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Research Article

Quantitation of propofol metabolites by LC–MS/MS demonstrating long detection window for urine drug monitoring

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ABSTRACT

Introduction: Chromatographic methods for analysis of propofol and its metabolites have been widely used in pharmacokinetic studies of propofol distribution, metabolism, and clearance. Application of chromatographic methods is also needed in clinical and forensic laboratories for detecting and monitoring propofol misuse.
Objective: We report a method for sensitive analysis of propofol, propofol 1-glucuronide (PG), 4-hydroxypropofol 1-glucuronide (1-QG), 4-hydroxypropofol 4-glucuronide (4-QG) and 4-hydroxypropofol 4-sulfate (4-QS) in urine by LC–MS/MS analysis. The method employs a simple dilute-and-analyze sample preparation with stable isotope internal standardization.
Results: Validation studies demonstrate a linear calibration model (100–10,000 ng/mL), with dilution integrity verified for the extended range of concentrations experienced in propofol use. Criteria-based validation was achieved, including an average coefficient of variation of 6.5 % and a percent bias of −4.2 ng/mL. The method was evaluated in 12 surgical patients, with monitoring periods lasting up to 30 days following intravenous propofol administrations of 100–3000 mg on the day of surgery. While the concentration ratio of PG to 4-hydroxy propofol metabolite decreased significantly in the days following surgery, PG maintained the highest concentration in all specimens. Both PG and 1-QG were detectable throughout the monitoring periods, including in a patient monitored for 30 days. Lower concentrations were determined for 4-QG and 4-QS, with evidence of detection up to 20 days. Propofol was not detectable in any urine specimens, thereby proving ineffective for identifying drug use.
Conclusion: The validated method for quantifying propofol metabolites demonstrates its applicability for the sensitive detection of propofol misuse over a long window of drug-use detection.

1. Introduction

Gas and liquid chromatographic methods of analysis, with varying detection techniques, have been instrumental in our understanding and use of propofol (2,6-diisopropylphenol, Diprivan®) as an intravenous anesthetic agent that was first synthesized in 1973 and introduced into practice in the late 1980s [1]. Chromatographic methods for quantifying propofol in blood and plasma have been widely applied in the pharmacokinetic determination of assessments, such as time-concentration profiles, half-life, volume-of-distribution, plasma binding, and drug clearance following the intravenous administration of propofol [2–5]. These assessments have served as the foundation for the pharmacokinetic models used in clinical practices. Our current understanding of propofol metabolism is largely based on the increasing use of high performance liquid chromatography (HPLC) analysis. The first HPLC study of propofol metabolites, in 1987, revealed major conjugated

Abbreviations: PG, propofol 1-O-glucuronide; 1-QG, 4-hydroxypropofol 1-O-glucuronide; 4-QG, 4-hydroxypropofol 4-O-glucuronide; 4-QS, 4-hydroxypropofol 4-O-sulfate; P-D17, propofol d17; PG-D17, Propofol-d17 1-O-β D-Glucuronide; UGT, uridine diphosphate glucuronosyltransferase enzymes; CYP, cytochrome P450 enzymes; SULF, sulfatase enzymes; HPLC, high performance liquid chromatography without detector specified; LC–MS/MS, high performance liquid chromatography interfaced with tandem mass spectrometry.

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metabolites of propofol, including propofol glucuronide (PG), along with the glucuronide and sulfate conjugates of 4-hydroxy propofol [6]. The structures of these four conjugated metabolites were later confirmed by proton nuclear magnetic resonance spectroscopy [7]. Fig. 1 illustrates the major biotransformation pathways of propofol in humans, which include direct glucuronidation of the administered drug via uridine diphosphate glucuronosyltransferase catalysis, and a separate pathway via cytochrome P450 catalysis to hydroxylpropofol with subsequent phase 2 conjugation to glucuronides and sulfate. Minor amounts of other phase 2 metabolites have been reported, such as direct sulfation of propofol to propofol sulfate, as verified by HPLC interfaced with high-resolution mass spectrometry [8], and alternate hydroxylation conjugates identified by HPLC – tandem mass spectrometry (LC-MS/MS), though their structural characterization remains to be confirmed [9].

HPLC methods have also been used to study physiological factors that modulate propofol metabolism. Extrahepatic glucuronidation of propofol has been shown in studies during the anhepatic phase of liver transplantation [10,11]. Correlating evidence of extra-hepatic metabolism of propofol to PG has been demonstrated directly in the kidney, intestines, lungs, and brain tissue [12–17]. This knowledge of extrahepatic metabolism has led to an important pharmacokinetic conclusion: rapid post-dose propofol inactivation occurs in organ systems throughout the body [18]. The additional importance of renal clearance is evident in renal failure studies, where HPLC-based measurements of propofol and its metabolites in blood have shown an increase in propofol's volume-of-distribution and in the accumulation of propofol metabolites [19]. HPLC analysis has also shown that propofol metabolism in neonates differs from adults, with a predominance of metabolism to the 4-hydroxypropofol conjugates in the early post-natal period. This suggests limitations in glucuronidation capacity during the early neonatal period [20–22]. Therefore, it is clear that the application of HPLC methods of analysis has revealed significant information regarding propofol metabolism throughout the body and the prominent role of renal clearance.

Advances in our understanding of propofol kinetics, along with progress in sensitive chromatographic analysis, are directly applicable to the current need for identifying and monitoring propofol misuse. While it is not the most common form of drug abuse, propofol misuse has gained notoriety due to highly publicized celebrity fatalities [23]. A recent review of propofol diversion and misuse by 88 healthcare professionals reveals that administering propofol outside of a controlled medical setting is frequently lethal [24]. The study indicates that anesthesiologists and certified registered nurse anesthetists are often the ones misusing the substance. A surprising and concerning finding of this study was that a fatal outcome was often the primary means of discovering propofol misuse. While workplace monitoring for other drugs of abuse is standard in the private employment sector and is mandated for federal workers in the United States [25], monitoring for propofol misuse in a medical population with a known incidence of misuse is rare, yet clearly necessary. Numerous methods for propofol testing in hair have been reported, aiming for a long detection period to identify drug misuse [26–30]. However, the method of hair sample collection and processing limits widespread use in most clinical and forensic toxicology laboratories. It is predicted that sensitive HPLC methods, specifically LC-MS/MS methods, of urine testing may also provide a prolonged post-dose interval of drug-use detection. This prediction is based on clinical pharmacokinetic studies and models that show a slow phase of propofol elimination from third compartment tissues like adipose and muscle [4,18]. This prediction of slow tissue release has been directly confirmed in medical examiner casework [31]. As metabolism with renal clearance is the primary route of elimination for propofol released from tissue stores, experience by others in urine metabolite monitoring for the glucuronides of propofol and 4-hydroxypropofol has demonstrated a detection window exceeding 60 h [32]. A case study monitoring only propofol glucuronide in a single patient suggests a much longer interval of detection [33].

The aim of this work is to validate a sensitive and routinely adaptable LC-MS/MS method for quantitative co-monitoring of the four major metabolites of propofol in urine, for application in either clinical or forensic toxicology laboratories as an aid in detecting and monitoring compliance for propofol misuse. What follows is a report on the methodology, validation studies, and an initial monitoring evaluation in surgical patients receiving intravenous propofol for anesthesia.

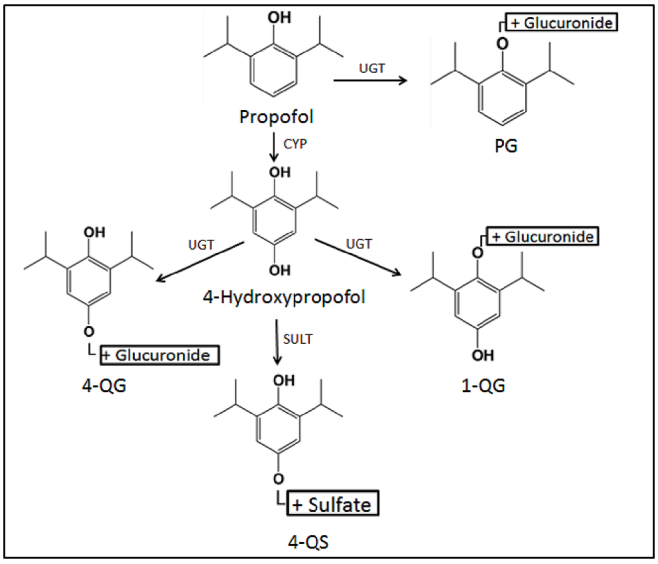

Fig. 1. Biotransformation of propofol to its major conjugated metabolites via direct glucuronidation or hydroxylation with additional glucuronidation or sulfation.

2. Materials and methods

2.1. Reference materials and reagents

Ammonium fluoride (Sigma-Aldrich) and HPLC grade methanol (Fisher Scientific) were used. Certified reference materials for propofol, propofol-d17 (P-D17) and propofol 1-O-β D glucuronide (PG) were from Cerilliant and propofol 1-O-β D glucuronide D17 (PG-D17), 4-hydroxypropofol-1-O-β glucuronide (1-QG), 4-hydroxypropofol-4O-β −glucuronide (4-QG) and 4-hydroxypropofol-4-sulfate (4-QS) were from Toronto Research Chemicals. Ammonium fluoride (1 mM) solutions in water and methanol were prepared for use as mobile phase-A (MP-A) and mobile phase-B (MP-B), respectively. A diluent (starting MP) was prepared with 1 mM ammonium fluoride in water and methanol (80:20). Reference standard and quality control (QC) stock solutions of analytes, supplied or prepared in methanol at concentrations of 0.1 mg/mL for PG and 1.0 mg/mL for P, 1-QG, 4-QG, and 4-QS were used to prepare stock multi-analyte calibrator and QC solutions (10 μg/mL) in methanol. The stock multi-analyte calibrator solution was diluted in analyte-negative urine as needed to prepare working calibrators at 0.1 (lower limit of quantitation; LLOQ), 0.5, 1.0, 2.0, 5.0, 10.0 (upper limit of quantitation; ULOQ) μg/mL concentrations. Working QC samples (0.2, 0.7, 8.0 μg/mL) were prepared from the stock multi-analyte QC solution with dilution in analyte-negative urine. Stock solutions (0.1 mg/mL) of P-D17 and PG-D17 were diluted 1:250 with starting MP to prepare an internal standard diluent reagent containing 0.4 μg/mL of each internal standard.

2.2. Clinical samples

To determine post-dose detection window sensitivity at a LLOQ cut-off concentration of 0.1 μg/mL, we analyzed urine samples obtained from 12 consenting patients who received propofol on Day 1 as part of

their surgical treatment at the Albany Medical Center Hospital. Total induction and maintenance infusion dose ranged widely from 100 to 3000 mg depending upon need to achieve anesthesia by bolus injection with or without maintenance of anesthesia by continuous infusion. Urine specimens were collected on Day 1 and daily thereafter, when available, until discharged from the hospital with the number of post-surgery days to discharge ranging from 1 to 30 (mean 8.1). The study protocol, including a consent form completed in all cases, was reviewed and authorized by the Institutional Review Board of Albany Medical College.

2.3. Propofol and propofol metabolite assay

Samples were prepared for analysis by adding 20 μL of urine calibrators, urine control, patient urine specimen, or diluted patient urine to a 96-well plate (Waters, 2-mL square well plates), followed by addition of 200 μL of internal standard diluent. Diluted patient samples were prepared in analyte negative urine for samples with analyte concentration exceeding ULOQ, with final concentration calculated by the dilution correction factor.

LC-MS/MS analysis was performed with a Waters ACQUITY UPLC I-Class (FTN) system interfaced with a Xevo® TQD tandem mass spectrometer detector. Chromatography was performed using a Waters ACQUITY UPLC BEH C18, 1.7 μm (2.1 × 50 mm) with a column temperature of 30 °C. Gradient elution conditions were: 20 % MP-B (0–0.2 min), 20–90 % MP-B (0.2–3.0 min), 90 % MP-B hold (3.0–3.8 min) and 20 % MP-B hold (3.8–4.0 min). Injection volume was 10 μL. Mass spectrometry was performed in electrospray negative ionization mode using the following conditions: source temperature 150 °C, capillary voltage 2.4 kV. Nitrogen was used as the desolvation gas (at 800 L/h, 500 °C) and as cone gas (at 10 L/h). Multiple reaction monitoring (MRM) conditions for analytes and internal standards are shown in Table 1. Acquisition data was processed using Waters TargetLynx™ software.

2.4. Method validation

The method was evaluated with reference to guidelines from the American Academy of Forensic Science Standards Board [34] and the New York State Department of Health's Clinical Laboratory Standards [35]. Minimum performance criteria established in the validation plan included precision (% coefficient of variation (CV) < 20 %); bias (within ± 20 %); weighted (1/x) least squares linear regression calibration ($r^2 \geq$ 0.999 and calibrator residuals within 20 %); carryover (negative control response following ULOQ calibrator analysis plus procedure requirement for repeat testing of positive case samples following a sample with a concentration greater than the ULOQ); matrix effect (acceptable detection sensitivity evaluation for matrix effect > ±20 %); processed sample stability (24 h stability within 20 % precision limits); 100-fold dilution integrity (within 20 % of target) and interference (<25 % of analyte LLOQ response for negative urine matrices, internal standards, and commonly encountered analytes). Interference was evaluated with other licit medications and illicit drugs, along with their metabolites, commonly tested at the National Toxicology Center. Matrix effects for each analyte and internal standard were determined in 12 analyte-negative matrices along with a matrix-free reference sample of water. Two multi-analyte and multi-internal standard reference materials were prepared in methanol at concentrations of 0.15 μg/mL and 1.0 μg/mL. A multi-internal standard reference material was prepared in methanol at concentrations of 0.4 μg/mL. Aliquots (20 μL) of the standard and internal standard preparations were then diluted with 160 μL of starting mobile phase in multiple wells, followed by the addition of 20 μL aliquots of the matrix-free sample (water) and separate well additions of 20 μL aliquots of each of the 12 analyte-negative matrix urine samples. The matrix (A) and non-matrix (B) samples were analyzed by the LC-MS/MS method, and the matrix effect was determined from the quantifier ion response using the formula ((A/B) −1) x 100. Criteria included matrix effect within ± 20 % or detection sensitivity at 150 % of LLOQ when matrix effects exceed ± 20 %.

Table 1
MS/MS ion acquisition conditions for MRM analysis of propofol analytes and internal standards.

Analytes and Internal standards	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Cone Voltage (V)	Collision Energy (eV)
PG	353.1	177.1 (quantifier)	40	29
		113.0 (qualifier)	40	16
1Q-G	369.1	193.1 (quantifier)	40	25
		113.0 (qualifier)	40	17
4Q-G	369.1	193.1 (quantifier)	40	25
		113.0 (qualifier)	40	17
4Q-S	273.1	193.1 (quantifier)	50	25
		150.1 (qualifier)	50	33
PG-D17 (Internal standard for PG, 1-QG, 4-QG, 4-QS)	370.1	194.1	40	30
Propofol	177.1	177.1 (quantifier)	50	3
P-D17(Internal standard for propofol)	194.1	194.1	50	3

3. Results and discussion

3.1. Assay performance

A chromatographic separation method was developed for optimized analyte selectivity, and an ammonium fluoride buffer was used in the chromatography mobile phase to increase ion response sensitivity, as reported by others [36]. A representative calibrator analysis in Fig. 2 shows the baseline resolution of each analyte, with the earliest elution of the least lipophilic glucuronide conjugates of 4-hydroxypropofol. This is followed by the progressively lipophilic sulfate conjugate and propofol glucuronide elutions. Propofol, due to its highly lipophilic properties — which necessitate intravenous administration in an emulsion formulation — is the last eluting peak. Chromatographic interferences were evaluated but not found in either analyte-negative urine or the internal standard preparation. Representative peak integration for analyte and internal standard MRM chromatograms, along with a representative calibration curve using TargetLynx™ software, is shown in Fig. 3. At the time of assay development, only two stable isotope labelled internal standards were available. Analytes were assigned to internal standards as shown in Table 1, based on similarities in matrix effects and then by retention time.

Validation of precision for all analytes was achieved, and the statistical analysis is shown in Table 2. The percent relative standard deviation (%CV) was determined at low (5.6–13.4 %), medium (7.2–9.9 %) and high (1.9–2.5 %) analyte concentrations based on within and

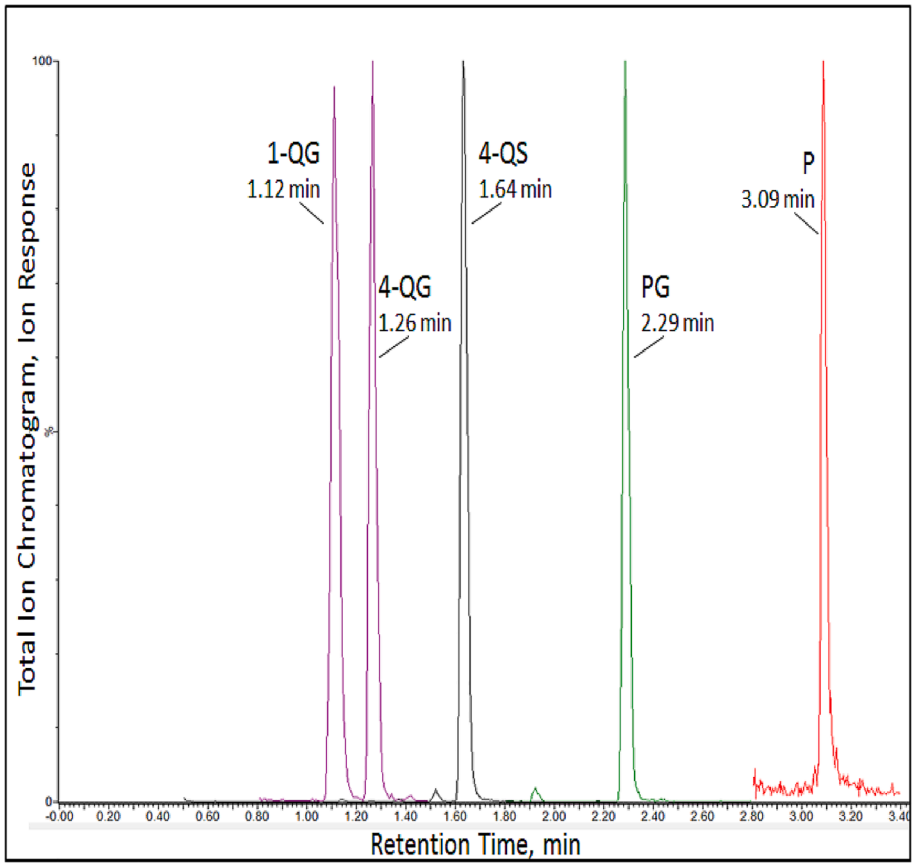

Fig. 2. Chromatographic separation of propofol and its metabolites for a multi-analyte calibrator (2.0 μg/mL).

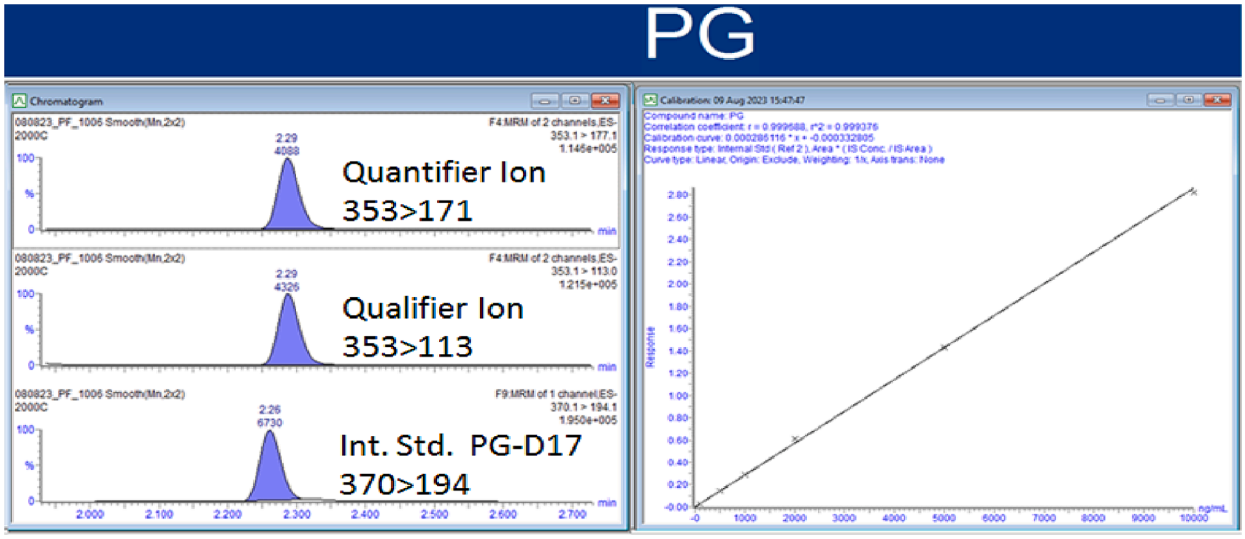

Fig. 3. Integrated MRM chromatograms (left panel) and linear regression calibration curve (right panel) for PG, with PG-D17 use as an internal standard. Ion responses for the analytes were calculated relative to the response for the internal standards. Concentration on the calibration curve is presented in ng/mL units.

between run replicate analyses (n = 12) of quality control pools over five analytical runs. Assay bias met criteria in the same study of the control pools and averaged 1.2 % with a range of −13.4 to 13.6 % (Table 3). In addition, a weighted (1/x) least-squares linear regression analysis of calibrators yielded $r^2 \geq 0.999$ with calibrator residuals within 20 %; carryover studies of the negative control demonstrated less than 25 % of LLOQ response following ULOQ calibrator analysis; processed sample stability after 24 h showed control analyses within 20 % of original analysis; matrix effect demonstrated acceptable detection sensitivity evaluation for matrix effect > ±20 %; 100-fold dilution integrity verified within 20 % of target and interference based on < 25 % of analyte LLOQ response for negative urine matrices, internal standards, and commonly encountered analytes was not found. Interference was evaluated with other drugs and metabolites commonly tested at the National Toxicology Center. Matrix effects with analyte addition (1.0 μg/mL) or internal standard addition (0.4 μg/mL) in water versus in analyte/

Table 2
Precision of propofol and metabolite analysis by LC–MS/MS.

	QC Pool Precision, %CV		
Analytes	0.20 μg/mL	0.7 μg/mL	8.0 μg/mL
PG	7.4	8.3	1.9
1-QG	6.9	7.2	2.0
4-QG	13.4	9.1	2.0
4-QS	9.9	8.7	2.0
P	5.6	9.9	2.5

Table 3
Bias assessment of propofol and metabolite analysis by LC-MS/MS.

	Mean QC Concentration, μg/mL (% Target Bias)		
Analytes	0.20 μg/mL	0.70 μg/mL	8.0 μg/mL
PG	0.19 (−4.1 %)	0.61 (−13.4 %)	8.3 (4.3 %)
1-QG	0.22 (7.9 %)	0.68 (−3.3 %)	8.3 (4.3 %)
4-QG	0.23 (13.6 %)	0.62 (−11.6 %)	8.1 (1.6 %)
4-QS	0.21 (3.0 %)	0.72 (2.8 %)	8.6 (7.7 %)
P	0.23 (13.3 %)	0.73 (3.8 %)	7.6 (−4.8 %)

internal standard addition in 12 analyte-negative urine specimens were determined. Average matrix effect results for propofol (1.9 %), P-D17 (−4.4 %), PG (13.7 %), PG-D17 (4.9 %), 1-QG (−12.0 %), 4-QG (0.2 %), and 4-QS (27.0 %) were within the ± 20 % criteria except for 4-QS, and matrices supplemented with a 0.15 μg/mL concentration of the analytes affirmed detection sensitivity in the analysis.

3.2. Clinical studies

Our validated LC-MS/MS method for monitoring propofol metabolites was applied in serial urine specimens obtained from 12 patients receiving propofol for surgical anesthesia. Monitoring was conducted from the day of drug administration (surgery) to hospital discharge, where possible. Table 4 summarizes the mean and range of metabolite concentrations determined for samples collected each day for the first seven days, with weekly averaging of concentrations thereafter. Both the mean and range of concentrations for all metabolites, but especially PG, declined rapidly in the first three to four days after propofol administration, with a slower yet parallel decline over the following weeks. PG maintained the highest metabolite concentration throughout the monitoring periods for all patients, generally followed in order by 1-QG, 4-QG, and 4-QS concentrations. The range of concentrations during the monitoring period was greatest for PG, with a maximal decline of over 3 log orders found between the times of surgery to discharge. However, unmetabolized propofol was not detectable in any urine samples, even in the early post-surgery period. This is consistent with the low to undetectable concentrations reported by others [6,37] and the highly lipophilic properties of the drug.

Propofol was not detected (n.d.) in any of the patient samples, with a detection limit of 0.1 μg/mL. Profiling individual patient metabolite levels over time enhances our understanding of the detectable window of drug use achievable with metabolite monitoring. The metabolite concentration profile over a 30-day post-dose period for the patient with the longest post-surgical hospitalization is presented in Fig. 4. Metabolite concentrations, plotted on a log scale, show both the early rapid decline and the slow terminal phase of metabolite clearance that continues for weeks after drug administration. This profile demonstrates that PG is detectable throughout a full 30-day monitoring period, consistent with a case report by others using PG monitoring only [33]. Extended monitoring over time in the post-dose period for the additional 4-hydroxypropofol conjugate metabolites has not been previously reported and indicates a parallel clearance and concentration decline for the three metabolites generated through the P450 pathway. Metabolite 1-QG is consistently the highest when comparing the P450 metabolite concentration, and the patient profile indicates that 1-QG monitoring may also be detectable throughout the 30-day monitoring period, suggesting a detection window similar to PG. Detection windows for 4-QG (20 days) and 4-QS (19 days) are also evident in the patient's metabolite profile. The relative metabolite clearance pattern observed for creatinine-normalized concentration (data not shown) was similar to the pattern shown in Fig. 4, due to a narrow intra-individual range in urine creatinine concentration. A progressive decline in metabolite levels toward the analytical detection limit is better determined and shown with actual concentration data, as displayed. As a correlating note, a prolonged detection window has also been known with metabolites of other lipophilic drugs. Δ9-tetrahydrocannabinol, for example, is another lipophilic drug that exerts its effects on the central nervous system within minutes to an hour after administration but requires days to weeks for clearance from body stores. Furthermore, it is important to note that detection windows are not fixed and may vary with the dose

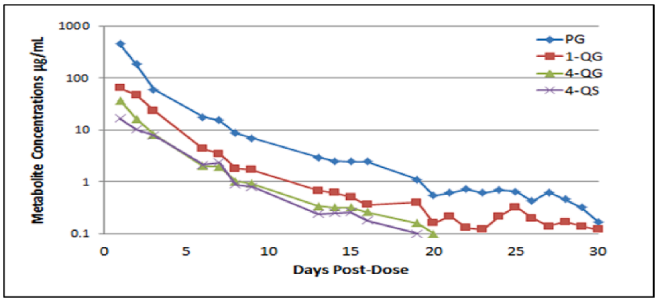

Fig. 4. Concentration profiles (log scale) of propofol metabolites over 30 days following surgical administration of propofol in a study patient with a long post-surgery hospitalization.

Table 4
Mean and range of propofol metabolite concentrations during the first 7 days and following weeks in 12 patients administered propofol for surgical anesthesia.

	Concentration, μg/mL							
Urine Collection	PG		1-QG		4-QG		4-QS	
	mean	range	mean	range	mean	range	mean	range
Day 1	350	58–840	59	13–132	40	8.6–97	28	8.7–93
Day 2	71	21–180	22	5.8–63	12	3.7—39	11	2.3–36
Day 3	28	6.3–92	14	1.3–52	7.8	0.92–35	5.5	0.52–21
Day 4	9.7	2.6–26	5.8	0.86–15	3.2	0.81–9.8	3.7	0.8–10
Day 5	5.9	0.32–18	2.8	0.18–6.3	1.8	0.05–4.0	1.7	0.05–4.2
Day 6	5.7	1.4–18	2.3	0.36–5.3	1.4	0.36–3.5	1.6	0.21–3.8
Day 7	5.3	1.4–16	1.6	0.36–3.5	0.91	0.36–2.0	0.88	0.21–2.4
Week 2	3.7	0.17–8.7	0.92	0.04–1.8	0.52	0.06–1.0	0.4	n.d.–0.89
Week 3	1.2	0.54–2.5	0.33	0.16–0.51	0.17	n.d.–0.32	0.11	n.d.–0.26
Week 4	0.34	0.27–0.7	0.19	0.12–0.33	n.d.	n.d.–n.d.	n.d.	n.d.–n.d.
Week 5	0.25	0.17–0.3	0.13	0.12–0.14	n.d.	n.d.–n.d.	n.d.	n.d.–n.d.

and frequency of drug use and the sensitivity of the monitoring method. However, the findings in this study indicate that monitoring major propofol metabolites in urine may be an effective aid in detecting propofol use in the days and weeks following therapeutic administration or drug misuse.

While current knowledge of propofol pharmacokinetics is extensive, this study provides new insight into the relative rates of drug clearance by direct glucuronidation versus the P450 metabolic pathways in the days following administration. General knowledge of propofol metabolism and renal clearance, based largely on studies performed within the first 24 h after administering propofol, suggests that 70 % of renal-cleared propofol is excreted as PG, while the P450 pathway accounts for less than 30 % of renal excretion [1,7]. In our study, we compared the contribution of each pathway daily from the day of surgery to hospital discharge. Relative clearance was based on the relative concentrations of PG to total metabolites and a similar calculation of total P450 metabolites (sum of 1-QG, 4-QG, and 4-QS concentrations) to total metabolites. The left panel of Fig. 5 shows the individual patient profiles of the relative contribution to clearance for PG over the first five-day period. A progressive reduction in percent clearance as PG was observed in the days following drug administration. Conversely, the study of P450 metabolite conjugates, displayed in the right panel of Fig. 5, shows an increasing contribution of the P450 pathway over the same period. The data for all patients was statistically evaluated and is graphically displayed in Fig. 6. The relative PG pathway clearance averaged 70 % on the day of drug administration but was reduced to less than 50 % on days four and five, with statistically significant post-surgery day reduction determined for days two through five. The study shows that by the fifth day post-dose, the P450 route of metabolism contributes to over 60 % of renal drug clearance in some patients.

This shift in metabolite clearance pathways suggests that the hydroxylation of propofol via the P450 metabolic pathway is increasing in the days following drug administration. This shift might also initiate during day 1, according to a study of propofol metabolite monitoring in urine collected in timed intervals during the first 24 h [38]. That study found that the PG contribution to drug clearance progressively declined as the sum of 1-QG, 4-QG, and 4-QS concentrations rose. Specifically, the contribution shifted from 33.7 % in the first 0–4 h collection interval to a 45.1 % contribution in the 12–24 h period following propofol administration. While the mechanism underlying this shift towards greater P450 metabolites is not currently known, a plausible hypothesis is a propofol-induced, concentration-dependent inhibition or saturation of the P450 metabolism pathway in the earlier post-infusion periods. This inhibition would then be reduced as the circulating concentration of propofol, or free propofol, declines through drug distribution and

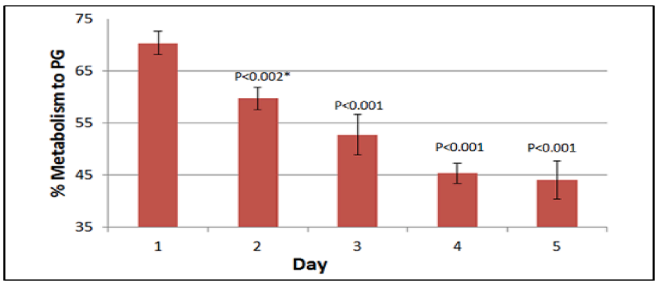

Fig. 6. All patients mean percent (±SD) contribution of PG metabolism to propofol renal clearance during the first 5 days. *Significant difference for data from each of days 2 through 5 compared to day 1 was determined by P value < 0.05 based on student's *t*-test analysis.

metabolism in the hours and days after drug administration. There are multiple reports of dose-dependent inhibitory effects of propofol on cytochrome P450 activity [39–42]. These inhibitory effects may occur with the gram-level dosing of propofol used in surgical procedures or in cases of drug misuse. The current findings of post-dose alterations in renal clearance of metabolites underscore the ongoing importance of chromatographic analysis. This analysis is now routinely interfaced with mass detection, providing enhanced sensitivity with molecular mass and fragmentation information.

Importantly, throughout our LC-MS/MS analysis of the patient samples, we consistently detected unknown ion chromatogram peaks consistent in molecular weight and collision-induced dissociation with hydroxypropofol glucuronide metabolites. These findings suggest that renal clearance of propofol may involve the clearance of additional metabolites. A representative chromatogram from an analysis of patient urine is shown in Fig. 7. Besides demonstrating the selective separation and identification of the four currently recognized metabolite conjugates of propofol and the consistent absence of propofol, the figure shows additional chromatographic peaks with a negative ion mass of 369.1 atomic mass units. The molecular and fragment ions determined for these structurally unknown metabolites are consistent with the elemental composition and fragmentation ions of additional hydroxylpropofol glucuronide metabolites. These appear, by mass spectroscopy analysis, to be constitutional isomers similar in elemental composition to the 1-QG and 4-QG isomers, but with hydroxylation on alternative propofol ring or side chain positions. The ion abundance of the additional metabolites detected in patient urine is low relative to the known 4-hydroxypropofol glucuronides, suggesting that they may be minor metabolites. Hydroxylation of a methyl group on an isopropyl side chain

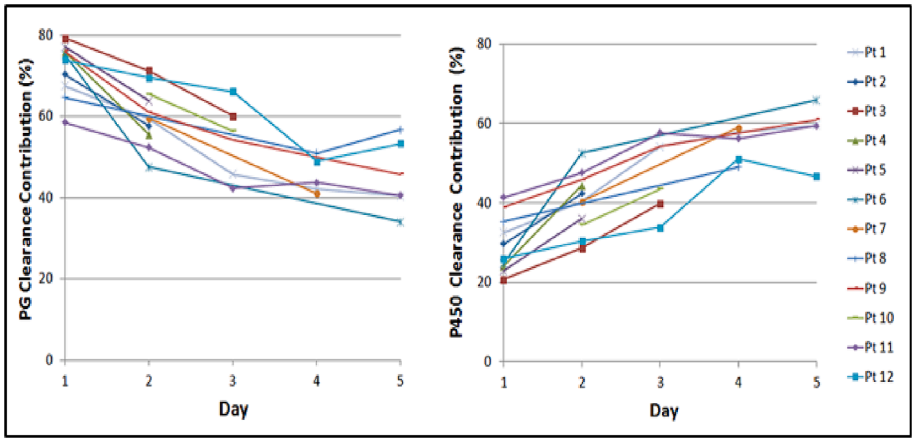

Fig. 5. Patient profiles of propofol renal contributions of PG (left panel) and conjugated P450 metabolite (right panel) on days one through five, based on concentration with percent contribution calculated for PG [PG/(sum of PG, 1-QG, 4-QG, 4-QS) x100] and total P450 metabolites [(sum of 1-QG, 4-QG, 4-QS)/(sum of PG, 1-QG, 4-QG, 4-QS) x100].

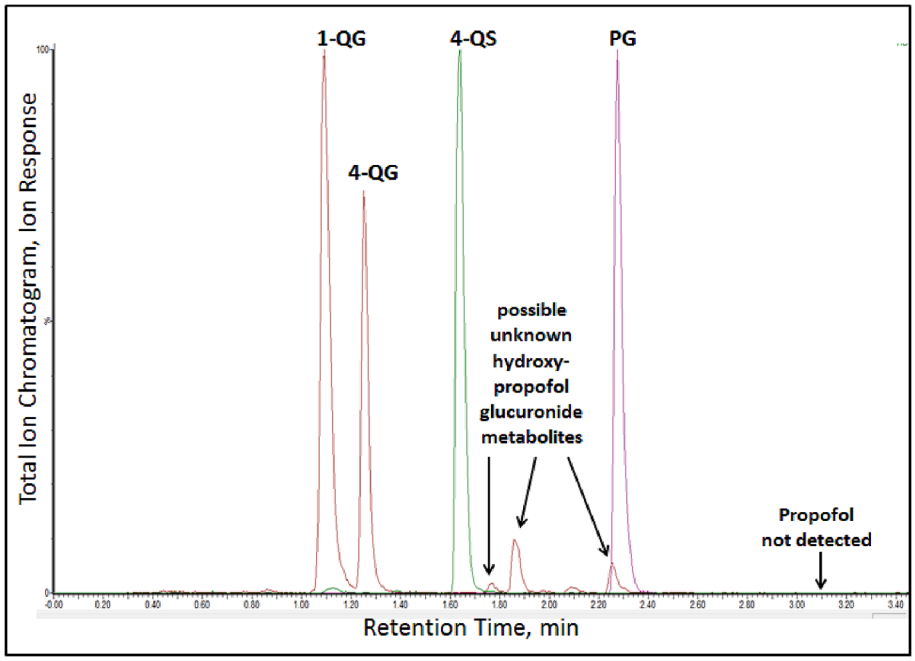

Fig. 7. Representative total ion chromatogram of urine propofol and metabolite analysis in a patient sample showing the major propofol metabolites and unknown metabolite peaks.

of propofol has been reported by others [9], but further work is required to structurally identify and quantify these additional propofol metabolites.

4. Conclusions

A sensitive and selective method for analyzing propofol and its major metabolites has been developed and validated for urine drug testing aimed at investigating propofol misuse. The assay has been validated based on current standards of practice, making it applicable to both forensic and clinical testing. The method's application to serial samples collected days and weeks after propofol administration for surgical procedures reveals a detection window of 30 days for PG or 1-QG monitoring, and 19–20 days for 4-QG and 4-QS. Moreover, the method of co-monitoring metabolites has unveiled changes in metabolite clearance patterns in the early post-dose period and the presence of additional, unknown metabolites involved in propofol elimination.

Ethics statement

The study protocol, including a consent form completed in all cases, was reviewed and authorized by the Institutional Review Board of Albany Medical College.

CRediT authorship contribution statement

Thomas G. Rosano: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Michelle Wood:** Conceptualization, Methodology, Writing – review & editing. **Kiley L. Scholz:** Conceptualization, Methodology, Validation, Writing – review & editing. **Kiera Whitely:** Conceptualization, Investigation, Visualization. **Nathaniel Kim:** Investigation. **Melissa Ehlers:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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