PO-CON1351E

SHIMADZU
Excellence in Science

Sensitive assay of free thyroid hormones by on line SPE-UHPLC-MS/MS in human plasma

ASMS 2013 WP-098

Maureen RAMERO[1], Stéphane MOREAU[1] and Mikaël LEVI[1]
[1]Shimadzu France SAS, Le Luzard 2, Boulevard Salvador Allende, 77448 Marne la Vallée Cedex 2

Sensitive assay of free thyroid hormones by on line SPE-UHPLC-MS/MS in human plasma

1. Introduction

Thyroid hormones are essential hormones involved in growth regulation and development. Free circulating thyroxin concentrations are constant and are correlated with the tissue hormone level. Highly sensitive and specific methods are needed because most circulating thyroid hormone is bound to plasma proteins and only a few pg/mL are in the unbound free form.

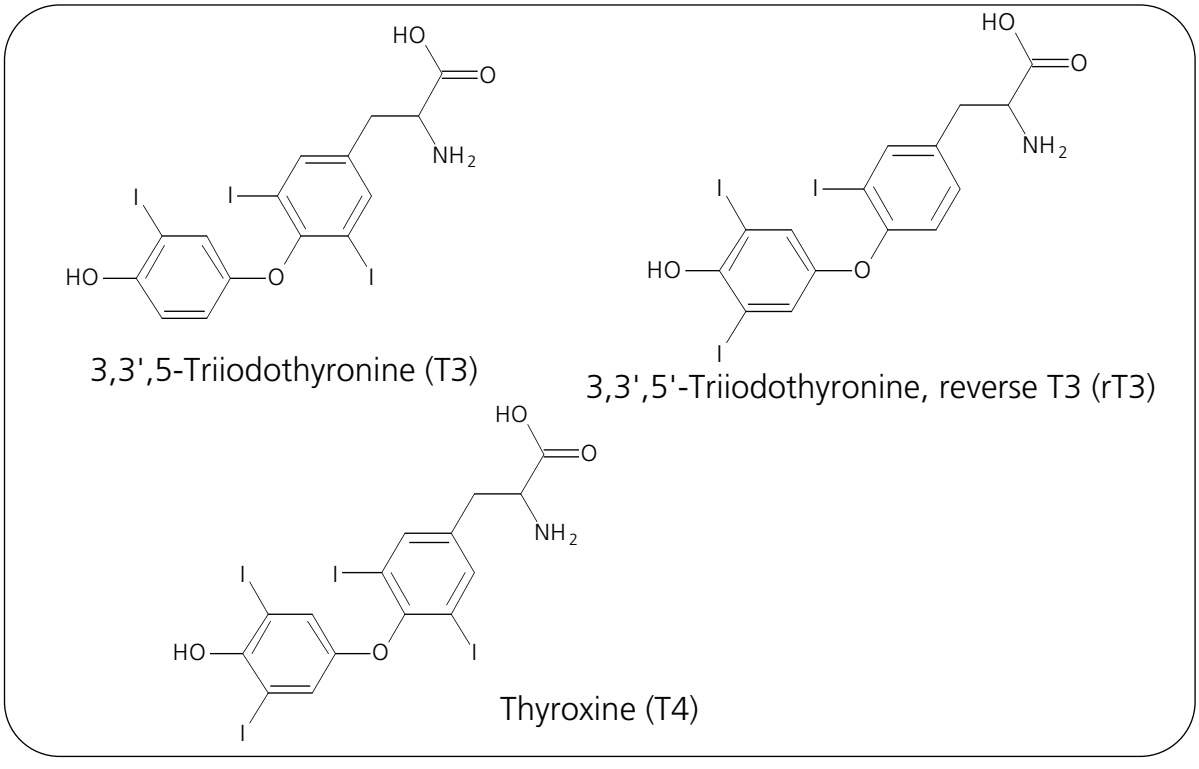

Fig. 1 Structure of studied thyroid hormones

2. Methods and Materials

Thyroxine (T4) and 3,3',5-Triiodothyronine (T3) were assayed by online SPE-UHPLC-MS/MS.
3,3',5'-Triiodothyronine, reverse T3 (rT3) is isobaric with T3, it was not assayed but was monitored in order to control that only T3 was effectively quantified. Free thyroid hormones were first isolated from plasma proteins by ultrafiltration. Then filtrate was injected on online SPE column. Free thyroid hormones were then separated and assayed by UHPLC-MS/MS.

Ultrafiltration

500 µL of plasma were ultra filtrated using an centrifree device (Millipore) at 2000 g for 20 minutes. About 150-200 µL of ultrafiltrate were obtained. 120 µL of ultrafiltrate were transferred to a vial with glass insert. Internal standards ($^{13}C_6$ analogues) were added at a final concentration of 10 pg/mL.

Sensitive assay of free thyroid hormones by on line SPE-UHPLC-MS/MS in human plasma

Online Solid Phase Extraction

Column : Phenomenex STRATA-XC 20 × 2 mm
Mobile phases : Sample loading (A): Water + 0.1% formic acid
Washing (B): Methanol/acétonitrile/formic acid (50/50/0.1 v/v)
Column cleaning (C): Methanol/acetonitrile/isopropanol/water/TFA (25/25/25/25/0.1 v/v)
Flow rate : 2 mL/min
Time program : 0-0.5 min : 100% A sample loading
0.5-1 min : 100%B interferences elution
1-1.5 min : column backflush with UHPLC mobile phase B'
1.5-3.5 min: 100%C column cleaning
3.5 – 8.5 min100% A column conditioning
Injection vol. : 100 µL

UHPLC conditions (Nexera MP system)

Column : Phenomenex Synergi Fusion-RP C18 50 × 2 mm 2.5 µm
Mobile phase A : Water + 0.05% Ammonia
B : Acetonitrile + 0.05% Ammonia
Flow rate : 0.4 mL/min
Time program : B conc. 10%(0-1.5 min) -10 to 100%(1.5 – 5.5 min) - 10%(5.51 - 8.5 min)
Column temperature : 30°C

MS conditions (LCMS-8040)

Ionization : ESI, Negative MRM mode
Ion source temperatures: Desolvation line : 250°C
Heater Block : 400°C
Gases : Nebulization : 3 L/min
Drying : 15 L/min

MRM Transitions:

Compound	MRM	Dwell time (msec)
T3	649.70>126.90 (Quan)	20
	649.70>632.75 (Qual)	20
T4	775.50>126.90 (Quan)	20
	775.50>604.80 (Qual)	20
rT3	649.50>126.90 (Quan)	20
	649.50>604.80 (Qaul)	20

Pause time : 3 msec
Loop time : 0.164 sec (minimum 15 points per peak for each MRM)

Sensitive assay of free thyroid hormones by on line SPE-UHPLC-MS/MS in human plasma

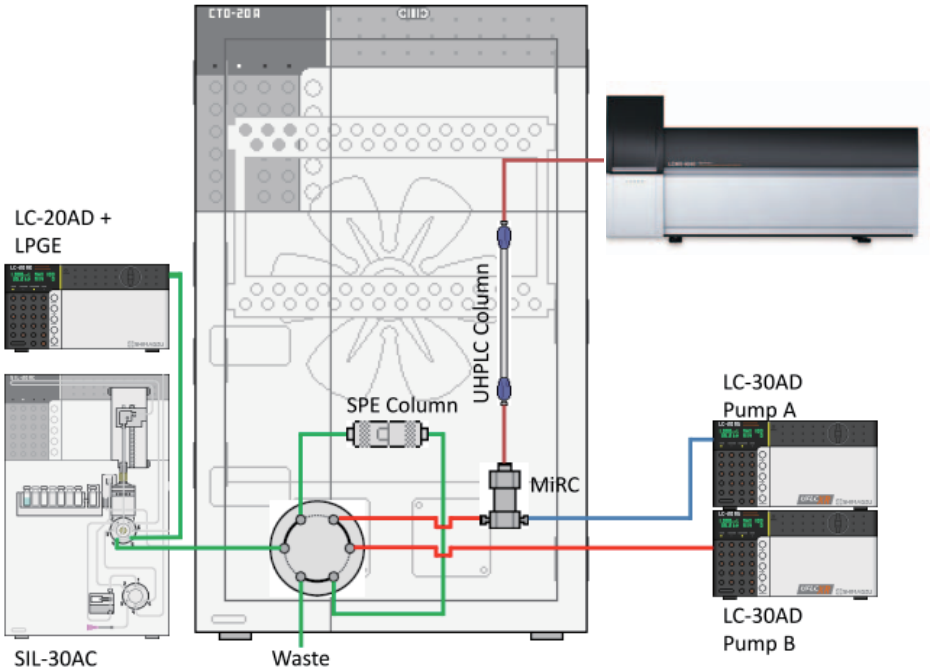

Fig. 2 Scheme of the analytical system

3. Results

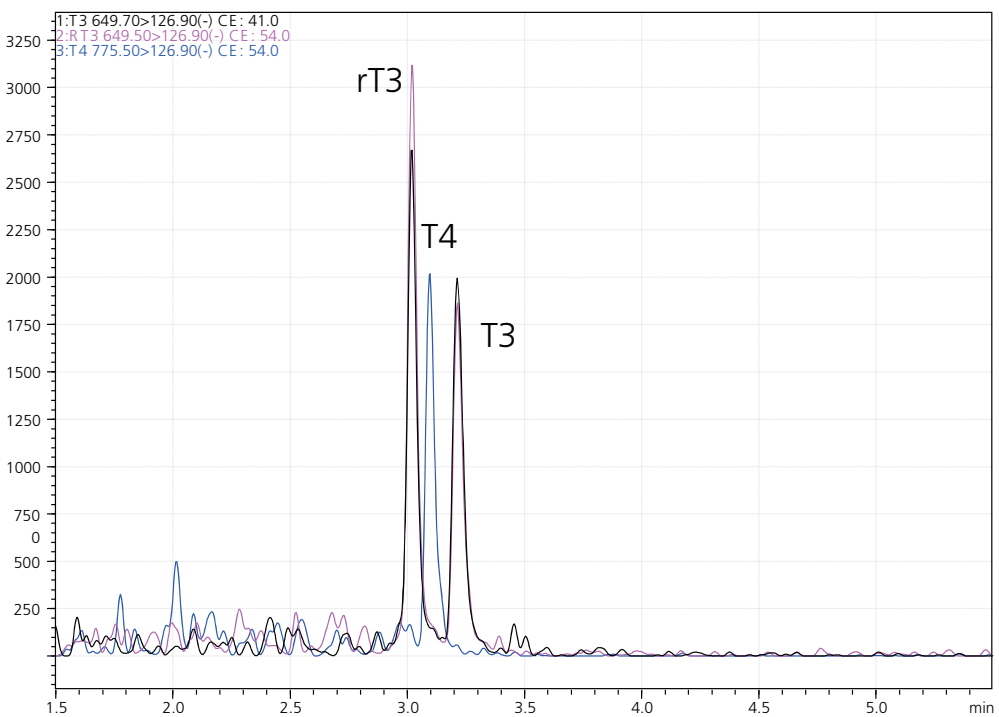

Fig. 3 Typical patient sample with hormone levels of about 10 pg/mL

Sensitive assay of free thyroid hormones by on line SPE-UHPLC-MS/MS in human plasma

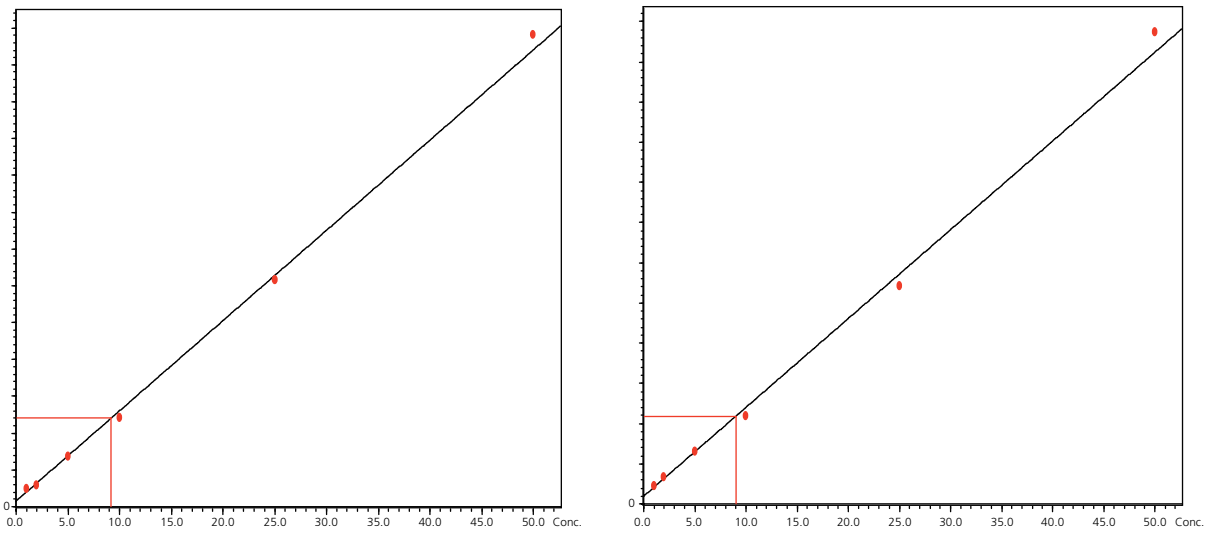

Fig. 4 Calibration curves of T3 (left) and T4 (right) from 1 to 50 pg/mL

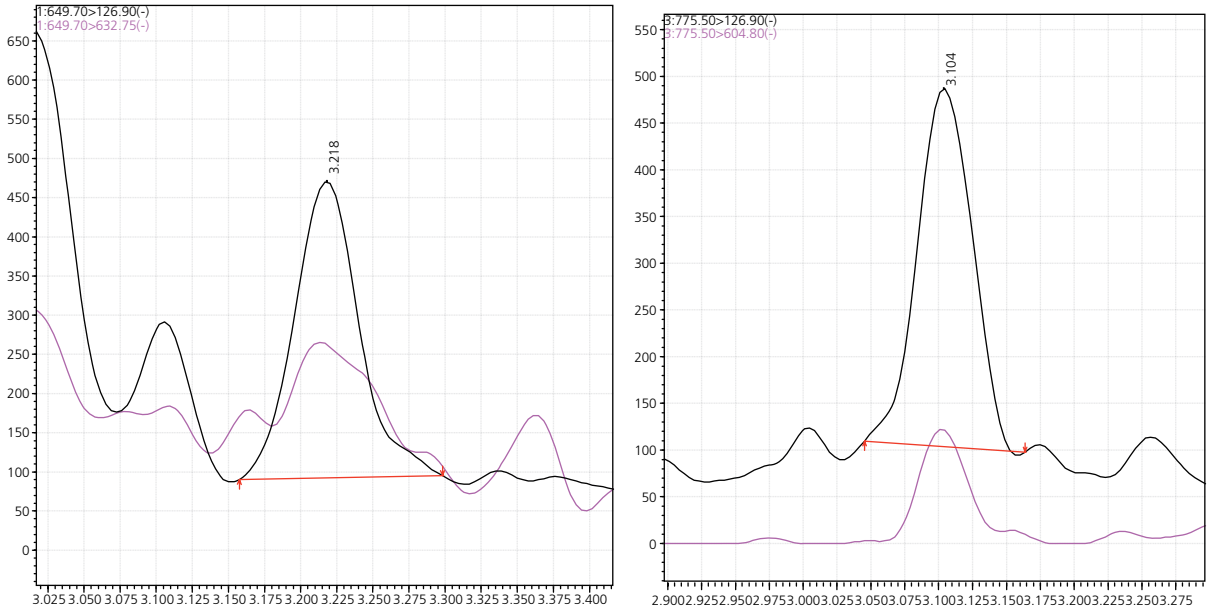

Fig. 5 Lowest calibration point for T3 (left) and T4 (right) at 1 pg/mL

Sensitive assay of free thyroid hormones
by on line SPE-UHPLC-MS/MS in human plasma

4. Conclusions

- The method enable fast and sensitive assay of free thyroid hormone levels in plasma sample,
- The sensitivity required to measure low levels is obtained and the matrix effect are limited by the use of a mixed-mode (cation exchange and hydrophobic) on line SPE column,
- The purification of small volumes of ultra filtrate is facilitated by on line SPE,
- The monitoring of the rT3 is required to be sure that there is no bias in T3 quantification.

First Edition: June, 2013

SHIMADZU

Shimadzu Corporation
www.shimadzu.com/an/