

Quantitative Analysis of a Thyroid Hormone Biomarker Extracted from Whole Blood Using a Mitra Microsampling Device

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Thyroid hormones are critical regulators of metabolism, growth, and development. Thyroxine (T4) and 3,3',5-triiodothyronine (T3) are produced in the thyroid gland and released into circulation where the more biologically active form, T3 exerts effects on peripheral tissues. The serum level of T4 is a useful biomarker of overall thyroid function. Low or high levels of circulating thyroid hormones are indicative of thyroid or pituitary gland dysfunction resulting from disease or malnutrition. Hyperthyroidism or hypothyroidism can result from autoimmune disorders, such as Graves' disease, certain medications, thyroid cancer, and can often occur during pregnancy. Individuals suffering from thyroid dysfunction suffer from a broad range of symptoms including weakness and fatigue. The Mitra Microsampling Device (MMD) enables convenient and accurate at-home sample self-collection from a simple finger prick. Here, T4 was extracted from whole blood collected using an MMD and analyzed by MFLC-MS/MS. Thyroxine (T4) was spiked into whole blood and 10 microliters absorbed onto a Mitra Microsampling Device. The MMD was dried at ambient temperature and then placed into 500 microliters of methanol containing internal standard. After incubation for 30 minutes on a shaker, 400 microliters of the extract was transferred to a 96 well plate, evaporated to dryness, and reconstituted with 100 microliters of 80:20 Methanol:H₂O with 0.1% NH₄OH. The extract was analyzed on an API-6500 mass spectrometer operating in positive ESI mode equipped with an Optiflow source. The MFLC system was a Waters M Class operating with binary gradient method and a flowrate of 50 microliters/minute. Separation was achieved using a Phenomenex Kinetex Biphenyl column (5 cm x 1.0 mm, 3 μm). The method is linear from 0.50 to 500 ng/ml with an r-value of 0.9973. Measurement of multiple lots of human whole blood using this method revealed endogenous T4 levels of 18.9 ng/ml. This method provides sensitive and accurate quantification of thyroid biomarkers utilizing a simple, at-home sample collection requiring only 10 microliters of sample.