

## Analysis of Thyroid Hormones in Gland and Serum Using Liquid Chromatography-Tandem Mass Spectrometry

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Thyroid hormones (THs), which are critical for growth and development in all vertebrates, can be impacted through chemical perturbation of the hypothalamic-pituitary-thyroid (HPT)-axis. Amphibian and mammalian models are being used to address this research priority within US EPA. Determining glandular levels of THs and precursors, and circulating THs is essential for understanding chemical effects on the HPT-axis. Although radio-immunoassays have historically been used to measure THs in serum, they are less suitable for gland samples or when serum volumes are limited and TH concentrations are low. Therefore we developed a sensitive and selective method to analyze THs and precursors using liquid chromatography (LC)-tandem mass spectrometry (MS/MS). Target compounds include: 3-monoiodotyrosine, 3,5-diiodotyrosine, 3,5-diiodothyronine, 3,3'-diiodothyronine, 3,3',5-triiodothyronine, 3,3',5'-triiodothyronine and thyroxine. To liberate THs from binding proteins, glands were digested with pronase and serum samples were treated with acid. Samples were processed through cation exchange solid phase extraction and analyzed by LC-MS/MS. The instrument calibration range for each analyte was 0.010 ng/mL to 10 ng/mL and was linear ( $r > 0.99$ ). Chromatographic separation of all 7 target compounds was achieved in 4 min. The method was capable of measuring low levels of iodotyrosines and iodothyronines in amphibian glands with lowest level of quantitation (LLOQ) of 3 to 16 pg/gland and LLOQs of 50 pg/mL for amphibian serum and 20 pg/mL for rat fetal serum. The use of LC-MS/MS for measuring THs in glands and serum will allow a more comprehensive evaluation of the HPT-axis. *This abstract does not necessarily reflect US EPA policy.*

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