Determination of serum D- and L-lactic acids in normal subjects and diabetic patients by column-switching HPLC with pre-column fluorescence derivatization

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Abstract

d-Lactic and l-lactic acids were simultaneously determined by means of a column-switching high-performance liquid chromatography (HPLC) with fluorescence detection. As a fluorescence reagent, 4-nitro-7-piperazino-2,1,3-benzoxadiazole (NBD-PZ) was employed for the fluorescence derivatization of lactic acid. The proposed HPLC system adopted both octylsilica (Cadenza CD-C8) and amylose-based chiral columns (CHIRALPAK AD-RH), which proved to give a sufficient enantiomeric separation of the lactic acid derivatives with a separation factor (alpha) of 1.32 and a resolution (R(s)) of 1.98. Moreover, the features of the first elution of d-lactic acid peak in the proposed HPLC were convenient for the determination of trace amount of serum d-lactic acid, which is known to increase under diabetes. Intra-day and inter-day accuracies were in the range of 90.5-101.2 and 89.0-100.7%, and the intra-day and inter-day precisions were 0.3-1.2 and 0.4-4.8%, respectively. The proposed method was applied to determine d-lactic and l-lactic acids in human serum of normal subjects and diabetic patients, showing that both d-lactic and l-lactic acid concentrations were significantly increased in the serum of diabetic patients (n=31) as compared with normal subjects (n=21). This fact was found for the first time owing to the development of the proposed HPLC method which is able to determine d-lactic and l-lactic acid simultaneously. Finally, serum d-lactic acid concentrations determined by the proposed HPLC method were compared with those from a reported enzymatic assay, and the smaller p value between normal subjects and diabetic patients was shown by the proposed HPLC method.