Quantitative Analysis of Carisoprodol and Meprobamate in Whole Blood Using Benzylcarbamate and Deuterated Meprobamate as Internal Standards

Delisa Downey1, Kelsie Simons1, Kenji Ota2, and Sarah Kerrigan1,*
1Forensic Science Program, College of Criminal Justice, Sam Houston State University, Box 2525, 1003 Bowers Blvd., Huntsville, Texas 77341 and 2California Department of Justice, Bureau of Forensic Services, Toxicology Bureau, 4949 Broadway, Sacramento, California 95820

Abstract
Carisoprodol and meprobamate are frequently encountered drugs in impaired driving casework. Deuterated internal standards, although preferred, were not available until recently. Earlier published studies report the use of a variety of non-deuterated internal standards, many of which lack the chemical and physical similarities that are desired for quantitative analysis. Carisoprodol and meprobamate were determined in whole blood using solid-phase extraction and gas chromatography–mass spectrometry with benzylcarbamate and meprobamate-d7 as internal standards. When benzylcarbamate was used as internal standard, the linear ranges for carisoprodol and meprobamate were 0–20 mg/L and 0–40 mg/L, respectively. The linear range increased to 100 mg/L when meprobamate-d7 was used. Limits of detection for carisoprodol and meprobamate were 0.2 and 0.4 mg/L, respectively, regardless of the internal standard selection. The limit of quantitation for both drugs using either internal standard was 0.4 mg/L. Accuracies using benzylcarbamate and meprobamate-d7 were 100–106% and 91–100%, respectively. Corresponding values for precision indicated intra-assay coefficients of variation of 2.6–4.3% for benzylcarbamate and 1.0–2.3% for meprobamate-d7. No carryover was evident at 100 mg/L, the highest concentration tested, and no interferences were observed. Results indicated that either benzylcarbamate or meprobamate-d7 is a suitable internal standard for quantitative determination of carisoprodol or meprobamate from whole blood.

Introduction
In an earlier published study that investigated drug testing practices in drug-impaired driving casework in the United States, 26 of the 40 laboratories included in the study reported that carisoprodol (Soma®) and/or meprobamate were among the 10 most frequently identified drugs (1). Carisoprodol is a widely prescribed and effective skeletal muscle relaxant. It produces well-documented central nervous system depressant effects and has the potential to impair driving (2). Carisoprodol has an elimination half-life of 0.9–2.4 h and is administered orally in divided doses of up to 2400 mg per day (3). Meprobamate, the principal active metabolite, has an elimination half-life of 6–17 h. Peak plasma concentrations following a 700-mg oral dose averaged 3.5 mg/L for carisoprodol (0.8 h) and 4.0 mg/L for meprobamate (3.8 h) (4).

Despite the prevalence of carisoprodol and meprobamate in forensic casework, the choice of internal standards in published studies to date is somewhat limited. Deuterated internal standards are recommended for forensic toxicology gas chromatography–mass spectrometry (GC–MS) assays whenever possible (5). However, these were not commercially available for either carisoprodol or meprobamate until very recently. Ideally, the internal standard should have physical and chemical properties that closely resemble the drug of interest during all stages of the analysis, including extraction from the biological matrix, chromatographic separation, derivatization (if necessary), and analytical detection. A variety of internal standards have been reported for the quantitative determination of carisoprodol and meprobamate including etidocaine (6), lidocaine (7), tybamate (8), vinylbarbital (9), felbamate (10), cyclopentobarbital (2), and carisoprodol (for meprobamate quantitation) (11,12). One chromatographic procedure without the use of internal standards has been reported (13). Meprobamate and carisoprodol are carbamate derivatives with neutral characteristics, which suggests that benzylcarbamate (Figure 1) might be a suitable internal standard. While we were developing a new quantitative GC–MS procedure for carisoprodol and meprobamate using benzylcarbamate, meprobamate-d7 became commercially available. A side-by-side comparison of both internal standards was undertaken, and this report summarizes the results.

* Author to whom correspondence should be addressed. Sarah Kerrigan, Ph.D., Sam Houston State University, Box 2525, 1003 Bowers Blvd, Huntsville, TX 77341. E-mail: sarah.kerrigan@shsu.edu.
Experimental

Materials and methods

Carisoprodol, meprobamate, and meprobamate-d7 were obtained from Cerilliant (Round Rock, TX). Benzylcarbamate and inorganic buffering salts were purchased from Sigma-Aldrich (St. Louis, MO). PolyCrom Clin II (3 cc) solid-phase extraction (SPE) columns containing 35 mg polymeric sorbent were obtained from SPEware (Baldwin Park, CA). Ethyl acetate, hexane, and methanol were obtained from Mallinckrodt-Baker (Hazelwood, MO). A DB-5MS capillary column (30 m × 0.25-mm i.d. × 25 µm) was purchased from VWR (West Chester, PA).

Benzylcarbamate and meprobamate-d7 internal standard solutions were prepared in deionized water at concentrations of 0.02 and 0.01 mg/mL. Working standards containing carisoprodol (0.1 and 0.01 mg/mL) and meprobamate (0.2 and 0.02 mg/mL) were used for the fortification of all calibrators and controls. Drug-free bovine blood containing potassium oxalate (0.2%, w/w) and sodium fluoride (1%, w/w) was purchased from Quad Five Material Bio (Ryegate, MT).

Sample preparation

Aqueous working standards were used to prepare whole blood calibrators in the ranges 2–20 mg/L carisoprodol and 4–40 mg/L meprobamate for routine purposes. A low positive control (4 mg/L carisoprodol and 16 mg/L meprobamate) and high control (18 mg/L carisoprodol and 32 mg/L meprobamate) were routinely included in each run. Internal standard solutions (100 µL meprobamate-d7, 25 µL benzylcarbamate) were added to whole blood (0.25 mL) and vortex mixed. Columns were then successively rinsed using 1 mL deionized water and 1 mL 0.1 M acetic acid. Columns were dried under full vacuum for 5 min, rinsed with hexane (1 mL), and the drugs of interest eluted using ethyl acetate (1 mL) into conical borosilicate glass tubes. Extracts were evaporated to dryness under nitrogen at room temperature, reconstituted in 40 µL of ethyl acetate, and transferred to autosampler vials for injection onto the GC–MS.

Instrumentation

GC–MS analysis was performed using an Agilent HP 5975 MSD/6890 GC with a DB-5MS (30 m × 0.25 mm × 0.25 µm) capillary column. The injector and interface were set at 250°C and 280°C, respectively. Injections (2 µL) were made in split mode with a 10:1 split ratio. The oven temperature was held at 140°C for 0.5 min, ramped to 290°C at a rate of 30°C/min with a final hold time of 2.5 min. The total run time was 8.0 min. Helium was used as the carrier gas at a flow rate of 1.3 mL/min. Data were acquired using selected ion monitoring of the following ions (quantitation ions are underlined): m/z 158, 245, 184 for carisoprodol (4.53 min); 144, 83, 114 for meprobamate (4.24 min); m/z 108, 151, 91 benzylcarbamate (2.78 min); and m/z 151, 89, 121 for meprobamate-d7 (4.22 min).

Assay performance

For the purposes of this study, the upper limit of linearity was defined as the concentration at which the gradient changed by 10% or more. Linear regression analysis was used throughout. The limit of detection (LOD) was defined as the lowest concentration of drug that met the following criteria: signal-to-noise ratio of 3:1 or more; ion ratios for both qualifiers within acceptable ranges (± 20%); and retention time within 2% of the expected value. The limit of quantitation (LOQ) was defined as the lowest concentration of drug that met the following criteria: signal-to-noise ratio of at least 10:1; ion ratios for both qualifiers within acceptable ranges (± 20%); retention time within 2% of the expected value; and a calculated concentration within 20% of the expected value. For the purpose of LOQ determination, whole blood calibrators were prepared and linear regression analysis conducted using low drug concentrations that bracketed the anticipated range of interest (0.2–2.0 mg/L).

Accuracy and precision were evaluated at two concentrations representing the low and high ends of the normal calibration range. Drug-free blood was fortified with carisoprodol at 3 and 15 mg/L and meprobamate at 6 and 30 mg/L. Intra-assay precision was based upon the mean of six determinations using whole blood calibrators as follows: 0, 2, 5, 10, and 20 mg/L (carisoprodol) and 0, 4, 10, 20, and 40 mg/L (meprobamate).

Results and Discussion

Linearity

When benzylcarbamate was used as the internal standard, the upper limits of linearity for carisoprodol and meprobamate were 20 and 40 mg/L, respectively. When meprobamate-d7 was used as the internal standard, calibrations for both drugs remained linear to 100 mg/L, the highest concentration tested. It should be considered that the high limit of linearity is, in large part, due to the small sample volume (0.25 mL) that is used. No carryover of drug was evident at the highest concentration tested (100 mg/L). For routine purposes, calibrators were prepared in the range 2–20 mg/L carisoprodol and 4–40 mg/L meprobamate, regardless of the internal standard selected. Although concentrations of the metabolite...
(meprobamate) may sometimes exceed 40 mg/L in antemortem casework, concentrations approaching 100 mg/L are unusual, and good laboratory practice would suggest that these should be diluted and re-extracted prior to the results being reported quantitatively.

Accuracy and precision

Accuracy was assessed using drug-free blood fortified with carisoprodol at 3 and 15 mg/L, and meprobamate at 6 and 30 mg/L. Results are summarized in Table I. When benzylcarbamate was used as the internal standard, accuracies for low and high concentrations of carisoprodol were 100% and 105%, respectively. Corresponding values for meprobamate were 102% and 106%. When meprobamate-d$_7$ was used as the internal standard, accuracy for carisoprodol was 97% and 91%. Corresponding values for meprobamate were 100% and 92%.

Intra-assay precision was evaluated by replicate analysis ($n = 6$) at two concentrations representing the high and low end of the normal calibration range. Results are summarized in Table I. When benzylcarbamate was used as the internal standard, coefficients of variation (CVs) were 3.2–4.0% for carisoprodol (3–15 mg/L) and 2.6–4.3% for meprobamate (6–30 mg/L). When meprobamate-d$_7$ was used as the internal standard, CVs for carisoprodol and meprobamate were slightly improved (1.3–2.1% and 1.0–2.3%) over the same concentration range.

To determine whether quantitative determinations were significantly different, the corresponding means and variances using benzylcarbamate and meprobamate-d$_7$ were compared using a $t$-test and F-test. The $t$-test revealed no significant difference ($P = 0.05$) for carisoprodol at 3 and 15 mg/L, or meprobamate at 6 mg/L. However, a statistical significance was evident for meprobamate at higher concentration (30 mg/L). The F-test was used to compare the ratio of the two sample variances in order to determine if one method was more precise than the other. Differences in precision were not significant ($P = 0.05$) for carisoprodol and meprobamate at low concentrations (3 and 6 mg/L, respectively). However, at higher drug concentrations (15 and 30 mg/L, respectively) the improved precision using deuterated internal standard was significant at the 5% level.

**LODs and LOQs**

The LODs for carisoprodol and meprobamate in blood were 0.2 and 0.4 mg/L, respectively. These are well below the recommended cutoff concentration of 0.5 mg/L carisoprodol in blood.

### Table I. Upper Limit of Linearity, LOD, LOQ, Precision, and Accuracy using Benzylcarbamate and Meprobamate-d$_7$ as Internal Standard

<table>
<thead>
<tr>
<th>Drug</th>
<th>IS</th>
<th>ULOL* (mg/L)</th>
<th>$R^2$</th>
<th>LOD (mg/L)</th>
<th>LOQ (mg/L)</th>
<th>Target Concentration (mg/L)</th>
<th>Calculated Concentration (mg/L)</th>
<th>Accuracy (%)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meprobamate</td>
<td>BZC</td>
<td>40</td>
<td>0.999</td>
<td>0.4</td>
<td>0.4</td>
<td>6</td>
<td>6.10 ± 0.26</td>
<td>102</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0–40 mg/L)</td>
<td></td>
<td></td>
<td></td>
<td>30</td>
<td>31.84 ± 0.82</td>
<td>106</td>
<td>2.6</td>
</tr>
<tr>
<td>Carisoprodol</td>
<td>BZC</td>
<td>20</td>
<td>0.999</td>
<td>0.4</td>
<td>0.4</td>
<td>3</td>
<td>2.99 ± 0.12</td>
<td>100</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0–20 mg/L)</td>
<td></td>
<td></td>
<td></td>
<td>15</td>
<td>15.80 ± 0.50</td>
<td>105</td>
<td>3.2</td>
</tr>
<tr>
<td>Meprobamate</td>
<td>M-d$_7$</td>
<td>100</td>
<td>0.999</td>
<td>0.4</td>
<td>0.4</td>
<td>6</td>
<td>5.99 ± 0.14</td>
<td>100</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0–40 mg/L)</td>
<td></td>
<td></td>
<td></td>
<td>30</td>
<td>27.59 ± 0.29</td>
<td>92</td>
<td>1.0</td>
</tr>
<tr>
<td>Carisoprodol</td>
<td>M-d$_7$</td>
<td>100</td>
<td>0.999</td>
<td>0.4</td>
<td>0.4</td>
<td>3</td>
<td>2.90 ± 0.06</td>
<td>97</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0–20 mg/L)</td>
<td></td>
<td></td>
<td></td>
<td>15</td>
<td>13.68 ± 0.18</td>
<td>91</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* Abbreviations: upper limit of linearity, ULOL; benzylcarbamate, BZC; and meprobamate-d$_7$, M-d$_7$.
suspected drug-impaired drivers (1). LODs were independent of internal standard because the criteria used to establish them did not involve the measurement of either benzylcarbamate or meprobamate-d7. The LOQ for both drugs, regardless of internal standard, was 0.4 mg/L (Table I). Signal-to-noise ratios for meprobamate and carisoprodol at the limit of quantitation were 40:1 and 186:1, respectively. Chromatograms for the whole blood calibrator at the LOQ are depicted in Figure 2. Calculated concentrations of carisoprodol and meprobamate at 0.4 mg/L were 0.39 and 0.44 mg/L, respectively, using meprobamate-d7 as the internal standard; corresponding values using benzylcarbamate were 0.38 and 0.42 mg/L, respectively.

Interferences

Extracts using the Polycrom Clin II columns were free from coextractive interferences. There were no observed interferences that resulted from the presence of other drugs that elute in the acidic/neutral fraction including barbiturates (butalbital, amobarbital, pentobarbital, phenobarbital) and others (e.g., carbamazein) at concentrations up to 40 mg/L.

Case samples

A total of 20 blood samples obtained from individuals apprehended for impaired driving were provided by the California Department of Justice, Bureau of Forensic Services, Toxicology Laboratory. Carisoprodol and meprobamate were determined quantitatively using the procedure described earlier, using both benzylcarbamate and meprobamate-d7 as internal standards. Chromatograms for a typical case sample are depicted in Figure 3. The range of concentrations for meprobamate and carisoprodol were 5–40 mg/L and 2–14 mg/L. Quantitative results using both internal standards are shown in Figure 4. Linear regression analysis indicated the correlation coefficient for carisoprodol to be 0.972 (y = 1.021x – 0.4352) and 0.952 for meprobamate (y = 1.0166x + 0.2451). The mean differences in concentration for carisoprodol and meprobamate were –0.6 and +0.3 mg/L, respectively. A paired t-test showed that differences in drug concentrations were not significant (P = 0.05).

Conclusions

This procedure describes the rapid extraction and quantitative determination of both carisoprodol and meprobamate using deuterated and non-deuterated internal standards. All assays performed within acceptable parameters in terms of linearity, LOD, LOQ, precision, and accuracy. Only nominal differences were observed. The advantages of meprobamate-d7 were increased linearity (up to 100 mg/L) and improved precision, particularly at higher concentrations. The main advantage of benzylcarbamate is its availability in powder form at less expense, compared to meprobamate-d7, which is commercially available in methanolic solutions at 100 µg/mL and 1 mg/mL. Overall, both internal standards provided excellent results in terms of assay performance.
References


Manuscript received November 6, 2008; revision received January 9, 2009.