Latin American Journal of Pharmacy
(formerly *Acta Farmacéutica Bonaerense)*
Lat. Am. J. Pharm. **37** (7): 1324-32 (2018)

Received: January 30, 2018
Accepted: May 11, 2018

Simultaneous Determination of the Plasma Concentrations of a Cocktail of 5 Cytochrome P450 Substrate Drugs and Their Metabolites by UPLC-MS/MS

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SUMMARY. **CYP450 enzyme is the most important drug metabolizing enzyme in the liver. In our work, we establish an UPLC-MS/MS method for simultaneous determination of 5 kinds of probe drugs (bupropion, metoprolol, midazolam, phenacetin and tolbutamide) and their metabolites (hydroxyburpropion, hydroxymetoprolol, 1-hydroxymidazolam, acteminophen and hydroxytolbutamide) in rat plasma. Five kinds of probe drugs are cytochrome P450 isozymes CYP2D6, CYP3A4, CYP1A2, CYP2B6, and CYP2C9 substrate. Using diazepam as an internal standard, the plasma samples were treatment by acetonitrile precipitation. The chromatographic separation was performed on a UPLC BEHC18 (2.1 × 50 mm, 1.7 µm) column using a mobile phase of acetonitrile-water with 0.1% formic acid. The multiple reactions monitoring in positive electrospray ionization was used for quantitative determination. The precision was less than 12%, the accuracy ranged from 90.3 to 111.4%. Plasma standard curves were in the range of 1-2000 ng/mL, correlation coefficients were greater than 0.995. The established UPLC-MS/MS method was applied to evaluate dextromethorphan on rat CYP450 enzyme activity.**

RESUMEN. La enzima CYP450 es la enzima metabolizadora más importante del hígado. En este trabajo desarrollamos un método de UPLC-MS/MS para la determinación simultánea de 5 tipos de fármacos sonda (bupropión, metoprolol, midazolam, fenacetina y tolbutamida) y sus metabolitos (hidroxibupropion, hidroximetolprolol, 1-hidroximidazolam, acteminofeno e hidroxitolbutamida) en plasma de rata. Cinco tipos de drogas sonda fueron las isoenzimas del citocromo P450 CYP2D6, CYP3A4, CYP1A2, CYP2B6 y CYP2C9. Utilizando diazepam como patrón interno, las muestras de plasma se trataron mediante precipitación con acetonitrilo. La separación cromatográfica se realizó en una columna UPLC BEHC18 (2,1 × 50 mm, 1,7 µm) usando una fase móvil de acetonitrilo-agua con ácido fórmico al 0,1%. El monitoreo de reacciones múltiples en ionización por electronebulización positiva se utilizó para la determinación cuantitativa. La precisión fue inferior al 12% y la precisión varió de 90.3 a 111.4%. Las curvas estándar de plasma estuvieron en el rango de 1-2000 ng/mL y los coeficientes de correlación fueron mayores a 0.995. El método establecido de UPLC-MS / MS se aplicó para evaluar el dextrometorfano en la actividad de la enzima CYP450 en ratas.

KEY WORDS: CYP450, metabolite, probe drug. UPLC-MS/MS.

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ISSN 0326 2383 (printed ed.)
ISSN 2362-3853 (on line ed.)