Separation and Low Level Determination of Thyroid Hormones From Human Serum by UHPLC-MS/MS Using a Novel C18-Based Stationary Phase

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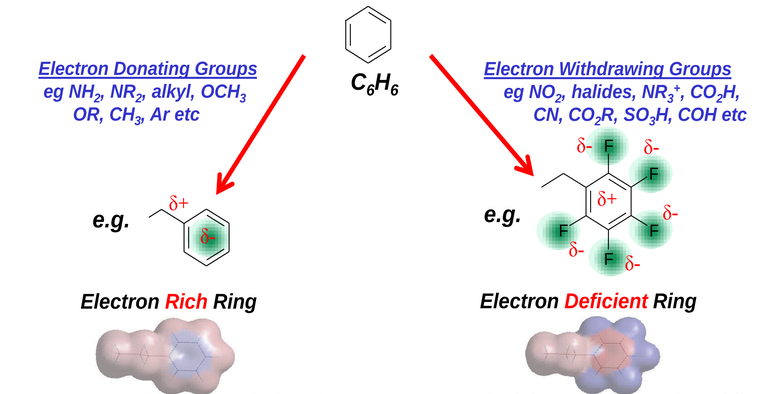
1. BACKGROUND

- Determination of thyroid hormones helps the clinical understanding of a variety of conditions and disease states that include metabolic disorders and depression.
- Analytes of interest include thyroxine (T4) which is the dominant prohormone in humans. The bioactive tri-iodothyronine (T3) results from deiodination of T4 whilst the minor isobaric metabolite reversed tri-iodothyronine (rT3) may also be present.
- A semi-automated solid phase extraction workflow for serum samples was devised. The novel ACE Excel 2µm C18-AR column provided separation with quantification by UHPLC-MS/MS.

2. RATIONAL PHASE DESIGN TO MAXIMISE SELECTIVITY



Robust C18-Based Phases Designed With Ultra-Low Bleed For MS

3. SERUM SAMPLE EXTRACTION & INSTRUMENT CONDITIONS

Format: EVOLUTE® EXPRESS AX 30 mg 96 Well Plate, part number 603-0030-PX01.

MS Conditions: XEVO TQS triple quadrupole (Waters Inc., USA). Desolvation temperature = 500C, Ion source temperature = 150C, positive ions acquired in MRM mode.

Compound	MRM Transition	Cone Voltage (V)	Collision Energy (eV)
T3	651.8 > 605.8 (651.8 > 507.8) (651.8 > 478.9)	50 (50) (50)	22 (22) (35)
rT3	651.8 > 605.8 (651.8 > 507.8) (651.8 > 478.9)	50 (50) (50)	22 (22) (35)
T3/rT3-d6 ISTD	657.8 > 611.8	50	22
T4	777.7 > 731.7 (777.7 > 351.0) (777.7 > 633.8)	50 (50) (50)	25 (45) (23)

Column: ACE Excel 2µm C18-AR, 2.1 x 100 mm
Mobile Phase A: 2mM ammonium acetate / 0.1% HCOOH (aq)
Mobile Phase B: 2mM ammonium acetate / 0.1% HCOOH in MeOH
Flow Rate: 0.4 mL/min
Gradient:

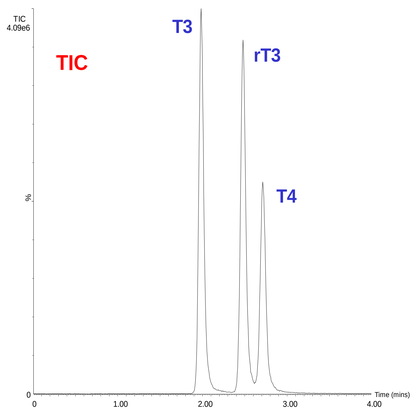
Time	% A	% B
0	40	60
3	23	77
3.1	40	60

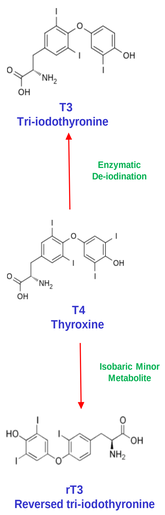
Injection Volume: 10 µL
Temperature: 40C

4. THYROXINE & METABOLITES: LOW LEVEL LC-MS/MS ANALYSIS

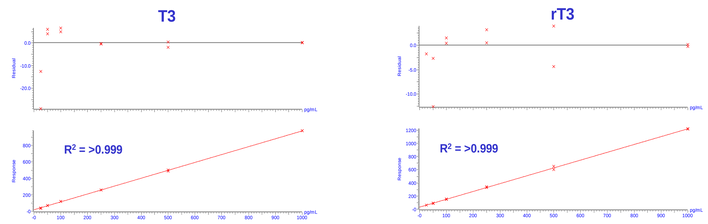
ACE Excel 2µm C18-AR 2µm, 100 x 2.1mm

Sample: 2.5 pg/µL

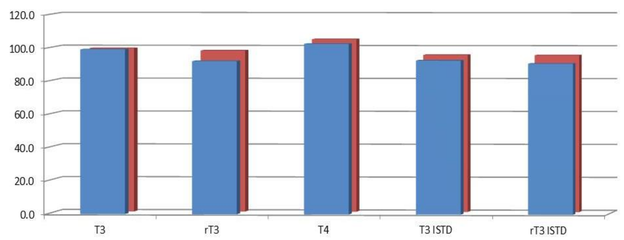




5. EXAMPLE LINEARITY AND RECOVERY DATA



Example spiked serum demonstrating linearity for T3 and rT3 from 25-1000 pg/mL (n=4)



Stripped serum was spiked at various levels from 25-1000 pg/mL. Highly reproducible recoveries > 80% across the range with RSDs < 10% were demonstrated.

6. SUMMARY AND CONCLUSIONS

- Quantification of thyroid analytes is helpful for clinical diagnostics and achievable using UHPLC-MS/MS.
- An extraction protocol using EVOLUTE® EXPRESS AX and a separation method using the novel ACE® Excel C18-AR column were developed to enable low level detection of the thyroid hormones.
- Recoveries were excellent and the method was found to be linear for each thyroid analyte across a wide concentration range of 25-1000pg/mL.