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QUANTITATIVE ANALYSIS BY LC-MS/MS OF THYROID HORMONE BIOMARKERS EXTRACTED FROM BLOOD COLLECTED WITH THE TASSO MICROSAMPLING DEVICE

Introduction

Thyroid hormones Thyroxine (T4) and 3,3',5-triidothyronine (T3) are regulators of metabolism, growth, and development. The levels of T3 and T4 are useful biomarkers of overall thyroid function. 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 Tasso-M20 device enables at-home, automated, self-collection of the sample. The device automatically collects four 17.5 microliter samples. The accurate quantitation of these hormones extracted from the Tasso-M20 device and analyzed using HPLC-MS/MS was performed in this study. The dynamic range is 0.100-500 ng/mL and is adequate to simultaneously detect T3 and T4 from samples.

Methods

T3/T4 was extracted from blood collected using a Tasso device and analyzed by HPLC-MS/MS. For standard preparation T4 and T3 were spiked into a surrogate matrix and 17.5 microliters of this solution was pipetted onto exposed Tasso-M20 tips. The tips were dried at ambient and placed into a DWP 96 and spiked with stable label internal standards and 500 microliters of methanol containing 1% formic acid. After 60 minutes of shaking, 400 microliters of the extract was transferred, evaporated to dryness, and reconstituted with 100 microliters of 80:20 Methanol:H2O containing 1% formic acid. The extract was analyzed on a SCIEX API-6500+ mass spectrometer. The binary HPLC method utilized an Agilent Pursuit Diphenyl column and Water/Acetonitrile mobile phases containing formic acid.

Data

The extraction of the Tasso-M20 tips was optimized using an extraction solvent of MeOH containing 1% formic acid. The recovery was 74% for T3 and 85% for T4. Soaking the tips in water or acidified water decreased the recovery by 20%. The recovery in non-acidified solvents was less than 50% for methanol and less than 1% in acetonitrile. An interferent peak for the T3 labeled internal standard was initially observed so the LC gradient was altered from 40% B (acetonitrile 0.1% formic acid) at 4 minutes to 40% B at 6 minutes. This successfully resolved the internal standard peak from the interferent. Since this is a biomarker assay a surrogate matrix was used for the standard curve. SigMatrix Serum Diluent containing 6% recombinant HSA (Human Serum Albumin) in

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PBS solution, pH 7.4 was used as the surrogate. To confirm the accuracy of the surrogate matrix QC samples were prepared in blood, aliquotted onto the tips, dried, extracted and analyzed along with standards prepared the same way in SigMatrix. The analysis confirmed that the surrogate is an accurate representation of the blood matrix. No interfering peaks were detected in blank SigMatrix extracts. The assay range is 0.100 to 500 ng/ml with r-values of 0.9996 for T3 and 0.9969 for T4. Preliminary measurement of human blood collected using the Tasso device, extracted and analyzed indicated average endogenous T4 and T3 levels of 30.5 and 0.360 ng/mL respectively. Further research will be conducted using a SCIEX API-7500 in order to decrease the LLOQ to 0.0500 ng/mL (or lower) in order to measure T3 levels in subjects with hypothyroidism.

Novel Aspects

Development of a sensitive/selective LC-MS/MS and extraction method for T3/T4 analysis from blood samples collected using a Tasso-M20 device.