Eupatilin is a major active component of Stillen?(Artemisia Herba Extract) having a potent antiagastotic effect. We investigated the physical properties of eupatilin using high performance liquid chromatography. Solubility, stability & partition coefficient of eupatilin were investigated. pH-stability of eupatilin was examined over the broad range through pH 1-9 at 37°C & it has good stability above the broad range pH. The solubility of eupatilin was extremely low but the value of logP was more than 2. Also, a high performance liquid chromatographic method was developed for the determination of eupatilin in rat plasma. The method involved deproteinization of biological sample with the same volume of acetonitrile, 0.2M zinc sulphate, and 0.15M barium hydroxide. The mobile phase employed was ammonium acetate buffer(1% ammonium acetate and 0.5% acetic acid) – acetonitrile (58.42, v/v) and the flow rate was 1.0 ml/min. The quantitation limit of eupatilin in rat plasma was 10 ng/ml. No interferences from endogenous substances were found.

[Cycloxygenase Inhibitory Activity of Ginsenosides from Panax ginseng](#) 
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P. ginseng C.A. Meyer is one of the most widely used herbal medicine in Asia. It has been used for the treatment of many disorders. Its major constituent is known to be ginsenosides, and there are many documents about bioactivities of ginsenosides such as anti-oxidant, anti-tumorogenic, anti-fatigue, and anti-inflammatory activities. Some of these activities are supposed to have some correlation with inhibitory action of cyclooxygenase (COX). Ginsenosides from P. ginseng and sapogenins were evaluated for their inhibitory effects against both cyclooxygenase-1 and -2 (COX-1 and -2). Inhibitory activity was evaluated by measuring prostaglandin E2 (PGE2) production from arachidonic acid with an ELISA reader. As a results, Rg5(S), and Rg3 and Rk1 showed COX-2 inhibitory activity in a selective manner (COX-1: IC50 = >100, 77.01 μg/mL, COX-2: IC50 = 35.47, 18.6 μg/mL). Protopanaxatriol (PPT) showed moderate activity on COX-1 and -2 (COX-1: IC50 = 39.16, COX-2: IC50 = 35.56 μg/mL), while Re, Rg3 (R), and protopanaxadiol (PPD) showed little activity.

[HPLC Analysis of Phytosphingosine and Its Metabolites in Mammalian Cells with TCPO- H2O2 Chemiluminescence Reaction](#) 
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Sphingolipids has been known to induce apoptosis, cell proliferation, differentiation and migration in a variety of cell types. Recently, its phosphate form was suggested that they may act both as an agonist ligand to SKIPs and a second messenger in intracellular action. Phytosphingosine (PHS) is not easily detected due to trace component of cellular lipids in mammalian and human tissues while this is a major sphingolipid in yeast and plants. We therefore developed highly sensitive and reproducible analytical method for PHS and its phosphate by oxalic acid bis(2,4,6-tri-chloropheny) ester(TCPO)-hydrogen peroxide(H2O2) chemiluminescence. The NDA derivatives of PHS exhibited stable fluorobenes and was enhanced their detectability at low concentrations by post-column chemiluminescence detection with TCPO - H2O2. The dried lipid extracts or sphingoid base standards for the calibration curve were dissolved in 40 ul of ethanol. NDA derivatization was accomplished by adding the following stock solutions: 40 ul 0.05M NaHCO3 / 0.1M NaOH buffer(pH 10.5), 20 ul 13% (w/v) NaCN, and 20 ul 0.5%(w/v) NDA. The tube was tightly sealed with PTFE film and heated at 67°C in a water bath for 90 min, glycine was added to stop the derivatization reaction. we successfully measured the amount of PHS and PHS-1-P in LLC-PK1 cells. Collectively, this method can be thus used to detect and distinguish PHS and PHS-1-P with high sensitivity from other sphingolipids in mammalian cells.
High Performance Liquid Chromatographic Analysis of Isoflavones in KUNBO
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Phytoestrogen has been used as supplement in order to treat osteoporosis. The representative phytoestrogen, isoflavones, are daidzein, genistein and formononetin which were present highly in legumes. We have studied the quantitative analysis of isoflavones in KUNBO by HPLC. KUNBO, a mixed herbal extract including Astragalus Radix and Rhynchosia nubilalis Semen (Leguminosae), etc., is a nutraceutical candidate for type I osteoporosis. Column used in HPLC was LUNA 5u C18 (250 x 4.6 mm) (Phenomenex Co., Torrance, CA, U.S.A.). Mobile phase used in HPLC was 5mM NaH2PO4 (pH 4.60) : MeOH (1:1) (Flow rate : 1.30 ml/min). We are measured at 260nm (UV/VIS detector). The content of aglycone daidzein was 20.04 ±0.40 mg/kg, and total daidzein was 417.82 ±8.71 mg/kg. The content of aglycone genistein was 14.80 ±0.09 mg/kg and total genistein 148.39 ±1.85 mg/kg. The content of aglycone formononetin was 21.84 ±0.11 mg/kg and total formononetin was 143.86 ±6.01 mg/kg. The retention times were 12.30 min for daidzein, 19.73 min for genistein and 37.99 min for formononetin. Thus, we have established the QC standard for KUNBO as the results. (Supported partially by a grant, # 02-PJ1- PG11-VN04-SV04-0004, from Health Technology Planning & Evaluation Board, Korea)

[PD4-14] [ 2003-10-10  14:00 - 17:30 / Grand Ballroom Pre-function ]

Determination of a Novel Antiangiogenic Agent KR-31831 in Rat Plasma by Liquid Chromatography-Tandem Mass Spectrometry
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A liquid chromatography-tandem mass spectrometric (LC/MS/MS) method was developed for the determination of a new anti-angiogenic drug KR-31831 in rat plasma. KR-31831 and internal standard, KR-31543 were extracted from rat plasma with dichloromethane at basic pH. A reverse-phase LC separation was performed on Luna C8 column with the mixture of acetonitrile-ammonium formate (10 mM, pH 4.5) (67:33, v/v) as mobile phase. The detection of analytes was performed using an electrospray ionization tandem mass spectrometry in the multiple-reaction-monitoring mode. The standard curve was linear (r = 0.999) over the concentration range of 1.0-500 ng/ml. The coefficient of variation of intra- and inter-assay ranged from 0.8-3.9% and 1.4-3.9%, respectively. The recoveries of KR-31831 ranged from 80.9 to 86.7%, with that of KR-31543 (internal standard) being 99.2 ± 2.7 %. The lower limits of quantification for KR-31831 was 1.0 ng/ml using 100 ml plasma sample. This method was applied to the pharmacokinetic study of KR-31831 in rats.

[PD4-15] [ 2003-10-10  14:00 - 17:30 / Grand Ballroom Pre-function ]

Determination of Acetyl-L-carnitine in human plasma by LC-ESI/MS/MS
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Acetyl-L-carnitine, a physiological component of the L-carnitine family, has been proposed for treating Alzheimer’s disease in pharmacological doses. Acetyl-L-carnitine and d3-acetylcaritnine (internal standard) were analyzed by electrospray ionization / tandem mass spectrometry (ESI/MS/MS) after derivatization to their butylesters through treatment with butanolic hydrogen chloride. Acetyl-L-carnitine produced a protonated precursor ion at m/z 260 and a corresponding product ion of m/z 85. Analytes were separated on a Capcell Pak C18 (2.0X150mm, 5 mm). The mobile phase was 40% acetonitrile with flow rate at 200 mL/min. Detection of acetyl-L-carnitine in human was accurate and precise, with a limit of quantitation of 0.1μg/mL. The calibration curves for acetyl-L-carnitine was linear in a concentration range of 0.1 ~ 20 μg/mL. This method has been successfully applied to a study of acetyl-L-carnitine in human plasma.