APPLICATION NOTE 44475

Determination of iodine-containing species in seaweed using IC-ICP-MS

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Goal

To demonstrate the use of a hyphenated IC-ICP-MS method for the quantitative determination of different iodine-containing species extracted from seaweed

Introduction

Iodine (I) is an essential element for humans and animals, as it is important for synthesis of thyroid hormones and hence normal growth and development[1]. Insufficient iodine intake may result in various health problems, which are well known. Much less emphasis is given on excessive iodine intake, which can also be harmful especially for people with a history of iodine deficiency[2].

Seaweeds are a rich natural source of iodine and can contain extremely high amounts of iodine (up to 8 g·kg^{-1} dry weight)[3]. Consumption of seaweed may therefore result in an excessive iodine intake. The possibilities for a reduction of iodine content in seaweed and seaweed products therefore need to be investigated.

Iodine can be present in inorganic forms (iodide, iodate) and organic forms [predominantly 3-iodo-L-tyrosine (monoiodotyrosine, MIT) and 3,5-diiodo-L-tyrosine dihydrate (DIT)]. The iodinated amino acids MIT and DIT are mainly integrated in proteins and are more difficult to release without enzyme involvement, while inorganic iodine species are more accessible and release may therefore be easier in environments in which enzymes are absent[4]. Knowing which iodine species are present in the seaweed may therefore assist in developing new tools and procedures to enable reduction of the iodine content. However, literature on methods for iodine speciation is limited.

The present work describes the optimization and validation of a new method for the determination of iodine species in seaweed by ion chromatography (IC) hyphenated to inductively coupled plasma mass spectrometry (ICP-MS; IC-ICP-MS). Different types of seaweed were included in the validation where LOD, LOQ, linearity, repeatability, spike recovery, and recovery of the total iodine were evaluated.

Experimental

Four seaweed samples were used for validation. NIST™ SRM 3232 (Kelp powder *Thallus laminariae*) is a standard reference material certified for the total iodine content[5].

EURL-MN PT-2019-01 was a material used in a proficiency test organized by the European Union Reference Laboratory for Metals and Nitrogenous Compounds in Feed and Food (EURL-MN), with an assigned concentration value for total I content[6]. Two other samples were used in validation, a sample of *Saccharina latissima* and retail Nori sheets that are used for making sushi. For the latter two, total iodine concentration was determined in this laboratory using the standard ICP-MS method for iodine determination in food by EN 15111:2007[7]. For all samples, the total iodine content is listed in Table 1. To the best of our knowledge, seaweed material with certified or informative values for iodine species is not available.

Table 1. Samples used for validation with total iodine content

Sample full name	Sample short name[a]	Seaweed species	Total I content (µg·g^{-1})
NIST SRM 3232 (Kelp powder)	NIST 3232	*Thallus laminariae*	944 ± 88[b]
EURL-MN PT-2019-01	PT-2019-01	*Fucus vesiculosus*	393 ± 10[c]
Saccharina latissima	*S. latissima*	*Saccharina latissima*	5141.3 ± 14.3[d]
Nori seaweed sheets for sushi	Nori	N/A[e]	47.7 ± 0.6[d]

[a] Short names are used in this document
[b] From Certificate of Analysis of Standard Reference Material™3232
[c] From Final PT report EURL-MN PT-2019-01
[d] Determined in our laboratory, average ± standard deviation, n = 2
[e] Not available—seaweed species not specified by manufacturer

Because the use of enzymes in the sample preparation procedure causes the transformation of iodate into iodide, iodate was not included in the method validation. Even if it were present in the sample, it would be transformed into iodide during extraction and would be determined as iodide. Therefore, when iodide is mentioned in the samples, it refers to inorganic iodine (sum of iodide and iodate) determined as iodide.

All samples were freeze-dried and ground if they were not already in the dried powdered form. A previously published extraction procedure[4] was used to extract iodine containing species. The extraction solution contained 40 mg pancreatin per 7 mL of 0.2 M KH_2PO_4/0.2 M NaOH buffer, pH 8. An aliquot of 0.2 g of sample was accurately weighed into 13 mL round base polypropylene tubes and mixed with 7 mL of extraction solution, then placed into an ultrasonic bath for 12 h at 50 °C. After extraction, samples were placed on ice to cool and then centrifuged for 15 min at 7000×g.

Supernatants were diluted as needed and transferred into the filter vials (0.45 µm pore size) in which test solutions were analyzed. The measurements were performed on the same day as extraction was finished, and sample extracts were stored at 4 °C between extraction and measurement.

A Thermo Scientific™ iCAP™ TQ ICP-MS was used for analysis in conjunction with a Thermo Scientific™ Dionex™ ICS-6000 Ion Chromatography System, consisting of the following:

- Dionex DP dual pump
- Dionex EG eluent generator
- Dionex DC detector/chromatography compartment
- Thermo Scientific™ Dionex™ AS-AP autosampler

The instrument was tuned regularly using the software-provided autotunes. The hyphenated IC-ICP-MS system was controlled using the ChromControl Plug-in in the Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution™ Software. ChromControl enables full hardware control, method definition and batch analysis of all samples using a single control software. Quantitative evaluation was accomplished using the tQuant feature set included in the Qtegra ISDS Software. An overview of the typical operating parameters can be found in Table 2.

Table 2. Operating conditions for ICP-MS and IC

ICP-MS	
Nebulizer	PFA-LC
Spraychamber	Quartz cyclonic, cooled at 2.7 °C
Injector	2.5 mm i.d., Quartz
Interface	Nickel sampler and nickel skimmer cone with high sensitivity insert
Plasma power	1550 W
Nebulizer flow	1.04 L·min^{-1}
Dwell time	0.1 s
Operation mode	SQ-KED
Monitored element	^{127}I

IC	
Columns	Thermo Scientific™ Dionex™ IonPac™ AG20 (2 × 50 mm) Thermo Scientific™ Dionex™ IonPac™ AS20 (2 × 250 mm)
Column temperature	30 °C
Injection volume	5 µL
Eluent	100 mM KOH in 5% (v/v) MeOH
Eluent source	Thermo Scientific™ Dionex™ EGC 500 KOH cartridge with Thermo Scientific™ Dionex™ CR-ATC 600 Continuously Regenerated Anion Trap Column
Elution	Isocratic
Flow rate	0.25 mL·min^{-1}

The only stable isotope of iodine is ^{127}I, which can normally be detected free from polyatomic or isobaric interferences, so that the use of a single quadrupole mode even without the collision/reaction cell (CRC) being active, would suffice for its analysis. However, kinetic energy discrimination (KED) mode with helium as a collision gas was previously tested (data not shown) and provided lower LODs and LOQs. KED mode was therefore used for performing the described method validation. Iodine may also occur as one of its radionuclides (e.g., ^{129}I or ^{131}I), which can be affected through increased backgrounds caused by xenon impurities in the argon gas used. These isobaric interferences can be removed efficiently using oxygen in triple quadrupole mode. However, since the radioactive isotopes were not subject to study, the use of oxygen was not deemed necessary.

Results and discussion

Different eluent concentrations were evaluated to determine the optimum chromatographic separation, allowing the separation of all relevant iodine species under investigation and their detection with high sensitivity. With an eluent concentration of 50 mM KOH, all peaks eluted in 35 min, but I^- and MIT peaks were not baseline resolved. With 60 mM KOH, MIT and I^- partially co-eluted, but with a reversed elution order compared to 50 mM KOH, i.e., I^- eluting after MIT. When the eluent concentration was increased to 70 mM KOH, MIT and I^- were well separated. At 100 mM the resolution was further improved and the run time decreased to approx. 13 min (Figure 1). Because 100 mM KOH is the highest concentration that can be produced with the eluent generator, higher KOH concentrations were not tested. The comparison is shown in Figure 1. Using a concentration of 70 mM KOH or more as an eluent, the elution order is IO_3^- – MIT – I^- – DIT, while with 50 mM KOH it was IO_3^- – I^- – MIT – DIT. Note, that IO_3^- can only be determined in the absence of enzyme because it causes the transformation of IO_3^- into I^-.

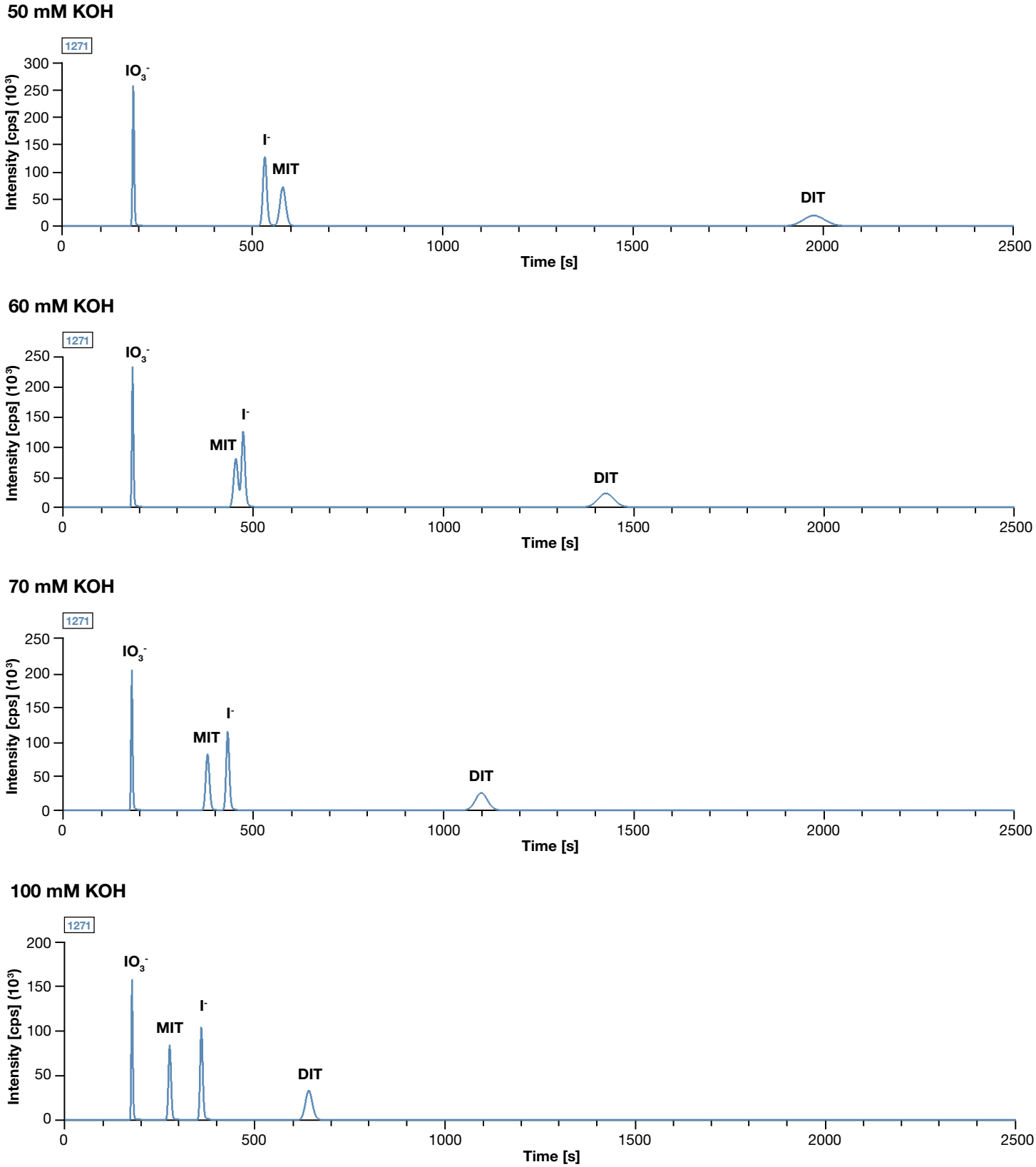

Figure 1. Separation of IO_3^-, I^-, MIT, and DIT standard mix in H_2O (100 ng·mL^{-1} iodine each) with 50, 60, 70, and 100 mM KOH as eluent

The addition of carbon is a well-known way to increase the observable detection sensitivity especially for elements with an elevated 1st ionization potential. Therefore, methanol was added to the eluent. Peaks were approximately doubled in height and area when 5% (v/v) methanol was added into the eluent, compared to 100 mM KOH in water alone. The addition of methanol might be useful if some species in the samples are present in low concentrations and high sensitivity is desired. When adding organic modifiers to the plasma, the use of platinum tipped cones in conjunction with oxygen is normally required; however, for solvents such as alcohols added at concentrations of not more than 5% (v/v), this can be omitted. Methanol in higher concentrations may on the other hand cause deposition of carbon on the cones, which then require more frequent cleaning. All of the following experiments were conducted using the optimized conditions detailed in the method section.

Instrumental limits of detection and quantification were calculated based on peak areas (I^-) or noise (MIT and DIT) in 10 blank samples. For calculation of the detection limit, three times the standard deviation observed in these 10 replicates was used, whereas ten times was taken for calculation of the quantification limit. As the dilution factor was different for each sample, LODs and LOQs were calculated as concentration in measured solution and not in the sample. The results are summarized in Table 3.

Table 3. LODs and LOQs calculated for measured solution

	Concentration in measured solution [ng·mL^{-1} of iodine]		
	MIT	I^-	DIT
LOD	0.013	0.037	0.040
LOQ	0.043	0.125	0.135

Linearity

Calibration standards were prepared in extraction solution in duplicates for each concentration level. Good linearity was found for MIT and I^- in the range between 0.1 and 100 ng·mL^{-1} iodine with $r^2 \geq 0.999$. For DIT, peaks at 0.1 and 0.2 ng·mL^{-1} iodine were difficult to accurately integrate, so linearity was evaluated as satisfactory in the range between 0.5 and 100 ng·mL^{-1} iodine with $r^2 > 0.9999$. The results are shown in Figure 2.

Good repeatability was obtained for all iodine species in all standards/samples. In most cases, the RSDs were ≤2%, except for very low concentrations resulting in smaller peaks (e.g., MIT and DIT in *S. latissima*), where RSDs up to 8.3% were obtained.

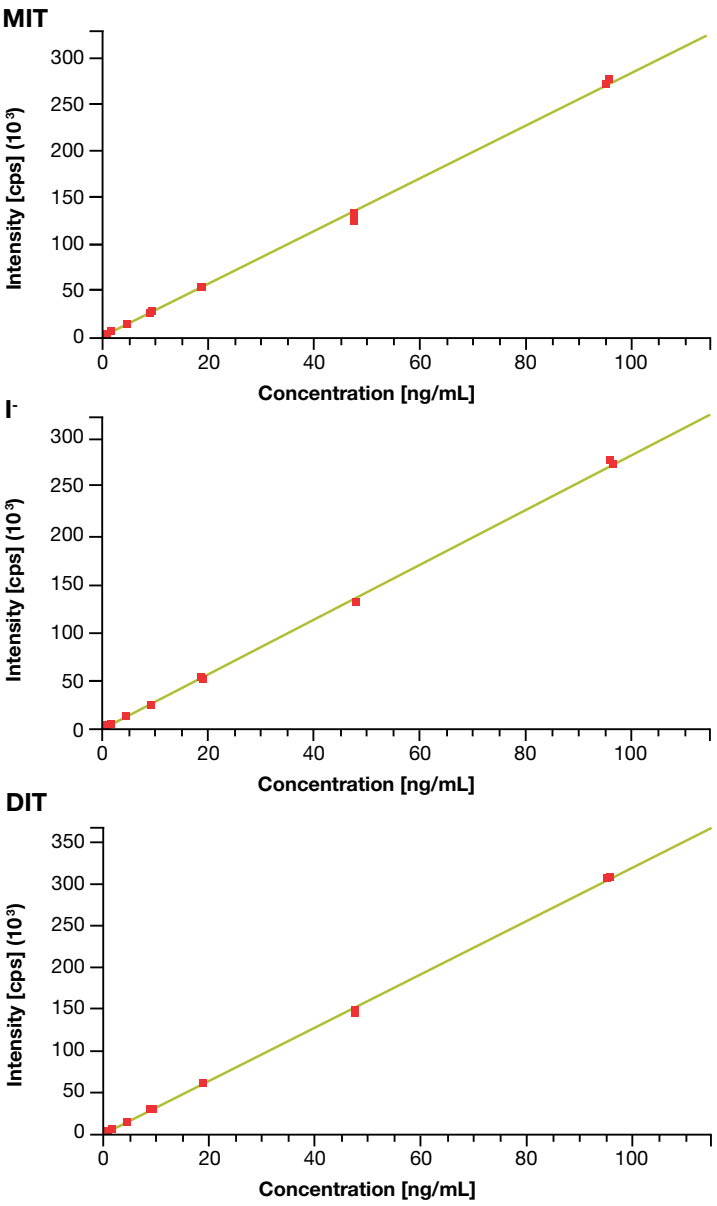

Figure 2. Calibration curves for MIT, I^-, and DIT (0.1–100 ng·mL^{-1} iodine)

Subsequently, all four samples were extracted and injected into the IC-ICP-MS system. Iodide and MIT were found in all samples. DIT was below the detection limit in EURL-MN PT-2019-01, but it was possible to quantify this compound in all other samples, although the concentrations were low and therefore the higher variation observed was expected. Iodide was the most abundant species in all samples except Nori, in which a higher proportion of iodine was found as MIT.

Chromatograms of the samples are shown in Figure 3. Additional peaks were also present in chromatograms but could not be identified by the standards available in our laboratory. These peaks were not quantified and were not included in the calculation of sum of the species and recovery.

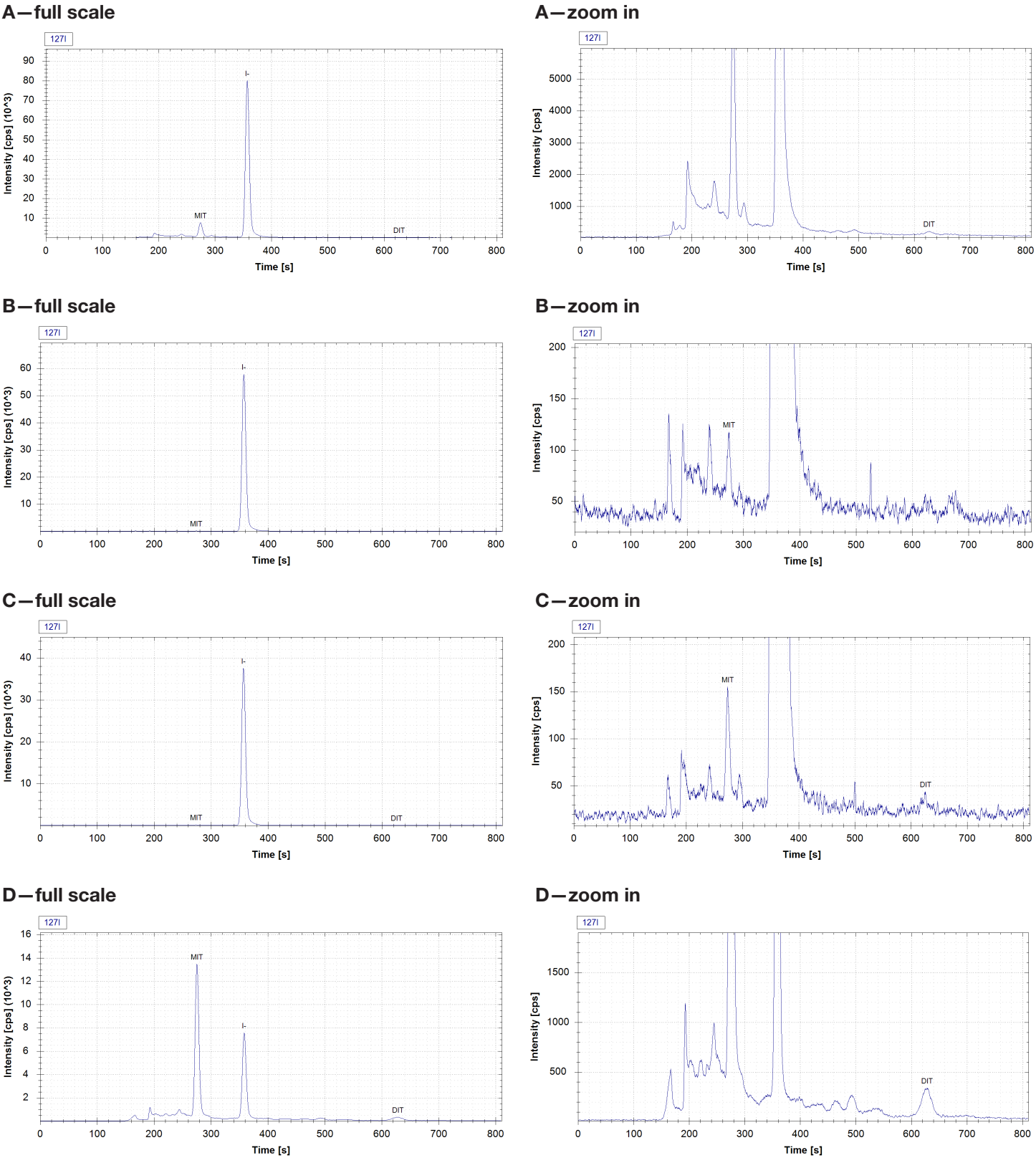

Figure 3. Chromatograms of samples: A—NIST 3232, B—PT-2019-01, C—*S. latissima,* and D—Nori

The concentrations of the species, a sum of the species, and the recovery of the total iodine are listed in Table 4, all of them as an average with standard deviation from three replicates.

Table 4. Iodine species content in different seaweed samples (average ± standard deviation, n = 3)

	µg·g^{-1} Iodine					
	MIT	I^-	DIT	Sum	Total I	Recovery (%)
NIST 3232	32.9 ± 0.4	383 ± 12	1.4 ± 0.1	417 ± 12	944 ± 88	44.2 ± 1.3
PT-2019-01	0.35 ± 0.05	289 ± 11	< LOD	289 ± 11	393 ± 10	73.6 ± 2.9
S. latissima	13.6 ± 0.4	4808 ± 178	4.5 ± 0.4	4826 ± 178	5141 ± 14	93.9 ± 3.5
Nori	12.8 ± 0.5	6.2 ± 0.2	0.65 ± 0.05	19.7 ± 0.7	47.7 ± 0.6	41.2 ± 1.5

The recoveries varied between samples, but they were consistent for the three replicates of each sample. As each sample represents a different type of seaweed, it is possible that iodine species are more easily extracted from some seaweed types than others. However, to investigate an alternative procedure that would provide higher extraction efficiency for all seaweed types was beyond the scope of the present work.

Method accuracy

Method accuracy was determined through recovery of spike studies. Samples were spiked before extraction by adding 0.1 mL of varying concentration of analytes to the sample and extraction solution mixture. The concentrations of analyte standards in the spiking solutions were determined based on previous analysis of samples and were different for each sample. With spike levels 1 and 2, the final concentration was approximately double and triple, respectively, of the endogenous concentration. Where MIT and DIT could not be determined in the samples or the concentration was very low, 1 and 2 µg·mL^{-1} iodine spike solutions were prepared, except for DIT in *S. latissima*, which required a higher fold dilution due to the large amount of I^-. The concentrations of iodine species in spike solutions for each sample are summarized in Table 5. The Nori sample was spiked with all three species together, while other samples were spiked with MIT and DIT separately from I^- so that the extracts were less diluted and, therefore, chromatographic peaks easier to integrate.

Table 5. Concentration of each iodine species (in µg·mL^{-1} iodine) in spike solutions

		µg·mL^{-1} Iodine		
		MIT	DIT	I^-
NIST 3232	Spike level 1	40	1	500
	Spike level 2	80	2	1000
PT-2019-01	Spike level 1	1	1	300
	Spike level 2	2	2	600
S. latissima	Spike level 1	20	10	5000
	Spike level 2	40	20	10000
Nori	Spike level 1	15	1	10
	Spike level 2	30	2	20

The recoveries of spikes were calculated using the following equation:

$$R_{spike}\ (\%) = \frac{(c_{found-spiked} - c_{found-non\text{-}spiked}) \times 100}{c_{spike}}$$

where R_{spike} is recovery of spike in percent, $c_{found-spiked}$ and $c_{found-non\text{-}spiked}$ are concentration (in µg·mL^{-1} iodine) of iodine species determined in spiked and non-spiked sample extracts, respectively, and c_{spike} is concentration in spike solution. The recoveries are summarized in Table 6.

Table 6. Recoveries of spikes (average ± standard deviation, analysis in duplicate)

		Recovery (%)		
		MIT	I^-	DIT
NIST 3232	Spike level 1	95.5 ± 11.6	113.8 ± 7.4	121.3 ± 7.6
	Spike level 2	101.0 ± 13.5	105.5 ± 9.1	89.0 ± 9.2
PT-2019-01	Spike level 1	74.9 ± 7.9	97.6 ± 2.5	100.1 ± 2.1
	Spike level 2	78.7 ± 5.5	97.5 ± 0.4	73.8 ± 0.8
S. latissima	Spike level 1	92.1 ± 11.0	102.5 ± 9.3	112.2 ± 9.8
	Spike level 2	102.5 ± 7.4	115.8 ± 0.1	110.3 ± 0.6
Nori	Spike level 1	90.9 ± 2.9	92.0 ± 1.6	95.0 ± 1.3
	Spike level 2	90.2 ± 0.9	96.5 ± 1.4	89.5 ± 1.9

The recoveries were between 74 and 121%, with variation from 0.1 to 13.5%. As expected, the variation was, in general, higher at lower concentrations.

Conclusion

This application note describes a method by which three iodine species (MIT, DIT, and iodide) were determined in 13 min by IC-ICP-MS. The determination of iodine species consisted of a previously published extraction procedure and a newly developed IC-ICP-MS method based on anion exchange chromatography using potassium hydroxide produced by eluent generation.

Low LODs (0.013–0.040 ng mL^{-1} iodine) in measured solution and LOQs (0.043–0.135 ng mL^{-1} iodine) were obtained by the 3-fold and 10-fold standard deviation approach. The response of the detector was linear in the range between 0.1 (0.5 for DIT) and 100 ng mL^{-1} iodine ($r^2 \geq 0.999$). Except for the lower analyte concentrations, for which repeatability of measurement was up to 8.3 %, the repeatability was primarily below 2%. The recoveries of spikes were between 73.8 and 121.3% and the recovery of total iodine (sum of the species compared to total iodine) were between 41 and 94%, showing that the extraction efficiency varies among different seaweed species. Other peaks were also present in the chromatograms of all four samples but could not be identified due to lack of available standards.

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