Latin American Journal of Pharmacy
(formerly *Acta Farmacéutica Bonaerense)*
Lat. Am. J. Pharm. **31** (10): 1464-8 (2012)

Regular Article
Received: October 13, 2012
Revised version: November 6, 2012
Accepted: November 6, 2012

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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ISSN 0326-2383