Penggunaan Konjugat Tiamin- Biotin dan Protein Ikat Tiamin dari Kacang Hijau pada Pengukuran Kadar Tiamin dalam Serum dengan Enzyme Labelled Protein Ligand Assay = Application of Thiamine-Biotin Conjugate and Mung Bean Thiamine Binding Protein for Serum Thiamine Level Measurement Using Enzyme Labelled Protein Ligand Assay

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Abstrak

Tiamin berfungsi sebagai koenzim untuk beberapa enzim yang terlibat dalam metabolisme karbohidrat. Mengingat pentingnya peran tiamin, maka dilakukan pengembangan teknik pengukuran tiamin yang analog dengan enzyme linked immunosorbent assay (ELISA), dimana antibodi diganti dengan protein pengikat spesifik yaitu protein ikat tiamin kacang hijau (PITKH). Teknik pengukuran ini dilakukan secara kompetitif, kompetitor akan dikompetisikan dengan tiamin bebas yang akan diukur. Kompetitor tersebut berupa konjugat antara tiamin-biotin. Tiamin murni diikatkan dengan biotin menggunakan senyawa perangkai yaitu glutaraldehid. Pada analisis LC-MS ditemukan 3 puncak, . Puncak ke 3 merupakan konjugat tiamin-biotin. Dibuat kurva standar dan diperoleh persamaan garis lurus dengan nilai R2= 0,9986. Uji validasi menggunakan konjugat tiamin-biotin menunjukan nilai coefficient of variation (CV) = 3,81%, nilai ini lebih kecil dari CV Horwitz = 8,12%, akurasi dengan nilai Recovery (R) =94 %-98%. Hasil ini menunjukan syarat pengukuran dengan teknik ELPLA sudah terpenuhi, dengan presisi dan akurasi yang baik. Aplikasi pengukuran kadar tiamin pada serum normal sebanyak 23 sampel didapatkan kadar tiamin berkisar 2,62-9,76 g/ml. Dengan demikian, teknik ELPLA dengan konjugat tiamin-biotin sebagai kompetitor dapat digunakan pada pengukuran kadar tiamin dalam serum

.....Thiamine has a coenzyme function in several enzymes involved in carbohydrate metabolism. Considering the important role of thiamine, a thiamine measurement technique analogous to the enzyme linked immunosorbent assay (ELISA) was developed, that the antibody was replaced by a specific binding protein named mung bean thiamine binding protein (MBTBP). The measurement technique was carried out competitively in which competitors would be competed with free thiamine to be measured. The competitor is a thiamine-biotin bond. Pure thiamine was bound to biotin using a coupling compound called glutaraldehyde. In the LC-MS analysis we found 3 peaks. The third peak was the thiamine-biotin conjugate. A standard curve was made and the value of its straight line equation was obtained R2= 0,9986. The validation test using thiamine-biotin conjugate showed coefficient of variation (CV) value = 3,81% which was smaller than Horwitz CV = 8,12%, with the accuracy of the Recovery (R) value = 94% – 98%. These results indicated that the measurement requirements for the ELPLA technique had been met with good precision and accuracy. The application of the serum measurements to 23 samples showed thiamine levels ranging from 2,62- 9,76 g/ml. Thus, the ELPLA technique with thiamine-biotin conjugate as a competitor could be used in the measurement of serum thiamine levels