Enantioselective Determination of Ornithine and Lysine in Human Physiological Fluids Using a Highly Selective Two-dimensional HPLC System Based on Intramolecular Excimer-forming Fluorescence Derivatization

R. Koga[a], M. Mita[b], T. Ide[c], H. Yoshida[a], H. Nohta[a], K. Hamase[d]

[a] Faculty of Pharmaceutical Sciences, Fukuoka University, 8-19-1 Nanakuma, Jonan-ku, 814-0180 Fukuoka/JP

[b] KAGAMI, Inc., 7-7-15 Saito-asagi, Ibaraki, 567-0085 Osaka/JP

[c] Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, 812-8582 Fukuoka/JP

[d] Graduate School of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, 812-8582 Fukuoka/JP

Ornithine (Orn) and lysine (Lys) are basic amino acids with two amino groups in the molecule. While their physiological functions and relations to diseases have been reported, their enantiomer-discriminated distributions and *in vivo* behaviors in diseases have mostly not yet been clarified due to the lack of analytical methods. Since there are a wide variety interfering substances in biological samples, highly selective methods are essential for the precise analysis of trace amino acid enantiomers. In the present study, a two-dimensional HPLC (2D-HPLC) system was developed based on the intramolecular excimer-forming fluorescence derivatization, which is useful for highly selective detection of compounds having multiple identical functional groups, and applied to human physiological fluids.

Target amino acids were derivatized with 4-(1-pyrene)butyric acid *N*-hydroxysuccinimide ester (PSE). To the CH_3OH supernatant of urine or plasma samples, 50 mM triethylamine and 100 mM PSE in DMSO were added and heated at 40ºC for 30 min. After cooling on ice, the reaction mixture was diluted with the mobile phase and an aliquot was subjected to the 2D-HPLC system combining a reversed-phase column and an enantioselective column. Dipyrene-labeled analytes were detected by their excimer fluorescence at 475 nm with excitation at 345 nm.

Dipyrene-labeled Orn and Lys were separated by a microbore-ODS column (Singularity RP18, 1.0 x 250 mm) in the 1st dimension and automatically fractionated into loops according to the retention times. The fractions were severally transferred to the enantioselective column (Singularity CSP-105S, 1.5 x 250 mm) in the 2nd dimension and the enantiomers were well separated (Rs>1.35). The developed method showed good performance in terms of linearity (R^2>0.999), precision (RSD<6.6%) and accuracy (98.7 to 114.2%). In the urine samples of healthy human volunteers, all of the 4 target enantiomers were clearly observed and the %D values of Orn and Lys were 12.0% and 4.3%, respectively. On the other hand, L-Orn, L-Lys and a trace amount of D-Lys was found in the human plasma. Highly selective determination of Orn and Lys enantiomers in biological samples was achieved using the present system, and further studies are in progress.