LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY (LC-MS/MS) FOR DETERMINATION OF BENZODIAZEPINES IN HUMAN PLASMA USING AN ODS COLUMN COMPATIBLE FOR HYDROPHILIC COMPOUNDS

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A liquid chromatography-tandem mass spectrometry (LC-MS/MS) method has been developed and validated for the determination of benzodiazepines (nitrazepam, clonazepam and clobazam) and their metabolites (7-acetamidonitrazepam, 7-aminonitrazepam, 7-aminoconazepam and N-desmethylclobazam) in human plasma, using a newly-developed ODS column (Unison UK-C18, 150 mm x 2 mm, Imtakt) compatible for hydrophilic compounds. Benzodiazepines were extracted from plasma by use of Waters Oasis® HLB cartridges (30 mg) as a solid phase extraction (SPE). Chromatographic separation was performed with the newly-developed ODS column, using a linear gradient of methanol-ammonium formate (10 mM, pH 3.0). Micromass Quattro micro™ API triple-quadrupole mass spectrometer with electrospray ionization source in the multiple-reaction-monitoring mode was used for detection of benzodiazepines and their metabolites. The newly-developed column was superior to the conventional ODS column (Cadenza CD-C18, 150 mm x 2 mm, Imtakt) in respect of the separation of hydrophilic metabolites. Chromatographic separation of the analytes was achieved within 30 min. Calibration curve for each of the above medicines and their derivatives was linear from 5 ng/mL to 500 ng/mL in plasma, respectively. The average recovery for the different analytes ranged from 78.1 % to 99.5 %. The intra-day precision of the methods was in the range of 3.1-12.4%.

C-Glucuronidation of phenylbutazone by human UDP-glucuronosyltransferase 1A9

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Glucuronidation is a major metabolic pathway in the biotransformation of many xenobiotics and endogeneous compounds. There have been many studies on the formation of O-, N- or S-glucuronides and identification of the UDP-glucuronosyltransferase (UGT) isoforms responsible for the formation of these glucuronides. The formation of C-glucuronides has been reported in only three types of compounds. Phenylbutazone (PB) C-glucuronide is formed by direct coupling of the dioxopyrazolidine ring to glucuronic acid via a C-C bond. The other two types of compounds are Δ⁴-tetrahydrocannabinol and ethchlorvynol. As mentioned above, C-glucuronides are rare metabolites, and the individual UGTs catalyzing their formation have never been identified. In the present study, 16 human UGTs (UGTs 1A1, 1A3, 1A4, 1A5, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B10, 2B11, 2B15, 2B17 and 2B28) were cloned and expressed in baculovirus-infected insect cells and investigated to determine their C-glucuronidating activity toward PB. Among the UGT isoforms investigated, only UGT1A9 catalyzed PB C-glucuronidation. Human liver and kidney microsomes, which are well known to express UGT1A9, had C-glucuronidating activity toward PB. However, the jejunum, which did not express UGT1A9, had no C-glucuronidating activity. These results demonstrate for the first time that PB C-glucuronidation is catalyzed by only UGT1A9.