Journal of Chromatography A, xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Determination of thyroid hormones in placenta using isotope-dilution liquid chromatography quadrupole time-of-flight mass spectrometry

Zhong-Min Li[a,*], Florian Giesert[b], Daniela Vogt-Weisenhorn[b,c], Katharina Maria Main[d], Niels Erik Skakkebæk[d], Hannu Kiviranta[e], Jorma Toppari[d,f], Ulla Feldt-Rasmussen[g], Heqing Shen[h], Karl-Werner Schramm[a,i], Meri De Angelis[a]

[a] Helmholtz Zentrum München-German Research Center for Environmental Health (GmbH), Molecular EXposomics, Ingolstädter Landstr. 1, 85764 Neuherberg, Germany
[b] Technische Universität München-Weihenstephan, Lehrstuhl für Entwicklungsgenetik, c/o Helmholtz Zentrum München, Ingolstädter Landstr. 1, 85764, Neuherberg, Germany
[c] Helmholtz Zentrum München-German Research Center for Environmental Health (GmbH), Institute of Developmental Genetics, Ingolstädter Landstr. 1, 85764 Neuherberg, Germany
[d] Department of Growth and Reproduction and EDMaRC, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark
[e] National Public Health Institute, Department of Health Security, Kuopio, Finland
[f] Institute of Biomedicine, University of Turku, Department of Paediatrics, Turku University Hospital, Turku, Finland
[g] Department of Medical Endocrinology PE, Rigshospitalet, University of Copenhagen, Denmark
[h] Key Lab of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences,1799 Jimei Road, Xiamen 361021, China
[i] Department für Biowissenschaftliche Grundlagen, Technische Universität München, Weihenstephaner Steig 23, 85350 Freising, Germany

ARTICLE INFO

Article history:
Received 7 September 2017
Received in revised form
18 December 2017
Accepted 18 December 2017
Available online xxx

Keywords:
Thyroid hormones
Placenta
Liquid chromatography
Quadrupole-time-of-flight mass spectrometry
Solid phase extraction
Matrix effect

ABSTRACT

The transplacental passage of thyroid hormones (THs) is of great significance since the maternal THs are vitally important in ensuring the normal fetal development. In this paper, we determined the concentrations of seven THs, viz. L-thyroxine (T_4), 3,3′,5-triiodo-L-thyronine (T_3), 3,3′,5′-triiodo-L-thyronine (rT_3), 3,3′-diiodo-L-thyronine (T_2), 3,5-diiodo-L-thyronine (rT_2), 3-iodo-L-thyronine (T_1) and 3-iodothyronamine (T_1AM), in placenta using isotope dilution liquid chromatography quadrupole time-of-flight mass spectrometry. We optimized the method using isotopically labeled quantification standards ($^{13}C_6$-T_4, $^{13}C_6$-T_3, $^{13}C_6$-rT_3 and $^{13}C_6$-T_2) and recovery standard ($^{13}C_{12}$-T_4) in combination with solid-liquid extraction, liquid–liquid extraction and solid phase extraction. The linearity was obtained in the range of 0.5–150 pg uL^{-1} with R^2 values >0.99. The method detection limits (MDLs) ranged from 0.01 ng g^{-1} to 0.2 ng g^{-1}, while the method quantification limits (MQLs) were between 0.04 ng g^{-1} and 0.7 ng g^{-1}. The spike-recoveries for THs (except for T_1 and T_1AM) were in the range of 81.0%–112%, with a coefficient of variation (CV) of 0.5–6.2%. The intra-day CVs and inter-day CVs were 0.5%–10.3% and 1.19%–8.88%, respectively. Concentrations of the THs were 22.9–35.0 ng g^{-1} T_4, 0.32–0.46 ng g^{-1} T_3, 2.86–3.69 ng g^{-1} rT_3, 0.16–0.26 ng g^{-1} T_2, and < MDL for other THs in five human placentas, and 2.05–3.51 ng g^{-1} T_4, 0.37–0.62 ng g^{-1} T_3, 0.96–1.3 ng g^{-1} rT_3, 0.07–0.13 ng g^{-1} T_2 and < MDL for other THs in five mouse placentas. The presence of T_2 was tracked in placenta for the first time. This method with improved selectivity and sensitivity allows comprehensive evaluation of TH homeostasis in research of metabolism and effects of environmental contaminant exposures.

© 2017 Elsevier B.V. All rights reserved.

1. Introduction

Thyroid hormones (THs) are a class of tyrosine-based hormones indispensable for normal development and metabolism. The production of L-Thyroxine (T_4) is regulated via the hypothalamic-pituitary-thyroid (HPT) axis. Peripheral deiodination of T_4 by type 1 (D1) and type 2 (D2) deiodinases produces biologically more active 3,3′,5-triiodothyronine (T_3). On the contrary, type 3 deiodinase (D3) converts THs into inactive metabolites, including 3,3′-diiodo-L-thyronine (T_2) and 3,3′,5′-triiodothyronine (rT_3). Besides, D_1 can convert T_3 and rT_3 into 3,5-T_2 (rT_2) or T_2 [1]. T_2 and rT_2, in turn, can give rise to monoiodothyronine (T_1) through further deiodination events. Decarboxylation is also involved in TH metabolism and

Abbreviations: IDL, instrument detection limit; IQL, instrument quantification limit; MDL, method detection limit; MQL, method quantification limit.

* Corresponding author.
E-mail address: zhong-min.li@helmholtz-muenchen.de (Z.-M. Li).

https://doi.org/10.1016/j.chroma.2017.12.048
0021-9673/© 2017 Elsevier B.V. All rights reserved.

Please cite this article in press as: Z.-M. Li, et al., Determination of thyroid hormones in placenta using isotope-dilution liquid chromatography quadrupole time-of-flight mass spectrometry, J. Chromatogr. A (2017), https://doi.org/10.1016/j.chroma.2017.12.048

yields decarboxylated metabolites, including 3-iodothyronamine (T_1AM) [2,3].

THs regulate a bulk of physiological processes and are important biomarkers. T_4 and T_3 are involved in the regulation of protein synthesis, oxygen consumption, carbohydrate metabolism, and fetal neurodevelopment [4]. rT_3 can inhibit the activity of T_3. Thus, the T_3/rT_3 concentration ratio is used to assess peripheral thyroid hormone metabolism [5,6]. T_2 and rT_2 have different activities in different tissues, including suppression of thyroid stimulating hormone (TSH) levels and increase in resting metabolic rate [7,8]. Although the exact contributions of T_1 and T_1AM are unclear, recent studies found that T_1AM administration in mice leads to a hypometabolic state that is opposite to the effects of excess T_4 [9].

During pregnancy, THs of maternal origin can be delivered to the fetus through placenta to support normal fetal brain development [10]. This is particularly important during early pregnancy, when the maternal T_4 is the sole source for the fetus [11]. Even minor changes in maternal TH circulation can produce various adverse outcomes including miscarriage, intrauterine growth restriction, hypertension, preterm birth, and a decreased child IQ [11,12]. Having the capacity to determine different TH analogues in placenta could provide a diagnostic tool and explain the association between maternal TH dysfunction and fetal metabolic abnormalities. Besides, prenatal exposure to environmental chemicals, such as polychlorinated dibenzo-*p*-dioxins and furans (PCDD/F), polychlorinated biphenyls (PCBs) and brominated flame retardants (PBDEs) are suspected to interfere with the maternal TH regulation system, and thereby affect the fetal development [13–16]. Therefore, the determination of a full suite of THs in placenta provides useful information to acquire a better understanding of the endocrine-disrupting properties of environmental chemicals.

Routine assessment of THs has long been achieved by the measurement of THs in blood using immunoassay (IA) methods [17]. This method is of high sensitivity, but lack selectivity due to nonspecific interferences [18–20]. Methods based on tandem mass spectrometry offer a better specificity and accuracy [21], and have been used in various samples such as human and animal serum/plasma and tissues [20,22–29]. Although it is of significant importance, only two methods were reported for TH analysis in human placenta using IA method or liquid chromatography with tandem mass spectrometry technique (LC–MS/MS) [30,31]. In addition, the LC–MS/MS method has certain limitations, for example, it can only give targeted MS/MS spectra and lack full-scan spectra. Recent studies use high resolution MS to obtain a full-scan spectrum of the thyroid hormones (THs) in blood and tissue samples [32,33]. The combination of HPLC with Q-TOF has received fast recognition since it is able to elucidate and screen target and non-target analytes from complex mixtures using extracted ion chromatograms (EIC) [34]. This can be used to identify and characterize the co-eluents that induce matrix effects, which is a valuable information for improving sample clean-up procedures and chromatographic optimization.

In this study, we aim to describe and evaluate an ultra performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF-MS) method which enables the analysis of T_4, T_3, rT_3, 3,3′-T_2, 3,5-T_2, T_1 and T_1AM in placenta. This method incorporates several modifications and improvements based on our previous method for other tissues [33] to obtain higher sensitivity and selectivity. Various sample clean-up techniques were investigated, including protein precipitation, phospholipid depletion and solid phase extraction (SPE). We evaluated this approach through validation of the accuracy, precision, matrix effect, and recovery using fortified samples. Finally, the optimized procedure was tested by analyzing human and mouse placenta samples relevant to TH research.

2. Experimental

2.1. Materials and chemicals

Fig. 1 showed the molecular structures of the investigated THs, quantification and recovery standards. Individual certified stock solutions of T_4, T_3, rT_3, $^{13}C_6$-T_3, and $^{13}C_6$-rT_3 at 100 μg mL^{-1} (dissolved in MeOH containing 0.1 M NH_4OH) were obtained from Sigma-Aldrich (St. Louis, MO, USA). 3,3′-T_2, T_1 and T_1AM were from Sigma-Aldrich (St. Louis, MO, USA). 3,5-T_2 and $^{13}C_6$-3,5-T_2 were from Santa Cruz Biotechnology (Dallas, Texas, USA). $^{13}C_6$-T_4 and $^{13}C_{12}$-T_4 were purchased from Cambridge Isotope Laboratories (Andover, MA). Primary individual stock solutions of these compounds (50 μg mL^{-1} for 3,3′-T_2, T_1, $^{13}C_6$-T_4 and $^{13}C_{12}$-T_4, 100 μg mL^{-1} for 3,5-T_2, T_1AM and $^{13}C_6$-3,5-T_2) were prepared in MeOH containing 0.1 M NH_4OH.

6-Propyl-2-thiouracil (PTU) was from Sigma-Aldrich (St. Louis, MO, USA). All other reagents and solvents were of ACS grade or LC–MS grade. Distilled water was obtained using a water distillation purification system and was used for the preparation of all aqueous solutions. Finally, an antioxidant solution consisting of 10 mg mL^{-1} citric acid monohydrate, L-(+)-ascorbic acid and R, R-dithiothreitol was prepared daily in water.

HybridSPE®-Phospholipid cartridges (30 mg/1 mL) were from Sigma-Aldrich (St. Louis, MO, USA), SampliQ OPT cartridges (60 mg/3 mL), Bond Elut Plexa cartridges (60 mg/3 mL), Bond Elut Plexa PAX cartridges (60 mg/3 mL) and Bond Elut Plexa PCX cartridges (60 mg/3 mL) were all obtained from Agilent Technologies (Santa Clara, CA, USA).

2.2. Tissue collection

We obtained the human placenta samples from a birth cohort study conducted in Finland and Denmark as described earlier [35,36]. The study was handled under the guidelines of the Finnish ethics committee (7/1996) and Danish ethics committee (kF01-030/97).

C57BL/6J mice were obtained from Charles River Laboratories Inc. (Kisslegg, Germany). The animals were housed in a room at 22 °C under a normal 12-h light: 12-h dark cycle, with regular food and water available *ad libitum*. The mice were killed via cervical dislocation. Placentas were dissected, weighted, snap-frozen in liquid nitrogen and kept at −80 °C. The animal studies were handled under the guidelines of the District of Upper Bavaria, Germany. In order to reduce the mice sacrificed, we used human placenta for method development.

2.3. Sample preparation

A 500-mg portion of wet placenta sample was weighted and placed into a 15-mL polypropylene centrifuge tube. After adding 500 μL of MeOH, the sample was homogenized by ultrasonication (Bandelin Electronics, Berlin, Germany) on ice. Next, standards (target analytes and quantification standards) were spiked. Samples fortified with THs were used to optimize and validate the sample clean-up procedure.

For TH extraction and protein precipitation, 1 mL $CHCl_3$ was added, vortexed vigorously, and the sample was kept on ice for 60 min. Afterwards, the mixture was centrifugated at 7000 rpm (5478 × g) for 10 min, and the liquid portion was decanted to another 15-mL tube. This process was repeated twice and the liquid was combined. Thereafter, 800 μL of 0.05% (w/w) $CaCl_2$ solution was added and vortexed. The homogenate was centrifuged (7000 rpm, 10 min), and the supernatant was decanted into a new centrifuge tube containing 300 μL of antioxidant solution. The extraction process was repeated twice. H_3PO_4 was added into the

Please cite this article in press as: Z.-M. Li, et al., Determination of thyroid hormones in placenta using isotope-dilution liquid chromatography quadrupole time-of-flight mass spectrometry, J. Chromatogr. A (2017), https://doi.org/10.1016/j.chroma.2017.12.048

Download English Version:

https://daneshyari.com/en/article/7609007

Download Persian Version:

https://daneshyari.com/article/7609007

Daneshyari.com