



Simultaneous Determination of Midazolam and 1'-Hydroxymidazolam in Rat Plasma by Protein Precipitation and LC-MS: Application to Pharmacokinetic Study

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SUMMARY. A sensitive and selective liquid chromatography–mass spectrometry (LC–MS) method for determination of midazolam and its metabolite 1'-hydroxymidazolam in rat plasma was developed and validated. After addition of carbamazepine as internal standard (IS), protein precipitation by acetonitrile was used as sample preparation. The chromatographic separation was performed on a Zorbax SB-C18 column (150 × 2.1 mm, 5 μm), using acetonitrile-0.1 % formic acid as the mobile phase with gradient elution, delivered at a flow-rate of 0.4 mL/min. Electrospray ionization (ESI) source was applied and operated in positive ion mode, and selected ion monitoring (SIM) mode used to quantify midazolam and its metabolite 1'-hydroxymidazolam. Calibration curves were linear in the concentration ranges of 5-2000 ng/mL for midazolam and 10-2000 ng/mL for 1'-hydroxymidazolam, with a lower limit of quantification (LLOQ) of 5 ng/mL for midazolam and 10 ng/mL for 1'-hydroxymidazolam, respectively. Intra- and inter-day precision were less than 13 % and the accuracy ranged from -10.7 to 9.5 %. This developed method was successfully used for determination of midazolam and its metabolite 1'-hydroxymidazolam in rat plasma for pharmacokinetic study.

KEY WORDS: 1'-hydroxymidazolam, LC–MS, Midazolam, Plasma.

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