



Determination of Phillyrin in Rat Plasma by High Performance Liquid Chromatography–Tandem Mass Spectrometry and its Application to a Pharmacokinetic Study

Huihui CAO, Jingdong PENG* & Lei ZHANG

Chemistry and Chemical Engineering, Southwest University,
Education Ministry Key Laboratory on Luminescence and Real–Time Analysis,
Chongqing 400715, China

SUMMARY. A simple, fast and sensitive high performance liquid chromatography-tandem mass spectrometry (HPLC–MS) method was developed and validated to quantitatively determinate phillyrin in rat plasma using reserpine as internal standard (IS). The sample preparation method was simple only using 0.6 mL acetonitrile as protein precipitant, the supernatant was dried at 50 °C in a vacuum drying oven and then dissolved in 1.0 mL of organic phase consisted with methanol and acetonitrile (1:9, v/v). The analytical samples were separated on a reversed-phase C₁₈ column (150 × 4.6 mm, 5 μm Phenomenex, CA, USA) at 30 °C, using 0.05 % acetic acid aqueous solution and acetonitrile (56:44, v/v) as mobile phase, at a flow rate of 0.3 mL/min. The detection was performed by electrospray ionization (ESI) mass spectrometry using extracted ion chromatogram (EIC) detection in positive ion mode while monitoring target molecular ions at [M+Na]⁺ *m/z* 557.57 for phillyrin and [M+H]⁺ *m/z* 609.45 for the I.S. Good linearity was generated over the concentration range of 0.069–8.97 μg/mL (*r* = 0.9995) with the limit of detection and quantification of 7.04 and 21.12 ng/mL, respectively. Intra-day and inter-day precisions were less than 6 % and the mean recoveries at three tested concentrations ranged from 96.27 to 100.63 %. Those results indicated that the proposed method was fast, sensitive and feasible enough to successfully apply to the pharmacokinetic study in rats after intravenous injection of phillyrin.

KEY WORDS: HPLC–MS, Pharmacokinetic study, Phillyrin.

* Author to whom correspondence should be addressed. *E-mails:* hxpengjd@swu.edu.cn, chh234@swu.edu.cn