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Pengembangan Metode Analisis Human Insulin secara Indirect ELISA Berbasis Antibodi Poliklonal Dibandingkan dengan KCKT Fase Terbalik = Development of Human Insulin Analysis Method by Indirect ELISA Based on Polyclonal Antibody Compared to Reverse Phase HPLC

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Abstrak

Diabetes melitus merupakan kelainan metabolik yang ditandai hiperglikemia dimana landasan terapinya masih menggunakan human insulin. Telah banyak sediaan yang beredar, namun metode analisis yang standar dan valid belum ditetapkan di dalam negeri. Penelitian ini bertujuan untuk mengembangkan dan memvalidasi metode analisis secara indirect ELISA dibandingkan KCKT UV fase terbalik untuk determinasi sediaan. Antibodi poliklonal IgG dihasilkan dari kelinci yang diimunisasi dengan 1 mg/mL antigen human insulin rekombinan, dimurnikan melalui presipitasi dan kromatografi afinitas, dikuantitasi dengan spektrofotometer UV280nm, dikarakterisasi dengan uji Dot blot menggunakan substrat BCIP-NBT, serta dikarakterisasi melalui uji SDS-PAGE dan Western Blot dengan konsentrasi gel 7,5% dan 17,5%. Sedangkan, metode KCKT menggunakan kolom ReliantTM C-18 (4,6 x 150 mm, 5 µm), fase gerak Na2SO4 pH 2,3 : Na2SO4 pH 2,3 dalam asetonitril (55:45,v/v) rasio 38:62 v/v, standar internal etilparaben 10 µg/mL, detektor UV 215 nm, suhu kolom 40°C, laju alir 1 mL/menit, dan volume injek 20 µL. Validasi kedua metode dilakukan terhadap larutan uji yang mengandung m-kresol dan gliserol. Metode ELISA pada rentang 80,11-200,28 µg/mL (r = 0,99) dan KCKT pada 9,735-146,025 µg/ml (r = 0,9997) terbukti linear. Rekoveri pada ELISA dan KCKT adalah 99,11% ± 5,01 dan 100,71% ± 1,11, sedangkan RSD 3,91% dan 0,64%. LOD dan LOQ metode ELISA 22,05 µg/mL dan 73,51 µg/mL, serta KCKT 0,193 µg/ml dan 0,643 µg/ml. Human insulin bersifat stabil pada suhu 2-8°C selama 24 jam (ELISA) dan suhu 23°C selama 48 jam (KCKT). Kesimpulan, hasil validasi kedua metode valid dan mampu mendeterminasi human insulin tanpa berbeda siginifikan (Uji T, a0,05).

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glycerol. ELISA method in the range of 80.11-200.28 µg/mL (r = 0.99) and HPLC at 9.735-146.025 µg/ml (r = 0.9997) was resulted to be linear. The recovery yields on ELISA and HPLC were 99.11%±5.01 and 100.71%±1.11. RSD on ELISA and HPLC were 3.91%, and 0.64%, respectively. The LOD and LOQ of the ELISA were 22.05 µg/mL and 73.51 µg/mL, while HPLC were 0.193 µg/ml and 0.643 µg/ml. Human insulin is stable at 2-8°C for 24 hours (ELISA) and 23°C for 48 hours (HPLC). In conclusion, the validation results of both methods are valid and able to determine human insulin with no significant difference (T test, a0.05). Cannot connect to Ginger Check your internet connection or reload the browser Disable in this text field Rephrase Rephrase current sentence Edit in Ginger Enable Ginger Cannot connect to Ginger Check your internet connection or reload the browser Disable in this text field Rephrase Rephrase current sentence Edit in Ginger.