Optimasi dan validasi metode analisis rabeprazol dalam plasma In vitro secara kromatografi cair kinerja tinggi = Optimization and validation of analytical method of rabeprazole in human plasma using high performance liquid chromatography

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Abstrak

Rabeprazol merupakan obat golongan penghambat pompa proton yang digunakan untuk pengobatan refluks gastroesophageal. Konsentrasinya sangat kecil dalam plasma sehingga diperlukan metode analisis yang sensitif, selektif, dan akurat. Pada penelitian ini, dilakukan optimasi dan validasi metode analisis rabeprazol dalam plasma in vitro menggunakan kromatografi cair kinerja tinggi UV-Vis dengan pantoprazol sebagai baku dalam. Pemisahan menggunakan kolom Kromasil® C18, 100-5, (4,6 × 250 mm, 5 m) dengan fase gerak isokratik yang terdiri dari 50 mM natrium dihidrogen fosfat pH 7,2 - asetonitril (55:45, v/v). laju alir 0,5 mL/menit dan dideteksi pada panjang gelombang 294 nm. Waktu retensi rabeprazol dan pantoprazol adalah 8,7 dan 9,8 menit dengan total waktu analisis adalah 12 menit. Sampel plasma (500 L) diekstraksi menggunakan dietil eter-diklorometan (90:10, v/v).
Metode ini spesifik karena tidak adanya puncak pengganggu plasma pada waktu retensi analit dan baku dalam. Metode ini valid dan linear pada rentang konsentrasi 10,08 - 1008,00 ng/mL dengan LLOQ 10,0 ng/mL (n = 6, koefisien variasi (KV) = 3,16%). Nilai % diff dan koefisien variasi untuk akurasi dan presisi intra hari dan antar hari tidak lebih dari 15%. Nilai perolehan kembali absolut dari rabeprazol sebesar 76 - 87% (KV = 6,54%) dan baku dalam sebesar 74% (KV = 3,13%). Rabeprazol dalam plasma dinyatakan stabil selama minimal 1 bulan pada suhu -20°C dan -80°C, stabil selama minimal 12 jam pada suhu kamar. Rabeprazol dinyatakan stabil selama 3 siklus beku dan cair. Metode ini memenuhi kriteria penerimaan seperti pada pedoman USFDA dan bisa diaplikasikan untuk analisis rabeprazol dalam plasma in vivo.
.....Rabeprazole is a proton-pump inhibitor, used in gastroesophageal reflux treatment. Its concentration is very small in human plasma, so it requires a sensitive, selective, and accurate method of analysis. In this study, carried out optimization and validation of rabeprazole analysis in human plasma using high performance liquid chromatography UV-Vis using pantoprazole as internal standard. Separation was performed on Kromasil® 100-5 C18, (4.6 × 250 mm, 5m) column with an isocratic mobile phase composed of 50 mM sodium dihydrogen phosphate pH 7.2 - acetonitrile (55:45, v/v), flow rate at 0.5 mL/min and was detected at 294 nm. Retention time of rabeprazole and pantoprazole were 8.7 and 9.8 minutes and total analytical run time was 12 minutes. Plasma sample (500 L) was extracted with diethyl eter - dichloromethane (90:10, v/v).
The method was specific as there were no interfering peaks of human plasma eluting at the retention times of the rabeprazole and the internal standard. The method was valid and linear within the concentration ranges of 10.08-1008.00 ng/mL with LLOQ 10,0 ng/mL (n = 6, coefficient variation (CV) = 3.16%). Intra-day and inter-day accuracy and precision was not more than 15% in both % diff and coefficient of variation. Absolute recovery were 76-87% (CV = 6.54%) for rabeprazole and 74% (CV = 3.13%) for internal standard. Rabeprazole was stable in human plasma for at least 1 month at -20°C and -80°C, and for 12 h at room temperature. Rabeprazole were stable to three freeze thaw cycles. This method also fulfil the

acceptance criteria following USFDA guidelines and suitable to be applied for analysis of rabeprazole in human plasma.