Pengembangan dan Validasi Metode Analisis Simultan Sefoperazon-Sulbaktam dalam Dried Blood Spots dan Volumetric Absorptive Microsampling secara KCKT-PDA = Method Development and Validation Simultaneous of Cefoperazone and Sulbactam in Dried Blood Spots and Volumetric Absorptive Microsampling by HPLC PDA

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Abstrak

Acinetobacter baumannii merupakan bakteri patogen gram negatif penyebab dominan infeksi nosokomial. World Health Organization (WHO) menerbitkan daftar prioritas pertama resistensi antibiotik terhadap Acinetobacter baumannii yang merupakan kategori kritis sebagai ancaman serius bagi kesehatan masyarakat global. Sefoperazon-sulbaktam merupakan salah satu antibiotik yang sensitif terhadap infeksi Acinetobacter baumannii. Berdasarkan penelitian di Rumah Sakit (RS) Dr. Soetomo Surabaya, telah terjadi resistensi terhadap antibiotik sefoperazon-sulbaktam sebanyak 27 %. Resistensi dapat terjadi karena konsentrasi di dalam darah tidak sesuai yang menyebabkan tidak efektifnya pengobatan. Metode bioanalisis dibutuhkan untuk penentuan konsentrasi obat di dalam darah. Penelitian ini dilakukan untuk memperoleh metode analisis sederhana, sensitif dan cepat yaitu analisis simultan sefoperazon sulbaktam dengan Kromatografi Cair Kinerja Tinggi (KCKT) detektor Photo Diodearray (PDA). Selain itu, untuk memperoleh metode preparasi biosampling untuk Dried Blood Spots (DBS) dan Volumetric Absorptive Microsampling (VAMS), serta melakukan perbandingan peak area ratio, selektivitas, recovery dan stabilitas dari DBS dan VAMS. Analisis kromatografi dilakukan dengan metode isokratik dengan fase gerak dapar fosfat 10 mM pH 3,2 – asetonitril (83 : 17), kolom C18 Xbridge (250 mm x 4,6 mm; 5 µm), laju alir 1,0 mL/menit, panjang gelombang 210 nm, suhu 35°C. Ekstraksi cair – cair dengan etil asetat sebagai metode preparasi untuk sampel dalam DBS dan VAMS. Hasil validasi metode bioanalisis memenuhi syarat LLOQ, linieritas, selektivitas, akurasi dan presisi, recovery, integritas pengenceran, carry over dan stabilitas yang mengacu pada Guideline on Bioanalytical Method Validation dari European Medicines Agency tahun 2011 dan FDA 2018, dengan nilai LLOQ untuk sulbaktam sebesar 1 µg/mL dan sefoperazon 5 µg/mL dan nilai r ³ 0,995. Hasil uji perbandingan menunjukkan bahwa metode biosampling VAMS memberikan hasil yang lebih baik, namun terdapat beberapa hasil yang tidak berbeda signifikan dari penggunaan dengan DBS.
.....Acinetobacter baumannii is a gram-negative pathogenic bacteria that causes the majority of nosocomial infection. The World Health Organization (WHO) published the first priority list of antibiotic resistance against Acinetobacter baumannii which is a critical category as a serious threat to global public health. Cefoperazone-sulbactam is an antibiotic combination that is susceptible to Acinetobacter baumannii infection. According to studies conducted at the Dr. Soetomo Surabaya Hospital, up to 27% of patients exhibited resistance to cefoperazone-sulbactam medicines. When the drug in the blood is under the required concentration, the treatment is ineffective and resistance can develop. For this reason, a simple, sensitive, and fast analytical method is needed for the analysis of cefoperazone-sulbactam. This research was conducted to obtain a simple, sensitive and fast analytical method using the High Performance Liquid Chromatography (HPLC) Photo Diode Array (PDA) detector. In addition was obtained preparation method for the Dried Blood Spots (DBS) and Volumetric Absorptive Microsampling (VAMS), as well as to

compare the peak area ratio, selectivity, recovery and stability of DBS and VAMS. Chromatographic analysis was carried out using the isocratic method with 10 mM phosphate buffer mobile phase pH 3.2 – acetonitrile (83:17), C18 Xbridge column (250 mm x 4.6 mm; 5 m), flow rate 1.0 mL/min, wavelength 210 nm, temperature 35ºC. Sample preparation method was performed by liquid-liquid extraction using ethyl acetate as a solvent. The results showed that the analytical method carried out meet the validation parameters requirements (LLOQ, linearity, selectivity, accuration and precision, recovery, dilution integrity, carry over and stabilitty) that refer to the Guideline on Bioanalytical Method Validation from the European Medicines Agency in 2011 and the FDA 2018, with an LLOQ value for sulbactam of 1 µg/mL and cefoperazone at 5 µg/mL and r ³ 0,995. The comparison test results demonstrated that the VAMS biosampling method provided better results, however certain results were not statistically different from those obtained using DBS in some cases