Validasi metode analisis ofloksasin dalam plasma in vitro secara kromatografi cair kinerja tinggi fluoresensi mengacu pada european medicines agency guideline = Validation of ofloxacin analytical method in in vitro plasma with high performance liquid chromatography fluorescence based on european medicines agency guideline/ Letitia Tania

Letitia Tania, author

Abstrak

Ofloksasin merupakan salah satu antibiotika golongan fluorokuinolon generasi kedua. Konsentrasi ofloksasin dalam plasma kecil, sehingga diperlukan metode analisis yang selektif, akurat, dan sensitif. Pada penelitian ini, dilakukan optimasi dan validasi metode analisis ofloksasin dalam plasma in vitro menggunakan kromatografi cair kinerja tinggi deteksi fluoresensi dengan siprofloksasin HCl sebagai baku dalam. Pemisahan dilakukan dengan menggunakan kolom C18 (Waters, SunfireTM 5 μm; 250 x 4,6 mm) dengan fase gerak isokratik yang terdiri dari trietilamin 1% dalam air pH 3,0–asetonitril (84:16), laju alir 1,0 mL/menit, suhu kolom 40oC, dan deteksi ofloksasin dilakukan pada panjang gelombang eksitasi 300 nm dan emisi 500 nm. Waktu retensi ofloksasin dan siprofloksasin HCl adalah 7,476 menit dan 8,446 menit dengan total waktu analisis 10 menit. Proses ekstraksi plasma dilakukan dengan metode pengendapan protein menggunakan metanol melalui proses vorteks selama 2 menit dan sentrifugasi pada kecepatan 10000 rpm selama 10 menit. Hasil penelitian menunjukkan bahwa spesifisitas dan selektivitas metode ekstraksi ditunjukkan dengan tidak adanya puncak pengganggu pada saat retensi analit dan baku dalam. Validitas dan linearitas metode analisis berada pada rentang konsentrasi 21,4 ng/mL – 4280 ng/mL dengan LLOQ 21,4 ng/mL. Nilai % diff dan koefisien variasi untuk akurasi dan presisi intra hari dan antar hari tidak lebih dari + 20% untuk konsentrasi LLOQ dan + 15% untuk konsentrasi rendah, sedang, dan tinggi. Ofloksasin dalam plasma dinyatakan stabil selama 3 siklus beku dan cair, stabil minimal 24 jam pada suhu kamar dan selama minimal 28 hari pada suhu -20oC. Metode analisis ofloksasin dalam plasma ini sudah memenuhi kriteria penerimaan sesuai persyaratan validasi pedoman EMEA.

<hr>

Ofloxacin is an antibiotic from second generation of fluoroquinolones group. Concentration of ofloxacin in human plasma is in low level, and due to that fact, it requires a selective, accurate, sensitive method of analysis. In this study, the optimization and validation of ofloxacin analysis in human plasma using high performance liquid chromatography-fluorescence with ciprofloxacin-HCl as an internal standard were carried out. Separation of ofloxacin was performed using C18 (Waters, SunfireTM 5 μm; 250 x 4.6 mm) column with an isocratic mobile phase consisted of triethylamine 1% in water pH 3.0–acetonitrile (84:16) in the flow rate of 1.0 mL/min, 40oC column temperature whereas the detection was carried out at excitation of 300 nm and emission of 500 nm. Retention time of ofloxacin and ciprofloxacin-HCl were 7.476 and 8.446 minutes respectively with total analytical run time was 10 minutes. Plasma extraction was done by eproteination using methanol, through the process of vortex and centrifugation (10000 rpm) for 2 minutes and 10 minutes consecutively. The results showed that the extraction method was specific and selective as there was no interfering peaks of human plasma at the retention times of the ofloxacin and the

internal standard. The method was valid and linear within the concentration ranged from 21,4 ng/mL to 4280 ng/mL with LLOQ of 21,4 ng/mL. Intra-day and inter-day accuracy and presicion was not more than + 20% for LLOQ and not more than + 15% for QCL, QCM, and QCH samples in both % diff and coefficient of variation. Ofloxacin was stable in human plasma at least three freeze and thaw cycle, for at least 24 hours in room temperature and 28 days at -20oC. This bioanalytical method fulfilled the acceptance criteria following EMEA guidelines.