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Short communication

A rapid quantitative method of carisoprodol and meprobamate by liquid chromatography–tandem mass spectrometry

Shannon Essler[a,1], Kerry Bruns[a,2], Michael Frontz[b,*], J. Rod McCutcheon[b,3]

[a] Southwestern University, 1001 E. University Avenue, Georgetown, TX 78626, USA
[b] Bexar County Medical Examiner's Office, 7337 Louis Pasteur Drive, San Antonio, TX 78229-4565, USA

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ABSTRACT

The identification and quantitation of carisoprodol (Soma) and its chief metabolite meprobamate, which is also a clinically prescribed drug, remains a challenge for forensic toxicology laboratories. Carisoprodol and meprobamate are notable for their widespread use as muscle relaxants and their frequent identification in the blood of impaired drivers. Routine screening is possible in both an acidic/neutral pH screen and a traditional basic screen. An improvement in directed testing quantitations was desirable over the current options of an underivatized acidic/neutral extraction or a basic screen, neither of which used ideal internal standards. A new method was developed that utilized a simple protein precipitation, deuterated internal standards and a short 2-min isocratic liquid chromatography separation, followed by multiple reaction monitoring with tandem mass spectrometry. The linear quantitative range for carisoprodol was determined to be 1–35 mg/L and for meprobamate was 0.5–50 mg/L. The method was validated for specificity and selectivity, matrix effects, and accuracy and precision.

1. Introduction

Carisoprodol (*N*-isopropyl-2-methyl-3-propyl-1,3-propanediol dicarbamate; *N*-isopropylmeprobamate) and its major metabolite meprobamate were first synthesized in the 1950s (Fig. 1). Although often prescribed clinically as a muscle relaxant or sedative, the abuse of carisoprodol and meprobamate are increasing [1,2]. Abusers become habituated to the subjective mood-altering properties of the drugs, such as relaxation and euphoria [3]. The number of emergency department episodes involving carisoprodol increased by almost 300% (6569–19,513 episodes) from 1994 to 2005 [4].

Carisoprodol has a half-life of 1.7 h and can be prepared alone, with active ingredients, such as aspirin and codeine, or in combination with CNS depressants, such as benzodiazepines, opiates and analgesics [4,5]. Carisoprodol (200–350 mg) can be taken up to four times per day and has an onset of action within 30 min [2,6]. The concentration of carisoprodol (700 mg) in blood after 1–2 h is 3.5 mg/L [6]. Alcohol, sedative antihistamines and drugs of abuse may potentiate its effects, and overdoses may induce myoclonic encephalopathy [1,2]. Dependence and withdrawal are common [3].

Meprobamate, an addictive Schedule IV controlled substance in the U.S., has a half-life of 11.3 h (up to 48 h with chronic usage). It can be taken up to six times per day at dosages ranging from 200 to 400 mg [2,5,7]. Therapeutic dosages of meprobamate and carisoprodol (MEPCAR)[4] decrease human performance and adversely affect driving safety [2,4,7]. The concentration of meprobamate (800 mg) in whole blood after 1–2 h is 16 mg/L [8]. Overdosing meprobamate can cause coma, heart failure and death.

Identification and quantification of MEPCAR in whole blood, urine, bile, muscle, liver, hair, vitreous fluid, plasma and serum have been adopted. Femoral blood is preferred because samples are less affected by postmortem artifactual release [9]. A variety of internal standards have been used in the quantitations of MEPCAR, including etidocaine, lidocaine, tybamate, vinylbarbital, felbamate, cyclopentabarbital and carisoprodol (for meprobamate quantitations). One published method did not use an internal standard [10]. The commercial availability of deuterated

* Corresponding author. Tel.: +1 210 335 4031; fax: +1 210 335 4009.
E-mail addresses: essler@medicine.tamhsc.edu (S. Essler), brunsk@southwestern.edu (K. Bruns), mefron@bexar.org (M. Frontz), rodmc05@yahoo.com (J.R. McCutcheon).
[1] Present address: Texas A&M Heath Science Center College of Medicine, 2401 S. 31st Street Temple, TX 76508, USA. Tel.: +1 512 560 4586.
[2] Tel.: +1 512 863 1628.
[3] Present address: 1001 Kiowa Dr. E, Lake Kiowa, TX 76240, USA. Tel.: +1 210 683 8062.

[4] MEPCAR, meprobamate and carisoprodol.

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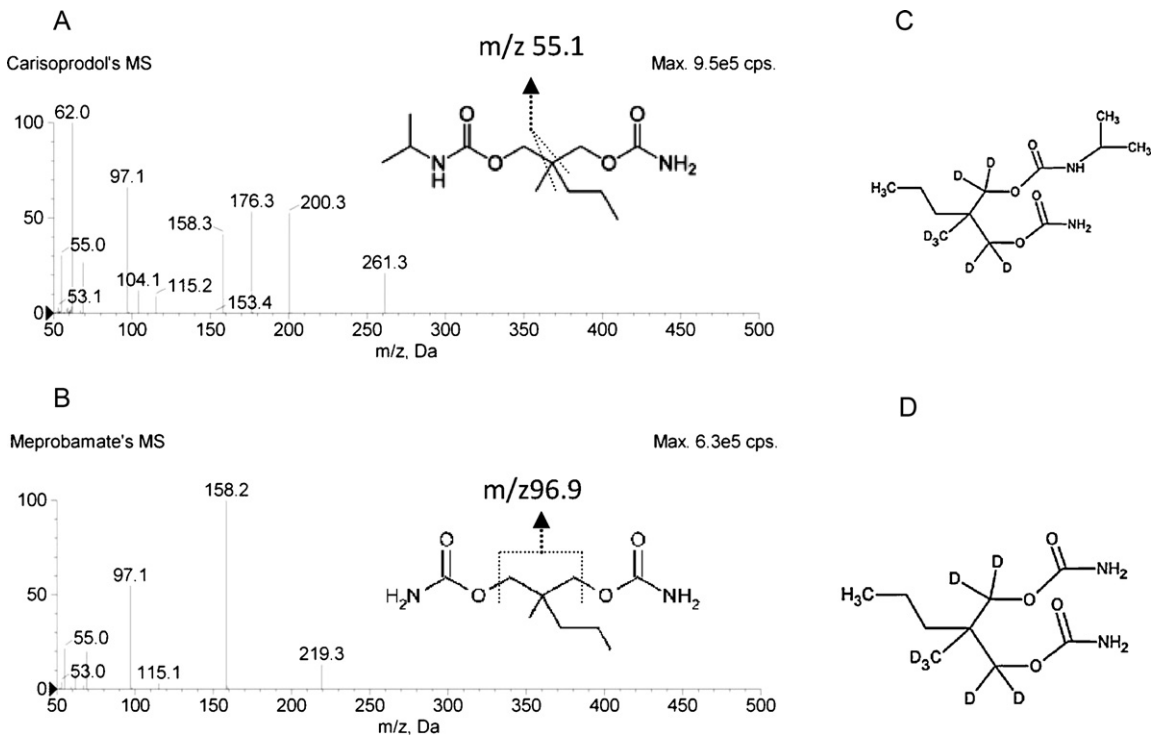

Fig. 1. Stuctures and enhanced product ion (EPI) spectra of carisoprodol (A) and meprobamate (B); structures of carisoprodol-D_7 (C) and meprobamate-D_7 (D).

meprobamate (MEP-D_7)[5] and carisoprodol (CAR-D_7)[6] in 2008 and 2009, respectively, prompted studies of their comparative efficacy in routine toxicological analysis. Downey et al. [10] found that the use of meprobamate-D_7, as opposed to benzylcarbamate, in a new quantitative MEPCAR GC/MS procedure, increased the linear range for meprobamate and carisoprodol from 20 mg/L and 40 mg/L, respectively, to 100 mg/L.

Researchers have typically used flame ionization detection (FID) for the quantitation of MEPCAR [8]. Derivatization of MEPCAR can be used to circumvent the thermal lability of the drugs at the injection port of the GC when it is coupled with MS [5]. However, the complicated derivatization is a time-consuming process that prolongs sample preparation and analysis time. Researchers have been quick to develop derivatization-free and highly sensitive methods using liquid chromatography as an alternative to GC [5]. Matsumoto et al. [5] developed a derivatization-free LC/MS method for the quantitative determination of MEPCAR in urine and plasma using positive electrospray ionization. Liquid chromatography–tandem mass spectrometry is quickly becoming the method of choice for the quantitative determination of drugs and metabolites in biological fluids. Liquid chromatographic methods employ simple sample preparations, are adaptable to rapid analyses and utilize mild working conditions. Mass spectrometric methods are ideal for use in multicompound analyses and can provide structural information about analytes [11].

Few methods have been reported which quantify MEPCAR using LC/MS/MS. A method using solid phase extraction followed by LC/MS/MS using electrospray ionization and multiple reaction monitoring of MEPCAR in equine urine and serum has been reported [12]. Hegstad et al. [13] developed and fully validated a method to quantify MEPCAR, among other drugs, in hair using LC/MS/MS and positive ion mode electrospray. An innovative multimethod for the simultaneous identification and quantification of 12 acidic and neutral compounds, including MEPCAR, in whole blood was developed using a simple liquid–liquid extraction and LC/MS/MS [14].

Prior to the validation of the LC/MS/MS meprobamate and carisoprodol quantitation method, a quantitation utilizing gas chromatograph/flame ionization detection (GC/FID) was used in the Bexar County Medical Examiner's Office (BCMEO[7]) Toxicology Lab. Although the GC method's linear calibration range was slightly greater, from 2.5 mg/L to 50 mg/L, the LC/MS/MS method offers the advantage of an expanded low-end linear range. The previous method used barbital as an internal standard for both meprobamate and carisoprodol, due to the prior unavailability of deuterated internal standards and their contraindicated use in a non-specific detector system such as FID. The GC/FID method involved a single step ethyl acetate extraction from blood buffered to a pH of approximately 4.4, and also required a derivatization step with bistrifluoroacetamide (BSTFA).

A validated and rapid LC/MS/MS method is described for the simultaneous quantitation of MEPCAR in whole blood using a simple protein precipitation, positive electrospray ionization and multiple reaction monitoring. Advantages of the method include shorter run time, use of deuterated internal standards, simple sample preparation procedure and expanded low limit of quantitation. The LC/MS/MS method performed within acceptable parameters in terms of linearity, limits of quantitation, precision, accuracy and stability. No other substances appeared to interfere with the detection and quantitation of MEPCAR. The LC/MS/MS MEPCAR quantitation method is now the method of choice for routine analysis of whole blood in postmortem and human performance toxicology casework at the BCMEO.

[5] MEP-D_7, deuterated meprobamate.
[6] CAR-D_7, deuterated carisoprodol.
[7] BCMEO, Bexar County Medical Examiner's Office.

2. Materials and methods

2.1. Chemicals

Carisoprodol and meprobamate were obtained from U.S. Pharmacopeia (Rockville, MD). Carisoprodol-D_7 and meprobamate-D_7 were purchased from Cerilliant (Round Rock, TX). Ammonium acetate (SigmaUltra, minimum 98%) and formic acid (≥96%, ACS) were obtained from Sigma–Aldrich (St. Louis, MO). Methyl alcohol (HPLC grade) was purchased from Burdick and Jackson (Morristown, NJ). Acetone (ChromAR, 99.5%) was obtained from Mallinckrodt (Hazelwood, MO).

2.2. Blood samples

Pooled drug-free human blood, obtained from a local blood bank (San Antonio, TX), was used for the development and validation of new LC/MS/MS method. The pooled blood was screened and confirmed to be drug-free. Authentic blood samples were collected from medical examiner cases, pathologists, coroners, and DUI, DWI and sexual assault cases.

2.3. Standard preparation

Carisoprodol-D_7 and meprobamate-D_7 internal standard (IS) solutions were prepared in acetone at a concentration of 0.4 mg/L. A reconstitution solvent was prepared by mixing formic acid (0.1 mL), acetonitrile (0.1 mL) and methanol (50 mL) and adding them to a solution of ammonium acetate (15.4 mg) and deionized water (50 mL).

Working standards containing meprobamate and carisoprodol (approximately 0.1 mg/mL and 1 mg/mL MEPCAR) were used to prepare calibrators. The actual concentrations were 101.6 mg/L and 1016 mg/L meprobamate and 96 mg/L and 960 mg/L carisoprodol. Working standards of meprobamate and carisoprodol (approximately 1 mg/mL and 0.1 mg/mL) were used to prepare controls (MEPCAR-C). Working standards were used to prepare calibrators in the range 1–50 mg/L MEPCAR and controls at 5, 10 and 20 mg/L MEPCAR-C.

Calibrators were prepared from samples of drug-free human blood spiked with meprobamate (1016 mg/L) and carisoprodol (960 mg/L). A set of calibrators was worked up with each MEPCAR quantitation and was used to create standard curves for meprobamate and carisoprodol. The calculated calibrator concentrations, using linear regression analysis, fell within 20% of their theoretical concentration. Samples with calculated MEPCAR concentrations outside of the accepted calibrator range were diluted and reanalyzed or designated as having concentrations too low for quantitation.

Controls were prepared using a different meprobamate and carisoprodol stock solutions than the calibrators. Calculated control concentrations fell within 20% of their theoretical value. Control samples were prepared for the accuracy and precision studies needed to validate the LC/MS/MS quantitation method for meprobamate and carisoprodol in whole blood.

2.4. Sample preparation

Whole blood (1 mL) was added to IS solution in acetone (2.5 mL) while vortexing. Tubes were centrifuged after sitting at room temperature for 10 min. The supernatant was decanted through a disposable filter into conical glass tubes and the filter was rinsed with acetone (1 mL). Extracts were evaporated to dryness under air at 75 °C, reconstituted in the reconstitution solvent (1 mL) and transferred to autosampler vials for injection onto the LC/MS/MS. To prepare a standard curve using calibrators or to prepare controls, working standard solutions were spiked into drug-free blood (2 mL) and samples (1 mL) were transferred into IS solution in acetone (2.5 mL) as before. The concentration of MEPCAR in casework was determined using linear regression analysis. The intensity, in counts per second, of the peaks present in casework samples was compared to the calibrators' peak intensities at different known concentrations.

2.5. Instrumentation

An Aligent 1100 series LC (Santa Clara, CA), a Luna 5 μm C18(2) 100 Å column (150 mm × 4.6 mm) from Phenomenex (Torrance, CA), and an Applied Biosystems/MDS Sciex 3200 QTRAP (Foster City, CA) were utilized for the development and validation of the new MEPCAR quantitation method. Ionization was achieved using a Turobo IonSpray electrospray in positive mode. Liquid chromatograph (LC) mobile phase A (5 mM ammonium acetate) with 0.1% formic acid was prepared by dissolving ammonium acetate (0.38 g) into deionized water (1 L) and adding formic acid (1 mL). Mobile phase B was prepared by adding formic acid (1 mL) to methanol (1 L).

Sample injections (10 μL) were accomplished following system equilibration with a 45:55 (A:B) isocratic mobile phase, at a flow rate of 0.5 mL/min. The total run time was 2.0 min; the sample needle was rinsed twice with methanol (10 μL) between runs. Data were acquired using multiple reaction monitoring (MRM) of the ions listed in Table 1. Retention times were carisoprodol (1.58 min), carisoprodol-D_7 (1.45 min), meprobamate (0.65 min) and meprobamate-D_7 (0.66 min), and MEPCAR had chromatographic peaks of acceptable symmetry. All aspects of data acquisition were controlled using Analyst 1.5 software.

2.6. Optimization

Method parameters were initially set to closely mimic an existing LC/MS/MS method for benzodiazepines within the laboratory.

Table 1
Transitions utilized for multiple reaction monitoring (MRM) of carisoprodol (CAR), and meprobamate (MEP) and their deuterated internal standards. Mass spectrometer parameters: declustering (DP), entrance (EP) and collision cell exit (CXP) potentials were optimized, as well as the collision energy (CE).

Analyte	Precursor ion (*m*/*z*)	Product ion (*m*/*z*)	DP (V)	EP (V)	CXP (V)	CE (V)
Carisoprodol	261.2	<u>55.1</u>	16	11	4	47
		176.1	16	11	4	13
		97.1	16	11	4	15
Carisoprodol-D_7	268.3	<u>183.2</u>	31	9	4	13
Meprobamate	219.2	<u>96.9</u>	26	8.5	2	19
		158.2	26	8.5	4	13
		54.7	26	8.5	32	25
Meprobamate-D_7	226.3	<u>165.4</u>	31	9.5	4	13

Underlined transitions were used for quantitation.

Table 2
The linear range over which meprobamate and carisoprodol could be quantitated. Calibrators were chosen for routine analysis (1, 5, 10, 20 and 35 mg/L) after determining the accuracy (%) and precision (CV%) of their preparation and analysis.

Target (mg/L)	Carisoprodol (R^2 = 0.9950, n = 6)			Meprobamate (R^2 = 0.9973, n = 6)		
	Calculated (mg/L)	Accuracy (%)	CV (%)	Calculated (mg/L)	Accuracy (%)	CV (%)
0.5	Calibrator dropped			0.4 ± 0.05	82	21.8
1	0.8 ± 0.04	84	17.6	0.8 ± 0.08	82	18.9
2	2.1 ± 0.06	107	11.3	2.1 ± 0.12	106	11.2
5	5.1 ± 0.08	102	6.5	5.1 ± 0.15	103	5.7
10	10.3 ± 0.26	103	10	10.4 ± 0.43	104	7.8
20	19.5 ± 0.16	98	3.3	19.6 ± 0.35	98	3.4
35	35.0 ± 0.43	100	5	34.7 ± 0.49	99	2.7
50	Calibrator dropped			50.3 ± 1.84	101	7.0

For expediency and to maximize instrument use while minimizing downtime, the analytical column and the mobile phase components remained the same as the benzodiazepines method. This also included maintaining the optimal capillary voltage (5.5 kV) and source block temperature (650 °C). By introducing an isocratic mobile phase rather than the ramp employed by the benzodiazepines method, it was noted that the resolution of the analytes was not compromised. In addition, the analysis time could be drastically shortened (from 12 min to 2 min) without the need to re-equilibrate the mobile phase ratio in the analytical column following each sample injection. The declustering (DP), entrance (EP), collision cell entrance (CEP) and collision cell exit (CXP) potentials were optimized to maximize the intensity of the protonated molecule (Table 1). Collision-activated dissociation (CAD) of each protonated species was performed. Nitrogen (60 psi) was used for the CAD gas and the collision energy (CE) was adjusted to optimize the signal for the most abundant product ions (Table 1).

2.7. Assay performance

Quantifications were performed using integration by dividing the area underneath an MRM peak of meprobamate or carisoprodol by the area underneath its deuterated analog. Limits of linearity were established when the calculated MEPCAR concentrations of the calibrators fell within 20% of the target value. Linear regression analysis was used throughout and the limit of detection was not tested for the purposes of this study. The lower limit of quantitation (LOQ) gave signal to noise ratios of 3:1 or more. For LOQ analysis, whole blood calibrators were prepared, six replicates per concentration level, and linear regression analysis was conducted using concentrations that bracketed the anticipated range of interest (0.5–50 mg/L).

The specificity and selectivity study tested for endogenous interferences from drug-free human blood and samples negative and positive for drugs besides MEPCAR. Samples were prepared without internal standard in aliquots of drug-free human blood (n = 5), drug-free medical examiner's cases (n = 10), and polydrug positive medical examiner cases that did not contain meprobamate or carisoprodol (n = 10). A test for interferences from the IS solution utilized drug-free medical examiner cases spiked with 2 mg/L MEPCAR and deuterated internal standards (n = 10). Calculated concentrations deviating greater than 20% from 2 mg/L would indicate an internal standard interference exists. In addition, the stability of sample extracts was tested 24, 48 and 72 h after an initial analysis by comparing the area ratios of analyte to internal standard of three replicates at low (5 mg/L) and at high (20 mg/L) concentrations between 4 days. The percent difference was documented.

Matrix effects, recovery and process efficiency were studied by determining the absolute peak areas of analytes in three different sets of samples. These samples consisted of a set of neat standards and two sets, prepared in blank matrix, spiked with MEPCAR post-extraction and pre-extraction, respectively.

The matrix effect calculation measures the degree of ion suppression or enhancement by dividing the peak response of neat solution standards by the response for standards spiked after extraction and multiplying by 100 to arrive at a percentage. Recovery is the extraction efficiency, calculated by dividing the response of samples spiked prior to the extraction process by the response of samples spiked following the extraction and multiplying by 100. The process efficiency reveals the percent of MEPCAR recovered after an entire sample preparation and analysis, and is calculated by multiplying matrix effect and recovery, then dividing the product by 100 [15].

Accuracy and precision were evaluated by spiking drug-free blood with MEPCAR-C at three concentrations representing low (n = 5), middle (n = 5) and high (n = 5) ends of the normal calibration range (5, 10 and 30 mg/L MEPCAR-C). Whole blood calibrators were also prepared at 1, 5, 10, 20 and 50 mg/L MEPCAR. Precision was evaluated as a coefficient of variation (CV%) and accuracy as the percent difference of experimental from the theoretical control concentrations. A sample's MEPCAR-C concentration was determined by dividing an analyte peak area by its internal standard peak area. The result was plugged into the calibrators' linear regression equation, used to find the analyte concentration/IS concentration and solve for the unknown analyte concentration. The accuracy and precision analysis was carried out on three separate occasions so that inter-assay precision and accuracy could be calculated.

3. Results and discussion

3.1. Linearity

The linear range of a LC/MS/MS quantitation method for carisoprodol (1–35 mg/L) and meprobamate (0.5–50 mg/L) was determined in whole blood. The average concentrations (n = 6 per level) of accepted calibrators fell within 20% of their theoretical concentration (Table 2). The accuracy of the calculated calibrator concentrations, measured as a percent similarity to the theoretical concentrations, was poorer at lower target concentrations (approximately 82%) than at higher concentrations (approximately 100%) for both meprobamate and carisoprodol. The results are congruent with the understanding that smaller volume micropipettes, such as those used to prepare the low calibrator concentration levels, have larger percent errors in volume delivery than larger volume micropipettes. The percent accuracy of the meprobamate and carisoprodol calibrators accepted for routine analysis ranged from 82% to 106% and 84% to 107%, respectively.

The precision between the six replicates at each concentration level, expressed as the percent coefficient of variation, was less than or equal to 10% for all calibrators prepared to a target concentration less than or equal to 5 mg/L of each analyte. The R^2 values for the linear regression analysis of meprobamate (y = 0.5250x + 0.0664) and carisoprodol (y = 0.2480x + 0.0664) were 0.9973 and 0.9975, respectively. For routine analyses, calibrators were prepared at 1, 5, 10, 20

Table 3
The accuracy of the MEPCAR LC/MS/MS quantitation method was determined at low, middle and high concentrations ($n = 5$) using the percent difference calculated between experimental and theoretical control concentrations.

Control concentration (mg/L)	Carisoprodol percent difference (%)				Meprobamate percent difference (%)			
	Within day accuracy			Between day accuracy	Within day accuracy			Between day accuracy
	Day 1	Day 2	Day 3		Day 1	Day 2	Day 3	
5	−4.5	3.8	10.0	7.2	−1.5	4.2	10.3	5.4
10	−1.4	−2.2	5.7	4.5	1.7	1.5	8.5	4.9
20	7.4	3.2	1.7	8.3	7.3	5.7	3.4	6.5

and 35 mg/L for meprobamate and carisoprodol. For routine analysis, samples determined to have concentrations exceeding the upper limit of quantitation (35 mg/L) are diluted, extracted, and reanalyzed prior to reporting their results quantitatively.

The calibrator range chosen for routine LC/MS/MS quantitations of meprobamate and carisoprodol is comparable to those prepared by Downey et al. [10]. Our use of carisoprodol-D_7 in place of benzylcarbamate can be characterized as a natural progression of methodology as an increasing library of deuterated internal standards are synthesized and become more widely available. Advantages to using meprobamate-D_7 include increased linearity and improved precision, particularly at higher concentrations.

The linear ranges of both MEPCAR in hair have been reported at 0.5–10.0 ng/mg ($R^2 = 0.995$) using GC/MS [16]. Alternatively, LC/MS/MS using electrospray ionization and multiple reaction monitoring has been used to quantify carisoprodol and its metabolites in equine urine and serum [12]. Calibration curves showed acceptable linearity in the range of 0.25–100 ng/mL for carisoprodol and 5–2000 ng/mL for meprobamate in equine serum ($R^2 > 0.995$) after solid phase extraction.

3.2. Accuracy and precision

Precision and accuracy of the LC/MS/MS MEPCAR quantitation method was determined using drug-free human blood calibrators spiked with MEPCAR to 1, 5, 10, 20 and 50 mg/L and using five replicates of drug-free human blood samples spiked with MEPCAR-C to 5, 10 and 30 mg/L. Control samples, spiked with MEPCAR-C, can be differentiated from samples spiked with MEPCAR by the stock solution of meprobamate and carisoprodol used to prepare the working solutions. The accuracy and precision analysis was carried out on three separate occasions so that inter-assay precision (reproducibility) and accuracy could be calculated.

Accuracies of the low, middle and high control concentrations intra- and inter-sample preparations have been reported as the average percent difference from the theoretical value (Table 3). In most cases, the average, calculated MEPCAR concentration was greater than the target concentration. Nonetheless, the intra- and inter-assay accuracy of the carisoprodol and meprobamate quantitations remained high. The greatest range of the average percent difference calculated in Days 1–3 was seen at 5 mg/L of carisoprodol (4.5–10.0%) and meprobamate (1.5–10.3%). The inter-assay accuracy of carisoprodol and meprobamate was lower at 5 mg/L (7.2% and 5.4%, respectively) than at 20 mg/L (8.3% and 6.5%), which had more precise calculated concentrations.

The intra- and inter-assay precision was evaluated using the coefficients of variation (CV) calculated for each control concentration (Table 4). The CVs calculated between days for replicates prepared at 20 mg/L were lowest for carisoprodol and meprobamate at 2.8% and 1.8%, respectively. Smaller volume micropipettes were used at 5 mg/L than at 20 mg/L to spike negative human blood with MEPCAR-C and the larger variation at the lower concentration is apparent (7.1% and 5.6% for carisoprodol and meprobamate), though not sufficient to cause concern.

The new LC/MS/MS method is accurate and precise, has a fairly simple sample preparation and quick run time of less than 2 min, as opposed to the GC/FID method that ran for 24 min.

Klintz et al. [17] documented an intra-assay precision of 4–8% in plasma samples analyzed for meprobamate and carisoprodol after a protein precipitation and 7-min GC/FID analysis, using vinylbarbital as the internal standard. Linnet et al. [14] completed MEPCAR quantitations in whole blood using liquid–liquid extraction and LC/MS/MS in 14 min. Inter-assay precision was less than 15%. Therefore, the LC/MS/MS MEPCAR quantitation method is also accurate and precise.

3.3. Matrix effects, recovery and process efficiency

Matrix effects were evaluated by the experimental method and calculations proposed by Matuszeski et al. [15]. The recovery (RE) calculation determines the extraction efficiency, whereas calculating matrix effects (ME) measures the degree of ion suppression or enhancement, and the process efficiency (PE) calculation reveals the percent of MEPCAR recovered after an extraction and analysis. The absolute peak area ratios of analyte to internal standard in the sets of neat standards, samples spiked pre-extraction and spiked post-extraction were used to calculated RE, ME and PE. The average percent recovery of MEPCAR and relative standard deviation (RSD) between replicates after extraction alone (RE), after analysis alone (ME), and after extraction and analysis (PE) were calculated in samples prepared to 5 mg/L and 20 mg/L MEPCAR (Table 4).

From the PE calculation, it was determined that the LC/MS/MS quantitation method recovers about 73% of carisoprodol present in whole blood at all concentrations tested. A greater percentage of the total meprobamate spike was recovered at 20 mg/L (63%) than at 5 mg/L (58%). The difference in total percent recovery of meprobamate and carisoprodol can be attributed to the fact that slight ion enhancement of carisoprodol and slight ion suppression of meprobamate was observed during analysis, according to the ME calculation at both concentrations. Acceptable matrix effect limits have not yet been defined and the response of individual drugs to sample matrix in the ionization chamber is not well understood. The percent recovery of carisoprodol and meprobamate after extraction alone (RE) was similar (approximately 62–70%) for both drugs and concentration levels. Therefore, the majority of signal loss occurs as a result of analyte loss during the extraction process, as opposed to ion suppression during analysis. Improper sample preparation can cause matrix effects, which may translate to inconsistent sensitivities, as well as decreased precision and accuracy [18].

3.4. Interferences and stability

Neither signals for meprobamate nor carisoprodol appeared in aliquots of drug-free human blood, drug-free medical examiner's cases, or polydrug positive medical examiner cases that did not contain meprobamate or carisoprodol. Therefore, endogenous interferences, as a result of the drug-free blood or the coextraction of MEPCAR with other drugs, were not present. The calculated

Table 4
The coefficients of variation (CVs) of MEPCAR at low, middle and high concentrations ($n = 5$), is reported for three separate analyses.

Control concentration (mg/L)	Carisoprodol coefficients of variation (CVs)				Meprobamate coefficients of variation (CVs)			
	Within day precision			Between day precision	Within day precision			Between day precision
	Day 1	Day 2	Day 3		Day 1	Day 2	Day 3	
5	6.1	4.5	2.6	7.1	8.6	4.7	3.9	5.6
10	9.8	5.2	2.6	4.3	10.4	5.7	4.7	3.9
20	4.1	3.8	5.5	2.8	4.3	6.0	6.4	1.8

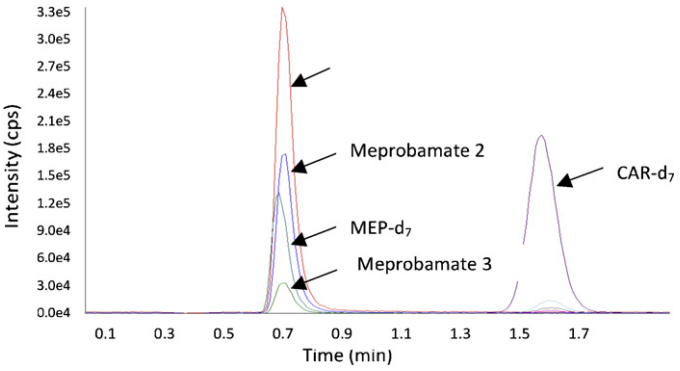

Fig. 2. HPLC chromatogram of multiple reaction monitoring for meprobamate-D_7 (0.65 min), meprobamate (0.67 min), carisoprodol-D_7 (1.45 min) and carisoprodol (1.58 min) transitions. This polydrug positive medical examiner's case also contained acetaminophen (<10 mg/L), diphenhydramine (0.61 mg/L), methadone (0.23 mg/L), methadone metabolite and hydrocodone (0.06 mg/L). Less than 2.5 mg/L meprobamate was found in the sample and carisoprodol was undetected.

concentration of samples prepared in drug-free medical examiner cases spiked with 2 mg/L MEPCAR and deuterated internal standards did not deviate greater than 20% from 2 mg/L. As a result, extracts were free from interferences that could have resulted from the internal standard's native drug contribution.

A chromatogram of a polydrug positive medical examiner's case known to have less than 2.5 mg/L meprobamate is shown (Fig. 2). While carisoprodol was not detected in the GC/FID MEPCAR quantitation, the LC/MS/MS quantitation method did reveal signals for the three carisoprodol transitions it monitors in MRM. The LC/MS/MS procedure yields a more sensitive response to the carisoprodol and meprobamate than the GC/FID method. In addition to carisoprodol and meprobamate, the polydrug positive case contained acetaminophen (<10 mg/L), diphenhydramine (0.61 mg/L), methadone (0.23 mg/L), methadone metabolite and hydrocodone (0.06 mg/L). Dihydrocodeine and EDDP were also detected in the sample, but less than the limit of quantitation. None of the drugs present in the sample matrix interfered with the quantitation of meprobamate and carisoprodol.

In a study of extract stability, the area ratios of analyte to internal standard for three replicates at low (5 mg/L) and at high (20 mg/L) concentrations was documented after being analyzed 0, 24, 48 and 72 h after sample preparation. The percent difference for the low and high concentrations between days was 0.60% and 1.43%, respectively, for carisoprodol and 1.69% and 2.51% for meprobamate. Carisoprodol and meprobamate were determined to be stable, non-volatile drugs. For routine analysis, both analytes can be analyzed up to 72 h after sample preparation.

3.5. Forensic casework

The toxicology lab of the BCMEO performed GC/FID MEPCAR quantitations on whole blood samples found to have carisoprodol, meprobamate or both. In the past year, blood concentrations reported for carisoprodol and meprobamate were approximately 1.5–70 mg/L, with median concentrations at 8.2 mg/L and 3.8 mg/L, respectively. One woman died of morphine and carisoprodol toxicity at a concentration of 74 mg/L.

4. Conclusions

This procedure describes a validated and rapid LC/MS/MS method for the simultaneous quantification of carisoprodol and meprobamate in whole blood. Its advantages include shorter run time, use of deuterated internal standards, easy workup and expanded lower limit of quantitation. All assays performed within acceptable parameters in terms of linearity, limits of quantitation, precision, accuracy and stability. No interferences were present as a result of the sample matrix, internal standards or the presence of other drugs in the sample. This meprobamate and carisoprodol quantitation method has been approved for routine analysis of whole blood casework in the toxicology lab of the BCMEO.

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