Journal of Chromatography B, 879 (2011) 783–788

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Enantiospecific determination of DL-methylphenidate and DL-ethylphenidate in plasma by liquid chromatography–tandem mass spectrometry: Application to human ethanol interactions

Hao-Jie Zhu [a], Kennerly S. Patrick [b], John S. Markowitz [a,*]

[a] Department of Pharmacotherapy and Translational Research and Center for Pharmacogenomics, University of Florida College of Pharmacy, 1600 SW Archer Road, RM PG-23, Gainesville, FL 32610-0486, USA
[b] Department of Pharmaceutical and Biomedical Sciences, Medical University of South Carolina, Charleston, SC, USA

ARTICLE INFO

Article history:
Received 4 October 2010
Accepted 19 February 2011
Available online 25 February 2011

Keywords:
Methylphenidate
Ethylphenidate
Ethanol
Enantiomer
LC–MS/MS
Drug interaction

ABSTRACT

In humans, concomitant DL-methylphenidate (DL-MPH) and ethanol results in the carboxylesterase 1 (hCES1) mediated biotransformation of MPH to the transesterification metabolite DL-ethylphenidate (DL-EPH). The separate enantiomers of MPH and EPH are found at low ng/ml to pg/ml plasma concentrations. Substantial pharmacological differences exist between D- and L-isomers of MPH and EPH, both in terms of pharmacological potencies and receptor selectivity, as well as in pharmacokinetic properties. Accordingly, a sensitive, accurate and precise enantiospecific analytical method is required in order to fully explore pharmacokinetic–pharmacodynamic correlations regarding the MPH–ethanol interaction. The present study describes a novel liquid chromatographic-tandem mass spectrometric method for simultaneous analysis of D- and L-MPH as well as D- and L-EPH concentrations from human plasma. This assay provides baseline resolution of the individual MPH and EPH isomers utilizing a vancomycin-based chiral column. The lower limit of quantification was 0.025 ng/ml for each isomer when extracting 0.5 ml plasma aliquots. Calibration curves were linear over the range from 0.025 ng/ml to 25 ng/ml for all analytes ($r^2 > 0.995$). Assay accuracy and precision were excellent and stability studies and assessment of potential matrix effects contributed to the validation of the method. Application of the method to human plasma samples collected after the administration of DL-MPH with or without ethanol is included, and the implications of this pharmacokinetic drug interaction discussed.

1. Introduction

DL-Methylphenidate (DL-MPH) is the most frequently prescribed drug for the treatment of the patients with attention-deficit hyperactivity/disorder (ADHD). This neurobehavioral disorder affects 4–8% of the population, and is increasingly found to span the lifecycle in contrast to earlier perceptions of ADHD as a condition that generally resolved upon reaching adulthood [1,2]. In humans, the major metabolic pathway of DL-MPH is the hydrolysis of the methyl ester catalyzed by carboxylesterase 1 (hCES1), which results in high circulating concentrations of the inactive metabolite ritalinic acid [3]. hCES1 mediated hydrolysis of DL-MPH is enantioselective, being substantially more efficient in hydrolyzing L-MPH compared to D-MPH. This results in significantly higher oral bioavailability of D-MPH relative to its L-isomer [4,5]. Importantly, the two isomers also differ in their pharmacological effects as D-MPH is responsible for the psychotherapeutic effects of the racemate [6–9]. Though DL-MPH therapy is generally considered to be effective and well tolerated, significant interindividual variability in efficacy and side effects have been well documented. The dramatic rise in DL-MPH use beginning in the early 1990s has made it a drug of high interest for a number of reasons. Among these is the concern for the diversion of this psychostimulant, and its known potential for recreational abuse alone or in combination with other substances. Indeed, DL-MPH has been reported to be co-abused with ethanol in 92% of those surveyed [10]. In view of the frequent dosing with the MPH–ethanol combination, studies have begun to address and define the pharmacokinetic–pharmacodynamic consequences of concomitant DL-MPH and ethanol use/abuse [5]. Co-administration of ethanol with MPH significantly increases the overall exposure to the parent drug MPH [5]. Potentially worrisome aspects of this interaction is the possibility for co-abuse in supra-therapeutic doses, including intranasal use of crushed tablets which avoids substantial first-pass metabolism. Established ethanol-induced elevations of systemic D-MPH may carry an attendant increase in the risk of cardiovascular accidents and central

* Corresponding author.
E-mail address: jmarkowitz@cop.ufl.edu (J.S. Markowitz).

1570-0232/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2011.02.033

nervous system toxicity. In addition to elevating blood levels of D-MPH, ethanol also serves as a substrate in the metabolic formation of L-ethylphenidate (L-EPH) and, to a more limited extent, the formation of pharmacologically active D-EPH [5]. These products of transesterification occur through the actions of hCES1 [5,11–13]. The relative concentrations of these isomers could be distorted (i.e. the ratio of D- to L-isomer) when hCES1 function is impaired due to *CES1* genetic variants and other factors [14,15]. Thus, it is essential to establish an enantioselective method for the quantification of each of these isomers for the investigation of therapeutic outcomes of MPH treatment and MPH–ethanol interactions. Further, the application of improved analytical methodologies offers a means to advance our understanding of the fields of drug abuse pharmacology and forensic medicine.

A number of analytical methods utilizing high-performance liquid chromatography (HPLC) coupled with various modes of detection including tandem mass spectrometry (MS/MS) as well as gas chromatography–mass spectrometry (GC–MS) has been developed for both non-enantiospecific and enantiospecific analyses of DL-MPH in biological matrices [16–23]. Additionally, several non-enantiospecific liquid chromatography–tandem mass spectrometry (LC–MS/MS) assays have been reported for simultaneous analysis of racemic MPH and EPH in human plasma [11–13]. An existing enantiospecific LC–MS/MS method for plasma MPH and EPH analyses was reported in a normal human volunteer study conducted by our group [5]. In the present study, a new method was validated which improves on existing methods in terms of chromatographic performance including both sensitivity and selectivity; enhanced by the use of the second generation vancomycin-based chiral column. This method was applied to the pharmacokinetic study of a human receiving DL-MPH (0.3 mg/kg) with or without ethanol (0.6 g/kg).

2. Materials and methods

2.1. Chemicals

DL-MPH, the internal standard (IS) d_3-DL-MPH (methyl labeled), LC–MS grade methanol, ammonium acetate, and trifluoroacetic acid were all purchased from Sigma–Aldrich (St. Louis, MO). DL-EPH was synthesized utilizing a previously described method [12]. All other chemicals were of analytical grade and commercially available.

2.2. Preparation of stock solutions, calibrator solutions, and quality controls

Stock solutions of DL-MPH were prepared in methanol while the IS d_3-DL-MPH and DL-EPH stock solutions were prepared in acetonitrile. The concentration of all stock solutions was 1 mg/ml (0.5 mg/ml for each isomer). Working solutions containing both DL-MPH and DL-EPH were prepared by diluting DL-MPH and DL-EPH in water at the following concentrations for each isomer: 250 ng/ml, 50 ng/ml, 10 ng/ml, 5 ng/ml, 2.5 ng/ml, 1 ng/ml, 0.5 ng/ml, and 0.25 ng/ml. All stock and working solutions were stored at −70 °C until use. Calibrator solutions were prepared by adding 50 μl of working solutions to 500 μl of blank human plasma. The calibrator concentrations were 25, 5, 1, 0.5, 0.25, 0.1, 0.05, and 0.025 ng/ml plasma for each isomer. Quality control (QC) samples were used at the concentrations of 10, 0.4, and 0.04 ng/ml plasma.

2.3. Instrumentation

The LC–MS/MS analysis was performed on a Shimadzu HPLC system (Shimadzu, Tokyo, Japan) including a degasser (DGU-14A), two pumps (LC-10ATvp), an autosampler (SIL-10ADvp) and a system control (SCL-10Avp), coupled to an Applied Biosystems-Sciex API 3000 triple quadrupole mass spectrometer (Foster City, CA, USA). The analytes were separated on an Astec Chirobiotic V2 column (5 μm, 250 × 2.1 mm, Sigma–Aldrich, St. Louis, MO). The mobile phase consisted of methanol containing 0.025% ammonium acetate (w/v) and 0.025% trifluoroacetic acid (v/v), and was delivered at a flow rate of 0.2 ml/min. The MS was operated in positive ion mode using turbo electrospray ionization. The MS tuning parameters were optimized for DL-MPH and DL-EPH by infusing 10 μg/ml of both analytes dissolved in mobile phase at a flow rate of 20 μl/ml. The following parameters were utilized for the MS analysis: curtain gas, 12 psi; nebulizer gas (gas 1), 12 psi; CAD gas, 4 psi; TurboIonSpray (IS) voltage, 5500 V; entrance potential (EP), 10 V; collision cell exit potential (CXP), 15 V; declustering potential (DP), 40 V; collision energy (CE), 30 eV; source temperature, 350 °C; and dwell time, 300 ms. The following transitions were monitored in the Multiple Reaction Monitoring (MRM) mode: both isomers of DL-MPH, *m/z* 234 > 84; both isomers of DL-EPH, *m/z* 248 > 84; both isomers of d_3-DL-MPH, *m/z* 237 > 84. Data were acquired and analyzed by AB Sciex Analyst software, version 1.4.2 (AB Sciex, Toronto, Canada).

2.4. Study subject and design

The subject provided written informed consent approved by the Medical University of South Carolina's (MUSC) Office of Research Integrity. He was a healthy 26 year-old, white male weighing 75 kg. Additionally, he was taking no prescription or over-the-counter medications or supplements and was a nonsmoker. The study was conducted in compliance with the current National Institute on Alcohol Abuse and Alcoholism Recommended Council Guidelines on Ethyl Alcohol Administration in Human Experimentation (June, 1996). The subject was admitted into the MUSC clinical research unit at 7:00 a.m. on each of two study days following an overnight fast. There were two phases to this study. During study phase I, the subject received a combination of DL-MPH and an alcoholic beverage while in study phase II, only DL-MPH was administered. For study phase I, following the subject's check-in to the unit, an intravenous catheter was placed into his forearm to facilitate serial blood sampling. The subject was then fed a standardized breakfast. One hour after finishing breakfast, a single oral dose of immediate-release DL-MPH (Ritalin®, Novartis Pharmaceuticals, Summit, NJ) was administered using a weight-based dosing regimen (0.3 mg/kg [22.5 mg using a tablet cutter]) [5]. Ethanol was mixed in orange juice and soda water and was dosed at 0.6 g/kg. The subject was asked to consume the drink mixture within a 15 min period. The subject remained in the study facility until all blood samples were collected, intravenous catheter removed and medically cleared for discharge. The second study phase was identical to the above described study phase I with regard to study timing, DL-MPH dosing and sampling protocol with the exception that no ethanol was consumed. However, an orange juice and soda mixture was provided at the identical time as the alcoholic drink in study phase I and also was consumed within a 15 min period. There was approximately a three month interval period between these two treatment regimens.

2.5. Collection of blood samples

A total of 13 samples were collected over the active study period at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10 and 12 h after MPH dosing. Blood collection tubes (Vacutainers® Becton Dickinson, Rutherford, NJ) containing sodium oxalate were previously stored in an ice bath to minimize both the potential of post-sampling MPH and EPH hydrolysis or transesterification. Venous catheter lines were flushed of residual heparin solution prior to sampling. Samples were promptly centrifuged at 4 °C for 5 min, and the plasma

was immediately aspirated into polypropylene vials and stored at −70 °C until analysis.

2.6. Plasma sample preparation

A liquid–liquid extraction method was utilized to extract both DL-MPH and DL-EPH from plasma. Aliquots (0.5 ml) of test subject plasma samples, calibrators, and QCs were used. Sodium carbonate buffer (0.5 ml, 10 mM, pH 11.3) containing 2 ng/ml of the IS d_3-DL-MPH was added to adjust the pH to 9.0. The samples were vortexed for 5 s followed by the addition of butyl chloride/acetonitrile (2 ml; 4:1). The samples were extracted by vortexing for 20 s, and then centrifuged at 2000 × *g* for 5 min at room temperature. The organic phase was transferred to clean glass tubes and evaporated to dryness under a gentle stream of nitrogen. The residue was reconstituted with 100 μl of mobile phase (methanol containing 0.025% ammonium acetate and 0.025% trifluoroacetic acid) and 10 μl of each were injected for analysis.

3. Results and discussion

3.1. Assay validation

3.1.1. Selectivity

Selectivity was evaluated by analyzing blank human plasma and plasma spiked with 0.25 ng/ml (for individual isomers) of DL-MPH or DL-EPH, or the IS d_3-DL-MPH. The blank plasma was obtained from 6 different sources and tested individually in the present selectivity study. As shown in Fig. 1A, no endogenous interfering peaks were observed in blank plasma for all three monitored *m/z* transitions (234 > 84, 237 > 84, 248 > 84). Excellent baseline separation was achieved for D- and L-isomers of MPH, EPH, and the deuterated IS (Fig. 1B–D). In addition to the column (Chirobiotic™ V2, 5 μm, 250 × 2.1 mm) utilized in the current study, a second chiral column with a similar stationary phase but shorter length (Chirobiotic™ V column [5 μm, 100 × 2.1 mm]), was initially assessed for its capability and performance in the separation and quantification of the respective isomers. Our results indicated that the use of this shorter column was not able to provide adequate baseline resolution of the analyte enantiomers under the otherwise identical LC conditions, though the retention times were significantly shorter than that using the 250 mm column.

3.1.2. Recovery

Recovery experiments were carried out by comparing the analytical results of extracted 3 QC samples (0.04, 0.4, and 10 ng/ml of each isomer of MPH and EPH) with unextracted QCs using 5 replicates. The recovery of IS was determined at the concentration of 2 ng/ml. The extent of recovery was found to be similar among the three different concentrations and the different compounds, ranging from 87.4% to 94.8%.

3.1.3. Linearity

Calibration curves were determined by plotting the concentration versus analyte-to-IS peak area ratio and were found to be linear within the range of 0.025–25 ng/ml for each isomer. This range of concentrations was used to bracket all anticipated concentrations of D-MPH in human plasma within the first 12 h of administration of the clinically relevant doses of DL-MPH (0.3 mg/kg). The correlation coefficients from five independent experiments were ≥0.995 for all four isomers.

3.1.4. Lower limit of quantification

Lower limit of quantification (LLOQ) of each analyte was determined to be 0.025 ng/ml using the criteria in the Guidance for Industry Bioanalytical Method Evaluation presented by the Center for Drug Evaluation and Research [24]. The accuracy of D-MPH, L-MPH, D-EPH, L-EPH was 91.6–104.4%, 91.6–109.6%, 92.0–106.8%, and 89.2–108.8%, respectively, while the %RSD of precision was 4.9%, 6.6%, 5.6%, and 7.5%, respectively, using five replicates at the LLOQ concentration.

Table 1
Intra-day and inter-day accuracy and precision of enantioselective LC–MS/MS method for the determination of D-MPH, L-MPH, D-EPH, L-EPH in human plasma.

Analyte (ng/ml)	Intra-day (n = 5)		Inter-day (n = 15)	
	Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)
D-MPH				
0.04	103.1	9.7	100.9	8.4
0.4	104.1	9.3	103.2	6.6
10	104.5	9.2	103.9	7.9
L-MPH				
0.04	101.6	13.2	105.2	11.0
0.4	97.4	4.7	99.1	7.0
10	103.0	8.6	105.6	8.0
D-EPH				
0.04	101.5	5.9	102.8	6.1
0.4	99.3	8.2	101.8	7.0
10	100.3	7.3	99.8	7.4
L-EPH				
0.04	95.4	10.1	97.1	9.7
0.4	100.5	4.8	98.4	6.4
10	100.1	6.9	100.4	8.2

3.1.5. Accuracy and precision

Three concentrations (0.04, 0.4, and 10 ng/ml) of QC samples in five replicates were utilized to validate the accuracy and precision of the developed method. The results showed that the intra- and inter-day accuracy ranged between 95.4% and 104.5%, respectively. The %RSD of intra- and inter-day precision was less than 13.2% (Table 1). All accuracy and precision results were within acceptable limits. The present LC–MS/MS method was thus found to meet accepted requirements of accuracy and precision [24].

3.1.6. Stability

The bench top, freeze–thaw, and autosampler stability of each enantiomer of MPH and EPH was evaluated using three QC samples (10, 0.4, and 0.04 ng/ml in plasma) with three replicates at each concentration. The bench top stability was assessed by measuring the concentration of each analyte after the samples remained at room temperature for 4 h. This time was chosen based on the expected duration that the samples could be maintained at room temperature during the sample preparation. The ratios of the concentrations of each analyte determined after 4 h exposure to room temperature to that measured immediately after preparation ranged from 0.925 to 0.987. Freeze and thaw stabilities were determined after three freeze–thaw cycles. The QC samples (1 ml) were allowed to thaw unassisted at room temperature, and left on bench for 1 h before being refrozen at −70 °C for 24 h. After three freeze and thaw cycles, the remained concentrations of each analyte were determined to be within 90.6–94.1% of that in freshly prepared QCs. To study the autosampler stability, the QC samples were prepared, transferred to autosampler sample vials, and kept in the autosampler (4 °C) for 24 h before the analysis. The results demonstrated that the concentrations determined in the samples stored in the autosampler for 24 h were between 96.3% and 101.8% of that from the samples measured immediately after preparation.

3.1.7. Matrix effect

In order to assess the assay for any potential matrix-induced ion suppression/enhancement, quantitative matrix effect studies were

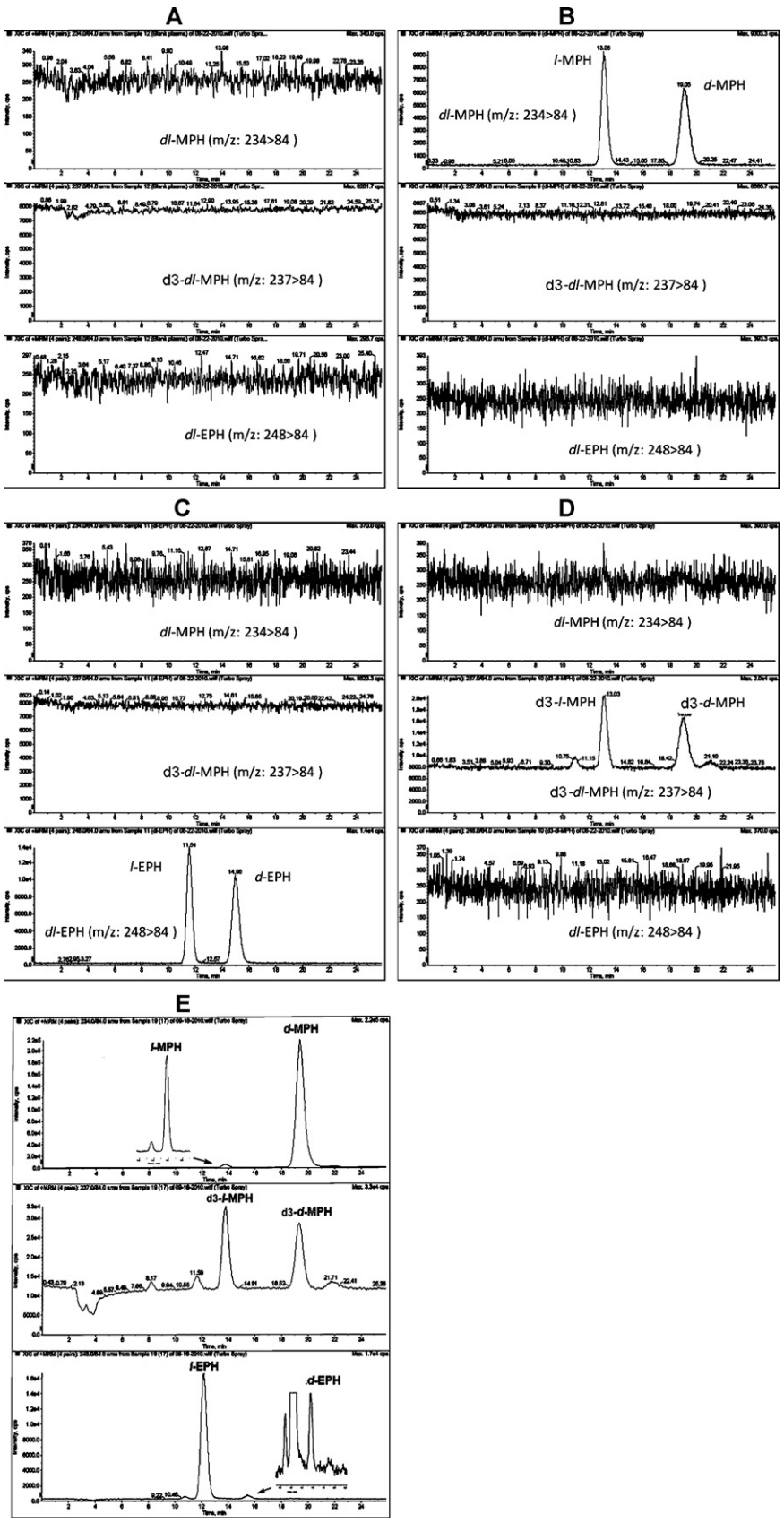

Fig. 1. Representative LC–MS/MS chromatograms of blank human plasma (A) and of plasma spiked with 0.25 ng/ml of DL-MPH (B), DL-EPH (C), and d_3-DL-MPH (D), and human plasma sample collected at 2 h after administration of 0.3 mg/kg DL-MPH, then 0.6 g/kg ethanol (E). The inserts in E show enlarged images of L-MPH and D-EPH chromatographic peaks. The *m/z* transitions of DL-MPH, DL-EPH, and d_3-DL-MPH were *m/z* 234 > 84, 248 > 84, and 237 > 84, respectively.

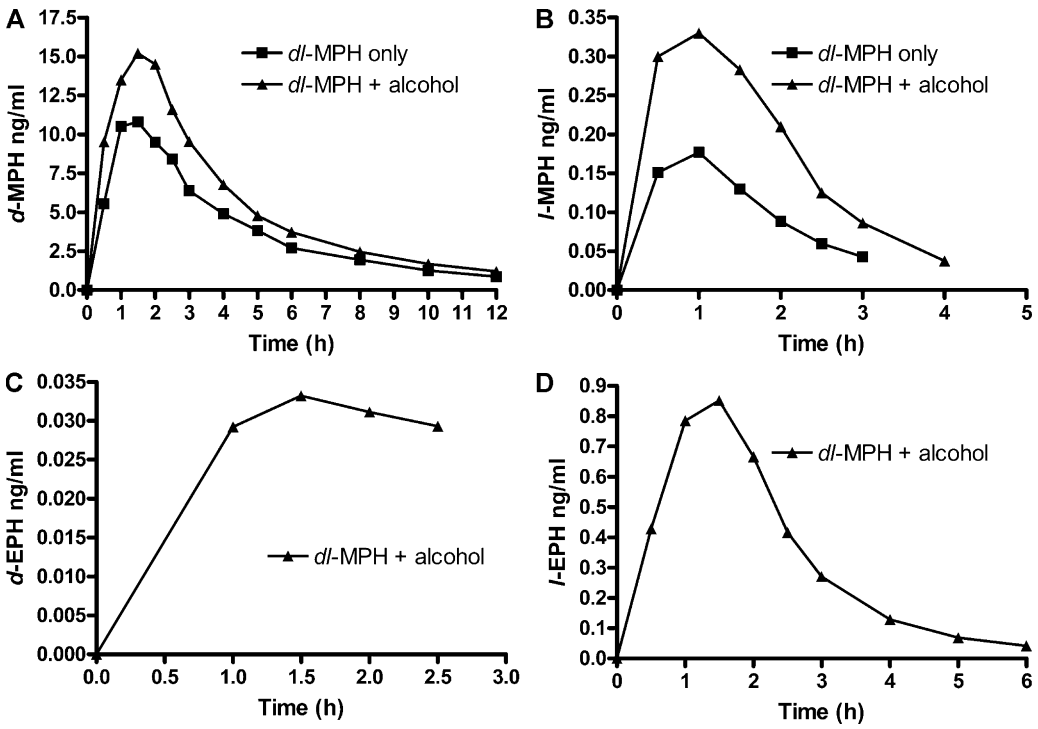

Fig. 2. Plasma concentration–time profile of DL-MPH and DL-EPH isomers from a healthy male volunteer administered 0.3 mg/kg DL-MPH with or without ethanol (0.6 g/kg).

conducted by comparing the absolute peak area of the analytes dissolved in the mobile phase to that of the same analyte solutions containing plasma extracts. Three concentrations (0.2, 2, 20 ng/ml) of each tested enantiomer and 5 ng/ml IS (2.5 ng/ml for d_3-D-MPH and d_3-L-MPH) were utilized in the test with 6 different sources of human plasma. As shown in Table 2, no significant ion suppression or enhancement was observed for all analytes and the IS under the present experimental conditions.

Table 2
Assessment of matrix effect of D- and L-MPH and EPH isomers and the IS.

Analyte (ng/ml)	Mean peak area ($\times 10^5$, $n = 6$)		Matrix effect[c] (%, mean ± SD)
	Without extracts[a]	With extracts[b]	
D-MPH			
0.2	4.94	4.83	97.9 ± 3.3
2	47.26	44.84	95.3 ± 8.9
20	526.20	515.41	97.9 ± 1.9
L-MPH			
0.2	5.09	4.85	98.8 ± 6.6
2	44.98	42.45	94.6 ± 5.9
20	516.14	510.07	98.8 ± 2.0
D-EPH			
0.2	6.26	5.99	95.6 ± 3.6
2	74.30	75.04	101.1 ± 4.7
20	696.29	713.46	102.4 ± 1.6
L-EPH			
0.2	6.18	5.99	97.0 ± 1.5
2	72.47	75.09	103.8 ± 5.6
20	687.69	711.46	103.8 ± 5.6
d_3-D-MPH			
2.5	63.43	60.18	95.1 ± 5.1
d_3-L-MPH			
2.5	60.99	57.47	94.3 ± 3.1

[a] Peak area of analytes solution without plasma extracts.
[b] Peak area of analytes spiked in plasma extracts.
[c] Matrix effect was expressed as the percentage of the mean peak area of the analytes prepared in the mobile phase relative to that of analytes spiked in plasma extracts.

3.2. Human DL-MPH–ethanol interaction study

A series of timed blood samples collected over a 12 h period in a controlled environment were utilized to establish the enantiospecific MPH and EPH plasma concentration–time profile for DL-MPH given alone or with ethanol in a single healthy male volunteer. Fig. 1E shows a representative chromatogram derived from the analysis of the plasma collected from the subject at the 2 h time point after co-administration of DL-MPH and ethanol. D-MPH plasma concentrations were significantly higher than L-MPH in both the DL-MPH dosed only phase, and those collected in which ethanol administration followed DL-MPH dosing (Fig. 2A and B). The data were found to be consistent with previously published studies demonstrating that L-MPH serves as a better substrate of hCES1 than D-MPH [4,5,15]. Additionally, in agreement with our previous findings [5], the co-administration of ethanol significantly increased the systemic exposure to both isomers of DL-MPH (Fig. 2A and B). The formation of D- and L-EPH was detected in the samples obtained during the DL-MPH and ethanol study phase (Fig. 2C and D). The plasma concentrations of L-EPH were considerably higher than those of D-EPH, indicating L-MPH is more extensively converted to L-EPH via hCES1 mediated transesterification. As expected, neither D-EPH nor L-EPH was observed in the samples when the subject was treated with DL-MPH only.

4. Conclusions

A sensitive and high quality enantiospecific LC–MS/MS method for the simultaneous analysis of DL-MPH and DL-EPH in human plasma has been developed and validated in the present study. The method has proven to exhibit molecular specificity as well as being robust, accurate and precise for all four isomeric analytes. Baseline resolutions of D- and L-isomers of MPH, EPH, and the IS d_3-MPH was excellent and achieved by employing the second generation vancomycin-based chiral column. In summary, the described ana-

lytical method was found to be readily applicable to the clinical study of DL-MPH pharmacokinetics following a typical therapeutic dose of DL-MPH with or without a moderate dose of ethanol. This investigative approach permits the establishment of metabolic interactions of DL-MPH with ethanol where the use of EPH serves as a unique biomarker for combined DL-MPH and ethanol exposure.

Acknowledgments

This work was supported by NIH grants R01DA022475-01A1 (J.S.M.) and R01AA016707 (K.S.P.).

References

[1] S. Okie, N. Engl. J. Med. 354 (2006) 2637.
[2] T.J. Spencer, L.A. Adler, Q. Meihua, K.E. Saylor, T.E. Brown, J.A. Holdnack, K.J. Schuh, P.T. Trzepacz, D.K. Kelsey, J. Atten. Disord. 14 (2010) 57.
[3] W. Wargin, K. Patrick, C. Kilts, C.T. Gualtieri, K. Ellington, R.A. Mueller, G. Kraemer, G.R. Breese, J. Pharmacol. Exp. Ther. 226 (1983) 382.
[4] Z. Sun, D.J. Murry, S.P. Sanghani, W.I. Davis, N.Y. Kedishvili, Q. Zou, T.D. Hurley, W.F. Bosron, J. Pharmacol. Exp. Ther. 310 (2004) 469.
[5] K.S. Patrick, A.B. Straughn, R.R. Minhinnett, S.D. Yeatts, A.E. Herrin, C.L. DeVane, R. Malcolm, G.C. Janis, J.S. Markowitz, Clin. Pharmacol. Ther. 81 (2007) 346.
[6] K.S. Patrick, R.W. Caldwell, R.M. Ferris, G.R. Breese, J. Pharmacol. Exp. Ther. 241 (1987) 152.
[7] J.S. Markowitz, C.L. DeVane, L.K. Pestreich, K.S. Patrick, R. Muniz, J. Child Adolesc. Psychopharmacol. 16 (2006) 687.
[8] J.S. Markowitz, K.S. Patrick, J. Clin. Psychopharmacol. 28 (2008) S54.
[9] D. Quinn, S. Wigal, J. Swanson, S. Hirsch, Y. Ottolini, M. Dariani, M. Roffman, J. Zeldis, T. Cooper, J. Am. Acad. Child Adolesc. Psychiatry 43 (2004) 1422.
[10] C. Darredeau, S.P. Barrett, B. Jardin, R.O. Pihl, Hum. Psychopharmacol. 22 (2007) 529.
[11] J.S. Markowitz, C.L. DeVane, D.W. Boulton, Z. Nahas, S.C. Risch, F. Diamond, K.S. Patrick, Drug Metab. Dispos. 28 (2000) 620.
[12] J.S. Markowitz, B.K. Logan, F. Diamond, K.S. Patrick, J. Clin. Psychopharmacol. 19 (1999) 362.
[13] M. Koehm, G.F. Kauert, S.W. Toennes, Arzneimittelforschung 60 (2010) 238.
[14] H.J. Zhu, D.I. Appel, Y.K. Peterson, Z. Wang, J.S. Markowitz, Toxicology 270 (2010) 59.
[15] H.J. Zhu, K.S. Patrick, H.J. Yuan, J.S. Wang, J.L. Donovan, C.L. DeVane, R. Malcolm, J.A. Johnson, G.L. Youngblood, D.H. Sweet, T.Y. Langaee, J.S. Markowitz, Am. J. Hum. Genet. 82 (2008) 1241.
[16] K.S. Patrick, K.R. Ellington, G.R. Breese, C.D. Kilts, J. Chromatogr. 343 (1985) 329.
[17] K.S. Patrick, E.J. Jarvi, J. Chromatogr. 528 (1990) 214.
[18] H.J. Zhu, J.S. Wang, K.S. Patrick, J.L. Donovan, C.L. DeVane, J.S. Markowitz, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 858 (2007) 91.
[19] E. Marchei, M. Farre, M. Pellegrini, S. Rossi, O. Garcia-Algar, O. Vall, S. Pichini, J. Pharm. Biomed. Anal. 49 (2009) 434.
[20] N. Barbarin, D.B. Mawhinney, R. Black, J. Henion, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 783 (2003) 73.
[21] D.R. Doerge, C.M. Fogle, M.G. Paule, M. McCullagh, S. Bajic, Rapid Commun. Mass Spectrom. 14 (2000) 619.
[22] S.N. Lin, D.M. Andrenyak, D.E. Moody, R.L. Foltz, J. Anal. Toxicol. 23 (1999) 524.
[23] L. Ramos, R. Bakhtiar, T. Majumdar, M. Hayes, F. Tse, Rapid Commun. Mass Spectrom. 13 (1999) 2054.
[24] U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Guidance for Industry: Bioanalytical Method Validation, 2001.