Evaluating the Relationship Between Carisoprodol Concentrations and Meprobamate Formation and Inter-Subject and Intra-Subject Variability in Urinary Excretion Data of Pain Patients

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Using urinary carisoprodol data from pain patients, our objectives were to determine the relationship between carisoprodol concentration and its conversion to meprobamate, and quantify the intra-subject and inter-subject variability in carisoprodol metabolism. Liquid chromatography–tandem mass spectrometry was used to quantitate carisoprodol and meprobamate concentrations in urine specimens. The log creatinine-corrected carisoprodol versus log creatinine-corrected meprobamate showed a marginal positive relationship ($R^2 = 0.395$), with a 29.1-fold variance between subjects at the mean carisoprodol concentration. The geometric mean carisoprodol and meprobamate urine concentrations were 0.519 ± 3.38 mg and 28.2 ± 2.34 mg analyte per gram creatinine, respectively. The log metabolic ratio (MR) versus log creatinine-corrected carisoprodol displayed a marginal positive correlation. A subpopulation of outliers with higher carisoprodol and lower meprobamate levels were considered poor metabolizers and represented 0.483% ($n = 21$) of the study population. Using a curve-fit mathematical model, we estimated 0.318% ($n = 10$) to be ultra-rapid metabolizers. The inter-subject population geometric standard deviation (SD) of the MR was 3.64. The intra-subject geometric median and mean SD of the MR were 1.60 (interquartile range: 1.28, 2.07) and 1.72 ± 1.60, respectively. Inter-subject variability was 2.27 times greater than the median intra-subject variability. With a better understanding of urine carisoprodol and meprobamate concentrations and variability, urine drug testing provides a useful monitoring reference for clinicians.

Introduction

Carisoprodol is a widely prescribed, centrally-acting musculoskeletal relaxant indicated as an intermittent adjunctive treatment for acute, painful musculoskeletal conditions. The precise mechanism by which carisoprodol acts is not completely understood. However, the action of carisoprodol is believed to result from its central nervous system sedative effects rather than its function as a direct muscle skeletal relaxant (1). In animal studies, carisoprodol appears to block interneuronal activity in the descending reticular formation and spinal cord, thereby altering pain perception (2). Carisoprodol is typically prescribed as 350-mg doses taken four times a day up to a maximum of 1,400 mg per day. Carisoprodol has a noted potential for substance abuse and dependence, and the duration of the carisoprodol regimen should not exceed two to three weeks (3). It is manufactured under both generic and brand (Carisoma, Soma, Somadril and Vanadom) formulations.

The majority of carisoprodol is metabolized, and only a trace amount is excreted unchanged. Animal studies have suggested that carisoprodol metabolizes into three primary metabolites by hepatic biotransformation: hydroxycarisoprodol, hydroxymeprobamate and meprobamate (4, 5). Figure 1 illustrates carisoprodol metabolism into its different metabolites. In a single 350-mg dose of carisoprodol, less than 1% is excreted unchanged and approximately 4.7% is excreted as meprobamate over 24 hours in urine (6). In humans, carisoprodol primarily metabolizes to meprobamate, an active metabolite, via the polymorphic CYP2C19 enzyme (7, 8). Meprobamate (Miltown, Equanil) is a schedule IV controlled substance used to treat anxiety and has a noted potential for abuse (9). Although studies attribute the side effects and abuse potential of carisoprodol to meprobamate formation, other studies have implicated carisoprodol alone as a major component in dependence and toxicity (10). Furthermore, carisoprodol has a high potential for drug diversion due to its ability to potentiate the effects of other illicit drugs (11, 12).

The addictive properties and abuse potential of carisoprodol make patient-monitoring useful for assessing appropriateness of the drug. One monitoring tool is urine drug testing (UDT) and the focus of our study is on the analysis of carisoprodol UDT. Our first objective was to evaluate meprobamate formation with respect to carisoprodol concentrations in urinary excretion data to establish markers as a reference guide for drug monitoring. The second objective was to determine the amount of intra-subject and inter-subject variability in carisoprodol metabolism as observed in urinary excretion data from pain patients prescribed carisoprodol. Literature regarding variability in carisoprodol metabolism is currently lacking. Understanding the variability between (inter-subject) and within (intra-subject) patients may help establish useful references for clinicians monitoring patients prescribed carisoprodol.

Experimental/Methods

Urine specimens were submitted to Millennium Laboratories (San Diego, CA) from pain management physicians’ offices for routine care purposes. The macro-database included 250,638 urine drug test results from 160,380 unique pain subjects. This retrospective study considered urine specimens collected from March 2008 to February 2010. Urine specimens from the macro-database were systematically sorted to include subjects who reported taking carisoprodol to fit the purposes of this study.
Inclusion criteria included: (i) valid urine specimens as defined by quantitated creatinine concentrations ≥20 mg/dL (13, 14); (ii) report of carisoprodol use based on the medication list; (iii) did not report taking meprobamate, the metabolite, in the medication list; (iv) having measured either carisoprodol or meprobamate concentrations at or above the lower limit of quantitation (LLOQ) of 100 ng/mL. Slight variations in sorting schematics for the different objectives are illustrated in Figures 2 and 3 for assessing metabolism and variability, respectively. In Part I (evaluation of carisoprodol metabolism), only specimens from a subject’s single and first visit were used in the analyses. In Part II, subjects with specimens collected over multiple visits (i.e., ≥2 visits) were used to analyze intra-subject variability, and only specimens from a subject’s single and first visit were included for inter-subject analysis.

Although CYP2C19 polymorphism information for each subject was not available, estimation of the proportion of ultra-rapid metabolizers was considered by examining subjects with detectable levels of meprobamate and low to non-detectable carisoprodol concentrations. A mathematical model was used to approximate the proportion of ultra-rapid metabolizers by plotting the frequency of subjects with measurable meprobamate concentrations and carisoprodol concentrations below the lower limit of quantitation. Approximation of poor metabolizers (measurable carisoprodol with no measurable meprobamate) was made by graphical analyses.

Carisoprodol and meprobamate were measured using liquid chromatography–tandem spectrometry (LC–MS-MS) techniques (15, 16). An Agilent 1200 series binary pump SL LC system, well plate sampler and thermostatted column compartment paired with an Agilent triple Quadrupole mass spectrometer and Agilent Mass Hunter software were used for analysis of carisoprodol and meprobamate. Chromatographic separation was performed using an acetonitrile–formic acid–water gradient running at 0.4 mL/min and a 2.1 × 50 mm², 1.8 µm Zorbax SB-C18 column. Mobile phase A was 0.1 percent formic acid in water, B was 0.1 percent formic acid in acetonitrile, and column temperature was set to 50°C. Samples were prepared for injection by incubating with 25 µL of urine with 50 units of β-glucuronidase Type L-II from Patella vulgata (keyhole limpet) (Sigma-Aldrich Corp, St. Louis, MO) in 50 µL 0.4M acetate buffer (pH 4.5) for 3 h at 45°C. Five microliters of the solution were injected for each sample.

All spectra were collected using positive electrospray ionization. The optimized instrumental parameters were as follows: gas temperature, 350°C; drying gas, 12 L/min; nebulizer gas (nitrogen), 35 psi (∼24,100 Pa); capillary voltage, 3,000 V; and fragmenter voltage, 60 V. Multiple reaction monitoring (MRM) mode was used for quantitation. Scan time was set to 500 milliseconds. In MRM mode, two transitions were used to identify and quantify a single compound. A quantitative transition was used to calculate concentration based on the quantifier ion and a second transition was used to ensure accurate identification of the target compound based on the ratio of the qualifier ion to the quantifier ion. From March 2008 to December 2008,
alpha-hydroxyalprazolam was used as the internal standard. After that time, deuterated carisoprodol-D7 and meprobamate-D7 were used as internal standards. The transitions used were as follows: carisoprodol-D7 268.2 → 183.3; carisoprodol 261.2 → 176.1; meprobamate-D7 226.2 → 165; meprobamate 219.1 → 158; and meprobamate 219.1 → 97. HPLC-grade water, acetonitrile, methanol, and formic acid were obtained from VWR (Westchester, PA). Carisoprodol and meprobamate were obtained from Cerilliant Corporation (Round Rock, TX). The deuterated internal standards were diluted to 1,000 ng/mL by adding them to synthetic urine (Microgenics Corporation, Fremont, CA).

Quantitative analysis was performed using Agilent Mass Hunter Quantitative Analysis software. A four-point calibration curve was created by using a linear fit and forcing the line to go through the origin. Accepted accuracy for calibrators was ±20 percent of the target value and the coefficient of determination ($R^2$) was required to be greater than or equal to 0.99 as verification of linearity and goodness-of-fit. The lower limit of quantitation for carisoprodol and meprobamate was 100 ng/mL. The upper limit of linearity for carisoprodol and meprobamate assays was 100,000 ng/mL.

**Graphical and Descriptive Statistical Methods**

Assays for carisoprodol and meprobamate concentrations were reported as nanograms of analyte per milliliter. Measured concentrations were creatinine-corrected to account for variable hydration status, water intake and body mass. Log-transformation was performed to approximate a Gaussian distribution, which normalizes the data to allow further statistical analyses (17). Both log-transformed and back-transformed data were reported. The median with noted 2.5- and 97.5-percentile ranges and mean ± standard deviation (SD) of log-transformed data were reported. Back-transformation was performed after all other statistical analyses were performed, and values were reported as geometric values: geometric median with noted 2.5- and 97.5-percentile ranges, and geometric mean ± geometric SD. The mean and median meprobamate concentration at the ±0.1-interval around the mean carisoprodol concentration was evaluated. The median and respective interquartile range (IQR) of the first and third quartiles were reported as: median (IQR: first quartile to third quartile).

The metabolic ratio (MR), which was used as the parameter to assess metabolism, was calculated by taking the ratio of the measured meprobamate–carisoprodol concentrations in the specimens. The MR is a ratio and therefore dimensionless. The geometric SD was used to assess variability. Because multiple specimens were provided for the same subject in the intra-subject population, the median and mean geometric SD of the MR was used to determine intra-subject variability. Use of the geometric SD of the MR provides a comparative marker to quantify the difference between intra-subject and inter-subject variability.

Due to the short half-life of carisoprodol relative to its metabolite, total carisoprodol load was of particular interest. Total carisoprodol load was used to account for the metabolism of carisoprodol to meprobamate. Because subjects only taking carisoprodol were considered for this study, measured meprobamate levels were considered to be a result of carisoprodol metabolism. The total carisoprodol load was calculated as the sum of the carisoprodol and meprobamate concentrations detected in urine specimens. Meprobamate concentrations were converted to carisoprodol concentrations by dimensional analysis (meprobamate MW: 218.25; carisoprodol MW: 260.33). Total load concentration was normalized and reported as milligrams per gram creatinine.

Graphical and descriptive statistical analyses were done using OriginPro v8.1 (OriginLab Corp., Northampton, MA) and Microsoft Excel 2007 (Microsoft Corp., Redmond, WA). Linear regression analyses were performed using OriginPro v8.1.

![Figure 3. Schematic of sorting methodology for inter-subject and intra-subject variability. Subject flowchart for determination of variability between and within subjects.](image-url)
software with correlation coefficients (R²-values) and slopes reported for analyses.

Ethics
This is a retrospective study of urinary excretion data results from de-identified patient reports. Study-specific subject and specimen identification numbers were assigned. Urine drug test reports only contained information regarding prescribed medication and quantitated urinary excretion data for tested drugs. This study was reviewed and granted IRB-exempt status by the University of California, San Diego (UCSD) Human Research Protections Program (HRPP).

Results
Part I: Evaluating the relationship between carisoprodol concentrations and meprobamate formation
Figure 2 summarizes the sorting schematic employed to obtain the study population. From the macro-dataset, 153,215 unique subjects had measured creatinine concentrations >20 mg/dL. The cohort was further stratified to only include specimens from subjects who reported taking carisoprodol and no other medications that would influence carisoprodol/meprobamate levels in first and single visits. This included 11,322 subjects. A total of 4,350 subjects met the inclusion criteria and were further analyzed to produce reference ranges established in Figure 4A and 4B, and tabulated in Table I. Urine specimens included in this study were from single and first-visit subjects only to prevent weighting the data towards subjects with multiple visits.

The minimum and maximum log carisoprodol concentrations without creatinine-correction were –4.00 to –0.165. Figure 4A is a histogram showing the distribution of log carisoprodol concentrations (milligrams of analyte per gram of creatinine) of the urine specimens that had carisoprodol and meprobamate concentrations ≥ 100 ng/mL. The distribution of the log-transformed concentrations approximately followed a Gaussian distribution. The mean log carisoprodol concentration was –0.284 ± 0.529, and the median was –0.310. The geometric mean carisoprodol concentration was 0.519 ± 3.38 mg per gram creatinine, and the geometric median was 0.490 mg per gram creatinine. The arrows from left to right indicate the 2.5- and 97.5-percentiles, which reflect the measured carisoprodol and meprobamate concentrations for 95% of the study population. The 2.5- to 97.5-percentiles were –1.18 to 0.750 (geometric values of 0.0657 to 5.62 mg per gram creatinine), respectively. The reference range at the 2.5- to 97.5-percentiles for log-transformed values without creatinine correction was –3.96 to –2.41, with a median –3.34. Table I summarizes the descriptive statistics for the creatinine-corrected carisoprodol concentrations.

The minimum and maximum log meprobamate concentrations without creatinine-correction were –3.99 to 0.119, respectively. Figure 4B is a similar histogram showing the distribution of the log meprobamate concentrations (milligrams per gram creatinine) for specimens with carisoprodol and meprobamate concentrations ≥ 100 ng/mL. The distribution of log concentrations approximately followed a Gaussian distribution. The mean log meprobamate concentration was 1.57 ± 0.454, and the median was 1.59. The geometric mean and geometric median were 37.0 ± 2.85 and 38.6 mg per gram creatinine, respectively. The

Table I
Descriptive Statistics of Log Carisoprodol and Meprobamate Concentrations (mg per gram creatinine)

<table>
<thead>
<tr>
<th></th>
<th>Descriptive statistics</th>
<th>Carisoprodol</th>
<th>Meprobamate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Log data*</td>
<td>Geometric data†</td>
<td>Log data</td>
</tr>
<tr>
<td>Mean</td>
<td>–0.284</td>
<td>0.519</td>
<td>1.57</td>
</tr>
<tr>
<td>SD</td>
<td>0.529</td>
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<td>0.454</td>
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<tr>
<td>95% CI</td>
<td>0.0157</td>
<td>1.04</td>
<td>0.0135</td>
</tr>
<tr>
<td>Lower 95% CI</td>
<td>–0.300</td>
<td>0.501</td>
<td>1.56</td>
</tr>
<tr>
<td>Upper 95% CI</td>
<td>–0.269</td>
<td>0.539</td>
<td>1.58</td>
</tr>
<tr>
<td>Median</td>
<td>–0.310</td>
<td>0.490</td>
<td>1.59</td>
</tr>
<tr>
<td>2.5-percentile</td>
<td>–1.18</td>
<td>0.0657</td>
<td>0.648</td>
</tr>
<tr>
<td>25-percentile</td>
<td>–0.668</td>
<td>0.215</td>
<td>1.30</td>
</tr>
<tr>
<td>75-percentile</td>
<td>0.0539</td>
<td>1.13</td>
<td>1.86</td>
</tr>
<tr>
<td>97.5-percentile</td>
<td>0.750</td>
<td>5.62</td>
<td>2.43</td>
</tr>
</tbody>
</table>

*Log-transformation was utilized for normalization of data set.
†Geometric data are reported values after back-transformation of the log-data.
arrows indicating the 2.5- to 97.5-percentiles were 0.648 to 2.43 (geometric values of 4.44 to 267 mg per gram creatinine), respectively. The reference range at the 2.5- to 97.5-percentiles for log-transformed values without creatinine correction was –2.36 to –0.564, with a median –1.43. Table I summarizes the descriptive statistics for the creatinine-corrected meprobamate concentrations.

Figure 5A shows the relationship between log meprobamate concentrations and log carisoprodol concentrations from all concentrations fitting the inclusion criteria. Apparent outliers (n = 21) observed upon graphical analyses were excluded from further linear regression analyses. A marginal positive correlation was observed (R² = 0.294, slope = 0.474). We selected the median log carisoprodol concentration to better assess the variance of meprobamate concentrations at a given carisoprodol concentration.

Figure 5B shows the histogram of the log meprobamate concentration at an interval ± 0.1 on either side of the median log carisoprodol concentration at –0.31; the range of the log carisoprodol concentration was –0.41 to –0.21. The corresponding mean and median log meprobamate concentrations were 1.45 ± 0.369 and 1.48 (IQR: 1.48 to 1.69), respectively. The geometric mean and geometric median were 28.2 ± 2.34 and 30.2 mg per gram creatinine (IQR: 30.2 to 49.0), respectively. Table II summarizes the descriptive statistics for the interval at the median log carisoprodol concentration. The calculated variance at this interval was 29.1 (antilog of 4 × SD or antilog of 1.464).

The MR was defined as the ratio of meprobamate to carisoprodol concentrations. Creatinine-correction was not taken into account because calculating MR was self-correcting and dimensionless. Figure 6A shows the relationship of log MR and the log creatinine-corrected carisoprodol concentrations. Graphical analyses showed apparent outliers (n = 21). This subpopulation was excluded from further linear regression analyses because they were considered a separate population of poor metabolizers. A marginal negative correlation between log MR and log carisoprodol concentrations was indicated by R² = 0.339 and slope = –0.526. The distribution of log MRs at the median interval of log carisoprodol concentration is shown in Figure 6B. The mean and median log MR at this given interval was 1.86 ± 0.365 and 1.90 (IQR: 1.63 to 2.13), respectively. The geometric mean and geometric median MR were 73.2 ± 2.32 and 79.7 [IQR: 42.7 to 136], respectively. The descriptive statistics are summarized in Table II.

A similar evaluation of the relationship between log MR was completed for log meprobamate concentrations, as shown in Figure 7. Graphical analyses also showed the same outliers (n = 21) as previously mentioned. These outliers were removed from further linear regression analysis for the same reasons mentioned previously. A marginal positive correlation between log MR and log meprobamate concentrations was observed (R² = 0.135, slope = 0.380). The mean and median log MR at this given interval was 2.06 ± 0.528 and 2.08 (IQR: 1.70 to 2.43), respectively. The geometric mean and geometric median MR were 114 ± 3.37 and 121 (IQR: 50.4 to 272), respectively. Table III summarizes the descriptive statistics for the log MR at the median interval log meprobamate concentration.

To estimate the proportion of ultra-rapid metabolizers, the frequency of carisoprodol concentrations below the lower limit

<table>
<thead>
<tr>
<th>Descriptive Statistics</th>
<th>Meprobamate Concentration</th>
<th>Log MR</th>
<th>Geometric data†</th>
<th>Log MR</th>
<th>Geometric data†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>1.45</td>
<td>1.86</td>
<td>28.2</td>
<td>73.2</td>
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<tr>
<td>SD</td>
<td>0.369</td>
<td>0.365</td>
<td>2.34</td>
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<tr>
<td>95% CI</td>
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<td>1.84</td>
<td>26.3</td>
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<td>Lower 95% CI</td>
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<td>1.89</td>
<td>29.5</td>
<td>78.0</td>
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<tr>
<td>Upper 95% CI</td>
<td>1.58</td>
<td>1.90</td>
<td>30.2</td>
<td>79.7</td>
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</tr>
<tr>
<td>Median</td>
<td>1.48</td>
<td>1.90</td>
<td>30.2</td>
<td>79.7</td>
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<tr>
<td>25%-percentile</td>
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<td>1.90</td>
<td>40.9</td>
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<tr>
<td>75%-percentile</td>
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<td>2.13</td>
<td>49.0</td>
<td>136</td>
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<td>97.5%-percentile</td>
<td>2.09</td>
<td>2.48</td>
<td>123</td>
<td>305</td>
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</tr>
</tbody>
</table>

*Log-transformation was utilized for normalization of data set.
†Geometric data are reported values after back-transformation of the log-data.
of quantitation was measured, as shown in Figure 8. The arrow indicates grouped specimens \((n = 10)\) with log meprobamate concentrations \(\geq 2.2\). The curve strongly follows a sigmoidal curve fit \(\left(R^2 = 0.996\right)\). The bottom asymptote was fixed at the lowest value \((0.318\%)\), which was used to estimate the proportion of ultra-rapid metabolizers among this study population. At meprobamate concentrations of 15.0 mg per gram creatinine (antilog 1.176), 50% of the observed specimens had carisoprodol concentrations below the lower limit of quantitation. The interpretation of this data is further elaborated on in the "Discussion" section.

**Part II: Assessing inter-subject and intra-subject variability in carisoprodol urinary excretion**

Figure 3 summarizes the sorting schematic employed to establish the inter-subject and intra-subject populations. From the macro-dataset containing urinary excretion data for over 250,000 specimens, 1,035 subjects (2,869 specimens) and 4,982 subjects (4,982 specimens) were sorted for the intra-subject and inter-subject populations, respectively. Histograms of the log total carisoprodol load and the log MR were graphed to compare the inter-subject and intra-subject populations (Figures 9 and 10, respectively). The overlap suggests that the intra-subject population is a good representation of the general (inter-subject) study population. Table IV summarizes the descriptive statistics performed for the log total carisoprodol load for both populations as graphed in Figure 9. The geometric median for the inter-subject population was 46.8 mg per g creatinine (IQR: 24.4, 86.8), and the geometric mean was 45.9 ± 2.73 mg per g creatinine. The geometric median of the total carisoprodol load in the urine for the intra-subject population was 44.9 (IQR: 25.1, 75.2). The geometric mean for the intra-subject population was 43.9 ± 2.33 mg per g creatinine. Table V summarizes the descriptive statistics performed for the log MR for inter-subject and intra-subject
populations associated with Figure 10. The geometric median for the inter-subject population was 77.1 (IQR: 38.3, 153), and the geometric mean was 70.8 ± 3.64. For the intra-subject population, the geometric median and geometric mean were 70.0 (IQR: 36.8, 127) and 63.0 ± 3.41, respectively.

Assessing intra-subject and inter-subject metabolic variability

The metabolic variability was assessed by evaluating the geometric SD. The inter-subject population geometric SD of the MR was 3.64 (Table V). For the intra-subject population, the median geometric SD was 1.60 (IQR: 1.28, 2.07) and the mean geometric SD was 1.72 ± 1.60. Five outliers were observed. These outliers had the following geometric SD of MR: 1005, 723.9, 95.1, 46.1 and 45.5. The inter-subject standard deviation was approximately 2.27 times greater than the median (and 2.11 times greater than the mean) of the intra-subject MR standard deviation. The associated descriptive statistics are summarized in Table VI.

Examples of variable metabolic capacity within subjects

To better illustrate the wide range of variability both within and among subjects, a graphical representation of the MRs from selected subjects over several visits is illustrated in Figure 11. Examples were chosen based on: (i) having three or more visits, and (ii) satisfying the criteria of the degree of the
not significantly glucuronidated under these conditions. Furthermore, the glucuronidase step did not significantly affect the analyses of our results.

**Discussion**

**Range of carisoprodol and meprobamate concentrations in urine drug tests**

The excretion histograms and the tabulated data in Tables I and II should give practitioners interpreting urinary excretion data a range of expected carisoprodol and meprobamate values. Interestingly, the log transformation of the data approximates a Gaussian distribution, as shown in Figures 3A and 3B.

Furthermore, as a means of validating the reasonability of this dataset, a simple justification of these data can be made by employing the following mathematical model. Assume that 1.0 to 1.5 mg of creatinine is normally excreted per day. Carisoprodol is extensively metabolized, and less than 1% of the 350-mg dose is found excreted unchanged in 24-h urine, whereas meprobamate accounts for 4.7% of the dose (6). Thus, the fraction excreted (FE) of 0.047 was used for this calculation. A mean amount of 37.0 ± 2.85 mg meprobamate per gram creatinine was observed in our study population. When taking FE into account, the mean dose was calculated to be 787 mg. Given that the normal dosing regimen for carisoprodol is 350 mg four times a day (1,400 mg), the amount in our data fit within a reasonable range. Although this mathematical equation gives us an estimate, one cannot predict the actual dose from urinary excretion data.

The use of FE is calculated as the total amount excreted unchanged divided by the dose. Some uncertainties associated with the use of FE include: (i) unknown hepatic and renal function for these subjects; and (ii) use of spot collections rather than collection over a 24-h period, which may better reflect literature values of FE. To account for these uncertainties, we used the mean meprobamate concentration that was calculated from over 4,000 subjects. The large sample size (and high power) used in this study was an attempt to overcome these potential limitations.

**Evaluating carisoprodol concentration and meprobamate formation**

A marginal positive correlation was observed between log meprobamate and log carisoprodol concentrations, as shown in Figure 5A. This relationship was strengthened when outliers were not included in the linear regression analysis. The observed linear relationship between carisoprodol and meprobamate formation was less than 1:1. Potential explanations for these observations include: (i) saturation and/or multiple metabolic and reabsorptive pathways; (ii) meprobamate accumulation and decreased carisoprodol levels over time; (iii) genetic variability in metabolizing enzymes; (iv) influences of variable urine pH; and (v) subjects attempting to falsely test positive.

**Saturation of metabolic pathway**

The lack of a 1:1 linear relationship in carisoprodol and meprobamate concentrations, as shown in Figure 5A, may be indicative of potential saturation of the primary carisoprodol

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**Table VI**

Descriptive Statistics of Intra-Subject Metabolic Variability (Standard Deviation of the Metabolic Ratio)*

<table>
<thead>
<tr>
<th>Descriptive statistics</th>
<th>Log data</th>
<th>Geometric data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.237</td>
<td>1.72</td>
</tr>
<tr>
<td>SD</td>
<td>0.216</td>
<td>1.60</td>
</tr>
<tr>
<td>Lower and upper 95% CI</td>
<td>0.224, 0.250</td>
<td>1.67, 1.78</td>
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<tr>
<td>Median</td>
<td>0.205</td>
<td>1.60</td>
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<tr>
<td>25-percentile</td>
<td>0.108</td>
<td>1.28</td>
</tr>
<tr>
<td>75-percentile</td>
<td>0.316</td>
<td>2.07</td>
</tr>
<tr>
<td>Mean</td>
<td>0.237</td>
<td>1.72</td>
</tr>
</tbody>
</table>

* Inter-patient geometric SD of the MR was 3.64 (antilog 0.561).
pathway. That is, at higher concentrations of carisoprodol, we observe an increase in meprobamate concentrations that is proportionally less than the meprobamate concentrations observed at lower carisoprodol concentrations. Further, saturation of the primary CYP2C19 metabolic pathway may lead to carisoprodol metabolism using an alternative pathway.

Accumulation of meprobamate
As described in the package insert, the half-life of carisoprodol and meprobamate are 2.0 ± 0.5 and 9.6 ± 1.5 hours, respectively (3). Over the duration of the carisoprodol regimen, meprobamate can accumulate while carisoprodol concentrations decrease over time. At 6-h dosing intervals, carisoprodol is decreased by three half-lives while meprobamate concentrations are still increasing. This is reflected in the urinary excretion concentrations. If a single dose is missed, that is, if the dosing interval is extended to 12 h, then carisoprodol would not be present in the urine sample, whereas meprobamate would be present. Undetectable levels of carisoprodol concentrations may be expected. Consequently, measurement of carisoprodol using urine drug testing must include measurements of both carisoprodol and meprobamate.

Allelic variations in the CYP2C19 enzymes
The ability and rate of metabolism may be due to pharmacogenetic differences in the polymorphic CYP2C19 enzyme. This point is further elaborated on in later sections that describe the observed ultra-rapid and poor metabolizers.

Influences of variable urinary pH
Differences in an individual’s ability to metabolize and reabsorb the drug and/or metabolite from the urine can further influence urinary excretion data (20). Factors affecting urine pH could potentially affect drug and metabolite concentration in the blood and urine by affecting excretion. Additionally, variability in reabsorption and secretion abilities (such as defects or effects in transporters) may influence carisoprodol concentrations.

Subpopulations of ultra-rapid and poor metabolizers
Two subpopulations were observed in this study. One subpopulation (n = 10; 0.318% of the study population) had a high metabolic capacity and were considered ultra-rapid metabolizers. The second subpopulation (n = 21) represented poor metabolizers and/or subjects attempting to falsely test positive. Graphical analyses of our data showed a subpopulation exhibiting higher concentrations of carisoprodol relative to meprobamate in Figures 5, 6, and 7. The observed outliers (n = 21; 0.483% of the study population) represent a subpopulation exhibiting lower metabolic capacity; that is, urine specimens from these subjects had lower MRs at higher carisoprodol concentrations.

Poor metabolizers were defined as subjects with high levels of carisoprodol and low levels of meprobamate. The possibility of true poor metabolizers may be better appreciated if time-after-dose is known; previous studies showed that poor metabolizers have a longer carisoprodol half-life than the normal population of extensive metabolizers. Other reasons for observing higher carisoprodol levels may include: (i) UDT collection immediately after the patient takes the parent drug, or (ii) concurrent use of medications that inhibit the CYP2C19 metabolizing enzyme, thereby interfering with carisoprodol’s metabolism to meprobamate.

This study did not assess the ethnicity of the subjects. Table VII summarizes the variable CYP2C19 phenotypes among different populations. The frequency of the CYP2C19 poor metabolizer (PM) phenotype varies among different ethnic groups and has been reported as follows: Caucasians, 12%; African-descent, 4%; Chinese, 15–17%; and Japanese, 18–23% (21, 22). Approximately 21 variant alleles for CYP2C19 have been described. The ‘1 (A, B, C) variants have been classified as the normal genotype associated with extensive metabolism (EM). Most of the other noted allelic variants have been associated with total absence of CYP2C19 activity, with ‘2 and ‘3 alleles as the most common alleles responsible for PM (23, 24). However, a ‘17 variant observed among Swedes, Ethiopians and Chinese individuals is believed to be associated with an ultra-rapid (UR) metabolic phenotype (23).

The classifications of ultra-rapid, extensive and poor metabolism phenotypes may contribute to the variability in metabolism we observe in this study population. However, one study noted a potential intermediate metabolizer (IM) phenotype with specific regards to carisoprodol metabolism in heterozygous individuals (25). Thus, such evidence further supports the heavy genetic component and its influence in the variability we observe in carisoprodol metabolism and meprobamate formation.

Furthermore, we employed a mathematical model in Figure 8 to estimate the meprobamate concentration at which 50% of the specimens had carisoprodol concentrations below the LLOQ and the proportion of ultra-rapid metabolizers, which was 0.318% of this study population. This was done by approximating the baseline of the sigmoidal-curve fit (26). Points at the bottom curve were more variable due to the small number of samples in the defined bins, and were therefore grouped. A total of 10 samples were grouped as ≥2.2 log meprobamate, which represents 0.318% of all specimens grouped at this log meprobamate concentration. The top and bottom curves were set constant (27). Fixing is justified for two reasons: (i) samples at this point were grouped together, (ii) removal of the fixing step resulted in a negative percentage, which is not biologically possible, and (iii) the sigmoidal curve model best fit the data as represented by the strong R²-value (R² = 0.996).

Furthermore, this model allows for interpretation and a range at which 50% of the specimens did not have detectable carisoprodol concentrations (this was detected at a log meprobamate concentration of 1.18). In the case of carisoprodol, this model allows us to calculate the meprobamate concentration in which 50% of the specimens did not have detectable carisoprodol concentrations. This may be important for practitioners

<table>
<thead>
<tr>
<th>Population</th>
<th>Phenotype</th>
<th>Phenotype frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasians</td>
<td>PM</td>
<td>12%</td>
</tr>
<tr>
<td>African descent</td>
<td>PM</td>
<td>4%</td>
</tr>
<tr>
<td>Chinese</td>
<td>PM</td>
<td>15–17%</td>
</tr>
<tr>
<td>Japanese</td>
<td>PM</td>
<td>18–23%</td>
</tr>
</tbody>
</table>

Evaluating the Relationship Between Carisoprodol Concentrations and Meprobamate 229
interpreting urine drug tests because it is reflective of the pharmacokinetics of carisoprodol.

**Comparing inter-subject and intra-subject variability**

The inter-subject and intra-subject variability was measured by assessing the geometric SD of the MR. The level of inter-subject variability in carisoprodol metabolism was approximately 2.27 times greater than the median (and 2.11 times greater than the mean) of the intra-subject MR SD.

**Variability within subjects**

Several factors may contribute to the variability observed within subjects. Some examples include time-after-dose, drug-drug interactions and circadian rhythm differences (20).

**Time-after-dose**

The time of specimen collection relative to the time of dose was not accounted for because this was a retrospective study. The pharmacokinetics of carisoprodol play an important role in understanding and interpreting urine specimen data and have been further elaborated upon in “Part I Discussion.” Carisoprodol has a half-life of approximately 2.0 ± 0.5 h, and meprobamate has a half-life of 9.6 ± 1.5h (3). Thus, depending on the time of collection, decreased carisoprodol and higher meprobamate concentrations may be detected over time. Due to the extensive metabolism and short half-life of carisoprodol, undetectable and/or zero-values for carisoprodol should be expected.

**Drug-drug interactions**

The use of unreported drugs interfering with the uptake, metabolism and reabsorption and/or excretion of the drug within a subject may be another potential explanation for some of the higher metabolic capacity we observed in the same subjects. Carisoprodol is extensively metabolized and is a significant substrate of CYP2C19 isoenzymes. For example, omeprazole, a proton-pump inhibitor drug, is also a substrate for the CYP2C19 enzyme. Concomitant use of omeprazole could act as a substrate inhibitor, competing and thereby decreasing the availability of CYP2C19 active sites for carisoprodol metabolism (23). In addition, CY2C19 inhibitors such as fluoxetine could increase carisoprodol plasma concentrations and decrease meprobamate concentrations (28).

**Circadian rhythm differences**

Drug intake at different times of day can affect the drug’s pharmacokinetic (PK) and pharmacodynamic (PD) properties. A drug’s absorption, distribution, metabolism and elimination are encompassed under a drug’s PK. Drug plasma concentrations have been affected by circadian rhythm differences as much as 50% over 24 h. The circadian changes also affect the active form of a drug and its target to determine response. Variable PDs have been observed in constant rate infusions over 24-h periods (29).

**Variability between subjects**

The level of inter-subject variability in metabolic capacity as measured by the geometric SD of the MR was 3.64. As previously discussed, factors such as time-after-dose and drug-drug interactions may influence the variability observed between subjects. Other factors may include a subject’s ability to absorb and reabsorb the drug and the subject’s pharmacogenetics, as discussed previously (20).

**Ability to reabsorb, secrete and renally eliminate the drug**

The ability to reabsorb, secrete and/or renally eliminate carisoprodol may affect both therapeutic and pharmacokinetic (e.g., reabsorption, excretion) outcomes. This parameter can vary with age and gender, and disease states may contribute to the observed variability (20). For example, a study observed an increase in plasma concentrations and a decrease in renal elimination of famotidine, a histamine H2-receptor antagonist, in elderly and renally impaired patients (30). With a decrease in creatinine clearance, as observed in both elderly and renal dysfunction patients due to decline in glomerular filtration, the ratio of famotidine clearance to creatinine clearance also declined. This was suggestive of potential deterioration of renal secretory mechanisms that may be present in both elderly and renally impaired patients.

**Limitations of urine drug testing**

Due to the nature of urine drug tests in pain management offices, the time of collection of urine specimens from patients could not be accounted for or controlled. However, a large sample size was used in this study design in an attempt to correct for random urine specimen collection times. Further attempts include evaluation of metabolic capacity (MR) and metabolite concentrations at an interval around the median log drug concentration.

**Conclusions**

Patient monitoring is important for improving therapeutic outcome and preventing potential drug diversion and abuse. The establishment of reference ranges for carisoprodol and meprobamate levels in urine specimens may facilitate physicians monitoring using non-invasive urine drug testing. Notably, values lying outside the suggested reference range should be given special consideration. Although diversion is a possibility, special consideration should also be given to individual pharmacogenetics, time-after-dose and the intrinsic pharmacokinetics of carisoprodol. One may expect to see undetectable levels of carisoprodol in urine drug tests even among patients consistent with their prescribed carisoprodol due to carisoprodol’s unique pharmacokinetics.

Due to the variability in the excretion and metabolism both within and among subjects, it would be difficult to predict the dose from urinary excretion data alone. Several factors contribute to the observed variability. However, urinary excretion data does provide a useful reference tool for clinicians to monitor consistency with prescribed medications and potentially evaluate and adjust the drug regimen for the most appropriate and effective therapy.
References


