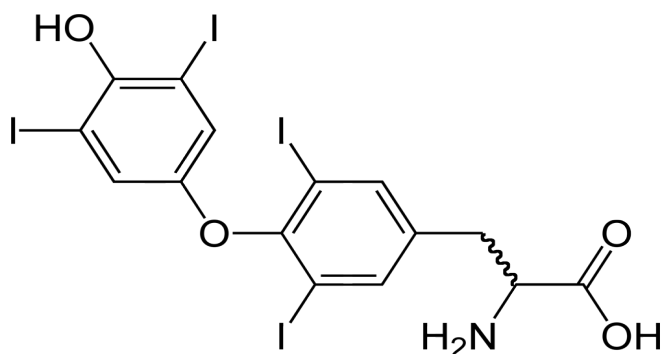


Thyroid Hormones in Serum and Plasma Using SPE Extraction and UHPLC-MS/MS Analysis



UCT Part Numbers

CSDAU203
Clean Screen® DAU
200mg / 3mL SPE Cartridge

SLGRDHLDR-HPOPT
Guard Column Holder

SLDA100ID21-18UM
Selectra® DA LC column
100 x 2.1 mm, 1.8 µm

SLDAGDC20-18UM
Selectra® DA guard column
10 x 2.1 mm, 1.8 µm

Summary:

Thyroid hormones are endogenous hormones that play an important role in many biological processes, including growth, development and metabolism. Most of the thyroid hormones circulating in the blood are bound to proteins and only a small fraction of circulating hormones are in their biologically active free form (unbound). Together with the thyroid stimulating hormone (thyrotropin), the concentrations of circulating thyroid hormones are used to assess thyroid function and diagnose thyroid disease, such as hyperthyroidism and hypothyroidism, or to monitor treatment status. It is therefore essential to have accurate and sensitive analytical methods that are capable of measuring low levels of thyroid hormones in serum or plasma. The thyroid hormones can be measured as free hormones, which are indicators of hormone activity in the body, or as total hormones (free hormones plus protein bound hormones).

This application note describes the development of an analytical method for the sensitive and accurate determination of free and total thyroid hormones in serum and plasma using a mixed-mode SPE extraction procedure and UHPLC/MS analysis. Chromatographic separation, including baseline resolution of triiodothyronine and reverse triiodothyronine, is conducted in 3 minutes using a Selectra® DA column. Ultra-low hormones and steroids serum (Golden West Biologicals, Inc.®) was used to generate accuracy and precision data. The method was evaluated at two concentrations (0.2 and 1 ng/mL) using procedures for free and total hormones. Recoveries of the three hormones ranged from 87 to 100% and reproducibility was $\leq 3.5\%$.



Sample Pretreatment:

Free Hormones:

1. To 1 mL of plasma add 1 mL of 0.1M HCl.
2. Vortex for 30 seconds.

Total Hormones:

1. To 1 mL of plasma add 2 mL acetonitrile.
2. Vortex briefly and let stand for 5 min.
3. Centrifuge at ≥ 1500 rcf for 5 minute.
4. Transfer supernatant to a clean glass tube.
5. Add 2 mL of 0.1M HCl and vortex for 30 seconds.

SPE Procedure:

1. SPE Conditioning

- a) 1 \times 3 mL methanol.
- b) 1 \times 3 mL 0.1M HCl.

2. Sample Extraction

- a) Apply the sample to the SPE cartridge (if required, use a low vacuum to draw the sample through at ≤ 3 mL/min).

3. Wash cartridge

- a) 1 \times 3 mL Ethyl Acetate.
- b) 1 \times 3 mL methanol
- c) Dry cartridges for ~ 30 seconds under a high vacuum to remove excess methanol.

4. Elution

- a) Elute with 1 \times 3 mL methanol containing 2% ammonium hydroxide (MeOH:NH₄OH, 98:2 v/v).
- b) Evaporate the sample to dryness under a gentle stream of nitrogen.
- c) Reconstitute in 100 μ L of methanol and vortex for 1 minute.
- d) Transfer sample to an autosampler vial containing a low volume insert.



LC-MS/MS Parameters:

Instrumentation	
HPLC system	Thermo Scientific™ Dionex™ Ultimate™ 3000 UHPLC
MS system	Thermo Scientific™ TSQ Vantage™ (MS/MS)
HPLC column	UCT Selectra® DA, 100 × 2.1 mm, 1.8 µm (p/n: SLDA100ID21-18UM)
Guard column	UCT Selectra® DA, 10 × 2.0 mm, 1.8 µm (p/n: SLDAGDC20-18UM)
Guard column holder	p/n: SLGRDHLDR
Column temperature	50 °C
Flow rate	400 µL/min
Injection volume	10 µL

Mobile Phase Gradient:

Time (min)	% Mobile Phase A (0.1% Formic Acid in Water)	% Mobile Phase B (0.1% Formic Acid in ACN)
0.0	70	30
2.5	0	100
3.5	0	100
3.6	70	30
6.0	70	30

MRM Transitions (ESI⁺)				
Compound	t_R (min)	Precursor ion	Product ion 1	Product ion 2
Triiodothyronine (T3)	2.61	651.7	605.7	197.0
Reverse triiodothyronine (Rt3)	2.77	651.7	605.8	507.8
Thyroxine (T4)	2.94	777.6	731.7	323.8
Thyronine-13C6	2.94	783.6	731.7	329.9



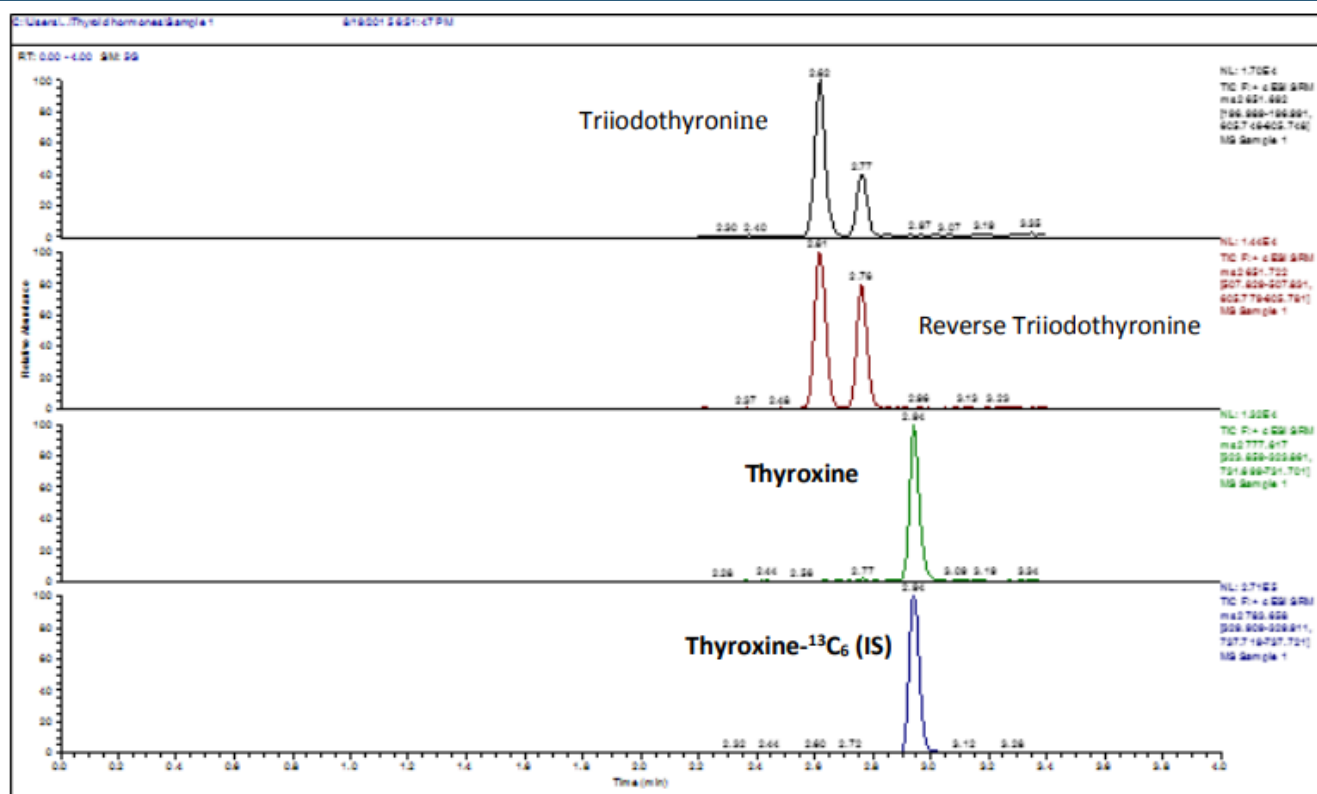


Figure 1: Chromatogram of an extracted plasma sample (0.2 ng/mL).

Results:

Analyte	0.2 ng/mL		1 ng/mL	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Thyroxine	94.4	2.5	95.2	1.4
Triiodothyronine	99.5	3.5	96.5	2.3
Reverse Triiodothyronine	99.8	2.3	91.5	1.8

Table 1: Accuracy and precision data in plasma using free hormone procedure (n=6 each).

Analyte	0.2 ng/mL		1 ng/mL	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Thyroxine	94.7	1.7	92.6	1.5
Triiodothyronine	96.5	0.8	91.1	3.5
Reverse Triiodothyronine	95.2	2.0	87.1	2.2

Table 2: Accuracy and precision data in plasma using total hormone procedure (n=6 each).

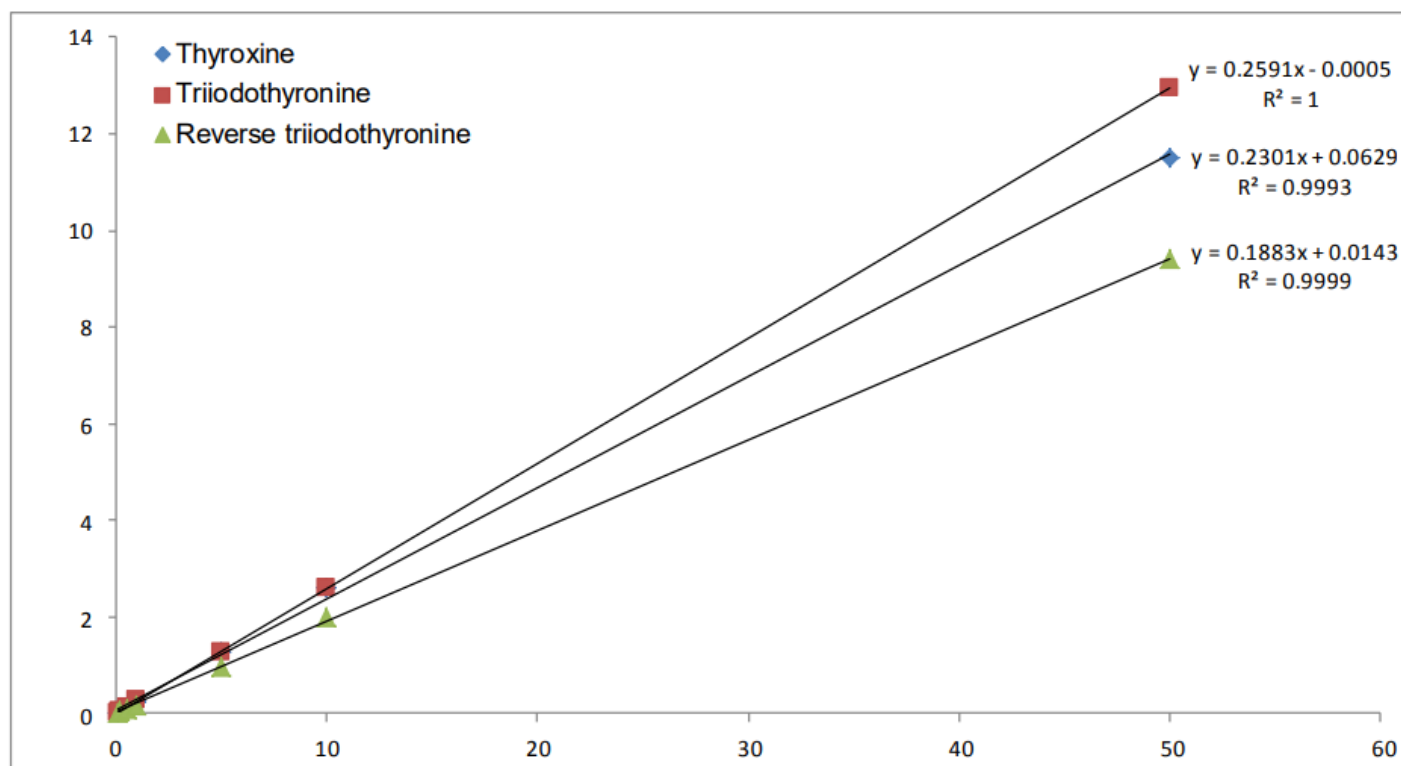


Figure 2: Example of extracted matrix-matched calibration curves (0.1, 0.2, 0.5, 1, 5, 10, and 50 ng/mL).

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