

# Improvement of Losartan Transdermal Permeation using Oleic Acid Pretreatment: *in Vitro* Observation and *in Vivo* Prediction

Annas Binarjo<sup>1\*</sup>, Akhmad Kharis Nugroho<sup>2</sup>

<sup>1</sup>Pharmaceutics and Pharmaceutical Technology Department, Faculty of Pharmacy, Ahmad Dahlan University, Indonesia

<sup>2</sup>Pharmaceutics and Pharmaceutical Technology Department, Faculty of Pharmacy, Universitas Gadjah Mada, Indonesia

## ABSTRACT

The effect of oleic acid on losartan transdermal permeation has been observed to explore its ability as chemical enhancer. Potassium losartan solutions in citric buffer pH 5.0 were made in two levels of concentration i.e. 2 mg/mL and 10 mg/mL using propylene glycol 15% as solubilizing agent. Losartan transport from such solutions with and without oleic acid one hour pretreatment were tested using male Wistar rat skin as a membrane for 30 hours in vertical diffusion cell. The transport profiles were analyzed based on the lag time diffusion method. It is showed that oleic acid pretreatment did not effect on losartan permeation rate and efficiency of 2 mg/mL potassium losartan concentration. However, such pretreatment enhanced losartan permeation rate and efficiency 21 and 23-fold higher respectively for 10 mg/mL the potassium losartan concentration. It is predicted that losartan minimum effective plasma concentration can be achieved in transdermal administration through this transport improvement in normal application area.

## ARTICLE HISTORY

Received: May 2018

Revised: September 2018

Accepted: December 2018

\*Corresponding author

Email: annasbinarjo@yahoo.co.id

**Keywords:** losartan; transdermal; oleic acid; *in vivo* prediction

## INTRODUCTION

Losartan(1-((2'-(2*H*-tetrazol-5-yl)bifenil-4-yl)methyl)-2-butyl-4-kloro-1*H*-imidazol-5-yl)methanol) is the first Angiotensin II Receptor Antagonist available in the market (Ripley and Hirsch 2010). The administration of losartan in diabetic-hypertension complication shows a significant advantages (Murthy, Kommineni, and Mayuren, 2013; Pan et al., 2015). Moreover, losartan also has an ability to protect the liver against ischemia (Koh, Yoon, and Lee, 2013).

This compound has low oral bioavailability (0.33) (Ripley and Hirsch 2010) due to the intensive first pass effect by CYP2C9 and CYP3A4 (Dina and Jafari, 2000) and *active efflux transporter activity* (Soldner et al., 2000). Transdermal delivery can be used to solve oral bioavailability problem (Tanner and Marks, 2008). Unfortunately, only few drugs can permeate through the skin due to the high resistance of stratum corneum. The epidermis, especially the outermost layer, the stratum corneum, consists of the nearly solid dead cell which is very difficult to be penetrated. The intercellular region of such dead cell comprises of lipid in lamellar organization (Bouwstra, Gooris, and Ponc, 2008). Such situation is an ideal barrier for many substances to be delivered via transdermal (Haftak, 2014). However, application of chemical enhancer can be developed to solve the barrier properties of skin (Paudel et al., 2010).

Vashisth and coworkers reported the potential chemical enhancer for transdermal delivery of losartan, such as tea tree oil, cumin oil, rose oil, and *Aloe vera* oil (Vashisth

et al., 2014). Capsaicin also can be used as enhancer of losartan from transdermal patch dosage form (Petkar and Kuchekar, 2007). The usage of proniosome carrier for losartan transdermal delivery also enhances the rate of losartan permeation across the skin (Thakur et al., 2009). Oleic acid is an enhancer which is frequently used. It has been proved that oleic acid can enhance skin permeation of many compounds i.e. piroxicam (Park et al., 2005), fisostigmin (Meshulam et al., 1993), and retinoid (Moghimi, Noorani, and Zarghi, 2010), both in *in vitro* as well as *in vivo* observation. Such enhancement activity is mediated by combination of two mechanisms: *lipid fluidization* and *lipid phase separation*. The intercalation of cis-structure oleic acid into lipid lamellar of stratum corneum induces the lipid disordering resulting the fluidity enhancement similar to the effect of temperature increasing. Additionally, oleic acid application can extract the lipid stratum corneum and decrease the membrane thickness (Naik, Pechtold, and Potts, 1995).

In the development of transdermal drug delivery, *in vitro* evaluation should be followed by an *in vivo* experiment to evaluate the efficiency of such delivery system. Prediction of *in vivo* results is required as an effort to select a system in the case of many formulation showing distinctive advantages regarding *in vitro* data of steady state *flux* and *lag time* diffusion. Some research conducted the prediction of *in vivo* data based on *in vitro* observation using exclusive software. Nakamura and coworkers for instance, succeeded to predict the tulobuterol pharmacokinetics profile from transdermal delivery based on *in vitro* observation using SKIN CAD software (Nakamura, Mori, and Tojo, 2012).

This research was aimed to enhance losartan *in vitro* skin permeation using oleic acid pretreatment, and use such data, in particular the most frequent parameters calculated in *in vitro* transdermal experiment, i.e. *steady state* flux and *lag* time diffusion to predict the pharmacokinetics profile. The prediction was constructed uncomplicatedly using equation derived from intra venous infusion equation and could be calculated easily using well-known office software.

## METHODS

### Instrumentation and Material

*In vitro* observation was conducted using vertical type diffusion cell (Figure 1) produced by Material Process Laboratory, Department of Engineering Physics, The Bandung Institute of Technology, with fresh male Wistar rat skin as membrane. The rats were purchased from Animal Handling Laboratory, Faculty of Pharmacy, Gadjah Mada University, Yogyakarta. By controlling the rat age (2 months) and weight (about 200 g), it was assumed that all membranes have the same thickness. Potassium losartan as active compound was purchased from PT Kalbe Farma Jakarta. Oleic acid as chemical enhancer (E Merk) was purchased from Asia Lab Yogyakarta. Losartan concentration in receptor compartment (calculated as potassium losartan) was measured according previous research (Binarjo and Nugroho, 2013). Briefly, HPLC (Shimadzu) controlled using *LC Solution software* (Shimadzu) was equipped with C-18 (Lichrospher RP 18 250-4 (5  $\mu$ m)) as stationary phase and UV detector set up at 223 nm. Acetonitrile-acetic buffer pH 4 in a volume ratio of 3:2 was used as mobile phase in an isocratic rate of 0.75 mL/minute.

The medium used for preparing drug formulation was 0.05 M citric buffer pH 5.0 (contain citric acid 0.37 g/L, sodium citric 0.96 g/L, sodium chloride 4

g/L; and mannitol 2 g/L). Propylene glycol was used as *solubilizing agent* in a concentration of 15%. As acceptor medium, 0.15 M phosphate buffer saline (PBS) pH 7.4 (contains NaCl 8 g/l, Na<sub>2</sub>HPO<sub>4</sub> 2.86 g/L, KH<sub>2</sub>PO<sub>4</sub> 0.2 g/L, and KCl 0.19 g/mL) was used. All substances used as mobile phase, acceptor medium as well as drug solution were produced by E Merk and were purchased from Asia Lab Yogyakarta.

## Methods

### Formulation of potassium losartan solution

In a 5 mL of volumetric flask, potassium losartan weighed accurately was filled in to get a concentration as listed in Table 1. About 0.75 mL of propylene glycol was added and followed by dissolving the drug using ultrasonicator. Citric buffer was added to get 5 mL drug solution.

### Transdermal transport observation

The male Wistar rat skin was excised after being sacrificed by introduced in chloroform saturated chamber. The hair was cut off by electric clipper, and the fat was removed. This clean skin was set up in the vertical diffusion cell (Figure 1) followed by receptor compartment filling in with PBS pH 7.4 and immersing magnetic bar prior to set up on thermoline (Cimarec). For formulation I and III the transports were started by filling the donor compartment with 2 ml formulation. For formulation II and IV, as listed in Table 1, the donor compartment was filled with 1 mL of oleic acid as a pretreatment. The magnetic bar was stirred and the temperature was maintained at 35°C. After pretreatment of formulation II and IV, the oleic acid was removed, and the skin was cleaned three times using distilled water. The water trace was absorbed by filter paper prior to transport started. The transport was begun by filling the donor compartment with 2 ml formulation. The aliquot volume of 1.0 mL was withdrawn from receptor compartment in scheduled time (0 (blank), 15,

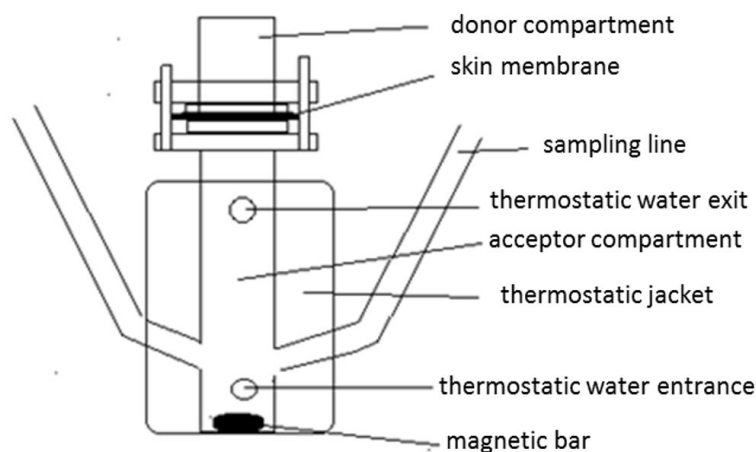


Figure 1. Schematic representation of vertical type diffusion cell

**Table 1. Concentration of potassium losartan and the pretreatment**

Group (Formulation)	Concentration of Potassium Losartan (mg/mL)	Oleic Acid Pretreatment
I	2	Without pretreatment
II	2	1 hour pretreatment
III	10	Without pretreatment
IV	10	1 hour pretreatment

**Table 2. Losartan transdermal transport parameters in mean  $\pm$  SD (n=3). Flux, lag time, permeability, diffusion coefficient, partition coefficient and transport efficiency are symbolized by J,  $T_{lag}$ , P, D, K and E respectively**

Group	J (ng/(hour xcm <sup>2</sup> ))	$T_{lag}$ (hour)	Px10 <sup>4</sup> (cm/hour)	Dx10 <sup>4</sup> (cm <sup>2</sup> /hour)	K	E x10 <sup>3</sup>
I	679.93 $\pm$ 534.49	17.02 $\pm$ 0.41	3.3 $\pm$ 1.0	3.9 $\pm$ 2.6	0.171 $\pm$ 0.055	2.7 $\pm$ 0.2
II	347.12 $\pm$ 147.5	15.04 $\pm$ 3.75	1.7 $\pm$ 0.8	4.7 $\pm$ 1.3	0.069 $\pm$ 0.011	2.3 $\pm$ 1.7
III	610.34 $\pm$ 79.88	14.18 $\pm$ 2.44	0.6 $\pm$ 0.1	4.8 $\pm$ 0.9	0.026 $\pm$ 0.007	0.8 $\pm$ 0.1
IV	12849.98 $\pm$ 4206.0	13.22 $\pm$ 3.19	12.8 $\pm$ 4.2	5.2 $\pm$ 1.2	0.525 $\pm$ 0.285	18.7 $\pm$ 5.0

20, 22, 24, 26, 28, and 30 hours), and the fresh receptor solution was added to maintain the constant volume of receptor compartment. The concentration of losartan (as potassium losartan) was measured using HPLC. Chromatograms of Potassium losartan solution in PBS in the range of concentration (ng/ml) 20.26, 50.65, 101.13, 202.26, 506.5, 1013, 2532.5, 5065, 10130, 20260 were recorded. The peak area at the retention time about 9 minutes has strong correlation against concentration. This concentration determination method has been validated (Binarjo and Nugroho, 2013).

#### Data analysis

Transport profile was analyzed using *Lag Time Diffusion* method. The transport parameters revealed from this method are *steady state* flux (J) and *lag time* ( $t_{lag}$ ). Permeability (P) was calculated ( $P=J/Cd$ ) whereas Cd is concentration of drug in donor compartment. Diffusion coefficient (D) was calculated using the thickness of membrane (h) and  $t_{lag}$  ( $D=h^2/6t_{lag}$ ). The data of P, D, and h were used to calculate partition coefficient (K) ( $K=hP/D$ ) (Martin, Bustamante, and Chun, 1993). Transport efficiency (E) was defined as a ratio of drug permeated after 30 hours of transport ( $M_t$ ) to available drug in donor compartment ( $M_d$ ). Statistical analysis *independent sample t-test* was carried out using *software SPSS 11.5*, (SPSS Inc, 2002). The difference is said to be significant if the critical p-value is less than 0.05.

## RESULTS AND DISCUSSION

### Calibration curve of potassium losartan in PBS pH 7.4

Calibration curve of potassium losartan in PBS pH 7.4

is shown in Figure 2. It is shown that this curve has strong linearity and can be used to calculate potassium losartan concentration since calculated r is higher than r table (0.95) (Muth 1999).

#### Transdermal transport profile

Figure 3 shows that the *steady state* condition has been reached after 15 hours of transport for all formulation. During 30 hours of experiment, there was not any post *steady state* signal detected. Therefore, the transport parameters were calculated from 15<sup>th</sup> to 30<sup>th</sup> hours of this profile and the data is listed in Table 2.

Based on *independent sample t-test* (SPSS 11.5 version), there was not any difference between *steady state flux* group I and III ( $p>0.05$ ), it means that losartan concentration in donor compartment did not influence the flux of transport without oleic acid pretreatment. This results was not agree with first Fick's law (Martin, Bustamante, and Chun, 1993). It is postulated that the higher of drug concentration in donor compartment would always enhance the rate of transport, but the transport efficiency would be decreased (Wester and Maibach, 1976). In present research, the rate of transport was not increased while the transport efficiency was decreased.

The transport rate which was not increased by the enhancement of drug concentration in donor compartment can be explained base on the hydrophilicity of permeant and the route of transdermal penetration. Showing the pKa value of 3.64 (Meloun, Syrový, and Vrána, 2005), based on Handerson-Hasselbach equation the losartan solution at pH 5.0 is more than 90% dissociated resulting in the low partition coefficient. As a result, the main

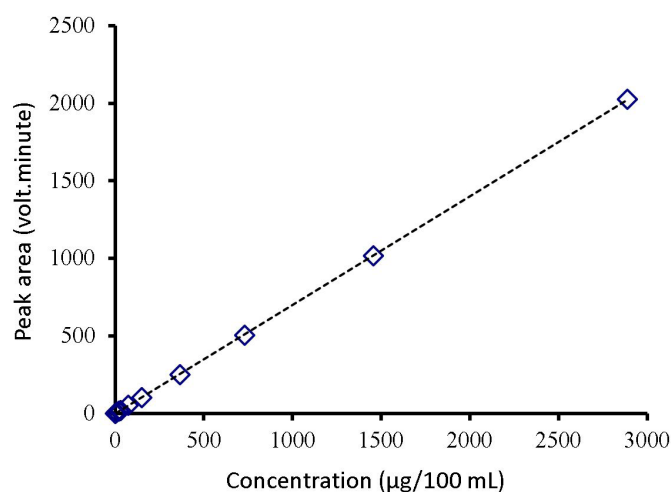


Figure 2. Calibration curve of potassium losartan in PBS pH 7.4. The linear equation is  $y = 0.7013x - 2.4466$  with  $r = 0.999991$

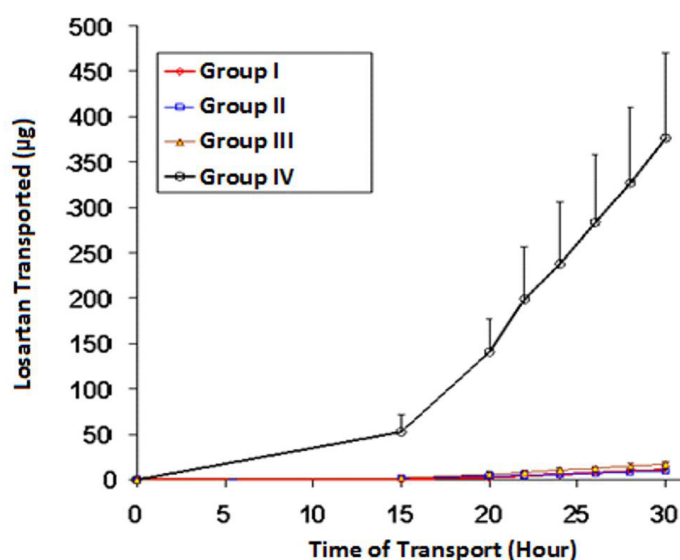


Figure 3. Losartan transdermal transport profile The transport parameters are shown in table 2. (based on 3 replicates, I: standard deviation (SD)).

route of transdermal permeation is transappendageal via hydrophilic route of sweat pore (Binarjo, 2014). The very small number and very little area of sweat pore was likely limited the number of permeant to cross over. Therefore, increasing the number of permeant, i.e. donor concentration, did not give any significant enhancement of absorption rate.

The oleic acid pretreatment increased the transport efficiency, resulting in the enhancement of *steady state flux* of group IV in comparison to group III by 21-fold. The ability of oleic acid to reduce the decreasing of transport efficiency in the higher concentration was also shown by the fact that in the low donor concentration, the oleic acid pretreatment did not enhance the flux (group I vs group II). Moreover, it was known that this transport efficiency enhancement in high drug concentration of donor solution was facilitated by the enhancement of

partition coefficient by 20-fold, resulting in the change of main route of permeation from transappendageal to intercellular route.

The diffusion coefficient which was not increased indicated that the lipid fluidization mechanism (Naik et al., 1995) was not appeared. If the lipid fluidization mechanism is occurred, based on Stoke-Einstein equation,  $D = RT / (6\pi\eta rN)$ , the diffusion coefficient will be increased (Achuthan et al., 2011). Lipid fluidization mechanism decreases the viscosity ( $\eta$ ) of membrane, and based on that equation above, the diffusion coefficient will increase.

#### In vivo prediction

The *in vivo* prediction of transdermal delivery was started by calculating the appropriate area of application to reach the desired plasma level in the therapeutics

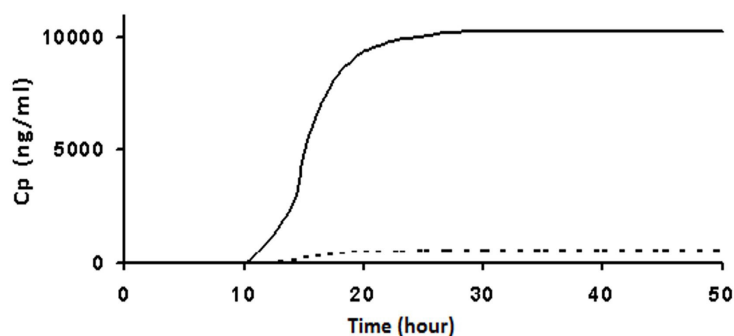


Figure 4. The prediction of losartan pharmacokinetics profile achieved from transdermal delivery, with oleic acid pretreatment (—) and without oleic acid pretreatment (---)

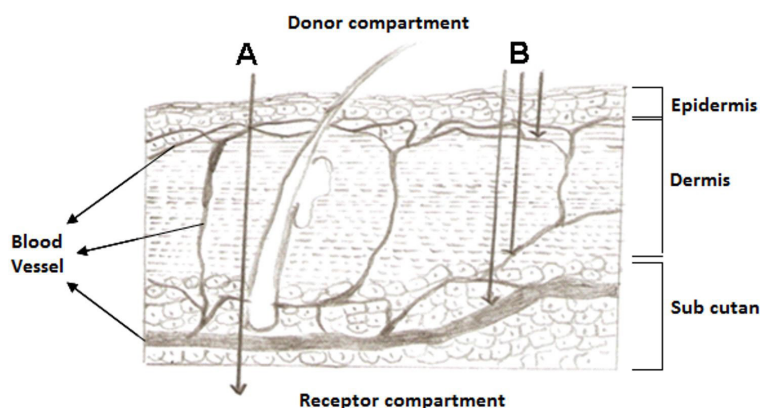


Figure 5. The movement of drug across the skin in *in vitro* observation (A) and *in vivo* study (B)

windows. Since the rate of drug entering to the body (*rate in*) is equivalent to the rate of elimination (*rate out*), the plasma level in desired level will be constant, similar to *intra vena* infusion administration. The *rate in* of delivery is the total flux ( $J_T = J \cdot A$ ), while the *rate out* is the zero order rate of elimination ( $k_0 = C_p \cdot V_d \cdot k$ ). The symbol of  $J$ ,  $A$ ,  $C_p$ ,  $V_d$ , and  $k$  represent *steady state flux*, surface area of application, desired plasma level in therapeutics window, distribution volume, and first order constant of drug elimination respectively.

Unfortunately, the effective plasma level of losartan has not been defined yet clearly, because its metabolite also has an antihypertensive activity (Choi et al., 2009). Lee and coworkers plotted the pharmacokinetics profile of losartan. By assuming the activity of losartan appear at 1 hour after administration, based on the pharmacokinetics profile the plasma level of losartan ( $C_p$ ) was 10 ng/mL. This work also suggested that the value of  $k$  is  $0.21 \text{ hr}^{-1}$  (Lee et al., 2003). The  $V_d$  of losartan is 32 L (Lacy, Armstrong, and Ingram 1998). Using these data, the application of formulation in group IV ( $\text{flux} = 12849.98 \text{ ng cm}^{-2} \text{ hr}^{-1}$ ) required an area of  $5.56 \text{ cm}^2$  (a square with the side of 2.36 cm) to reach the desired plasma level of 10 ng/mL. This area is reasonable in topical application.

The pharmacokinetics profile of transdermal delivery can

be predicted from *in vitro* data, which is analyzed using lag time diffusion method resulting lag time and flux.  $C_p$  as a function of application time can be calculated based on the equation of *intra vena* infusion as shown in equation 1.

$$C_p = \frac{R(1 - e^{-kt})}{V_d \cdot k} \quad (1)$$

The symbol of  $R$  represents the rate of delivery of *intra vena* infusion (in ng/hour) which is similar with total flux in transdermal delivery. The physical properties of the skin require the *lag time* in transdermal transport (delivery). This condition suggests the derivation of equation 1 to equation 2, to be applicable for transdermal delivery.

$$C_p = \frac{J_{\text{total}}(1 - e^{-k(t - t_{\text{lag}})})}{V_d \cdot k} \quad (2)$$

Pharmacokinetics profile can be predicted using equation 2. For the application of formulation in group IV, the pharmacokinetics profile prediction is shown in figure 4, by using the total flux ( $J_{\text{total}}$ ) and lag time ( $t_{\text{lag}}$ ) are 71445.9 ng/hr (calculated from  $12849.98 \text{ ng hr}^{-1} \text{ cm}^{-2} \cdot 5.56 \text{ cm}^2$ ) and 13.22 hours respectively.

Figure 4 indicates that the drug occurs in the blood after 10 hours of administration. The plateau condition which



was started at about 30 hours happened because the constant rate in absorption (zero order) was equal to the rate constant of elimination. The first order of elimination is prompted to zero order because of the constant of plasma level. It is predicted that 2 ml of dosage form can be administrated for 48 hours in the constant of rate absorption. Such time is reasonable since for 48 hours of application, the drug absorbed was 3.43 mg (calculated from 71445.9 ng/h x 48 h), while the available drug in dosage form was 20 mg (calculated from 10 mg/ml x 2mL).

Comparing pharmacokinetics profile in figure 4 with pharmacokinetics profile of oral delivery in the dosage of 50 mg (Lee et al., 2003), it is sound that transdermal delivery is better than oral delivery after 10 hours of application. The long duration of lag time in *in vitro* observation is an obstacle, but it is not always happened in *in vivo* condition. The membrane in *in vitro* observation is thicker since the sampling is withdrawn from the receptor compartment. It is not absolutely right that receptor compartment represents the blood vessel, as the blood vessel occurs in the dermis, the part of the membrane. The schema in Figure 5 shows such condition.

Figure 5 proposes that the drug movement is farther in *in vitro* observation. In *in vivo* observation the drug reaches the capillary vessel in the dermis tissue, or farthest to vein blood vessel in sub cutis tissue. Moreover, the fluidity of membrane in viable tissue, as in *in vivo* observation, is higher than that in dead skin. In the dead skin, the membrane is more viscous, and the implication is the lower of diffusion coefficient (D). According to the lag time equation,  $t_{lag} = h^2/6D$ , the increasing of h (the thickness of the membrane) as well as the decreasing of D, affect the increase of lag time.

## CONCLUSION

Oleic acid pretreatment to the rat skin increased the rate of transdermal permeation 21-fold as well as transport efficiency of 23-fold for 30 hours of experiment. It is predicted that using such pretreatment, losartan transdermal delivery can reach the effective plasma level at the ideal surface area of topical application.

## ACKNOWLEDGEMENT

The research was supported by Hibah Kompetensi DIKTI batch II from The Ministry of Education Council Republic of Indonesia, year 2009-2011. AB thanks to Marlyn D Laksitorini for the discussion and Panji for HPLC assistance

## REFERENCES

- Achuthan, S., Chung, B. J., Ghosh, P., Rangachari, V., & Vaidya, A. (2011). A modified Stokes-Einstein equation for A $\beta$  aggregation. *BMC Bioinformatics*, 12(SUPPL. 10). <https://doi.org/10.1186/1471-2105-12-S10-S13>
- Binarjo, A. (2014). Faktor Penentu permeasi transdermal: Tinjauan berdasarkan hukum fick I. *Pharmaceutical Sciences and Research (PSR)*, 9(3), 121-135-135. <https://doi.org/10.7454/psr.v9i3.3348>.
- Binarjo, A., Nugroho, A.K. (2013). The study on losartan concentration determination using spectrophotometric and hplc method, and its application to transdermal transport in vitro. *Pharmaciana*, 3(1), 31-48.
- Bouwstra, J. A., G. S. Gooris, and M. Ponc. (2008). Skin lipid organization, composition and barrier function. *International Journal of Cosmetic Science*, 30(5), 388-388. [https://doi.org/10.1111/j.1468-2494.2008.00430\\_1.x](https://doi.org/10.1111/j.1468-2494.2008.00430_1.x).
- Choi, J. H., Shin, S., Park, D., Jeon, J. H., Choi, B. H., Jang, M. J., ... Kim, Y. B. (2009). Comparative antihypertensive activities of losartan and hm70186 in rats with hepatic dysfunction. *Archives of Pharmacol Research*, 32(7), 1005-11. <https://doi.org/10.1007/s12272-009-1705-0>.
- Dina, R., and Jafari, M. (2000). Angiotensin II-receptor antagonists: An overview. *Am J Health Syst Pharm*, 57, 231-41.
- Haftek, M. (2014). Memory' of the Stratum Corneum: Exploration of the Epidermis' Past. *British Journal of Dermatology*, 171, 6-9. <https://doi.org/10.1111/bjd.13243>.
- Koh, E. J., Yoon, S. J., & Lee, S. M. (2013). Losartan protects liver against ischaemia/reperfusion injury through PPAR- $\gamma$  activation and receptor for advanced glycation end-products down-regulation. *British Journal of Pharmacology*, 169(6), 1404-1416. <https://doi.org/10.1111/bph.12229>.
- Lacy, C.F., L.L. Armstrong, and N.B. Ingram. (1998). *Drug Information Handbook*. 6th Ed. Cleveland: Lexi-Com Inc.
- Lee, Craig R., John A. Pieper, Alan L. Hinderliter, Joyce A. Blaisdell, and Joyce A. Goldstein. (2003). Losartan and E3174 Pharmacokinetics in Cytochrome P450 2C9\*1/\*1, \*1/\*2, and \*1/\*3 Individuals. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*, 23, 6, 720-25. <https://doi.org/10.1592/phco.23.6.720.32187>.

- Martin, A, P Bustamante, and A.H.C. Chun. (1993). *Physical pharmacy, physical chemical principles in The Pharmaceutical Sciences*. 4th Ed. Philadelphia: Lea and Febiger.
- Meloun, Milan, Tomáš Syrový, and Aleš Vrána. (2005). The thermodynamic dissociation constants of losartan, paracetamol, phenylephrine and quinine by the regression analysis of spectrophotometric data. *Analytica Chimica Acta*, 533, (1), 97–110. <https://doi.org/10.1016/j.aca.2004.11.007>.
- Meshulam, Y., Kadar, T., Wengier, A., Dachir, S., and Levy, A. (1993). Transdermal penetration of physostigmine: effects of oleic acid enhancer. *Drug Development Research*, 28(4), 510–15. <https://doi.org/10.1002/ddr.430280410>.
- Moghim, H. R., N. Noorani, and A. Zarghi. (2010). Stereoselective permeation of tretinoin and isotretinoin through enhancer-treated rat skin. II. Effects of lipophilic penetration enhancers. *Iranian Journal of Pharmaceutical Research*, 3(0), 17–22.
- Murthy, T.E.G.K., Manogna K. Kommineni, and Candasamy Mayuren. (2013). Influence of losartan on the hypoglycemic activity of glimepiride in normal and diabetic rats. *Therapeutic Advances in Endocrinology and Metabolism*, 4(5), 133–38. <https://doi.org/10.1177/2042018813509397>.
- Muth, J.E.D. (1999). *Basic statistics and pharmaceutical statistical application*. New York: Marcel Dekker Inc.
- Naik, A, L.A.R.M Pechtold, and R.O Potts. (1995). Mechanism of oleic acid-induced skin penetration enhancement in vivo in humans. *Journal of Controlled Release*, 37(3), 299–306. [https://doi.org/10.1016/0168-3659\(95\)00088-7](https://doi.org/10.1016/0168-3659(95)00088-7).
- Nakamura, A., Mori, D., & Tojo, K. (2012). Evaluation of the predicted time-concentration profile of serum tulobuterol in human after transdermal application. *Chemical & Pharmaceutical Bulletin*, 60(3), 300–305.
- Pan, Y., Q. Y. Qiao, L. H. Pan, D. C. Zhou, C. Hu, H. F. Gu, S. K. Fu, X. L. Liu, and H. M. Jin. (2015). Losartan reduces insulin resistance by inhibiting oxidative stress and enhancing insulin signaling transduction. *Experimental and Clinical Endocrinology & Diabetes: Official Journal, German Society of Endocrinology [and] German Diabetes Association*, 123(3), 170–77. <https://doi.org/10.1055/s-0034-1395658>.
- Park, Eun-Seok, Yu Cui, Bum-Jin Yun, In-Ja Ko, and Sang-Cheol Chi. (2005). Transdermal delivery of piroxicam using microemulsions. *Archives of Pharmacol Research*, 28(2), 243–48.
- Paudel, Kalpana S, Mikolaj Milewski, Courtney L Swadley, Nicole K Brogden, Priyanka Ghosh, and Audra L Stinchcomb. (2010). Challenges and Opportunities in Dermal/Transdermal Delivery. *Therapeutic Delivery* 1 (1): 109–31.
- Petkar, K.C, and S.B Kuchekar. (2007). In-Vitro Percutaneous Absorption of Losartan Potassium in Human Skin and Prediction of Human Skin Permeability. *DARU Journal of Pharmaceutical Sciences* 15 (2): 53–60.
- Ripley, Elizabeth, and Ari Hirsch. (2010). Fifteen Years of Losartan: What Have We Learned about Losartan That Can Benefit Chronic Kidney Disease Patients? *International Journal of Nephrology and Renovascular Disease* 3 (June): 93–98.
- Soldner, Andrea, Leslie Z Benet, Ernst Mutschler, and Uwe Christians. (2000). Active Transport of the Angiotensin-II Antagonist Losartan and Its Main Metabolite EXP 3174 across MDCK-MDR1 and Caco-2 Cell Monolayers. *British Journal of Pharmacology* 129 (6): 1235–43. <https://doi.org/10.1038/sj.bjp.0703150>.
- Tanner, T., and R. Marks. (2008). Delivering Drugs by the Transdermal Route: Review and Comment. *Skin Research and Technology: Official Journal of International Society for Bioengineering and the Skin (ISBS) [and] International Society for Digital Imaging of Skin (ISDIS) [and] International Society for Skin Imaging (ISSI)* 14 (3): 249–60. <https://doi.org/10.1111/j.1600-0846.2008.00316.x>.
- Thakur, Reena, Md Khalid Anwer, Mohammad S. Shams, Asgar Ali, Roop K. Khar, Faiyaz Shakeel, and Ehab I. Taha. (2009). Proniosomal Transdermal Therapeutic System of Losartan Potassium: Development and Pharmacokinetic Evaluation. *Journal of Drug Targeting* 17 (6): 442–49. <https://doi.org/10.1080/10611860902963039>.
- Vashisth, Indu, Abdul Ahad, Mohd. Aqil, and Suraj P. Agarwal. (2014). Investigating the Potential of Essential Oils as Penetration Enhancer for Transdermal Losartan Delivery: Effectiveness and Mechanism of Action. *Asian Journal of Pharmaceutical Sciences* 9 (5): 260–67. <https://doi.org/10.1016/j.ajps.2014.06.007>.
- Wester, Ronald C., and Howard I. Maibach. (1976). Relationship of Topical Dose and Percutaneous Absorption in Rhesus Monkey and Man. *Journal of Investigative Dermatology* 67 (4): 518–20. <https://doi.org/10.1111/1523-1747.ep12664543>.