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Towards tributyltin quantification in natural water at the Environmental Quality Standard level required by the Water Framework Directive

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ABSTRACT

The European Union (EU) has included tributyltin (TBT) and its compounds in the list of priority water pollutants. Quality standards demanded by the EU Water Framework Directive (WFD) require determination of TBT at so low concentration level that chemical analysis is still difficult and further research is needed to improve the sensitivity, the accuracy and the precision of existing methodologies. Within the frame of a joint research project "Traceable measurements for monitoring critical pollutants under the European Water Framework Directive" in the European Metrology Research Programme (EMRP), four metrological and designated institutes have developed a primary method to quantify TBT in natural water using liquid–liquid extraction (LLE) and species-specific isotope dilution mass spectrometry (SSIDMS). The procedure has been validated at the Environmental Quality Standard (EQS) level (0.2 ng L^{-1} as cation) and at the WFD-required limit of quantification (LOQ) (0.06 ng L^{-1} as cation). The LOQ of the methodology was 0.06 ng L^{-1} and the average measurement uncertainty at the LOQ was 36%, which agreed with WFD requirements. The analytical difficulties of the method, namely the presence of TBT in blanks and the sources of measurement uncertainties, as well as the interlaboratory comparison results are discussed in detail.

1. Introduction

Organotin compounds (OTCs) are well known global pollutants, toxic for living organisms at extremely low concentration levels. They are responsible for endocrine disruption, genetic disorder, shell deformation, mammalian reproductive and metabolic disorders and act as obesogen [1]. The most toxic forms are the tri-substituted derivatives [2]. Since 1980s their effects on dogwhelk, oyster, mussel, gastropods and other invertebrates have been studied. Due to the persistence of OTCs in the environment their presence in living organisms and bioavailability to the food chain is still a current issue [3,4].

After the 2008 global ban of the use of tributyltin (TBT) as antifouling paint on large vessels, the decrease of TBT concentrations in sediments, water, marine mammals and low-ertrophic-level species such as mussels and oysters has been observed in the coastal environment, e.g. [5–9]. Although TBT concentrations are decreasing, historical contamination in the water body is still important. As an example, TBT exceeded the quality standards up to 4400 fold in Belgian harbors, coastal and estuarine water samples [10]. In 2009, TBT concentrations were extensively at, or below, the Environmental Quality Standards (EQS) for waters of the English Channels [11]. 52 of the 206 water samples of a Norwegian harbor, contained TBT above the limit of detection (LOD), which varied from 0.5 to 1.0 ng L^{-1} [12]. The European Water Framework Directive 2000/60/EC (WFD) and Directive 2013/39/EU defined specific measures for pollution control and EQS levels for priority substances, expressed as annual average values (AA-EQS) and maximum allowable concentrations (MAC-EQS). The MAC-EQS of TBT compounds was set up at 0.2 ng L^{-1} (as tributyltin cation). The WFD daughter directive 2009/90/EC

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Table 1
Characteristics of existing methodologies for TBT quantification in diverse types of water.

Water medium	LOD (ng L^{-1})	LOQ (ng L^{-1})	Repeatability (%)	Recovery (%)	Analytical technique	Reference
Seawater	1.2	–	11	103 ± 7	SPME-GC-FPD	[32]
Freshwater	0.001	–	16	–	SPME-GC-ICP-MS	[33]
Seawater	0.00005–0.002	–	–	95–105	LLE-PTV-GC-ICP-MS	[34]
Lake water	50	–	3–6	89–102	SPME-HPLC-ES-MS	[35]
Mineral water, waters through PVC pipes	14.4	48	< 10	104 ± 9	SPE-ETAAS	[36]
Milli-Q water	0.2	–	19	80	SPME-GC-PFPD	[37]
Seawater	16.8	–	20	76–100	SPME-GC–MS	[38]
Deionized water	7.3	–	4	–	SDME-GC–MS	[39]
Pure water, seawater	0.4	–	11	97 ± 9.7	LPME-GC–MS-MS	[40]
Buffered Milli-Q water	0.4	1.3	2–9	–	SPME-ID-GC–MS	[41]
Seawater	–	3.3–40	–	93 ± 12	SPE (imprinted polymers) GFAAS or GC-FPD	[42]
Seawater	35,000	79,000	15–31	72–87	LC-APCI-MS	[43]
Seawater	0.8	–	3–6	96–113	SDME-GC-ICP-MS	[44]
Milli-Q water or Estuarine water	0.8	–	4–17	80	SBSE-TD-GC–MS	[45]
River water, seawater	0.2	–	2–5	94–105	DLLME-GC-FPD	[46]
Lake water, seawater	20	–	13	81–92	SPE-LC-ESI-MS	[47]
Seawater, waste water	0.03–1	–	< 20	–	SPME-GC–MSSPME-GC-FID	[48]
Tap water, freshwater, seawater	7	–	–	85–123	MCR-ALS-EEFMs	[49]
Harbor waters, waste water, tap water, mineral water	0.01	0.05	11–19	92–111	SBSE-GC–MS-MSGC-GC–MS-MS	[20]
Seawater, ultrapure water	0.2	0.5	4.8	90–99	LLE-GC-ICP-MS	[25]
Seawater	0.8	2.5	< 16U (k=2): 41%	67–123	SBSE-LC-MS-MS	[22]
Freshwater, seawater	–	1000	7–25	70–130	SPME-GC–MS-MS	[50]
Ultrapure water, river water	23	77	< 9	68 ± 3	SBSE-LVI-GC–MS	[51]
Mineral water	–	0.06	13	90–110	SPE-GC-ICP-MS	[21]
Mineral water	–	0.06	3–22	80–120 at LOQ	LLE-GC-ICP-MS	This work
Tap water, freshwater, groundwater, seawater	**MAC-EQS:0.2 ng L^{-1}**	**LOQ: 0.06 ng L^{-1}**	***U*(k=2) at EQS: ≤ 50%**	–	–	**2015 EU WFD**

Analytical techniques: SPME: Solid Phase Micro Extraction; FPD: Flame Photometric Detection; PTV: Programmed Temperature Vaporization; HPLC: High Performance Liquid Chromatography; ES: Electrospray; ETAAS: Electrothermal Atomic Absorption Spectrometry; PFPD: Pulsed Flame Photometric Detection; SDME: Single-Drop Microextration; LPME: Liquid-Phase Microextraction; GFAAS: Graphite Furnace Atomic Absorption Spectrometry; APCI: Atmospheric Pressure Chemical Ionization; SBSE: Stir Bar Sorptive Extraction; TD: Thermal Desorption; DLLME: Dispersive Liquid–Liquid Microextraction; ESI: Electrospray Ionization; FID: Flame Ionization Detector; MCR-ALS-EEFMs: Excitation–Emission Fluorescence Matrices processed by Multivariate Curve Resolution/Alternating Least-Squares; LVI: Large Volume Injection; LLE: Liquid Liquid Extraction.

demands technical specifications for analytical methods with a limit of quantification (LOQ) equal to or lower than 30% of the EQS, and a measurement uncertainty (95% confidence) of 50% or less at EQS. Analytical methodologies for TBT are well represented and reviewed in the literature [13,14], but concentrations required by quality standards are so low that chemical determination is from the analytical point of view still very difficult [15–18]. Current commonly used analytical methods show typical LOQ in the 1–10 ng L^{-1} concentration range [15]. The standardized analytical method proposed by the ISO standard 17353:2004 [19] allows quantification of TBT at about 10 ng L^{-1}, which is two orders of magnitude above the EQS. Table 1 gives an overview of the state of the art for TBT quantification in diverse types of water samples. TBT recoveries are in the 70–130% range. Most of the studies report only the limit of detection (LOD) of the methodologies. It is worth noting that, when the LOD is determined as three times the standard deviation of the noise, no information is given on the extend of procedural blanks, which is of the utmost importance at such low concentration levels. Between papers giving the LOQ of the method, only two works report a LOQ that meets WFD requirements [20,21]. Repeatability varied between 2% and 30%. Repeatability depends on the concentrations at which it was evaluated and it is hardly comparable across the different methodologies. Only one study reports a measurement uncertainty of 41%, with a repeatability lower than 16%, which shows that the repeatability represents just one contribution to the uncertainty budget [22]. There is a need for further research to improve the sensitivity of existing methodologies, to obtain results by applying the validated methods at EQS and LOQ levels of concentration demanded by the WFD, and to assess the uncertainty associated to the measurements, not only the method repeatability.

The purpose of this paper is to present a primary method for the quantification of TBT in water using liquid-liquid extraction (LLE) and species-specific isotope dilution mass spectrometry (SSIDMS). A primary method of measurement is a reference measurement procedure used to obtain a measurement result without relation to a measurement standard for a quantity of the same kind [23]. The method was developed in natural mineral water and validated at EQS (0.2 ng L^{-1}) and LOQ (0.06 ng L^{-1}) concentration levels, by four designated and metrological institutes within the frame of a joint research project "Traceable measurements for monitoring critical pollutants under the European Water Framework Directive" of the European Metrology Research Programme (EMRP). This work presents for the first time a method at EQS level, showing the analytical difficulties, addressing in particular the problem of the blanks and discussing the sources of measurement uncertainties and metrological traceability issues. Moreover the paper presents the results of an interlaboratory comparison work aiming to validate the analytical method. All these aspects will be helpful for analysts performing the TBT analysis in a variety of aqueous samples. The method is carefully described, addressing every detail of the procedure working at low concentration levels.

2. Experimental

2.1. Reagents and standards

Tributyltin chloride (96.2%), n-hexane CHROMASOLV (97%) and methanol (MeOH) were purchased from Sigma-Aldrich (USA). Glacial acetic acid (HAc), tetrahydrofuran, (THF; 99.8%) and sodium

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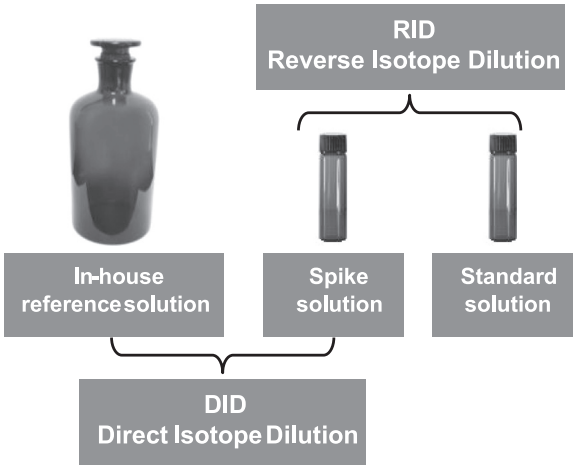

Fig. 1. Purpose of the solutions prepared for the isotope dilution procedure.

tetraethylborate ($NaBEt_4$) were purchased respectively from Merck (Germany), Carlo Erba (Italy) and Strem Chemicals (USA) (Institute 1), or from Merseburger Spezialchemikalien (Germany) (Institute 2) or from Acros Organics (Institute 4). Stock solutions of 10% $NaBEt_4$ in THF were prepared monthly, 1% solutions in ultrapure water were prepared daily. Sodium acetate (NaAc) was purchased from Merck (Germany). The ^{119}Sn-enriched butyltin mix or ^{119}Sn-enriched TBT was purchased from ISC Science (Spain). Institute 3: Tributyltinchloride (TBTCl, 96%) was purchased from Aldrich (Milwaukee, WI, USA). Tripropyltin chloride (TPrTCl, 98%), acetic acid and sodium acetate were obtained from Merck. Hexane and methanol were from J.T. Baker (Deventer, Holland). Sodium tetraethyl borate ($NaBEt_4$, 98%) was obtained from Strem Chemicals (Newburyport, MA, USA). The aqueous solution of $NaBEt_4$ (2% (w/v)) was prepared just before derivatization.

All stock and intermediate solutions were prepared in disposable 12 mL amber glass vials with PTFE caps from Chromacol Limited (UK). The solvent used for stock solutions and for all intermediate solutions, including spike solutions, was the mixture HAc/MeOH 3:1 (v/v). Stock solutions were stored at −20 °C and protected from light: solutions with concentration higher than 1 g kg^{-1} as tin are stable for 1 year, and solutions between 1 and 100 mg kg^{-1} as tin are stable for three months. The stability of TBT standards is of primary importance, therefore solutions at lower concentrations were prepared daily. The number of intermediate solutions depends on the organotin concentration that is to be assayed. However, the dilution factor never exceeded 100: the dilutions used usually amount to 0.1 g of the initial solution to be diluted in 10 g of the final solution. The last two dilutions were prepared in purified water from a Milli-Q system Q-POD Element (Millipore, USA) to avoid the addition of any organic solvent to the final working solutions.

2.2. The analytical method by species-specific isotope dilution (SSID) coupled to LLE-GC-ICP-MS

The SSID-LLE-GC-ICP-MS analytical method consists of several steps: preparation of all the solutions needed for the ID approach, including the in-house reference solutions; derivatization of the TBT in a buffered media; liquid-liquid extraction with an organic solvent, GC-ICP-MS analysis. All the solutions in this procedure were prepared by gravimetry, except for the solution containing the derivatizing agent that was prepared by volumetric dilution. The solutions used to implement the procedure are (1) tributyltin solutions with natural abundances (*standards*) and (2) tributyltin internal standards solutions enriched in the tin isotope 119 (*spikes*), in different dilutions. The mass fractions of the working solutions of spike and standards depend on the expected concentration of TBT in the sample to quantify. Usually the concentration of the spike to be prepared is evaluated imposing in the direct isotope dilution (DID) equation (refer to Eq. (2)) the isotope ratio of the blend, the amounts of sample and spike used and the expected concentration of TBT in the sample. Once the concentration of the spike is decided, the same approach is used to evaluate the concentration of the standard needed to quantify the spike (using Eq. (1)).

1) **Preparation of the in-house reference solution**. No certified reference material for organotin in water is commercially available until today. To validate the accuracy of the method, a reference water with a gravimetric reference value was used in this work. The water was prepared daily by diluting the working standard solution of TBT in 1 L of commercially available mineral water (purchased in glass bottles) at the mass fraction of 0.2 ng L^{-1} and 0.06 ng L^{-1} (as cation). After addition of the TBT the water was agitated for 30 min on a rotary table, before ID analysis. For comparison aims, the average TBT mass fraction calculated for each series of measurements was normalized to the reference solution daily prepared. All the institutes prepared the in-house reference solutions in the same way.
2) **Direct isotope dilution (DID).** The in-house reference water samples were spiked with 1 g of spike to be quantified by DID (Fig. 1). After spike addition, samples were agitated for 15 min and left standing for 24 h before derivatization and extraction. Four blanks were prepared in parallel using the same volume of mineral water as for the samples. The signal of the blanks and the way this information is used in data treatment is a very important topic that is addressed further in the discussion.
3) **Reverse isotope dilution (RID).** To perform the RID, the ID approach is applied a second time: in this case the spike is the unknown sample and a standard is used to quantify the amount content of the labeled compound in the spike solution (Fig. 1) [24]. For this purpose 5 mL of spike solution were mixed with 5 mL of standard (4 replicates) and the isotope ratio of the blend was measured. Three blanks were prepared using the same amount and same volume as for the RID samples (10 mL of HAc/MeOH 3:1). The average values of the signals of the blanks (isotopes 119 and 120) were subtracted from each sample signal during data treatment.
4) **Derivatization and LLE procedure.** Organotin derivatization was performed in an environment buffered at pH 5 using 10 mL of acetate buffer solution (HAc-NaAc; 1 mol L^{-1}). Solutions of 0.5% $NaBEt_4$ in milliQ water were prepared right before being used. 1 mL and 0.2 mL of $NaBEt_4$ 0.5–1% were added respectively to the in-house reference water and to the solutions for RID. LLE was performed by adding 1 mL of n-hexane and shaking the samples on a rotary table for 30 min at 230 rpm. The organic solvent was recovered using glass microseparators. Samples and blanks were analysed by GC-ICP-MS at once; otherwise they were stored at –20 °C for a maximum of 48 h before being analysed.
5) **GC-ICP-MS analyses.** Instrumentation parameters for GC, ICP-MS and the interface are given in Table 2 for the four institutes.
6) **Quantification by SSID.** The isotope ratio measured was the ratio of the isotope 120 to the isotope 119. The selected ratio was $R^{120/119}$=0.4-0.5. The mass fraction of the labeled compound in the spike solution, w_{spike}, was determined by RID:

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Table 2
GC-ICP-MS parameters.

	Parameters	GC Agilent 7890A/ICP-MS Agilent 7700x – Institute 1	GC Agilent 7890A/ICP-MS Agilent 7500cx – Institute 2	GC Agilent 6890/ICP-MS Agilent 7500ce - Institute 3	GC Thermo Trace Ultra/HR-ICPMS Thermo Element 2 - Institute 4
GC	Column	HP-5.30 m; 0.25 mm ID; 0.25 μm film thickness; 5% phenyl and 95% methylpolysiloxane	DB-5 MS UI.30 m; 0.25 mm ID; 0.25 μm film thickness; 5% phenyl and 95% methylpolysiloxane	DB-5MS15 m; 0.25 mm ID; 0.25 μm film thickness; 5% phenyl and 95% methylpolysiloxane	Trace TR5.30 m; 0.25 mm ID; 0.25 μm film thickness; 5% phenyl and 95% methylpolysiloxane
	Carrier gas	Helium	Helium	Helium	Helium
	Carrier gas flow	2 mL min^{-1}	2 mL min^{-1}	1 mL min^{-1}	7 mL min^{-1}
	Injected volume	2 μL	2 μL	2 μL	2 μL
	Injector temperature	250 °C	250 °C	280 °C	250 °C
	Injection mode	Splitless	Splitless	Splitless	Splitless
	Furnace temperature	Initial temperature of 50 °C, 40 °C min^{-1} up to 250 °C. Then 0.9 min at 250 °C.	Initial temperature of 60 °C, 30 °C min^{-1} up to 300 °C.	For the first 0.5 min the column temperature was held at 60 °C, then raised to 110 °C at a heating rate of 20 °C min^{-1}, then raised to 200 °C at a heating rate of 25 °C min^{-1} and held there for 0.5 min, then raised to 220 °C at a heating rate of 40 °C min^{-1} and, in a final step, raised to 280 °C at a heating rate of 40 °C min^{-1} and held at this temperature for 3 min	Initial temperature of 40 °C, 45 °C min^{-1} up to 250 °C. Then 1 min at 250 °C.
Interface	Transfer line temperature	250 °C	290 °C	280 °C	300 °C
	Injector temperature	250 °C	290 °C	280 °C	300 °C
ICP-MS	RF Power	1550 W	500 W	1500 W	900 W
	Plasma-forming gas flow	15 L min^{-1}	15 L min^{-1}	15 L min^{-1}	16 L min^{-1}
	Carrier gas flow	1.5 mL min^{-1}	1.1 mL min^{-1}	0.69 mL min^{-1}	1 mL min^{-1}
	Addition of oxygen	Yes	No	Yes	Yes
	Integration time	30 ms	18 ms	100 ms	20 ms
	Isotopes measured	^{119}Sn and ^{120}Sn	^{119}Sn and ^{120}Sn	^{119}Sn and ^{120}Sn	^{119}Sn and ^{120}Sn

Table 3
Isotopic composition of the ISC standard solution.

Sn isotope	Measured TBT abundances (%) ($k=2$)	Certified TBT abundances (%)	TBT abundances and uncertainties (%) as reported in [52]	TBT abundances and uncertainties (%) as reported in [52] (mean of inorganic tin – MBT, DBT, TBT)
116	0.0060 ± 0.0004	0.03	0.013 ± 0.009	0.029 ± 0.008
117	0.103 ± 0.003	0.1	0.105 ± 0.004	0.114 ± 0.005
118	14.1 ± 0.2	14.3	14.3 ± 0.3	14.3 ± 0.1
119	82.8 ± 0.3	82.4	82.5 ± 0.4	82.4 ± 0.2
120	2.96 ± 0.08	3.1	3.10 ± 0.07	3.13 ± 0.03
Molar mass ($g\ mol^{-1}$)	118.7893 ± 0.0021			

$$w_{spike} = w_{std}\frac{m_{std}}{m_{spikeRID}}\frac{M_{spike}}{M_{std}}\frac{R_{expRID}A_{nat}^{119} - A_{nat}^{120}}{A_{spike}^{120} - R_{expRID}A_{spike}^{119}} \quad (1)$$

w_{std} = mass fraction of the standard solution used for RID
m_{std} = mass of the standard solution for RID
$m_{spikeRID}$ = mass of the isotope-enriched standard for RID
M_{spike} = tin atomic mass in the isotope-enriched standard
M_{std} = tin atomic mass in the standard
R_{expRID} = isotope ratio of the mixture 120/119 for RID
A_{nat119} = natural abundance of the isotope 119 (known from the IUPAC tables)
A_{nat120} = natural abundance of the isotope 120 (known from the IUPAC tables)
$A_{spike120}$ = abundance of the isotope 120 in the isotope-enriched standard
$A_{spike119}$ = abundance of the isotope 119 in the isotope-enriched standard
The normalized TBT mass fraction, w_x^*, was calculated using the DID equation, normalized to the gravimetric mass fraction of the in-house reference solution:

$$w_x^* = w_{spike}\frac{m_{spikeDID}}{m_x}\frac{M_x}{M_{spike}}\frac{R_{expDID}A_{spike}^{119} - A_{spike}^{120}}{A_{nat}^{120} - R_{expDID}A_{nat}^{119}}\frac{1}{w_x^{ref}} \quad (2)$$

$m_{spikeDID}$ = mass of the isotope-enriched standard for DID
m_x = sample mass
M_x = tin atomic mass in the sample
R_{expDID} = isotope ratio of the mixture 120/119 for DID
w_x^{ref} = TBT mass fraction within the sample as calculated by gravimetry.

RID and DID measured isotope ratios were corrected by (i) subtraction of tin average blank signals ($B_{RID}^{120}, B_{RID}^{119}, B_{DID}^{120}, B_{DID}^{119}$) to the respective tin signals ($Sn_{RID}^{120}, Sn_{RID}^{119}, Sn_{DID}^{120}, Sn_{DID}^{119}$) and (ii) application of the mass bias factor, k:

$$R_{expRID} = \frac{Sn_{RID}^{120} - B_{RID}^{120}}{Sn_{RID}^{119} - B_{RID}^{119}} * k \quad (3)$$

$$R_{expDID} = \frac{Sn_{DID}^{120} - B_{DID}^{120}}{Sn_{DID}^{119} - B_{DID}^{119}} * k \quad (4)$$

k was calculated as the ratio between the isotope ratio measured using a TBT standard, and the theoretic isotope ratio calculated from IUPAC tables.

7) **Metrological traceability and purity of standards.** The purity of a commercial batch of neat TBT chloride (TBTCl) has been assessed using GC-ICP-MS, 13C-qNMR (Bruker AVANCE 600) and coulometric Karl-Fischer titration (Metrohm Karl-Fischer coulometer 756 KF). Three batches of neat TBTCl were purchased from Sigma Aldrich (USA) (TBTCl 96% and "pestanal") and Supelco (USA) (TBTCl "analytical standard"). Two subsamples of each material were analysed with GC-ICP-MS after derivatization using $NaBEt_4$ and LLE. The obtained chromatograms did not reveal any considerable difference in the organotin pattern of the three batches (data not shown). Sigma Aldrich TBTCl with a certified purity of 96% was selected for further purity assessment by qNMR and Karl-Fischer titration. Six independent 13C-qNMR experiments were performed. The mass fraction of TBTCl was quantified using Benzoic acid (NIST SRM 350b) as internal standard. The resulting TBTCl mass fraction was $(977.3 \pm 5.3)\ mg\ g^{-1}$. The mass fraction of water in the sample was found to be $(215 \pm 2)\ mg\ kg^{-1}$. The study confirmed the purity value given by the supplier. The same TBTCl standard from Sigma-Aldrich was used by the four partners for this work and the purity value of 97.73% was used for calculations in order to assure the metrological traceability to the International System of Units. With the same aim, the isotopic composition of the ^{119}Sn-enriched butyltin mix solution purchased from ISC Science was measured using GC-HR-ICP-MS (Table 3). TBT abundances for the isotopes of interest in this work were respectively, $A_{spike119} = (82.82 \pm 0.26)$ and $A_{spike120} = (2.96 \pm 0.08)$ (coverage factor, $k=2$).
8) **Laboratory working conditions.** Glassware was washed, filled or soaked with nitric acid 2–10% overnight and rinsed out three times with deionized water. When possible, the equipment was calcinated in a furnace at 450 °C for at least 5 h. All solutions for standards and spikes were prepared in glass bottles with PTFE caps "single use" to avoid any contamination problem and a time-consuming cleaning procedure requiring a large quantity of nitric acid. Special attention was paid to the working place: high and low TBT concentrations were never prepared together under the same hood; accurate cleaning of the working place under the hood was performed before preparation of the samples at very low concentrations and blanks. The derivatization agent was prepared in a different fume hood.

3. Results and discussion

3.1. Method development

For the development of a measurement procedure for TBT in whole water samples at low concentration level required by the EU WFD, it was necessary to evaluate the extraction, derivatisation and pre-concentration methods. Therefore three different extraction techniques, liquid-liquid extraction (LLE), headspace solid phase microextraction (HS-SPME) and solid phase extraction (SPE) were evaluated. TBT can not be analysed using gas

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chromatography without derivatisation; hence the derivatisation procedure was evaluated as well, using sodiumtetraethylborate ($NaBEt_4$) for ethylation. The influence of amount and concentration of $NaBEt_4$, and pH dependence were investigated. As TBT analysis is burdened with procedural blanks, different treatments for glassware and purification of $NaBEt_4$ were tested. All experiments for method development were done using a rather simple water matrix (ultrapure water, mineral water and tap water) and were performed using concentrations above the EQS level and using a sample volume in the range 100 mL to 1 L. Deuterated TBT was used as the internal standard (IS) for most of the experiments when the isotope dilution calibration method was not used.

3.1.1. Extraction methods

3.1.1.1. SPE. The SPE method has been previously published [21]. A chemometric approach based on the use of factorial fractionary plan was defined. A linear model describing the studied factors (phase, eluent, phase mass, eluent volume, pH, ethylation and sample volume) was optimized and led to a list of best conditions. SPE appeared to be a convenient technique for TBT pre-concentration at pico-trace levels. Nevertheless, compared to LLE, the approach resulted in unhandy long methods, with many steps needed that increased the risk of contamination.

3.1.1.2. HS-SPME. The success of a SPME method is crucial dependent on selecting the suitable polymeric coating of the fiber to adsorb the relevant analyte. The polarity of the fiber must be aligned with the polarity of the analyte to enable a maximum of adsorption tendency. Two different fiber materials with different polarities were evaluated: (i) polydimethylsiloxane (PDMS) and (ii) divinylbenzene/carboxene/polydimethylsiloxane (DVB/CAR/PDMS). PDMS gave the best performances (Supplementary material Fig. S1) and was selected for further HS-SPME experiments. The following tests were performed: samples of ultra-pure water were spiked with commercial available ethyltributyltin (EtTBT) and were extracted with HS-SPME and analysed using GC-ICP-MS; samples with different TBT concentrations were ethylated varying the concentration and amount of $NaBEt_4$. Results (not shown) led to a poor repeatability, with relative standard deviations always higher than 20%. Because of the bad repeatability, the unhandy long method due to the long extraction time (40 min) and the fact that each sample can only be analysed once, LLE was preferred to SPME as a way to quantify TBT in this project.

3.1.1.3. LLE. This extraction method is based on the solubility of analytes in two different immiscible liquids. In case of TBT an organic solvent is added to the water phase and after extraction and phase separation the organic solvent is recollected and analysed. Prepared samples were extracted with different amounts of hexane to evaluate the adequate amount of organic solvent. Portions larger than 1 mL were pre-concentrated with nitrogen to a final volume of 1 mL before GC-ICP-MS analysis. One milliliter of hexane was found to be sufficient for extraction of TBT (Supplementary material Fig. S2). Repeated extractions with hexane showed that an extraction with 1 mL is depletive: second extraction resulted in a retrieval of TBT $< 10\%$ based on the first extraction. Isooctane was also tested as an alternative solvent: because of its lower volatility it is easier to handle, but experiments showed higher blanks values. For further experiments hexane was used as the extraction solvent. To evaluate the time for extraction, samples were shaken during increasing times before the separation of the extraction solvent. Thirty minutes was selected as a good compromise for extraction time (Supplementary material Fig. S3). These preliminary results and the work done using SPE and HS-SPME led to the choice of LLE as the most appropriate extraction method to meet WFD needs. The other parameters involved in LLE were then carefully evaluated. Large volume injection has also been tested to further decreases the LOQ of the method: the sample volume and temperature programme were studied. It was concluded that with a sample volume of 1 L or more and ICPMS detection an additional preconcentration step using large volume injection is not necessary to meet the EQS value.

3.1.2. Optimization of the derivatisation

To investigate the optimum amount of $NaBEt_4$, samples in triplicate were ethylated with a different amount of 1% $NaBEt_4$ solution. Only 1% solutions of NaBEt4 were investigated as a result of the SPME experiments. 500 μL of a 1% NaBEt4 solution were found sufficient for complete ethylation (data not shown).

Phosphate, carbonate and tris-citrate buffers were used to optimize the pH of TBT ethylation in water samples. Their applicability was critically evaluated in the pH range from 3.0 to 10.0 and compared to acetate buffer and results were published in [25]. The results of these experiments show a more or less constant ethylation yield over the whole pH range investigated. It should be stressed that studies were carried out in Milli-Q water due to the fact that in salty water (artificial salt water with 3.8% NaCl or sea water) at pH higher than 6, as expected, visible precipitates in phosphate and carbonate buffers, and bubbles in carbonate buffer at pH lower than 4 have started to form. Precipitates or bubbles were not observed when tris-citrate or sodium acetate – acetic acid buffers were applied. Among buffers studied tris-citrate buffer gave similar results concerning ethylation yields as sodium acetate – acetic acid. Therefore, for further analysis sodium acetate – acetic acid buffer was used.

To investigate the stability of the derivatization reaction in the sample medium, samples were left standing for 10, 30, 45 and 60 min respectively after the addition of $NaBEt_4$, before hexane addition and recovery of the organic phase. For waiting times < 45 min, no influence on the TBT/IS peak area ratio is visible in the chromatograms. This test showed that it is possible to prepare multiple samples simultaneously (adding buffer, checking pH value, adding $NaBEt_4$) before the samples have to be extracted with hexane.

3.1.3. Procedural blanks

The adsorption of TBT to the container walls has been studied in the EMRP Joint Research Project and published in [26]. Amber glass was clearly the best material for TBT samples storage. In fluorinated polyethylene (FPE) bottles, less than half of the added TBT was recovered after 192 h. Cleaning of laboratory glassware was then optimized in order to minimize blank values. The efficiency of acidic cleaning was similar when 10 or 20% HNO_3 was used. Slightly lower blanks were obtained when glassware was heated at 400 °C for 4–5 h. Addition of ethanol (5–10 mL in 1 l of sample) or 10% isopropanol to reduce wall effects resulted in higher blanks. $NaBEt_4$ and extraction solvent are supposed to be the main sources of contamination; hence they have been carefully evaluated. The derivatization solution has been cleaned by adding a solvent, mixing, and removing the solvent. However, lower blanks were obtained without cleaning the derivatization agent. The use of isooctane, as an alternative extraction solvent to hexane, resulted in higher blanks. When working with SPME, venting the $NaBEt_4$ solution with argon for 30 min reduced blanks of about 10 times. For LLE venting had not the same impact and could be omitted.

The final laboratory best working conditions are described in the experimental section. Blank values could be reduced by about one order of magnitude.

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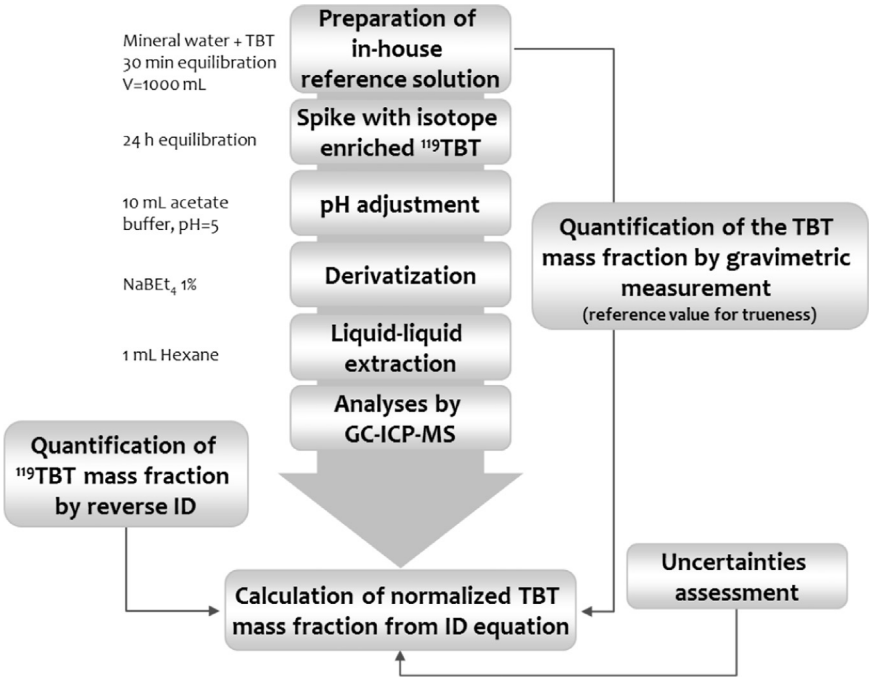

Fig. 2. Analytical procedure.

3.2. Results of the interlaboratory comparison (ILC)

The Directive 2013/39/EU defines specific measures for pollution control and environmental quality standards (EQS), expressed as annual average values (AA-EQS) and maximum allowable concentrations (MAC-EQS). The MAC-EQS of tributyltin compounds has been set up at 0.2 ng L^{-1} (as cation). The QA/QC Directive (Commission Directive 2009/90/EC) regulates the measurements uncertainties and the LOQ: measurement uncertainties should not exceed 50% ($k=2$) at EQS level and LOQ should not reach more than 30% of the EQS value. In order to meet these requirements, the most important validation parameters of the developed method within the EMRP project were:

(1) trueness;
(2) measurement uncertainty;
(3) LOQ.

The method has been validated at EQS and LOQ levels. The analytical procedure developed in this work is presented in Fig. 2. Each institute prepared its own in-house reference solution. Derivatization was performed in a buffered environment, followed by LLE and analysis by GC-ICP-MS. Three measurement replicates (3 injections in the GC-ICP-MS system) were performed to analyse each sample. The spike concentration was assessed by RID. The validation plan is described in Table 4. To ensure comparability, the average TBT mass fraction calculated for each series of measurements was normalized to the daily prepared reference solution (see Eq. (2)). The mass fractions are given as TBT cation, to be consistent to the limits required by the WFD.

The presented analytical procedure addresses the information resulting from analytical blanks in data treatment. This information can be treated in different ways: (i) the blank average mass fraction calculated by IDMS can be subtracted to the average TBT mass fraction; (ii) the average blank signal can be subtracted to the signal used to calculate the TBT mass fraction. At EQS level the second approach was applied. On the other hand, at LOQ level, in presence of high blank contents, we expected the first approach being more accurate: Institute 1 and Institute 2 applied the two approaches, Institute 3 used the first approach and Institute 4 used the second approach.

Table 4
Validation plan adopted by the four institutes at EQS and LOQ levels.

EQS level		LOQ level	
Sample	**Number of replicates**	**Sample**	**Number of replicates**
Evian water spiked at EQS level	6	Evian water spiked at LOQ level	6
Blanks of Evian water (no ^{119}TBT spike added)	4	Blanks of Evian water spiked with ^{119}TBT	4
Samples for RID	4	Samples for RID	4
Blanks for RID	3	Blanks for RID	3
Standards for mass bias factor evaluation	2	Standards for mass bias factor evaluation	2

TBT quantification at EQS level (0.2 ng L^{-1}). Each Institute prepared 6 samples spiked at EQS level, 4 blanks (using only mineral water) and the samples for RID and mass bias calculation (Table 4). The results obtained (Fig. 3 and Table 5) show very good agreement between the participants to the ILC with an average normalized TBT mass fraction, w_x^*, of 1.04 and an average uncertainty ($k=2$) of 17.3%.

TBT quantification at LOQ level (0.06 ng L^{-1}). Each Institute prepared 6 samples spiked at LOQ level and the samples for RID and mass bias. Then, 4 blanks were prepared using mineral water spiked with the enriched ^{119}TBT in order to quantify the TBT mass fraction in blanks, for LOQ evaluation. Institute 1, Institute 2 and Institute 4 prepared also blanks using only mineral water. The results obtained (Fig. 3,Table 6) show good agreement between the participants to the ILC with an average normalized TBT mass fraction, w_x^*, of 1.27 and an average uncertainty ($k=2$) of 36.4%.

3.2.1. Trueness

The trueness has been evaluated for TBT in mineral water at EQS and LOQ levels. In the absence of any certified reference

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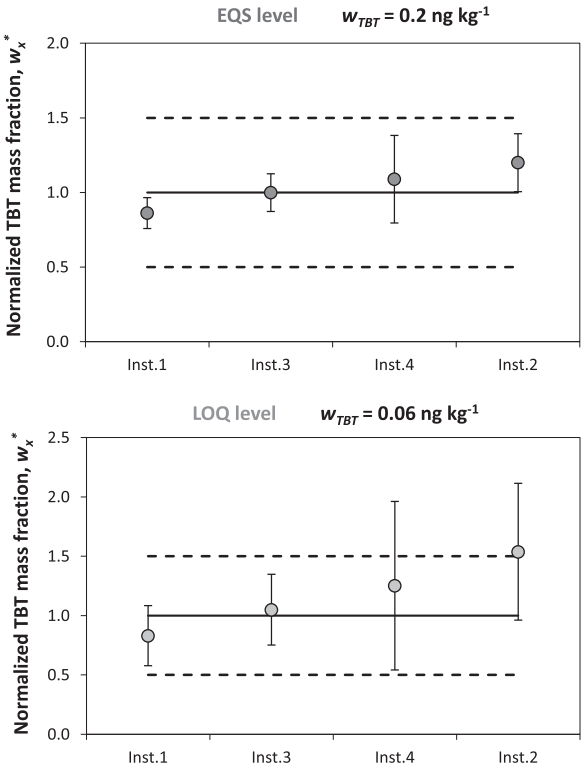

Fig. 3. Normalized TBT mass fraction, w_x^*, as measured by the four institutes at EQS and LOQ levels. The error bars show the expanded uncertainty, U ($k=2$). The black line represents 100% recovery. Dashed lines represent an uncertainty of 50%.

Table 5
Normalized TBT mass fractions, w_x^*, expanded uncertainty, U ($k=2$) and relative expanded uncertainty, $U\%$ ($k=2$), as measured by the four institutes at EQS level.

EQS							
	Inst.1	**Inst.2**	**Inst.3**	**Inst.4**	**Average**	**s**	**CV (%)**
w_x^{norm}	0.86	1.20	1.00	1.09	**1.04**	0.14	**13.8**
U (k=2)	0.10	0.19	0.13	0.29	0.18		
U% (k=2)	11.9	16.2	12.6	26.9	**17.3**		

material for organotin in water, the method accuracy was quantified by using a reference water prepared gravimetrically by each Institute, according to the defined procedure. The optimization criterion was the closeness to 1, which is the value that the normalized TBT mass fraction should have when the recovery is 100%.

3.2.1.1. EQS level

The normalized TBT mass fraction was in the range 0.86–1.20 (Table 5). The average of the values measured by the four laboratories was 1.04, with a coefficient of variation (CV) of 13.8%.

3.2.1.2. LOQ level

The normalized TBT mass fraction was in the range 0.83-1.94 with a CV of 37.8% (Fig. 3, Table 6). The average normalized TBT mass fraction was 1.27 and was calculated on the measurements obtained using the approach "subtraction of the blank mass fraction" (Table 6, **in bold**), except for Institute 4 which used the approach "subtraction of the blank signal".

The normalized TBT mass fraction values of all the partners at both levels of concentration were within the range 0.5-1.5, which is the range obtained when a measurement uncertainty of 50% ($k=2$) is associated to the theoretic normalized mass fraction of 1. In conclusion, the trueness was satisfactory at EQS and LOQ levels.

3.2.2. Measurement uncertainty

An Ishikawa diagram has been designed to identify the potential factors influencing the measurement uncertainty associated to the developed method (Fig. 4). The standard uncertainty associated to the normalized TBT mass fraction, w_x^*, was obtained by propagating the variances of the coefficients associated to the variables of the model, following the *Guide to the expression of uncertainty in measurement* (GUM) [27]. Uncertainty calculation was performed by one institute: the information needed was collected from the partner institutes and the same data treatment was applied to all data series. In such a way it was possible to compare the predominant factors of the uncertainty budget among the participants of the ILC, overcoming the calculation approaches, which often differ from a National Metrology Institute (NMI) to another.

3.2.2.1. EQS level

The relative expanded uncertainty evaluated by the different institutes varied between 12% and 27% (Table 4). The uncertainty of the method met the WFD requirements with $U\%$ ($k=2$) $<$ 50%.

3.2.2.2. LOQ level

The relative expanded uncertainties evaluated by the different institutes varied between 18% and 57% (Table 5). Institute 4 had a contamination problem during the procedure at LOQ level that influenced the measurement uncertainty. Both approaches, "subtraction of the blank mass fraction" and "subtraction of the blank signal", led to method uncertainties around 36%.

Between the potential factors influencing the measurement uncertainty represented through the Ishikawa diagram, the following parameters were predominant both at EQS and LOQ level (Fig. 5): the measurement repeatability of the DID and the RID and the uncertainty associated to the experimental isotope ratio. Furthermore, also the TBT mass fraction in blanks, in the in-house reference water, and in the standard used for the RID, as well as the mass of the standard and the mass of the spike, play a significant role in the uncertainty building. As expected, the relative uncertainty, $U\%$, increases with the decrease of the TBT mass fraction (from EQS to LOQ), for all participants. At LOQ level the repeatability associated to the RID become more important: f_{RID} increased from 1% to 5% for Institute 2, from 5% to 15% for Institute 3, and from 0.3% to 23% for Institute 1. In the case of Institute 1, the uncertainty associated to the isotope ratio of the RID also increased from 3% to 47%. This is due to the low concentration of the

Table 6
Normalized TBT mass fractions, w_x^*, expanded uncertainty, U ($k=2$) and relative expanded uncertainty, $U\%$ ($k=2$), as measured by the four institutes at LOQ level. Bold values are the ones used for average calculations. bk conc: subtraction of the blank mass fraction; bk signal: subtraction of the blank signal.

LOQ									
	Inst.1 - bk conc	**Inst.1 - bk signal**	**Inst.2 - bk conc**	**Inst.2 - bk signal**	**Inst.3 - bk conc**	**Inst.4 - bk signal**	**Average**	**s**	**CV (%)**
w_x^*	**0.83**	0.96	**1.94**	1.54	**1.05**	**1.25**	1.27	0.48	37.8
U (k=2)	**0.25**	0.17	**0.58**	0.58	**0.30**	**0.71**	0.46		
U% (k=2)	**30.5**	17.8	**30.1**	37.5	**28.4**	**56.7**	36.4		

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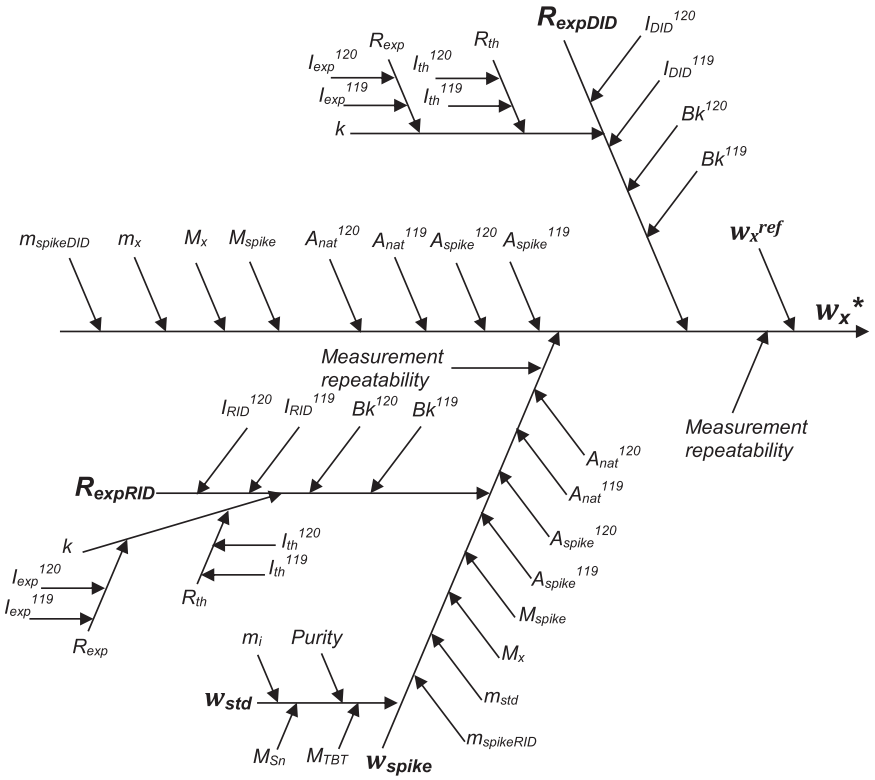

Fig. 4. Ishikawa diagram for the establishment of the uncertainties associated to the ID-GC-ICP-MS method.

spike that should be used at LOQ level to quantify the endogenous TBT: the RID became also a critical step in the procedure. Institute 4 showed a repeatability problem in the DID: R_{expDID} represented 3–18% of the final *U* budget and f_{DID} represented 80–97%. When a problem of repeatability is present, the contribution of other factors becomes negligible. This is fundamentally due to contamination problems that become critical at LOQ level, where the *U%* increased for Institute 4 from 27% at EQS level to 57% at LOQ level. At LOQ level, the influence of the blank quantification and subtraction (w_x^{Bk}) was 4% for Institute 2, 17% for Institute 1% and 19% for Institute 3. Data treatment with subtraction of the blank mass fraction, which is fundamental for the trueness as discussed previously, contributes to the final *U* budget.

3.2.3. *The limit of quantification and the blank issue*

The way to determine the limit of detection/quantification of an IDMS method is not described in standard documents. The signal detected by the ICP-MS is the response of the blend constituted by the analyte and the labeled compound. The detection limits for IDMS found in literature are often estimated empirically, using the formulation "three times the standard uncertainty of the concentration of a blank measured at the mass of the reference isotope, using a linear calibration function", e.g. [28]. Yu and collaborators proposed in 2002 a novel formulation for the determination of IDMS detection limit, which is a function of the enrichment of the isotopic spike and the linear calibration detection limits measured at the masses for the isotope ratio measurement [29]. They showed that the empirical formulation fails to account for the enrichment of the spike and for the uncertainties in the measurement of the spiked isotope, resulting in underestimation of IDMS detection limits. In the case of TBT quantification at subfemtogram levels, the quantification limit must deal with important analytical blanks, which can be reduced but not completely avoided during the analytical procedure. Instead of a statistical formulation, in this work we preferred to adopt a pragmatic approach described in the CITAC/EURACHEM *Guide to Quality in Analytical Chemistry* [30], which defines the LOQ as "the lowest concentration of analyte that can be determined with an acceptable level of uncertainty". Therefore, the LOQ of the IDMS method here developed has been evaluated as the minimal quantity that could be correctly quantified by IDMS, in term of trueness and uncertainty. As previously shown, TBT quantification was successful at LOQ level (Fig. 3 and Table 6), which is 3 times lower than the EQS limit: 0.06 ng L^{-1}. Thereby, this was assumed as the LOQ of the method presented here.

The analytical blanks are of crucial importance because they are obligatorily involved in the data treatment: if taken into account by *subtraction of the blank mass fraction* or *subtraction of the blank signal*, they would not degrade the TBT quantification at subfemtogram level. On the contrary, without the application of one of these approaches, the results are incorrect (Fig. 6). In fact, when the TBT mass fraction was measured without blank subtraction, the obtained values were higher than the expected gravimetric value, both at EQS and LOQ levels. Practical laboratory experience shows that, even when all the precautions described in the experimental section (*Laboratory working conditions*) are respected, the procedural blanks often have an unpredictable behavior, resulting in unexpected contaminations. It is appropriate to prepare at least 3 blanks in order to discharge one dataset, when one blank is contaminated. When the contamination is systematic and the ICP-MS signal of the blank is comparable to the sample signal, the reason should be researched in the analytical procedure: all the critical steps should be checked, as well as the reagents used, and the analysis must be repeated. Otherwise, when the ICP-MS signal of the blank is systematic, but lower than the sample signal, the presence of a TBT background in blanks could be accepted and included in data treatment.

Two institutes have compared the approaches *subtraction of the blank mass fraction* and *subtraction of the blank signal* (Table 6): Institute 1 found a better trueness and uncertainty when

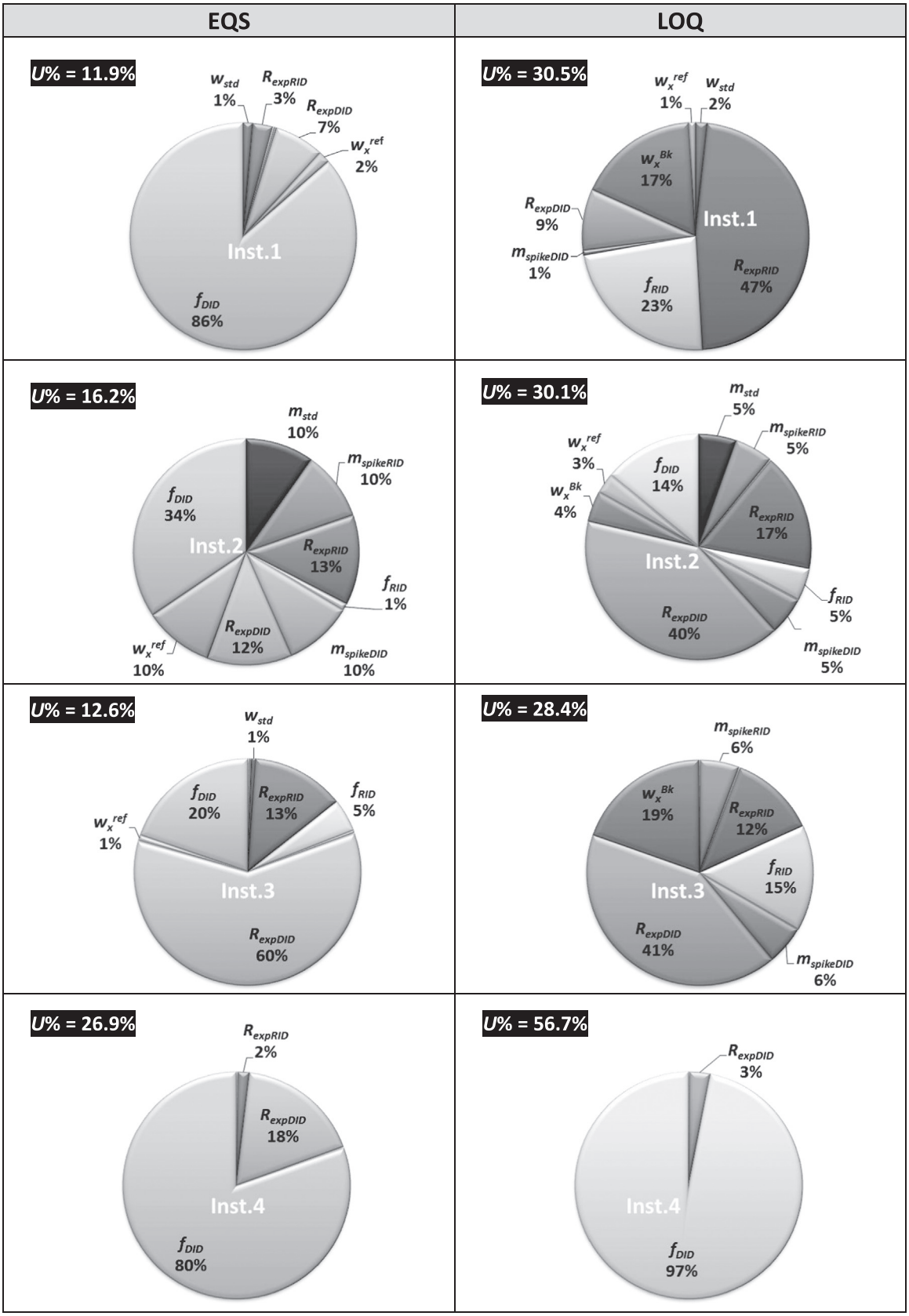

Fig. 5. Predominant factors of the uncertainty budget at EQS and LOQ level, for each participant. f_{RID} and f_{DID}: measurement repeatability of the RID and DID methods, respectively. w_x^{bk}: TBT mass fraction in blanks calculated by ID. Factors with impact $<1\%$ are not represented. Relative expanded uncertainty, $U\%$ ($k=2$).

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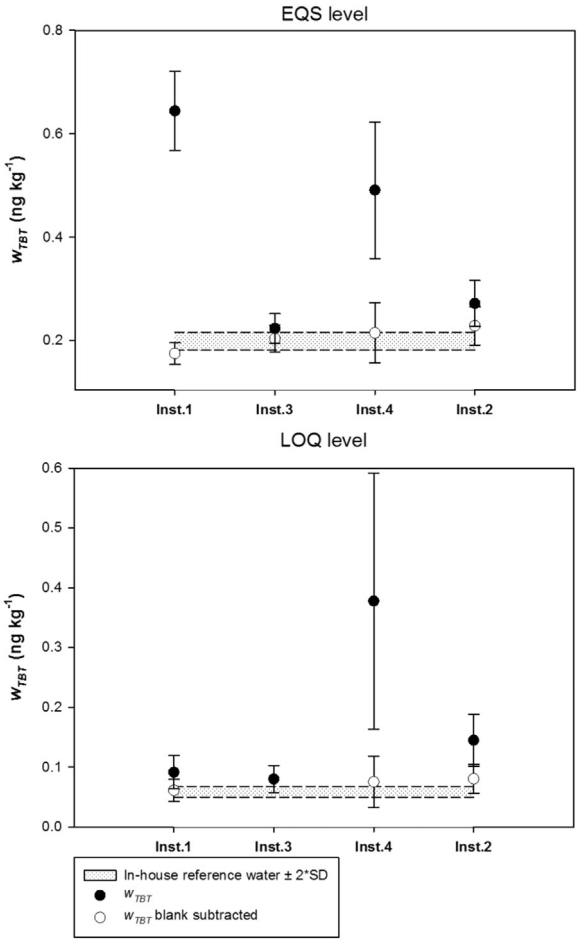

Fig. 6. TBT mass fractions measured by the four institutes at EQS and LOQ levels, with/without blank subtraction. Expanded uncertainty, U (k=2). Dotted area fills represent the average value of the in-house reference water in the range given by two times the standard deviation (SD) of the reference waters prepared by the partners.

Table 7
TBT mass fractions measured in blank samples, w_x^{bk}, expanded uncertainty, U (k=2) and relative expanded uncertainty, U% (k=2).

(ng kg^{-1})	Inst.1	Inst.2	Inst.3	Average	s	CV (%)
w_x^{bk}	0.039	0.042	0.021	0.034	0.011	33.2
U (k=2)	0.006	0.007	0.007	0.007		
U% (k=2)	14.4	16.0	35.3	21.9		

subtracting the blank signal. The trueness improved also for Institute 2, but the uncertainty was lower, 30% instead of 38%, when the blank mass fraction was subtracted. Therefore, the two approaches can be considered equivalent. However, the "subtraction of the blank signal" is a faster approach as it requires less preparation steps (no spike addition to blank samples and equilibration) and less data treatment.

The TBT mass fraction was measured in blanks by IDMS. Three partners found a w_x^{bk} in blank samples in the range 0.021–0.042 ng kg^{-1}, with U% between 15% and 35% (Table 7). The average w_x^{bk} was 0.034 ng kg^{-1}. Hence, the LOQ of the method was only 1.8 times higher than the background, but results show that this was not precluding a correct evaluation of the TBT content at LOQ.

3.2.4. Method validation

A test based on E_N score [31] was applied to the difference of the normalized TBT mass fraction, w_x^* and the theoretic value of 1, which is the value that w_x^* should have when the recovery is 100%. The expected ideal uncertainty was imposed equal to 50% of 1, which gave rise to a range of TBT mass fractions between 0.5 and 1.5. The E_N score was applied to the results obtained by the four institutes at EQS and LOQ levels and was always lower than 1 (Table 8). Thus, in the confidence range of 95%, the obtained results were not significantly different from the expected theoretic values. Moreover, the uncertainty associated to the method was lower than the criterion imposed by the WFD, as previously discussed. Therefore, the two prerequisites, trueness and uncertainty, were met and the method was validated.

4. Conclusions

A primary method to quantify TBT in water samples was developed using LLE and SSID coupled to mass spectrometry. The method was validated by an inter-laboratory comparison organized among four European NMIs and designated Institutes, in mineral water at EQS level (0.2 ng L^{-1}) and LOQ level (0.06 ng L^{-1}).

The measurement accuracy of the method has been evaluated at both levels of concentration: the trueness satisfied the E_N score, and the uncertainty met the WFD requirements with U% (k=2) $<$ 50%. The LOQ of the method was 0.06 ng L^{-1}.

The study of the factors influencing the measurement uncertainty evidenced among all, the following critical factors: f_{DID}, f_{RID}, R_{expDID} and R_{expRID}. Analytical blanks were found to be crucial for trueness and they should be included in the data treatment.

This method is today one of the rare published analytical procedures for TBT quantification meeting WFD requirements. The procedure is simple as any unnecessary manipulation which could be time-consuming and potential source of contamination has been avoided.

Table 8
Normalized TBT mass fractions, w_x^*, expanded uncertainty, U (k=2), normalized theoretic TBT mass fractions, $w_{xtheoretic}^*$, theoretic expanded uncertainty, $U_{theoretic}$ (k=2) and E_N score, for the four institutes at EQS and LOQ levels. bk conc: subtraction of the blank mass fraction; bk signal: subtraction of the blank signal.

	EQS				LOQ			
	Inst. 1	Inst. 2	Inst. 3	Inst. 4	Inst. 1 - bk conc	Inst. 2 - bk signal	Inst. 3 - bk conc	Inst. 4 - bk signal
w_x^*	0.86	1.20	1.00	1.09	0.83	1.54	1.05	1.25
$w_{xtheoretic}^*$	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
$U(k=2)$	0.10	0.19	0.13	0.29	0.25	0.58	0.30	0.71
$U_{theoretic}(k=2)$	0.20	0.20	0.20	0.20	0.10	0.10	0.10	0.10
E_N	**0.61**	**0.72**	**0.003**	**0.25**	**0.62**	**0.92**	**0.16**	**0.35**

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2016.07.056.

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