Determination of Carisoprodol and Meprobamate in Oral Fluid

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The determination of carisoprodol and its metabolite meprobamate in oral fluid using solid-phase extraction and liquid chromatography with tandem mass spectral detection (LC–MS-MS) and its application to authentic specimens is described. The method employs collection of oral fluid with the Quantisal device, extraction using cation exchange/hydrophobic solid-phase columns, and LC–MS-MS in positive ion electrospray mode. The method was fully validated using various parameters, including selectivity, linearity, accuracy, intra-day and inter-day imprecision, drug recovery from the collection pad, limit of quantitation and matrix effects. The method was applied to both routine research specimens and an authentic specimen taken from an individual prescribed a daily dose of 350 mg carisoprodol following surgery.

Introduction

Carisoprodol (Soma) is a muscle relaxant, prescribed to relieve pain and discomfort caused by strains, sprains and other muscle injuries. The onset of action is rapid and effects last 4 to 6 h. The major metabolic pathway of carisoprodol involves its conversion to meprobamate, which may be prescribed as a pain reliever in its own right. At therapeutic levels it causes sedation, drowsiness and fatigue in addition to its intended use of pain relief. Both drugs are generally prescribed for short periods, but in a recent review of forensic testing laboratories, carisoprodol and meprobamate were seventh out of the top 10 drug classes encountered in cases of driving under the influence of drugs (1). With prolonged abuse at high dosage, carisoprodol can lead to tolerance, dependence and withdrawal symptoms in humans. Clinicians have reported a possible withdrawal syndrome consisting of insomnia, nausea, vomiting, tremors and anxiety in patients who discontinue the use of carisoprodol (2) and in at least one case, withdrawal has been mistaken for a psychotic disorder (3). The abuse of carisoprodol has increased substantially in many areas in the United States (4, 5) and there have been recommendations to place it into the Controlled Substances Act as a scheduled drug (6).

The literature is sparse regarding the concentration of these drugs in oral fluid. Only two publications, both using the Intercept collection device, describe the determination of carisoprodol and meprobamate in oral fluid as part of a general screening protocol (7, 8). The first paper did not specifically discuss carisoprodol or meprobamate; in the second, an oral fluid and blood pair were described resulting in an oral fluid–whole blood ratio but no specific drug concentrations. In this study, we show the viability of the addition of carisoprodol to an oral fluid testing profile when Quantisal is used. Keeping in mind the advantages of oral fluid as a sample matrix over conventional biological matrices (i.e., blood and urine), specifically the ease of collection and difficulty of adulteration, the purpose of this research was to develop a sensitive and specific quantitative method for detection of carisoprodol and meprobamate in oral fluid.

Materials and Methods

Supplies and reagents

Quantisal devices for the collection of oral fluid specimens were obtained from Immunalysis Corporation (Pomona, CA). The devices contain a collection pad with a volume adequacy indicator, which turns blue when one milliliter of oral fluid (±10%) has been collected. The pad is then placed into transport buffer (3 mL), allowing a total specimen volume available for analysis of 4 mL (3 mL buffer + 1 mL oral fluid). Because the oral fluid concentration is diluted 1:3 when using Quantisal collection devices, detected drug concentrations were adjusted accordingly. Deuterated d7-carisoprodol (internal standard) and the unlabelled carisoprodol and meprobamate drug standards were obtained from Cerilliant (Round Rock, TX). Solid-phase extraction columns (C18 II, 691-03535T) were obtained from SPEWare (San Pedro, CA). All solvents were HPLC grade or better, and all chemicals were ACS grade.

Extraction

The internal standard solution contained deuterated d7-carisoprodol at a concentration of 2,500 ng/mL. A calibration curve was prepared in the transport buffer associated with the Quantisal device. The concentrations were equivalent to neat oral fluid volumes of 25, 50, 100, 250 and 500 ng/mL of carisoprodol and meprobamate. Internal standard (20 µL) and 0.1M potassium phosphate buffer, pH 6.0 (1 mL) were added to each calibrator, control or specimen. Solid-phase extraction columns were conditioned with methanol (2 mL) and 0.1M potassium phosphate buffer, pH 6.0 (2 mL). Samples were allowed to flow through the phase slowly, then the columns were washed with deionized (DI) water (2 mL) and methanol–DI water (25:75 v/v, 1 mL). The columns were dried at 30 psi for 5 min, and then hexane (1 mL) was added. After further drying for 5 min, the drugs were eluted in ethyl acetate–hexane (50:50 v/v, 3 mL). Specimens were evaporated to dryness under nitrogen at 40°C and reconstituted in methanol (50 µL) for liquid chromatography–tandem mass spectrometry (LC–MS-MS) analysis.

LC–MS-MS

An Agilent Technologies 1200 Series liquid pump connected to a 6410 triple quadrupole mass spectrometer, operating in positive electrospray mode (ESI) was used for analysis. The liquid chromatographic column was a Zorbax Eclipse XDB-C18 Rapid
Resolution HT (4.6 × 50 mm × 1.8 μm). The column temperature was 40°C and the injection volume was 5 μL. The mobile phase consisted of 20 mM ammonium formate (pH 6.4, Solvent A) and methanol (Solvent B). At the beginning of the run, the mobile phase composition was 85% A–15% B at a constant flow rate of 0.7 mL/min. After 4 min, the percentage of solvent B was 100%, and at 5 min, the percentage of B returned to 15%. The equilibration time was 3 min. The gas temperature was 350°C, the gas flow was 10 L/min and the nebulizer pressure was 50 psi. Nitrogen was used as the collision gas and the capillary voltage was 4,000 V. For d7-carisoprodol, the precursor ion 268.2 was fragmented to 183.2 at optimized fragment voltage of 60 V and collision energy of 2 V. Two transitions were selected and optimized for carisoprodol and meprobamate (quantifying transition is bold): Carisoprodol: 261.2 > 176.1; 261.2 > 158.1 (qualitative transition). Meprobamate: 219.1 > 158.1; 219.1 > 97.1 (qualitative transition) at fragment voltages of 60 V and collision energy of 2 V. Each subsequent analysis required the ratio between the quantitative transition and the qualifying transition to be within ±20% of that established by calibration standards, to meet the criterion for a positive result. For carisoprodol, the acceptable range for the qualifying transition was 30.0–40.9%; meprobamate, 26.9–40.3%. The retention times were 4.6 and 3.9 min for carisoprodol and meprobamate, respectively.

**Method Validation**

**Linearity and sensitivity**
 Calibration using deuterated internal standards was calculated using linear regression analysis over a concentration range of 25–500 ng/mL. A survey of forensic testing laboratories showed a general testing range for carisoprodol and/or meprobamate in blood to be 2–5,000 ng/mL with a mode of 1,000 ng/mL (1). Because carisoprodol has a saliva to plasma ratio much less than one, the selected calibration range focused on the ability to determine lower drug concentrations.

Peak area ratios of the target analyte and the internal standard were calculated using Mass Hunter software (Agilent). The data were fit to a linear least-squares regression curve with a 1/x weighting and not forced through the origin. The linearity of the assays was established with five calibration points, excluding the drug-free matrix. The limit of quantitation (LOQ) of the method was determined using serial dilutions to the lowest point where the acceptable criteria for the quantitation of a compound were met. Criteria included chromatographic peak retention time (±2% of calibration standard) and mass spectral qualifier transition ratio (±20%) compared to a mean value determined from replicate analyses (n = 5) of the 250 ng/mL calibration standard. The quantitative value of the LOQ had to be within ±20% of the target concentration and replicate analyses were required to have low variation in response (n = 5; CV, 15%). Specimens with quantitative values greater than 500 ng/mL were diluted into the linear range.

**External quality control specimens**
 There are few commercial vendors of oral fluid control specimens, and none which offer carisoprodol and/or meprobamate. Therefore, external positive controls at high or low concentrations (100 and 25 ng/mL) were prepared in-house from a different lot number of source material than the calibration curve. Positive or negative controls were included in every batch to consist of at least 10% of the batch size.

**Drug recovery from the pad, accuracy and imprecision**

**Recovery**
 The efficiency of extraction from the collection device was determined. Synthetic oral fluid was fortified with drugs at a concentration of 50 ng/mL. A collection pad was placed into the fluid until the volume adequacy indicator turned blue showing that 1 mL (±10%) of oral fluid had been absorbed. The pads were then placed into the Quantisal buffer (3 mL), capped and allowed to remain at room temperature overnight, to simulate transportation to the laboratory. The following day, the pads were removed after separation from the stem, and an aliquot (1 mL) of the specimen was analyzed. The procedure was repeated five times.

**Accuracy**
 The accuracy of the procedure was determined by the analysis of six separate specimens prepared at concentrations of 75 and 150 ng/mL, using different lot numbers than the calibration materials. Accuracy was calculated as mean measured concentration divided by the fortified concentration × 100%.

**Imprecision**
 Inter-day and intra-day imprecision of the assays was determined at two concentrations: 75 and 150 ng/mL. Intra-day data were obtained from six replicate analyses performed on one day (n = 6); inter-day data were obtained by analyzing six specimens each day for five days (n = 30).

**Selectivity**

**Ion suppression**
 Oral fluid specimens were obtained from drug-free volunteers, extracted and analyzed according to the described procedures to assess interference from extraction or matrix, or potential ion suppression. The protocol from Matuszewski (9) was used to assess matrix effects and process efficiency. To perform experiments according to these protocols, a non-extracted drug standard at a concentration of 50 ng/mL was prepared in addition to drug free matrix extracts and negative controls (extracts containing only internal standard).

The recovery of carisoprodol and meprobamate from oral fluid was determined by first assessing the response of the extracted samples (n = 6) at a concentration of 50 ng/mL (RES). Next, oral fluid was extracted and drug was added post-extraction at a concentration of 50 ng/mL (n = 6) (RPES). The percentage recovery was then calculated from the equation (RES / RPES) × 100.

The percentage reduction / improvement in response due to matrix effects (ion suppression / ion enhancement) was determined by assessing the peak area response of a non-extracted neat drug standard (n = 6) at a concentration of 50 ng/mL.
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Exogenous interference
To assess potential problems arising from exogenous sources, commonly encountered drugs were added to the drug-free oral fluid specimens and subjected to the same extraction and analysis procedures. The following drugs were analyzed at a concentration of 10,000 ng/mL: morphine, codeine, 6-acetylmorphine, oxycodone, oxymorphone, hydrocodone, hydromorphone, buprenorphine, norbuprenorphine, cocaine, benzoylcegonine, cocaethylene, norcocaine, tramadol, fentanyl, 11-nor-Δ⁹-carboxy-tetrahydrocannabinol, tetrahydrocannabinol, amphetamine, methamphetamine, methylenedioxymethamphetamine (MDMA), methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDEA), nortriptyline, amitriptyline, methadone, diazepam, nordiazepam, oxazepam, alprazolam, chloralidepoxide, bromazepam, temazepam, lorazepam, flurazepam, nitrazepam, triazolam, tramadol, secobarbital, pentobarbital, butalbital and phenobarbital.

Stability
The stability of the drugs in the extracts was assessed by allowing the vials to remain inside the auto-sampler, maintained at 7°C. The extracts were re-analyzed after 24 and 48 h against a fresh calibration curve.

Results and Discussion

Method validation

Linearity and sensitivity
The LC–MS-MS procedure developed was validated according to accepted protocols. The limit of quantitation was 10 ng/mL for both drugs and linearity was obtained with an average correlation coefficient of 0.998.

Recovery, accuracy and imprecision
Oiestad et al. reported a carisoprodol recovery of 91% and meprobamate of 76% using the Intercept device (7). The recovery of the drugs from the Quantisal collection pad was 88% for carisoprodol and 92% for meprobamate.

The developed assay showed an accuracy of 100% for both drugs at both concentrations. Intra-day imprecision for carisoprodol and meprobamate at 75 ng/mL was 3.5 and 3.9%, respectively; at 150 ng/mL, the variation was 1.5 and 2.2%, respectively. For inter-day, carisoprodol imprecision was 4% at 75 ng/mL; 2.5% at 150 ng/mL. Meprobamate was 4.4% at 75 ng/mL and 4.8% at 150 ng/mL.

Selectivity

Ion suppression
Because the oral fluid is diluted during in collection, deuterated internal standards are added and specific solid-phase procedures are employed, minimal ion suppression is associated with the method. The percentage matrix effect and process efficiency were determined as follows: carisoprodol –17%, 77%; and meprobamate –20%, 75%.

Interference
Oral fluid specimens collected from drug-free individuals showed no interference with the assay. For exogenous interference, commonly encountered drugs of abuse were studied as described and no interference was observed.

Stability
The extracts were stable for at least two days when kept inside the auto-sampler, which was maintained at 7°C. Less than a 5% difference was found in the quantitation after 24 and 48 h.

Application to research specimens
During the course of research studies, oral fluid specimens are received into our facility. Although the number of positives for carisoprodol and/or meprobamate is small, there seems to be no definite trend in concentration or relationship of carisoprodol to meprobamate. For example, in six positive samples detected within the last year, one contained meprobamate (1,394 ng/mL); three had only carisoprodol (180, 915 and 1,628 ng/mL) and two had high concentrations of meprobamate (3,735, 5,000 ng/mL) combined with low levels of carisoprodol (88, 356 ng/mL) (Figure 1). It is also possible that in some cases, meprobamate was prescribed as a drug itself rather than formed via a metabolic pathway.

To attempt to interpret the various detectable concentrations, an oral fluid specimen was collected using the Quantisal device from an individual prescribed carisoprodol following in-patient surgery. The subject reported no prior use of prescription medications (including carisoprodol). The subject took 350 mg of carisoprodol once daily for two weeks. The oral fluid sample was collected 12 h after ingestion of the final carisoprodol dose and showed no carisoprodol to be present.

Figure 1. Concentrations of carisoprodol and meprobamate detected in oral fluid specimens routinely received into the test facility (n = 6).
but meprobamate was detected at a concentration of 1,015 ng/mL (Figure 2). This may indicate that in therapeutic dosing, a higher concentration of meprobamate is present, in contrast to other drugs in oral fluid, which generally tend to concentrate the parent compound rather than the metabolite.

Very limited information is available on the oral fluid–blood (OF–B) ratio for carisoprodol and meprobamate. In 2010, Gjerde et al. (8) reported that the OF–B ratio for carisoprodol was approximately 0.07 and for 0.2 meprobamate. Although this was based on only one pair of specimens, it does indicate that high levels of carisoprodol in oral fluid may correspond to even higher concentrations in blood. Carisoprodol is given in relatively high doses, and reported overdose values for blood are in the range of 20–75,000 ng/mL for carisoprodol; 18–50,000 ng/mL for meprobamate (10, 11). More recently, in cases of driving impairment in which carisoprodol was the sole drug involved, the strongest symptoms of intoxication were detected when the combined blood concentration of carisoprodol and meprobamate was over 10,000 ng/mL (12). Even with a low OF–B ratio, the described procedure is adequately sensitive for therapeutic and toxic concentrations to be determined in oral fluid.

Summary

The method is precise, accurate and sensitive, and allows the detection of meprobamate, a metabolite of carisoprodol in authentic oral fluid samples taken from an individual prescribed the painkiller following surgery. The detection of measurable amounts of meprobamate 12 h following ingestion of carisoprodol indicates potential applications of the method, which may include therapeutic drug monitoring, driving under the influence of carisoprodol, pain management and overdose detection.

References