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Quantitative measurement of propofol and in main glucuroconjugate metabolites in human plasma using solid phase extraction–liquid chromatography–tandem mass spectrometry

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Abstract

A high-performance liquid chromatographic method coupled with tandem mass spectrometry detection has been developed for the determination of propofol and its main glucuroconjugate metabolites (propofol–glucuronide (PG), 1-quinol-glucuronide (1-QG) and 4-quinol-glucuronide (4-QG) in human plasma. All compounds were extracted with a single solid phase extraction procedure using Max Oasis© cartridges. Propofol and thymol (internal standard) were analyzed using a C8 reversed-phase column with a mobile phase consisting of methanol–water (75:25, v/v) containing 0.025% NH_4OH. Chromatography of glucuroconjugate metabolites and phenyl-β-D-glucuronide (internal standard) was performed using a hydrophilic interaction liquid chromatography (HILIC) and a mixture of acetonitrile/water/ammonium acetate buffer (100 mM, pH 5, 87/1/12, v/v/v). Both chromatographic separations were achieved in isocratic mode allowing a rapid analysis without re-equilibration of the phase. The method is specific and sensitive with a range of 10–1500 ng mL^{-1} for propofol and 1-QG, 20–3000 ng mL^{-1} for PG and 25–3750 ng mL^{-1} for 4-QG. The regression curves were linear for all compounds. The method is accurate and precise with intra-assay and inter-assay precision <8% and bias ≤6% for all compounds. This assay has allowed the successful measurement of propofol and its main glucuroconjugate metabolites in human plasma from 24 patients undergoing anaesthesia for elective partial hepatectomy surgery.

Keywords: Propofol; Metabolites; Tandem mass spectrometry; Blood

1. Introduction

Propofol is an anaesthetic drug which has been widely used for numerous years in the induction and maintenance of anaesthesia [1]. Propofol undergoes rapid metabolism yielding glucuronide metabolites directly from parent drug or after hydroxylation reaction of the phenyl ring [2]. The drug's high substrate affinity for UDP-glucuronosyltransferase has led recently to the testing of propofol as a competitive inhibitor to modulate the anticancer drug resistance in colon cancer cells [3,4]. Other studies have shown that propofol and its metabolites possess an antioxidant capacity in vitro [5].

The quantification of propofol in human whole blood or plasma commonly employs liquid chromatography (coupled with UV, fluorescence, or electrochemical detection) although several assays using gas chromatography have also been described [6–9]. Recently, liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) techniques were described for the determination of propofol using either APCI without derivatization or ESI with derivatization step [10,11]. As

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far as we know, no validated LC–MS/MS assay has been proposed for the quantification of glucuronide metabolites in human blood. The aim of the present work is to propose a simple and sensitive method for the quantification of propofol and its main glucuronide metabolites in human blood using LC–MS/MS.

2. Experimental

2.1. Reagents and chemicals

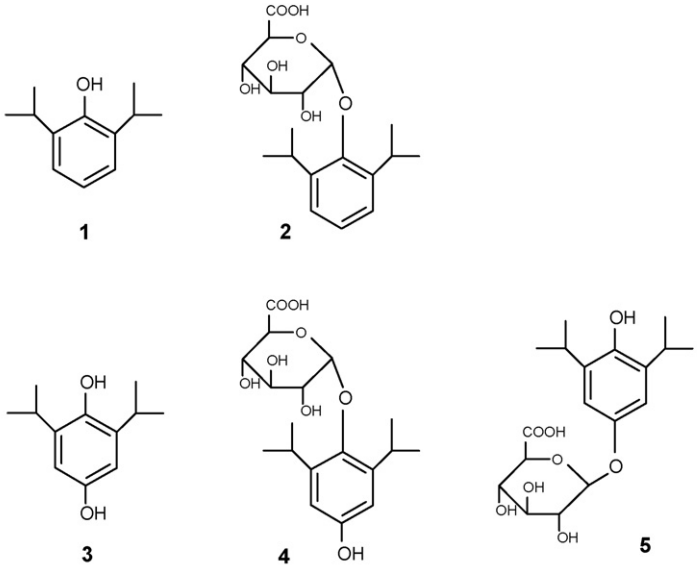
Propofol was purchased from RBI (Natick, USA). The 4-hydroxylated metabolite of propofol was kindly provided by Zeneca Pharma (Cergy, France). Glucuroconjugated metabolites were prepared and characterized by Favetta et al. [12] as described previously. The chemical structures of propofol and propofol metabolites are presented in Fig. 1. Thymol, phenyl-β-D-glucuronide, formic acid and ammonium hydroxide were obtained from Sigma (St. Quentin Fallavier, France). HPLC-grade acetonitrile and methanol used in chromatography were purchased from Merck (Darmstadt, Germany).

2.2. Standard solution and calibration standards

Stock solutions (1 mg mL^{-1}) of propofol and thymol were prepared in methanol and stored at +4 °C. Propofol metabolites and phenyl-β-D-glucuronide were prepared in water and stored at −20 °C. Under these conditions, the solutions were found to be stable for at least 6 months (data not shown). Standard solutions were made daily by further dilution of stock solutions with methanol/water (50/50, v/v). For calibration curves, blank plasma samples (450 μL) were spiked with 50 μL of the appropriately diluted standard solutions to give final concentrations of 10, 25, 100, 250, 750 and 1500 ng mL^{-1} for propofol; 20, 50, 200, 500, 1500 and 3000 ng mL^{-1} for PG; 10, 25, 100, 250, 750 and 1050 ng mL^{-1} for 1-QG and 25, 62.5, 250, 625, 1875 and 3750 ng mL^{-1} for 4-QG. Blank plasma samples containing no added propofol and metabolites were also prepared. All samples were subjected to the sample preparation procedure described below.

2.3. Sample preparation

A 500 μL volume of plasma and 50 μL of a mixture of internal standard solution (thymol 4 μg mL^{-1} and phenyl-β-D-glucuronide 20 μg mL^{-1}) were mixed vigorously for 30 s. An Oasis® MAX (30 mg), extraction cartridge (Waters, Milford, USA) was conditioned under vacuum with 1 mL of methanol and 1 mL of deionized water. The plasma (500 μL) was applied to the SPE column. After a slow percolation, the cartridge was washed with 1 mL of a mixture of methanol and ammonium hydroxide 2% (5/95, v/v). The first elution was carried out with 1 mL of acetonitrile/methanol (75/25, v/v). The second elution was performed with 1 mL of acetonitrile/methanol (75/25, v/v) containing 2% of formic acid. Glucuroconjugate metabolites were directly analyzed from the second elution. For propofol quantification, 200 μL of both elution fractions were mixed with



1: 1-hydroxy-2,6-diisopropyl-benzene (propofol). **2**: 3,4,5-trihydroxy-6-(2,6-diisopropyl-phenoxy)-oxane-2-carboxylic acid (propofol glucuronide; PG). **3**: 2,6-diisopropyl-1,4-dihydroxy-benzene (4-hydroxy-propofol; 4-OH-P). **4**: 3,4,5-trihydroxy-6-(4-hydroxy-2,6-diisopropyl-phenoxy)-oxane-2-carboxylic acid (1-quinol glucuronide; 1-QG). **5**: 3,4,5-trihydroxy-6-(1-hydroxy-2,6-diisopropyl-phenoxy)-oxane-2-carboxylic acid (4-quinol glucuronide; 4-QG).

Fig. 1. Chemical structure of propofol and metabolites.

200 μL of ammonium hydroxide 0.025% in order to obtain a mixture close to the composition of mobile phase. The solution was transferred to a glass vial kept at 10 °C into the autosampler and 10 μL were injected.

2.4. HPLC conditions

The high-performance liquid chromatographic system consisted of a ThermoElectron Surveyor MS pump equipped with a Surveyor autosampler injector (ThermoElectron, San Jose, USA).

Propofol and thymol were separated on a X-Terra© MS C8 column (Waters, Milford, USA), 3.5 μm, (100 mm × 2.1 mm i.d.) with a X-Terra© guard cartridge (10 mm × 2.1 mm i.d.). The separation was isocratic using a mixture of methanol/water (75/25, v/v) both solutions containing NH_4OH (0.025%). The mobile phase was delivered through the column (temperature maintained at +30 °C) at a flow rate of 200 μL min^{-1}. The mobile phase was switched to waste for 3.75 min in order to avoid matrix components passing through the detector.

Glucuroconjugated metabolites and phenyl-β-D-glucuronide were analyzed on an Atlantis© Hilic column, (Waters, Milford, USA), 3.0 μm, (150 mm × 2.1 mm i.d.) with an Atlantis© Hilic guard cartridge (10 mm × 2.1 mm i.d.). The separation was isocratic using a mixture of acetonitrile/water/ammonium acetate buffer (100 mM, pH 5, 87/1/12, v/v/v). The column was kept at +25 °C and the flow rate was 200 μL min^{-1}.

In an alternative method, a column X-Terra® RP18 (5 μm, 250 mm × 2.1 mm i.d.) was eluted under isocratic conditions (85% water/15% methanol, v/v, both with 0.025% NH_4OH) for 4 min and then a rapid linear gradient of methanol (5% methanol at 4.5 min and held for 8 min) was set for the elution of others compounds.

2.5. Mass spectrometric conditions

MS/MS data were acquired using a Quantum-Ultra (ThermoElectron, San Jose, USA) triple quadrupole mass spectrometer equipped with an Ion Max API source. The instrument was operated in negative ion mode with an electrospray ionization (ESI) source. The position (x, y, z) of the ESI probe was optimized with propofol and PG. Argon was used as collision gas at 1.5 mTorr. For all compounds, spray voltage and capillary temperature were set at 3.0 kV and 300 °C, respectively. The pressures for the nitrogen sheath gas, auxiliary gas and sweep gas were maintained at 30, 10 and 5 units. The $[M-H]^-$ ions of different compounds were passed through the first (Q1) and the third (Q3) quadrupole with full-width at half maximum height of 0.7 Th. Compounds were quantitated in selected reaction monitoring (SRM) mode. SRM was performed with 500 ms dwell time per channel. The collision energy was set at 10 eV for propofol and thymol, and at 22 eV for PG, 1-QG, 4-QG and phenyl-β-D-glucuronide. Argon was used as collision gas at 0.2 Pa.

To investigate suppression of the ESI, a post-column infusion system with syringe pump (flow rate 5 μL min^{-1}) was used to deliver solution at 10 μg mL^{-1} of the different compounds. Effluent from the HPLC column combined with the infused analytes entered into the detector. Mobile phase and extract sample from blank human plasma were successively injected.

2.6. Calibration curve and validation procedure

Calibration curves were constructed by plotting the ion abundance peak area ratio propofol/thymol or glucuroconjugated metabolites/phenyl-β-D-glucuronide as function of plasma propofol or metabolite concentration. The linearity of this assay was tested using an analysis of variance. The significance of the slope and the validity of the linear calibration curves were tested using Fisher–Snedecor's F-test ($p < 0.05$). Homocedasticity was statistically determined using Cochran's test ($p < 0.05$). Data were fitted by weighted (1/concentration) for least-squares regression, and standard curves were determined using linear regression analysis.

A total of seven calibration curves (prepared as a single replicate and analyzed in 7 different days) were generated during the entire validation process using freshly prepared samples. At 4 different days, run included a calibration curve and quality control (QC) samples at three different levels (20, 150 and 1050 ng mL^{-1} for propofol and 1-QG; 40, 300 and 2100 ng mL^{-1} for PG and 50, 375 and 2625 ng mL^{-1} for 4-QG.). For each level, six different samples have been extracted and for each only one injection was performed. The limit of quantitation (LOQ) was chosen as the concentration of the lowest calibration standard. The upper limit of quantitation was chosen as the concentration of the upper calibration standard.

The accuracy and precision of the assay were assessed by the mean relative percentage deviation from the nominal concentrations and the within-run precision (WRP) and between-run precision (BRP), respectively. The within-run precision was determined as $\mathrm{WRP} = 100 \times (\sqrt{\mathrm{MS_{wit}}}/\mathrm{GM})$. The between-run precision was estimated as $\mathrm{BRP} = 100 \times (\sqrt{\mathrm{MS_{bet}} - \mathrm{MS_{wit}})/n}/\mathrm{GM}$. $\mathrm{MS_{wit}}$, $\mathrm{MS_{bet}}$, n and GM, represented the within-groups mean square, the between-groups mean square, the number of replicate observations within each run and the grand mean, respectively. These parameters were calculated using the software Statview for windows version 5.0 (SAS institute, Cary, USA).

The extraction efficiency for propofol and its metabolites at two different plasma concentrations (50 and 850 ng mL^{-1} for propofol and 1-QG, 100 and 1600 ng mL^{-1} for PG, 125 and 2000 ng mL^{-1} for 4-QG) were determined by comparing the mean peak areas from spike samples obtained through the complete SPE procedure with those obtained by spiking blank extracts from the SPE procedure. The recovery of the extraction procedure corresponds to the ratio of the responses from extract sample and the responses from post-extract spiked sample. This calculation normalizes any matrix effects [13].

2.6.1. Plasma samples

Clinical samples were obtained from 24 patients undergoing elective partial hepatectomy surgery for hepatocellular carcinoma or liver metastases resection after giving their informed consent. Anaesthesia was induced with intravenous injection of propofol, remifentanil and cisatracurium. Propofol infu-

sion was performed with a target-controlled infusion system: Diprifusor™. The hypnotic effect of propofol was measured with an automatic multifactorial electroencephalogram analyzer (Bispectral index), Bis® monitoring (Aspect Bis-4xp, Aspect Mediccal systems, Newton, MA). The initial target plasma concentration of propofol was 4 μg mL^{-1}. The infusion rate of propofol was varied to maintain the BIS value between 45 and 55 through out the entire surgical procedure. Three blood samples were collected for each patient: (T0) 30 min before the vascular exclusion or the liver metastases resection, (T1) 60 min after partial hepatectomy or the liver metastases resection, (T2) at the end of the anaesthesia procedure when BIS values reached 80 indicating a conscious patient. An arterial catheter was placed in the controlateral arm for collection of 5 mL of blood samples in glass tubes containing potassium oxalate. Blood samples were immediately centrifuged, and plasma aliquots were stored at +4 °C whilst awaiting analysis. Samples from patients were systematically diluted (1/15) with blank human plasma prior to extraction. The blank plasma for the dilution was the same lot used to prepare calibration standards.

3. Results and discussion

3.1. Detection

3.1.1. Glucuroconjugate metabolites

The mass spectra of PG and 1-QG showed deprotonated molecules ($[M-H]^-$) at *m/z* 353.2 and 369.2, respectively. The prominent ions observed at *m/z* 177.2 for PG and *m/z* 193.2 for 1-QG, corresponding to the loss of glucuronide moiety, were selected in the third quadrupole for the quantification. 4-QG presents the same transition as 1-QG and should be consequently separated by chromatography. The mass spectrum of the β-D-phenyl glucuronide (internal standard) showed a ($[M-H]^-$) at *m/z* 269.0 and a major product at *m/z* 93.0.

3.1.2. Propofol

Ionization of propofol and thymol was enhanced when methanol was used in the mobile phase in high proportion. The use of acetonitrile decreases the response dramatically. Moreover, as previously described, only the negative ion mode allows the detection of both underivatized compounds [10].

The mass spectral fragmentation of propofol and related phenols such as thymol was thoroughly studied by Bajpai et al. [14] who used transitions 177.2 → 161.2 and 149.2 → 133.0 for propofol and thymol, respectively. These transitions correspond to the loss of methane by the sequential loss of CH_3 and H radicals. From the work of Bajpai et al. and our own experience, propofol presents a poor stability of the intermediate radical ions, leading to a weak abundance and reproducibility. Consequently, propofol do not constitute an "ideal" candidate for tandem mass spectrometry detection. Bajpai et al. preferred APCI to ESI in order to enhance the response due to non-polar nature of propofol. This ionization mode allows to compensate, at least in part, a loss of response due to the selection of a transition (*m/z* 177.2 → 161.2) with the weak ion abundance [10]. Beaudry et al. [11] performed a derivatization step with dansyl chloride to improve ESI signal by the production of compound easily protoned. The detection was performed in positive ion mode with the transition *m/z* 412 → 171 [11]. This last approach, although time consuming, allows to decrease significantly the LOQ of propofol in biological samples.

In the present study, the transitions 177.2 → 161.2 and 149.2 → 133.0 for propofol and thymol were not used due to the intensity ratio product/parent ions very low (<3%) as indicated above. Nitrogen was also tested as collision gas, but this configuration did not produce higher product ion abundance. Consequently, the quantification of propofol and thymol was performed in MRM mode with a "false" transition 177.2 → 177.2 and 149.2 → 149.2 for propofol and thymol, respectively. These transitions were preferred to the SIM mode because the ratio signal to noise was enhanced with SRM mode. This is due, at least in part, because the electron multiplier is set to a gain of 3.10^5 in MS mode and 2.10^6 in MS/MS mode.

3.2. Chromatographic conditions

Representative chromatograms of plasma samples from a patient are shown in Fig. 2. The retention time of propofol and thymol were 6.7 and 4.2 min, respectively (MS C8 column). The retention time of PG, 4-QG, 1-QG and β-D-phenyl glucuronide were 4.8, 5.5, 6.3 and 9.4 min, respectively (Hilic column). Fig. 3 shows the chromatograms obtained from spiked plasma for the lowest point of the calibration curve.

Chromatographic conditions for all compounds were isocratic in order to avoid a period of re-equilibration between runs. For glucuroconjugate metabolites, the chromatographic conditions were set to separate 1-QG and 4-QG since both molecules exhibit the same SRM transition. This was obtained using Hilic column and a run time of 12 min.

Faster elution may be obtained, for propofol and thymol, by increasing the proportion of methanol in mobile phase. However, as shown in Fig. 4, a region of ionization suppression was observed at the beginning of the chromatogram and the composition of the mobile phase was modified to avoid the effects of this phenomenon. Ionization suppression was not observed for PG (Fig. 4) and 1-QG and 4-QG (data not shown).

An X-Terra© RP18 column (5 μm, 250 mm × 2.1 mm i.d.) was also tested in an attempt to analyze all the compounds in the same run. The separation of 1-QG and 4-QG was achieved using isocratic condition (95% water/5% methanol, v/v, both with 0.025% NH_4OH). Then a rapid gradient of methanol was set for the elution of others compounds. This procedure was rejected as having too low a throughput because of the equilibration period that was necessary between runs to maintain a good separation of 1-QG and 4-QG. Moreover, in this condition, β-D-phenyl glucuronide was poorly retained and the retention of the molecule was in the region where interferences were observed leading to poor signal reproducibility. Thus, this I.S. could not be used for the quantification of glucuronide metabolites. Consequently, two isocratic chromatographic assays were preferred using the same extraction procedure.

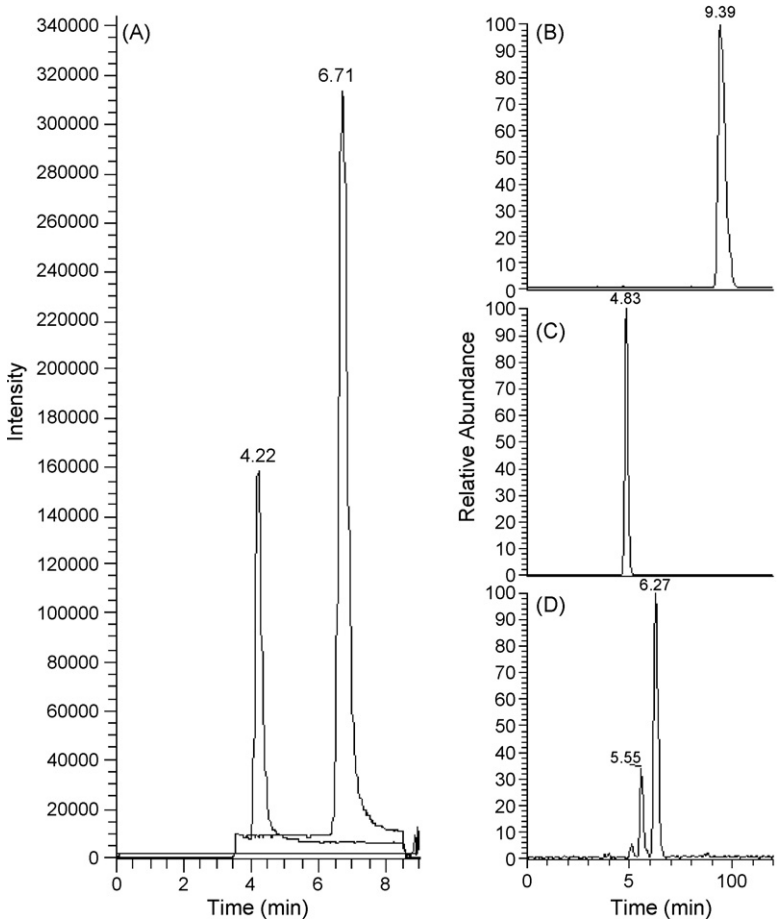


Fig. 2. Chromatograms from patient sample after dilution with blank human plasma (1/15). (A) Thymol (I.S. 4.22 min) and propofol (6.71 min) at 1.34 μg mL^{-1}, (B) phenyl-β-D-glucuronide (I.S. 9.39 min), (C) propofol–glucuronide (4.83 min) at 9.68 μg mL^{-1}, (D) 4-quinol-glucuronide (5.55 min) at 3.06 μg mL^{-1} and 1-quinol-glucuronide (6.27 min) at 3.77 μg mL^{-1}. Final concentrations are indicated.

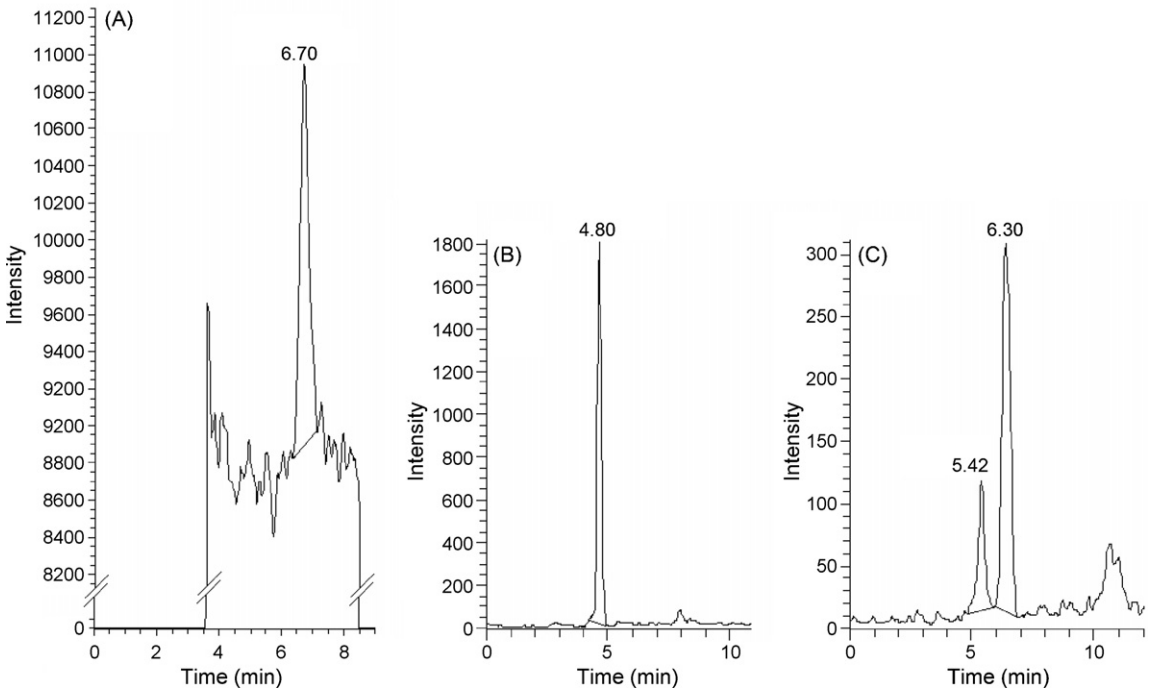


Fig. 3. Chromatograms obtained from spiked human plasma with propofol at 10 ng mL^{-1} (A, Tr: 6.70 min), propofol–glucuronide at 20 ng mL^{-1} (B, Tr: 4.80 min), 4-quinol-glucuronide at 25 ng mL^{-1} (C, Tr: 5.42 min), 1-quinol-glucuronide at 10 ng mL^{-1} (C, Tr: 6.30 min).

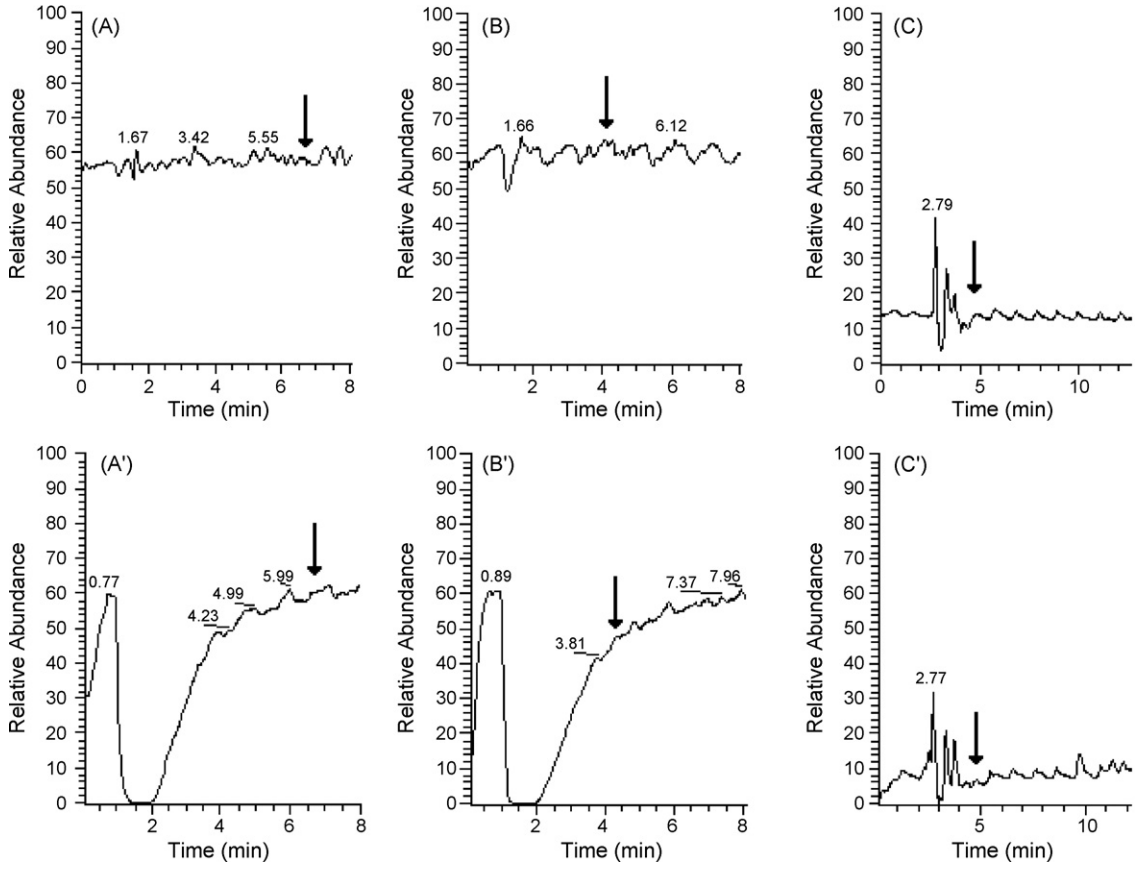


Fig. 4. Ionization suppression study of propofol (A and A′), thymol (B and B′) and propofol–glucuronide (C and C′). Experiment was carried out using a post-column infusion of a 10 μg mL^{-1} solution (speed infusion: 5 μL min^{-1}). Chromatograms A, B and C were obtained after injection of 10 μL of mobile phase. Chromatograms A′, B′ and C′ were obtained after injection of 10 μL of blank plasma after extraction procedure. Arrow indicates the retention time of compounds.

3.3. Calibration curves, accuracy, precision and LOQ

A linear relationship was obtained between the peak area ratio of propofol to thymol against the spiked concentration of propofol in human plasma over the range of 10–1500 ng mL^{-1}. The same relationship was observed between glucuroconjugate metabolites and β-D-phenyl glucuronide over the range of 20–3000 ng mL^{-1} for PG, 10–1500 ng mL^{-1} for 1-QG and 25–3750 ng mL^{-1} for 4-QG. For all compounds a weighting factor (1/concentration) was applied and a mean least-squares linear regression correlation coefficient of greater than 0.992 was obtained. Precision was less than 8% and accuracy was less than 5% when concentrations were back calculated from the equation of the regression analysis (Table 1). The within-run and between-run variability (precision) and the mean predicted concentration (accuracy) analyzed at the various concentrations in sextuplicate on 4 separate days are reported in Table 2. All parameters fulfilled the respective requirements of the Washington consensus conference on analytical methods validation [15]. Numerous techniques have been proposed for the quantification of propofol in blood samples and for the most sensitive of them the LOQ was from 2 to 10 ng mL^{-1}. In the present study, the LOQ was set at 10 ng mL^{-1}. The LOQ of the glucuronides was set at 20, 10 and 25 ng mL^{-1} for PG, 1-QG and 4-QG, respectively. These values are 10–100 times lower than those obtained by Vree et al. [16].

3.4. Extraction procedure

A rapid preparation method based on protein precipitation with acetonitrile was tested but gave variable results. This problem was solved when the SPE method was applied. Although SPE is more time consuming than a simple precipitation method, our extraction procedure is fast since no evaporation was performed. Avoiding this step ensured that thymol and propofol were protected from evaporative losses. Oasis^{TM} Max cartridge is based on mixed mode anion exchange and reversed-phase interaction enable extraction of weak acid and lipophilic compounds such as propofol and the glucuroconjugate metabolites. So, in our conditions using this cartridge, the extraction of all compounds was performed in the same procedure. The full extraction of propofol and thymol was obtained after a first elution with acetonitrile/methanol (75/25, v/v) and a second elution with acetonitrile/methanol (75/25, v/v) containing 2% of formic acid. Glucuroconjugate metabolites were only recovered in the second elution fraction. The extraction efficiency of propofol and its glucuroconjugate metabolites was concentration inde-

Table 1
Inter-day validation of the determination of propofol and its main metabolites

Concentration (ng mL^{-1})			
Spiked	Found (mean ± S.D., 7 days)	Precision (%) (between-run)	Accuracy (%)
Propofol			
10	10.3 ± 0.3	3.4	102.9
25	24.9 ± 0.7	2.9	99.5
100	97 ± 5	4.9	97.1
250	251 ± 11	4.4	100.4
750	749 ± 3	0.3	99.8
1500	1503 ± 7	0.5	100.2
PG			
20	19.9 ± 1.3	6.7	99.4
50	47.6 ± 2.1	4.5	95.2
200	205 ± 13	6.3	102.3
500	513 ± 13	2.6	102.5
1500	1534 ± 42	2.8	102.3
3000	2951 ± 45	1.5	98.4
1-QG			
10	10.1 ± 0.8	7.8	100.6
25	24.8 ± 1.2	5.0	99.1
100	98 ± 5	4.6	98.3
250	253 ± 7	2.6	101.2
750	750 ± 5	0.7	100.1
1500	1499 ± 12	0.8	99.9
4-QG			
25	24.7 ± 1.1	4.4	98.8
62.5	63.5 ± 1.9	3.1	101.5
250	246 ± 9	3.8	98.3
625	631 ± 5	0.9	100.9
1875	1876 ± 31	1.7	100.0
3750	3749 ± 25	0.7	100.0

Data from seven calibration curves prepared as a single replicate and analyzed in seven different days. (PG: propofol–glucuronide, 1-QG: 1-quinol-glucuronide, 4-QG: 4-quinol-glucuronide).

Table 2
Assessment of accuracy and precision

Concentration (ng mL^{-1})		Precision (%)		Accuracy (%)
Spiked	Found (mean ± S.D.)	Within-run	Between-run	
Propofol				
20	19.8 ± 1.2	5.2	3.4	98.8
150	148 ± 6	3.1	2.7	98.8
1050	1042 ± 16	1.5	0.6	99.2
PG				
40	41.4 ± 3.8	6.9	6.9	103.5
300	307 ± 26	4.8	7.7	102.3
2100	2076 ± 50	2.1	1.4	98.9
1-QG				
20	21.2 ± 1.8	5.6	7.5	106.0
150	157 ± 13	3.4	8.8	104.6
1050	1051 ± 17	1.1	0.5	100.1
4-QG				
50	50.5 ± 2.5	3.5	4.0	101.1
375	374 ± 13	3.0	2.3	99.6
2625	2628 ± 12	0.4	0.1	100.1

Data from six replicates for each concentration and analyzed in four different days. (PG: propofol–glucuronide, 1-QG: 1-quinol-glucuronide, 4-QG: 4-quinol-glucuronide).

pendent and averaged 100% for propofol, 90% for 1-QG and 85% for PG and 4-QG.

3.5. Analysis of clinical samples

Drug and metabolite concentrations in clinical samples were found to range from 0.42 to 5.5 μg mL^{-1} for propofol, 4.8–45.9 μg mL^{-1} for PG, 2.1–11.4 μg mL^{-1} for 1-QG and 1.5–11.9 μg mL^{-1} for 4-QG. Due to the large range of the concentration found in these samples, a dilution (1/15) was systematically performed. This procedure allows, with a single

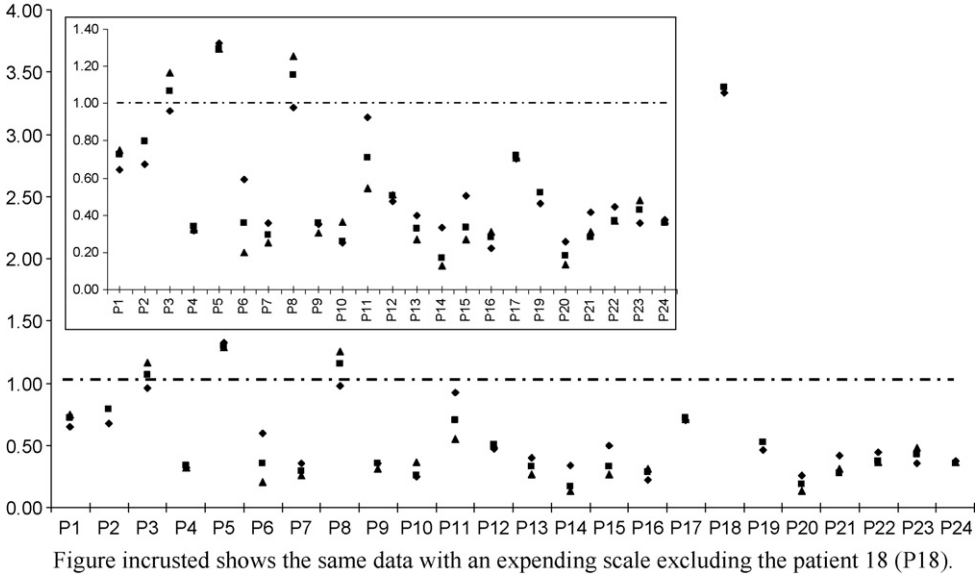


Fig. 5. Measure of plasma metabolic ratio (1-QG + 4-QG)/PG during propofol infusion in 24 patients. For each patient, three determinations during anaesthesia were performed (see text for detail and Fig. 1 for abbreviation).

extraction, the quantification of all target compounds. Patient plasma concentrations were within the standard curves. Our results confirm previous data on human metabolic pathway of propofol although all pathways were not quantified since we only focused on main glucuroconjugate metabolites [12,17]. Firstly, the ratio between propofol and the sum of the glucuroconjugate metabolites varied from 1 to 15% indicating the rapid and extensive biotransformation of propofol. Secondly, PG is the main metabolite of propofol i.e. the direct glucuronidation from propofol corresponds to the major pathway. Thirdly, we confirmed the relative interindividual variation between phases I and II metabolism for the propofol biotransformation. This is investigated by the mean metabolite ratio (1-QG + 4-QG)/PG which varied from 0.21 to 3.36 as shown in Fig. 5. Actually, the numerator corresponds to the sum of the metabolites for which the CYP450 activity is the rate-limiting step for their further formation via the 4-OH-P synthesis. At last, the mean ratio 1-QG/4-QG varied from 0.55 to 1.95, and for 19 patients 1-QG was more abundant than 4-QG. These results were not to carry out extensive investigation of metabolic pathway of propofol, but to show the usefulness and the quality of the present analytical method.

The 4-OH-P was also monitored using the same chromatographic conditions as propofol with the transition m/z 193.2 → 193.2 (energy collision 10 eV) and a retention time of 6.06 min. However, this metabolite was not observed from human plasma samples. This point corroborates a previous study in which the metabolite was only identified when hydrolysis pretreatment was performed [9]. However, this data is not in agreement with a previous study which identifies the presence of 4-OH-P in human plasma [16].

4. Conclusion

Numerous techniques have been proposed for the quantification of propofol and recently two methods have been described with LC–MS/MS. In the present work, we focused on the development of an assay for the measurement of propofol and its main glucuroconjugate metabolites in blood. Our method is the first validated LC–MS/MS method to be published for the quantification of propofol metabolites. Although two different chromatographic procedures were performed for propofol and its glucuroconjugate metabolites both were rapid and achieved in isocratic mode. To compensate the relative drawback to use two analytical columns, we developed an assay using only the ESI probe and a SPE method allowing extraction of all compounds in the same procedure. The present method does not require time-consuming step such as derivatization or evaporation of the eluate after the SPE. Propofol is analyzed on a C8 analytical column and chromatographic separation of the metabolites was performed with Hilic column. The Hilic chromatography allows injection of an organic solvent and gave excellent retention and a peak shape for these polar compounds. The assay is accurate and reproducible yielding precision and accuracy <10% over 4 days of validation. The described method permits the analysis of patient samples for propofol and its main glucuroconjugate metabolites.

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