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Optimization and validation of a fluorescent kinetic analysis for the measurement of enzymatic activity of plasma DPP4

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In the course of a clinical study developing evoglipitin (DA-1229) tartrate, a specific dipeptidyl peptidase 4 (DPP4) inhibitor for the treatment of type 2 diabetes, an analytical method of fluorescent kinetic assay was optimized and verified to determine an enzymatic activity of soluble DPP4 in human plasma using a spectrofluorometer. The validation was performed for the parameters including the accuracy, the precision, the Limit of Detection (LOD), the linearity, the dynamic range, the short/long-term stability, the freezing-thawing stability, the Km constant, the dilution effect, and the recovery efficiency. The plasma DPP4 enzymatic activity (mU/min) was measured as the Initial Velocity (VO) of enzymatic reaction over time. After the reaction, the deviation of the mean from the nominal value, the Coefficient of Variation (CV) within/between runs, and the relative determinant constant (R2) were calculated. Accuracy and precision were within the deviation of the mean ≤15%, CV ≤15%, R2>0.99 except for LOD, where it did not exceed the deviation of the mean ≤20%, CV ≤15%, R2>0.95, respectively. The linearity of VO and the dynamic range of DPP4 values were reliable in the range of 6.06x103–5.13 x 105 mU/min and 62.5-1,500 ng/mL, respectively. Plasma DPP4 was stable under the various temperatures and even after three cycles of the freezing-thawing. The Km constant of plasma DPP4 was similar to that of the recombinant DPP4. Evoglipitin (DA-1229) tartrate effectively inhibited the DPP4 enzymatic activity in a dose-dependent manner without the dilution effect of sample. Due to the limited recovery efficiency of DPP4 in sample larger than 10 uL, the volume of sample was determined to be 10 uL for reliable assays. The optimized and validated analysis method of the DPP4 activity was successfully set up and employed for the measurement of the DPP4 activity in human plasma.

Biography

Hyunyee Yoon has expertise in developing the bio-analytic methods in the field of clinical studies. She has been operating the protein immunology core facility to support a number of clinical researchers at Seoul National University Hospital and Biomedical Research Institute since 1999. The major assays that she has worked on include protein quantification by an ELISA and a fluorescent multiplex bead array, protein qualification by a western blot and a Simple Western™ assays, enzymatic kinetic assay using a multi-detection analyzer, and immunogenicity analysis using a Meso Scale Discovery immunoassay. She has accumulated in-depth knowledge and experiences on various assays that are essential for the development and validation of the pharmacokinetic and pharmacodynamics analysis of clinical researches. Her continuous effort to resolve the complicated study derives the precise and reliable results.

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