

[PD4-14] [ 04/21/2000 (Fri) 14:50 - 15:50 / [1st Fl, Bldg 3] ]

**Configurational Analysis of Organic Acids and Amino Acids in Beakgangjam and Dongchunghacho by Achiral Gas Chromatography**

Kim KR, \*Lee J, Ha D, \*Won S<sup>o</sup>, \*Park J, \*Kim JH

College of Pharmacy, Sungkyunkwan University, \*Dept. of Biotechnology & Bioproducts Research Center, Yonsei University

Recently, particular attention has been paid to the absolute configurations of naturally occurring optically active organic acids and amino acids because of their different biological activities. In this study, we determined absolute configuration of organic acids and amino acids present in Beakgangjam and Dongchunghacho as diastereomeric derivatives by achiral gas chromatography (GC). After their extraction, amino acids were converted into diastereomeric N(O,S)-ethoxycarbonylated (S)-(+)-3-methyl-2-butyl esters and the organic acids into diastereomeric O-trifluoroacetylated (S)-(+)-3-methyl-2-butyl esters, followed by GC analysis on achiral DB-5 and DB-17 dual-capillary columns. The absolute configurations of all amino acids and organic acids from Beakgangjam and Dongchunghacho were positively determined to be in their 100% L-form.

[PD4-15] [ 04/21/2000 (Fri) 14:50 - 15:50 / [1st Fl, Bldg 3] ]

**Determination of the Absolute Configurations of Urinary Chiral Acids from Patients Suffering from Short & Long Chain 3-Hydroxy Acyl-CoA Dehydrogenase Deficiency by Achiral Gas Chromatography**

Kim KR, \*Lee J, Ha D<sup>o</sup>, \*Won S, \*\*Yoon HR, \*Kim JH

College of Pharmacy, Sungkyunkwan University, \*Dept. of Biotechnology & Bioproducts Research Center, Yonsei University, \*\*Seoul Medical Science Institute, Seoul Clinical Laboratories

Chiral acids occurring in metabolic pathways are known as important biochemical indicators of specific enzyme deficiencies in inborn errors of metabolism and their accurate chiral determination is thus of utmost importance for the correct diagnosis. After extraction from urine samples of patients suffering from short chain 3-hydroxy acyl-CoA dehydrogenase (SCHAD) & long chain 3-hydroxy acyl-CoA dehydrogenase (LCHAD) deficiency, chiral acids such as lactic, 2-hydroxybutyric and 3-hydroxybutyric acids were converted to diastereomeric O-trifluoroacetylated (1S, 2R, 5S)-(-)-menthyl esters for the direct GC analysis on achiral dual-capillary column system. In cases of SCHAD & LCHAD, the absolute configurations of lactic and 2-hydroxybutyric acids were positively determined to be in their S-form, while 3-hydroxybutyric acid was in R configuration.

[PD4-16] [ 04/21/2000 (Fri) 14:50 - 15:50 / [1st Fl, Bldg 3] ]

**Collaborative study for the establishment of somatropin reference standard of KFDA**

Sohn YW, Shin W, Joung JW<sup>o</sup>, Jin JH, Oh HJ, Choi YJ, Oh IU and Bristow AF \*

Division of biotechnology, KFDA, Korea, \* Department of Endocrinology, NIBSC, UK

The complexity and variability of both the biologicals and the bioassays used to test them lead to the use of reference standard. It is very important for the national regulatory authority to establish

national reference standard with a defined specific activity for use in biological and physico-chemical assays to enable the harmonized quality control of biological products. The candidate reference standard of KFDA for somatotropin 98/674 was evaluated to determine the suitability of serving as the standard for somatotropin by the collaborative study, in which 10 laboratories were participated. Physico-chemical analysis and in vivo bioassay were performed by direct comparison with the international somatotropin standard 88/624. Data of identification by SDS-PAGE, IEF, peptide mapping, and HPLC indicated KS 98/674 was almost identical to IS 88/624. Determination of somatotropin content by SE-HPLC yielded a mean estimate of 2.01 µg somatotropin per ampoule. Data from the study also yielded mean values of 0.39±0.26 % for high molecular weight impurities by SE-HPLC and mean values of 2.13±1.29 % for somatotropin related proteins by RP-HPLC. Estimates of relative potency IU by weight gain bioassay in the hypophysectomised rats showed one IU of IS 88/624 was equivalent to one IU of Korean Standard 98/674. Based on the results of the study, the candidate standard 98/674 is suitable to serve as a somatotropin reference standard of KFDA.

[PD4-17] [ 04/21/2000 (Fri) 14:50 - 15:50 / [1st Fl, Bldg 3] ]

### Determination of Hair Polyamines as N,N-Ethylloxycarbonyl-Pentafluoropropionyl Derivatives by Gas Chromatography-Mass Spectrometry

^\*Choi MH<sup>o</sup>, \*Kim KR, ^Chung BC

^Bioanalysis & Biotransformation Research Center / Korea Institute of Science & Technology,  
\*College of Pharmacy / Sungkyunkwan University

A noble method is described for the simultaneous determination of hair polyamines such as 1,3-diaminopropane, putrescine, cadaverine, spermidine, and spermine by gas chromatography-mass spectrometry (GC-MS). The method is based on the extractive two-phase ethylloxycarbonylation of amino functions in aqueous solutions combined with further pentafluoropropionylation of the remaining active protons for the direct analysis by GC-MS with the selected ion-monitoring (SIM) mode. This method showed a good overall accuracy (% bias) and precision (% CV) as 3.32~11.05 and 5.88~14.71, respectively. When applied to hydrolysates of human head hair samples from 11 male and 19 female normal subjects, all 5 polyamines were positively detected at the concentrations of 8.82~871.87 ng/g. The detection limits for SIM of the polyamines ranged from 0.02 to 0.2 ng, while their recovery rates varied in the range of 76.42~93.38%. The levels of polyamines except for cadaverine in hair specimens studied were found to be higher in men than in women.

[PD4-18] [ 04/21/2000 (Fri) 14:50 - 15:50 / [1st Fl, Bldg 3] ]

### Determination of the Absolute Configurations of Urinary Chiral Acids from Patients Suffering from Ornithine Transfer Carbamylase Deficiency, β-Ketothiolase Deficiency & Lactic acidosis by GC

Kim KR, \*Lee J, Ha D<sup>o</sup>, \*Won S, \*\*Yoon HR, \*Kim JH

College of Pharmacy, Sungkyunkwan University, \*Dept. of Biotechnology & Bioproducts Research Center, Yonsei University, \*\*Seoul Medical Science Institute, Seoul Clinical Laboratories

Chiral acids occurring in metabolic pathways are known as important biochemical indicators of specific enzyme deficiencies in inborn errors of metabolism and their accurate chiral determination is thus of utmost importance for the correct diagnosis. After extraction from urine samples of patients suffering from Ornithine Transfer Carbamylase (OTC) Deficiency, β-Ketothiolase Deficiency & Lactic Acidosis, chiral acids such as lactic, 2-hydroxybutyric and 3-hydroxybutyric acids were converted to diastereomeric O-trifluoroacetylated (1S, 2R, 5S)-(-)-menthyl esters and p-hydroxyphenyllactic acid was converted to diastereomeric O-trifluoroacetylated (S)-(+)-3-methyl-2-butyl ester for the direct GC analysis on achiral dual-capillary column system. In all cases of OTC, β-Ketothiolase and Lactic Acidosis, the absolute configurations of lactic, 2-hydroxybutyric and p-hydroxyphenyllactic acids were positively determined to be in their S-form, while 3-