Simultaneous determination of D-lactic acid and 3-hydroxybutyric acid in rat plasma using a column-switching HPLC with fluorescent derivatization with 4-nitro-7-piperazino-2,1,3-benzoxadiazole (NBD-PZ)

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Abstract

A highly sensitive method for the determination of D-lactic acid and 3-hydroxybutyric acid (3-HB) in rat plasma was developed using high-performance liquid chromatography with octadecylsilica (ODS) connected to a chiral column. At first, (D + L)-lactic acid and 3-HB in the plasma were derivatized with a fluorescent reagent, 4-nitro-7-piperazino-2,1,3-benzoxadiazole (NBD-PZ), separated on the ODS column and determined fluorimetrically at 547 nm with 491 nm of excitation wavelength. During the separation step on the ODS, the peak fraction of (D + L)-lactate derivative was introduced directly to a phenylcarbamoylated beta-cyclodextrin chiral column by changing the flow of the eluent via a six-port valve. Then, D-lactate derivative was separated enantiomerically from the L-lactate derivative, and the enantiomeric ratio was determined from the chromatogram. Intra- and inter-day accuracy values for the determination of D-lactic acid in 10 microL of rat plasma were 97.8-109.2 and 98.4-109.9%, and those for 3-HB were 99.8-108.4 and 99.8-103.8%, respectively. The intra- and inter-day precision values were within 4.6 and 5.1% for D-lactic acid, and 2.7 and 2.4% for 3-HB, respectively. The detection limits for D-lactic acid and 3-HB were approximately 2.0 and 0.04 microM, respectively (signal-to-noise ratio 3). The proposed method was applied to the plasma of diabetic rats induced by intraperitoneal administration of streptozotocin, and the significant increases of both D-lactic acid and 3-HB concentrations were observed in the diabetic rats as compared to the normal rats.