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Determination and Validation of Imperialine in Rat Plasma by Ultra-Performance Liquid-Chromatography with Tandem Mass Spectrometry

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***SUMMARY*. Imperialine is the major biologically active isosteroidal alkaloid present in Bulbus Fritillaria. In this work, a sensitive and selective UPLC-MS/MS method for determination of imperialine in rat plasma was developed and validated. After addition of hupehenine as an internal standard (IS), protein precipitation by acetonitrile-methanol (9:1, v/v) was used to prepare samples. Chromatographic separation was achieved on a UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 μm) with 0.1% formic acid and acetonitrile as the mobile phase with gradient elution. An electrospray ionization source was applied and operated in positive ion mode; multiple reaction monitoring (MRM) mode was used for quantification using target fragment ions *m/z* 430.3→138.2 for imperialine, and *m/z* 416.3→98.0 for IS. Calibration plots were linear throughout the range 2-2000 ng/mL for imperialine in rat plasma. Mean recoveries of imperialine in rat plasma ranged from 93.9 to 97.0%. RSD of intra-day and inter-day precision were both < 6%. The accuracy of the method was between 96.5% and 106.5%. The method was successfully applied to pharmacokinetic study of imperialine after either oral or intravenous administration. The bioavailability of imperialine was reported as 48.5%.**

RESUMEN. Imperialina es el principal alcaloide isosteroidal biológicamente activo en Bulbus Fritillaria. En este trabajo fue desarrollado y validado un método de UPLC-MS/MS sensible y selectivo para la determinación de imperialina en plasma de rata. Después de la adición de hupehenina como un estándar interno (IS), se precipitaron las proteínas con acetonitrilo-metanol (9: 1, v/v) para preparar las muestras. La separación cromatográfica se logró en una columna de UPLC BEH C18 (2,1 × 100 mm, 1,7 μm) con 0,1% de ácido fórmico y acetonitrilo como fase móvil, con gradiente de elución. Se aplicó una fuente de ionización por electrospray operada en modo de ion positivo y para la cuantificación se usó el modo de seguimiento de reacción múltiple (MRM), utilizando fragmentos de iones diana *m/z* 430,3→138,2 para imperialina y 416,3→98.0 *m/z* para IS. Los gráficos de calibración fueron lineales en todo el rango de 2-2000 ng/mL para imperialina en plasma de rata. Las recuperaciones medias de imperialina en plasma de rata oscilaron entre 93,9 y 97,0%. La precisión RSD intra-día y entre días fueron ambas < 6%. La precisión del método estuvo entre 96,5 y 106,5%. El método se aplicó con éxito para el estudio farmacocinético de imperialina después de la administración oral o intravenosa. La biodisponibilidad de imperialina fue del 48,5%.

KEY WORDS: imperialine, pharmacokinetics, plasma, rat, UPLC-MS/MS.

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