Simultaneous determination of talniflumate and its metabolite in human plasma by high-performance liquid chromatography

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Talniflumate is a potent analgesic and anti-inflammatory drug widely prescribed in rheumatoid diseases. The purpose of this work was to develop and validate a specific and robust method for the simultaneous determination of talniflumate and its metabolite, niflumic acid, in human plasma. Indomethacin was used as an internal standard (IS). To simultaneously determine the plasma concentration of talniflumate and niflumic acid, IS solution and methanol were added to plasma samples and the mixture were centrifuged at 3000 g for 30 min. Then, 60 μl of supernatant was injected onto the HPLC reversed-phase column (C18). The signals were monitored by UV detector at 288 nm. The run time was 20 min per sample and analyte was quantified by linear regression of peak area ratio. This assay was validated at a nominal range of 0.1 to 5 μg/ml. Linear over the calibration range was > 0.9972. The interday accuracy ranged from 92.3 to 99.09 % with precision ranging from 91.44 to 94.62 %. The intraday accuracy ranged from 90.65 to 99.29 % with precision ranging from 91.36 to 94.19 %. The retention times of the IS, niflumic acid and talniflumate were 6.5, 7.5 and 13.5 min, respetively. This analytical method was shown to be accurate and reproducible. This method could be suitable for the simultaneous determination of talniflumate and its metabolite in human plasma.

[PD4-25] [04/18/2003 (Fri) 13:30 - 16:30 / Hall P]

Comparison of liquid chromatographic enantiomer resolution of racemic amino compounds on chiral stationary phases of crown ether type

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ChiroSil RCA(+) and SCA(-) HPLC chiral stationary phases (CSPs) developed by covalently bonding (+)- and (-)-(18-crown-6)-2,3,11,12-tetracarboxylic acid (18-C-6-TA) to silica gel were employed for enantioresolution of racemic amino compounds, respectively. Also, these 18-C-6-TA covalently bonded CSPs were compared to a commercially available Crownpak CR CSP prepared by coating chiral crown ether as a chiral selector on ODS column. It was shown that these ChiroSil RCA(+) and SCA(-) columns have the advantage of the reversal of elution order. For the resolution of diphenylalanine enantiomers, especially, it was observed that the chromatographic parameters such as separation factors and retention times are greatly influenced by mobile phase conditions.

[PD4-26] [04/18/2003 (Fri) 13:30 - 16:30 / Hall P]

Identification of Impurities in a Sample of Illicitly Synthesized Methamphetamine

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Analysis of illicit methamphetamine samples seized in Korea is discussed. The samples are extracted with the small portion of ethyl acetate under neutral conditions and the extracts are analyzed by GC-MS. Several impuritiy peaks are found in each chromatrogram. Eight

compounds (1,2-Dimethyl-3-phenylaziridine, amphetamine oxime, ephedrine, N-formylmethamphetamine, N-acetylmethamphetamine, acetylephedrine, 3,4-dimethyl-5-phenyl-2-oxazolidone, methamphetamine dimer) are identified impurities in illicit methamphetamine and the identity of the impurity is conformed synthesis. Identification of other impurities found on the chromatrogram is under investigation. These impurities revealed that most of the seized methamphetamine in Korea was synthesized from ephedrine as a starting material.

[PD4-27] [04/18/2003 (Fri) 13:30 - 16:30 / Hall P]

MODULATION OF THE ACTIVITY OF PRO-INFLAMMATORY ENZYMES, COX-2 AND iNOS, BY CHRYSIN DERIVATIVES

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Chrysin, a natural flavone compound contained in plants, has anti-inflammatory activity. Its anti-inflammatory effect has been previously explained in part by the suppression of promoter activities of inducible pro-inflammatory enzymes (cyclooxygenase-2 (COX-2) and inducible nitrogen synthase (iNOS)). Nitrate production triggered by the activation of lipopolysaccharides (LPS) was most highly suppressed by the treatment of chrysin, followed by 5-hydroxy-7-methoxyflavone (Ch-2), 5,7-diacetylflavone (Ch-4) in cultured Raw 264.7 cells. Here we tested the inhibitory activity of chrysin derivatives on COX-2 and iNOS enzymes. Interestingly COX-2 enzyme was strongly inhibited by Ch-2 (IC50 = 2.7 mM) but not by other derivatives. Furthermore, the inhibition of COX enzyme by Ch-4 was selective on COX-2 isoenzyme over COX-1. The three-dimensional interaction models of Ch-4 complexing with COX-2 showed comparatively good fit into the topology of the binding pocket. There was a hydrogen bond between oxygen of ketone group of the 7-positon of Ch-4 and the hydroxyl group of Tyr355. Docked conformation of Ch-4 into V523I mutant of COX-2 indicated that Ile523 of COX-1 might contribute to the selectivity of COX-2/COX-1. Ch-4 showed no effect on iNOS activity. Chrysin and Ch-2 weakly inhibited iNOS enzyme activity in hemoglobin assay. However, the underlying mechanisms of the inhibition of iNOS by chrysin are not clear.

[PD4-28] [04/18/2003 (Fri) 13:30 - 16:30 / Hall P]

In vitro metabolism of a new protective agent, KR-31543 in human liver microsomes

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The purpose of this paper was to identify the metabolic pathway of a new neuroprotective agent, KR-31543 for ischemia-reperfusion damage in human liver microsomes and characterize cytochrome P450 (CYP) enzymes involved in the in vitro metabolism of KR-31543. KR-31543 generates two metabolites in human liver microsomes: M1, N-(4-chlorophenyl)-N-(2-methyl-2H-tetrazol-5-ylmethyl)amine and M2, hydroxy-KR-31543. From a combination of chemical inhibition, immunoinhibition, correlation analysis in human liver microsomes and metabolism by expressed recombinant CYP enzymes, KR-31543 is metabolized predominantly by CYP3A4 and is mainly converted to M1, N-(4-chlorophenyl)-N-(2-methyl-2H-tetrazol-5-ylmethyl)amine. KR-31543 was found to be a potent inhibitor of human CYP2D6 and 3A4 in human liver