Validasi Metode Analisis Asaro Valproat yang Diderivatisasi dengan 2,4-Dibromoasetofenon dalam Plasma Manusia In-vitro Secara Kromatografi Cair Kinerja Tinggi-Photo Diode Array dan Aplikasinya Secara In-vivo

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Abstrak

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Asaro valproat adalah satu dari banyak obat yang digunakan sebagai antiepilepsi dan memiliki banyak efek samping, sehingga direkomendasikan untuk menentukan konsentrasinya di dalam plasma. Penelitian ini bertujuan untuk memvalidasi metode analisis asam valproat setelah diderivatisasi dengan 2,4-dibromoasetofenon di dalam plasma in-vitro dan in-vivo, menggunakan kromatografi cair kinerja tinggi-Photo Diode Array.
Asam valproat dan asam nonanoat sebagai baku dalam diekstraksi dari plasma dengan etil asetat. Supematan yang diperoleh dinetralkan dan diuapkan, kemudian residu kering direkonstitusi dengan larutan penderivat-katalis dalam asetonitril kemudian diderivatisasi pada suhu 75°C selama 25 menit. Pemisahan dilakukan menggunakan kolom C18 Sunfire ® (250 mm x 4,6, 5 Jlm) dengan elusi isokratik menggunakan fase gerak asetonitril-air (73 :27). Pengukuran dilakukan pada panjang gelombang 294 nm dengan kecepatan alir 1,5 mL/menit. Metode ini valid berdasarkan hasil LOQ 4,75 flg/mL, perolehan kembali relatif konsentrasi rendah, sedang dan tinggi berturut-turut 100,67%, 99,78%, dan 93,16%. Koefisien variasi intra dan inter day dan persen penyimpangan (SD) dari metode ini masuk dalam kriteria penerimaan, yaitu dibawah ± 15%. Kurva kalibrasi linier dalam plasma in-vitro (Y = 0,0123 + 0,0085X) pada konsentrasi 4,75-237,75 Jlg/mL dengan nilai r =0,9999. Metode yang dihasilkan dapat diaplikasikan untuk menetapkan kadar asam valproat dalam plasma setelah pemberian secara oral tablet natrium divalproat 500 mg.
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ABSTRACT
Valproic acid is one of mostly used antiepileptic drug which have side effects, so it is highly recommended to evaluate its plasma concentration The aim of the research was to validate a method for the determination valproic acid in plasma in-vitro and in-vivo after derivatization with 2,4-dibromasetofenon using high performance liquid chromatography-photo diode array. Valproic acid and internal standard nonanoic acid were extracted from plasma sample with ethyl acetate. Then supematan was neutralizatied and evaporated. dried residue reconsituted in derivate-catalyst solution then derivatized at 75°C for
25 minutes. The resulting derivatives were separated on a Sunfire C18 (250 mm x 4.6, 5 Jlm) reverse phase column with acetonitrile-water (73:27) as
mobile phase, were detected at 294 nm and analysis were tun at flow rate 1.5 mL/minute. The calibration curve in plasma in-vitro (Y =0.0123 + 0.0085 x) presented good linier (r = 0.9999) between 4.75-237.75 Jlg/mL with LLOQ
4.75 Jlg/mL. The mean of relative recovery at low concentration, middle concentration and high concentration are 100.67%, 99.78%, and 93.16 %, respectively. Intra- and inter- day coefficient of variation

and percent error value of the assay method were all acceptable range ± 15%. The presented method was might be applied to the determine of the valproic acid concentration in plasma after oral administration of 500 mg sodium divalproate.

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