

The effects of obesity on anesthetic drug distribution – absolute quantitation of propofol in human plasma by GC-MS/MS

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Overview

- Fast isocratic GC-MS/MS method for efficient analysis of large numbers of samples
- Development of an improved pharmacokinetic model for administration of propofol to obese and lean patients

Introduction

Dosing of anesthetics in obese patients is challenging due to the physiologic and anthropometric changes associated with obesity. Obesity is associated with increases in total body weight and fat mass. The increases in fat mass may theoretically alter the distribution of lipophilic drugs such as propofol. These changes are likely to alter the pharmacokinetics of drugs in this patient population, which makes the drug administration challenging. Propofol is the most commonly used anesthetic induction agent in both lean and obese subjects presenting for surgical procedures. To date, little information about the PK/PD of propofol in the obese population exists.

The goal of this study was to determine propofol pharmacokinetics in the obese population. Our methodology included high-resolution sampling in order to capture the early drug distribution kinetics. We developed the GC-MS/MS based assay to obtain highly selective and sensitive quantitative analysis of propofol levels in plasma.

Sample preparation

Sample collection

Morbidly obese subjects (n=20, BMI>40) and lean control subjects (n=10, BMI<25) presenting for surgery were enrolled. Subjects were excluded from enrollment if they had any significant cardiovascular, pulmonary, hepatic or renal impairment that could interfere with pharmacokinetics. Total weights ranged from 54kg to 176 kg.

For induction of anesthesia, subjects were administered propofol at a rate of 100 mg/kg/hr. Whole blood samples were collected from an arterial line catheter at time 0 (baseline), and after beginning the infusion, were collected every 5 seconds until 2 minutes, at 2.5, 3, 4, 5, 7, 9, 11, 13, 15, 30, 45 minutes, and at 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, and 16 hours. In total there were 46 samples collected from each subject. Immediately after collection, samples were placed on ice and centrifuged to separate plasma. Plasma was immediately frozen at -80° C until analysis.

Sample preparation

To 50 µl of plasma sample, 10 µl of stock solution of propofol-d17 (IS) (25 µg/mL) was added. To enhance the extraction efficiency, 100 µl of 100 mM KCl was added to each sample. The analytes were extracted with 500 µl of heptane. The upper, organic phase was transferred to the autosampler vial and submitted for GC-MS/MS analysis.

GC-MS/MS system

GC System: Bruker 450-GC
Injection: 1 µL, split ratio 10:1
Inlet temperature: 300°C
Column: BR-5ms, 30 m x 0.25 mm ID, 0.25 µm film thickness
Column He flow: 1.1 mL/min
Oven temperature program: Isobaric 195°C
Transfer line temperature: 280°C
MS detection: Bruker Scion TQ triple quadrupole MS
Ionization mode: EI, 70 eV, 230°C
Scanning mode: Selected Reaction Monitoring (SRM)
Analysis time: 2.3 min injection-to-injection

Data analysis/ PK modeling

Quantitative data was processed using MSWS 8.0 software from Bruker Daltonics Inc. Non-linear mixed effects modeling using NONMEM VI® was used to fit the data. General additive models were used to identify potentially significant covariates. Bootstrapping confirmed the final model.

Results

FIGURE 1. MS and MS/MS spectra. Two SRM transitions were selected for propofol and for d17-propofol which was used as the IS.

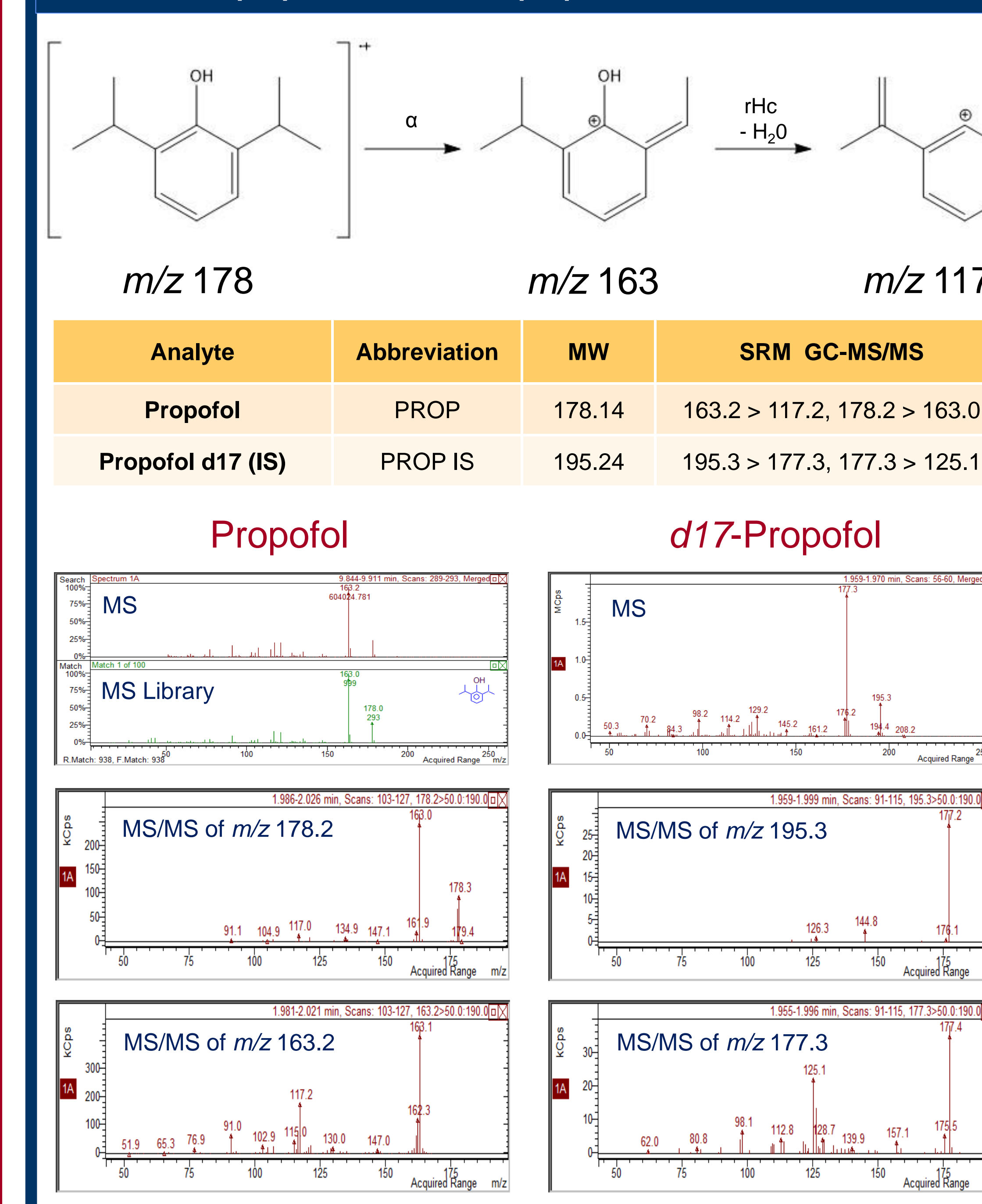


FIGURE 2. Extraction. Several extraction methods were evaluated for highest analyte recovery and robustness: protein precipitation with MeOH (PP); liquid-liquid extraction with either ethyl acetate (EtAc) or with heptane (HEP). Extraction efficiency with PP was very poor and about 85% with EtAc. Addition of 100mM KCl to samples treated with heptane increased the extraction efficiency from 90 to 95%.

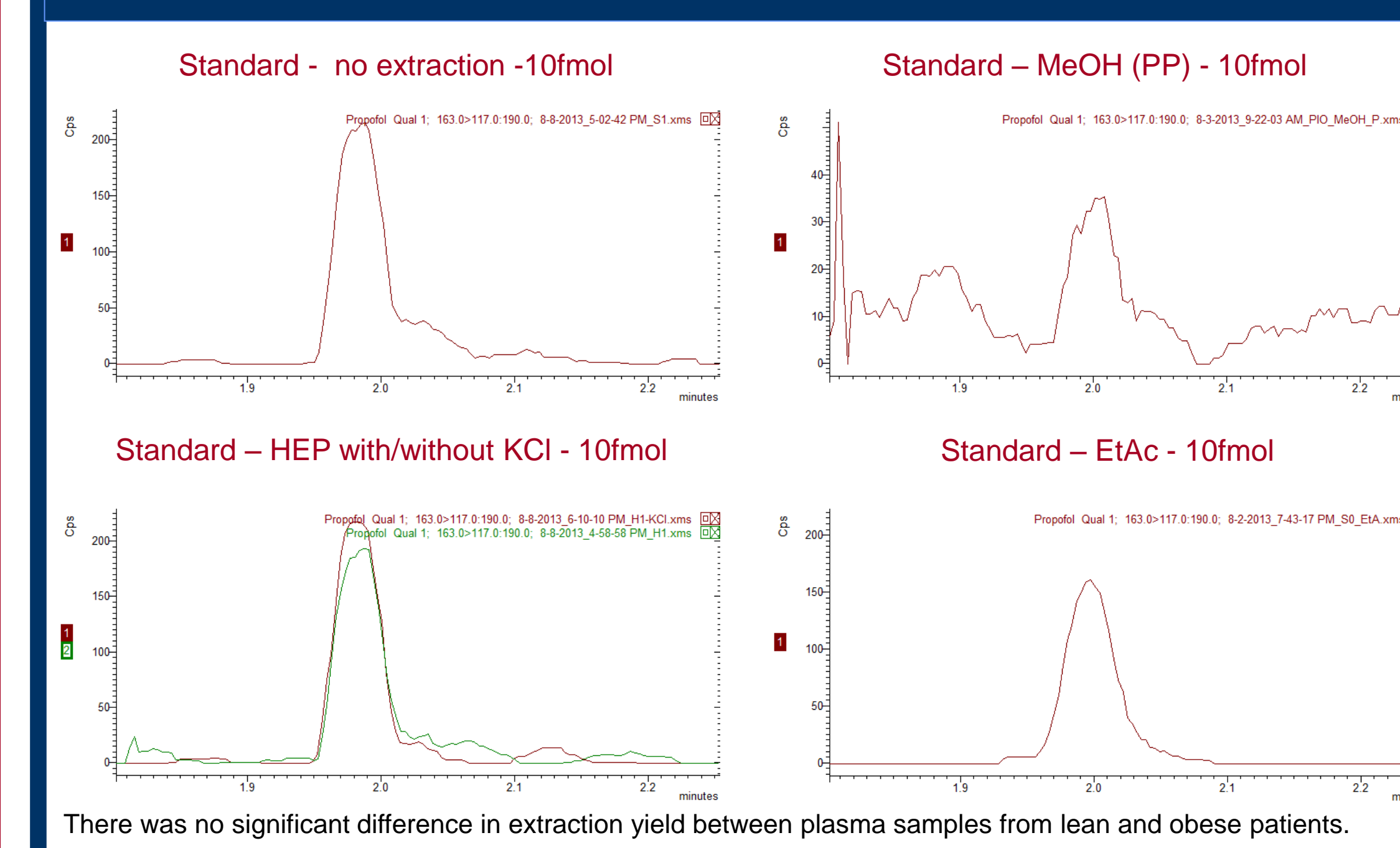


FIGURE 3. Calibration curve range & linearity. Calibration curves and QCs were run with each batch of samples and demonstrated consistent method performance over time. LLOQ, defined as signal to noise ratio (S/N) of 10 to 1, was 4 nM (400 amol on column), LLOD 2 nM; response between 1 nM and 4 µM was linear.

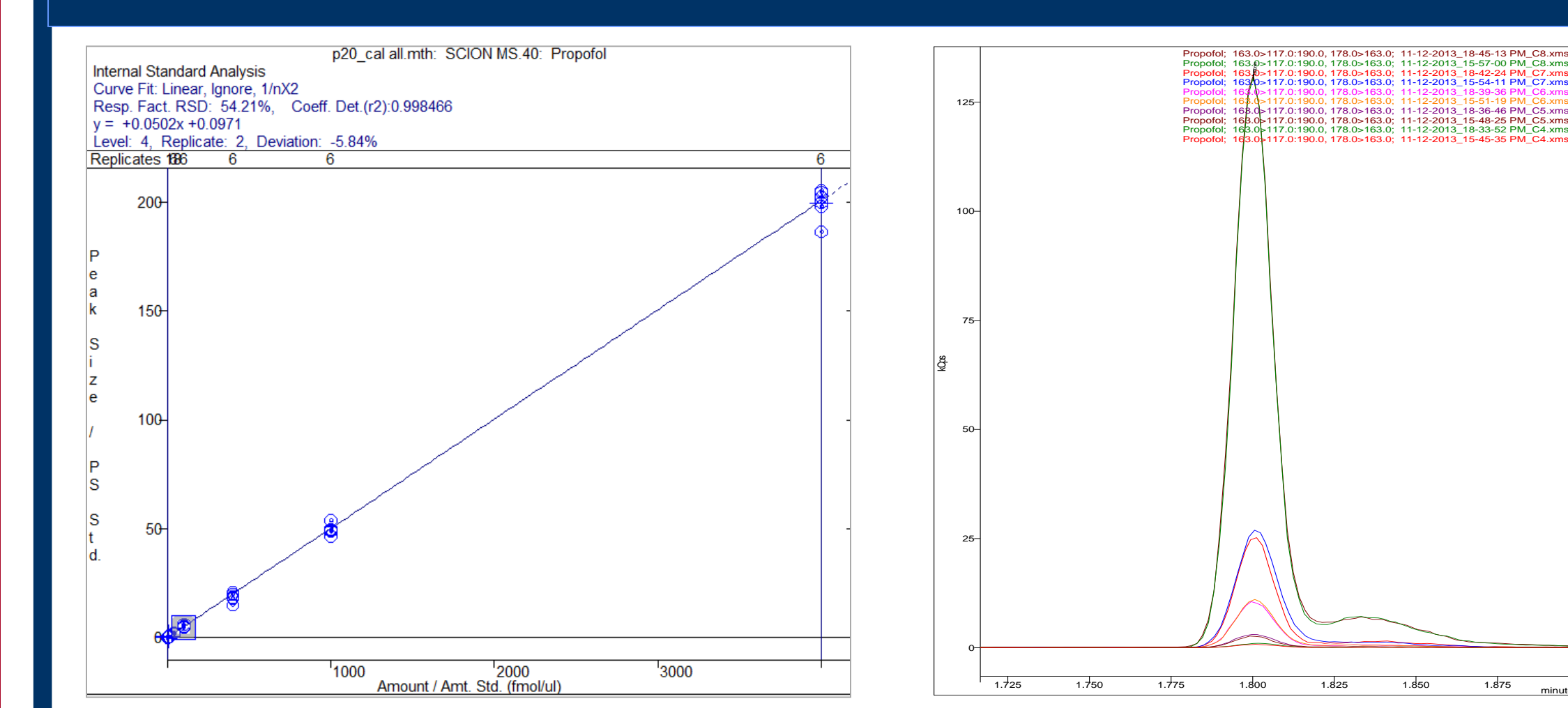


FIGURE 4. Compartment based pharmacokinetic model. A 3-compartment pharmacokinetic model best describes the propofol data. Significant covariates are as follows: Lean body weight best describes volumes 1 and 2; total body weight best describes clearance 2. Age and cardiac output are significant covariates for clearance 1. There are no significant covariates for volume 3 and clearance 3.

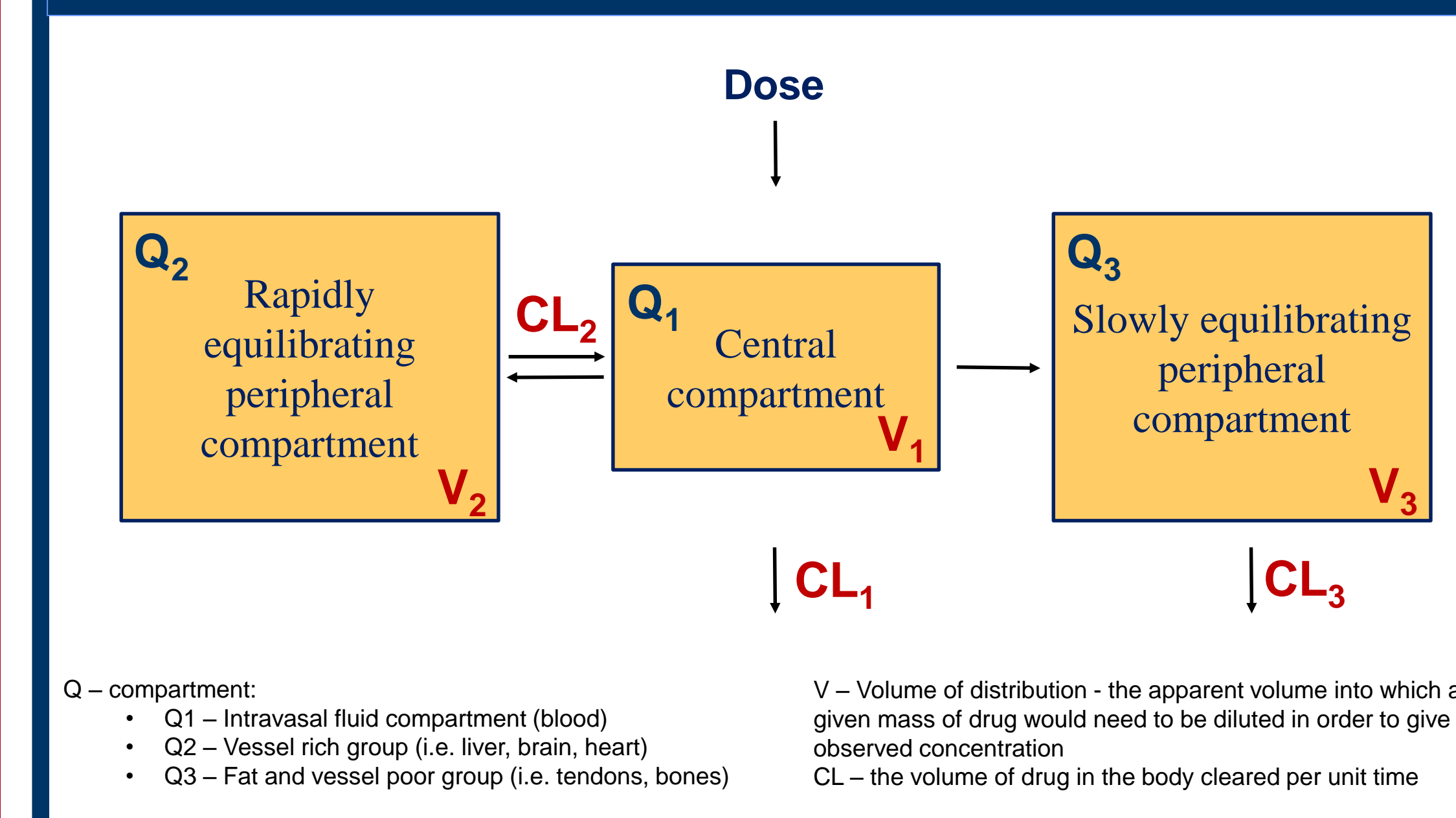
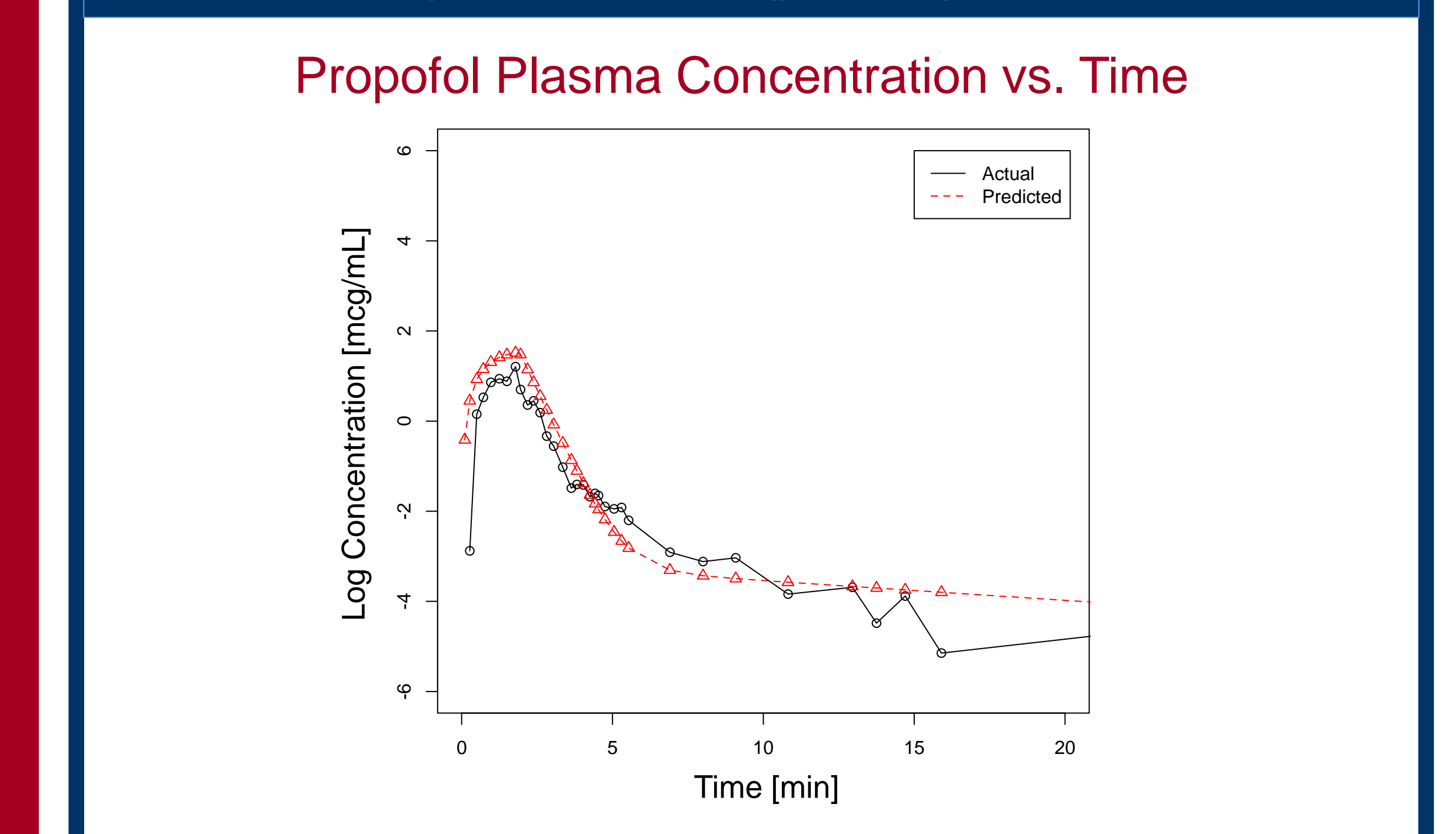


FIGURE 5. Predicted vs. actual propofol plasma concentration during the first 20 min. for a representative patient. The model uses multiple patient parameter inputs to generate the predicted curve. Median performance error (bias) was 1% and median absolute performance error (precision) was 19%.



Conclusions

- In this study we developed an fast isocratic GC-MS/MS method with injection-to-injection time 2.3 min., enabling efficient analysis for propofol in human plasma.
- The highest yield of extraction yield of propofol from plasma was achieved using heptane with addition of 100mM KCl.
- The pharmacokinetic data for propofol can be best described with a 3-compartment model.
- Lean body weight (V1 and V2) and cardiac output (CL1) and age (CL1) were significant covariates.

Future work

- We are currently conducting a study analyzing the pharmacokinetics of anesthetics (opioids and intravenous induction agents) specific to the bariatric population. We will analyze both plasma and tissue samples to construct a combined physiologic and compartmental model.

References

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Acknowledgements

Thanks to the Vincent and Stella Coates Foundation. Thanks to Yuxi (Rachel) Wu for sample preparation assistance and to Helen (Qingyu) Sun, Ed George, Keifei Wang and Brinker Gildersleeve from Bruker Chemical and Applied Markets for help with GC-MS/MS analysis setup and support.

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