Quantification of Raloxifene in Mouse Plasma and Tissues by Ultra-High Performance Liquid Chromatography-Tandem Mass Spectrometry

Keywords
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Abstract
Raloxifene, a selective estrogen receptor modulator, is used for the treatment or prevention of osteoporosis and breast cancer in postmenopausal women¹. Due to lack of a sensitive and fast analytical method for the quantification of raloxifene in plasma and tissues², we developed and validated an ultra-high performance liquid chromatography-tandem mass spectrometric (UPLC–MS/MS) method to quantify raloxifene levels in mouse plasma and tissues. Chromatographic separation was carried out on an UPLC C₁₈ column with a gradient elution of a mixture of 0.1% formic acid in water and acetonitrile. MS/MS analysis was performed using positive electrospray ionization with multiple reaction monitoring mode to quantify raloxifene (m/z 474.3 → 112.1) and bazedoxifene (internal standard; m/z 470.8 → 126.1). Raloxifene eluted at 1.75 min, whereas bazedoxifene eluted at 1.77 min. The lower limit of detection and quantification of raloxifene was 0.3 and 1.2 ng/ml, respectively. The assay was linear from 1.2 to 600 ng/ml. Intra-day accuracy and precision were <14.4%. The matrix (plasma, liver homogenate, or intestinal homogenate) did not affect raloxifene quantification, and the recovery of raloxifene from these matrices was >95%. After intraperitoneal administration of raloxifene (10 mg/kg) to mice, raloxifene level in plasma, liver, and intestine was highest at 5 min post-dosing and decreased at 30 and 60 min post-dosing. When 12.5, 25, or 50 mg/kg of raloxifene was administered for a 5-min duration, raloxifene concentration in plasma, liver, and intestine increased in a dose-dependent manner. At the highest dose of 50 mg/kg, raloxifene was distributed predominantly to the intestine (90.82 ± 11.95 µg/g), followed by liver (76.59 ± 5.41 µg/g) and plasma (7.9 ± 0.26 µg/ml). In conclusion, this UPLC–MS/MS approach allows specific, sensitive, and rapid quantification of raloxifene in mouse plasma, liver, and intestine. This method may be applied to pharmacokinetic studies of raloxifene in humans.

References