LC-MS/MS quantitative analysis of 11 total thyroid hormones and metabolites in serum for clinical research use

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ABSTRACT

Introduction: Liquid chromatography triple quadrupole mass spectrometry is suited for rapid analysis of multiple analytes of interest and different structures and physiochemical properties. Thyroid hormone and related compounds are of great interest in the field of clinical research. A comprehensive method for the analysis of Thyroid hormones and related compounds in serum and other matrices is essential. There are several companies and academic laboratories that provide methods for the analysis of Thyroid hormones and metabolites in serum. Single sample preparation technique and reagents are generally preferred for routine clinical use. The current work was aimed at developing a sensitive, early detection, and rapid method for the analysis of Thyroid hormones and metabolites in serum.

RESULTS

The Thyroid hormones, 3,3’,5-Triiodothyronine (rT3), 3,3’,5-Diiodothyronine (3,5-T2), 3-Iodothyronine (T3), Triiodothyronine-13C6 (rT3-13C6), 3-Iodothyronine-13C6 (T3-13C6), Triiodothyroacetic Acid (TAC), Triiodothyroacetic Acid-13C6 (TAC-13C6), Triiodothyroacetic Acid-13C6 (TAC-13C6), and Triiodothyroacetic Acid-13C6 (TAC-13C6) were detected in serum. The calibration curves ranged from 1 pg/mL to 1000 ng/mL and various pooled donor samples were used to test the method. The extract was reconstituted in 200 µL of Thyroid ISTD mixture at 1000 ng/mL. Four in-house samples were extracted and the following were found within the determined calibration range: Triiodothyronine and Thyronine in 3 samples, 3-Iodothyronamine and 3,3’-Diiodothyronine in samples and samples respectively. The extract was transferred to a test tube and 200 µL of Thyroid ISTD mixture at 1000 ng/mL were added to each and 3-Iodothyronamine, Diiodothyroacetic acid, Triiodothyroacetic acid and Tetraiodothyroacetic acid were found in samples. The samplers temperature was 4 oC. The LC-MS/MS conditions were as follows: Vanquish Horizon HPLC binary pump, well plate, thermostatted column compartment, LC-MS/MS conditions – Vanquish Horizon, Vanquish LF 500 HPLC binary Pump, 5 µm 100 µg/mL 4.6x100 mm column, 200 µg/mL 3,3’,5-Triiodothyronine (rT3): 100 mg/mL 3,3’,5-Triiodothyronine-13C6: 100 µg/mL Thyroxine (T4): 100 µg/mL Thyroxine-13C6: 100 µg/mL. The calibration curves ranged from 1 pg/mL to 1000 ng/mL and various pooled donor samples were used to test the method.

CONCLUSIONS

• Further evaluate other sample preparation techniques for improved total Thyroid and metabolite determination and maximize the efficiency of the method as well as evaluate potential for the analysis of Thyroid hormones and metabolites.

REFERENCES


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