Analysis of iodine and its species in animal tissues

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ABSTRACT
A method allowing the determination of the total iodine content and iodine species in samples of animal tissues using inductively coupled plasma mass spectrometry (ICP-MS) as an element-specific detector was developed. The total iodine content was determined after microwave digestion with 25% (w/w) water solution of tetramethylammonium hydroxide. The detection limit was 26.9 μg kg⁻¹ I, and the accuracy of the determination was proven through the analysis of SRM “Non-Fat Milk Powder,” porcine liver, and Atlantic Cod muscle samples using standard addition methods. The extracts for the speciation analysis were prepared through sample dispersion in water using an Ultra-Turrax® T10. The extraction yields ranged from 46 to 84% for different types of tissues. The determination of the inorganic iodine species was performed using ion-exchange chromatography (PRP X100, mobile phase 100 mmol L⁻¹ ammonium nitrate, pH 7.4) coupled to ICP-MS. A detection limit of 1.1 μg kg⁻¹ I was obtained for both species. The organic iodine species were separated using size-exclusion chromatography (Superdex 75 column, mobile phase 20 mmol L⁻¹ Tris–HCl, pH 7.5) and also detected using ICP-MS. Samples of porcine muscle, liver, kidney and thyroid gland, chicken muscle and liver and Atlantic Cod muscle were analyzed. The porcine thyroid gland and Atlantic Cod muscle samples were the richest in iodine (a more than 10× greater content of iodine than the other samples). With respect to the inorganic species, only iodide was found in the sample extracts. Conversely, many organic iodine species were found in the extracts.

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1. Introduction
Iodine is an essential micronutrient that plays a crucial role in the healthy development of animals.[1] Iodine is obtained especially from food and water. In water, iodine occurs mainly in form of inorganic salts (i.e. iodate and iodide) with a content from tenths to tens of micrograms of iodine per liter; organically bound iodine (e.g. humic substances released from soils) usually represents only a small part.[2] In contrast, the abundance of inorganic and organic iodine species in food depends on its origin. In food with an animal origin, iodine is present in the form of inorganic iodide and is bound to many complex organic compounds (e.g. thyroid hormones, high molecular weight proteins).[3–5] In food with a plant origin, such as forage, hay or vegetables, iodine is primarily present in form of iodide.[6] The bioavailability of iodide for mammals is 96%, while the bioavailability of organic species, such as monoiodotyrosine (MIT), is only 80%.[7] In the case of livestock, iodine is not only received in natural forms, but its intake is also controlled by fortified feedstock. The fortification of feedstock proves to be necessary because the iodine concentration in plants is generally low (0.17–0.70 mg kg⁻¹ dry matter for forage and hay) and also extremely variable depending on the iodine content in soil.[8,9]

Dietary iodine is digested and absorbed in the stomach and small intestine, where most of the ingested iodine is reduced to iodide, which passes into the blood.[10,11] Only amino acids, present in some feed with animal origins, such as 3,3′,5-triiodothyronine (T3) and thyroxine (T4), or short-chain iodopeptides, may be transported intact across the intestinal wall into the blood.[12] From the blood, iodide is transported into the follicular cells in the thyroid gland[13] and subsequently to colloid. There is rapidly oxidized to elemental iodine, which is bound to tyrosine residues in the thyroglobulin molecule to form MIT and diiodotyrosine.[11] These organics species are then either converted to T3 and T4 or deiodinated in follicular cells to form iodide. Thus, released iodide is again transported to the colloids.[13] In addition to thyroid gland iodine, iodide is also concentrated in the salivary glands, gastric mucosa, placenta, ciliary body, plexus choridoeus, and mammary glands. [14] Moreover, iodide can be found in body fluids, such as blood, urine, sweat or breast milk, and in the case of birds also in eggs.[4,15]
The determination of individual iodine compounds is possible using speciation analysis, which is frequently realized by a combination of capillary electrophoresis (CE) or liquid chromatography (LC) as a separation technique and inductively coupled plasma mass spectrometry (ICP-MS) as an element-specific detector.[16,17] However, LC has a wider range of application than CE due to the possible selection of variable stationary phases and mobile phases. Speciation analysis of iodine using LC and ICP-MS coupling, especially in water samples, was recently described by many authors. Zheng [18] analyzed inorganic iodine species in seawater using size-exclusion chromatography (SEC) with mixture of 50 mmol L−1 malonic acid and 37.5 mmol L−1 tetramethylammonium hydroxide (TMAH) as the mobile phase. The same type of sample was analyzed by Chen [19] using ion-exchange chromatography (IEC) with 20 mmol L−1 NH₄NO₃ as the mobile phase. The iodine species in rain and river water were analyzed by Gilfedder [20] using IEC with 35 mmol L−1 NaOH as the mobile phase, Pantsar-Kallio [21] separated inorganic species in drinking water using IEC with KNO₃ and HNO₃ solutions as the mobile phase. The next type of frequently analyzed sample is body fluids. Michalke [22,23] analyzed iodide, thyroid gland hormones, and their precursors in serum and urine using reverse-phase chromatography (RPC) with a gradient mobile phase (10 mmol L−1 Tris–HCl pH 7.3 in 1% methanol, 10 mmol L−1 Tris–HCl pH 7.6 in 50% methanol). The iodine species in serum and urine were also analyzed by Wang [24] using IEC with gradient elution (15 mmol L−1 NH₄NO₃ and 100 mmol L−1 NH₄NO₃, pH 10), by Gika [25] using RPC with gradient elution (0.1% trifluoroacetic acid and acetonitrile), by Han [26] using RPC with gradient elution (tetrabutylammonium hydroxide, L-phenylalanine, deoxycholic acid, and NH₄Cl), and by Makarov and Szpunar [27] using SEC with 30 mmol L−1 Tris–HCl buffer. Milk can also be considered to be a body fluid. The inorganic species in milk were analyzed by Leiterer [1] using IEC with mixture of 3.5 mmol L−1 Na₂CO₃ and 1.0 mmol L−1 NaHCO₃ as the mobile phase, and iodine bound to biomolecules in milk was determined by Fernandez Sanchez [5] and by de la Flor St. Remy [28] using SEC with 30 mmol L−1 Tris–HCl and 0.1 mol L−1 Tris–HCl as the mobile phases.

The preparation of the above mentioned types of samples does not have serious difficulties, whereas water or body fluids can be analyzed directly, after dilution or after centrifugation; in the case of solid samples, it is necessary to prepare a sample extract prior to analysis. Several methods of extraction of iodine species from plant samples for speciation analysis have been described: solid–liquid extraction with NaOH, Tris/HCl, ethanol, HCl, and enzymatic hydrolysis were applied to seaweed samples,[29–32] and a similar extraction procedure (NaOH and HCl) was used for mushrooms.[33] However, in the case of animal tissues, all of the described methods of sample preparation were used almost exclusively for the determination of the total iodine content.[3,8,34,35] Only two papers concerning iodine speciation in animal tissue samples were recently published. In the work of Simon [36], entire bodies of Zebrafish and African clawed frog were homogenized in buffer (250 mmol L−1 sucrose, 10 mmol L−1 Tris at pH 7.4), and after centrifugation, the homogenates were analyzed using RPC–ICP-MS. Gika [25] extracted iodine species from thyroid tissue with a mixture of acetonitrile and NaOH, and the extract was analyzed using the RPC–UV method.

The aim of our research was to prepare the necessary analytical background for the investigation of the influence of animal feed fortified with iodine or with other elements that impact iodine metabolism, for example, selenium is such an element that plays a role in the synthesis of thyroid hormones.[37] To investigate the bioavailability and distribution of iodine in animal tissues, it was necessary to prepare methods for the determination of the total iodine content as well as for the description of iodine speciation in animal tissues. These methods comprise considerable sample preparation, determination of total iodine concentration by ICP-MS, and determination of iodine species using several modes of LC–ICP-MS coupling. The use of IEC and SEC coupled to ICP-MS was chosen because it represents an efficient and sensitive technique for the simultaneous separation and quantification of both inorganic and organic iodine species.

2. Experimental
2.1. Instrumentation
ICP-MS measurements were carried out using an ELAN DRC-e instrument (Perkin Elmer, Concord, Canada) equipped with a concentric nebulizer, a cyclonic glass spray chamber, and a peristaltic pump for sample aspiration. Samples were decomposed using a microwave decomposition unit UniClever (Plazmatronika, Wrocław, Poland) (digestion program: max. power 90 W for 1 min, max. power 105 W for 1 min, max. power 120 W for 1 min, max. power 135 W for 7 min, and 10 min cooling), or they were homogenized using an Ultra-Turrax® T10 basic disperser with an S10N-10G dispersing element (IKA, Germany).

A high-pressure pump Series 200 (Perkin Elmer, Shelton, USA) was used for the mobile phase delivery to the chromatographic column. Two chromatographic columns were optionally used as follows: an ion-exchange column, PRP X100 (250 × 4.6 mm, 5 μm, Hamilton) and a size-exclusion column, Superdex 75 10/300 GL (300 × 10 mm, 13 μm, GE Healthcare Life Sciences) with an optimum fractionation range of 3000–70,000 Da and an exclusion limit of 100,000 Da. The samples and standards were injected via Rheodyne 9010 injectors equipped with PEEK sample loops.
2.2. Reagents

Alkaline digestion was performed with 25% (w/w) solution of tetramethylammonium hydroxide pentahydrate (TMAH) prepared by dissolution of solid TMAH in water (Sigma-Aldrich, Steinheim, Germany). A standard solution of 1000 ± 2 mg L\(^{-1}\) Ge (CertiPur, Merck, Darmstadt, Germany) was used for the preparation of a stock solution of the internal standard containing 5 mg L\(^{-1}\) Ge (IS). A standard solution of iodide (1000 ± 2 mg L\(^{-1}\) I, Analytika, Prague, Czech Republic) and solid potassium iodate (GR ACS, Merck, Darmstadt, Germany) were used for preparation of the calibration solutions. The certified reference material SRM 1549 “Non-Fat Milk Powder” (NIST, Gaithersburg, MD, USA) was used for the method verification. Demineralized water Milli-Q (MilliPore, Bedford, MA, USA) was used for the preparation of all solutions.

Solution of 100 mmol L\(^{-1}\) ammonium nitrate (extra pure, Merck, Darmstadt, Germany) served as the mobile phase for IEC [38]; the pH was adjusted via the addition of a 25% ammonia solution (SupraPur, Merck, Darmstadt, Germany) to a value of 7.4. As a mobile phase for the SEC, a buffer solution of 0.02 mol L\(^{-1}\) tris-(hydroxymethyl)-aminomethane (Fluka-Chemie, Neu-Ulm, Germany), adjusted with HCl (Suprapur, Merck, Darmstadt, Germany) to pH 7.5, was used.[39,40] For the Superdex column calibration, an SEC marker kit (Sigma-Aldrich, Steinheim, Germany) was used as follows: thyroglobulin (669 kDa), apoferritin (443 kDa), β-amylose (200 kDa), alcohol dehydrogenase (150 kDa), bovine serum albumin (BSA) (66 kDa), carbonic anhydrase (29 kDa), cytochrome c (12.4 kDa), and aprotinin (6.5 kDa). A complex of blue dextran (2000 kDa) and BSA was used for determination of the exclusion volume because blue dextran itself cannot be detected by ICP-MS. Other standards used for species identification were iodide (Analytika, Prague, Czech Republic), iodate (Merck, Darmstadt, Germany), 3,3′,5-triiodo-L-thyronine (Sigma-Aldrich, St. Louis, USA), and L-thyroxine (Sigma-Aldrich, Steinheim, Germany).

2.3. Samples

A sample of porcine thyroid gland was obtained from domestic breeding, and all other samples of animal tissues were purchased in a market. The samples were stored at -18 °C until analysis.

The thawed samples (0.5–1 g) were decomposed using microwave digestion with 3 mL of 25% (w/w) TMAH solution in 210-mL PTFE vessels. After cooling, the samples were transferred into 25-mL volumetric flasks. These solutions were filtered through 0.45-μm filters.
The linearity of the response of ICP-MS to both investigated inorganic iodine species was tested in the concentration range 0–100 μg L\(^{-1}\) I. Solutions containing iodide or iodate (0, 20, 40, 60, 80, 100 μg L\(^{-1}\) I, \(n = 2\)) were prepared in a matrix containing TMAH solution. Linearity was proven using Mandel’s test of linearity \([41]\) for both species. Both response lines had almost identical slopes: 0.01201 ± 0.00026 cps for iodate and 0.01247 ± 0.00027 cps for iodide (estimated uncertainties comprise both solution preparation and signal measurement).

The accuracy of the determination was verified through the analysis of SRM 1549 (see Table 2) and using the standard addition method. The porcine liver was the first analyzed sample. The iodine concentration was 54.3 ± 8.2 ng g\(^{-1}\) I. After the addition of iodide to the sample in an amount corresponding to 10 ng g\(^{-1}\) I, an iodine content of 65.6 ± 8.1 ng g\(^{-1}\) I was found. The recovery of the spike was 113 ± 22%.

Similarly, the Atlantic Cod muscle contained 1487 ± 72 ng g\(^{-1}\) I, and after the addition of iodide in an amount corresponding to 300 ng g\(^{-1}\) I, an iodine content of 1801 ± 90 ng g\(^{-1}\) I was found. The recovery of the spike was 105 ± 7%.

### 3.2. Speciation analysis using IEC

This method was used for the determination of inorganic iodine species – iodide and iodate. The separation and quantification were performed using an IEC with 100 mmol L\(^{-1}\) ammonium nitrate as the mobile phase. First, the pH value of the mobile phase was optimized to obtain suitable conditions for separation and detection of the iodine species. Values of 7.4, 8.2, 9.0, and 10.0 were tested. The shift in the retention time caused by the change in the pH was negligible for iodate because it eluted in the dead time. In the case of iodide, the...
minimal dilution of the sample. The porcine liver sample was analyzed during optimization. The sample was chopped using a knife prior to the extraction and then dispersed in water using the Ultra-Turrax® for one minute at 20,500 rpm. A longer duration was not possible because after one minute the mixture began to foam. Table 3 shows that the recovery of the extraction varies slightly (from 69 to 82%, average 76%) depending on the water volume/sample mass ratio; however, it varied for individual ratios, but there was no obvious dependence between recovery and this ratio. Finally, a water volume/sample mass ratio 2:1 was chosen because it allows for minimal sample dilution.

The limits of detection and accuracy of determination were determined using IEC–ICP-MS coupling through the analyses of iodide and iodate solutions \((n = 6)\) with low concentrations (both approximately 0.1 μg L\(^{-1}\)) and were estimated to be triple the standard deviation. It was 0.11 μg L\(^{-1}\) (1.1 μg kg\(^{-1}\) I for 1 g of sample) for both iodide and iodate. The linearity of the calibration curve was tested in the range 0–100 μg L\(^{-1}\) (0, 20, 40, 60, 80, and 100 μg L\(^{-1}\), \(n = 2\)). Mandel’s test proved the linearity for both analyzed species. The accuracy of determination was verified only by analysis using the standard addition method because a certified reference material with a certified content of iodine species is not available. The iodide concentration found in the porcine liver sample was 20.8 ± 3.0 ng g\(^{-1}\). After the addition of iodide to the sample in an amount corresponding to 20 ng g\(^{-1}\), retention times were slightly affected by the pH (Figure 1). In addition, a high pH of the mobile phase leads to a significant depression of the intensity of the measured signal of \(^{127}\)I. This effect was observed for both iodide and iodate and was most likely caused by the ammonia in the mobile phase. To achieve a high pH in the mobile phase, it is necessary to add a relatively large amount of ammonia, which significantly increases the amount of dissolved agents and introduces contaminants into the plasma. The peak areas at pH 7.4 are more than twice as large as at pH 10.0. The same effect occurred to the \(^{72}\)Ge signal in the internal standard, and consequently, the ratio of the analyte signal to the internal standard signal is not affected by the pH of the mobile phase. However, a higher intensity of the individual signals leads to a better signal/noise ratio (S/N), see Figure 2. The S/N values were calculated as signal intensity at the peak maximum divided by the noise range for analyses of solutions containing 50 μg L\(^{-1}\) I. The pH value of 7.4 was chosen as optimum. Moreover, the pH value of most body fluids is very close to this value. Therefore, this choice prevents possible changes in speciation due to changes in pH. It was also taken into account during the preparation of sample, and we preferred water extracts to TMAH extracts.

The sample preparation using an Ultra-Turrax® disperser was optimized to obtain the maximum extraction efficiency of the analyte and, at the same time, a minimal dilution of the sample. The porcine liver sample was analyzed during optimization. The sample was chopped using a knife prior to the extraction and then dispersed in water using the Ultra-Turrax® for one minute at 20,500 rpm. A longer duration was not possible because after one minute the mixture began to foam. Table 3 shows that the recovery of the extraction varies slightly (from 69 to 82%, average 76%) depending on the water volume/sample mass ratio; however, it varied for individual ratios, but there was no obvious dependence between recovery and this ratio. Finally, a water volume/sample mass ratio 2:1 was chosen because it allows for minimal sample dilution.

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3.4. Real samples analyses

Using the developed methodologies, real samples of various animal tissues were analyzed. The total iodine content after microwave digestion and the content of iodide after dispersion using the Ultra-Turrax® were quantified (see Table 4). Additionally, some of the organic species were identified using coupled SEC–ICP-MS.

The Atlantic Cod muscle sample was the richest in total iodine content; its level was greater than the other samples by more than one order of magnitude. This result met the expectations because saltwater fishes are usually characterized by high iodine contents. The porcine thyroid gland was the next richest sample. This result was also predictable because it is the organ where the primary metabolism of iodine occurs. The liver and kidney samples contained higher amounts of iodine than the muscle samples (approx. twice as much) with the exception of bovine muscle. The increased content of iodine in the liver sample is also caused by its metabolism here. A small portion of the iodine is distributed from the liver to the bile, and the remainder passes through the kidneys into the urine and is excreted from the body, which explains the relatively high content of iodine present in the porcine kidney.

The water-soluble iodine obtained through the analysis of extracts after dispersion of the samples in water was lower than the total iodine content obtained after microwave digestion. The efficiency of the procedure was quite different for individual tissue samples (38–84%). The analyses of the extracts using IEC–ICP-MS found only iodide, whereas the presence of iodate was not confirmed. This result is consistent with the fact that iodate is immediately reduced to iodide in the gastrointestinal tract. Moreover, if the sample extracts were spiked with iodate (10 μg L⁻¹ I), it was totally or partially reduced to iodide depending on the type of tissue (Figure 4). The rate of reduction, measured immediately after iodate addition, was 100% for porcine liver and porcine kidney, 47% for porcine muscle (leg), 75% for chicken muscle (breast) and 24% for Atlantic Cod muscle. The reduction was most likely caused by glutathione or other thiol group-containing proteins. The reduction rate corresponded with the published [44] high content of glutathione in liver (guinea-pig 0.29%; rat 0.23%; rabbit 0.33%) and kidneys (guinea-pig 0.19%; rat 0.18%; rabbit 0.15%), and with the low content of glutathione in muscle (guinea-pig 0.037%; rat 0.033%; rabbit 0.038%).

The highest concentration of iodide was found in the Atlantic Cod muscle extract. Iodide represents almost one hundred percent of soluble iodine content here. A somewhat lower abundance of iodide was found in porcine muscle and chicken muscle (72 ± 16% and 61.1 ± 7.4%, respectively) and in porcine liver and turkey.
CHEMICAL SPECIATION & BIOAVAILABILITY

The estimation of the iodine concentration in individual chromatographic fractions.

First, the thyroid gland was analyzed. Based on the metabolism of iodine in the thyroid gland, the presence of iodide, thyroglobulin, thyroid hormones, and their precursors could be expected in the extract. The first peak (see chromatogram in Figure 5(A)) at $t_R = 16.92$ min belongs to excluded species in the high molecular region. This retention time corresponds to thyroglobulin. The other high molecular weight fraction eluted at $t_R = 20.53$ min is identical to the BSA standard. However, in animal bodies, there are three transport proteins with close molecular weight: albumin (66 kDa), transthyretin (55 kDa), and thyroid binding globulin (54 kDa). Due to the broadening of the peak, it is not possible to resolve these species. We assume that this fraction represents a mixture of all three mentioned species. The chromatographic pattern from $t_R = 30$ to $t_R = 50$ min obtained for the thyroid gland extract (Figure 5(A)) is similar to a water solution of the T3 and T4 standards (Figure 6). Thus, it is probable that T3 and T4 were also present in the original thyroid gland sample, and they underwent hydrolysis during sample preparation or analysis. The last peak in the chromatogram ($t_R = 56.8$ min) corresponds to iodide.

A completely different and very poor pattern was observed for the muscle samples. The major peak in all chromatograms of muscle extracts was assigned to iodide. Moreover, small protein peaks with retention time $t_R = 20.5$ min and a small peak at $t_R = 35.3$ min were observed in the case of porcine, bovine, chicken (Figure 5(B)), and Blue shark muscle. The previously mentioned fraction ($t_R = 35.3$ min) also occurred in the Atlantic Cod muscle extract together with species eluted at retention time $t_R = 42.75$ min and in the Blue shark muscle extract together with species eluted at retention time $t_R = 39.45$ min. It is worth noting that iodide abundance as well as total iodine content in the Atlantic Cod muscle liver (48.2 ± 8.1% and 50.2 ± 6.5%, respectively). A high proportion of iodide was found also in porcine kidneys and chicken gut. The rest of the soluble iodine content can be reasonably attributed to the organic species, although none were recorded even when the analysis was prolonged to forty minutes and 2% methanol was added to the mobile phase. A verification of iodide in the extracts was also performed using coupled SEC–ICP-MS. The results obtained from both methods were in agreement, see Table 4.

Using SEC–ICP-MS, we also identified iodine bound in the organic species and determined its abundance. The real concentrations of the individual organic iodine species could not be correctly evaluated because standards were not available for all species. In addition to the sorption of some species on the stationary phase, another difficulty is caused by the undefined number of iodine atoms in the molecule. Especially in proteins, the number of bonded iodine residues can be quite variable. Therefore, only relative peak area (Table 5) was used for the estimation of the iodine concentration in individual chromatographic fractions.

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![Figure 4. Chromatograms of the chicken muscle extract obtained using ion-exchange chromatography (column PRP X100, 250 × 4.6 mm, 5 μm; mobile phase 100 mmol L⁻¹ NH₄NO₃, pH 7.4): 1-original extract, 2-original extract spiked with iodate.](image-url)

Table 4. Determination of the total iodine and recovery of sample homogenization.

| Tissue                          | Total iodine after microwave digestion (ng g⁻¹) | Water-soluble iodine (ng g⁻¹) | Extraction yield (%) | Iodide abundance determined using IEC (%) | Iodide abundance determined using SEC (%) |
|--------------------------------|-----------------------------------------------|------------------------------|----------------------|------------------------------------------|------------------------------------------|
| Porcine liver                  | 51.4 ± 4.8                                    | 43.2 ± 3.6                   | 84 ± 10              | 48.2 ± 8.1                               | 35 ± 11                                  |
| Porcine kidney                 | 103 ± 11                                       | 61.9 ± 5.7                   | 60.2 ± 8.6           | 71 ± 16                                  | 78 ± 13                                  |
| Porcine muscle (leg)           | 34 ± 13                                        | 26.9 ± 5.2                   | 80 ± 35              | 72 ± 16                                  | 74.2 ± 7.1                               |
| Porcine thyroid gland          | 513 ± 160                                      | 370 ± 91                     | 72 ± 28              | 38 ± 16                                  | 30.0 ± 7.9                               |
| Chicken liver                  | 68.3 ± 9.4                                     | 31.5 ± 3.9                   | 46.1 ± 8.5           | 35.3 ± 8.6                               | 51.9 ± 8.5                               |
| Chicken muscle (breast)        | 29 ± 13                                        | 14.30 ± 0.75                 | 50 ± 22              | 61.1 ± 7.4                               | 56.4 ± 4.4                               |
| Chicken heart                  | 25.4 ± 2.1                                     | 21.1 ± 1.7                   | 82.9 ± 9.4           | 39.8 ± 2.4                               | 63.5 ± 2.9                               |
| Chicken gut                    | 123 ± 16                                       | 46.8 ± 1.5                   | 38.1 ± 5.1           | 66.8 ± 6.7                               | 57.2 ± 2.4                               |
| Turkey liver                   | 105.5 ± 7.3                                    | 83.5 ± 9.5                   | 79 ± 11              | 50.2 ± 6.5                               | 51.9 ± 2.1                               |
| Bovine muscle (short loin)     | 86 ± 12                                        | 33.3 ± 7.1                   | 38.7 ± 9.8           | 40.3 ± 1.6                               | 52.3 ± 3.7                               |
| Atlantic Cod muscle            | 1484 ± 72                                      | 1006 ± 75                    | 67.8 ± 6.0           | 96.3 ± 9.9                               | 97 ± 12                                  |
| Blue shark muscle              | 50.6 ± 4.7                                     | 36.2 ± 4.7                   | 72 ± 12              | 34.7 ± 1.4                               | 30.5 ± 6.9                               |

*Based on 3 individual determinations, expanded uncertainty.

*bResult ± combined uncertainty of water-soluble iodine and of total iodine after microwave digestion.

*bBased on three individual determinations, combined uncertainty of water-soluble iodine and of iodide content in water-soluble iodine.

*bBased on two individual determinations, combined uncertainty of water-soluble iodine and of iodide content in water-soluble iodine.
molecular weight fraction at $t_R = 20.5$ min (Figure 5(C)).
Moreover, some other fractions were found in extracts: fractions at $t_R = 22.58$ min, $t_R = 24.60$ min, $t_R = 28.28$ min and a small fraction at $t_R = 29.75$ min. While the first one ($t_R = 22.58$ min) was also found in the porcine kidney extract, the others were not present in any other samples. Thus, it is most likely a metabolic product specific to the liver metabolism of iodine compounds.

The chromatographic profile of iodine in porcine kidney extracts (Figure 5(D)) is very similar to the porcine liver extract. Moreover, one high molecular weight species at $t_R = 15.27$ min was observed. In the extract of chicken gut, one high molecular weight species at $t_R = 16.60$ min, a fraction at $t_R = 20.43$ min, and iodide were found. Abundance of iodide was approximately 60% because whole gut was analyzed, not only mucosa where iodide is concentrated. Similar chromatographic pattern was observed for the extract of chicken heart. Two high molecular weight species at $t_R = 16.47$ min and $t_R = 19.85$ min and iodide were found.

4. Conclusion
The developed methodology allows for the reliable determination of the total iodine and iodide contents in animal tissues, even at the very low concentrations and in the Blue shark muscle is very different although both tissues originate from sea fishes.

The liver samples contained the minor iodide. Furthermore, they contained high molecular weight fractions at $t_R = 14.8$, $t_R = 15.5$, $t_R = 16.2$, and $t_R = 19.7$ min and a middle molecular weight fraction at $t_R = 20.5$ min (Figure 5(C)).

Figure 5. Chromatograms of tissues obtained using size-exclusion chromatography (column Superdex 75 10/300 GL, 300 × 10 mm, 13 μm; mobile phase 0.02 mol L$^{-1}$ Tris–HCl, pH 7.5): (A) porcine thyroid gland, (B) chicken muscle, (C) chicken liver, and (D) porcine kidney.

Figure 6. Chromatogram of thyroxine dissolved in water (1) and 3,3′,5-triiodo-L-thyronine dissolved in water (2) obtained using size-exclusion chromatography (column Superdex 75 10/300 GL, 300 × 10 mm, 13 μm; mobile phase 0.02 mol L$^{-1}$ Tris–HCl, pH 7.5).
Table 5. Relative peak areas and estimated molecular weights M of iodine species eluted using size-exclusion chromatography.

| Tissue                        | $t_R$ (min) | $M$ (kDa) | Relative peak areaa (%) |
|-------------------------------|-------------|-----------|-------------------------|
| Porcine liver                 | 16.17       | >100      | 15.3 ± 8.4              |
|                               | 20.53       | 78.4      | 26.0 ± 4.5              |
|                               | 22.58       | 26.6      | 18.0 ± 3.7              |
|                               | 29.75       | <3        | 11.9 ± 1.1              |
|                               | 57.48       | b         | 28.8 ± 6.7              |
| Porcine kidney                | 15.27       | >100      | 6.16 ± 0.38             |
|                               | 16.53       | >100      | 7.4 ± 1.6               |
|                               | 20.47       | 80.9      | 19.9 ± 1.1              |
|                               | 22.33       | 30.4      | 15.8 ± 5.1              |
|                               | 56.97       | b         | 50.8 ± 3.2              |
| Porcine muscle                | 20.47       | 80.9      | 32.3 ± 2.5              |
|                               | 56.73       | b         | 67.7 ± 3.4              |
| Porcine thyroid gland         | 16.92       | >100      | 14.3 ± 3.4              |
|                               | 20.53       | 78.4      | 9.85 ± 0.57             |
|                               | 33.33       | <3        | 8.1 ± 3.2               |
|                               | 35.18       | b         | 10.2 ± 1.6              |
|                               | 42.42       | b         | 8.7 ± 1.4               |
|                               | 56.80       | b         | 46.2 ± 2.9              |
| Chicken liver                 | 15.47       | >100      | 33.3 ± 2.3              |
|                               | 20.60       | 75.6      | 25.7 ± 1.5              |
|                               | 56.63       | b         | 41.0 ± 3.3              |
| Chicken muscle                | 20.58       | 76.4      | 25.0 ± 2.1              |
|                               | 35.13       | b         | 19.6 ± 2.3              |
|                               | 56.67       | b         | 55.5 ± 2.2              |
| Chicken heart                 | 16.47       | >100      | 9.24 ± 0.19             |
|                               | 19.85       | >100      | 42.0 ± 2.9              |
|                               | 57.42       | b         | 48.8 ± 2.3              |
|                               | 16.60       | >100      | 18.2 ± 1.0              |
|                               | 20.43       | 82.4      | 22.2 ± 1.2              |
|                               | 58.78       | b         | 59.6 ± 6.8              |
| Turkey liver                  | 14.83       | >100      | 21.1 ± 1.1              |
|                               | 19.72       | >100      | 35.42 ± 0.84            |
|                               | 24.60       | 9.2       | 4.60 ± 0.17             |
|                               | 28.28       | <3        | 4.70 ± 0.21             |
|                               | 57.73       | b         | 34.2 ± 2.3              |
| Bovine muscle (short loin)    | 20.73       | 70.4      | 41.7 ± 6.9              |
|                               | 57.48       | b         | 58.3 ± 9.7              |
| Atlantic Cod muscle           | 35.33       | b         | 2.14 ± 0.24             |
|                               | 42.75       | b         | 1.41 ± 0.27             |
|                               | 57.53       | b         | 96.5 ± 3.9              |
| Blue shark muscle             | 20.57       | 76.8      | 22.4 ± 2.3              |
|                               | 35.60       | b         | 22.2 ± 2.3              |
|                               | 39.45       | b         | 18.4 ± 1.8              |
|                               | 57.73       | b         | 37.0 ± 2.3              |

*aBased on two individual determinations, expanded uncertainty.

*bThe estimation is out of the range of the calibration curve.

Frequently present in many tissues. Moreover, it also enables an estimate of the abundance of organic iodine species. Therefore, this methodology may be used for the evaluation of the impact of feed fortification on animal health and also for the examination of iodine bioavailability in foodstuffs with an animal origin.

Microwave digestion using 25% TMAH was proven to be an effective technique for the quantitative extraction of iodine and can be used for the determination of the total iodine content. The efficiency of iodine extraction into water using an Ultra-Turrax® disperser varied from 38 to 84% for different tissues. However, for the speciation analysis, this method represents a useful tool because proteins are not denatured by high temperature. The speciation analysis using IEC–ICP-MS found only iodide in the extracts; iodate was not present, and no organic species were eluted in this way. Conversely, SEC–ICP-MS coupling revealed many iodine-containing organic compounds in the extracts.

Disclosure statement

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