Development of a three-dimensional HPLC system for the simultaneous determination of lactate and 3-hydroxybutyrate enantiomers and the applications to various food and clinical samples

謝, 金玲

氏　　名　：謝　金玲

論文題名　：**Development of a three-dimensional HPLC system for the simultaneous determination of lactate and 3-hydroxybutyrate enantiomers and the applications to various food and clinical samples**（乳酸および 3-ヒドロキシ酪酸鏡像異性体を対象とした三次元 HPLC 法開発と食品・臨床サンプルへの適用）

区　　分　：甲

論　文　内　容　の　要　旨

Enantioselective studies of lactate (LA) and 3-hydroxybutyrate (3HB) have received attention because their presence in high animals and relationships to various diseases are gradually elucidated recently. In the past, due to the homochirality features, the trace levels of the minor enantiomers were thought as unnatural compounds and LA/3HB were determined without chiral discriminations. With the advances in analytical technologies, the minor forms of enantiomers have been found in mammals and shown to have physiological significance. Their diagnostic values as novel biomarkers are gathering high interest since different levels of enantiomers were observed along with the progress of diseases such as diabetic mellitus. To precisely determine chiral hydroxy acids, multi-dimensional high-performance liquid chromatography (HPLC) is considered as one of the most powerful methods and a three-dimensional HPLC (3D-HPLC) system was reported for the determination of LA and 3HB enantiomers. However, due to uncountable intrinsic substances in the biological matrices, specificity is still a matter of concern. Therefore, in the present study, a highly selective 3D-HPLC system has been developed/validated and applied to various food and clinical samples.

In Chapter 1, a 3D-HPLC system composing a reversed-phase (1D), a mixed-mode (2D) and an enantioselective (3D) column have been optimized with the use of human urine as real matrices. The standard solutions and samples containing LA and 3HB enantiomers were pre-column derivatized with 4-nitro-7-piperazino-2,1,3-benzoxadiazole and introduced to the 3D-HPLC system. To separate the unknown substances, various types of reversed-phase stationary phases and mobile phases were tested in the first and second dimensions. As results, the utilizations of tandemly connected Singularity RP18 columns (1.0 mm x 500 mm) in 1D and tandemly connected Singularity MX-005 columns (1.0 mm x 500 mm) in 2D with ethanol-containing solutions as mobile phases gave the best separations. In 3D, several polysaccharide-type columns were checked and LA/3HB enantiomers could be well separated using the tandemly connected Chiralpak IG columns (2.0 mm x 500 mm). The method was validated by the

calibration curves, precision and accuracy and sufficient values were obtained. Consequently, the present method was applied to human plasma and urine, and the trace levels of LA and 3HB enantiomers were clearly observed.

In Chapter 2, the presence of LA enantiomers in our daily life was checked by applying the 3D-HPLC system to various food and beverage samples. Because LA enantiomers are considered as possible biomarkers to diseases, the effect of dietary intake on the concentrations of LA enantiomers in human bodied are the matter of interest. Various fermented products including lactic fermenting beverages, yogurts, cheeses, soy sauces and vinegars were analyzed. As a result, high portions of D-LA were found in the vinegars and further determinations in various vinegars were performed. In addition, the developmental changes of a traditional amber rice vinegar were performed. Moreover, healthy volunteers were recruited to ingest the vinegars, and the effect of dietary intake for 1 h and 24 h were investigated. As a result, although LA enantiomers in the human plasma increased after ingestion, they returned to the basal concentrations for a while and the utilization of LA enantiomers as the biomarkers are considered possible.

In Chapter 3, the relationships between LA/3HB enantiomers and chronic kidney disease (CKD) were studied. Because LA and 3HB are reported to be related with diabetic mellitus and kidney damage, their concentrations in non-CKD and CKD subjects are worthy investigating. Concerning CKD, it is almost no symptom in its early stage, and it leads to end-stage kidney disease, cardiovascular disease and death along with the progression of disease. Therefore, the prevention and early detection of CKD is necessary. In this Chapter, non-CKD and CKD subjects were recruited and the concentrations of LA and 3HB enantiomers in the plasma were determined. As a result, the significant differences were found in the total LA and D-LA levels between non-CKD and CKD subjects, while there was no significant difference of 3HB enantiomers. When plotting the ROC curves, the AUC of a value calculated using D-LA and eGFR were higher than that of a single parameter such as eGFR, total LA and D-LA. It indicates that D-LA may be related with CKD and the determination of LA with chiral discrimination would be benefit to the diagnose of CKD.

In the present thesis, a highly selective 3D-HPLC system has been designed/developed for the simultaneous analysis of LA and 3HB enantiomers, and it is applicable to various matrices including food/beverages and clinical samples. The presence of the target hydroxy acids, the effect of dietary intake and their relationships to disease were revealed. These results are helpful to elucidate the biological meaning of LA and 3HB enantiomers in the health science. The further studies for the determination of LA and 3HB with chiral insight in the clinical applications are expected.