European Pharmacopoeia (EP) and the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis based on the reliability, convenience and simplicity of the chromogenic assay. A correlation study was carried out with a one-stage factor VIII:C clotting assay and the performance of the chromogenic assay was evaluated using two test kits that fulfilled the requirements of EP for factor VIII concentrates test. Although chromogenic assay has partly differences in measurement principle and standardization, this assay has a high correlation with clotting assay in various types of factor Ⅷ concentrates and factor Ⅷ standard. We conclude that the chromogenic assay for factor VIII:C concentrates correlates well with the clotting assay and shows good analytical performance.

[PD4-17] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

Capillary Electrophoretic Analysis of PEGylated Interferon Alpha

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Capillary electrophoretic method for characterization of PEGylated interferon alpha (IFN) was developed. IFN was modified by the reaction of amine residues with an active ester of monomethoxy polyethylene glycol at various molar ratios. As a CE method, capillary electrophoresis sodium dodecyl sulfate nongel sieving (CE-SDS-NGS) was performed using an uncoated capillary filled with a hydrophilic replaceable polymer network matrix. The results were compared to those obtained using SDS-PAGE with barium iodide staining and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). CE-SDS-NGS showed good resolution between each PEGylated IFN species as well as the native IFN. The total amount and distribution of PEGylated IFN species were directly measured and the relative standard deviation (RSD) was around 1-3%. The distribution profile of PEGylation determined by CE-SDS-NGS was found to be consistent with that obtained by SDS-PAGE. CE-SDS-NGS provides a novel approach for the analysis of PEGylated proteins and shows the advantages of speed, high resolution, automation, and quantitation over SDS-PAGE.

[PD4-18] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

Diagnosis of Organic Acidurias by GC-MS combined with Solid-Phase Extraction and Methoxime-tert.-Butyldimethylsilylation

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Organic acidurias are inherited metabolic disorders generally caused by the diminished activity or absence of specific enzymes involved in the metabolic pathway. Solid-phase extraction of urinary organic acids using Chromosorb P was performed after methoximation of keto acids in alkalinized urine samples, followed by conversion to stable tert.-butyldimethylsilyl (TBDMS) derivatives for the profiling analysis by gas chromatography-mass spectrometry. Each organic acid was identified through home-built TBDMS library matching. The diagnostic usefulness of the present organic acid profiling analysis was demonstrated by comparing urinary profile of normal subject to those of patients with methyl malonicaciduria, isovaleric aciduria and propionic aciduria.

[PD4-19] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

Validation of Ethoxycarbonylation combined with tert.-Butyldimethylsilylation for the

Simultaneous Gas Chromatographic Analysis of Amino Acids and Organic Acids

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N(O,S)-ethoxycarbonylation combined with tert.-butyldimethyl- silylation was optimized and validated for the simultaneous gas chromatographic (GC) analysis of amino acids and organic acids. Ethoxycarbonlyation of amino, phenolic and sufhydry groups with ethyl chlorofomate in aqueous solution was followed by tert.-butyldimethylsilylation of carboxyl and remaining polar groups for the direct GC analysis after solvent extraction. The present method was found to be potentially useful for the biochemical diagnosis of inherited metabolic disorders.

[PD4-20] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

Simultaneous Quantitative Analysis of Sphingoid Base 1-Phosphates in Biological Samples by o-Phthalaldehyde Precolumn Derivatization after Dephosphorylation with Alkaline Phosphatase

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This paper describes a simultaneous analytical method for the measurement of sphingoid base 1-phosphates and sphingoid bases from a variety of biological samples. This method consists of two steps of sample pretreatment: the enzymatic dephosphorylation of sphingoid base 1-phosphates by alkaline phosphatase and the subsequent analysis of OPA derivatives of the liberated sphingoid bases by HPLC. By introducing C_{17}-sphingosine 1-phosphate and C_{17}-sphingosine as internal standards, not only phytosphingosine 1-phosphate, sphingosine 1-phosphate, and sphinganine 1-phosphate but also phytosphingosine, sphingosine, and sphinganine present in a sample could be quantified in 12 min on a C_{18} reversed-phase column with a simple mobile phase of acetonitrile : water (90 : 10, v/v). With this HPLC method, we could reproducibly analyze the levels of sphingoid base 1-phosphates over a broad range of concentrations from 0.5 to 100.0 pmol from various biological samples including serum, cultured cells and rat tissue homogenates. The conversion of sphingoid base 1-phosphates into sphingoid bases increased the stability of the OPA adducts. Thus, this indirect measurement of sphingoid base 1-phosphates increased the sensitivity and reproducibility of the method. This HPLC method was also used to measure the changes in the levels of sphingoid base 1-phosphates in cultured cells
after treatment with 1,25-$(OH)_2D_3$, a sphingosine kinase activator, or with fumonisin B_1, a sphinganine N-acyltranferase inhibitor.

[PD4-21] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

Diagnostic Patterns for Capillary Electrophoretic Urinary Nucleoside Profiles from Patients with Liver Diseases

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An efficient capillary electrophoretic profiling method in micellar electrokinetic capillary chromatography (MEKC) mode was combined with simple pattern recognition methods for the correlation between urinary