Enantioselective Separation and Simultaneous Determination of Tolperisone and Eperisone in Rat Plasma by LC-MS/MS

R. NAGESWARA RAO,[1]* AND S. SATYANARAYANA RAJU[2]

[1]*Analytical Chemistry Division, Indian Institute of Chemical Technology, Tarnaka, Hyderabad, India -500 007*
[2]*Natural Products Chemistry Division, Indian Institute of Chemical Technology, Tarnaka, Hyderabad, India 500 007*

ABSTRACT Tolperisone and eperisone used as muscle relaxants possess one chiral center each and exist as two optical isomers for each drug. Therefore, enantioselective assays to measure each enantiomer in biological matrices are of great importance. In the present study a simple and complete reverse-phase liquid chromatography tandem mass spectrometric method for separation and enantioselective determination of tolperisone and eperisone in rat plasma was developed. The analytes were extracted from rat plasma by a simple protein precipitation method with acetonitrile as the extraction solvent. The enantioselective separation of analytes was achieved on a Cellulose Tris (4-chloro-3-methylphenylcarbamate) chiral column with a mobile phase of acetonitrile: 10 mM ammonium acetate in an isocratic mode of elution and mass spectrometric detection. The calibration curve for each enantiomer was found to be linear over 0.2 to 20 ng/mL for each enantiomer. The proposed method exhibited good intra- and interday precision (% CV) ranged between 0.95–6.05% and 1.11–8.21%, respectively. The intra- and interday accuracy for the proposed assay method ranged between 94.0–100.5% and 92.7–102.1%, respectively. The proposed method was validated as per regulatory guidelines. *Chirality 25:622–627, 2013.*

KEY WORDS: muscle relaxants; enantiomers; rat plasma; tandem mass spectrometry; reversed phase

INTRODUCTION

Tolperisone, known as 2-methyl-1-(4-methylphenyl)-3-(1-piperidinyl)-1-propanone (TOL) is a centrally acting muscle relaxant agent that has been in therapeutic use for decades. It is mainly used for treating muscle spasticities of neurological origin and painful muscle spasms due to rheumatologic conditions. Besides being an effective antispastic agent,[1,2] tolperisone also has analgesic activity in rodents[3] and humans[4] with relatively few side effects. Tolperisone enantiomers have different pharmacodynamic properties.[5,6] (+)-Tolperisone has higher muscle relaxant activity than the (−)-isomer, whereas the later shows higher broncho- and peripherial vasodilatatory activities than the dextrorotatory enantiomer.[7] Chiral inversion of tolperisone enantiomers was observed by Yokoyama et al.[6] Eperisone (EPI) (4-ethylphenyl-2-methyl-3-piperidinopropiopenone) is also a central muscle relaxant,[8,9] widely used for the treatment of spastic paralysis. Eperisone has a short muscle relaxant activity on account of its extensive first-pass metabolism after oral administration.[10,11] No reports were available in the literature for the pharmacodynamic and pharmacokinetic properties of eperisone enantiomers. Ono et al.[12] have shown that tolperisone and eperisone exert a local anesthetic-like (membrane-stabilizing) action both on motoneurons and primary afferents in vivo as well as on peripheral nerves of rats in vitro. The local anesthetic action of tolperisone and eperisone was implicated in their antinociceptive effects on acute pain in mice.[3]

A few analytical and bioanalytical methods have been reported for the estimation of tolperisone and eperisone individually or in pharmaceutical combined dosage forms or in biological matrixes by high-performance liquid chromatography (HPLC) using different detection methods, viz. ultraviolet (UV)[13–17] and mass spectrometry (MS).[18–20] Some of the researchers have worked on gas chromatography (GC) and GC-MS for the estimation of eperisone in human serum.[21,22] Velmurugan et al.[23] reported the reverse-phase HPLC separation of the enantiomers of tolperisone on a Heptakis(6-azide-6-deoxy) perphenylcarbamated β-cyclodextrin column. Haginaka et al.[24,25] studied the chiral recognition ability of ovoglycoprotein obtained from chicken egg white and the effect of protein aggregation on chiral resolution of some drugs, including TOL. Further, they compared the chiral recognition ability of ovoglycoprotein obtained from chicken egg white with that of Japanese quail egg white. Williams et al.[26] compared the separation of TOL enantiomers on Cyclobond I 2000 SN on liquid chromatography (LC) and supercritical fluid chromagography (SFC). However, these studies did not focus on quantitative determination of TOL enantiomers in rat plasma. Yokoyama et al.[6] reported on an HPLC method for determination of TOL enantiomers in rat plasma. To the best of our knowledge, no method has been reported for enantioselective determination of either tolperisone or eperisone by reverse-phase HPLC or LC-MS in rat plasma.

The present article describes a simple, accurate, and sensitive method for the enantioselective quantification of tolperisone and eperisone in rat plasma using LC-MS/MS. The present method can be used for the study of chiral inversion of TOL and EPI in rat plasma, as this is the first enantioselective method for both drugs. The bioanalytical

*Correspondence to: R. Nageswara Rao, Analytical Chemistry Division, Indian Institute of Chemical Technology, Tarnaka, Hyderabad, India -500 007. E-mail: rnrao@iict.res.in; rnrao55@yahoo.com
Received for publication 26 November 2012; Accepted 1 April 2013
DOI: 10.1002/chir.22187
Published online 9 July 2013 in Wiley Online Library (wileyonlinelibrary.com).

procedure involves extraction of analytes from the rat plasma by a simple protein precipitation method, a complete reverse-phase chiral LC analysis with tandem mass detection operated in the electrospray ionization (ESI) mode. The assay was validated in accordance with the regulatory requirements.

MATERIALS AND METHODS

Materials

Tolperisone and eperisone were obtained as gift samples from Micro Labs (Bangalore, India). The chemical structures of tolperisone and eperisone are shown in Figure 1. Rat blood was procured from the animal house, College of Pharmacy, Andhra University (Visakhapatnam, India). Acetonitrile and methanol of HPLC grade were purchased from Fisher Scientific (Delhi, India). Analytical grade ammonium acetate was obtained from S.D. Fine Chemicals (Mumbai, India). Ultrapure water was obtained from a Millipore synergy ultrapure water purification system (Stanford, CA). All the solvents and solutions were filtered through a 0.45-μm filter obtained from PAL Millipore (Bangalore, India).

Preparation of Stock and Working Solutions

Stock solutions of TOL and EPI racemates at 1.0 mg/mL in acetonitrile and water at a 1:1 ratio were prepared separately. Mixed working solutions of TOL and EPI at the desired concentration for the calibration curve and QC samples were made by serial dilution with acetonitrile/water (1/1, %v/v) starting with the respective stock solutions. All the stock and working standard solutions were stored at 2–8 °C.

Preparation of Calibration Standards and Quality Control Samples

Fresh calibration standards were prepared by spiking an appropriate amount of the working standard solutions into pooled rat plasma. Seven calibration standards of TOL and EPI were prepared at 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, and 20.0 ng/mL (concentration of each enantiomer of both the analytes). Quality control samples were prepared by spiking an appropriate amount of working standard solutions into rat plasma to reach the desired concentration. Lower limit of quantitation QC (LLOQ), medium QC (MQC), and high QC (HQC or ULOQ) were prepared at 0.2, 2.0, and 20.0 ng/mL for each enantiomer of TOL and EPI. All the calibration standards and QC's were stored at –20 °C.

Sample Preparation

Rat plasma was collected from blood by centrifugation at 5000*g* for 5 min, and stored at –20 °C prior to analysis. An aliquot of 200 μL plasma was transferred into a 2.0 mL Eppendorf tube, together with 50 μL mixed working solution of analyte of the desired concentration; 200 μL acetonitrile was added to precipitate plasma proteins, the mixture was vortex for 1 min, and centrifuged at 5000*g* for 10 min. The supernatant was transferred to an autosampler vial and 20 μL was injected into the instrument for analysis by LC-MS/MS.

Chromatographic Conditions

The chromatographic separation was carried out using Agilent 1100 series HPLC system (Waldbronn, Germany), consisting of a G1312A binary pump, G1379A degasser, G1329A autosampler, and G1329B thermostat. The separation of analytes was performed on Phenomenex Lux-4 (250 × 4.6 mm, 5 μm). The mobile phase consisted of acetonitrile / 10 mM ammonium acetate in 30:70(v/v), pumped at a flow rate of 0.8 mL/min. The injection volume was 20 μL and the total analysis time per sample was 15.0 min.

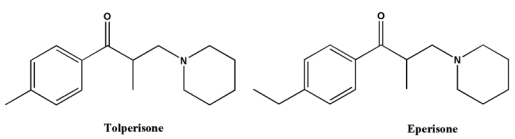

Fig. 1. Chemical structures of tolperisone and eperisone.

Mass Spectrometric Conditions

Ionization and detection of analytes were carried out on Agilent LC-MSD Trap SL mass spectrometer equipped with an electrospray ion interface, operating in positive ion polarity. Quantification was performed using multiple reaction monitoring (MRM) mode to monitor precursor → product ion transitions *m/z* 246 → 98, for TOL enantiomers, and *m/z* 260 → 98 for EPI enantiomers (Fig. 2). Nitrogen gas was used as nebulizer and curtain gas. Collision-induced dissociation was achieved using helium gas (collision gas). The ion source conditions were set as follows: temperature, 320 °C; nebulizer gas, 40 psi; dry gas, 11.0 L/min; ion spray voltage, 4500 V; collision energy, 35.0 V; electron multiplier voltage, 2300 V; declustering potential, 60 V; focusing potential, 400 V; entrance potential, 10 V; collision exit potential, 30 V. All quantification data were processed using Quantanalysis v. 1.5 software.

Racemization Test

Individual enantiomers of TOL and EPI were collected separately using the proposed method on preparative HPLC. The separated enantiomers were spiked into blank plasma individually and injected into the LC-MS/MS system after the extraction procedure.

Assay Validation

Method validation was performed according to U.S. Food and Drug Administration (FDA) guidelines.[27] The assay method was validated for specificity, linearity, precision, accuracy, matrix effect, carryover, and stability. Specificity was assessed by screening six batches of blank rat plasma for the presence of interference as well as the batch-to-batch variation. Linearity of calibration curves based on peak areas was assessed by weighted least-squares analysis. Three separate batches of rat plasma samples were analyzed to asses the intra- and interday precision and accuracy of the method. Each batch consisted of one blank, one set of seven calibration standards, and QC samples. The intra- and interday precision was calculated as the coefficient of variation (%CV) and accuracy as percent recovery. The matrix effect was evaluated by comparing the peak areas of spiked-after-extraction samples with those from injections of the respective neat standard solution at the same concentrations. Carryover was evaluated by injecting a blank plasma extract immediately after the upper limit of quantitation (ULOQ) of the calibration curve. For sufficient accuracy at the LLOQ, any carryover peak must be less than 20% of the LLOQ response. Stability was assessed for LLOQ and HQC samples subjected to three freeze-thaw cycles (free-thaw stability) and bench top stability at room temperature (23 ± 1 °C) for 24 h. To evaluate the storage and reinjection reproducibility of the processed samples, the QC samples were stored in an autosampler for approximately 48 h before reinjection in the LC-MS system.

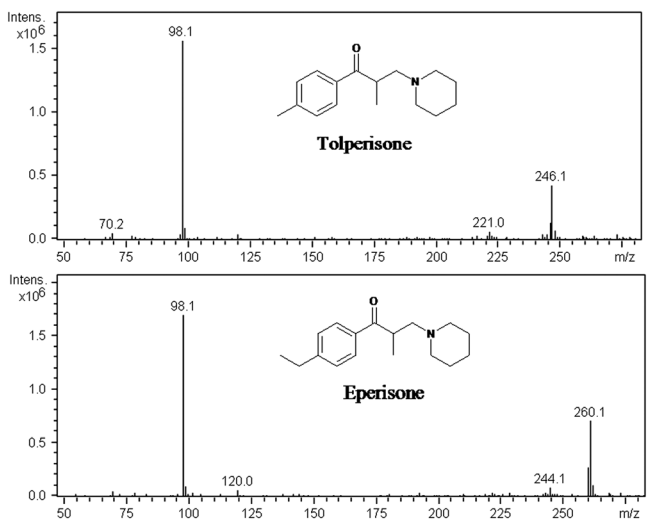

Fig. 2. Typical mass spectra showing product and precursor ions of TOL and EPI.

RESULTS AND DISCUSSION

The primary aim of the present study was to develop a complete reverse-phase LC-MS/MS method. Thus, MS-compatible solvents and buffers were tried during the study. Various combinations of acetonitrile, methanol, ammonium acetate, ammonium formate, acetic acid, and formic acid were investigated to optimize the mobile phase composition for resolution of four enantiomers, sensitivity, speed, and peak shape. Ten mM ammonium acetate was used instead of pure water for better resolution and peak shape. Resolution of the enantiomers was decreased with the use of acid additives like formic acid and acetic acid. Elution time of the analytes was prolonged with the use of ammonium formate instead of ammonium acetate. After evaluation of a number of reverse-phase chiral columns with different stationary phases like Chiral AGP, Chiral CBH, Eurocel 01, and Phenomenex Lux-4, the best chromatogram in terms of resolution and peak shape using isocrate elution with 0.8 mL/min flow rate was obtained on the Phenomenex Lux-4 column. Enantiomeric separation of TOL and EPI enantiomers on different chiral columns could be compared in the chromatograms shown in Figure 3. The enantioselectivity data of the analytes is given in Table 1. Under optimized chromatographic conditions, TOL and EPI enantiomers were eluted at retention times of 8.5, 9.4, and 12.4, 13.6 min, respectively. Absolute configuration of tolperisone enantiomers were assigned by measuring the optical rotation of the individual enantiomers separated by HPLC and with support from the previous studies reported on the absolute configuration of tolperisone[23] (Velumurugan et al.). The optical rotation was recorded at 25 °C with a concentration of 1.0 mg/mL of each analyte and the values are –25.4, +24.9, –30.5 and +29.2 of R-(–)-TOL, S-(+)-TOL, R-(–)-EPI and R-(+)-EPI, respectively. Accordingly, the EPI configuration was assigned. No racemization was observed in the analysis of the TOL and EPI enantiomers in rat plasma, including the steps of extraction and LC-MS analysis (Fig. 4).

One-step protein precipitation, which is economical and convenient, was adopted to simplify sample preparation.

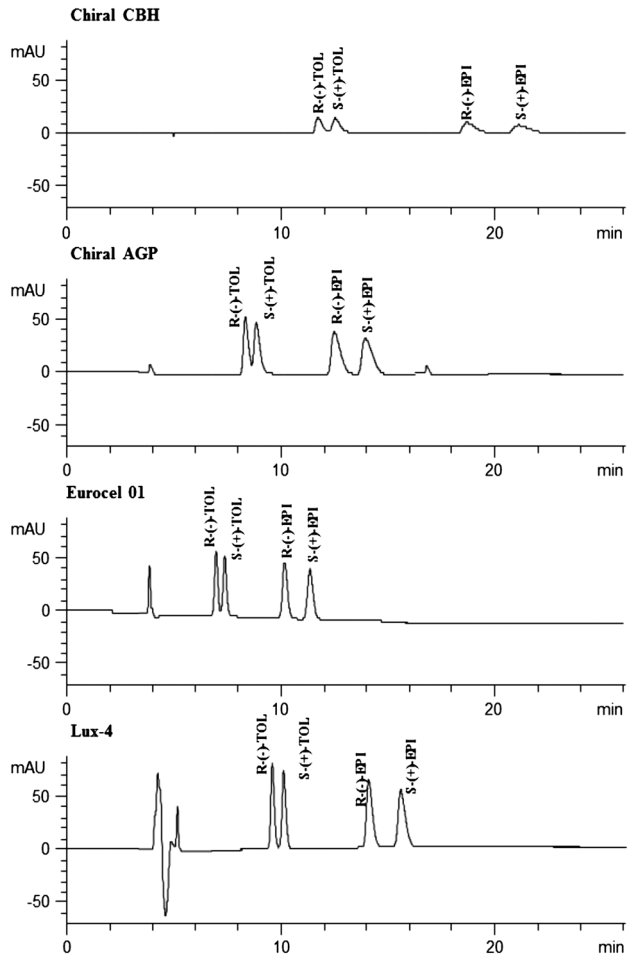

Fig. 3. Overlaid chromatograms of TOL and EPI enantiomers on (1) Chiral CBH, (2) Chiral AGP, (3) Eurocel 01, and (4) Lux-4 using ACN:10 mM ammonium acetate (30:70 % v/v) as mobile phase.

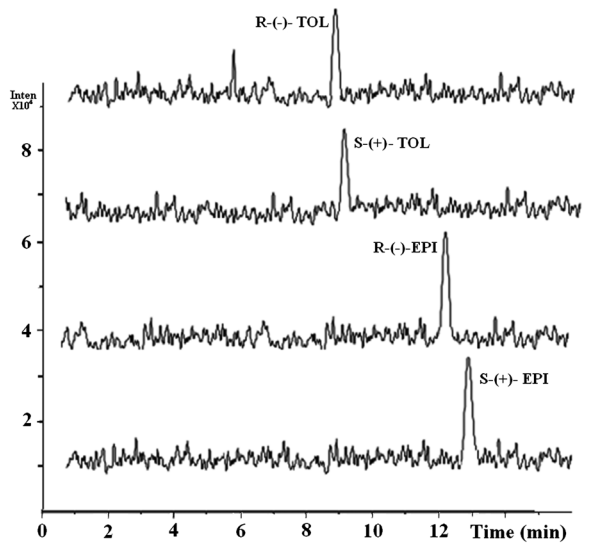

Fig. 4. Typical mass chromatograms of TOL and EPI enantiomers after racemization test.

TABLE 1. Enantioselectivity data of TOL and EPI on different columns used

	TOL				EPI			
Column	k'_1	k'_2	α	R_s	k'_1	k'_2	α	R_s
Chiral CBH	1.64	1.83	1.11	1.29	3.22	3.77	1.16	1.84
Chiral AGP	1.14	1.27	1.11	1.08	2.22	2.59	1.16	1.69
Eurocel 01	0.81	0.91	0.89	1.38	1.64	1.95	1.18	2.65
Lux-4	1.24	1.39	1.12	1.67	2.32	2.68	1.15	2.69

Where k'_1: retention factor of R (–)- TOL/ R(–)- EPI; k'_2: retention factor of S(+)- TOL /S(+)- EPI α: separation factor or selectivity; R_s: resolution factor.

Compared with ethyl acetate and methanol, acetonitrile was selected as the protein precipitant due to excellent precipitation and fewer matrix effects. The recovery rate was high and the analytes were stable under these conditions. The matrix effect was evaluated by comparing the average absolute peak areas of TOL and EPI enantiomers in six batches of blank plasma extracts spiked postextraction with these enantiomers at the MQC level and in three blank water extracts spiked postextraction at the same concentration level, which were very close (< 5.1%). Therefore, the matrix effect was minimal for the proposed method (Table 2).

The sensitivity was assessed by comparing the chromatograms for six different blank rat plasma with those for the corresponding standard spiked samples (Fig. 5). There was no significant interference from endogenous substances observed at the retention times of the analytes. The results suggested that no considerable endogenous contribution from plasma interferes with the measurement of the analytes, demonstrating specificity of the MRM technique.

The assay was linear over the concentration range of 0.2 to 20.0 ng/mL for plasma for each enantiomer. Good linearity was observed in the stated linearity range. LLOQ was determined to be 0.2 ng/mL for each enantiomer. The coefficient of determination of the four enantiomers was better than 0.9996. the lnearity range of previous reported methods for TOL was 0.5–300 ng/mL[20] and EPI was 0.02-20.0 ng/mL.[18] These methods successfully demonstrated the pharmacokinetics of both drugs. The linearity of the present method was well within the reported linearity range, indicating that the studied range covers the expected concentrations.

The precision was calculated by using the coefficient of variation (%CV) and the accuracy was evaluated using percentage recovery. The intra- and interday CV of the QC samples was 0.95–6.05% and 1.52–8.21%, respectively, for rat plasma. The above values were within the acceptable range, and the method was thus judged to be accurate and precise. The intra- and interday accuracy was calculated by percent recovery of each enantiomer and ranged between 94.0–100.5% and 92.7–102.1%, respectively (Table 3). The present method uses an external standard method for the quantification of TOL and EPI enantiomers in rat plasma. The assay precision and accuracy data were well within the acceptable validation limits. Thus, an internal standard was not used in the present study. No peaks were observed in the chromatogram of blank plasma sample analyzed immediately after ULOQ. As a result, carryover from the previous concentrated samples of analyte should be negligible.

The results obtained for the stability experiments in processed samples left for 48 h in the LC autosampler at 4 °C indicates that the enantiomers were stable, given that the accuracy and precision of the assay were not >20% at the lower limit of quantification and not >15% at the medium and high concentrations. After three freeze/thaw cycles, the QC samples showed <6.8% CV in precision and –3.0 to 1.0 % bias for four enantiomers. The stability results are summarized in Table 4.

CONCLUSION

A simple and rapid reserve-phase chiral LC–MS/MS method for enantioselective determination of TOL and EPI enantiomers in rat plasma was developed and validated. The method involves only a one-step protein precipitation procedure, which

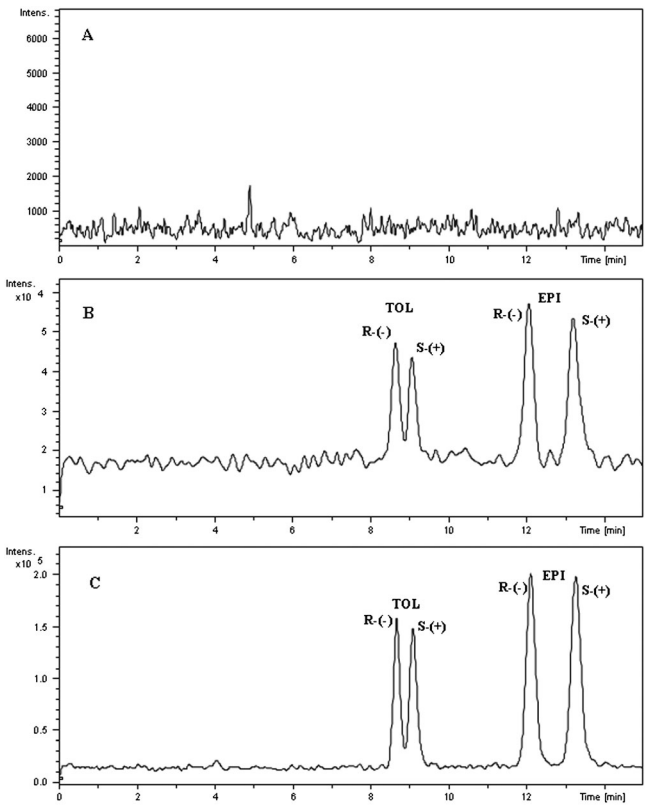

Fig. 5. Representative mass chromatograms of extracted rat blank plasma (**A**), plasma spiked with TOL and EPI at 0.2 ng/mL (LLOQ) (**B**), and 20.0 ng/mL (ULOQ) (**C**) of each enantiomer.

TABLE 2. Results of matrix effect

	R(–)- TOL (2.0 ng/mL)		S(+)- TOL (2.0 ng/mL)		R(–)- EPI (2.0 ng/mL)		S(+)- EPI (2.0 ng/mL)	
	a	b	a	b	a	b	a	b
Mean (peak area)	26196.0	26972.3	27036.7	28520.0	39184.3	40069.0	38840.3	40133.0
SD	297.61	493.16	349.46	1101.31	238.01	261.54	379.58	954.27
% CV	1.14	1.83	1.29	3.86	0.61	0.65	0.98	2.38
% Matrix effect	2.9		5.2		2.2		3.2	

a = samples prepared from control blank plasma; b = samples prepared from neat solutions.

TABLE 3. Precision and accuracy data

		Intra-day			Interday		
Analyte	Conc. (ng/mL)	Mean	%CV	% Bias	Mean	%CV	% Bias
R(–)- TOL	0.200	0.201	3.98	0.5	0.195	4.56	-2.5
	2.000	2.055	4.31	-2.6	1.940	3.06	-3.0
	20.000	19.762	4.18	-1.2	19.473	4.38	-2.6
S(+)- TOL	0.200	0.201	4.67	-2.8	0.187	4.28	-6.5
	2.000	1.915	6.05	-4.3	1.886	2.38	-5.7
	20.000	19.611	2.08	-1.9	19.212	4.25	-3.9
R(–)- EPI	0.200	0.196	1.84	-2.0	0.189	2.38	-5.3
	2.000	1.954	4.79	-2.5	0.977	8.21	-2.4
	20.000	19.771	0.95	-1.1	19.770	1.52	-1.5
S(+)- EPI	0.200	0.188	5.13	-6.0	0.186	5.93	-6.8
	2.000	1.950	3.03	-2.5	1.949	5.26	-2.5
	20.000	19.703	0.96	-1.5	19.64	1.11	-1.8

TABLE 4. Stability data

	LLOQ (0.2 ng/mL)				HQC (20.0 ng/mL)			
	R(–)- TOL	S(+)- TOL	R(–)- EPI	S(+)- EPI	R(–)- TOL	S(+)- TOL	R(–)- EPI	S(+)- EPI
Stability after three freeze-thaw cycles								
Mean	0.181	0.185	0.186	0.182	19.110	19.043	19.358	18.779
% CV	3.98	3.54	4.02	5.24	4.26	3.62	3.27	2.22
% Bias	-5.8	-7.5	-6.7	-9.0	-4.4	-4.8	-3.2	-6.1
Room temperature stability 24 h								
Mean	0.182	0.175	0.183	0.177	18.927	18.704	18.567	19.146
% CV	4.99	5.76	4.37	2.54	1.56	4.36	3.85	4.75
% Bias	-9.2	-12.7	-8.3	-11.2	-5.4	-6.5	-7.2	-4.3
Autosampler stability 48 h								
Mean	0.192	0.185	0.190	0.192	19.417	19.497	19.995	18.635
% CV	2.35	5.21	3.70	2.35	2.84	2.66	3.29	3.34
% Bias	-4.2	-7.1	-4.8	-4.2	-2.9	-2.5	0.0	-6.8

reduces the sample preparation time and allows quantification of all four enantiomers for the concentration range 0.2-20.0 ng/mL for plasma with an LLOQ of 0.2 ng/mL. The precision, sensitivity, and selectivity of the method were sufficient to determine the drug in rat plasma.

ACKNOWLEDGMENTS

The authors thank Dr. J.S. Yadav, Director, IICT, for constant encouragement. Mr. S.S.N. Raju thanks the Department of Science and Technology (DST) New Delhi, India, for financial support.

LITERATURE CITED

1. Pratzel HG, Alken RG, Ramm S. Efficacy and tolerance of repeated oral doses of tolperisone hydrochloride in the treatment of painful reflex muscle spasm: results of a prospective placebo-controlled double-blind trial. Pain 1996;67:417–425.
2. Dulin J, Kovacs L, Ramm S, Horvath F, Ebeling L, Kohnen R. Evaluation of sedative effects of single and repeated doses of 50 mg and 150 mg tolperisone hydrochloride. Results of a prospective randomised double-blind placebocontrolled trial. Pharmacopsychiatry 1998;31:37–42.
3. Sakaue A, Honda M, Tanabe M, Ono H. Antinociceptive effects of sodium channel-blocking agents on acute pain in mice. J Pharmacol Sci 2004;95:181–188.
4. Svensson P, Wang K, Arendt-Nielsen L. Effect of muscle relaxants on experimental jaw-muscle pain and jaw-stretch reflexes: a double-blind and placebo-controlled trial. Eur J Pain 2003;7:449–456.
5. Furuta Y, Nakamura K, Tashiro Y. Tolperisone optical isomers and their salts. Japan Patent 40779, 1978.
6. Yokoyama T, Fukuda K, Mori S, Ogawa M, Nagasawa K. Determination of tolperisone enantiomers in plasma and their disposition in rats. Chem Pharm Bull 1992;40:272–274.
7. Furuta Y, Yoshikawa A. Reversible adrenergic alpha-receptor blocking action of 2, 48-dimethyl-3-piperidino-propiophenone (tolperisone). Jpn J Pharmacol 1976;26:543–550.
8. Kuroiwa Y, Sobue I, Tazak Y, Nakanishi T, Ohtomo E, Itahara K. Effects of E-0646 on cases of spasticity — a double blind comparision using tolperisone hydrochloride. Clin Eval 1981;9:391–419.
9. Iwase S, Mano T, Saito M, Ishida G. Effect of a centrally-acting muscle relaxant eperisone hydrochloride, on muscle symmpathetic nerve activity in humans. Funct Neurol 1992;7:459–470.
10. Fujita T, Takamatsu T, Hisamoto T, Tsutsumi J, Kinoshita K, Kanai T. Studies on the metabolic fate of 4'-ethtyl-2- methyl-3-piperidinopropiopenone hydrochloride(1):absorption, disposition and excretion in rat and guinea pigs. Pharmacometrics 1981;21:835–846.
11. Mihara K, Masumura M, Yoshioka E, Hanada K, Nakasa H, Ohmori S, Kitada M, Ogata H. Intestinal first-pass metabolism of eperisone in the rat. Pharm Res 2001;18:1131–1137.
12. Ono H, Fukuda H, Kudo Y. Mechanisms of depressant action of muscle relaxants on spinal reflexes, participation of membrane stabilizing action. J Pharm Dyn 1984;7:171–176.

13. Saisunee L, Boonsom L. High performance thin layer chromatographic determination of tolperisone hydrochloride. J Pharm Biomed Anal 1999;20:401–404.
14. Saisunee L, Boonsom L, Piyaporn P. Simultaneous determination of tolperisone and lidocaine by high performance liquid chromatography. J Pharm Biomed Anal 2001;26:865–872.
15. Napaporn Y, Boonsom L, Saisunee L. Simultaneous micellar LC determination of lidocaine and tolperisone. J Pharm Biomed Anal 2003;31:629–638.
16. Bernadett S, Kovács-Kiss D, Ludányi K, Bódis A, Klebovich I, Antal I. LC-UV assay of tolperisone HCl from sustained release matrix tablets. Chromatography 2010;71:109–113.
17. Bae JW, Park YS, Sohn UD, Myung CS, Ryu BK, Jang CG, Lee SY. HPLC determination of tolperisone in human plasma. Arch Pharm Res 2006;29:339–342.
18. Li D, Xin W, Shengqiang Z, Jianpin S, Yindi Z. Rapid and sensitive liquid chromatography-electrospray ionization mass spectometry method for the determination of eperisone in human plasma. J Chromatogr Sci 2004;42:254–258.
19. Jeoung MK, Jeong ES, Kim NH, Kim CS, Chung YB, Lee YM, Ahn SY, Cho HE, Lee YH, Hong JT, Moon DC. Determination of eperisone in human plasma by liquid chromatography-ESI-tandem mass spectrometry. Arch Pharm Res 2007;30:1174–1178.
20. Choi CI, Park JI, Lee HI, Lee YJ, Jang CG, Bae JW, Lee SY. Determination of tolperisone in human plasma by liquid chromatography/tandem mass spectrometry for clinical application. J Chromatogr B 2012;911:59–63.
21. Miskolczi P, Vereczkey L, Frenkl R. Gas-liquid chromatographic method for the determination of tolperisone in human plasma: pharmacokinetic and comparative bioavailability studies. J Pharm Biomed Anal 1987;5:695–700.
22. Takenaka F, Hasui M, Ohkawa T. Determination of eperisone in human plasma by gas chromatography - mass spectrometry. J Chromatogr 1992;584:261–266.
23. Velumurugan T, Ching CB, Ng SC, Bai ZW, Ong TT. Optimisation of reversed phase high-performance liquid chromatographic separation of the enantiomers of tolperisone on a Heptakis(6-azide-6-deoxy) perphenylcarbamated β-cyclodextrin column. Chromatographia 2002;56:229–232.
24. Haginaka J, Matsunaga H, Tsukamoto T. Separation of enantiomers on a chiral stationary phase based on ovoglycoprotein III. Effect of aggregation of ovoglycoprotein on chiral resolution. J Chromatogr A 1999;830:81–89.
25. Haginaka J, Kagawa C, Matsunaga H. Separation of enantiomers on a chiral stationary phase based on ovoglycoprotein. VII. Comparison of chiral recognition ability of ovoglycoprotein from chicken and Japanese quail egg whites. J Chromatogr A 1999;858:155–165.
26. Williams KL, Sander LC, Wise SA. Comparison of liquid and supercritical fluid chromatography for the separation of enantiomers on chiral stationary phases. J Pharm Biomed Anal 1997;15:1789–1799.
27. Food and Drug Administration, Center for Drug Evaluation and Research (May 2001). Available at: http://www.fda.gov/cder/guidance