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Comparative Bioavailability Study of Two Imidapril Tablet Formulations in Indonesian Healthy Subjects

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ABSTRACT

This study aimed to compare the bioavailability of two 10-mg Imidapril HCl tablet formulations using TENSIMID® as the test formulation and TANAPRESS® as the reference formulation. Twenty-seven healthy subjects completed a single-dosed, open-label, randomized, two-way crossover bioequivalence study under overnight fasting condition with one week wash-out period. The blood samples were collected from the subjects prior to administration and up to 12 hours after dosing. Plasma concentrations of imidapril were determined using LC-MS/MS method with TurboIon Spray mode. Pharmacokinetic parameters of AUC_{0-t}, $AUC_{0-\infty}$and C_{max} were tested for bioequivalence after log-transformation of data and ratios of t_{max} was evaluated non-parametrically. The estimated points and 90% confidence interval (CI) for AUC_{0-t}, $AUC_{0-\infty}$and C_{max} of imidapril were 93.04% (82.63-104.76%), 93.12% (82.84-104.67%), and 94.00% (80.96-109.14%), respectively. There was no statistically significant difference of t_{max} and $t_{1/2}$ detected in both formulations ($p<0.05$). The result indicated that the two formulations of imidapril were bioequivalent and thus may be prescribed interchangeably.

Keywords: ACE inhibitor, bioequivalence and bioavailability, Imidapril, LC-MS/MS, Pharmacokinetic

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INTRODUCTION

Treatment of hypertension has been associated with reductions in the incidence of stroke and coronary heart disease, risk of major cardiovascular event, cardiovascular death, and total mortality which are apparently dependent on the magnitude of blood pressure (BP) reduction, particularly systolic BP reduction [1, 2, 3, 4]. For many years, a variety of angiotensin-converting enzyme (ACE) inhibitors have been effective in reducing BP by inhibiting the renin-angiotensin-aldosterone system [5].

One of drugs included in ACE inhibitor therapeutic class is imidapril ($C_{20}H_{27}N_3O_6$, CAS 89371-37-9, molecular weight 405.4 g/mol). Based on the chemical structure (Figure 1. Chemical structure of imidapril.), imidaprilis a member of imidazolidines class that is (4S)-1-methyl-2-oxoimidazolidine-4-carboxylic acid in which the hydrogen of the imidazoline nitrogen has been substituted by (1S)-1-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino}ethyl group[6].

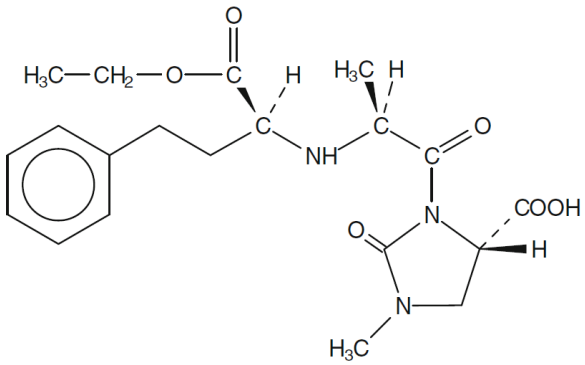

Figure 1. Chemical structure of imidapril.

Imidapril is indicated for the treatment of essential hypertension as well as for the treatment of renal parenchymal hypertension, diabetic nephropathy associated with type 1 diabetes mellitus and/or chronic heart failure in some countries[7]. As a prodrug, imidapril is converted by hydrolysis in the liver into its active metabolite imidaprilat. Imidaprilat competitively binds to and inhibits ACE, thereby blocking the conversion of angiotensin I to angiotensin II. The potent vasoconstrictive actions of angiotensin II are subsequently prevented and results in vasodilation. The secretion of angiotensin II-induced aldosterone by adrenal cortex is also decreased by Imidaprilat leading to an increase in sodium excretion and subsequently to an increase in water outflow. Imidapril does not affect heartrate and contractility [6].

The most commonly reported adverse events during clinical trials and post-marketing surveillance were cough, hypotension, dizziness, and pharyngeal discomfort [5].The persistent dry cough which is a common adverse event associated with the use of ACE inhibitor drugs is sometimes severe enough to require drug withdrawal. However, among other ACE inhibitors, imidapril is generally well tolerated, with a lower incidence of dry

cough compared to enalapril or benazepril. In patients whom treatment with an ACE inhibitor, imidapril is considered as a first choice option in the treatment of mild to moderate essential hypertension based on its efficacy and tolerability[5]. In the essential hypertension treatment, once-daily oral imidapril is initiated at 5mg/day. The dosage may be titrated to 10 mg/day or 20 mg/day in some cases depending on efficacy. Dosage adjustment is needed in elderly patients, those with impaired renal or hepatic function, those at an increased risk of first-dose hypotension, and in patients with renal parenchymal hypertension[7]. Like other ACE inhibitor drugs, imidapril may inhibit aldosterone production and decrease secretion of potassium. Therefore, concomitant use of any medications that may increase the potassium levels, like potassium sparing diuretics or potassium supplements, is not recommended. Co-administration imidapril with lithium preparations may increase reabsorption of lithium in the renal tubules and subsequently may become toxic [5].

The absorption of imidapril is significantly reduced when it is administered with high-fat meal. Imidapril is recommended to be taken ≈15 minutes before meal at about the same time of a day[5]. After single oral dose administration of imidapril 2.5-20 mg, Imidapril and imidaprilat reach its peak plasma concentrations (C_{max}) of 5.8-54.9 ng/mL and 1.2-28.8 ng/mL after median time (t_{max}) of 2 hours and 5 hours, respectively[5,7]. The respective areas under the imidapril and imidaprilat mean plasma concentration-time curve from 0 to 24 hours (AUC_{0-24h}) were 30.2-238.2 ng×h/mL and 18-304.1 ng×h/mL, respectively. It increased linearly in a dose-related manner [7]. The plasma protein binding of imidapril and imidaprilat is 85% and 53%, respectively. Imidapril is absorbed from the gastrointestinal tract in proportionally to dose and is rapidly converted to the active metabolite, imidaprilat. It is also metabolized into three inactive metabolites in the liver. Single dose of imidapril is primarily eliminated through excretion in the urine and faeces with terminal elimination half-life of imidaprilat is ≈24 hours. The absolute bioavailability of imidapril is ≈42% [7].

The present study was conducted to investigate the pharmacokinetic and bioavailability of two imidapril tablet formulations in order to prove the bioequivalence between both formulations.

MATERIALS AND METHODS

Subjects and Study Design

A-single dose, open-label, randomized, two-way crossover study with an overnight fasting and one-week wash-out period was conducted in compliance with the ethical principles of the Declaration of Helsinki for biomedical research involving human volunteers and Good Clinical Practice (GCP). The study protocol was reviewed by the Committee of the Medical Research Ethics of the Faculty of Medicine, University of Indonesia (Jakarta, Indonesia) and was approved by National Agency of Drug and Food Control (Jakarta, Indonesia). All participants signed a written informed consent after they had been informed of the nature and details of the study in accordance with Indonesian Guidelines for Bioequivalence Studies[8].

Twenty-seven selected Indonesian subjects (16 males, 11 females) participated in this study. Demographic of the subjects are shown in Table 1. Demographic data for Imidapril bioequivalence study in 27 volunteers.. Subjects were selected after passing clinical screening procedures which included physical examination, hematology test (hemoglobin, hematocrit, erythrocyte, leukocyte, mean corpuscular (MC) values, leukocyte differential, platelets count, and Erythrocyte Sedimentation Rate(ESR)), laboratory test (serum Glutamic Oxaloacetic Transaminase (sGOT), serum Glutamic Pyruvic Transaminase (sGPT), alkaline phosphatase, total bilirubin, total protein, albumin, globulin, fasting glucose, total cholesterol, blood urea nitrogen, ureum, and creatinine), urine analysis, serological test (Hepatitis B antigen (HBsAg), Hepatitis C (anti HCV), and HIV (anti-HIV)), drug abuse test, and additional pregnancy test for female subjects. Subjects were excluded if they were smoker, pregnant, had history or condition of hepatic, cardiovascular, cerebrovascular, renal, or gastrointestinal disease, potentially hypersensitive to Imidapril, had history of drug abuse, and donated or lost more than 300 mL of blood within 3 months prior to screening of the study. All subjects were required not to take any drugs for at least two weeks prior to the study until completion of the study. They also refrained from consuming alcohol, caffeine, chocolate, tea or coke-containing beverages at least 48 h before each dosing and until the last blood sampling. Subjects were randomized to one of the two sequences to receive the formulations according to the randomization scheme. The test formulation was TENSIMID® (10-mg Imidapril HCl tablet) manufactured by PT. Novell Pharmaceutical Laboratories (Jakarta, Indonesia) and the reference formulation was TANAPRESS® (10-mg Imidapril HCl tablet) manufactured by PT. Tanabe Indonesia (under license from Mitsubishi Tanabe Pharma Corporation, Japan).

Subjects were confined to clinical unit of Clinisindo Laboratories for one night prior to the study to assure the fasting condition (10 h before drug administration), the day of the study, and 24 h after dosing. On the study day, subjects were given one tablet of either product with 240 ml of water. No food was allowed until 4 h after dose administration. Water intake was allowed 1 h before and after dosing. Standard meals were served at 4 h (±1163.04kcal) and 11 h (±1226.07kcal), snacks were served at 8 h(±290.83kcal) after drug administration. Total calories were calculated by a nutritionist. Strenuous exercise was not allowed during the sampling days. Blood pressure, heart rate, body temperature and adverse events were monitored during blood sampling. Approximately 5 mL blood samples were collected prior to dosing and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10 and 12 h after drug administration in the Li-Heparin collection tubes. The blood plasma was separated and kept frozen at -20°C until analysis. After one-week wash-out period, subjects returned to Clinisindo Laboratories and the study was repeated in the same manner to complete the crossover design.

Table 1. Demographic data for Imidapril bioequivalence study in 27 volunteers.

	Mean (±SD)	Range
Age (years)	34.9 ± 9.0	19-51
Weight (kg)	59.6 ± 8.6	45-70
Height (cm)	162.3 ± 8.4	148-181
BMI (kg/m^2)	22.5 ± 2.0	18.0-24.9

Analytical Method

The plasma concentration of imidapril was determined using LC-MS/MS method with TurboIon Spray mode. Perindopril (CAS 107133-36-8, MW 368.468) was used as the internal standard. The assay method has been already validated in terms of selectivity, sensitivity, linearity, precision and accuracy, recovery, stability, matrix effect, and carry-over; and has been also verified before being used in the study according to Guideline on Bioanalytical Method Validation, European Medicines Agency 2011[9].

Briefly, the plasma samples (300 µL) were added with the internal standard. After mixing, 600 µL of methanol was added. Subsequently, the mixture was vortex-mixed for 30 seconds and centrifuged at 3000 rpm for 10 minutes. The supernatant (200 µL) was transferred into vial and added with 800 µL of methanol-water (1:1) and vortex-mixed for 5 seconds. The aliquot (5 µL) was injected into the LC MS/MS system.

The analytical separation was performed on a Synergi™ 4µm, Polar-RP-80Å, 50 x 2.0 mm, 4 µm column (Phenomenex®, Torrance, CA, USA) preceded by a AQ C18, 4 x 2.0 mm guard column (Phenomenex®, Torrance, CA, USA). Mobile phase was 0.1% formic acid in water and 0.1% formic acid in acetonitrile set as gradient. Flow rate used was 0.6 mL/min. Column temperature was maintained at 40°C. Multiple Reaction Monitoring (MRM) in positive ion mode was used to monitor transitions at m/z 406.140 → 234.100 and m/z 369.172 → 172.100 for Imidapril and the IS, respectively.

Safety Evaluation

Analysis of safety-related data was considered usingcommon adverse events, which occurred after initiation ofthe study and supported by detailed tabulation and analysis.

Pharmacokinetic and Statistical Analysis

The bioequivalence evaluation was determined using parameters of AUC_{0-t}, $AUC_{0-\infty}$, and C_{max}. The AUC_{0-t}truncated was calculated using the trapezoidal rule as the extent of absorption. The $AUC_{0-\infty}$values were calculated by adding the quotient of C_t (estimated last plasma concentration) and the appropriate K_{el} (elimination rate constant) to the corresponding AUC_{0-t}, that was $AUC_{0-\infty} = AUC_{0-t} + C_t/K_{el}$. K_{el} was calculated by least-squares regression from the data of at least 4 non-zero observations (or 3, if it is not possible) during the terminal elimination phase. The maximum plasma concentration (C_{max}) and the time to reach maximum plasma concentration (t_{max}) were obtained by observing of the individual imidapril plasma concentration time data and were used as a measurement of absorption rate. The t_{max} and the apparent elimination half-life ($t_{1/2}$) would be analyzed as additional evaluation. The $t_{1/2}$ of Imidapril was calculated by using the equation of $t_{1/2} = (\ln 2)/K_{el}$.

90% Confidence Interval (CI) of the geometric mean ratio Test/Reference (T/R) for AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} were calculated assuming a multiplicative model and Analysis of Variance (ANOVA) was applied using the respective logarithmic-transformed data. The accepted bioequivalence range for the parameters was 80.00-125.00%[8, 10]. For $t_{1/2}$, data would be analyzed for normality of data distribution prior to statistical calculation. $T_{1/2}$ would becalculated without logarithmic-transformed data using paired t-test (parametric method) if it wasnormally distributed or using Wilcoxon Signed rank test (non-parametric method) if itwas not normally distributed. T_{max} was analyzed by non-parametric method using Wilcoxon signed rank test without logarithmic transformation. Statistical analysis was performed using EquivTest® version 2.0 (Statistical Solution, Cork, Ireland) and SAS® version 9.1 (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Bioanalytical Method Validation

The best linear fit and least-squares residual for the calibration curve were achieved with $1/x^2$ weighing factor. The peak-area ratios between imidapril and the internal standard were linear over the within the concentration range of 0.2002-80.08 ng/mL and was created at the following concentration of 0.2002, 0.4004, 1.001, 2.002, 5.005, 10.01, 20.02, 40.04, and 80.08 ng/mL. The limit of quantification (LOQ) of imidapril was established at 0.2002 ng/mL and the precision and accuracy at the LOQ were 11.38% and –(17.48)-11.49%, respectively. Table 2. Precision and accuracy of the method. summarizes the precision and accuracy of the quality control samples during pre-study validation.

Table 2. Precision and accuracy of the method.

Concentrations (ng/mL)	Intra-batch CV			Inter-batch CV		
	Mean ± SD (ng/mL)	RSD (%)	% defference	Mean ± SD (ng/mL)	RSD (%)	% defference
0.60	0.59 ± 0.05	8.40	-(12.96) – 8.60	0.60 ± 0.04	7.04	-(2.52) – 3.75
32.06	31.20 ± 0.72	2.30	-(4.62) – 1.06	31.11 ± 0.70	2.24	-(3.20) – -(2.69)
64.13	60.87 ± 3.31	5.44	-(9.71) – 3.74	61.05 ± 2.31	3.79	-(5.29) – -(4.02)

The mean recoveries of imidapril at low, medium, and high concentration were 92.40%, 94.42%, and 93.45%, respectively. The mean recovery of the IS was 99.87%. Matrix effect of human plasma on ionization efficiency was assessed by comparing the peak response of 6 determinations in low and high concentrations spiked in extracted drug-free human plasma samples with that of neat standards at corresponding concentration. The same evaluation was also performed for the IS. The coefficient variation (CV) of the IS-normalized matrix factor calculated from the 6 lots of matrix of low and high concentrations were 8.84% and 3.97%, respectively. Percent interference in the blank sample was found not more than 20% and 5% for imidapril and the IS, respectively, indicating that there was no carry-over effect during validation. The stability study showed that imidapril in human plasma was stable at room temperature for 6 hours, at -20°C for 61 days, and after 3 freeze-thaw cycles. The autosampler stability showed that imidapril was stable for 26 hours.

Clinical Observation

Both formulations of imidapril were well tolerated at the administered dose and no significant adverse clinical events were observed. All subjects experienced in total of 31 adverse events during the study. All the adverse events were mild, and the subjects recovered without using any medications. There were no serious adverse

events. The disposition of adverse events is shown in Table 3. Disposition of adverse events.

Table 3. Disposition of adverse events

Casual relation to study drug	Events	Test Formulation	Reference Formulation	Total
Related	Somnolence	4	4	8
	Hypotension	2	5	7
	Dizziness	1	0	1
Probable	Bradycardia	7	4	11
Unrelated	Hypertension	0	2	2
	Bradycardia	1	1	2
Total		15	16	31

Pharmacokinetic and Statistical Evaluation

A total of twenty-eight subjects were enrolled in the study. One subject withdrew the consent before period I due to personal reasons. Thus, a total of twenty-seven subjects were available for pharmacokinetic evaluation. The imidapril mean plasma concentration-time profiles are presented in Figure 2. Mean plasma concentration-time profiles of imidapril after a single dose of 10-mg imidapril tablet formulations in 27 subjects..

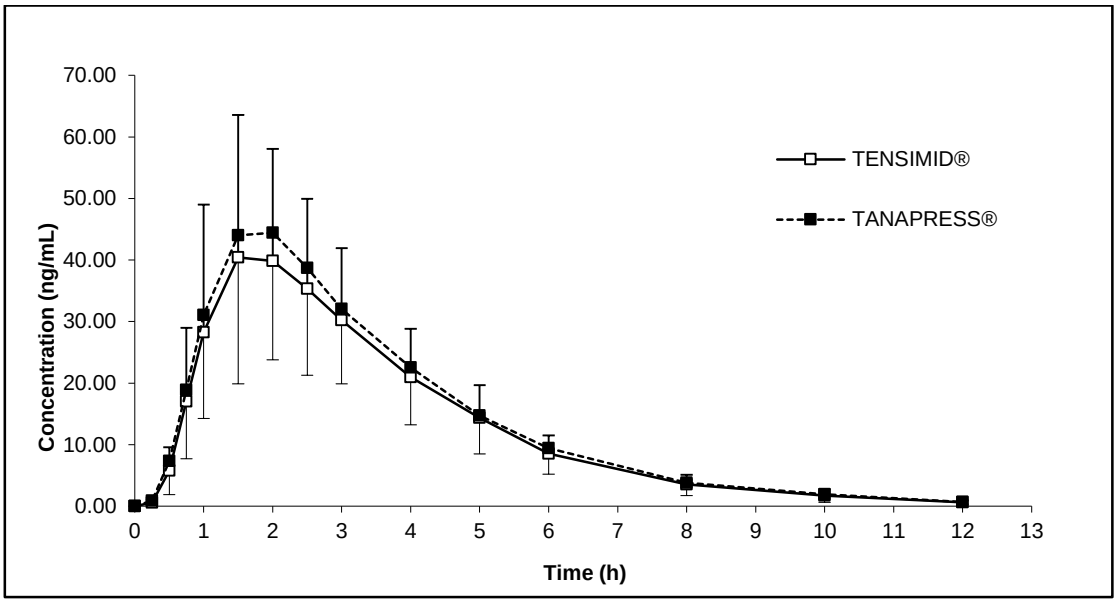

Figure 2. Mean plasma concentration-time profiles of imidapril after a single dose of 10-mg imidapril tablet formulations in 27 subjects.

Descriptive statistics of the pharmacokinetic parameters of imidapril test and reference formulations are summarized in Table 4. Pharmacokinetic parameters of imidapril after administration of two tablet formulations in 27 subjects., where geometric and range values for the parameters of AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, and $t_{1/2}$ and median and range value for the parameter of t_{max} obtained for both formulations are presented.

Table 4. Pharmacokinetic parameters of imidapril after administration of two tablet formulations in 27 subjects.

Parameters	Test Formulation		Reference Formulation	
	Mean ± SD	Range	Mean ± SD	Range
AUC_{0-12h} (ng×h/mL)	156.23 ± 59.11	58.36-293.24	169.61 ± 67.41	66.78-320.45
$AUC_{0-\infty}$(ng×h/mL)	157.81 ± 59.32	58.90-294.27	171.20 ± 67.75	67.28-321.87
C_{max} (ng/mL)	44.31 ± 21.04	12.20-102.77	48.08 ± 24.29	16.88-118.22
$t_{1/2}$ (h)	1.66 ± 0.32	1.22-2.58	1.60 ± 0.23	1.26-2.11
t_{max} (h)	2.00 ± 0.51	1.00-3.00	2.00 ± 0.39	1.00-2.50

The obtained mean values for test and reference formulations were 156.23 ng×h/mL and 169.61 ng×h/mL for AUC_{0-12h}, 157.81 ng×h/mL and 171.20 ng×h/mL for $AUC_{0-\infty}$,44.31 ng/mL and 48.08 ng/mL for C_{max}, and 1.66 hours and 1.60 hours for $t_{1/2}$, respectively. The t_{max} median value for both formulations was 2 hours.

In this study, AUC_{0-12h}, $AUC_{0-\infty}$, and C_{max} were defined as the primary parameters in order to assess bioequivalence conclusion between both formulations. The results of bioequivalence statistical analysis of imidapril are presented in Table 5. Statistical evaluation and intra-subject CV of imidapril.. The 90% CI for the ratio of T/R ranged from 83.63-104.76% with 93.04%-point estimate for AUC_{0-12h}, 82.84-104.67% with 93.12%-point estimate for $AUC_{0-\infty}$, and 80.96-109.14% with 94.00-point estimate for C_{max}. The result of the statistical evaluation for the 90% CI of the primary parameters were all within the bioequivalence acceptance limit of 80-125%.

Additionally, for both t_{max} and $t_{1/2}$, as the secondary pharmacokinetic parameters, there were not statistically differences detected between test and reference formulations ($p<0.05$) as calculated using Wilcoxon sign rank test.

Table 5. Statistical evaluation and intra-subject CV of imidapril.

	AUC_{0-12h} (%)	$AUC_{0-\infty}$(%)	C_{max} (%)
Ratio (T/R point estimate)	93.04	93.12	94.00
90% geometric CI	83.63-104.76	82.84-104.67	80.96-109.14
Intra-subject CV	25.50	25.14	32.09

The mean ratio of AUC_{0-t}/ $AUC_{0-\infty}$ for all individuals on test and reference formulations were around 98%, indicating that the sampling time was adequate since the extrapolated portion of the total AUC was less than 20%. The intra-subject CV of imidapril for pharmacokinetic parameters of AUC_{0-12h}, $AUC_{0-\infty}$, and C_{max} as determined by ANOVA were 25.50%, 25.14% and 32.09%, respectively. Considering on the intra-subject CV of AUC_{0-12h} parameter which was 25.05%, the sample size in this study (n=27) was sufficient in order to conclude bioequivalence with nominal power of 80% at significance level of 5%[11, 12, 13].

CONCLUSION

In conclusion, the two imidapril formulations were equivalent with respect to the rate and extent of absorption and it can be assumed to be therapeutically equivalent and exchangeable in clinical practice.

FUNDING

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CONFLICT OF INTEREST

The authors have declared that no competing interests exist.

REFERENCES

1. *Collins R, Peto R, MacMahon S, et al.* Blood pressure, stroke, and coronary heart disease. Part 2, Short-term reductions in blood pressure: overview of randomised drug trials in their epidemiological context.Lancet. 1990; *335 (8693)*: 827–38. doi: 10.1016/0140-6736(90)90944-z.
2. *Chobanian AV, Baris G, Black H,et al.*Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure.Hypertension. 2003; *42(6)*: 1206–1252. doi: 10.1161/01.HYP.0000107251.49515.c2.
3. *WHO World Health Organization.*The World Health Report 2002: Reducing Risk, Promoting Healthy Life.Geneva, Switzerland: WHO; 2002. http//www.who.int/whr/2002/en/index.html [assessed on May 5, 2020]
4. *Turnbull F, Neal B, Algert C,et al.* Effects of different blood-pressure-lowering regimens on major cardiovascular events: results of prospectively-designed overviews of randomised trials.Lancet. 2003;*362 (9395)*: 1527–1535. doi: 10.1016/S0140-6736(03)14739-3.
5. Imidapril: a guide to its use in essential hypertension.Drugs Ther Perspect. 2007; *23 (12)*: 1–4. doi: 10.2165/00042310-200824010-00001.
6. *National Center for Biotechnology Information*. PubChem Database. Imidapril, CID=5464343.https://pubchem.ncbi.nlm.nih.gov/compound/Imidapril [accessed on May 5, 2020]
7. *Robinson DM, Curran MP, Lyseng-Williamson KA.* Imidapril. A review of its use in essential hypertension, type 1 diabetic nephropathy and chronic heart failure. Drugs. 2007;*67 (9)*: 1359–1378. doi: 10.2165/00003495-200767090-00008.
8. *BPOM Badan Pengawas Obat dan Makanan Republik Indonesia*.Pedoman Uji Bioekivalensi (Guidelines on Bioequivalence Study). Jakarta, Indonesia: BPOM; 2015.
9. *CHMP Committe for Medicinal Products for Human Use*.Guideline on bioanalytical method validation. London, United Kingdom: EMEA; 2011.EMEA/CHMP/EWP/192217/2009 Rev. 1 Corr. 2**
10. *CHMP Committe for Medicinal Products for Human Use*.Guideline on the Investigation of Bioequivalence. London, United Kingdom: EMEA;2010.CPMP/EWP/QWP/1401/98 Rev. 1/ Corr
11. D*iletti E, Hauschke D, Steinijans VW*. Sample size determination for bioequivalence assessment by means of confidence intervals.Int J Clin Pharmacol Ther Toxicol. 1991; 29 (1): 1–8.
12. *Hauschke D, Kieser M, Diletti E, Burke M*. Sample size determination for proving equivalence based on the ratio of two means for normally distributed data.Stat Med. 1999; *18 (1)*: 93–105. doi: 10.1002/(SICI)1097-0258(19990115)18:1<93::AID-SIM992>3.0.CO;2-8.
13. *CDER Center for Drug Evaluation and Research*. Guidance for Industry Statistical Approaches to Establishing Bioequivalence. Maryland, USA:FDA; 2001.