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# Development of a rapid and sensitive LC–ESI/MS/MS assay for the quantification of propofol using a simple off-line dansyl chloride derivatization reaction to enhance signal intensity

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**Abstract**

A rapid, selective and sensitive method was developed for the determination of propofol concentration using an off-line dansyl chloride derivatization step to enhance signal intensity. The method consisted of a protein precipitation extraction followed by derivatization with dansyl chloride and analysis by liquid chromatography ionspray tandem mass spectrometry (LC–ESI/MS/MS). The separation was achieved using a 100 mm × 2 mm $C_8$ analytical column combined with an isocratic mobile phase composed of 80:20 acetonitrile: 0.5%formic acid in water. Signal intensity of the propofol-dansyl chloride derivative was increased up to 200-fold as compared to the underivatized propofol in positive electrospray mode. An analytical range of 20–20,000 ng/mL was used in the calibration curve of plasma and blood samples. The novel method met all requirements of specificity, sensitivity, linearity, precision, accuracy and stability. A pharmacokinetic study was performed in rats and the novel analytical method was used as a routine analysis to provide enhanced measurements of plasma and blood concentrations of propofol. Blood and plasma pharmacokinetic results show that a very important fraction of propofol distributes into red blood cells. In conclusion, a rapid and sensitive LC–ESI/MS/MS method using a derivatization agent was developed to enhance signal intensity of propofol. Routine analysis with the novel method provided accurate results and enhanced the detection levels of plasma and blood concentrations of propofol to better characterize the in vivo biodiposition of propofol.


*Keywords:* Propofol; Dansyl chloride; Mass spectrometry; HPLC; Blood; Plasma; Rats; Pharmacokinetics

## 1. Introduction

Propofol (2,6-diisopropylphenol) is rapidly gaining popularity within the veterinary medicine community for anesthesia of different species including cats and dogs. It is used for both induction [1] and maintenance of anesthesia [2]. Moreover, propofol is widely used during minor surgery of laboratory animals (rat, mice and dog). The major advantage of this drug over other injectable anesthetic agents is the rapid and complete recovery that occurs even after prolonged intravenous infusion [3]. This property is attributed to the rapid and extensive biotransformation of the parent compound, primarily by the liver, to multiple inactive metabolites, which are excreted in the urine. The relative abundance of individual metabolic pathways has been found to vary between different species, which is a typical finding for drugs mainly metabolized by the liver. The pharmacokinetic profile of propofol is characterized by a multi-exponential decline in plasma and blood concentrations so that concentration levels can be measured over a long period of time [4].

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In order to properly characterize the biodiposition of propofol, it is necessary to develop a precise and accurate analytical method suitable for the routine analysis of propofol concentrations in blood and plasma. Various methods have been reported in the literature based on HPLC equipped with either ultraviolet (UV) [5] or fluorescence [6,7] detectors. Most of these methods require extensive sample preparation procedures and various steps to concentrate the product. These methods typically use solid phase extraction or liquid-liquid extraction to isolate propofol from the biological matrix. Although these preparation steps are necessary to improve selectivity of the method and increase the sensitivity of the detection, they require intensive labor and manipulations [5–7].

In recent years, liquid chromatography coupled with atmospheric pressure ionization and mass spectrometry has progressed to become the method of choice in bioanalysis [8]. Simple, rapid and automated analysis methods can be developed to provide high precision and accuracy, improved sensitivity and selectivity, and finally provide molecular information (e.g. full scan MS/MS on ion trap). However, due to its non-polar nature and the absence of an ionizable group on the molecule, the ionization efficiency of propofol in electrospray is low and leads to poor detection limits. Propofol has a phenol group that can be modified chemically by a single step reaction with common derivatization agents used in HPLC with fluorescence detector [9]. Dansyl chloride has been used recently to improve ESI signal intensity [10] and more specifically for ultra trace analysis of ethinyl estradiol in human plasma [11]. As depicted in Fig. 1, the reaction of dansyl chloride and propofol results in a product containing a tertiary amine, which is easily protonated in positive electrospray mode and can significantly improve the limit of detection of propofol.

The objective of the current study was to develop a novel LC–ESI/MS/MS method using a simple off-line dansyl chloride derivatization reaction of propofol. The derivatization step is expected to significantly enhance signal intensity and improve the limit of detection of the assay. Propofol was administered intravenously to rats and the novel method was used for routine analysis of plasma and blood samples in order to characterize its pharmacokinetics.

## 2. Experimental

### 2.1. Reagents

Propofol and the internal standard eugenol [2-methoxy-4-(2-propenyl)phenol] were purchased from Sigma–Aldrich (St. Louis, MO, USA). The derivatization agent, dansyl chloride, was purchased from Sigma–Aldrich. Other chemicals, including acetonitrile, methanol, sodium hydroxide and formic acid were purchased from J.T. Baker (Phillipsburg, NJ, USA).

### 2.2. Instrumentation

The HPLC system consisted of an autosampler Varian 9100 (Palo Alto, CA, USA) and a Water 625 pump (Milford, MA, USA). The LC–MS/MS system used was a PESciex API $3^+$ (Applied Biosystem/MDS Sciex, Concord, Ont., Canada). Data was acquired on an Apple™ Macintosh® (Silicon Valley, CA, USA) equipped with operation system 7.4. Data acquisition and analysis were performed using MassChrom 1.0 (Concord, Ont., Canada). Calibration curves were calculated from the equation $y = ax + b$, as determined by weighted ($1/x$) linear regression of the calibration line constructed from the peak-area ratios of the drug and the internal standard.

### 2.3. Sample preparation

Using a simple protein precipitation method, propofol was extracted from rat plasma (or blood). A total of 50 μL of sample was mixed with 250 μL of internal standard solution (250 ng/mL of eugenol in acetone) in a 1.5 mL centrifuge tube. The sample was then vortexed vigorously and the samples were allowed to rest 10 min at room temperature prior to centrifugation. Samples were centrifuged at approximately 12,000 × g for 10 min and 250 μL of the supernatant was transferred into a 650 μL injection vial. One hundred microliters of dansyl chloride solution (1 mg/mL in acetone) and 20 μL of 100 mM NaOH solution were added to injection vials and the sample was heated at 60 °C for 10 min. The vial was vortexed briefly and transferred to an autosampler for analysis.

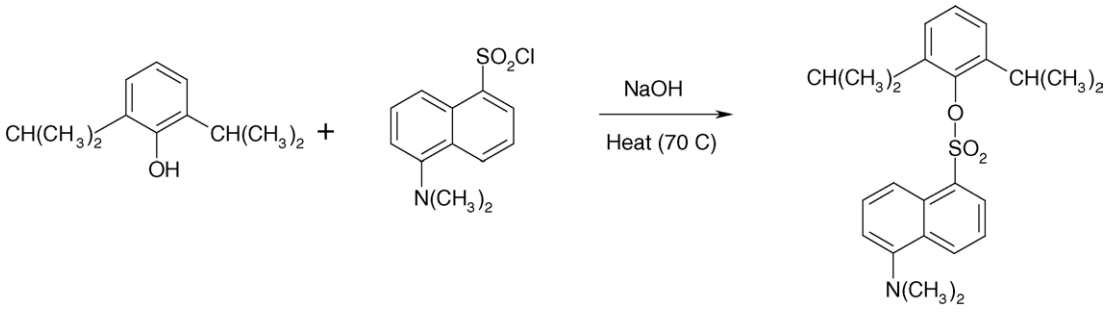


Fig. 1. Reaction scheme of propofol with dansyl chloride.

### 2.4. Chromatographic conditions

An isocratic mobile phase was used with a Keystone Scientific (Thermo) BDS Hypersil C8 100 mm × 2 mm with particle size of 5 μm column. The mobile phase consisted of acetonitrile and 0.5% formic acid in water at a ratio of 80:20, respectively. The flow rate was fixed at 0.55 mL/min and propofol eluted at 2.7 min and the internal standard at 1.8 min. The eluent was split 1:10 prior to introduction into the electrospray source. Ten microliters of the extracted sample was injected and the total run time was set at 3.2 min.

### 2.5. Mass spectrometry conditions

The mass spectrometer was interfaced with the HPLC system using a pneumatic assisted electrospray ion source. The $N_2$ pressure of the nebulizer gas was set at 40 psi and the ESI electrode was set to 4000 V. The declaustering potential was set at 15 V and the collision energy ($E_{lab}$) at 25 V. The collision gas used was argon at $2.7 \times 10^{14}$ molecules/cm$^2$. The SRM transitions were $m/z$ 412 → 171 and 398 → 171 for propofol and eugenol, respectively. The dwell time was set at 200 ms and the pause time at 5 ms.

### 2.6. In vivo study design

Male Fisher rats (average weight 300 g) were purchased from Charles River (St. Constant, Canada) for a pharmacokinetic study of a commercially available lipid emulsion formulation of propofol (Diprivan®, Astra Zeneca, USA) in plasma ($n = 6$ rats) or blood ($n = 6$ rats). Rats received a 10 mg/kg dose of propofol administered intravenously at a dose volume of 1 mL/kg over a period of 1 min via jugular venipuncture under isoflurane anesthesia. Blood samples (0.3 mL) were collected from the contralateral jugular vein at 1, 2, 3, 5, 7, 10, 15, 30 and 60 min following dosing.

Blood samples were collected into heparinized tubes and immediately placed on ice pending further processing. For the pharmacokinetic study of propofol in plasma, blood samples were centrifuged at 4 °C for 10 min at 3200 × g and plasma aliquots were stored at −80 °C pending analysis. For the pharmacokinetic study of propofol in blood, blood samples were stored at −80 °C pending analysis. Concentrations of propofol in plasma and blood were assessed using the novel LC–ESI/MS/MS method.

Pharmacokinetic parameters of propofol in plasma and blood were calculated using non-compartmental methods [12]. The area under the curve from time 0–60 min ($AUC_{0–60}$) was calculated using the linear trapezoidal rule. A terminal rate constant of elimination ($k_{el}$) was calculated using a minimum of three measurable plasma concentrations and a terminal elimination half-life ($T_{1/2}$) was calculated using $0.693/k_{el}$. The area under the curve extrapolated to infinity ($AUC_{INF}$) was calculated using $AUC_{0–60} + C_{last}/k_{el}$, where $C_{last}$ was the last measurable blood or plasma concentration. Clearance (CL) was calculated by dividing the actual dose administered by the $AUC_{INF}$. The mean residence time (MRT) was obtained by dividing the area under the first moment-time curve ($AUMC_{INF}$) by the $AUC_{INF}$. The total volume of distribution ($V_{ss}$) was calculated using CL × MRT.

Propofol distribution between blood, plasma and RBC can be described by the following equation:

$$\mathrm{CONC_{BLOOD}} \times V_{\mathrm{BLOOD}} = \mathrm{CONC_{PLASMA}} \times V_{\mathrm{PLASMA}} + \mathrm{CONC_{RBC}} \times V_{\mathrm{RBC}} \quad (1)$$

where $\mathrm{CONC_{BLOOD}}$, $\mathrm{CONC_{PLASMA}}$ and $\mathrm{CONC_{RBC}}$ represent the concentrations of propofol in blood, plasma, and RBC, respectively and $V_{\mathrm{BLOOD}}$, $V_{\mathrm{PLASMA}}$ and $V_{\mathrm{RBC}}$ represent the volume of blood, plasma, and RBC, respectively. The volume occupied by RBC and plasma are related to hematocrit ($H$) and blood volume by the following equations:

$$V_{\mathrm{RBC}} = H \times V_{\mathrm{BLOOD}} \quad (2)$$

$$V_{\mathrm{PLASMA}} = (1 - H)V_{\mathrm{BLOOD}} \quad (3)$$

By substituting Eqs. (2) and (3) in Eq. (1), propofol concentrations in RBC were calculated with the following equation [12]:

$$\mathrm{CONC_{RBC}} = \frac{[(\mathrm{CONC_{BLOOD}} - \mathrm{CONC_{PLASMA}})(1 - H)]}{H} \quad (4)$$

In this study, the hematocrit for male rats was fixed to a nominal value of 0.45 based on the literature [13].

## 3. Results and discussion

### 3.1. Sample preparation and extraction

The protein precipitation approach was the simplest method available for preparation and the observed recovery was >90%. One drawback of using a protein precipitation method is the impact of ionization suppression and matrix effect on the quantification precision and accuracy due to possible competition for ionization from extracted endogenous substances. In order to test the potential matrix effect and ionization suppression, we fortified six different samples to obtain 20, 5000 and 20,000 ng/mL, and compared the signal intensity versus a pure reference and a fortified blank extract. No significant differences were observed, since the signal intensity corresponded to 86–97% those of the reference for all blank samples. The experiment described by King et al. [14] could not be performed in the current study since the reference solution included residues of derivatization agent and this could have impacted the efficiency of ionization. The derivatization is the most critical part of the methodology. A kinetic experiment of the reaction of propofol with dansyl chloride showed no significant increase in signal intensity after 5 min. In order to be certain that the reaction went

to completion, the reaction was conducted over a 10-min period.

### 3.2. Tandem mass spectrometry

Full scan and product ion mass spectra for propofol and eugenol were obtained in positive ion mode. The full scan spectra of the dansyl chloride derivative of propofol and eugenol showed an intense signal for the protonated molecular ion ($[M+H]^+$) at $m/z$ 412 and 398, respectively. The reactions lead to a mass increase of 233 Da for each compound. The product ion spectra of propofol and eugenol have predominant fragment ions at $m/z$ 171 (Fig. 2). The mass transition in selected reaction monitoring mode was set for best sensitivity at 412 → 171 and 398 → 171 for propofol and eugenol, respectively. The product ion spectra were compatible with the molecular structure of derivatized products. With the propofol-dansyl chloride derivative, signal intensity was increased up to 200-fold as compared to underivatized propofol in positive electrospray mode and up to 50-fold when compared to underivatized propofol in negative electrospray mode. In both positive and negative electrospray modes, the ion abundance of underivatized propofol was weak and not reproducible. The limit of detection, defined as the quantity of analyte required to obtain three times the signal to noise ratio response, was established at 25 fmol injected on column for the propofol-dansyl chloride derivative in the positive electrospray mode.

### 3.3. Sensitivity and selectivity

The precision (coefficient of variance, %CV) and accuracy (%NOM) at the LLOQ level were determined in six replicates of plasma samples fortified at 20 ng/mL of propofol. The precision obtained was 5.7% and the accuracy was 95.0%. A representative chromatogram of LLOQ samples is shown in Fig. 3A. Extracted blank plasma did not show any interference from endogenous substances, as shown in a representative chromatogram of an extracted blank sample (Fig. 3B). During all experimentations, no interference at the mass transition and retention times of propofol and eugenol were observed in any of the rat plasma samples tested.

### 3.4. Calibration curve analysis

A linear regression (weighted 1/concentration) was judged to produce the best fit for the concentration-detector relationship. The regression model used was determined using the sum of the squares of the deviation [15]. By convention, the regression line is considered to properly fit the

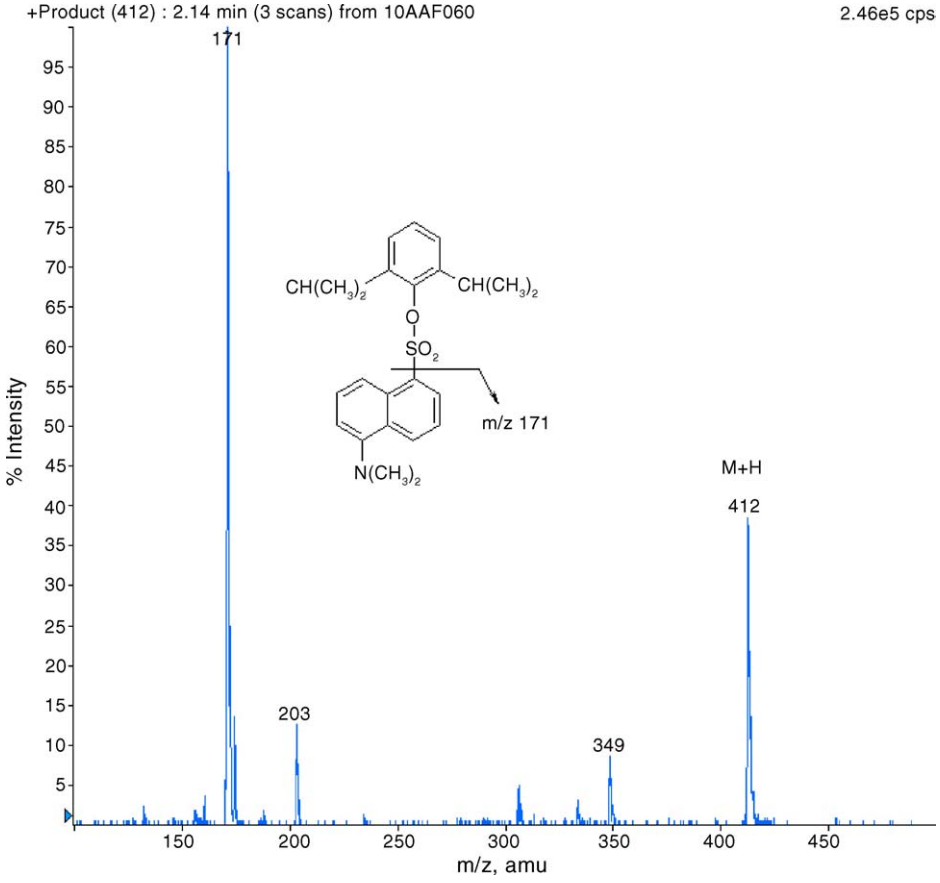


Fig. 2. Product ion spectrum of the propofol-dansyl chloride derivative.

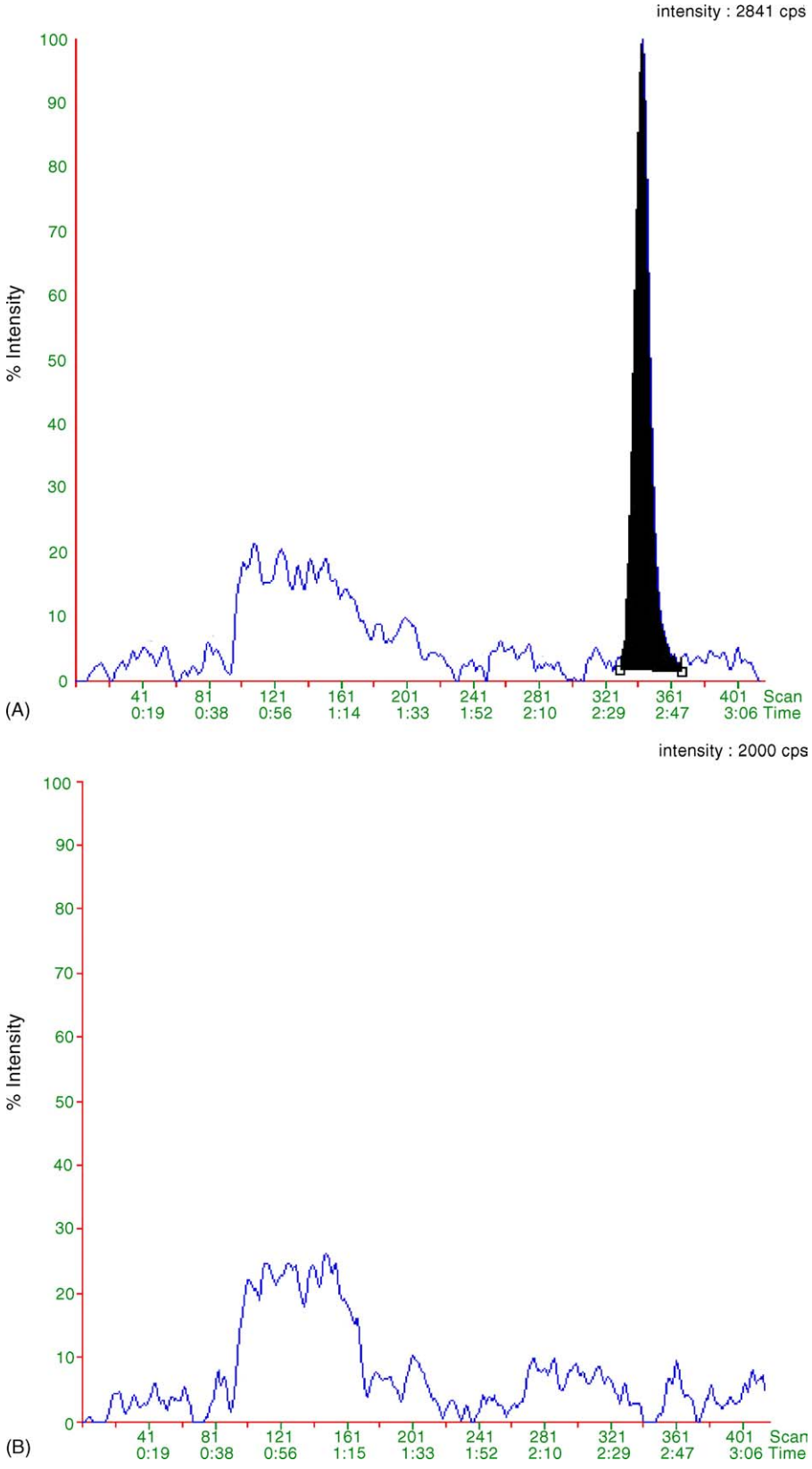


Fig. 3. (A) Representative extracted LLOQ (20 ng/mL) chromatogram of propofol in rat plasma. (B) Representative chromatogram of an extracted control (blank) rat plasma.

Table 1
Summary of inter-batch precision and accuracy data for propofol in plasma

| | 20 ng/mL | 1000 ng/mL | 20000 ng/mL |
|---|---|---|---|
| *n* | 18 | 18 | 18 |
| Mean | 19.2 | 1060 | 19895 |
| S.D. | 1.292 | 113.3 | 1737.9 |
| %CV | 6.7 | 10.7 | 8.7 |
| %NOM | 95.8 | 106.0 | 99.5 |
| 95% CI | 0.6 | 52 | 803 |

calibration set when the sum of squares of the deviation is minimized. The calculated coefficients of correlations ($r$) were better then 0.995 for an analytical range set from 20 to 20,000 ng/mL.

### 3.5. Precision and accuracy

The reproducibility of the method was evaluated by analyzing six replicates of plasma samples fortified with propofol at the nominal concentration of 20, 1000 and 20,000 ng/mL in three individual runs. The inter batch precision and accuracy statistical results are shown in Table 1. With the determination of intra batch precision, the %CV and accuracy were 6.0, 7.7, 5.7% and 99.0, 97.3, 99.8% at 20, 1000 and 20,000 ng/mL, respectively.

A set of quality-control (QC) samples were prepared in rat blood at nominal concentrations of 20, 1000 and 20,000 ng/mL and were analyzed with a calibration curve from rat blood samples. The observed intra batch precision and observed accuracy were 9.7, 6.5, 5.0% and 97.9, 93.6, 91.4% at 20, 1000 and 20,000 ng/mL, respectively.

The limit of quantification was set at 20 ng/mL and, according to the bioanalytical validation guideline published by the FDA [16], acceptable precision and accuracy results were achieved. In most bioanalytical reports, the precision of an analytical assay is usually measured using %CV (standard deviation/mean × 100), even though this does not provide a direct estimation of the analytical error. A better statistical estimate of the error is provided by using confidence intervals (CI) around the mean value. As a result, the acceptance criteria can be defined as a function of the confidence levels instead of an arbitrary number. This method has allowed the quantification of propofol down to $20.0 \pm 0.6$ ng/mL with a 95% confidence level.

### 3.6. Stability

Stability experiments were performed to demonstrate whether or not propofol is stable under typical sample storage and analysis conditions. Bench-top stability was performed at room temperature and propofol was stable in rat plasma and blood for at least 4 h. Freeze and thaw stability after 3 cycles was verified and no significant differences were observed versus freshly prepared samples (<6% difference). Storage stability at $-80\,^{\circ}$C was performed and propofol was stable for at least 7 weeks in rat plasma.

### 3.7. In vivo study

Mean propofol concentrations in plasma, blood and RBC (ng/mL) following an intravenous dose of 10 mg/kg in rats are depicted in Fig. 4. Overall, mean concentrations of propofol declined in a multiexponential manner. It has been suggested that plasma concentrations of propofol greater than 2800 ng/mL are associated with anesthesia in rats [17]. Mean plasma concentrations of propofol remained higher than 3000 ng/mL within the first 7 min following intravenous administration, whereas those observed in blood remained higher than 3000 ng/mL until 30 min post-administration. The higher exposure of propofol in blood as compared to plasma suggests a preferential distribution of the drug into red blood cells. This fraction is likely to be distributed into the RBC or to bind directly on the RBC membrane [18].

Mean pharmacokinetic parameters of propofol in plasma and blood are presented in Table 2. The clearance of propofol in plasma was markedly higher than that observed in blood (CL: 130 mL/min/kg versus 39 mL/min/kg). This resulted in a significantly lower $AUC_{INF}$ in plasma (94,254 ng min/mL) as compared to blood (330,650 ng min/mL). The total vol-

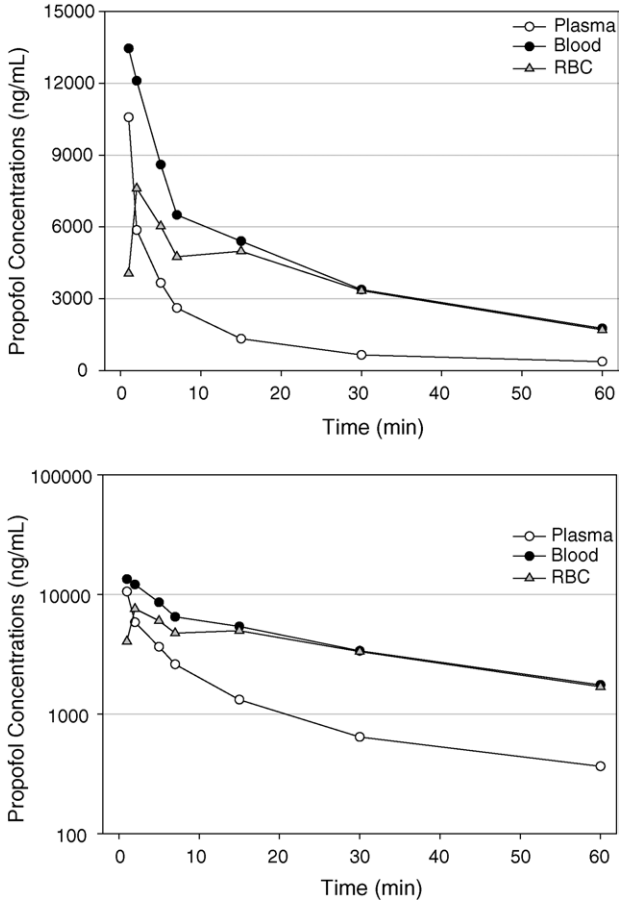


Fig. 4. Mean propofol concentrations (ng/mL) in plasma, blood and RBC following a single intravenous dose (10 mg/kg) of a commercially available emulsion formulation of propofol in male Fisher rats (linear (top) and semi-log scale (bottom)).

Table 2
Mean ± S.D. pharmacokinetic parameters of propofol in plasma and blood following a single intravenous dose (10 mg/kg) of a commercially available emulsion formulation in male Fisher rats

| Parameters | Plasma | Blood |
|---|---|---|
| $AUC_{0–60}$ (ng min/mL) | 79668 ± 18962 | 256102 ± 22839 |
| $AUC_{INF}$ (ng min/mL) | 94254 ± 23515 | 330650 ± 56637 |
| CL (mL/min/kg) | 130 ± 29.6 | 39.3 ± 3.1 |
| MRT (min) | 23.4 ± 10.3 | 40.1 ± 8.0 |
| $T_{1/2}$ (min) | 21.4 ± 6.3 | 28.8 ± 7.1 |
| $V_{ss}$ (mL/kg) | 2989 ± 1311 | 1564 ± 262 |

Table 3
Mean ± S.D. partition coefficient (*K*p) of propofol in blood following a single intravenous dose (10 mg/kg) of a commercially available emulsion formulation in male Fisher rats

| Time (min) | Mean $K_p$ (RBC:plasma) |
|---|---|
| 1 | 3.2 ± 1.9 |
| 2 | 0.8 ± 0.5 |
| 5 | 1.3 ± 2.1 |
| 7 | 0.6 ± 0.2 |
| 15 | 0.3 ± 0.2 |
| 30 | 0.2 ± 0.1 |
| 60 | 0.2 ± 0.2 |

ume of distribution of propofol in plasma was approximately two-fold higher than that observed in blood (2989 mL/kg versus 1564 mL/kg). MRT of propofol in plasma was lower than than blood (23.4 versus 40.0 h, respectively) as well as $T_{1/2}$ (21.4 min versus 28.8 min, respectively). Partition coefficient ($K_p$) between RBC and plasma at each time points were presented in Table 3. The RBC:plasma partition coefficient decreased over time, suggesting a re-distribution of propofol from red blood cells to plasma, where it is cleared from the system by liver metabolism.

## 4. Conclusion

A novel high-performance liquid chromatographic–mass spectrometric method was developed using a simple off-line dansyl chloride derivatization reaction of propofol. The derivatization of propofol with dansyl chloride improved significantly the detection limit and signal stability in positive electrospray ionization for the determination of plasma and blood concentrations of propofol. The novel method met all requirements of specificity, sensitivity, linearity, precision, accuracy and stability generally accepted in bioanalytical chemistry. An in vivo study was performed in rats and the novel analytical method was used to assay plasma and blood concentrations of propofol in order to characterize the biodisposition of propofol.

## References

[1] A. Shafer, V.A. Doze, S.L. Shafer, P.F. White, Anesthesiology 69 (1988) 348–356.
[2] P. Dailland, D.I. Cockshott, J.D. Lirzin, P. Jacquinot, J.C. Jorrot, J. Devery, J.L. Harmey, C. Conseiller, Anesthesiology 71 (1989) 827–834.
[3] R.E. Mandsager, C.R. Clarke, R.V. Shawley, C.M. Hague, Am. J. Vet. Res. 56 (1995) 95–99.
[4] B. Marsh, M. White, N. Morton, G.N.C. Kenny, Br. J. Anaesth. 67 (1991) 41–48.
[5] D. Teshima, H. Nagahama, K. Makino, Y. Kataoka, R. Oishi, J. Clin. Pharm. Ther. 26 (2001) 381–385.
[6] T.B. Vree, A.J. Lagerwerf, C.P. Bleeker, P.M.R.M. de Grood, J. Chromatogr. B 721 (1999) 217–228.
[7] N.H. Kay, J.W. Sear, J. Uppington, I.D. Cockshott, J.E. Douglas, Br. J. Anaesth. 58 (1986) 1075–1079.
[8] M. Jemal, Biomed. Chromatogr. 14 (2000) 422–429.
[9] A.T. Rhys Williams, S.A. Winfield, R.C. Belloli, J. Chromatogr. 240 (1982) 224–229.
[10] J.M. Quirke, C.L. Adams, G.J. Van Berkel, Anal. Chem. 66 (1994) 1302–1315.
[11] W.Z. Shou, X. Jiang, W. Naidong, Biomed. Chromatogr. 18 (2004) 414–421.
[12] M. Rowland, T.N. Towzer, Clinical Pharmacokinetics: Concepts and Applications, Lippincott Williams & Wilkins, PA, 1995.
[13] T. Matsuzawa, M. Nomura, T. Unno, J. Vet. Med. Sci. 55 (1993) 351–362.
[14] R. King, R. Bonfiglio, C. Fernandez-Metzler, C. Miller-Stein, T. Olah, J. Am. Soc. Mass Spectrom. 11 (2000) 942–950.
[15] F. Beaudry, Pharm. Sci. 1 (2000) 2437.
[16] Guidance for Industry. Bioanalytical Method Validation. Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM). May 2001, http://www.fda.gov/cder/guidance/4252fnl.pdf.
[17] H.K. Adams, J.B. Glen, P.A. Hoyle, Br. J. Anaesth. (1980) 743–746.
[18] K. Samii, J.X. Mazoit, Br. J. Clin. Pharm. 47 (1999) 35–42.