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Short communication

HPLC MS/MS method for quantification of meprobamate in human plasma: Application to 24/7 clinical toxicology

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ABSTRACT

We described the development and full validation of rapid and accurate liquid chromatography method, coupled with tandem mass spectrometry detection, for quantification of meprobamate in human plasma with [^{13}C-^{2}H$_3$]-meprobamate as internal standard. Plasma pretreatment involved a one-step protein precipitation with acetonitrile. Separation was performed by reversed-phase chromatography on a Luna MercuryMS C18 (20 mm × 4 mm × 3 μm) column using a gradient elution mode. The mobile phase was a mix of distilled water containing 0.1% formic acid and acetonitrile containing 0.1% formic acid. The selected reaction monitoring transitions, in electrospray positive ionization, used for quantification were 219.2 → 158.2 *m/z* and 223.1 → 161.1 *m/z* for meprobamate and internal standard, respectively. Qualification transitions were 219.2 → 97.0 and 223.1 → 101.1 *m/z* for meprobamate and internal standard, respectively. The method was linear over the concentration range of 1–300 mg/L. The intra- and inter-day precision values were below 6.4% and accuracy was within 95.3% and 103.6% for all QC levels (5, 75 and 200 mg/L). The lower limit of quantification was 1 mg/L. Total analysis time was reduced to 6 min including sample preparation. The present method is successfully applied to 24/7 clinical toxicology and demonstrated its usefulness to detect meprobamate poisoning.

1. Introduction

Meprobamate is a carbamate used as an anxiolytic and sedative since the early 50's [1]. This compound is also the main active metabolite of carisoprodol [2]. It is still widely prescribed although it presents toxic effects in case of misuse. Meprobamate poisoning can lead to drowsiness, stupor, ataxia, hypertonic coma, hypotension, cardiovascular shock, respiratory depression and death [1–3]. For these reasons, assaying meprobamate 24/7 is of great interest in clinical toxicology.

Several methods have been reported for the quantification of meprobamate in human plasma, high-performance liquid chromatography [4] and gas chromatography [5–15]. These methods present the following drawbacks: long analysis time and/or complex sample pre-treatment (derivatization, solid or liquid phase extraction).

We present here, the development and validation of a rapid and simple method for meprobamate quantification in human plasma by LC–MS/MS after simple deproteinization using isotopically labeled analogue.

2. Materials and methods

2.1. Chemicals and reagents

A certified solution of meprobamate at 1 mg/mL in methanol was provided from LGC Standards (Illkirch, France). The internal standard was [^{13}C-^{2}H$_3$]-meprobamate (98%) and was purchased from Alsachim (Strasbourg, France). LC–MS grade methanol was provided by Sigma–Aldrich (St.-Quentin Fallavier, France) and LC–MS grade distilled water by Aguettant (Lyon, France). Analytical grade formic acid was obtained from Sigma–Aldrich (St. Quentin Fallavier, France). Working solutions containing meprobamate and internal standard were prepared at concentrations of 1 mg/L in methanol to optimize specific mass spectrometer detection parameters, and stored at −20 °C. Working solutions of both molecules at 1 g/L in methanol were used for the preparation of calibrators and quality control (QC) samples. Calibration standards and QC were prepared by serial dilution of the working solution in blank plasma. Calibrator concentrations were 0, 1, 10, 50, 100, 150 and 300 mg/L for meprobamate and QC levels were 5, 75 and 200 mg/L. Calibration curves and QC samples were prepared from separate working solutions. Internal standard (IS) solution was prepared at a concentration of 2 mg/L by diluting [^{13}C-^{2}H$_3$]-meprobamate in methanol. A 0.2 μm polyvinylidene fluoride filter was used for mobile phases and was provided by Interchim (Montluçon, France).

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2.2. Sample preparation

An aliquot (50 μL) of plasma, calibrator or QC was combined with 950 μL of IS working solution for protein precipitation. The mixture was vortexed and centrifuged at 12,000 rpm for 4 min. Then 50 μL of the supernatant was diluted with 2 mL of distilled water. The injected volume in the ultra performance liquid chromatography (UPLC) system was 10 μL.

2.3. Liquid chromatography–tandem mass spectrometry

The Acquity ultra-performance liquid chromatograph system was coupled to a Quattro Micro triple quadrupole mass spectrometer from Waters (Saint-Quentin en Yvelines, France). Chromatographic separation was performed at 40 °C on a Luna MercuryMS C18 column (20 mm × 4 mm × 3 μm) from Phenomenex (Saint-Quentin en Yvelines, France). The mobile phase was a mix of A: distilled water containing 0.1% formic acid and B: acetonitrile containing 0.1% formic acid. The gradient was: 0–0.1 min: 5% B; 0.1–0.5 min: linear from 5 to 70% B; 0.5–1.2 min: 70% B; 1.21 min return to initial conditions until 1.5 min. Mobile phase flow was set to 0.5 ml/min. Positive electrospray ionization mode was used. Acquisition was performed in selected reaction monitoring (SRM) mode and the protonated molecular ion of each compound was chosen as precursor ion. Mass spectrometer parameters were: capillary voltage 1.5 kV; lens voltage 0.3 V; source temperature 140 °C; desolvatation temperature 400 °C; cone gas flow 50 L/h; desolvatation gas flow 600 L/h. Dry nitrogen (≥99.5%) produced by N_2 generator F-DBS (Courtaboeuf, France) was used as desolvatation and nebulization gas and argon (>99.999%, Mecer, France) was used as collision gas. Mass spectrometry parameters were optimized by direct infusion of 1 mg/L solutions. Inter-channel and inter-scan delays were 0.01 s and 0.02 s. Dwell time was set to 0.2 s. All data were acquired and processed using MassLynx v 4.1 software (Waters).

2.4. Quantification and method validation

Meprobamate was quantified using [^{13}C-$^{2}H_3$]-meprobamate as an internal standard; peak area ratio was used for calculation. Intra- and inter-day precisions were determined by quantification of QC samples at 5, 75 and 200 mg/L ten times during the same day and on seven consecutive days. Accuracy of the method was determined by injecting the 3 level QC samples ten times. The mean analytical recovery was determined by comparing the measured concentrations against the theoretical concentration (mean concentrations/theoretical concentration × 100) for the 3 level QC samples. Matrix effect (ME) was evaluated by comparing the peak areas of meprobamate and IS between plasma (n = 10) and spiked aqueous solution (ME% = plasma area/aqueous area × 100). Lower limit of detection (LLOD) and lower limit of quantification (LLOQ) were determined by direct injection of decreasing amounts of meprobamate in plasma samples and were calculated as the concentration giving peaks with a signal-to-noise ratio of 3 and 10 respectively. Specificity was evaluated by analysis of 8 different blank batches of plasma.

2.5. Clinical toxicology

The method was used 24/7 in our laboratory to evaluate meprobamate exposition of emergency unit patients from university hospital of Saint-Etienne (France). From July 2009 to July 2010, the distribution of meprobamate plasma levels was evaluated and the frequency of negative (<1 mg/L), therapeutic or subtherapeutic (between 1 and 60 mg/L), toxic (between 60 and 120 mg/L), highly toxic (between 120 and 150 mg/L) and potentially lethal (>150 mg/L) was discussed.

3. Results

3.1. Liquid chromatography

An example of representative chromatograms of drug free human plasma and post intoxication human sample are shown in Fig. 1. Retention, peak shape and retention time stability of meprobamate and IS were excellent on the column (data not shown). Retention time was near 0.95 min for meprobamate and IS, total run time of 1.5 min allowed return to initials conditions.

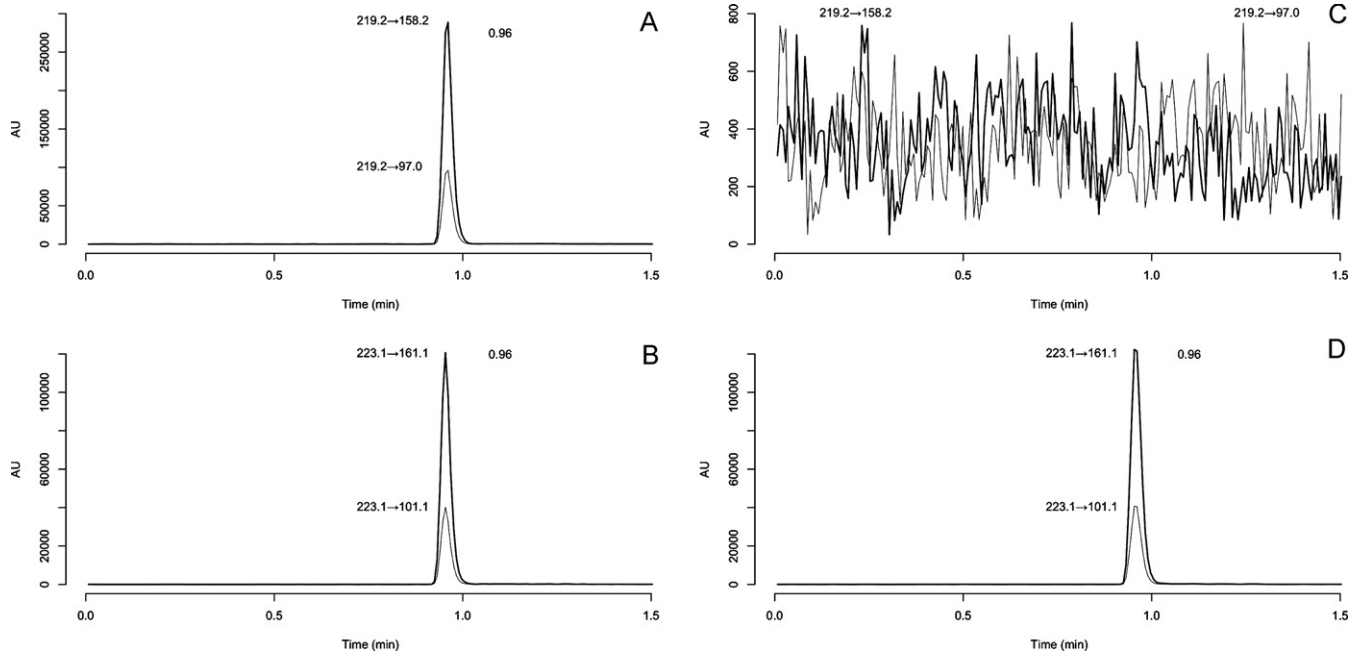

Fig. 1. Representative chromatograms of a patient with 77.8 mg/L of meprobamate (A and B) and negative sample (C and D). (A) and (C) represent meprobamate chromatograms; (B) and (D) represent [^{13}C-$^{2}H_3$]-meprobamate chromatograms.

Table 1
MS/MS parameters.

	Transition (*m*/*z*)	Cone voltage (V)	Collision energy (V)	Dwell time (s)
Meprobamate	219.2 → 158.2	10	10	0.2
	219.2 → 97.0	10	10	0.1
[^{13}C-^{2}H$_3$]-meprobamate	223.1 → 161.1	15	10	0.2
	223.1 → 101.1	15	10	0.1

3.2. Mass spectrometry

The SRM transitions, in positive electrospray ionization, used for quantification were 219.2 → 158.2 *m*/*z* and 223.1 → 161.1 *m*/*z* for [meprobamate + H]$^+$ and [[^{13}C-^{2}H$_3$]-meprobamate + H]$^+$ respectively. Qualification transitions were 219.2 → 97.0 and 223.1 → 101.1 *m*/*z* for meprobamate and internal standard, respectively. Optimized mass spectrometer parameters for each compound are shown in Table 1.

3.3. Method validation

The meprobamate calibration curve was linear between 1 and 300 mg/L. The typical equations of the calibration curves were $y = 0.032x + 0.009$, where y represents the ratio of meprobamate peak area to that of IS and x the plasma concentration. The relative standard deviations of slopes and intercepts for 10 calibration curves were below 7% and mean r^2 was above 0.999. The inter- and intra-day precision and accuracy measured at the three QC levels were summarized in Table 2. The relative standard deviations were less than 6.4% for both inter- and intra-day precision. The accuracy was within 95.3% and 103.6%. The above values were within the acceptable range, the method is considered accurate and precise. The LLOD was 0.5 mg/L and LLOQ was 1 mg/L with an injection volume of 10 μL. The meprobamate and the IS concentrations were stable in matrix at the end of three consecutive freeze–thaw cycles, after 24 h at room temperature and after 1 month at −80 °C with a bias below 3%. No significant matrix effect was observed, the peak area ratios of meprobamate and IS compared with aqueous standard solutions ranged from 95 to 101% ($n = 10$). No interference peak was observed in eight different blank plasmas (data not shown).

3.4. Clinical toxicology

From July 2009 to July 2010, over the 42,790 visits in emergency unit, 1414 patients requested toxicological screening. Among them, 535 meprobamate quantifications were realized for 358 suspicious poisoning cases. Data from intoxications' follow up were excluded from the study. Intoxication was confirmed in 80 (22%) cases and severe in 18 (5%) of them. One hundred and seventy one (48%) meprobamate screening were negative. Fig. 2 presents the distribution of clinical data.

Table 2
Precision and accuracy of the method.

	Meprobamate		
Theoretical concentration (mg/L)	5	75	200
Intra-day ($n = 10$)			
Precision (%)	5.1	6.0	4.4
Accuracy (%)	98.5	100.1	102.7
Inter-day ($n = 7$)			
Precision (%)	6.2	6.4	4.5
Accuracy (%)	101.4	95.3	103.6

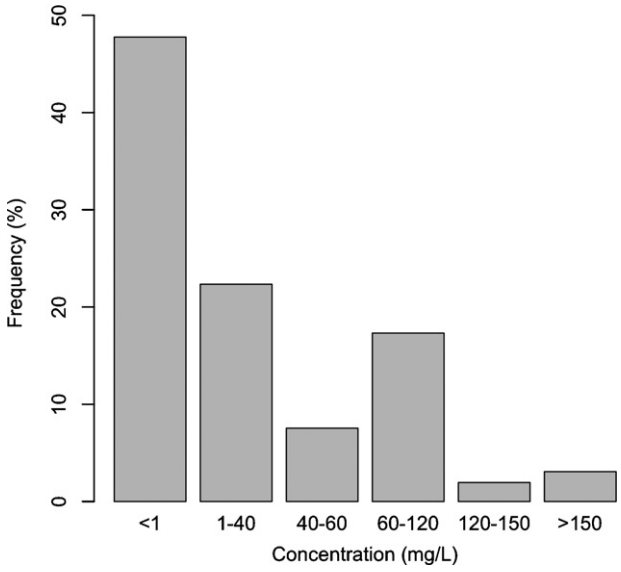

Fig. 2. Histogram of meprobamate clinical data from July 2009 to July 2010.

4. Discussion and conclusion

Several assays using chromatographic method have been published. Most of these methods use CPG [5–15] or HPLC-UV [4]. All of them required solid or liquid phase extraction and some of them derivatization of meprobamate to enhance detection. Considering 24/7 activity, complex sample preparation is the major drawback associated with these methods. Our objective was to develop a one step preparation with fast analysis assay suitable for 24/7 clinical toxicology. To our knowledge, we present here the first fully validated LC MS/MS assay for quantification of meprobamate with analogue isotopically labeled internal standard. Total analysis time was reduced to 6 min including sample preparation.

Even though, meprobamate is an old drug, it is still widely used as sedative and more recently in the treatment of alcoholic withdrawal syndrome. During the last year, we observed 80 poisoning cases in which 18 severe cases required intensive care unit hospitalization.

In conclusion, we described the development and full validation of a one-step preparation, rapid, high throughput sensible and accurate liquid chromatography method using tandem mass spectrometry detection for meprobamate quantification in human plasma. The present method is successfully applied to 24/7 clinical toxicology and demonstrated its usefulness to detect meprobamate poisoning.

References

[1] J. Denisson, J.N. Edwards, G.N. Volans, Hum. Toxicol. 4 (1985) 215.
[2] C. Bismuth, F. Baud, M. Galliot, X.H. Du Fretay, E. Kerviller, J. Toxicol. Clin. Exp. 5 (1985) 321.
[3] C. Bismuth, F. Baud, F. Conso, J.P. Fréjaville, R. Garnier, Toxicologie Clinique, 4ème edition, Flammarion, Paris, 1995, p. 149.
[4] R.N. Gupta, F. Eng, HRC&CC 3 (1980) 419.
[5] D. Downey, K. Simons, K. Ota, S. Kerrigan, J. Anal. Toxicol. 33 (2009) 278.

[6] S. Daval, D. Richard, B. Souweine, A. Eschalier, F. Coudore, J. Anal. Toxicol. 30 (2006) 302.
[7] J.Y. Kim, M.K. In, K.J. Paeng, B.C. Chung, Rapid Commun. Mass Spectrom. 19 (2005) 3056.
[8] T. Gunnar, S. Mykkänen, K. Ariniemi, P. Lillsunde, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 806 (2004) 205.
[9] Y. Gaillard, F. Billault, G. Pépin, Forensic Sci. Int. 86 (1997) 173.
[10] Y. Gaillard, G. Pépin, B. Cabrera, Ann. Biol. Clin. 53 (1995) 361.
[11] Y. Gaillard, J.P. Gay-Montchamp, M. Ollagnier, J. Chromatogr. 577 (1992) 171.
[12] P. Kintz, P.J. Mangin, Anal. Toxicol. 17 (1993) 408.
[13] T. Trenque, D. Lamiable, H. Millart, R. Vistelle, H. Choisy, J. Chromatogr. 615 (1993) 343.
[14] J. Stidman, E.H. Taylor, H.F. Simmons, J. Gandy, A.A. Pappas, J. Chromatogr. 494 (1989) 318.
[15] I. Bechet, A. Ceccato, P. Hubert, P. Herné, J. Crommen, J. Pharm. Biomed. Anal. 10 (1992) 995.