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Development and Validation of Liquid Chromatographic Tandem Mass Spectrometry for Determination and Pharmacokinetic Study of Glimepiride in Rat Plasma

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***SUMMARY*. A new ultra performance liquid chromatography-tandem triple-quadrupole mass spectrometry method for determination and pharmacokinetic study of glimepiride in rat plasma has been developed and validated. The samples were extracted using liquid-liquid extraction with ethyl acetate. The chromatographic separation was performed on a reversed-phase BEH C18 column (2.1 × 100 mm, 1.7 μm, Waters, USA) kept at 30 °C using a mobile phase consisted of acetonitrile and water (containing 0.01% formic acid and 0.05% ammonia) (40:60,V /V), pumped at a flow rate of 0.2 mL/min. The analytes were detected using a Waters triple quadrupole mass spectrometer with positive electrospray ionization in multiple reaction monitoring (MRM) mode for target fragment ions *m/z* 492.1 for glimepiride, *m/z* 507.3 for hydroxyglimepiride, and *m/z* 277.1 for the IS. Good linearity for hydroxyglimepiride and for glimepiride was achieved using weighted (1/x2) least squares linear regression over a concentration range of 10-1000 ng/mL with a lower limit of quantification (LLOQ) of 1 ng/mL for glimepiride and 0.5-50 ng/mL with a (LLOQ) of 0.1 ng/mL for hydroxyglimepiride. Mean recovery of glimepiride and hydroxy glimepiride from plasma were better than 92.2%. RSD of the intra- and inter-day precisions were both lower than 7.93%. The method was successfully applied to a kinetic study in order to assess the main pharmacokinetic parameters of glimepiride.**

RESUMEN. Se ha desarrollado y validado un nuevo método de cromatografía líquida de ultra rendimiento-tándem triple-espectrometría de masa cuadrupolo para la determinación y estudio farmacocinético de glimepirida en plasma de rata. Las muestras se obtuvieron mediante extracción líquido-líquido con acetato de etilo. La separación cromatográfica se realizó en una columna BEH C18 de fase inversa (2,1 × 100 mm , 1,7 μm, Waters, EE.UU.) a 30 °C usando una fase móvil consistente en acetonitrilo y solución acuosa de 0,01 % de ácido fórmico y 0,05 % de amoníaco (40:60, V/V) , bombeado a una velocidad de flujo de 0,2 mL/min. Los analitos se detectaron usando un espectrómetro de masas triple cuadrupolo Waters con ionización por electronebulización positiva en el modo de monitorización de reacción múltiple (MRM) para los iones diana fragmentados de *m/z* 492.1 para glimepirida, *m/z* 507,3 para hydroxyglimepiride y *m/z* 277,1 para el IS . Buena linealidad para hidroxiglimepirida y para glimepirida se logró utilizando pesos (1/x2) de cuadrados mínimos de regresión lineal en un intervalo de concentración de 10-1000 ng/mL con un límite inferior de cuantificación (LLOQ) de 1 ng/mL para glimepirida y 0,5-50 ng/mL con un LLOQ de 0,1 ng/mL para hydroxyglimepiride. La recuperación media de glimepirida e hidroxiglimepirida del plasma superaron el 92,2 %. Las RSD de las precisiones intra -e inter-día fueron inferiors al 7,93 %. El método se aplicó con éxito a un estudio cinétic con el fin de evaluar los principales parámetros farmacocinéticos de glimepirida.

KEY WORDS: CYP2C9, Glimepiride, Pharmacokinetics, UPLC-ESI-MS/MS.

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