Quantitative Analysis of the Thyroid Hormone T4 Biomarker Extracted From Whole Blood Using a Mitra® Microsampling Device

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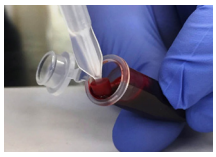
OVERVIEW



Purpose:

Develop extraction and LC-MS/MS methods for the determination of Thyroxine (T4).

Methods:

Human blood containing T4 was collected and allowed to dry. The dried blood was extracted and analyzed by microflow LC-MS/MS (MFLC-MS/MS). Samples were quantified against standards extracted from an artificial matrix.

Results:

The method is linear from 0.500 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 approximately 18.9 ng/ml.

INTRODUCTION

Thyroid hormones are critical regulators of metabolism, growth, and development. Thyroxine (T4) and 3,3',5-triidothyronine (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.

METHODS

Extraction:

- Sample volume: 10 µl absorbed on MMD
- Dry 2 hours
- Add 500 µl methanol containing T4$^{13}C_6$
- Remove device, centrifuge and collect 400 µl of supernatant
- Evaporate to dryness under nitrogen gas
- Reconstitute with 80:20 methanol:water

LC-MS/MS:

- Waters ACQUITY UPLC® M-Class MFLC
- Binary gradient using acetonitrile and water with 0.1% formic acid
- Flow Rate: 50 µl/min
- Column: Phenomenex Kinetex® biphenyl column (50 x 1.0mm, 1.7 µm)
- Sciex QTRAP® 6500 or 6500+ operating in MRM mode
- Sciex OptiFlow™ Source
- Positive ion mode

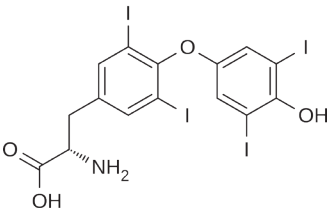


Figure 1: Thyroxine

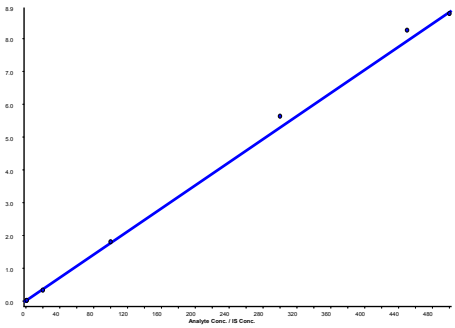


Figure 2: Linear (1/x²) response of Thyroxine (T4) from 0.500 to 500 ng/mL in artificial matrix.

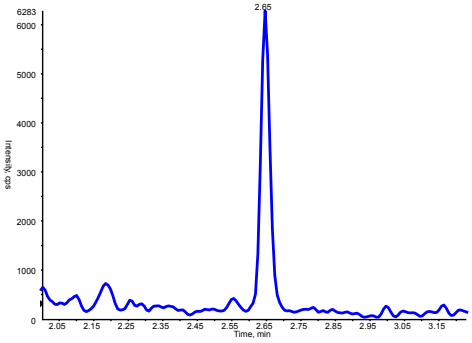


Figure 3: Thyroxine (T4) 0.500 ng/mL extracted from artificial matrix.

CONCLUSION

- Method provides sensitive and accurate quantification of thyroid biomarkers.
- At-home sample collection requiring only 10 microliters of sample.

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