DETERMINATION OF ZONISAMIDE AND ITS METABOLITE, 2-SULPHAMOYLACETYLPHENOL, IN HUMAN LIVER CYTOSOL AND MICROSOmes USING LC-MS/MS
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The reduction of zonisamide (ZNS) to 2-sulphamoylacetethylphenol (SMAP) was catalyzed by human liver microsomal cytochrome P450. Recent studies showed that aldehyde oxidase in monkey liver cytosol played a major role as a reductase toward ZNS. We examined the drug-reducing ability of human liver cytosol and microsomes. We developed a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the determination of ZNS and SMAP. The method involved a solid phase extraction (SPE) with Waters Oasis® HLB cartridges (30 mg). Chromatographic separation was performed with an ODS column compatible for hydrophilic compounds (Cadenza UK-C18, Intakt), using a mixture of methanol-ammonium acetate (10 mM). Determination was performed with a Micromass Quattro micro™ API triple-quadrupole mass spectrometer with electrospray ionization source in the multiple reaction monitoring (MRM) mode. The m/z transitions 213.0→132.0 (ZNS) and 216.0→199.2 (SMAP) were used for quantification. The in vitro metabolism of ZNS was characterized under aerobic and anaerobic conditions. ZNS was reduced to SMAP with human liver microsomes under anaerobic condition. Furthermore, ZNS was reduced to SMAP with human liver cytosol in anaerobic condition with 2-hydroxypyrimidine, an electron donor to aldehyde oxidase. Both of cytochrome P450 and aldehyde oxidase may be involved in the reductive metabolism of ZNS in human liver.

DETERMINATION OF VARDENAFIL IN SMALL VOLUMES OF RAT PLASMA AND BILE USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH FLUORESCENCE DETECTION
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A simple and sensitive HPLC method with fluorescence detection was developed for the determination of the phosphodiesterase-5 inhibitor vardenafil in small volumes of plasma and bile. Plasma samples (50 µl) were treated with 150 µl of the internal standard solution (cisapride, 10 mg/ml in acetonitrile). An aliquot of the supernatant was injected onto the column. Bile samples (10 µl) were added with 190 µl of cisapride solution and diluted with equal volume of mobile phase before injected into the chromatographic system. Chromatographic separation was achieved on a C18 column with the mobile phase of acetonitrile-water containing 50 mM ammonium acetate (40/60, v/v), at a flow rate of 1 ml/min. Fluorescence was measured with excitation and emission set at 280 and 470 nm, respectively. The retention time was about 14 min for vardenafil and 16.5 min for cisapride. No endogenous substances were found to interfere. Calibration curves were linear from 10 to 1000 ng/ml and 0.2-100 µg/ml for plasma and bile samples, respectively. The limit of quantitation was 10 ng/ml for plasma and 0.2 µg/ml for bile. The intra- and inter-day imprecision (expressed as coefficient of variation, C.V.) did not exceed 17.8%, and the accuracy was within 9.3% deviation of the nominal concentration. The method was used to investigate the disposition and biliary excretion of vardenafil in rats.