The mass spectrometric study for the elucidation of cyclofenil's metabolites in human urine was performed by the electrospray ionization (ESI)-tandem mass spectrometry (MS/MS), electron impact (EI) and chemical ionization (CI)-mass spectrometry (MS). Cyclofenil, 4,4'-(cyclohexylidenemethylene) bis(phenyl acetate), is used to stimulate gonadotrophin release by inhibiting the negative feedback effects of endogenous oestrogen. It works as an antiestrogen, by binding with the receptors and preventing gynecomastia. Athletes also use this one, as it has effects similar to both clomiphene and hCG. The cyclofenil and metabolites were extracted from urine and characterized by the high performance liquid chromatography (HPLC) with ESI-tandem mass spectrometry. The metabolites were found by comparing chromatograms of the control urine and the dosed urine. The structures of the metabolites were identified by the MS/MS which can take place secondary fragmentation. And also the metabolites were identified using the gas chromatography /mass spectrometry (GC/MS). The extracted metabolites after hydrolysis from urine were derivatized and separated by the GC and subsequently identified by both of the EI and CI-MS. A di-deacetylated metabolite (m.w. 280) was a major metabolite and the other was a di-deacetylated-hydroxylated form (m.w. 296) of the cyclofenil. The parent was not detected from the urine and the metabolites were identified with the glucuronide metabolites (m.w. 456 and 472) by the HPLC/MS/MS. The identified metabolites can be used to confirm the fact of the cyclofenil's dose in the drug analysis.

[PD4-13] [04/19/2001 (Thr) 13:30 - 14:40 / Hall 4]

Quantitative determination of clarithromycin in human plasma by high-performance liquid chrpmatography using C18 reverse column with flourescence detection chrpmatography

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A validated, highly sensitive and precise high-performance liquid chromatographic method for the determination of the clarithromycin in human plasma is described. A diethyl ether extract, obtained from plasma using a saturated sodium carbonate solution, was treated with 9-fluorenylmethyl-oxycarbonyl chloride FMOC-CL for 40 min at 40℃ and chromatographed capcell Pak C18 MG maintained at 50℃ during elution, using an eluent composed of acetonitrile-hydrogen phosphate buffer, pH 5.0, with 0.05 v/v% triethylamine. Flourescence detection was used at an excitation wavelength of 255nm and an emission wavelength of 315 nm. These results suggest that pre-treated clarithromycin was well separated with internal standard erythromycin on chromatogram file and the lower limit of quantitation was 0.1 ㎍/ml for clarithromycin.

[PD4-14] [04/19/2001 (Thr) 13:30 - 14:40 / Hall 4]

Analysis of Phenylalanine and Tyrosine by Tandem Mass Spectrometry for PKU Screening

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Phenylketonuria (PKU) is an autosomal recessive genetic disorder caused by a deficiency of hepatic phenylalanine hydroxylase (PAH) activity. As a result, phenylalanine is not converted to the amino acid tyrosine. This causes an excessive amount of phenylalanine and toxic metabolites to accumulate in the body, including the brain, blood and urine. Recently, an important development for PKU screening has been the measurement of analytes by tandem mass spectrometry. Seoul Medical Science Institute has begun development of a PKU screening system using this new technology. Analysis for PKU by

tandem mass spectrometry reduces sample preparation time (non-derivatized procedure) and specificity is enhanced by using ratios of phenylalanine (Phe) to tyrosine (Tyr) concentrations. The specificity and sensitivity of the procedure is provided largely by the selectivity of the Multiple Reaction Monitoring (MRM) scan functions performed by the mass spectrometer. The method is very sensitive, with detection limits well below the normal concentrations of the amino acids found in blood.

[PD4-15] [04/19/2001 (Thr) 13:30 - 14:40 / Hall 4]

Determination of Recombinant Human Epidermal Growth Factor (rhEGF) in Pharmaceutical Formulation by High Performance Liquid Chromatography with Electrochemical Detection

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An improved determination method of recombinant human epidermal growth factor (rhEGF) using high performance liquid chromatography with electrochemical detection (ECD) has been developed. The factors affecting the response of rhEGF, have been studied the pH and kinds of buffer, polarisation voltage of the working electrode, cell temperature, and flow rate of eluent.
rhEGF was separated from other components in its formulation on a reversed-phase C18 column with 24 % acetonitrile in 100 mM phosphate buffer (pH 4.6) at a flow rate of 1.0 mL/min. Electrochemical oxidation of rhEGF at a glassy carbon electrode occurred at 0.90 V relative to the Ag/AgCl reference electrode. The limit of quantitation was approximately 0.5 ng and the calibration curve was linear in the concentration range between 1.0-20 ng of rhEGF (the correlation coefficient was 0.995). The method was successfully applied for the determination of rhEGF in a pharmaceutical formulation.

[PD4-16] [04/19/2001 (Thr) 13:30 - 14:40 / Hall 4]

Analysis of Acylcarnitines by Tandem Mass Spectrometry for Newborn Screening

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The acylcarnitines are a homologous group of metabolites derived from mitochondrial acyl-coenzyme A (CoA) intermediates of fatty acid and branched chain amino acid catabolism by the action of a family of enzymes, the carnitine acyltransferases. The diagnostic importance of acylcarnitines was not realized until recently, owing to the lack of specific method for their analysis. In this reason, Seoul Medical Science Institute has begun development of Acylcarnitines screening system using this new technology. In the triple quadruple, collision-induced dissociation (CID) of acylcarnitine buthyl ester M+ ions produces a common fragment of m/z 85, usually the base peak in the product ion spectrum. By scanning the first quadruple (Q1) over the mass range m/z 200-550 while Q3 transmits only ions of m/z 85, acylcarnitines are detected simultaneously and with high sensitivity.

[PD4-17] [04/19/2001 (Thr) 13:30 - 14:40 / Hall 4]

Improved Gas Chromatography-Mass Spectrometry Detection of Five Corticosteroids in Serum and Urine

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