



Sensitive quantitation of free triiodothyronine (FT3) and free thyroxine (FT4) in human serum

In this technical note, the combination of a rapid sample preparation procedure consisting of a liquid-liquid extraction with a robust and sensitive LC-MS/MS method using the SCIEX QTRAP 6500+ system enabled sensitive and accurate quantitation of FT3 and FT4 extracted from human serum. Excellent linearity was observed across the calibration series [0.5-100 pg/mL], with r^2 values of 0.9995 for FT3 and 0.9993 for FT4. Low-level sensitivity was achieved at the lowest calibrator [0.5 pg/mL] with a signal-to-noise ratio [S/N] of 7:1 for FT3 and 8:1 for FT4. In addition, the method showed excellent precision [%CV] and % accuracy [12.3% and 95-108% for FT3 and 11.2% and 95-100% for FT4] at the lowest calibrator [0.5 pg/mL], demonstrating the quantitative performance of the assay.

Key benefits of FT3 and FT4 analysis from human serum using the QTRAP 6500+ system

Low-pg/mL level sensitivity and excellent quantitative performance:

Sensitive quantitation of FT3 and FT4 was performed with excellent precision [12.3% for FT3 and 11.2% for FT4] at the lowest calibrator level [0.5 pg/mL]

Rapid sample preparation: FT3 and FT4 were extracted from human serum samples by centrifugal filtration using filters with a molecular-weight cutoff [MWCO] of 10 kDa

Chromatographic separation: Optimized LC conditions enabled [10 minutes] chromatographic separation of FT3 and FT4

Excellent linearity: Calibration curves for FT3 and FT4 showed r^2 values above 0.99 across the calibration ranges

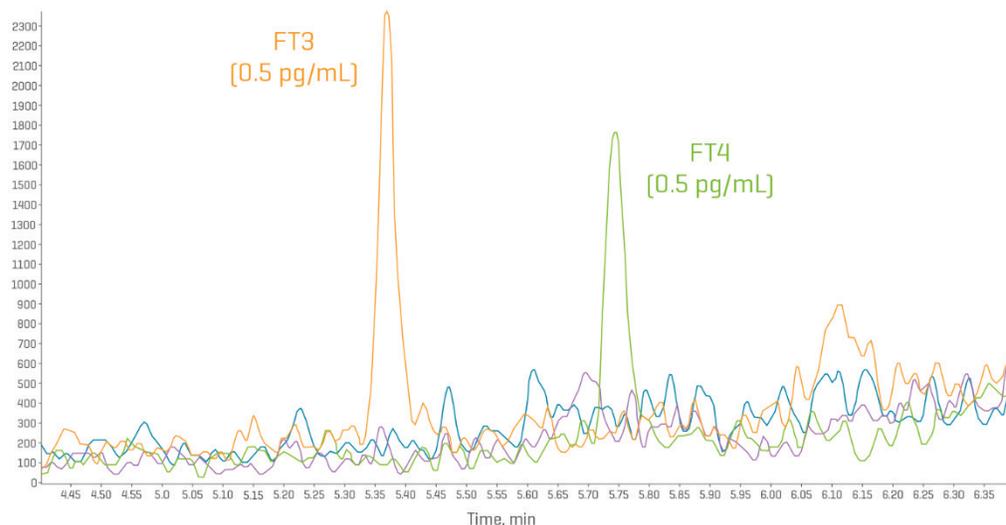


Figure 1. Extracted ion chromatograms [XICs] of FT3 [orange] and FT4 [green] extracted from serum matrix. Overlaid XICs show calibration standard for FT3 and FT4 at 0.5 pg/mL [0.77 and 0.64 pmol/L, respectively] using the QTRAP 6500+ system following the sample preparation and LC-MS/MS conditions.

Introduction

Free triiodothyronine (FT3) and free thyroxine (FT4) are key thyroid hormones that play essential roles in regulating metabolism, growth, and development. Produced by the thyroid gland, FT4 serves primarily as a precursor that is converted into the more biologically active FT3 in peripheral tissues. Because FT3 and FT4 circulate at low concentrations and are tightly bound to carrier proteins, accurate quantitation in biological samples is critical for clinical research.

Methods

Sample preparation: FT3 and FT4 were extracted from charcoal-stripped serum by centrifugal filtration using filters with a molecular-weight cutoff (MWCO) of 10 kDa.

Liquid chromatography: Chromatographic separation was achieved using a [Phenomenex Kinetex C18 column](#) [50 x 3 mm, 2.6 μ m, 00B-4462-Y0]. Mobile phase A was acetic acid in water, and mobile phase B was acetic acid in methanol. The total run time was 10 minutes.

Mass spectrometry: Data was collected using a [QTRAP 6500+ system](#) with an IonDrive Turbo V ion source and operated in electrospray ionization (ESI) positive mode. The Scheduled MRM algorithm was used in [SCIEX OS software](#) (version 3.1.6) to collect 10-12 data points for quantifiable data. Compound-dependent parameters were optimized by infusion.

Data processing: Data processing was performed using [SCIEX OS software](#) (version 3.1.6). Peak integration was achieved using the MQ4 algorithm. Quantitative analysis was conducted in the Analytics module of SCIEX OS, where calibration curves, concentration calculations, and assay precision statistics were automatically generated.

Results and discussion

Figure 1 shows the chromatographic separation of FT3 and FT4 into a control, charcoal-stripped serum sample at the lowest calibrator level (0.5 pg/mL). The extracted ion chromatograms showed a signal-to-noise (S/N) ratio of 7:1 for FT3 and 8:1 for

FT4 at the lowest calibrator level (0.5 pg/mL), as calculated using a peak-to-peak algorithm.

The quantitative performance of the method was investigated by injecting a series of calibrator samples spiked at concentrations from 0.5-100 pg/mL. **Figure 2** shows the calibration curves for FT3 and FT4 across the calibration range (0.5-100 pg/mL). The plot shows excellent linear responses across the calibration series, with r^2 values of 0.9995 for FT3 and 0.9993 for FT4.

The precision and accuracy values were calculated from 3 replicate injections of the lowest matrix calibrators analyzed (0.5 pg/mL). The precision (%CV) was 12.3% for FT3 and 11.2% for FT4 and the %accuracy ranged between 95-100% for FT3 and 95-100% for FT4 at the lowest calibrator (0.5 pg/mL).

Conclusions

A fast and sensitive LC-MS/MS method for the detection of FT3 and FT4 extracted from human serum samples was developed. The method demonstrated:

- Fast sample preparation, which consisted of centrifugal filtration using filters with a molecular-weight cutoff
- Chromatographic separation of FT3 and FT4
- Excellent linear responses across the calibration series, with r^2 values of 0.9995 for FT3 and 0.9993 for FT4
- Good sensitivity resulting in S/N of 7:1 for FT3 and 8:1 for FT4 at the lowest calibrator level (0.5 pg/mL)
- High quantitation performance of the method, resulting in excellent precision and accuracy at the lowest calibrator (0.5 pg/mL).

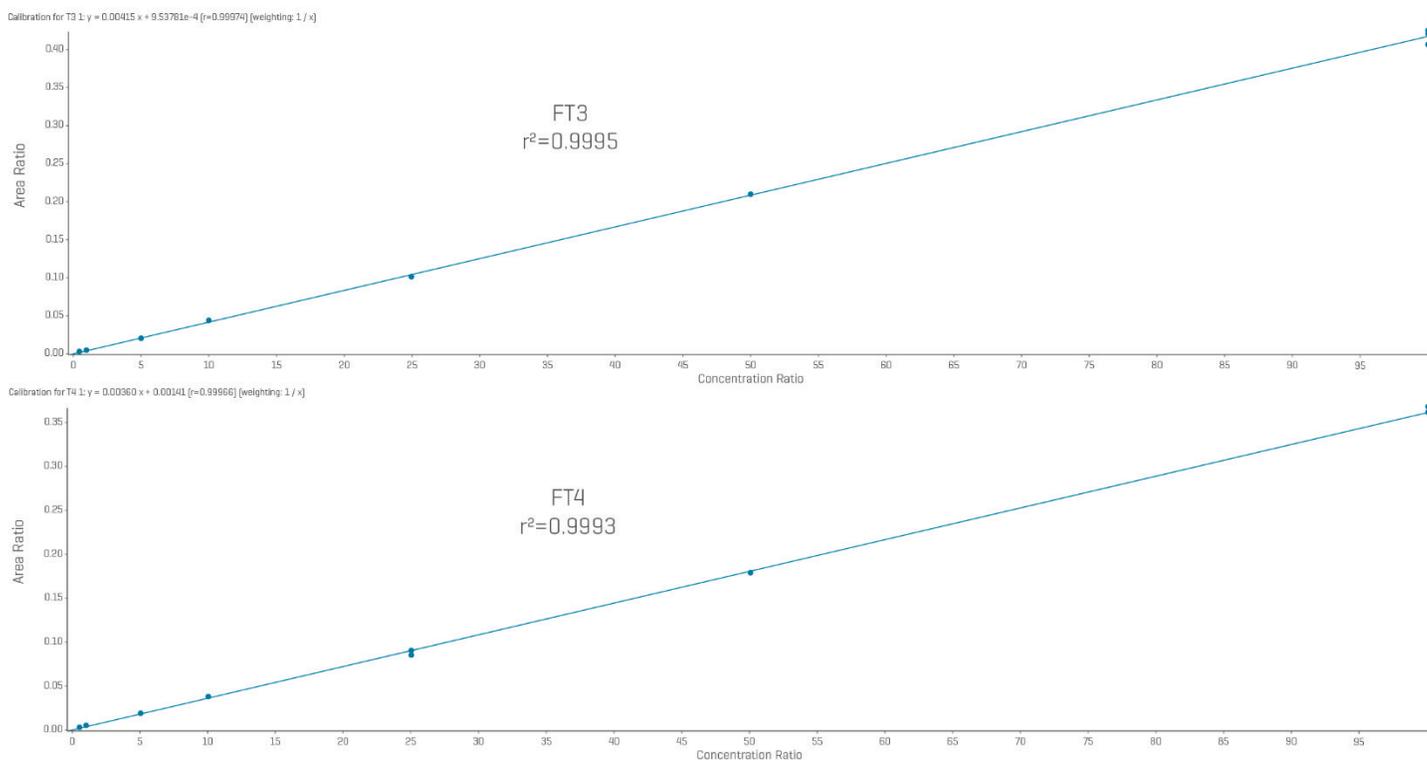


Figure 2. Linear calibration curves for FT3 and FT4 extracted from serum. The calibration curves were run in triplicate across the range (0.5-100 pg/mL). The curves were generated using linear regression and 1/x weighting for FT3 and FT4 in serum, resulting in r^2 values of 0.9995 and 0.9993, respectively.

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