Fluorimetric determination of d-lactate in urine of normal and diabeticrats by column-switching high-performance liquid chromatography

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Abstract

A highly sensitive method for the fluorimetric determination of D-lactate in urine of normal and diabetic rats was developed using column-switching high-performance liquid chromatography (HPLC) with an octadecylsilica (ODS) column connected to a chiral column, an amylose tris(3,5-dimethylphenylcarbamate) coated on silica gel (Chiralpak AD-RH). During the separation step on the ODS column, the peak fraction of the (D+L)-lactate derivative with a fluorescence reagent, 4-nitro-7-piperazino-2,1,3-benzoxadiazole (NBD-PZ), was introduced directly to the chiral column by changing the flow of the eluent via a six-port valve. The D-lactate derivative was separated enantiomerically from the 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%, and the intra- and inter-day precision values were within 0.80 and 14.44%, respectively. The detection limit for D-lactate was approximately 10 nM (with a signal-to-noise ratio of 3). The proposed HPLC method was applied to the urine of normal and diabetic rats induced by intraperitoneal administration of streptozotocin, and significant increases in D-lactate excreted into the urine were observed in diabetic rats compared to normal rats. In diabetic rats, D-lactate concentrations showed a rising tendency from the seventh day and then remained stable from the 28th day after induction, suggesting that urinary D-lactate may be used as an indicator to determine the diabetic stage and the level of kidney damage.