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Direct high-performance liquid chromatography determination of propofol and its metabolite quinol with their glucuronide conjugates and preliminary pharmacokinetics in plasma and urine of man

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Abstract

Propofol (P) is metabolized in humans by oxidation to 1,4-di-isopropylquinol (Q). P and Q are in turn conjugated with glucuronic acid to the respective glucuronides, propofol glucuronide (Pgluc), quinol-1-glucuronide (Q1G) and quinol-4-glucuronide (Q4G). Propofol and quinol with their glucuronide conjugates can be measured directly by gradient high-performance liquid chromatographic analysis without enzymic hydrolysis. The glucuronide conjugates were isolated by preparative HPLC from human urine samples. The glucuronides of P and Q were present in plasma and urine, P and Q were present in plasma, but not in urine. Quinol in plasma was present in the oxidised form, the quinone. Calibration curves of the respective glucuronides were constructed by enzymic deconjugation of isolated samples containing different concentrations of the glucuronides. The limit of quantitation of P and quinone in plasma are respectively 0.119 and 0.138 μg/ml. The limit of quantitation of the glucuronides in plasma are respectively: Pgluc 0.370 μg/ml, Q1G 1.02 μg/ml and Q4G 0.278 μg/ml. The corresponding values in urine are: Pgluc 0.264 μg/ml, Q1G 0.731 μg/ml and Q4G 0.199 μg/ml. A pharmacokinetic profile of P with its metabolites is shown, and some preliminary pharmacokinetic parameters of P and Q glucuronides are given.

Keywords: Propofol; Quinol; Glucuronide

1. Introduction

Propofol (2,6-di-isopropylphenol) in soya bean oil–water emulsion is an intravenous anesthetic agent in clinical use [1–4]. The pharmacokinetics of the compound itself after a single bolus injection can be described in a three compartment model with α, β and gamma $t_{1/2}$ values of respectively 1.8, 34 and 180 min [1]. It has been suggested that the short anesthetic activity of propofol after a single bolus dose is the result of rapid metabolism [2]. Propofol-1-glucuronide (Pgluc; 40%) and the 1- and 4-glucuronide (Q1G, Q4G) of quinol (2,6-di-isopropylquinol; Q) and 4-sulphate conjugate of 2,6-di-isopropyl-1,4-quinol are described as the major metabolites in man (Fig. 1) [5]. After a subanesthetic dose of ^{14}C propofol, 88% of the isotope is excreted in the urine as metabolites and less than 0.3% is excreted as propofol [6].

Adam et al. [7] described an HPLC analysis of propofol in blood following coupling with Gibbs'

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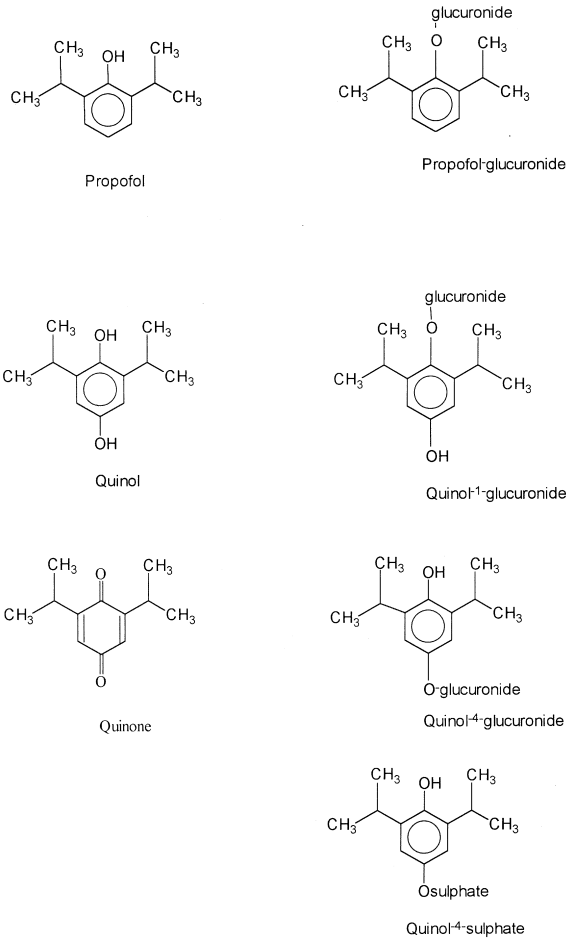

Fig. 1. Structures of propofol, its metabolites quinol and quinone with the corresponding glucuronide conjugates.

reagent. This technique has a detection limit of 0.2 μg/ml, but fails to measure the metabolites. Kay et al. [3] analysed propofol by HPLC with fluorescence detection following extraction into cyclohexane. The limit of sensitivity of this assay is approximately 2 ng/ml. Both methods are time consuming and do not include the metabolites in their analysis. A HPLC method with electrochemical detection with a sensitivity limit of 20 ng/ml was described by Mazzi and Schinella [8]. A similar detection limit was obtained with fluorescence detection [9]. Vree et al. [10] described a HPLC analysis with UV detection enabling the analysis of propofol and the metabolites quinone and quinol. At that time propofol conjugates were known to the innovators (ICI, Zeneca, UK). Metabolites of propofol were detected in urine of patients and cumulative percentages were reported [2,4–6]. The existence of propofol glucuronides in plasma and urine was demonstrated after hydrolysis of the biological sample by a few groups [11] and analysis with GC–MS of the aglycons [12,13]. A direct HPLC analysis of propofol and its glucuronide metabolites is not reported. A direct measurement must be able to discriminate between the glucuronide of propofol and the 1-glucuronide and the 4-glucuronide of its metabolite quinol.

Therefore, the aim of this study was to develop a direct gradient HPLC analysis for propofol with its quinol metabolite and glucuronide conjugates in blood and urine of man.

2. Experimental

2.1. Chemicals

Propofol (Diprivan®) was obtained from Zeneca (Ridderkerk, The Netherlands). Propofol (2,6-di-isopropylphenol, $C_{12}H_{18}O$, MW 178.27; CAS number 2078-54-8), 1,4-quinone $C_{12}H_{16}O_2$, MW 192.27, and 1,4-quinol (2,6-di-isopropyl-1,4-quinol) ($C_{12}H_{18}O_2$, MW 194.27) were obtained from Zeneca (Ridderkerk, The Netherlands).

2.2. HPLC analysis

The HPLC system consisted of a Marathon autosampler (Separations, H.I. Ambacht, The Netherlands), a Spectra Physics quaternary P4000 HPLC pump, a Spectra Physics UV 1000 UV detector, a Spectra Physics Fluorimeter FL 2000 (Spectra Physics, Thermo Separations, Breda, The Netherlands) and a Hitachi D 2500 integrator.

The column was C_{18} Spherisorb ODS 5-μm, 250×4.6 mm I.D. (Chrompack, Cat. No. 28812, Bergen op Zoom, The Netherlands) with a guard column 75×2.1 mm, packed with pellicular reversed-phase (Chrompack Cat. No. 28603).

2.2.1. Glucuronides

2.2.1.1. *Plasma and urine*. The *gradient* eluent was a mixture of 6 g/l orthophosphoric acid in water–acetonitrile with a solvent flow of 1 ml/min, at a pressure of 23.30 MPa. The gradient was 80/20 (v/v) water–acetonitrile at the start and changed in 25 min to 40/60 (v/v) and stayed for 20 min at this composition.

The injection volume was 50 μl. The chromatographic analysis was carried out at room temperature. UV detection was achieved at 270 nm. The fluorimeter operated at 270 nm excitation and 310 nm emission.

2.2.2. Propofol and quinone

2.2.2.1. *Plasma and urine*. The *isocratic* eluent was a mixture of 6 g/l orthophosphoric acid in water–acetonitrile–methanol (40/50/10, v/v/v) with a solvent flow of 1 ml/min, at a pressure of 23.30 MPa. The injection volume was 50 μl. The chromatographic analysis was carried out at room temperature. UV detection was achieved at 270 nm and the fluorimeter operated at 270 nm excitation and 310 nm emission.

The capacity factors of propofol with the metabolites are given in Table 1.

Table 2 shows the UV absorption and fluorescence excitation and emission spectra of propofol and its metabolites.

Table 1
Retention times (t_r) and capacity factor (k') of propofol, its metabolites and glucuronide conjugates

Compound	t_r min	k'	Ratio k' glucuronide/aglycon
Gradient mode			
t_0	1.1		
Hippuric acid?	7.04	5.40	
Quinol-4-gluc	10.93	8.94	0.387
Quinol-1-gluc	13.06	10.79	0.468
Propofol-1-gluc	19.09	15.78	0.636
Quinol	26.45	23.05	
Propofol	30.02	24.81	
Quinone	30.02	24.81	
Co-medication			
Bupivacaine	50.0		
PPX	>50		
4OH-bupivacaine	n.d.		
Morphine	5.6		
Morphine-3-gluc	<1		
Morphine-6-gluc	<1		
Isocratic mode			
t_0	2.0		
Quinol	8.18	3.09	
Propofol	11.82	4.91	
Quinone	12.72	5.36	

2.3. Mass spectrometry

A LCQ™ mass spectrometer (ThermoQuest, Finnigan MAT Benelux, Veenendaal, The Netherlands) was used for the identification of the glucuronides of propofol, and quinol in human urine samples. Injection volume was 50 μl. Flow-rate was 3 μl/min, volume was 250 μl. API source: source voltage 4.19 kV, source current 5.08 μA, vaporizer temp. 499.5°C.

2.3.1. HPLC for LC–MS

The gradient eluent was a mixture of 6 g/l acetic acid in water–acetonitrile with a solvent flow of 1 ml/min, at a pressure of 23.30 MPa. The gradient was 80/20 (v/v) water–acetonitrile at the start and changed in 25 min to 40/60 (v/v) and stayed for 20 min at this composition.

2.4. Isolation of the propofol glucuronides

The urine was concentrated as follows. To urine (pH 5) was added 20 g/l Celite 545 and the mixture was filtered. The compound was extracted on an XAD-2 column at pH 5 and eluted with methanol. The fractions that contained the glucuronides were evaporated to dryness by a gentle stream of nitrogen, dissolved in water, filtered and injected onto the column of the preparative HPLC.

The preparative Gilson system consisted of a Gilson 302 sample pump (Gilson, Meyvis, Bergen op Zoom, The Netherlands), two 305 Gilson gradient pumps, an 811 B Dynamic mixer, a Kratos 757 UV detector (Separations), an LKB 2211 superrac (LKB, Woerden, The Netherlands), and a BD7 recorder (Kipp & Zonen, Delft, The Netherlands). The col-

Table 2
UV absorption and fluorescence excitation–emission spectra (nm) of propofol and its metabolites

	UV_{max}		Fluorescence[a]	
			Excit	Emission
Quinol-4-gluc	265−	285	270	310
Quinol-1-gluc	268+	285	270	310
Propofol-1-gluc	260			
Quinol		282		
Propofol	270		270	310
Quinone		282		

[a] Fluorescence when one free phenolic hydroxyl group is present.
Glucuronidation UV_{max} at 265 nm; shift −10 nm.
Hydroxylation shift from 270 to 285 nm, +15 nm.

umn was a C_8 Rainin Dynamax 60 Å (250×10 mm I.D., particle size 8 μm) from Meyvis.

Concentration of the trapped samples was carried out by a IKA rotavapor (Janke and Kunkel, Staufen, Germany) equipped with a Trivac vacuum pump (Leybold-Heraeus, Woerden, The Netherlands).

2.5. Sample treatment

2.5.1. Glucuronides

Plasma samples (0.3 ml) were deproteinized with 0.3 ml 2 *M* trichloroacetic acid, centrifuged at 3000 *g*, and 50 μl of the supernatant was injected onto the column.

Urine samples were centrifuged at 3000 *g*, the supernatant was diluted 1:9 with 0.02 *M* KH_2PO_4 buffer pH 6.8 and 50 μl was injected onto the column.

2.5.2. Propofol and quinone

Plasma: to 0.3 ml of plasma were added 0.1 ml ammonium sulphate (saturated) and 0.3 ml acetonitrile. The mixture was vortexed for 30 s, and centifuged at 3000 *g* for 5 min. The organic layer was injected onto the column (50 μl).

Urine was centrifuged at 3000 *g*, the supernatant was diluted 1:9 with 0.02 *M* KH_2PO_4 buffer pH 6.8 and 50 μl was injected onto the column.

2.6. Deconjugation of the propofol glucuronides

Deglucuronidation was carried out with 100 μl of human urine containing propofol and propofol glucuronide, 100 μl of β-glucuronidase and 800 μl of 0.2 *M* (Na_2H-KH_2) phosphate buffer at 37°C for 5 h (HPLC check disappearance glucuronides as shown in Fig. 2).

Four different β-glucuronidase enzymes were tested: (a) 100 000 *u*/ml β-glucuronidase type B1 (bovine liver, Sigma, Cat. No. G-0251) and phosphate buffer pH 5.0; (b) 107 200 *u*/ml β-glucuronidase type H2 (*Helix pomatia*, Sigma, Cat No. G-0876) and phosphate buffer pH 5.0; (c) 100 000 *u*/ml β-glucuronidase type LII (lyophilized powder from limpets *Patella vulgata*, Sigma, Cat. No. G-8132) and phosphate buffer pH 3.8; (d) 20 000 *u*/ml

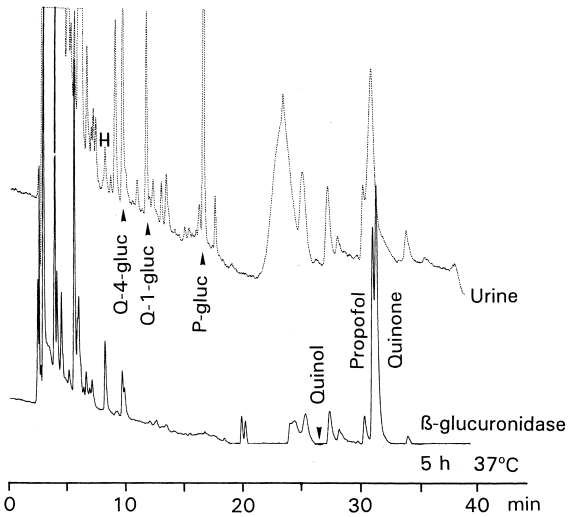

Fig. 2. Chromatogram (gradient mode) of an actual human urine sample containing propofol glucuronide (P-gluc), quinol-1-glucuronide (Q-1-gluc) and quinol-4-glucuronide (Q-4-gluc) before and after deglucuronidase (5 h, 37°C) treatment. Liberated quinol is immediately oxidised in urine to quinone. H is hippuric acid.

β-glucuronidase type VIIA (*Escherichia coli*, Sigma, Cat. No. G-7646) and phosphate buffer pH 6.8.

2.6.1. In urine

Deglucuronidation was carried out with 50 μl of diluted human urine containing propofol and propofol glucuronide, 50 μl of β-glucuronidase (20 000 *u*/ml β-glucuronidase type VIIA (*Escherichia coli*, Sigma, Cat. No. G-7646) and 400 μl of 0.2 *M* (Na_2H-KH_2) phosphate buffer pH 6.8 at 37°C for 5 h (specific β-glucuronidase, clean chromatograms and complete deconjugation). 50 μl was injected onto the column.

2.6.2. In plasma

Deglucuronidation was carried out with 100 μl of human plasma containing propofol and propofol glucuronide, 100 μl of β-glucuronidase (20 000 *u*/ml β-glucuronidase type VIIA (*Escherichia coli*, Sigma, Cat. No. G-7646) at 37°C for 5 h. Fifty μl was injected onto the column.

2.7. Calibration curves

Samples containing different concentrations of propofol glucuronide, quinol-1-glucuronide and quinol-4-glucuronide isolated from human urine by preparative HPLC were deconjugated using system D.

The increase in the concentration of propofol, quinol (aglycon) represented the concentration of the conjugate propofol glucuronide and quinol glucuronide. A calibration curve was constructed with the help of the following formula

$$[P, Qgluc] = d[P,Q] \times M_{P,Q\text{-gluc}}/M_{p,q} \quad (1)$$

where d[P,Q] is the difference in concentration of propofol, quinol after and before deconjugation and M is the relative molecular mass (all $r = 0.95$).

2.8. Limits of quantitation

The limits of detection in water and quantitation of propofol and its metabolites in plasma and urine were determined at a signal-to-noise ratio of 3, and shown in Table 3.

2.9. Recovery

A calibration curve of five concentrations (0.10–12 μg/ml) in 0.02 *M* Na_2HPO_4 was compared with a calibration curve of the same concentrations in plasma.

Recovery of propofol, quinone, propofol glucuronide, quinol-1-glucuronide and quinol-4-glucuronide from plasma was compared with that from standards treated as in 2.5.1 and 2.5.2 as shown in Table 3.

2.10. Linearity of dilution of the glucuronides

The samples of isolated glucuronides of quinol and propofol were diluted with water, 0.002 *M* phosphate buffer pH 6.8, 0.02 *M* and 0.2 *M* in order to check linearity of the diluted samples.

2.11. Reference solution

The concentration of the isolated propofol-1-glucuronide and quinol-1-glucuronide and quinol-4-glucuronide were determined after deglucuronidation applying the above mentioned formula. The concentration represents a corresponding peak height in the chromatogram.

2.12. Stock solution

A urine sample of a patient, containing suitable concentrations of the three glucuronides was considered as a stock solution. The concentration of the three glucuronides were determined by comparison of *peak heights* of the reference solution.

2.13. Stability

The stability of propofol- and quinol glucuronides in urine and plasma was tested as follows:

Urine

Stock solution: three samples of 2 ml of urine were brought to pH 5.0, 6.0 and 8.0 and incubated at 37°C for 0–6 and 24 h. Each hour a 100-μl sample was taken, and the reaction stopped with 900 μl of

Table 3
Limits of detection and quantitation and recovery of propofol, quinone, and the glucuronide conjugates in plasma and urine ($n=6$)

Compound	Detection limit (μg/ml in water)	Quantitation limit (μg/ml in matrix)
Plasma		
Propofol	0.10	0.119
Propofol glucuronide	0.10	0.370
Quinone	0.10	0.138
Quinol-1-glucuronide	0.20	1.024
Quinol-4-glucuronide	0.10	0.278
Quinol	0.01	0.014
Urine		
Propofol	0.10	0.100
Propofol glucuronide	0.20	0.264
Quinone	0.10	0.100
Quinol-1-glucuronide	0.20	0.731
Quinol-4-glucuronide	0.10	0.199
Percentage recovery from plasma ($n=6$)		
Propofol	102	
Propofol glucuronide	82	
Quinone	92	
Quinol	101	
Quinol-1-glucuronide	67	
Quinol-4-glucuronide	52	

0.01 *M* H_3PO_4. From this mixture, 20 μl were injected onto the column.

Autosampler

The stability of propofol- and quinol glucuronides in the autosampler in water and 0.01 *M* H_3PO_4 was tested during 24 h. Samples were taken every 1 h and injected onto the column.

2.14. Stability of the glucuronide reference compounds

The concentration of the reference compounds was tested monthly for 6 months and compared to the initial concentration (%).

The concentration of the glucuronide stock solutions was tested monthly for 6 months and compared to the initial concentration (%).

2.15. Intra- and inter-day variations

Intra- and inter-day coefficients of variations (% C.V.) were obtained with plasma and urine samples of known concentrations of the analytes.

2.16. Patients

Fifteen premedicated patients undergoing lung surgery gave their informed consent to participate in the study, which was approved by the hospital ethics committee.

One patient of this number of patients (male, 35 y, 85 kg) received a 3.5-g propofol infusion over a 5 h-period and was used for the pharmacokinetic figures and analysis.

2.17. Sampling

Blood samples of 3 ml were taken from an arterial cannula into blood gas syringes immediately before and after the dose of propofol at predetermined times. The blood samples were centrifuged and the plasma was stored at −20°C pending analysis.

Urine was collected over predetermined time intervals and the volume and pH measured. Urine samples were stored at −20°C pending analysis.

The total time of sample collection was 60 h. Three samples of 7 ml of each void were stored at −20°C pending analysis.

2.18. Pharmacokinetics

The pharmacokinetic parameters were calculated using the MediWare® computer package [14].

3. Results and discussion

The search for drug and metabolite glucuronide conjugates proceeds according to several steps.

The first step is to check whether glucuronides of the drug/metabolite are present in the urine or plasma, by measuring the difference in concentration before and after deglucuronidation. This step was followed by the HPLC method described before [10]. It turned out that large concentrations of glucuronides were present in the urine of a patient who received a 5 h continuous infusion of propofol (3g/5 h).

3.1. Presence of glucuronides in urine

The retention time of propofol and quinol was decreased step by step and a gradient mobile phase developed which showed compounds that were only present in the urine of patients receiving propofol.

Fig. 2 shows the chromatograms (UV detection) of a human urine sample after intravenous infusion of propofol before and after β-glucuronidase treatment. This sample shows the presence of propofol glucuronide, and two quinol glucuronides. No free propofol or quinol were present in urine.

When there is one free phenolic hydroxy group available in the propofol and quinol molecule it shows fluorescent properties. This means that propofol-1-glucuronide has lost its fluorescence, but that quinol-1-, and -4-glucuronide have retained this property. Quinone and quinol show a low fluorescent activity. Fluorescent detection was used as a control measurement for the presence of propofol (Table 2).

The presence of glucuronides in urine is demonstrated by the chemical method, but it can also be demonstrated by the pharmacokinetic method. If a series of urine samples is collected with the aim to reconstruct the renal excretion rate-time profile, it must show an absorption phase, maximum in the excretion rate, followed by an elimination phase. The three compounds identified chemically, showed the pharmacokinetic profile as shown below in Fig. 9.

3.2. Identification of the isolated glucuronide conjugates

The isolated glucuronide conjugates were hydrolysed with β-glucuronidase (system D, clear chromatograms, pure β-glucuronidase activity and 100% result). Propofol-1-glucuronide was hydrolysed to propofol (UV + fluorescence).

Quinol-1-glucuronide and quinol-4-glucuronide (UV + fluorescence) were both hydrolysed to quinol (quinone)(UV) (see Fig. 2).

3.3. Mass spectrometry

3.3.1. Isolated glucuronides

Quinol-4-glucuronide: MW + 1 = 387.9 = 100%. MS–MS of this peak revealed two fragments, m/z 193.8 of the glucuronide and m/z 194.9 of the quinol (m/z 151.2 = MW quinol-isopropyl).

Quinol-1-glucuronide: MW + 1 = 387.9 = 100%. MS–MS of this peak revealed two fragments, m/z 193.9 of the glucuronide and m/z 194.9 of the quinol. Quinol glucuronide m/z 387.9 fragments into m/z 352.5 (6%)(M-35.4), m/z 334.8 (12%) (M-35.4-18), m/z 315.3 (4%) (M-35.4-18-18).

Propofol glucuronide: MW + 1 = 371.8 = 100%. MS–MS of this peak revealed two fragments, m/z 193.8 of the glucuronide and m/z 178.9 of propofol.

Propofol glucuronide fragments into m/z 336.7 (100%)(M-35.1), 318.9 (58%) (M-35.1-18), m/z 301 (10%) (M-35.1-18-18), m/z 257 (10%) (M-114.8), m/z 193.8 (65%) glucuronide), m/z 178.9 (75%) propofol.

No difference in atomic pressure ionization mass spectra between quinol-1-glucuronide and quinol-4-glucuronide was observed.

3.3.2. Propofol glucuronides in urine

In human urine of patients, two chromatographic peaks were present with m/z 388, from which following MS–MS, the parent ion fragmented into m/z 193.9 (glucuronide) and m/z 194.9 (quinol). propofol glucuronide (m/z 372.0) fragmented into m/z 178.9 (propofol) and m/z 193.8 (glucuronide).

3.3.3. Reference solution of the glucuronides

The reference concentrations of the glucuronide compounds were: propofol glucuronide 105.7 μg/ml, quinol-1-glucuronide 1172 μg/ml, and quinol-4-glucuronide 193.7 μg/ml.

3.3.4. Stock solutions

The stock concentrations of the glucuronide compounds were: propofol glucuronide 105.7 μg/ml, quinol-1-glucuronide 292.5 μg/ml, and quinol-4-glucuronide 79.4 μg/ml.

3.3.5. Calibration curves

The calibration curves of isolated quinol-1-glucuronide and quinol-4-glucuronide differed in their slope as shown in Fig. 3.

The quinol glucuronide with the calibration curve identical to that of propofol-1-glucuronide was identified as quinol-1-glucuronide.

3.3.6. Identification of quinol glucuronides by group contribution to retention behaviour.

Table 1 shows the retention times and capacity factors of propofol, the quinol metabolite and the corresponding glucuronides. Quinol-1-glucuronide was identified due to analogy in retention behaviour to propofol glucuronide.

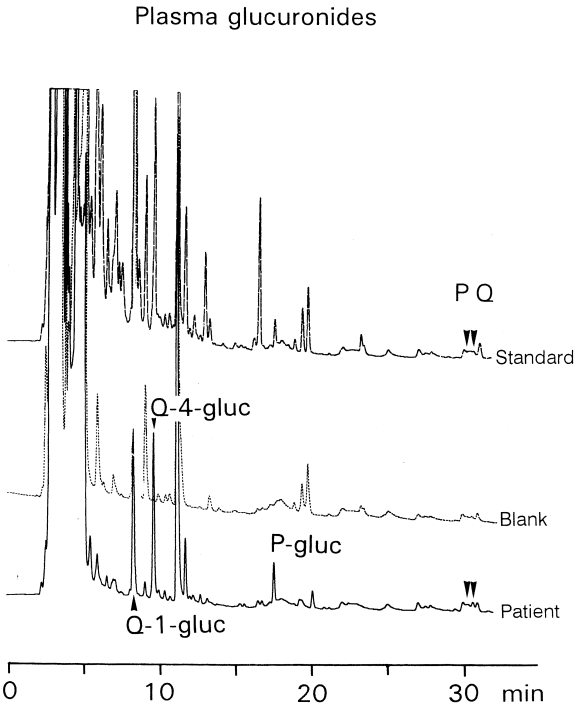

Fig. 4. Chromatogram (gradient mode) of an actual human plasma sample ($t=3$ h) containing propofol glucuronide (P-gluc), quinol-1-glucuronide (Q-1-gluc) and quinol-4-glucuronide (Q-4-gluc). Propofol (P) and quinone (Q) are not visible due to protein precipitation with trichloracetic acid (TCA).

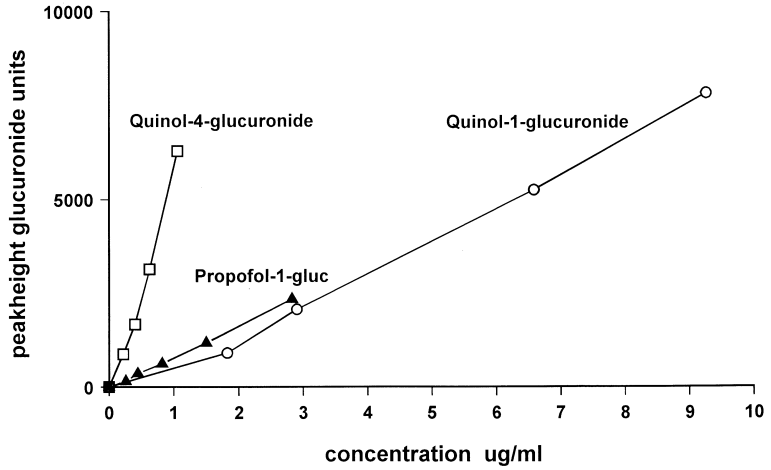

Fig. 3. Calibration curves of propofol glucuronide, quinol-1-glucuronide and quinol-4-glucuronide in urine. Similarities in calibration curves between propofol-1-glucuronide and quinol-1-glucuronide are an indirect proof of structure similarity.

Thus retention behaviour and molar extinction coefficient (calibration curves) revealed the difference between quinol-1- and quinol-4-glucuronide.

3.3.7. Linearity of the dilution of the glucuronides.

Dilution of propofol- and quinol glucuronides in water resulted in a non-linear calibration curve. Dilution in phosphate buffer resulted in linear calibration curves; the phosphate buffer 0.02 *M* pH 6.8 gave the best results for linearity and peak height, and thus reduced the saltload in the column.

3.2. Presence of glucuronides in plasma

When the glucuronide conjugates are present in urine samples, it must be investigated whether they are also present in plasma. Gradient HPLC with UV detection at high sensitivity able to detect anticipated low plasma concentrations of the glucuronides may reveal many interfering endogenous compounds (Fig. 4). High sensitivity fluorescence detection selectively revealed the presence of the quinol-glucuronides. When the plasma concentrations of the glucuronides are high, the plasma concentrations of propofol and quinone are low. With gradient analysis, propofol and quinone are not well separated. For this reason the isocratic HPLC analysis was used (Fig. 5) as published earlier [10].

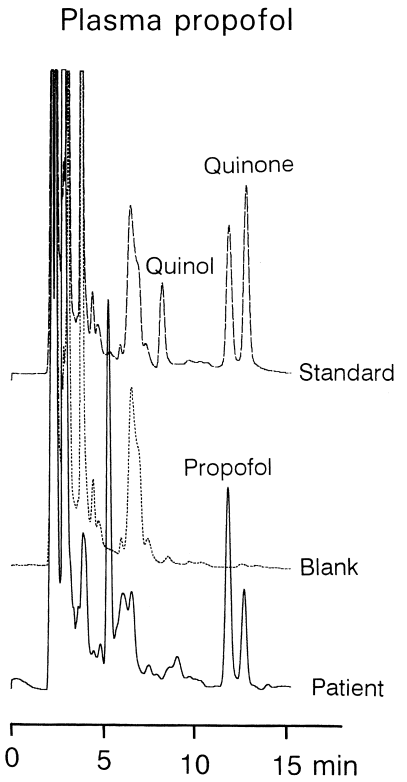

Fig. 5. Chromatogram (isocratic mode) of an actual human plasma sample (+human blank; human blank+standard) containing propofol, quinol and quinone. Quinol is immediately oxidised in plasma to quinone.

3.2.1. Stability of propofol, quinol and quinone in plasma

Fig. 6 shows that propofol and quinol, when added to fresh and old plasma were stable during the test period of 6 h. Quinone, when added to fresh plasma, decomposed during 6 h to 20.3% of the original concentration, and to 50% when added to 'old' plasma (Fig. 7). The figures shows that the decomposing enzyme or factor wears off in activity over time. The glucuronides in plasma and urine are stable for at least 6 months.

Table 4 shows the intra- and inter-day variations of propofol and its metabolites in plasma and in urine.

Fig. 8 shows the plasma concentration–time curve and Fig. 9 also the renal excretion rate-time profiles of propofol with its quinol metabolite and their respective glucuronide conjugates in one exemplary patient after a 5-h infusion with 3510 mg of propofol.

Excess quinol that is not immediately conjugated to glucuronic acid is oxidised to quinone, which in turn is decomposed (or conjugated) due to plasma enzyme activity. As shown by Fig. 6, this decomposition ended at a concentration of approximately 20% of the initial concentration. If the decomposition reaction were linear, no quinone metabolite could have been identified in plasma. This instability of quinone was already demonstrated before [10].

The elimination of propofol after a 5-h infusion of 3510 mg can be described by a two compartment model with a $t_{1/2\alpha}$ of 0.9 h and a terminal $t_{1/2\beta}$ of 14 h. The elimination of propofol glucuronide can be

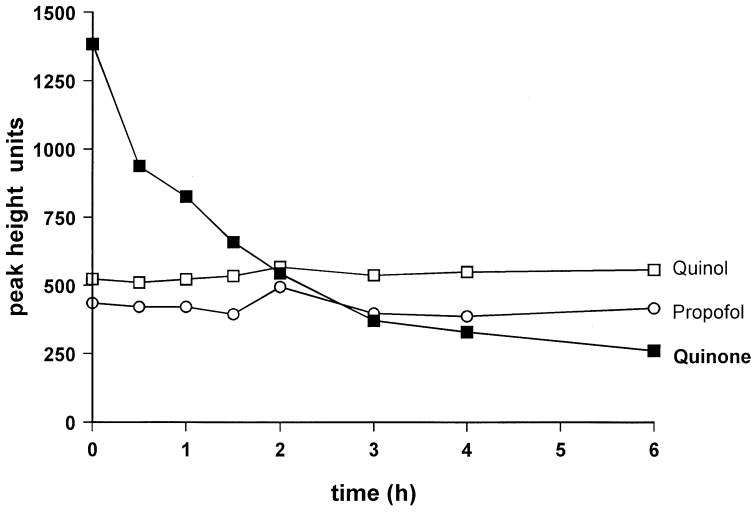

Fig. 6. Stability of propofol, quinol and quinone in fresh plasma (room temperature).

described by a one compartment model and is characterised by a $t_{1/2\beta}$ of 4 h. The elimination of the metabolite quinol-4-glucuronide mirrors that of propofol and shows a $t_{1/2\alpha}$ of 3 h and a $t_{1/2\beta}$ of 17 h.

Identification and recognition of metabolites depend on the instrumentation used. Considering the recovery of the glucuronide metabolites (40–50%) there is ample room for further metabolism and formation of unrecognised metabolites by our HPLC method. Nakao et al. [15] and Sneyd et al. [16,17] reported by NMR analysis of a 24-h urine sample the presence of quinol-4-sulphate (5% of the dose). This minor metabolite can be found in our chromatogram eluting just before quinol-4-glucuronide. It was

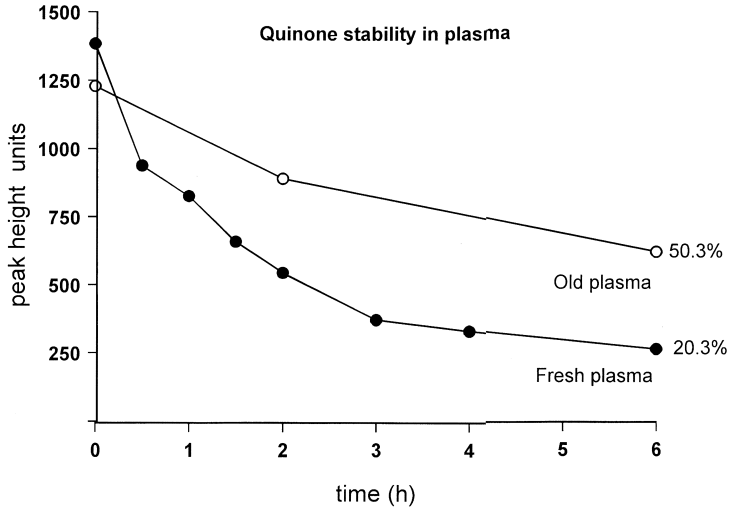

Fig. 7. Stability of quinone in fresh and old plasma (room temperature).

Table 4
Inter-day and intra-day coefficient of variation (C.V.) of spiked propofol glucuronide, quinol-1-glucuronide and quinol-4-glucuronide in plasma and urine ($n=6$, in vitro)

Concentration (μg/ml)	C.V. (%)			
	Inter-day		Intra-day	
	Plasma	Urine	Plasma	Urine
Gradient mode				
Propofol glucuronide				
0.37	0.61	12.9	22.8	4.60
0.74	4.66	5.50	16.2	7.77
3.70	5.14	3.90	3.60	2.70
Quinol-1-glucuronide				
1.02	1.77	28.7	4.50	10.4
2.05	1.88	4.70	6.40	4.50
10.2	6.24	3.40	1.00	1.90
Quinol-4-glucuronide				
0.28	1.61	2.62	2.20	3.35
0.56	1.00	4.70	3.60	0.88
2.78	2.07	6.90	2.60	2.24
Isocratic mode				
Propofol				
0.12	8.85		4.11	
0.24	3.45		1.95	
0.95	1.02		0.64	
3.80	0.24		0.99	
Quinone				
0.14	11.6		1.80	
0.28	4.02		4.50	
1.10	4.78		4.08	
4.40	0.38		1.32	

expected that the tentative quinol-4-sulphate would give m/z fragments of quinol m/z 194 and m/z 98 of the sulphate group. This behaviour could not be confirmed, so the compound must have a different structure and is not related to the metabolism of propofol, or the mass–mass spectrometry behaves in a different way [15].

Further metabolism of (^{14}C) propofol by oxidation of the isopropyl group in rabbits and rat was reported by Simons et al. [18]. Also this compound in MS–MS analysis must reveal a glucuronide group and a recognisable propofol/quinol fragment.

In conclusion, the analysis of propofol and its metabolites can be performed with gradient HPLC with UV (and fluorescence) detection. For the analysis of the glucuronides a gradient eluent is required, while for propofol itself, an isocratic mobile phase is still preferred.

Acknowledgements

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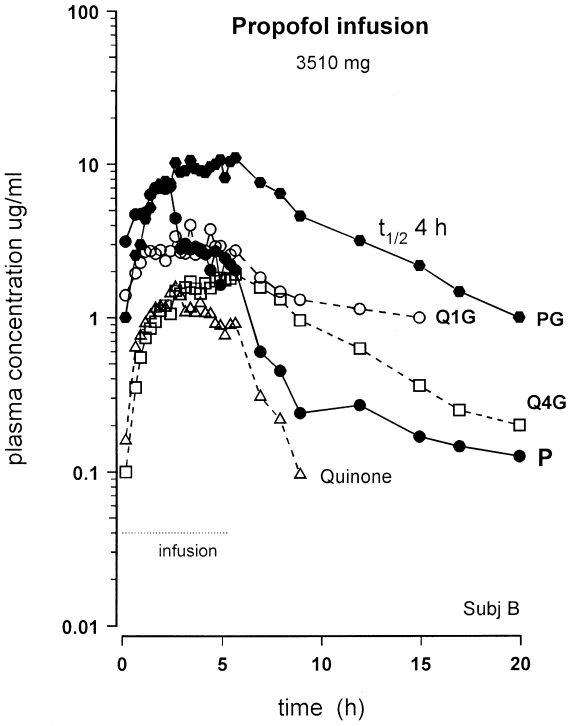

Fig. 8. Plasma concentration–time curve of propofol (P), propofol glucuronide (PG), quinone, quinol-1-glucuronide (Q1G) and quinol-4-glucuronide (Q4G) in a patient during and after an 5-h infusion with 3510 mg of propofol.

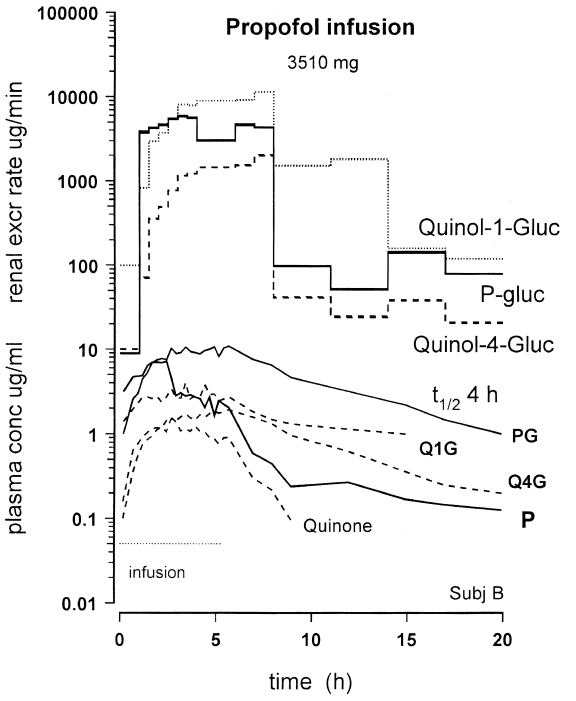

Fig. 9. Plasma concentration–time curve and renal excretion rate-time profiles of propofol (P), propofol glucuronide (PG), quinone, quinol-1-glucuronide (Quinol-1-Gluc) and quinol-4-glucuronide (Quinol-4-Gluc) in the same patient (Fig. 8) during and after an 5-h infusion with 3510 mg of propofol.

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