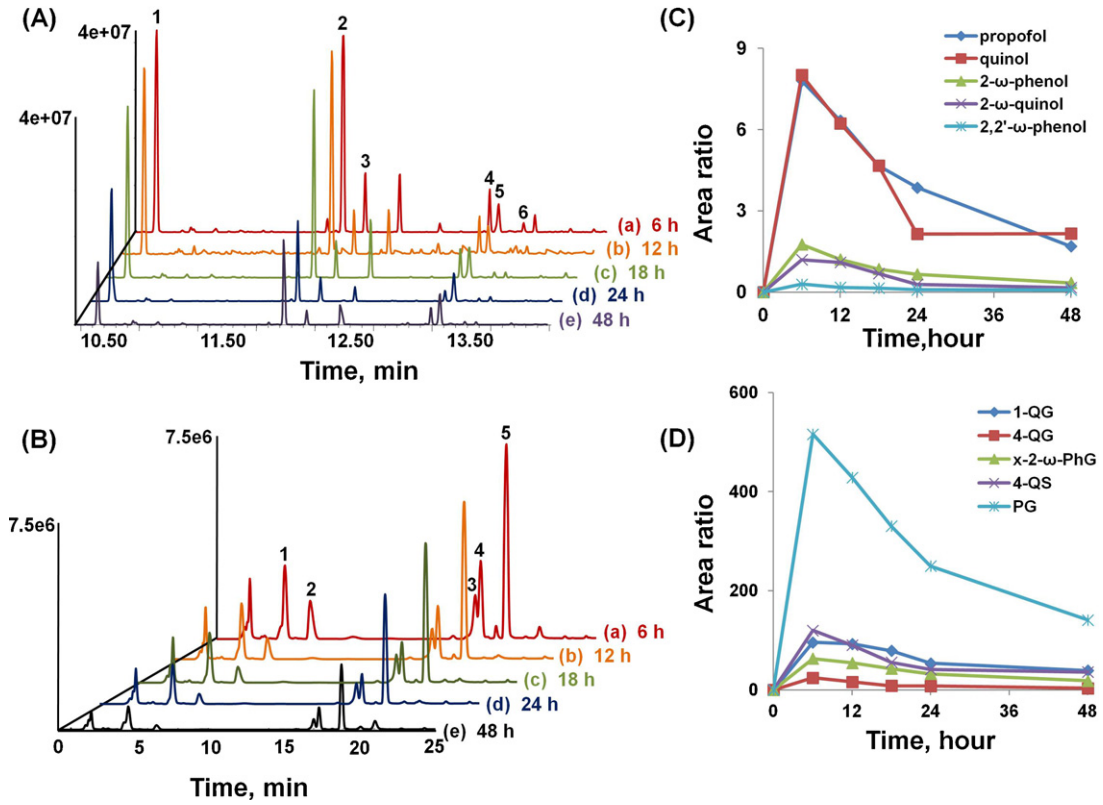


Fig. 7. TICs of urine at different collection times following propofol administration and excretion profiles for propofol obtained by GC/MS and for its metabolites obtained by LC/MS methods. Peak identities are as same as Figs. 3 and 6, respectively.

In this study, GC/MS and LC/MS methods were compared in terms of the sensitivity, convenience of sample preparation, and analysis time. Both methods have their own advantages and disadvantages, as shown in Table 1. The sample preparation for LC/MS analysis was very simple compared to what was required for GC/MS analysis. Overall, the LC/MS method provided a shorter total analysis time. The detection with LC/MS was more sensitive for conjugated metabolites, but was not sufficiently sensitive for intact propofol and its unconjugated hydroxyl metabolites. The GC/MS method, coupled with TMS derivatization was very sensitive for detection of propofol and its hydroxylated metabolites, although extensive sample pretreatment was required. The LLOQs for propofol-TMS and PG obtained by GC/MS-SIM mode and LC/MS-MRM mode, respectively, were about 0.51 and 2.01 ppb. These LLOQs were over two orders lower than those reported previously [9,12,13]. The correlation coefficients for the calibration curves of propofol and PG were over 0.995 and 0.990, respectively, indicating good linearity. These results showed that both methods are suitable for the determination of propofol and its metabolites although each method had its own limitations.

4. Conclusion

Propofol and its metabolites can be suitably determined in urine using either GC/MS or LC/MS methods. Overall, the LC/MS method provided a shorter total analysis time with simpler sample preparation, while GC/MS provided better separation and easier identification of metabolites, by producing structurally characteristic ions in EI spectra of TMS derivatives. The LC/MS method provided high-sensitivity for phase II metabolites but could not sensitively detect the parent propofol compound or its phase I metabolites under present experimental conditions. The addition of TEA to the mobile phase significantly improved the detection sensitivity of propofol phase II metabolites. In contrast, although information on phase II metabolites could not be directly obtained, GC/MS combined with TMS derivatization provided high-sensitivity for detection of propofol and its hydroxylated metabolites as well as reliable identification of minor metabolite. Another advantage of TMS derivatization was the conversion of the quinone form to a quinol form *via* an enolization process, which would provide reasonable information about the propofol metabolism in further studies. Thus, both GC/MS and LC/MS methods should be used as complementary methods to provide a better understanding of propofol metabolism in human. The high sensitivity of both methods could be applied in clinical medicines and forensic science.

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