Fasted state bioavailability of two delayed release formulations of divalproex sodium in healthy Iranian volunteers

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Abstract

The purpose of this study was to compare the pharmacokinetics and bioavailability of two commercial brands of delayed release divalproex sodium (CAS 76584-70-8) tablets in healthy male Iranian volunteers in fasted state. Each single-dose, randomized, open-label, blind study was conducted according to a crossover design in subjects. A washout interval of 14 days separated the doses in each study. Serial venous blood samples were obtained over 24 h after each administration to measure drug in serum, and placed into tubes containing sodium heparin. Then the separated plasma was kept frozen at –20 °C for subsequent analysis. The plasma concentrations of drug were analyzed by a validated sensitive HPLC method with UV detection. Mean maximum serum concentrations of 124.5 ± 34.8 µg/ml and 134.2 ± 31.1 µg/ml were obtained for the test and reference products, respectively. The AUC_0^t and AUC_0^∞ were 2023.8 ± 578.8 1 µg h/ml and 2705.3 ± 792.1 µg h/ml for the test and 2068.2 ± 526.4 µg h/ml and 2729.6 ± 698.2 µg h/ml for the reference formulation, respectively. The calculated 90 % confidence intervals for the ratio of C_{max} (87.2–101.5 %), AUC_0^t (92.1–108.6 %) and AUC_0^∞ (93.1–110.6 %) values for the test and reference products were all within the 85–120 % interval proposed by the FDA and EMA. Therefore the divalproex sodium tablets of the test and reference products are bioequivalent in terms of rate and extent of absorption.

Key words

- Bioavailability
- Delayed release tablets
- Divalproex sodium
- Fasted state

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1. Introduction

Divalproex sodium (DVX, CAS 76584-70-8) is a unique combination of equal proportions of sodium valproate and valproic acid which dissociates in the gastrointestinal tract into the active valproate ion. DVX is indicated for use as sole and adjunctive therapy in the treatment of patients with complex partial seizures that occur either in isolation or in association with other types of seizures. It is also indicated in the treatment of simple and complex absence seizures, and adjunctively in patients with multiple seizure types that include absence seizures [1]. DVX is an effective anticonvulsant, antimanic and migraine prophylaxis agent [2–6]. The extended-release form of DVX appropriate for once-daily dosing was approved by the U.S. Food and Drug Administration for migraine and is approved for epilepsy in Canada [7]. Valproic acid is characterized by dose-limited absorption, nonlinear plasma protein binding, and multiple metabolic pathways of elimination. Once absorbed, valproic acid is largely bound to plasma proteins and has a relatively small volume of distribution [8]. In the fasting state, peak plasma concentration is reached approximately 3–4 h after oral administration. Administration of DVX under nonfasting conditions can delay the systemic absorption of drug. The plasma half-life of valproate is in the range of 6–16 h. However, in patients concurrently taking other enzyme-inducing medications, the plasma half-life of valproate is usually in the lower part of the mentioned range. Valproate is primarily metabolized in the liver and is approximately 90 % bound to plasma proteins [1, 3]. It has been reported that the absolute oral bioavailability of divalproex is 89 % [4]. DVX is available in several formulations including the traditional, enteric coated, delayed-release (DR) tablet, the sprinkle capsule, and the extended-release

(ER) tablet. Divalproex ER is approximately 8–20 % less bioavailable than divalproex DR. Proportional dosing with 8–20 % higher daily doses of divalproex ER compared with divalproex DR has been demonstrated to produce equivalent levels of bioavailable serum valproate in healthy volunteers and in epileptic patients [5, 9]. Obtaining an equivalent area under the curve (AUC) while slowing the gastrointestinal transit and avoiding food effects and dose dumping among a population with epilepsy with individual variability requires extensive engineering of the formulations [1]. DVX is made and marketed by several pharmaceutical manufacturers. Availability of different formulations of the same drug substance, at the same strength, and dosage form could be different and is important for health care professionals. If separate formulations have different bioavailabilities, switching over to a new brand may lead to inadequate therapeutic effect, toxicity or even cases of drug resistance can arise [10, 11]. In the present study the rate and extent of absorption of two commercial brands of DVX delayed release (enteric coated) tablets were determined and compared following oral administration in healthy fasted Iranian volunteers.

2. Methods

2.1 Study design

Two brands of DVX delayed release tablets were used. The innovator product (Batch no. 54031) was selected as reference. The test product (Experimental Batch no. 01) was manufactured by Exir (Boroujerd, Iran), a local company in Iran. Both formulations were obtained from the respective manufacturers and they contained 500 mg DVX. The study was a fasted state, open label, single-dose, blind randomized study with a crossover design carried out in accordance with the guidelines of the Declaration of Helsinki (World Medical Assembly 1964) as revised in Edinburgh (2000). The study was also reviewed and approved by the local ethical review board of Tabriz University of Medical Sciences in Iran. Twenty-four adult Iranian male volunteers (mean age ± SD, 23.1 ± 3.1; range 20–31 years and mean weight ± SD, 70.0 ± 8.2; range between 56–85 kg) were selected for enrollment in this study. None of them had a history of hypersensitivity to medications and they were non-smokers. There was two-week washout period between studies. Prior to the study, a written consent form was obtained from each individual subject participating in the study. An equivalent to 500 mg DVX tablet of the test and reference products was administered to each subject as a single dose. Blood samples of 5 ml were collected at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 h after each administration via an intravenous cannula placed in the subject's forearm. All samples were centrifuged in heparinated tubes. The plasma samples were separated and promptly frozen at –20 °C until assay.

2.2 Analytical methodology

There have been several methods for determining valproic acid plasma concentrations in the literature, including gas chromatography method with flame ionization detection (GC/FID) [1, 2, 12, 13], capillary gas chromatography [14], fluorescence polarization immunoassay [7, 15] and enzyme immunoassay (EIA) [8]. However, in this study plasma samples were analyzed using a modified reversed-phase high performance liquid chromatography (RP-HPLC) method. The analytical procedure was validated for specificity, accuracy, precision and sensitivity. On 1 ml of plasma 20 µL of the internal standard (10 mg/ml of otanoic acid, Sigma, St Louis, MO, USA) and 25 µL of perchloric acid 60 % (Merck, Darmstadt, Germany) was added, then all samples were extracted with 3 ml hexane (Merck, Darmstadt, Germany) by vortexing for 5 min. After centrifugation for 5 min at 1000 g, the upper organic phase was transferred to a 5-ml glass tube. 500 µL of 0.5 % triethylamine (Merck, Darmstadt, Germany) was added. The mixture was vortex-mixed for 2 min and then, some of the upper organic phase was discarded and the remaining mixture (about 1 ml) was transferred into a 1.5 ml microcentrifuge tube. After centrifugation for 2 min the upper organic phase was discarded completely. Five µL of HCl 2 N (Merck, Darmstadt, Germany) was added to the aqueous phase and 150 µL of the resulting solution was injected onto the HPLC column. A liquid chromatographic system (Knauer, Germany) comprising of Knauer K1000 solvent delivery module equipped with a Rheodyne (Cotati, CA, USA) injector and a variable wavelength ultraviolet spectrophotometric detector (Knauer smartline 2500) set at 210 nm. EZ Chrom Elite version 2.1.7 was used for data acquisition, data reporting and analysis. The mobile phase consisted of citrate buffer (2.5 mM citric acid, Merck, Darmstadt, Germany +2.5 mM K_2HPO_4 (Merck, Darmstadt, Germany)) – phosphate buffer (50 mM KH_2PO_4, Merck, Darmstadt, Germany pH = 7.4) – acetonitrile, (35 : 35 : 30, %v/v). The analytical column used for chromatographic separations was MZ Phenyl (150 × 4.6 mm) 5 µm with a MZ Phenyl (10 × 4 mm) 5 µm precolumn guard; the flow rate was 1.5 ml/min; the detector wavelength was set at 210 nm. Under these conditions the retention times for valproic acid and the internal standard (octanoic acid) were 6.65 and 8.47 min respectively. All plasma samples of a given volunteer collected in the two treatment periods were measured in the same chromatographic run (analytical own control). Each run had a separate daily calibration. Calibration curves were obtained by plotting the valproic acid to octanoic acid peak area ratio against the concentrations of the standard solutions. The within-day and between-days accuracy and precision values of the method were determined using the quantitation of quality control samples with different concentrations during 3 consecutive days [16–18].

2.3 Pharmacokinetic calculations

The plasma concentration-time profile of each individual treatment was constructed. Pharmacokinetic parameters, including maximum observed plasma concentration (C_{max}), time to C_{max} (T_{max}), and area under the plasma concentration-time curve from time 0 to infinity (AUC_0^∞) were calculated using non-compartmental methods [8, 12, 19]. The area under the plasma concentration-time curve from time zero to t (AUC_0^t) was calculated using the linear trapezoidal rule. The terminal first order constant (k_{el}) was determined by a least squares fit of the terminal plasma concentrations (using Excel 1 for Windows) [10, 11]. The constant k_{el} was used to extrapolate AUC_t^∞. AUC_0^∞ was calculated according to the following equation: $AUC_0^\infty = AUC_0^t + C_t/K_{el}$ and elimination half-life (t) was calculated by the quotient of $0.693/k_{el}$ [2, 18, 20–22]. Bioequivalence between the products was determined by calculating 90 % confidence intervals (90 % CI) for the ratio of C_{max}, AUC_0^t, and AUC_0^∞ values for the test and reference products. Analysis of variance (ANOVA) was used to assess group and period effects.

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3. Results and discussion

3.1 Method validation

Injection of blank plasma samples at each run revealed no interference of valproic acid and internal standard with different endogenous compounds. Representative chromatogram of a typical blank plasma sample and different valproic acid standard concentrations in plasma samples is shown in Fig. 1. The method was linear in the 10 to 150 µg/ml range ($r^2 > 0.99$). The limit of quantitation for valproic acid was 2 µg/ml, with a within-day reproducibility of ± 2.9 % for 100 µg/ml and a day-to-day reproducibility of ± 2.6 % for the same concentration (Table 1). The recovery of valproic acid was 93.26, 94.98, and 95.39 % at concentration levels of 25, 100, and 150 µg/ml, respectively. Recovery values for the extraction procedure were calculated by comparing chromatographic responses obtained from spiked extracted plasma and drug free plasma samples spiked with the same concentration immediately after extraction. Recovery of the internal standard was 91 %.

3.2 Data analysis

The mean concentration-time plots after administration of reference and test products to 24 healthy volunteers are shown in Fig. 2. In Table 2 the mean pharmacokinetic parameters after administration of both products to 24 healthy volunteers are reported. It shows that mean maximum serum concentrations of 124.5 ± 34.8 µg/ml (90 % CI: 112.8–136.1) and 134.2 ± 31.1 µg/ml (90 % CI: 123.7–144.6) were obtained for the test and reference formulation, respectively. The respective values for T_{max}, were 6.58 ± 1.25 h (90 % CI: 6.16–7.0) and 6.58 ± 0.93 h (90 % CI: 6.27–6.90). Parameters that measured the extent of absorption are AUC_0^t and AUC_0^∞. The AUC_0^t and AUC_0^∞ for the test formulation were 2023.8 ± 578.8 µg h/ml (90 % CI: 1829.5–2218.2) and 2705.3 ± 792.1 µg h/ml (90 % CI: 2439.4–2971.3), respectively, the calculated values for the reference formulation were 16 732.4–4971.3 µg h/ml (90 % CI: 15 063.3–18 401.6) and 2705.3 ± 792.1 µg h/ml (90 % CI: 2439.4–2971.3). The results of the analysis of variance (ANOVA) for the assessment of product, group and period effects and the 90 % CI for the ratio of AUC_0^t, AUC_0^∞ and C_{max} values for the test and re-

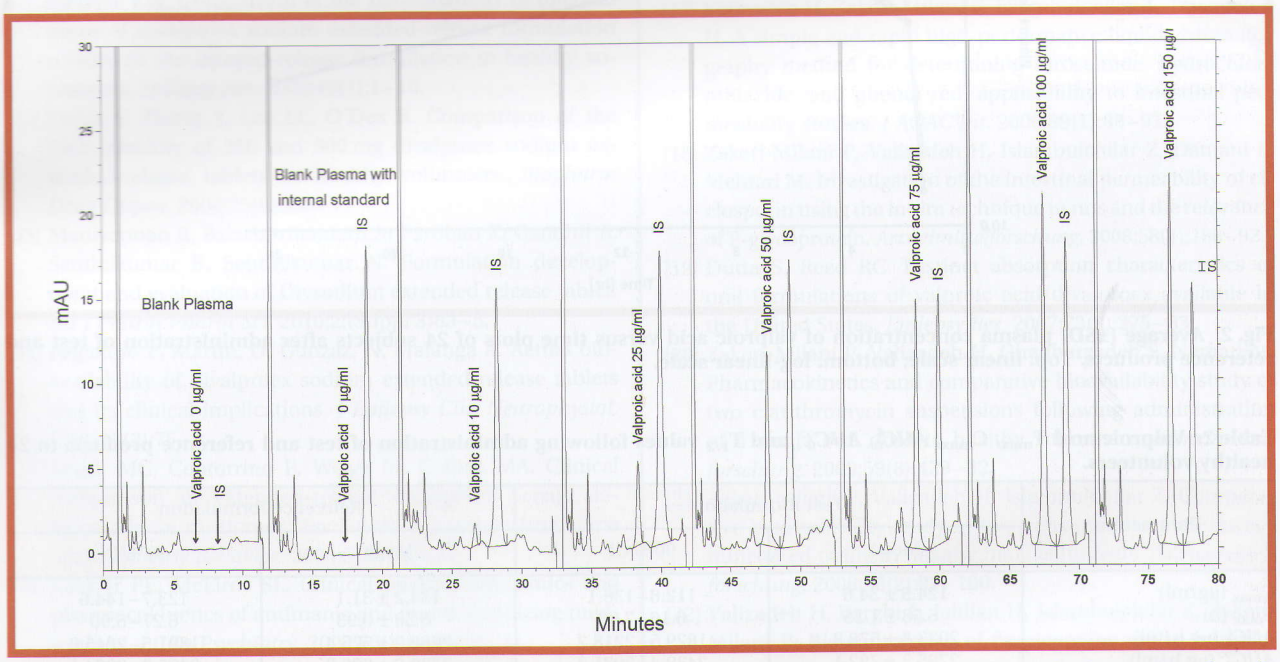

Fig. 1: Representative chromatogram of a typical blank plasma sample and different valproic acid standard concentrations in plasma samples.

Table 1: Inter-assay and intra-assay precision obtained from 4 levels of QC samples.

Added concentration (µg/ml)	Inter-assay				Intra-assay			
	Mean Measured concentration (µg/ml)	SD (µg/ml)	CV (%)	Accuracy (%)	Mean Measured concentration (µg/ml)	SD (µg/ml)	CV (%)	Accuracy (%)
25	25.1	1.5	6.0	100.6	24.5	1.9	7.6	97.8
75	75.6	1.1	1.5	100.8	76.2	1.6	2.1	101.6
100	101.9	3.0	2.9	101.9	103.3	2.7	2.6	103.3
150	153.1	4.0	2.6	102.1	152.0	5.1	3.3	101.4

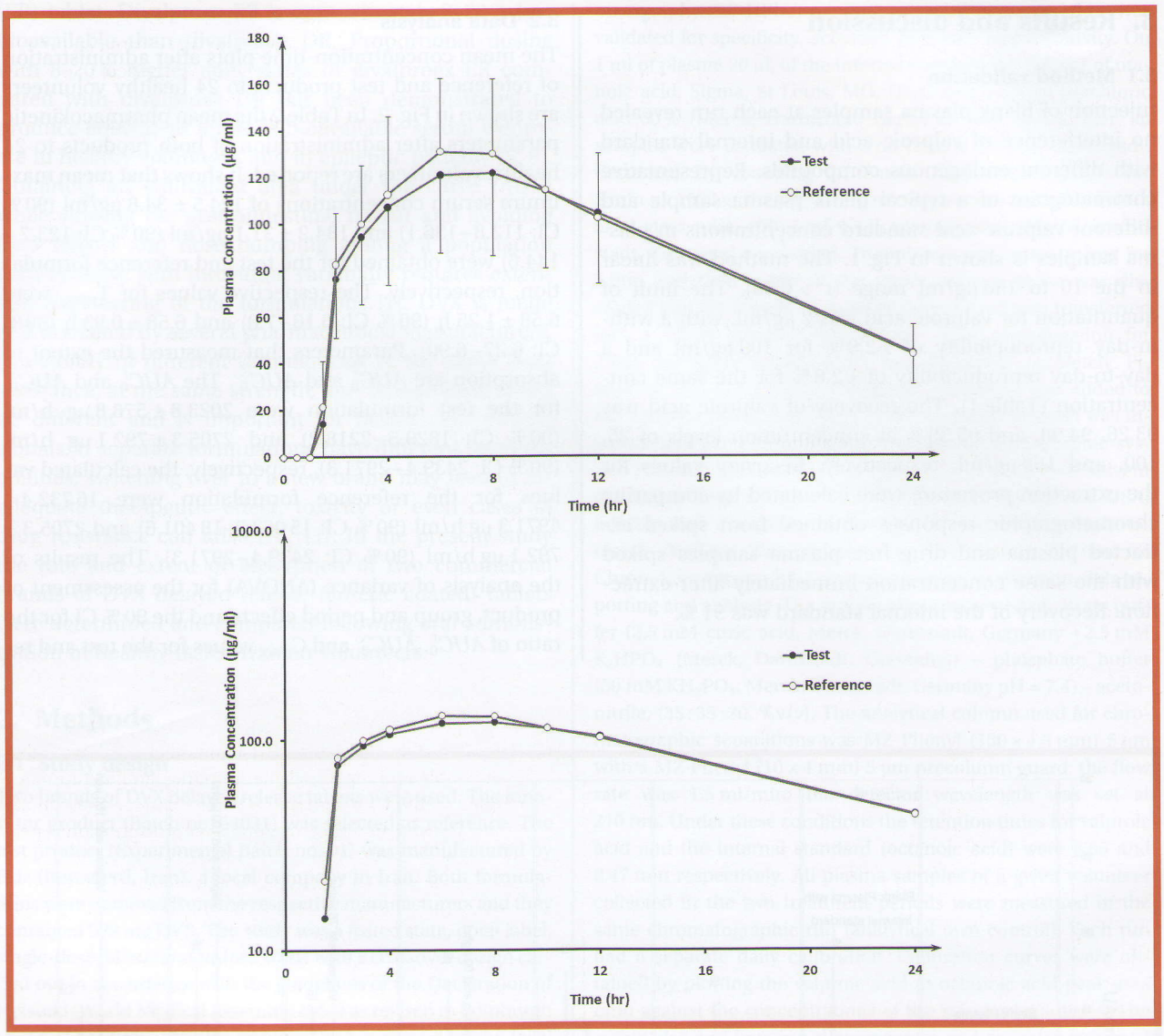

Fig. 2: Average (±SD) plasma concentration of valproic acid versus time plots of 24 subjects after administration of test and reference products. Top: linear scale; bottom: log-linear scale.

Table 2: Valproic acid T_{max}, C_{max}, AUC_0^t, AUC_0^∞, and $T_{1/2}$ values following administration of test and reference products to 24 healthy volunteers.

Parameters	Test formulation		Reference formulation	
	Mean	90 % CI	Mean	90 % CI
C_{max} (µg/ml)	124.5 ± 34.8	112.8–136.1	134.2 ± 31.1	123.7–144.6
T_{max} (h)	6.58 ± 1.25	6.16–7.00	6.58 ± 0.93	6.27–6.90
AUC_0^t (µg h/ml)	2023.8 ± 578.8	1829.5–2218.2	2068.2 ± 526.4	1891.5–2245.0
AUC_0^∞ (µg h/ml)	2705.3 ± 792.1	2439.4–2971.3	2729.6 ± 698.2	2495.2–2964.1
$T_{1/2}$ (h)	10.58 ± 0.95	10.26–10.90	10.39 ± 1.77	9.70–10.89

Table 3: Results of the statistical evaluation of the bioequivalence study (α = 0.05).

Pharmacokinetic parameter	ANOVA (P-value)			90 % CI for the ratios
	Variation source			
	Product	Group	Period	
C_{max}	0.1222	0.7029	0.4090	87.2–101.5
AUC_0^t	0.6982	0.4978	0.3347	92.1–108.6
AUC_0^∞	0.8772	0.7974	0.1530	93.1–110.6

ference products are shown in Table 3. According to the FDA and EMEA, bioequivalence for most dosage forms requires that the mean pharmacokinetic parameters of the test dosage form should be within 80 % to 125 % of the reference dosage form using the 90 % CI [23, 24]. The calculated 90 % CI values for the ratio of C_{max}, AUC_0^t and AUC_0^∞ for the test and reference products were 87.2–101.5 %, 92.1–108.6 % and 93.1–110.6 %, respectively. The findings reveal that no statistical differences were

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found between the pharmacokinetic parameters for the test and reference products, therefore no relevant differences were found for T_{max}, k_{el} and $T_{1/2}$. Thus, from the results obtained it can be concluded that the divalproex tablets of test and reference formulations are bioequivalent in terms of rate and extent of absorption.

4. Conclusion

Using a fully validated RP-HPLC method and according to the obtained results it is concluded that the divalproex enteric coated test and reference products are bioequivalent in terms of rate and extent of absorption.

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Conflict of Interest

The authors received financial support for this study from Exir Pharmaceutical Co, Boroujerd (Iran).

References

[1] Dutta S, Zhang Y, Selness DS, Lee LL, Williams LA, Sommerville KW. Comparison of the bioavailability of unequal doses of divalproex sodium extended-release formulation relative to the delayed-release formulation in healthy volunteers. *Epilepsy Res.* 2002;49(1):1–10.

[2] Dutta S, Zhang Y, Lee LL, O'Dea R. Comparison of the bioavailability of 250 and 500 mg divalproex sodium extended-release tablets in healthy volunteers. *Biopharm Drug Dispos.* 2004;25(8):353–7.

[3] Manivannan R, Balasubrmaniam A, Partiban K, GandhiI R, Senthilkumar B, Senthilkumar N. Formulation development and evaluation of Divsodium extended release tablet. *Int J Pharm Pharm Sci.* 2010;2:(Suppl 3)83–5.

[4] Fagiolino P, Martin, O, Gonzaz, N, Malanga A. Actual bioavailability of divalproex sodium extended-release tablets and its clinical implications. *J Epilepsy Clin Neurophysiol.* 2007;13(2):75–8.

[5] Smith MC, Centorrino F, Welge JA, Collins MA. Clinical comparison of extended-release divalproex versus delayed-release divalproex: Pooled data analyses from nine trials. *Epilepsy Behavior.* 2004;5(5):746–51.

[6] Keck Jr PE, McElroy SL. Clinical pharmacodynamics and pharmacokinetics of antimanic and mood-stabilizing medications. *J Clin Psychiatry.* 2002;63(Suppl 4):3–11.

[7] Centorrino F, Kelleher JP, Berry JM, Salvatore P, Eakin M, Fogarty KV, *et al.* Pilot comparison of extended-release and standard preparations of divalproex sodium in patients with bipolar and schizoaffective disorders. *Am J Psychiatry.* 2003;160(7):1348–50.

[8] Fujii A, Yasui-Furukori N, Nakagami T, Niioka T, Saito M, Sato Y, *et al.* Comparative in vivo bioequivalence and in vitro dissolution of two valproic acid sustained-release formulations. *Drug Design Develop Ther.* 2008;(2):139–44.

[9] Thibault M, Blume WT, Saint-Hilaire JM, Zakhari R, Sommerville KW. Divalproex extended-release versus the original divalproex tablet: Results of a randomized, crossover study of well-controlled epileptic patients with primary generalized seizures. *Epilepsy Res.* 2002;50(3):243–9.

[10] Islambulchilar Z, Valizadeh H, Zakeri-Milani P. Rapid HPLC determination of pioglitazone in human plasma by protein precipitation and its application to pharmacokinetic studies. *J AOAC Int.* 2010;93(3):876–81.

[11] Valizadeh H, Nemati M, Hallaj-Nezhadi S, Ansarin M, Zakeri-Milani P. Single dose bioequivalence study of alpha-methyldopa tablet formulations using a modified HPLC method. *Arzneimittelforschung.* 2010;60(10):607–11.

[12] Dutta S, Zhang Y, Conway JM, Sallee FR, Biton V, Reed MD, *et al.* Divalproex-ER pharmacokinetics in older children and adolescents. *Pediatr Neurol.* 2004;30(5):330–7.

[13] Dutta S, Reed RC, Cavanaugh JH. Pharmacokinetics and safety of extended-release divalproex sodium tablets: Morning versus evening administration. *Am J Health Syst Pharm.* 2004;61(21):2280–3.

[14] Cloyd JC, Kriel RL, Jones-Saete CM, Ong BY, Jancik JT, Remmel RP. Comparison of sprinkle versus syrup formulations of valproate for bioavailability, tolerance, and preference. *J Pediatr.* 1992;120(4 I):634–8.

[15] Rha JH, Jang IJ, Lee KH, Chong WS, Shin SG, Lee N, *et al.* Pharmacokinetic comparison of two valproic acid formulations–a plain and a controlled release enteric-coated tablets. *J Korean Med Sci.* 1993;8(4):251–6.

[16] Zakeri-Milani P, Barzegar-Jalali M, Tajerzadeh H, Azarmi Y, Valizadeh H. Simultaneous determination of naproxen, ketoprofen and phenol red in samples from rat intestinal permeability studies: HPLC method development and validation. *J Pharm Biomed Anal.* 2005;39(3–4):624–30.

[17] Valizadeh H, Zakeri-Milani P, Islambulchilar Z, Tajerzadeh H. A simple and rapid high-performance liquid chromatography method for determining furosemide, hydrochlorothiazide, and phenol red: applicability to intestinal permeability studies. *J AOAC Int.* 2006;89(1):88–93.

[18] Zakeri-Milani P, Valizadeh H, Islambulchilar Z, Damani S, Mehtari M. Investigation of the intestinal permeability of ciclosporin using the in situ technique in rats and the relevance of P-glycoprotein. *Arzneimittelforschung.* 2008;58(4):188–92.

[19] Dutta S, Reed RC. Distinct absorption characteristics of oral formulations of valproic acid/divalproex available in the United States. *Epilepsy Res.* 2007;73(3):275–83.

[20] Zakeri-Milani P, Valizadeh H, Ghanbarzadeh S, Nemati M. Pharmacokinetics and comparative bioavailability study of two clarithromycin suspensions following administration of a single oral dose to healthy volunteers. *Arzneimittelforschung.* 2009;59(8):429–32.

[21] Zakeri-Milani P, Valizadeh H, Islambulchilar Z. Comparative bioavailability study of two cefixime formulations administered orally in healthy male volunteers. *Arzneimittelforschung.* 2008;58(2):97–100.

[22] Valizadeh H, Barghi L, Jalilian H, Islambulchilar Z, Zakeri-Milani P. Bioequivalence of fexofenadine tablet formulations assessed in healthy iranian volunteers. *Arzneimittelforschung.* 2009;59(7):345–9.

[23] FDA. Guidance for industry, bioavailability and bioequivalence studies for orally administered drug products; General considerations. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), USA, *http://www.fda.gov/cder/guidance/5356fnl.pdf.* 2003.

[24] EMA. Guideline on the Investigation of Bioequivalence. European Medicines Agency, Pre-Authorisation Evaluation of Medicines for Human Use, Committee for Medicinal Products for Human Use (CHMP), London, UK, *http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2010/01/WC500070039.pdf*