Purification and Chracterization of fibrinolytic enzymes from Vegetable warms

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A thrombus is a mass formed from the constituents of the blood within the vessels or the heart during life. The process of formation is known as thrombosis. A vegetable warms producing fibrinolytic enzyme was isolated from chines traditional medicinal mushrooms. Cordyceps militairs and Paecilomyces tenuipes. The fibrinolytic enzyme of Cordyceps militaris and Paecilomyces tenuipes was purified from fruiting body by -70 prechilled ethanol precipitation, ion-exchange chromatography, gel filtration. The purified fibrinolytic enzyme isolated, showed a molecular mass of 52 kDa and 46 kDa on SDS-PAGE and fibrin zymography. Analysis of fibinolysis and fibrinogenolysis by SDS-PAGE have high substrate specificity. The hydrolysis rate of fibnogen subunit was A , B and chain in order.
The optimum pH and temperature for the enzyme activity were pH 8.0 and 42, respectively. The enzyme activity was highly inhibited by Cu2+, Co2+, and
PMSF, indicating that the enzyme is a serine protease. The purified fibrinolytic enzyme activity is about 1.5 folds higher than that 1.0 unit of plasmin. These might be developed as a therapuetic agent for the treatment of thrombic disease.

Poster Presentations - Field D4. Analytical Chemistry

[PD4-1] [10/18/2002 (Fri) 13:30 - 16:30 / Hall C]

Quantitative determination of pseudoephedrine in human plasma by reversed-phase liquid chromatography-electrospray ionization mass spectrometry

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A sensitive and selective reversed-phase LC-ESI-MS method to quantitate pseudoephedrine in human plasma was developed and validated. Phenacetin was used as an internal standard. Samples were prepared simply by acetonitrile precipitation without an evaporation step. Chromatographic separation was achieved on a XTerra MS C18 column (150 x 2.1 mm I.D., 3.5 μm particles), using gradient elution with 0.5% (v/v) trifluoroacetic acid (TFA) in water and 0.5% (v/v) TFA in methanol at a flow-rate of 0.1 ml/min. The detection utilized selected ion monitoring in the positive-mode at m/z 166.3 and 180.2 for the protonated molecular ions of pseudoephedrine and internal standard, respectively. The lower limit of quantitation of pseudoephedrine in human plasma was 10 ng/ml and good linearity was observed in the concentration range of 10-500 ng/ml. The reversed-phase LC-ESI-MS method was successfully applied for the quantitation of pseudoephedrine in human plasma from healthy volunteers dosed with pseudoephedrine hydrochloride tablets.

[PD4-2] [10/18/2002 (Fri) 13:30 - 16:30 / Hall C]

Concentrations of Dextromethorphan in Urine and Blood of Two Crime Suspects

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Dextromethorphan (Romilar, DEX) is a synthetic analogue of codeine, is not classified as a narcotic and is used only for its antitussive effects in Korea. The daily intake by adults range up to 120mg. Usually in the case of

traffic accident, alcohol concentration of blood is analyzed in Korea, but drug tests (medicine, narcotic, alcohol) are submitted in Australia. In crimes of violence (2 examples), a traffic and a murder accident, drug testing in urine and blood was performed. Alcohol, methamphetamine, heroin, cocaine, cannabis, barbiturate derivatives and benzodiazepine derivatives were not detected, but DEX and its metabolite dextrorphan were detected in urine or blood. Quantitication of DEX in urine and blood were quantitated by GC/TSD and GC/MS, respectively. First, in a murder-suspect (29-year-old, male) the quantitative contents of DEX were 8.9㎍/㎖ in urine, 0.6 ㎍/㎖ in blood. Second, in a heavy traffic accident (34-year-old, male), the quantitative contents of DEX were 38.1㎍/㎖ in urine, 2.1 ㎍/㎖ in blood. Therefore, drug testing of medicine and narcotic as well as alcohol have to been forced in crimes of violence, murder and traffic accidents.

[PD4-3] [10/18/2002 (Fri) 13:30 - 16:30 / Hall C]

Development of economic preparative method of (S)-(+)-enantiomer of arylpropionic acids

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Many of the chiral NSAIDs are marketed as racemates. There is an increasing interest in developing the enantiomerically pure forms of the NSAIDs because the anti-inflammatory activity of NSAIDs have previously been shown to be largely sterospecific for the (S)-(+)-enantiomer.Therefore, simple and economic preparative method to identify the (S)-(+)-enantiomer of NSAIDs (arylpropionic acids) as diastereomeric solvation complex was developed using several chiral solvating agents by recrystallization of racemate and solvent fractionation. Enantiomeric purity was determined by chiral HPLC system using Chiralcel OD-H and Chiralpak AD column and by 1H-NMR.

[PD4-4] [10/18/2002 (Fri) 13:30 - 16:30 / Hall C]

Simultaneous determination of corticosteroids in a herbal medicinal preparation by GC-MS

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The determination method for 11 corticosteroids (betamethasone, cortisol, cortisone, cortisone acetate, dexamethasone, cortisol acetate, isoflupredone acetate, methylprednisolone, prednisone, prednisolone, and triamcinolone acetonide) in a herbal medicinal preparation (Sibjeondaibotang) by a gas chromatography-mass spectrometric (GC-MS) method with selected ion monitoring (SIM) mode is described. Samples (4 mL) were extracted by liquid-liquid extraction with diethyl ether. The residues were then evaporated, purified, derivatized, and injected into the GC-MS system. This report exhibits recovery range (38.2 ~ 67.9 %), quantitation limits (0.1 ~ 1.2 μg/mL), and correlation coefficients (0.9685 ~ 0.9999) for corticosteroids, which estimated from validation data using cortisol-d_4 as the internal standard.

[PD4-5] [10/18/2002 (Fri) 13:30 - 16:30 / Hall C]

Assay Validation of Lansoprazole in Human Plasma

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A simple, rapid and reliable high performance liquid chromatography (HPLC) method has been developed for the