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Editorial

Ultra Performance Liquid Chromatography Tandem Mass Spectrometry to Quantify Thyroid Hormones

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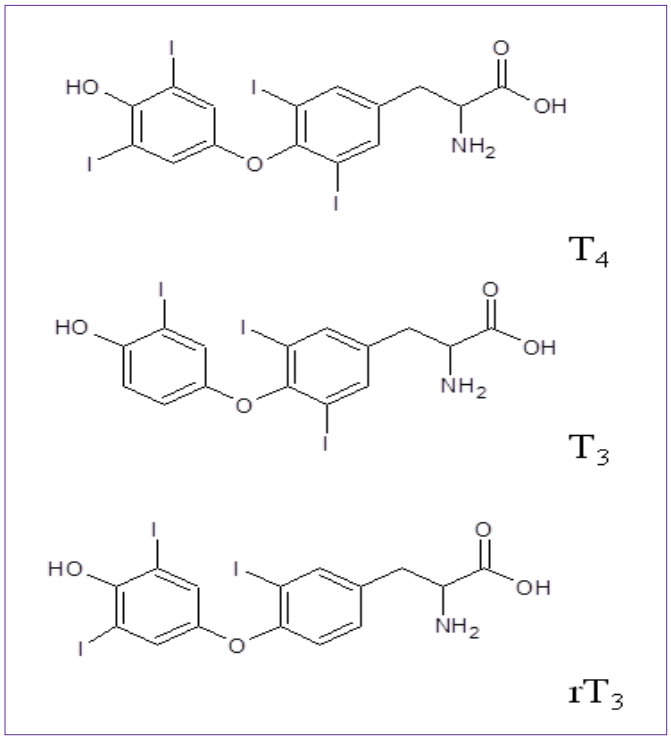
Editorial

Thyroid hormones are tyrosine-based small molecules produced by the thyroid gland that are responsible for the regulation of metabolism and some protein synthesis. Clinically significant thyroid hormones in the blood include Thyroxine (T_4), Triiodothyronine (T_3) and reverse Triiodothyronine (rT_3). The thyroid hormone T_4 is the most abundant form and has the longest half life in the blood followed by T_3 which is approximately 20 times less than T_4 followed by rT_3 which is approximately 10 times less than T_3 [1-5]. Thyroxin and triiodothyronine can be measured as free, which are indicators of thyroxine and triiodothyronine activities in the body, or total, which also depend on the thyroxine and triiodothyronine that is bound to thyroxine-binding globulin [6]. Thus to fully characterize thyroid hormones, a sensitive, selective and rapid analytical method is required to quantify (free and total) thyroid hormones in serum.



Traditionally, High-Performance Liquid Chromatography coupled to a tandem quadrupole Mass Spectrometer (HPLC-MS/MS) utilizing Multiple Reaction Monitoring (MRM) and Isotope Dilution (ID) is one of the most widely used sensitive and selective quantification techniques for small molecules [7-9] and metabolites [1,10] in clinical laboratory environments. These quantification methods are often performed using large 2.1- or even 4.6-mm-i.d.columns, which easily accommodate high flow rates to keep analysis times short but comprise sensitivity. In recent years, the use of 1 mm or smaller capillary HPLC columns with low flow rates has become a common way of enhancing the sensitivity of HPLC-MS/MS methods [11,12]. However, on many conventional HPLC systems, the use of small diameter columns with low flow rates results in longer analysis and re-equilibration times. The main factor that impedes rapid quantitative analysis on capillary HPLC columns at higher flow rates is the inability of most HPLC systems to perform at pressures exceeding 6000 psi. The use of sub-2-µm particle sizes to achieve higher separation efficiency is not realistic because of these pressure constraints [12]. To accommodate pressure limitations at higher flow rates in conventional HPLC, increased column lengths and larger particle sizes are generally utilized [13].

However, a new class of chromatography called Ultra Performance Liquid Chromatography (UPLC) has permitted system pressures to reach as high as 15,000 – 17,000 psi thereby enabling the use of sub-2-µm particle sizes and flow rates as high as 2 mL/min [14]. Efficiency is the primary separation parameter behind UPLC technology since it relies on the same selectivity and retentivity as HPLC. In the fundamental Resolution (Rs) equation:

$$Rs = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k}{k+1}\right)$$

Resolution is proportional to the square root of *N* (theoretical plate number). Since *N* is inversely proportional to particle size (*dp*):

$$N \propto 1/dp$$

As the particle size is lowered by a factor of three, from, for example, 5 µm (HPLC scale) to 1.7 µm (UPLC scale), *N* is increased by three and resolution by the square root of three or 1.7. *N* is also inversely proportional to the square of the peak width:

$$N \propto 1/w^2$$

N illustrates that the narrower the peaks are, the easier they are to separate from each other. In addition, peak height is inversely proportional to the peak width:

$$H \propto 1/w$$

As the particle size is decreased to increase *N* and subsequently *Rs*, an increase in sensitivity is obtained, since narrower peaks are taller peaks. Illustrated below are extracted ion chromatograms for free T_3 and T_4 from rat serum utilizing an Agilent Zorbax Stable Bond

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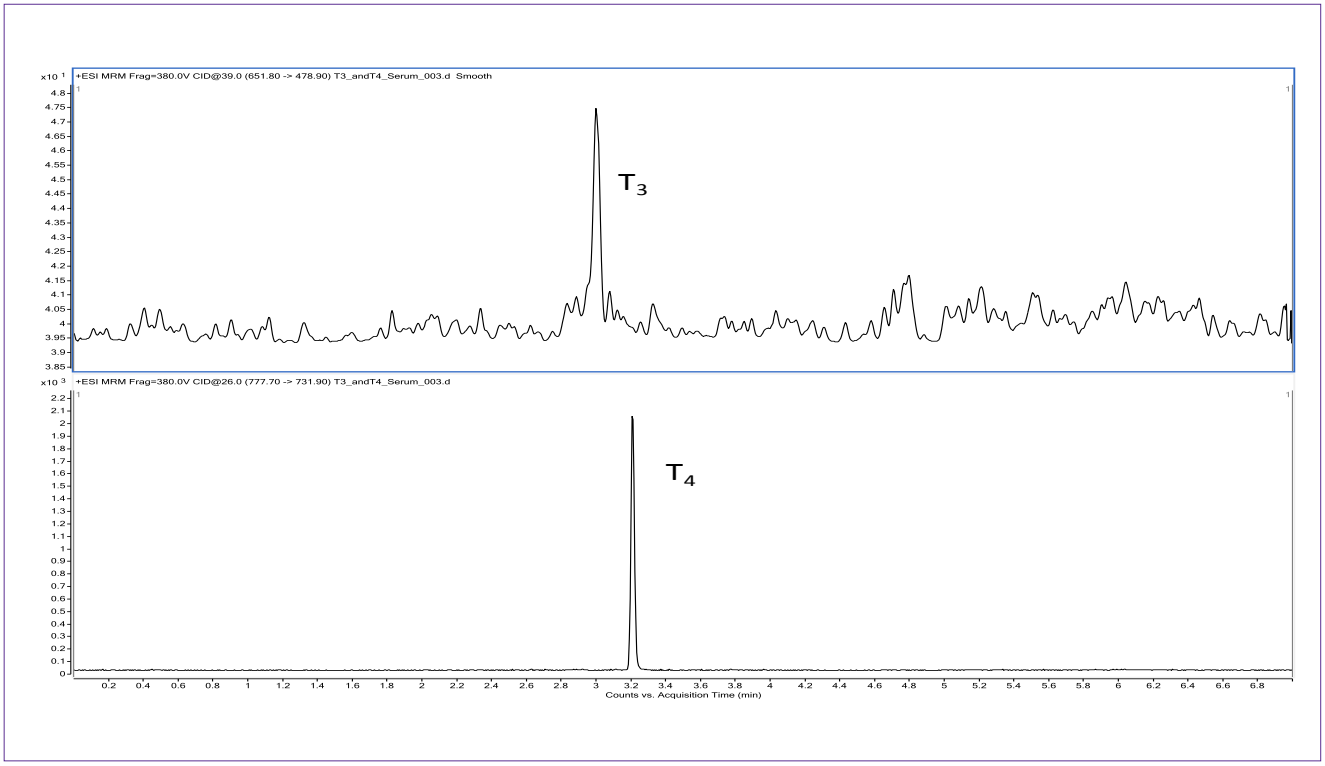

C_{18}, 2.1x50 mm, 1.8 µm particle size columns. Total run time with column wash and re-equilibration was 7-minutes.

In conclusion coupling UPLC to tandem mass spectrometry provides a sensitive, selective and rapid analytical method to characterize and quantify thyroid hormones in serum at relevant concentrations with improved resolution, sensitivity and speed with no comprises.

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