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Bioequivalence assessment of two pregabalin capsules in healthy Mediterranean Arab volunteers

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Key words
capsule – bioanalytical method – pregabalin – bioequivalence

Abstract. Background: Treatment of neuropathic pain has always been challenging, not only from the pharmaco-therapeutic/toxicological point of view, but also due to the unpredictable pharmacokinetic (PK) variations among different generic formulations of the same drug, which require further dose optimization. Objectives: This progressive work aims to evaluate the bioequivalence (BE) of a generic product of 150 mg pregabalin capsule (antineuropathic drug) vs. the reference brand drug Lyrica®. Method: An LC-MS/MS bio-analytical method was developed and validated according to the International Conference on Harmonization (ICH) guidelines in order to be used for the analysis of pregabalin in plasma. BE of capsules was tested by comparison against the reference brand capsules in accordance with the requirements of the declarations of Helsinki, the current Good Clinical Practice (GCP) Guidelines and the ICH. The resulting data were compared against corresponding pregabalin data published on other human races. Results: The relationship between concentration and peak area ratio was found to be linear within the range 0.096 – 6.068 µg/mL for pregabalin. The correlation coefficient (r) was equal to 0.9983. Statistical comparison of the main PK parameters showed no significant difference between test and reference. The mean C_{max} values for test and reference were 4.290 and 4.164 µg/mL, and the mean $AUC_{0–last}$ values were 24.275 h×µg/mL and 23.674 h×µg/mL, respectively. The 90% CIs of geometric mean ratios (test/reference) for pregabalin were 100.34 – 104.78%, 100.34 – 104.70%, and 95.65 – 110.96% for $AUC_{0–last}$, $AUC_{0–\infty}$, and C_{max}, respectively, thus fall within the international specified BE limit (80 – 125%). Both products were well tolerated by all the volunteers and there were no significant differences on physical examination or in vital signs and laboratory tests between groups. All volunteers completed the study and were discharged in good health. Conclusion: The tested generic capsules appear to be bioequivalent to the reference brand and are expected to have a similar efficacy and safety profile.

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Introduction

Neuropathy is a major health problem affecting many patients and can cause a significant decline in life quality. Usually neuropathic pain occurs secondarily to peripheral nerve damage in adult individuals [1]. This kind of pain is often difficult to manage [2]. However, there are several pharmacological treatment options to start with. One of the commonly used drugs is pregabalin [3], which was initially known as S(+)-3-isobutyl-γ-aminobutyric acid. Pregabalin is a derivative of the inhibitory neurotransmitter γ-aminobutyric acid (GABA). It is also structurally related to the anti-convulsant drug gabapentin. In 1993, an anticonvulsant activity for pregabalin through a similar pharmacological mechanism as gabapentin was reported for the first time [4]. Later, in 1997, Field et al. [5] reported an antihyperalgesic activity for pregabalin, which was further confirmed by other groups [6, 7]. Clinically, pregabalin has been approved for the treatment of neuropathic pains associated with diabetes mellitus [8], spinal cord injury [9], fibromyalgia [10], and postherpetic neuralgia [11]; in addition it is approved as adjunct therapy for the treatment of partial seizures in adults [12]. Recently, it has been approved for the treatment of anxiety disorders in Europe [13]. Despite its structural similarity

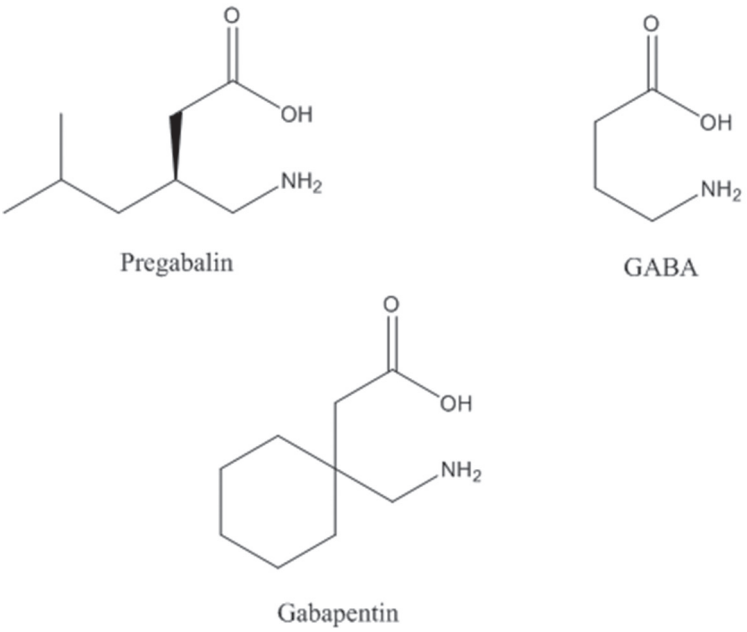

Figure 1. Chemical structure of GABA, gabapentin, and pregabalin.

with GABA and gabapentin (Figure 1), this drug does not bind to GABA or benzodiazepine receptors. Rather, pregabalin is believed to exert its pharmacological actions via specific targeting of the α2-δ subunit of the neural presynaptic calcium channels, thus interfering with the hyperexcitability of the target neurons [14, 15]. In addition, there were other reports that pregabalin can inhibit the release of norepinephrine and the excitatory neurotransmitters glutamate and aspartate [16, 17, 18]. However, the exact mechanism of action is still unknown. Normally patients start the treatment using relatively low doses that are increased gradually to the maximum effective and tolerable dose. Like many drugs, pregabalin is associated with various side effects such as dizziness, somnolence, ataxia, abnormal thinking, balance disorder, weight gain, edema, impotence, dry mouth, and constipation. These side effects could be harmful to the patients and may negatively influence the compliance, therefore, the adjustment of the dose and thereof the plasma concentration of pregabalin is a critical issue that is dependent to a great extent on the dosage form and the formulation. Pregabalin is absorbed rapidly and efficiently following oral administration with peak plasma concentration (t_{max}) of 0.7 – 1.3 hours, a bioavailability of 90%, an elimination half life time ($t_{1/2}$) of ~ 5 – 7 hours, and an apparent volume of distribution of 0.5 L/kg [19, 20]. In fact, it was found that taking pregabalin with food can reduce plasma maximal concentration (C_{max}) by 25 – 30%, and increase the t_{max} to 3 hours [21]. Due to structural similarity to amino acids, pregabalin is supposed to utilize the L-amino acid transport system for absorption [22]. Pregabalin poorly binds plasma proteins and does not influence liver enzymes [23]. The use of generic drugs including antiepileptic drugs has increased in many low-income countries as well as globally during the past two decades. Although these less-expensive generic drugs may represent an important option for many patients, it is very important to investigate whether the generic forms are comparable and accordingly interchangeable with the brand, not only for pharmacoeconomic reasons but also as a prerequisite for the health authorities to approve these generic preparations. In fact, any respectable pharmaceutical company conducts several deep preformulation, formulation, and stability studies in order to ensure high quality of the developed generic product. In addition, all these studies have to be in accordance with international guidelines such as International Conference on Harmonization (ICH), Food and Drug Administration (FDA), and European Medicines Agency (EMA). The Saudi Food and Drug Administration (SFDA) as well as the above international regulatory bodies examine the dossiers and perform controls to ensure safety and efficacy of these developed generic drugs. Accordingly, SFDA indicates the requirement for new data and tight application of regulations that ensure in-vivo safety and efficacy. As a contribution from our side, the main objective for this study was to investigate the interchangeability of two pregabalin 150 mg capsule formulations in healthy Mediterranean/Arab male volunteers using pharmacokinetic (PK) parameters. Herein a comparative bioequivalence (BE), randomized, two-period, two-treatment, two-sequence, single dose, open label, double blinded, crossover BE study was conducted in healthy volunteers under fasting conditions, where the key PK parameters were assessed for two drug products, pregabalin 150 mg capsule of Avalon Pharma (Middle East pharmaceutical indus-

tries Co ltd, KSA) vs. Lyrica® 150 capsule of Pfizer Manufacturing Deutschland GmbH (Freiburg, Germany).

Materials and methods

Volunteers and clinical protocol

The study was conducted in the labs of Arab Pharmaceutical Industry Consulting Co. Ltd./Jordan in accordance with the requirements of the declarations of Helsinki [24], the current Good Clinical Practice (GCP) guidelines [25], and the ICH guidelines [26]. The study protocol and the informed consent forms were approved by the Institutional Review Board (IRB). 26 adult volunteers participated in the study, all of them completed the study and they were evaluated for PK data.

The volunteers were healthy, mixed skin Arab and Mediterranean aged between 18 and 50 years, with a body mass index from 18.5 to 30.0 kg/m^2 and an average weight of 76 kg. The volunteers were subjected to a full medical and physical inspection to confirm their healthy status and were not using any other medication during the period of the study. A written informed consent letter, which explained the nature of the study, was given to each volunteer.

The number of enrolled subjects was determined using references on intra-subject variability, which does not need more than 26 subjects. The study was single center, and the subjects were randomly assigned into two groups, each of 14 volunteers (**■■■ 14 × 2 is not 26!**) . The volunteers entered the center 16 hours before the administration of the first dose, and they were asked to fast, at least 10 hours prior to drug intake. They were not allowed to eat for 4 hours after drug administration, but after that they were instructed to consume only the food that was specified in the protocol. Any detected violation was documented in the standardized meal and/or fluid intake by registration forms and deviation form. The first group was given the brand capsules and the second group was given the generic capsules. Each volunteer received an oral capsule of the assigned formulation given with 240 mL of water in sitting position. 20 blood samples (8 mL each) were collected from each volunteer over 36 hours, where the first sample was withdrawn 1 hour before dosing, followed by subsequent sampling at the following time points: 0.33, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.50, 3.00, 3.50, 4.00, 6.00, 8.00, 10.00, 12.00, 16.00, 24.00, and 36 hours after capsule administration. The blood samples were collected in 10-mL heparin blood tubes, centrifuged at 4,000 rpm for 5 minutes within max. 15 minutes. Each plasma sample was transferred, using disposable polypropylene droppers into two labeled polypropylene tubes; then capped and stored in an Ultra freezer (–70 °C) until analysis. The second period of the crossover was performed after a washout period of 1 week. During that week, the volunteers were away, and they came back to the center 16 hours prior to administration of the second period. Here, the same protocol as in the first treatment period was exactly followed with the exception that the treatment was switched.

Chemicals

Pregabalin powder was obtained from Jordanian Pharmaceutical Manufacturing Co. (Aman, Jordan) while pregabalin-$^{13}C_3$, the internal standard (IStd) was obtained from **■■■ Manufacturer?** (Strasbourg, France). HPLC-grade methanol and acetonitrile were from Romil (Cambridge, UK), isopropanol was from Carbon Group, extra pure formic acid was from Scharlab (Barcelona, Spain), and diethylether from JHD (**■■■ city, company?**). HPLC-grade water was supplied by Sartorius (Göttingen, Germany) purified water. Pregabalin 150 mg capsules (Avalon Pharma Middle East pharmaceutical industries Co.Ltd. KSA), lyrica 150 mg capsules of Pfizer Manufacturing (Freiburg, Germany).

Instruments and chromatographic separations

The analysis was performed using an HPLC system (Agilent auto sampler model 1100, **■■■ company, city, country?**) coupled with MS detector (Applied Biosystem API Sciex 5000, **■■■ city, country?**). The

stationary phase was Gemini C18 110A (50 × 3.00 mm), 3 µm. The mobile phase was composed of (80 : 20) (V/V) (ACN: 0.002 M ammonium acetate) and the pH was adjusted up to 8. The injection volume was 10 µm, and the flow rate was 0.300 mL/min. The defection (**■■■ defection = default?**) settings were M/z (160.095/142.100) for pregabalin and M/z (163.178/144.943) for pregabalin-$^{13}C_3$.

Preparation of stock & working solutions for pregabalin and internal standard (IStd)

Stock solution for calibrators and quality control samples (QCs) were prepared by dissolving 0.10001 g pregabalin, assay (99.9%) using methanol up to 25.00 mL final volume to make up a stock solution containing 3992.403 µg/mL. An IStd solution was prepared by dissolving 0.002501 g pregabalin-^{13}C3, assay (99%) using methanol up to 200.00 mL final volume in order to make up a stock solution containing 12.380 µg/mL. For drug extraction, 1 mL of acidified acetonitrile was added in order to precipitate proteins, and the supernatant was removed for analysis.

Sample preparations for HPLC injection

Prior to the extraction, each sample including calibrators, quality control (QC) samples and authentic samples were spiked with the IStd solution. A 0.200 mL volume of each sample was spiked with a 50 µL of IStd in order to get 4.410 µg/mL as final concentration of pregabalin-^{13}C3. Each sample solution was vortexed for 30 seconds.

Validation procedures

A simple, fast, selective, accurate, and precise HPLC method coupled with MS-MS detector, for the determination of pregabalin in human plasma has been developed and validated. In brief, the linearity study was carried out in the range of concentrations from 0.096 to 6.068 µg/mL. The lower limit of quantification (LLOQ) was estimated by analyzing known samples of pregabalin at progressively lower concentrations. The coefficient of variation (CV) was used to determine assay precision. Stock solution stability in mobile phase was assessed using two standard mixtures that are equivalent to LLOQ and upper limit of quantification (ULOQ) concentrations with IStd. Short and long-term matrix based stability were assessed using two quality control samples with low concentration and two others with high concentration pregabalin concentrations. Stability after freeze and thaw cycles (FTC) was assessed using two sets of QC samples which were subjected to three freeze-thaw cycles (stability samples). Stability samples were processed with a freshly prepared calibration curve and analyzed in a single run with comparison QC sample (comparison sample).

Whole-blood stability was assessed by spiking whole-blood samples with two different concentrations. Recovery for the drug and internal standard was assessed using six extract at three concentrations levels (low, medium, and high).

Matrix effect was investigated for pregabalin and the IStd. The matrix factor (MF) was calculated in each lot of matrix, by calculating the ratio of the peak area in the presence of matrix, to the peak area in absence of matrix (pure solution of the pregabalin).

The QC samples were used to evaluate the performance of the assay. They were prepared by spiking blank plasma with pregabalin. The QC samples were prepared to have low, medium, and high concentrations (pregabalin: QCL: 0.299 µg/mL, QCM: 8.975 µg/mL, QCH: 13.462 µg/mL). The concentration in each QC sample was determined from the calibration curve and it was compared with the nominal concentration. The analysis run was accepted if at least 3 out of 4 QC samples were within 15% of nominal concentration.

Pharmacokinetic and statistical analysis

The PK parameters were calculated using standard non compartmental methods. The peak plasma concentration (C_{max}) and the corresponding time of maximum plasma concentration (t_{max}) were taken directly from

Table 1. Summary of validation procedure and stability determination.

Summary of validation					
Parameter	Results				
Sensitivity	LLOQ is (0.096 µg/mL). Accuracy of mean calculated concentration at LLOQ (110.42) Precession of calculated concentration at LLOQ is (2.83%)				
Recovery	Analyte: Mean recovery 102.34% IS: recovery = 39.61%				
Accuracy precision	QC's	LLOQ	QCL	QCM	QCH
Within run	Precession (CV%)	2.83	6.35	2.03	5.51
	Relative error (%)	10.59	5.19	–0.62	–2.19
Between runs	Precession (CV%)	8.74	8.84	3.67	6.56
	Accuracy (%)	107.29	98.33	98.61	97.04
Stability					
Short-term stability (plasma)		Up to 24 hours (RT)			
Short-term stability (stock solution in mobile phase		Up to 8 hours (RT) for pregabalin and pregabalin-^{13}C3			
FTC stability		Up to 4 cycles at temperature range –85 °C to –55 °C			

the data according to international guidelines [27, 28]. The elimination rate constant (Ke) was evaluated from the slope of the semi-logarithmic plot of the terminal elimination phase of the plasma concentration-time curve calculated by linear regression. The elimination half-life time ($t_{1/2}$) was calculated using the formula $t_{1/2}$ = ln2/Ke. The areas under the drug blood concentration time curves ($AUC_{0–last}$) and the area to the infinity ($AUC_{0–\infty}$) were calculated by using the linear trapezoidal method. For bioequivalence analysis, two-way analysis of variance (ANOVA) was used to assess the effect of formulations, periods, sequences (fixed effects), and subjects (random effect) on $AUC_{0–last}$, $AUC_{0–\infty}$, and C_{max} using Thermo Scientific Kinetica, (version 5.1) a commercially available software package. As Kinetica cannot be used for the calculation of the 90% CI for the unbalanced design, SAS was used in the generation of the statistical report including the 90% CI for all pharmacokinetic parameters. As per the FDA and the EMA guidelines for the conduction of the bioequivalence studies, the 90% CI for pharmacokinetic parameters C_{max} and AUC should lie within 80 – 125% [27, 28].

Results

Results of validation procedures

A summary of the results of validation parameters for the assay and stability studies are provided in Table 1. Briefly, the relationship between concentration and peak area ratio was found to be linear within the range 0.096 – 6.068 µg/mL for pregabalin. The correlation coefficient (r) was close to 0.9983. The method was found sensitive with lower limit of accuracy (LLOQ) of 0.096 µg/mL, analyte mean recovery was 102.34%, recovery of IStd was 39.61%. The result of the accuracy and precession within run and between runs are also summarized in Table 1.

The drug was found to be stable for up to 24 hours at room temperature (RT), whereas the stability in the mobile phase at RT was up to 8 hours for pregabalin and pregabalin-^{13}C3. In addition, the FTC study has shown that the drugs could withstand up to 4 cycles of temperature fluctuations.

Results of pharmacokinetic study

Both pregabalin 150 mg capsules of Avalon Pharma vs. Lyrica® 150 mg capsules of Pfizer Manufacturing Deutschland GmbH showed no serious adverse events, and only the usual side effects of pregabalin were observed for both preparations, such as dizziness, headache, dry mouth, feeling drunk, sedation, numbness, somnolence, decreased platelets, and increased ALT. The subjects were discharged in good health. Figure 2 shows plasma pregabalin geometric mean concentration (ng/mL) vs. time (hour) curves (A) and log plasma pregabalin geometric mean concentration vs. time (hour) curves

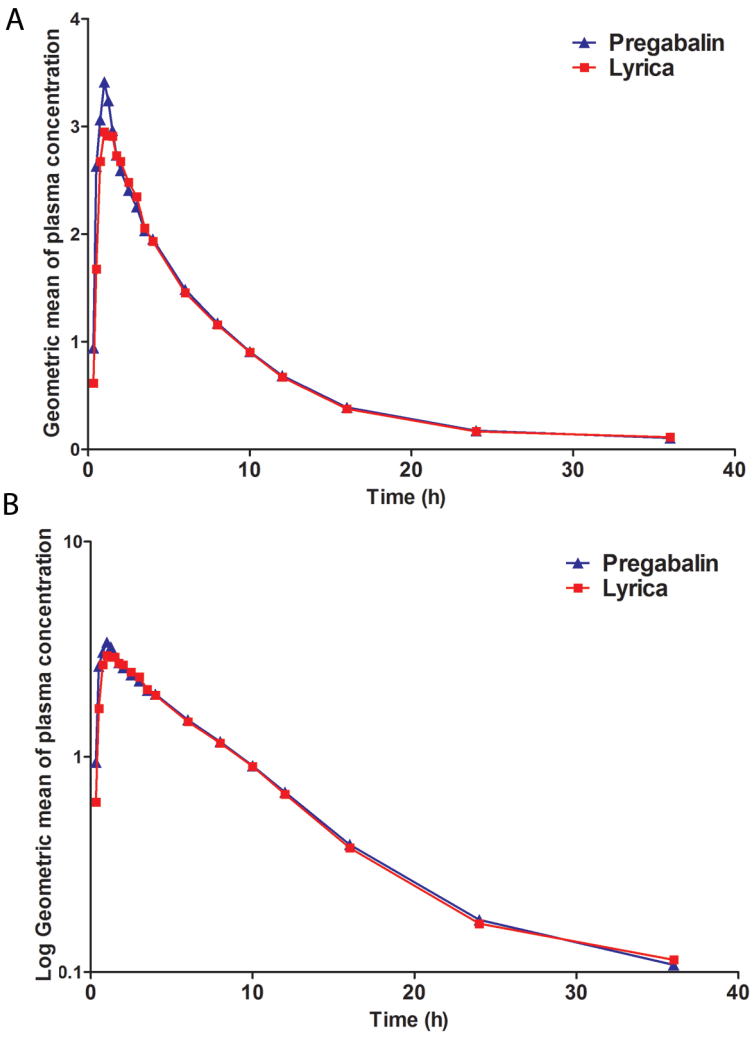

Figure 2. Plasma concentration profile for a single oral dose of pregabalin 150 mg capsule. A: Mean drug plasma concentration (ng/mL) vs. time (h); B: log mean drug plasma concentration vs. time (h).

(B) following a single oral dose pregabalin 150 mg capsule.

The plasma concentrations of both brands indicate that mean profiles after administration of the two formulations were almost superimposable. As per the statistical report there was no significant difference on the effect of period, sequence and formulation. All estimated PK parameters were in agreement with reported values. Table 2 shows a summary of the PK parameters for the two formulations of pregabalin 150 mg. As per the guidelines and the protocol of the clinical study, the 90% CI is calculated for all PK parameters. The 90% CIs of geometric mean ratios (test/reference) for pregabalin were 100.34 – 104.78%, 100.34 – 104.70%, and 95.65 – 110.96% for AUC_{0-last}, $AUC_{0-\infty}$, and C_{max} respectively. Positive correlation was observed since the p-value was less than 0.05. No statistically significant difference between the two formulations was found. These PK parameter values lie within the FDA- and EMA-specified BE limit (80 – 125%) [27, 28]. Our results in this part of the study suggest that for the investigated 150 mg dose both formulations were equivalent and thus similar extent of efficacy should be expected.

Discussion

According to the FDA, brand name drug is defined as a drug marketed under a proprietary, trademark-protected name and generic drug is the same as a brand name drug in active ingredient, dosage form, safety, strength, route of administration, quality, performance, intended use, and contains the same salt, ester, or chemical form. Generic versions of a drug can vary in shape, scoring configuration, packaging, and excipients. If all the previous criteria are met, then the two drugs are considered to be therapeutically equivalents [29].

During the development phase of an oral solid dosage form, several preformulation and formulation trials and tests are carried out in order to achieve a generic product that can be interchangeable with the original brand in terms of efficacy and safety. Accordingly, in-vitro dissolution in different pH media is conducted on the generic product and it must show similar dissolution profile or overlapping to the reference brand. These tests cannot generally replace the in-vivo tests, which demonstrate the efficacy and safety of the generic product since it may contain different excipients.

Several previous studies on pregabalin preparations have tested the BE of dosage forms other than capsules [30], others investigated the BE of generic pregabalin capsules in volunteers from South- America, Europe, middle-east, and far Asia [31, 32, 33, 34].

The aim of this study was to test the BE of pregabalin 150 mg capsule produced by Avalon Pharma vs. the reference pregabalin 150 mg capsule (Lyrica) produced by Pfizer Manufacturing Deutschland GmbH. The two dosage forms were administered to 26 fast-

Table 2. Summary of calculated pharmacokinetic parameters of pregabalin in the bioequivalence study (n = 26).

Summary of pharmacokinetic analysis					
Efficacy results summary	Parameters (unit)	Test pregabalin		Reference Lyrica	
As geometric means (ranges) for C_{max} and AUC ratio	C_{max} (µg/mL)	4.290		4.164	
		2.549	7.032	2.888	8.022
	AUC_{0-last} (µg×h/mL)	24.275		23.674	
		16.035	38.052	15.970	38.511
	$AUC_{0-\infty}$ (µg×h/mL)	25.611		24.986	
		17.103	39.466	17.155	39.936
As medians (ranges) for t_{max}, and $t_{1/2}$	t_{max} (h)	0.75		1.00	
		0.50	3.50	0.50	3.00
	$t_{1/2}$ (h)	5.59		5.49	
		4.07	7.60	4.21	7.49
Bioequivalence results summary	Parameter	Point estimate (ratio of geometric mean %)	Lower limit %	Upper limit %	CV%
	C_{max}	103.02	95.65	110.96	16.40
	AUC_{0-last}	102.54	100.34	104.78	4.75
	$AUC_{0-\infty}$	102.50	100.34	104.70	4.66
Safety results	Dizziness, headache, dry mouth, feeling drunk, sedation, numbness, somnolence, decreased platelets, and increased ALT had occurred as adverse events in this study. No serious adverse events had occurred in this study.				

Table 3. Review on previous pharmacokinetic studies on pregabalin carried out in different countries.

	Dosage form	Mean AUC_{0-t} µg×h/mL	Mean $AUC_{0-\infty}$ µg×h/mL	C_{max} µg/mL	t_{max} hours	$t_{1/2}$ hours	Country	Reference
Test	100-mg capsule dissolved in water	26.9	26.7	3.8	0.577	7.31	USA	[30]
Reference	100-mg capsule	26.6	27.0	3.7	0.615	6.92		
Test	Capsules 150 mg	24.3	25.6	4.3	0.75	5.59	Jordan	Current study
Reference formulation Lyrica®	Capsules 150 mg	23.7	25.0	4.2	1.00	5.49		
Test	Capsules 150 mg	27.9	28.3	4.0 ± 0.80	1.00	5.66	Indonesia	[33]
Reference formulation Lyrica®	Capsules 150 mg	27.4	27.9	3.9 ± 0.80	1.00	5.87		
Test	Capsules 150 mg	10.35	13.92	2.10	0.75	5.67	Chile	[32]
Reference formulation Lyrica®	Capsules 150 mg	10.31	13.78	2.15	0.63	5.56		
Test	Hard capsule 300 mg	54.81	56.1	7.4	1.03	6.47	Portugal	[31]
Reference formulation Lyrica®	Hard capsule 300 mg	54.0	55.2	7.4	1.25	6.49		
Test	Capsules 300 mg	42.852	44.996	6.348	1.39	5.34	Jordan/ Lebanon	[34]
Reference formulation Lyrica®	Capsules 300 mg	42.971	45.352	6.393	1.43	5.39		

ing male volunteers in order to eliminate the influence of food on drug absorption.

The validated analytical methods described above were utilized for quantification of the plasma concentrations of pregabalin after administration of 150 mg of pregabalin as immediate-release capsules. Analysis was successfully applied without interference with the used excipients in the capsule formulation. They provided the appropriate accuracy, sensitivity, and selectivity with high sample throughput and economically-convenient procedure required for PK studies. In this study, several PK parameters were tested using a validated HPLC-MS method. In fact, all validation parameters were tested according to the international guidelines and they were within the accepted limits as reported in Table 2.

The statistical comparison of the main PK parameters, AUC_{0-last}, $AUC_{0-\infty}$, C_{max}, and t_{max} clearly indicated no significant dif-

ference between test and reference capsules, in any of the calculated PK parameters. The obtained values were compliant with the FDA and EMA requirements for bioequivalence of generic drugs since the $AUC_{0-\infty}$, AUC_{0-last}, and C_{max} mean ratios are within the 80 – 125% interval [27, 28]. It can be noticed that the C_{max} was higher for the test (4.290 µg/mL) vs. reference (4.164 µg/mL) but still within the therapeutic range. The obtained PK parameters such as t_{max} and $t_{1/2}$ were comparable to those of previously published similar studies that were conducted on volunteers from other races (Table 3). However, there were some minor differences in terms of AUC_{0-last}, $AUC_{0-\infty}$, C_{max}, t_{max}, and even $t_{1/2}$, but it was not possible to conclude whether these variations are due to differences in methodological settings or different genetic backgrounds, especially that currently there are no hints in the literature on the influence of pharmacogenetic polymorphism/race on the PK of pregabalin.

Conclusion and recommendations

The test and reference capsules are bioequivalent and are expected to show a similar safety and efficacy profile. We recommend a future study to investigate whether genetic background could influence the PK of pregabalin.

Conflict of interest

The authors report no conflicts of interest in this manuscript.

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