

A Rapid and Sensitive LC-MS/MS Method for the Analysis of Free Thyroid Hormones

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Thyroid hormones are essential for the regulation of development and growth in humans and animals. The thyroid gland produces thyroxin (T4) and tri-iodothyronine (T3) and quickly releases into the circulation system. The concentration of circulating T4 is 50-60 times higher than T3 and the majority of them are bound to blood proteins. The unbound or “free” T3 and T4 are the active forms of the hormone which only represent a small portion (less than 1%) of total thyroid hormones. Accurate and sensitive measurement of low levels of free hormones is necessary to assess thyroid function for both veterinary and human clinicians. Reverse tri-iodothyronine (rT3) is an inactive form resulted from T4 biotransformation. Since rT3 functions as the feedback inhibitor of thyroid hormone production, the measurement of rT3 can be an important diagnostic marker in clinical implication.

The intent of this application was to develop an LC-MS/MS method for the analysis of thyroid hormones (T3, rT3, and T4) at the free form levels using the highly efficient and selective Raptor™ Biphenyl column. The clinical applicability of the method was demonstrated by analyzing the fortified thyroid hormone in phosphate buffer saline containing 4% human albumin.

Human albumin was dissolved in PBS solution to a final concentration of 4%. This solution was used to prepare calibration standards ranging from 2 to 400 pg/mL. Standard solutions (0.5 mL) were spiked with 5µL of internal standard, T4-¹³C₆ (1 ng/mL), and mixed with 1 mL of acetonitrile in a 4-mL glass vial. A 2 mL aliquot of ethyl acetate was added, stirred for 2 min, and then centrifuged for 10 min at 4,300 rpm. The organic phase was removed and placed into a 4-mL glass vial, evaporated to dryness at 55°C under a gentle stream of nitrogen. The dried extract was reconstituted with 80 µL of a 30:70 water:methanol solution and injected (10 µL) into the Raptor™ Biphenyl column (100x2.1mm, 2.7µm) for the analysis with the Waters

ACQUITY UPLC® I-Class System coupled with a Waters Xevo® TQ-S mass spectrometer using electrospray ionization in positive ion mode. .

Good linearities (1/x weighted) were obtained for all 3 forms of thyroid hormones with coefficients of variation (r^2) > 0.990 from 2 to 400 pg/mL (for T3) or 5 to 400 pg/mL (for T4 and rT3). The %deviation was < 15%. Simultaneous analysis of all 3 analytes was performed with a fast 3.5 minute of total run time for each injection with a complete separation of T3 and rT3 isobars.

It was demonstrated that the Raptor™ Biphenyl column is excellent for rapid and sensitive analysis of thyroid hormones. With the established method, as low as 2 pg/mL (T3) or 5 pg/mL (T4 and rT3) of thyroid hormones can be accurately determined with less than 3.5 minutes of analysis time. The analytical method is thus applicable to the clinical analysis of free thyroid hormone at low pg/mL levels.