Title	Quantification of Raloxifene in Mouse Plasma and Tissues by Ultra-High Performance Liquid Chromatography-Tandem Mass Spectrometry
Keywords (up to 5)	Raloxifene, Ultra-High Performance Liquid Chromatography-Tandem Mass Spectrometry, Bioanalytical Method Validation, Tissue Distribution, Mouse Plasma and Tissues
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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 woman[1]. Due to lack of a sensitive and fast analytical method for the quantification of raloxifene in plasma and tissues[2], 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 C_{18} 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	1. J.V. Pinkerton, et al., J Steroid Biochem Mol Biol, 142 (2014) 142-154. 2. J. Trontelj, et al., J Chromatogr B Analyt Technol Biomed Life Sci, 855 (2007) 220-227.