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Iodine speciation in dog foods and treats by high performance liquid chromatography with inductively coupled plasma mass spectrometry detection

Robert A. Wilson [a,*], Enrique G. Yanes [a], Robert J. Kemppainen [b]

[a] U.S. Food and Drug Administration (FDA), Forensic Chemistry Center (FCC), Cincinnati, OH 45237-3097, USA
[b] Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, Auburn, AL 36849, USA

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ABSTRACT

An analytical method for determination of the iodine species 3-monoiodotyrosine (MIT), iodide, 3,5-diiodotyrosine (DIT), 3,5-diiodothyronine (3,5-T2), 3,5,3′-triiodothyronine (T3), and thyroxine (T4) in dog foods and treats is reported. Iodine speciation was carried out using a HPLC method capable of both anion-exchange and reversed-phase retention coupled with inductively coupled plasma mass spectrometry detection (LC-ICP-MS). The method was evaluated by the analysis of the iodine species concentrations in twelve dog foods and treats following enzymatic digestion. The concentrations of MIT, iodide, DIT, T3, and T4 in the samples ranged from 0.64–59.5 μg/g, 0.86–4.05 μg/g, <MDL–74.7 μg/g, <MDL–4.66 μg/g, and <MDL–29.2 μg/g, respectively. The average recoveries based on sample fortification for MIT, iodide, DIT, T2, T3, and T4 were 97%, 114%, 89%, 102%, 90%, and 80%, respectively. An additional analysis was done for DIT, T3, and T4 in the enzyme digest by LC-triple-quadrupole mass spectrometry (LC–MS/MS) to cross-validate the results obtained by LC-ICP-MS. Both methods were in good agreement for the concentrations of DIT, T3, and T4.

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1. Introduction

Iodine, an essential element, is transported into the thyroid gland as iodide and binds with tyrosine present in thyroglobulin to form iodotyrosines. Through a coupling mechanism the precursors 3-monoiodotyrosine (MIT) and 3,5-diiodotyrosine (DIT) combine to form the thyroid hormones 3,5,3′,5′-tetraiodothyronine commonly known as thyroxine (T4) and 3,5,3′-triiodothyronine (T3) [1]. These hormones play an important role in regulating cellular activity, growth, and brain development [2,3]. The most common iodine-associated problems are the product of dietary deficiencies, resulting in goiter (an enlargement of the thyroid gland) and various disorders associated with growth and development commonly referred to as iodine deficiency disorders (IDD) [3]. However, excessive iodine intake has also been shown to lead to iodine-induced hypothyroidism [4] or hyperthyroidism [5,6]. As a result, a recommended dietary allowance (RDA) of 150 μg/day and a tolerable upper intake level (UL) of 1100 μg/day for iodine has been established for adults in the U.S. [7]. For dogs, a RDA of 29.6 μg/kg body weight has been established for iodine [8], and no UL has been set.

The majority of ingested iodine is reduced to iodide in the gut and absorbed. However, some iodine containing compounds, including the thyroid hormones, are not reduced and are absorbed intact. Due to their orally active nature, ingestion of excessive amounts of thyroid hormones has been shown to lead to thyrotoxicosis in humans [9]. Multiple incidents of thyrotoxicosis in humans from gullet contaminated beef in the U.S. [10] lead to a ban on the use of gullet trimmings in foods for human consumption. In general, exogenous thyrotoxicosis in dogs is rare, though consumption of gullet contaminated foods [11,12] and excessive doses of thyroid medication through excessive administration [13] or consumption of feces from medicated pets [14] has been shown to lead to thyrotoxicosis in dogs.

Iodine species specific monitoring in food is important because of the potentially harmful nature of excessive consumption of total iodine, T3, and T4. Total iodine in solids is commonly determined by an alkaline extraction with tetramethylammonium hydroxide (TMAH) [15–21] or an acid digestion [22–25] followed by inductively coupled plasma mass spectrometry (ICP-MS) analysis. An alkaline extraction approach is preferred for iodine because the presence of iodate and iodide in acidic solutions can lead to the for-

* Corresponding author.
E-mail address: Robert.Wilson@FDA.HHS.gov (R.A. Wilson).

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mation of molecular iodine, resulting in analyte loss [26]. T4 and T3 concentrations are routinely determined by radioimmunoassay (RIA) [27], though high-performance liquid chromatography with ultraviolet detection (HPLC-UV) [28–30] and reverse-phase liquid chromatography coupled with ICP-MS detection (RPC-ICP-MS) have also been used [31–33]. For species specific analysis of a solid matrix that may contain thyroglobulin, an enzymatic hydrolysis step is often required to liberate the bound hormones and other analytes [30].

In addition to measuring the orally active thyroid hormones and total iodine, it is beneficial to analyze for the inactive thyroid hormone precursors MIT and DIT, as well as other iodine containing compounds which could potentially be found in samples such as diiodothyronine (3,5 T2 or 3,3′ T2) or 3,3′,5′-triiodothyronine (rT3). The identification of the hormone precursors can be an additional indicator of gullet meat contamination in foodstuffs because approximately 2/3 of the iodine in the thyroid is present as thyroid hormone precursors [2]. Previously, RPC-ICP-MS has been used for the quantification of iodide, the active thyroid hormones T4 and T3, and the inactive precursors in urine, serum, and thyroglobulin [31–33]. However, this approach requires quantification of iodide in the void volume and an additional post column dilution of the sample to reduce the methanol (MeOH) concentration [31,32] prior to analysis by ICP-MS, or multiple analysis at different MeOH concentrations to ensure plasma stability [33].

In this work, a multimode HPLC method capable of both anion-exchange and reversed-phase retention coupled with ICP-MS detection (operated in organic solvent mode) was developed. This method allows for the separation of the inorganic iodine species as well as the organic iodine containing species MIT, DIT, 3,5-T2, T3, and T4 in a single separation without the need for void volume quantification or post column dilution. This method was used for the quantification of the iodine species present in dog jerky treats and food following an enzymatic digestion. A secondary analysis for cross-validation of some organic iodine containing constituents in the dog foods and treats was done by LC-triple-quadrupole mass spectrometry (LC–MS/MS). Additionally, the total concentration of iodine present in the dog jerky was determined by an alkaline extraction followed by ICP-MS quantification. The extraction efficiency of the enzymatic digestion was determined by a comparison of the total iodine concentration from the enzymatic digestion and alkaline extraction. Finally, a mass balance approach was applied to determine the on-column recovery of the iodine species from the enzymatic digestion.

2. Experimental

2.1. Instrumentation

Chromatographic separations for LC-ICP-MS were performed using an Agilent 1100 liquid chromatographic system (Agilent Technologies, Tokyo, Japan). The chromatographic column used was an OmniPac PAX-500 (4 × 250 mm) multimode column (Thermo Scientific, Sunnyvale, CA, USA) with a matching guard column OmniPac PAX-500 (4 × 50 mm). The outlet of the separation column was connected directly to an Agilent 8800 ICP-MS (Agilent Technologies, Tokyo, Japan), which served as the element-specific mass detector for ^{127}I. The instrument was operated in organic solvent ignition mode with platinum interface cones, a brass base, the spray chamber temperature adjusted to −5 °C, a 1.5 mm inner diameter torch, a reduced carrier gas flow of 0.76 L/min, the RF power set to the instrument maximum of 1600 W, and a 20% oxygen in argon mixed gas added to the argon carrier gas flow at an instrument controlled rate of 10% of the carrier gas flow rate. Total iodine was determined by an Agilent 7500ce or 7900 series ICP-MS (Agilent Technologies, Tokyo, Japan). All ICP-MS instrumentation was equipped with a micromist nebulizer and a Scott-type double-pass quartz spray chamber.

The chromatographic separations for LC–MS/MS were performed on an Agilent 1200 Series Liquid Chromatograph (Agilent Technologies, Tokio, Japan) equipped with a binary pump, autosampler and a temperature controlled column compartment. Reverse phase chromatography was performed on a Phenomenex Luna 3 μ C_8 2.0 × 150 mm column (Phenomenex, Torrance, CA, USA). An AB Sciex API 5000 Triple Quadrupole LC–MS/MS mass spectrometer (AB Sciex, Foster City, CA) equipped with a Turbo Spray interface was used for analyte detection.

2.2. Reagents and standards

All reagents used were analytical grade or better. All solutions were prepared in 18 MΩ cm^{-1} doubly deionized water (DIW) generated by a Milli-Q Advantage ultrapure water system (Millipore, Billerica, MA, USA). Ammonium nitrate, ammonium hydroxide, acetonitrile (ACN), MeOH and formic acid Optima LC/MS (all from Fisher Scientific, Fair Lawn, NJ, USA) were used for preparation of mobile phases and samples for LC-ICP-MS and LC–MS/MS. Tetramethylammonium hydroxide 25% w/w (Alfa Aesar, MA, USA) was used for total iodine determination by alkaline extraction. Ammonium bicarbonate, glacial acetic acid (both from J.T. Baker, Phillipsburg, NJ, USA) and the enzyme protease (pronase E) from *Streptomyces griseus* (Sigma-Aldrich, Saint Louis, MO, USA) were used for the preparation of the proteolytic enzyme solution.

Single analyte, 500–2000 μg/g stock standard solutions of T4 (L-thyroxine sodium salt hydrate, Acros Organics, NJ, USA), T3 (3,3′5-Triiiodo-L-thyronine sodium salt, Sigma-Aldrich, Saint Louis, MO, USA), and 3,5-T2 (Sigma-Aldrich, Saint Louis, MO, USA) were prepared by dissolving appropriate amounts of each in MeOH. Single analyte, 300–2000 μg/g stock standard solutions of MIT and DIT (both from Sigma-Aldrich, Saint Louis, MO, USA) were prepared by dissolving appropriate amounts of each in DIW. A 100 μg/mL stock standard of rT3 (Cerilliant, Round Rock, TX, USA) was diluted in mobile phase A and 5% ACN to the desired concentration. A 50 μg/g iodate stock standard solution was prepared by dissolving appropriate amounts of potassium iodate (Fisher Scientific, Fair Lawn, NJ, USA) in DIW. A certified 1000 μg/mL iodide (SPEX CertiPrep, Metuchen, NJ, USA) standard was used for both speciation and total iodine analysis. A 1001 μg/mL iodide (Inorganic Ventures, Christiansburg, VA, USA) check standard traceable to NIST was used for verification of the iodide calibration standard concentrations for total iodine determination.

2.3. Samples

Seventeen dog jerky treats and foods for sale in the US were collected. Three samples identified SJ-1 to SJ-3 were purchased from a local pet food retailer. These samples consisted of two treats identified by the manufacture as lamb jerky treats and one identified as lamb strips. Four lamb jerky dog treats were collected directly from the manufacture and identified as SJ-4 through SJ-7. Ten dog treats and foods were received for independent evaluation following an investigation into exogenous thyrotoxicosis in dogs attributable to consumption of all-meat commercial dog treats [12]. These samples were identified as RK-01 through RK-10 and consisted of five beef, two chicken, one lamb, and two unknown meat-based foods and treats. A total of eight manufactures of dog jerky foods and treats were represented in this work. All of the dog foods and treats were ground in a food processor prior to analysis. A one gram portion of each ground sample was freeze dried for 24 h in a FreeZone 6, freeze dry/shell freeze system (Labconco, Kansas City, MO, US) to determine the moisture content. All sample and NIST SRM concen-