Determination of Tolbutamide and its Metabolite in Human Plasma by High Performance Liquid Chromatography and its Application to Pharmacokinetics

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SUMMARY. Tolbutamide, an oral sulfonylurea hypoglycemic drug used in the treatment of Type II diabetes mellitus, was selected as the probe substrate for cytochrome P450 2C9 in vitro and in vivo. To investigate the pharmacokinetics of tolbutamide and its metabolite 4-hydroxytolbutamide, a sensitive and selective method of high performance liquid chromatography for the determination of tolbutamide and its metabolite 4-hydroxytolbutamide in human plasma was developed and validated. Plasma samples were extracted using liquid-liquid extraction with diethyl ether. Tolbutamide, 4-hydroxytolbutamide and internal standard carbamazepine were separated on ZORBAX SB-C18 column (150 × 4.6 mm, 5 μm) with gradient elution and detected by UV at 230 nm. The mobile phase was water, acetonitrile and 0.1 % trifluoroacetic acid in water at a flow rate of 1mL/min. The calibration curves were linear over the concentration ranges of 0.5-100 μg/mL for tolbutamide and 0.01-2.0 μg/mL for 4-hydroxytolbutamide. RSD of inter-day and intra-day for three quality control levels (QCs) were less than 9.30 % for tolbutamide and less than 7.55 % for 4-hydroxytolbutamide, respectively. The validated method was proved to be applicable to a pharmacokinetic study after a single oral administration of 500 mg tolbutamide to healthy subjects.

KEY WORDS: HPLC-DAD detection, Human plasma, 4-hydroxytolbutamide, Pharmacokinetics, Tolbutamide.

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