Quantitative Determination of Levonorgestrel in Fish Plasma using UPLC-MS/MS

S. Jayaraman, M. Cantwell, D. Borsay Horowitz and L. J. Mills. U.S.EPA, NHEERL, Narragansett, RI, USA.

In this study, a sensitive high-performance liquid chromatography electrospray tandem mass spectrometric method was developed for the determination of levonorgestrel in fish plasma using levonorgestrel-d_6 as an internal standard (IS). In the laboratory, the fish cunner, (*Tautogolabrus adspersus*) was dosed by oral gavage with 7.5 mg/kg levonorgestrel in a methylcellulose carrier. Blood was taken from the caudal vein of three individual fish at each sampling time after gavage (15 minutes, 1, 3, 6, 18, 24, 48, 72 and 96 hours), plasma was separated, and samples were stored at -80°C until chemical analysis. To quantify levonorgestrel, 150 µL plasma was extracted by an ether extraction procedure, followed by solid phase purification. Waters Xevo tandem quadrupole mass spectrometer (TQD) with positive electrospray ionization was operated in the multiple reaction monitoring (MRM) for the analysis of levonorgestrel. Extract (10 uL) was injected on a Waters BEH C18, 50 mm x 2.1 mm, 1.7 um using water and acetonitrile as the mobile phase. The method was validated over a concentration range of 0.93 and 238 ng mL^{-1}. The transitions of precursor ions were *m/z* 313→109 for levonorgestrel and *m/z* for 319→251 for IS. The mean recovery of levonorgestrel from plasma (*n* =3) was 83.5% and 88.2% for IS. Matrix Effect (ME) was measured using the post-extraction spiked method and was calculated to be 70%. Results show the levonorgestrel concentration in plasma peaked at 0.8% of the gavage amount at three hours after treatment, and then gradually declined.