Enantiomeric determination of D-, L-lactate in diabetic rat urine using a column-switching HPLC

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Abstract

A highly sensitive method for the determination of D-lactate in rat urine was developed by using a high-performance liquid chromatography (HPLC) with an octadecylsilica (ODS) connected to a chiral column. At first, (D+L)-lactate in the urine were derivatized with a fluorescent reagent, 4-nitro-7-piperazino-2,1,3-benzoxadiazole (NBD-PZ), and 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 an amylose tris (3,5-dimethylphenylcarbamate) (Chiralpak AD-RH) chiral column by changing the flow of the eluent via 6-port valve. Then, D-lactate derivative was separated enantiomerically from L-lactate derivative, and the enantiomeric ratio was determined from the chromatogram. The accuracy values for the determination of D-lactate in 20 μL of rat urine were 96.93% -104.85%. The intra- and inter-day precision values were within 0.80% and 14.44%. The proposed method was applied to the urine of diabetic rats induced by intraperitoneal administration of streptozotocin, and the significant increases of D-lactate was observed in the diabetic rats as compared to the normal rats.