Development of a three-dimensional HPLC system with fluorescence detection for the simultaneous determination of lactate and 3-hydroxybutyrate enantiomers in human clinical samples

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Introduction

Lactate (LA) and 3-hydroxybutyrate (3HB), typical chiral hydroxy acids in living beings, are considered to have relationships with diseases such as metabolic disorders. Because both of them are chiral compounds, their enantioselective analysis in our bodies is the matter of interest since they might become the new drug candidates or clinical biomarkers. In order to determine the trace amounts of LA and 3HB enantiomers in various biological matrices containing uncountable intrinsic interfering compounds, we have developed a two-dimensional HPLC system following the fluorescence labeling of the carboxylic acid with 4-nitro-7-piperazino-2,1,3-benzoxadiazole (NBD-PZ). Although the 2D-HPLC is one of the most suitable methods for the trace analysis of chiral compounds in complicated matrices[1], the selectivity is still insufficient in some cases. Therefore, in the present study, an online 3D-HPLC system with fluorescence detection has been designed combining reversed-phase, mixed-mode and enantioselective separations and applied to human physiological fluids.

Materials and Methods

To the plasma or urine (10 μL), 5 μL of water and 85 μL of acetonitrile (MeCN) were added. The mixture was centrifuged at 3,300 rpm at 4˚C for 10 min, and the supernatant was collected. To 10 μL of the supernatant, 20 μL of an MeCN solution containing 5 mM NBD-PZ, 50 mM 2,2'-dipyridyl disulfide, and 50 mM triphenylphosphine was added. The mixture was stored at 25˚C for 60 min, and 220 μL of 0.1% trifluoroacetic acid (TFA) aqueous solution was added. An aliquot (100 μL) of the reaction mixture was subjected into the 3D-HPLC system and the elution of NBD-LA and 3HB in all 3 dimensions was monitored by fluorescence detectors (emission at 530 nm with excitation at 470 nm).

Results and Discussion

In the first dimension, a reversed-phase column (KSAARP, 1.0 mm i.d. x 250 mm) was used to separate the NBD-derivatives of LA and 3HB. For the mobile phase, various concentrations of MeCN (10 to 20% in water) at different temperatures were investigated. As a result, NBD-LA and NBD-3HB were separated well using an aqueous solution containing 15% MeCN and 0.05% TFA at 40˚C. The peaks of NBD-LA and NBD-3HB enantiomers were fractionated respectively as their scalemic D plus L mixtures and introduced into the next (second) dimension. In the second dimension, NBD-LA or NBD-3HB were isolated again from other interfering compounds by a mixed-mode column (KSAAMX-001, an originally designed column by the collaboration with Shiseido having 3,5-dinitrophenylaminocarbonyl-Gly as a selector, 1.5 mm i.d. x 250 mm). The mobile phase conditions in this dimension were tested with the organic solvents including MeCN, methanol and ethanol. As a result, 100% ethanol was selected as a mobile phase for both NBD-LA and NBD-3HB. The peaks of their D+L mixtures were collected again and transferred to the final (third) dimension. In the third dimension, the enantiomers of NBD-LA and NBD-3HB were separated into the D-form and L-form by a polysaccharide type chiral column (Chiralpak AD-H, 2.0 mm i.d. x 250 mm). As the mobile phase, the mixture of MeCN, methanol and ethanol were investigated in detail and both NBD-LA and NBD-3HB enantiomers were completely separated by 100% ethanol with resolution values higher than 2.01. The present 3D-HPLC system was applied to human plasma and urine and trace levels of LA and 3HB enantiomers were successfully determined.

Conclusion

The 3D-HPLC system is suitable to evaluate the amounts/alternations of LA and 3HB enantiomers in real world matrices, and various clinical applications including metabolic disorders, cardiovascular diseases are ongoing.

Bibliography

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