INTISARI

Obat anti hipertensi golongan inhibitor *Angiotensin Converting Enzyme* (ACE) adalah salah satu dari 48 jenis obat yang menjadi prioritas BPOM RI untuk dilakukan uji bioekivalensi. Lisinopril adalah salah satu contoh inhibitor ACE. Umumnya, HPLC-Tandem Spektra-Massa digunakan untuk uji bioekivalensi lisinopril, dengan hasil yang sensitif dan akurat, akan tetapi berbiaya relatif mahal. Oleh karena itu, perlu dikembangkan metode lain yang lebih murah. Penetapan kadar obat dalam darah (plasma) dari waktu ke waktu diperlukan pada uji bioekivalensi, sehingga suatu metode penetapan kadar obat dalam darah yang valid mutlak diperlukan.

Lisinopril di daerah UV-Vis mempunyai serapan rendah karena gugus kromofor yang sangat minim. Derivatisasi biasanya dilakukan untuk meningkatkan sensitivitas. Salah satu contoh senyawa penderivat adalah 1-fluoro 2,4 dinitro benzen (FDNB). Penelitian ini bertujuan mencari dan memvalidasi metode penetapan kadar lisinopril dalam *spiked* plasma secara HPLC atau UPLC melalui derivatisasi dengan FDNB. Metode yang telah divalidasi, diaplikasikan pada plasma tikus, untuk mengetahui apakah terdapat senyawa metabolit dan plasma endogen yang mengganggu.

Metode ini berdasarkan reaksi lisinopril dalam akuades dengan FDNB, pada komposisi pelarut bufer borat (pH 9,5):asetonitril = 10:90 (v/v), suhu reaksi 70°C, waktu reaksi 25 menit dengan detektor pada λ 353,0 nm. Kolom Nova-pack® C_{18} (250 mm x 4,6 mm, 4 µm, Waters) digunakan untuk analisis lisinopril secara HPLC dengan gabapentin sebagai standar internal. Fase gerak yang digunakan adalah bufer asetat (pH 3,5; 0,02 M) : asetonitril = 50 : 50 (v/v) dan diukur pada λ 353,0 nm. Metode analisis yang digunakan menghasilkan resolusi (Rs) > 2,5 dengan waktu analisis 25 menit. Mengingat total waktu analisis yang diperlukan, untuk analisis rutin metode tersebut kurang efisien.

Metode lain, yaitu reaksi lisinopril dalam pelarut metanol dengan FDNB. Reaksi ini optimum dengan menggunakan bufer borat (pH 11,0) pada suhu kamar dengan waktu kestabilan produk derivatisasi 60 menit. Selanjutnya, dilakukan analisis lisinopril dalam *spiked* plasma melalui derivatisasi dengan FDNB secara *Ultra Performance Liquid Chromatography* (UPLC) menggunakan kolom Acquity BEH C_{18}. Fase gerak yang digunakan untuk sistem UPLC ini adalah bufer asetat (0,01 M, pH 3,5) : asetonitril : metanol = 70 : 10 : 20 (v/v/v) pada λ 296,0 nm. Metode ini telah memenuhi persyaratan validasi. Liniearitas kadar lisinopril berada pada rentang 5,0-100,0 ng/mL (r^2 =0,9987), rekoveri 88,59±6,01–101,70±2,56% dan nilai RSD 2,57-8,16 %. Batas deteksi instrumen = 0,73 ng/mL dan batas kuantitasi 2,44 ng/mL. Ketika metode tersebut diaplikasikan pada plasma tikus, analisis lisinopril dalam plasma tidak terganggu oleh adanya metabolit lisinopril dan senyawa endogen dalam plasma. Selain itu, kadar lisinopril dalam plasma tikus memiliki nilai RSD 5,15-13,22% < 15% telah memenuhi persyaratan. Dengan demikian, metode ini berpotensi digunakan untuk uji bioekivalensi lisinopril.

Kata kunci: lisinopril, FDNB, derivatisasi, HPLC, UPLC.

ABSTRACT

A bioequivalency test is required to be conducted for 48 priority types of drugs of BPOM RI. A hypertension drug, group of Angiotensin Converting Enzyme (ACE), is one of the drugs which should be tested. Thus, lisinopril as one of the member of ACE inhibitor should be tested. HPLC MS-MS is a method which generally used for the test, resulted a sensitive and accurate result. However, this method is costly. Therefore, an alternative method which is cheaper needs to be developed. In addition, determination of drugs concentration in plasma needs to be monitored time to time. Hence, a method to determine drugs concentration in plasma is required.

Lisinopril has minimum functional group of chromophore, thus its absorbance at ultraviolet-visible region is low. Increasing the sensitivity, a derivatization step of reaction needs to be conducted. A 1-fluoro-2,4-dinitro benzene (FDNB) is one of the compound which usually used as a derivatized reagent. A validated method for determination of lisinopril at spiked plasma with HPLC or UPLC method was developed in this research. Subsequently, it was applied to analyze lisinopril at mice plasma; studying whatever the metabolite compounds or endogenous compounds of plasma interfere the analysis or not.

Derivatization reaction of Lisinopril which is dissolved at aquades was conducted. The optimum condition was found at borate buffer (pH 9.5) and acetonitrile (10 : 90, v/v) at 70^{o}C within 25 minutes of reaction time, detected at λ 353.0 nm. Gabapentin-DNB, was used as an internal standard when Lisinopril-DNB was analyzed with HPLC. A Nova-pack® C_{18} 4 µm (250 mm x 4,6 mm, Waters) was used as a stationary phase and buffer acetate (pH 3.5; 0.02 M) : acetonitrile = 50 : 50 (v/v) as a mobile phase at λ 353.0 nm. A well separation of lisinopril was obtained at high resolution (Rs>2.5). However, it seems that this HPLC method doesn't efficient due to a long analysis time and more preparation steps. Therefore, another method needs to be developed and validated in order to eliminate these drawbacks. Thus, derivatization of Lisinopril with FDNB in methanol was also developed.

It was found, that room temperature with borate buffer (pH 11.0) was the optimum reaction condition. The derivatization product was still stabile upon 60 minute of reaction. Analysis was further conducted with *Ultra Performance Liquid Chromatography* (UPLC) using an Acquity BEH C_{18} column. Acetate buffer (0.01 M, pH 3.5): acetonitrile: methanol (70: 10: 20, v/v/v) was used as mobile phase, and was detected at λ 296.0 nm. Method validation was shown that the developed method has a good linearity at concentration range of Lisinopril 5.0-100.0 ng/mL (r^2 = 0.9987), 88.59±6.01–101.70±2.56% of recovery, 2.57-8.16% of RSD, LOD of instrument = 0.73 ng/mL and LOQ of instrument = 2.44 ng/mL. When it was applied on the rat plasma, both lisinopril metabolite and endogenous compounds of plasma were not interfere the analysis of lisinopril, as lisinopril is a targeted compound. Resulting a fullfilled of RSD, ca. 5.15-13.22%, this is far the lower limit of RSD, i.e. < 15%. Hence, a developed analysis method of Lisinopril with UPLC is reliable to use for bioequivalensi test.

Keywords: lisinopril, FDNB, derivatization, HPLC, UPLC.