B were not different and accepted by KP disintegration test criteria.
So, the disintegration test in soft capsules is applicable in present KP disintegration test.

Poster Presentations - Field E2. Pharmacokinetics

[PE2-1] [04/19/2001 (Thr) 15:30 - 16:30 / Hall 4]

HPLC, BLOOD PARTITION AND PROTEIN BINDING OF A NEW REVERSIBLE PROTON PUMP INHIBITOR, DBM-819

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A high-performance liquid chromatographic method was developed for the determination of a new proton pump inhibitor, DBM-819, in human plasma and urine, and rat tissue homogenates using KR-60461 as an internal standard. A 100-ml aliquot of acetonitrile (containing 0.5 mg/ml of the internal standard) and a 200-ml aliquot of 0.1 M Na2HPO4 (adjusted pH 11 with 1 N NaOH) were added to a 100-ml aliquot of biological sample. After vortex-mixing, the mixture was extracted with 1 ml of ethylacetate. After centrifugation at 12,000 g for 3 min, the organic layer was collected and evaporated under nitrogen gas. The residue was then reconstituted with a 100-ml aliquot of mobile phase, and a 40-ml aliquot was injected onto the HPLC column. The mobile phase, 0.02 M phosphate buffer (pH 5) : acetonitrile : methanol (46:44:10, v/v/v), was run at a flow rate of 0.5 ml/min and the column effluent was monitored by the fluorescence detector set at an excitation wavelength of 340 nm and an emission wavelength of 470 nm. The retention times for DBM-819 and the internal standard were approximately 10.5 and 12 min, respectively. The detection limits of DBM-819 in human plasma and urine, and rat tissue homogenates (including blood) were 0.01, 0.02 and 0.02 mg/ml, respectively. The coefficients of variation of the assay (within-day and between-day) were below 10.6% for human plasma and urine, and rat tissue homogenates. No interferences from endogenous substances were found.
The blood partition of DBM-819 between plasma and blood cells, and the factors influencing the binding of DBM-819 to 4% human serum albumin (HSA) were also evaluated. DBM-819 reached equilibrium rapidly between plasma and blood cells of rabbit blood. The equilibrium plasma/blood cells partition ratios were independent of initial rabbit blood concentrations of DBM-819, 0.5, 2, and 10 mg/ml; the values were in the range of 0.376 ?2.30. Binding of DBM-819 to 4% HSA was dependent on HSA concentrations, DBM-819 concentrations, incubation temperature, 멿he buffer?pHs, alpha-1-acid glycoprotein concentrations, and addition of acetylsalicylic acid. However, the binding of DBM-819 was independent of heparin concentration and buffers containing various concentrations of chloride ion.

[PE2-2] [04/19/2001 (Thr) 15:30 - 16:30 / Hall 4]

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS, BLOOD PARTITION AND PROTEIN BINDING OF OF A NEW NEUROPROTECTIVE AGENT FOR ISCHEMIA-REPERFUSION DAMAGE, KR-31378

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A neuroprotective agent for ischemia-reperfusion damage, KR-31378, in human plasma and urine and in rat tissue homogenates. The method involved deproteinization of the the biological samples with 0.5 volumes of saturated Ba(OH)2, 0.5 volumes of 0.04 M ZnSO4 and 1 volume of acetonitrile. A 80-ml aliqout of the supernatant was injected onto a reversed-phase C18 column. The mobile phase, 50 mM triethylamine acetate : acetonitrile : tetrahydrofuran (65:30:5, v/v/ A high-performance liquid chromatographic method was developed for the determination of a v), was run at a flow rate of 1.0 ml/min. The column effluent was mornitored by a ultraviolet detector set at 310 nm. The retention time of KR-31378 was approximately 6.5 min. The detection limits of KR-31378 in human plasma and urine and rat tissue homogenates were 0.2, 0.5 and 0.5 mg/ml, respectively. The coefficients of variation (within-day and between-day) were below 13.6% for human plasma and urine and rat homogenates. No interferences from endogenous substances were found.
The blood partition of KR-31378 between plasma and blood cells and the factors influencing the binding of the drug to 4% human serum albumin (HSA) using an equilibrium dialysis technique were evaluated. KR-31378 reached an equilibrium rapidly between plasma and blood cells of rabbit blood. The equilibrium plasma/blood cells concentration ratios were independent of initial rabbit blood concentrations of KR-31378, 1, 10 and 50 μg/ml; the values were in the range of 1.42-2.33. It took approximately 12-h jncubation to reach an equilibrium between plasma and isotonic Sfrensen phosphate buffer of Ph 7.4 containing 3% Dextran ('the buffer') compartments. The mean binding of KR-31378 to 4% HSA was dependent on HSA concentrations (the binding values were 25.3, 25.8 was 25.4% at KR-31378 concentrations ranging from 1 to 100μg/ml. Binding of KR-31378 and 32.0% for HSA concentrations of 2, 4 and 5%, respectively), incubation temperature (the binding values were 48.8, 29.0 and 25.8% for 4, 22, and 37°C, respectively), isotonic Sfrensen phosphate buffer of Ph 7.4 containing 3% Dextran ('the buffer') pHs (the binding values were 17.7, 20.6, 22.8, 25.6 and 29.5% for buffer pHs of 5.8, 6.4, 7.0, 7.4 and 8.0, respectively) and alpha-1-acid glycoprotein (AAG) concentrations (the binding value were 25.6, 29.9, 34.4 and 50.3% for AAG concentrations of 0, 0.08, 0.16 and 0.32%, respectively).

[PE2-3] [04/19/2001 (Thr) 15:30 - 16:30 / Hall 4]

Identification of the cytochrome P450 isoenzymes involved in the metabolism of KR-60436 in the human liver microsomes

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The metabolism of KR-60436, a new proton pump inhibitor, has been studied in incubations with human liver microsomes and purified bacterial recombinant cytochrome P450 (CYP) isoforms using liquid chromatography-mass spectrometry.
In the in vitro studies with rat and human liver microsomes, KR-60436 was extensively metabolized to produce at least seven metabolites, which exhibited different H+/K+ ATPase inhibitory activities. Results obtained by using chemical inhibitors or antibodies selectively active against specific CYPs and purified bacterial recombinant CYP isoforms provide a direct evidence for the involvement of CYP3A4, CYP2C9 and CYP2D6 in KR-60436 oxidative metabolism. KR-60436 inhibited CYP1A2-, CYP2E1- and CYP2C9-catalyzed activity, while CYP3A4-related activity was unaffected.

[PE2-4] [04/19/2001 (Thr) 15:30 - 16:30 / Hall 4]