Talanta 134 (2015) 576–586

Contents lists available at ScienceDirect

Talanta

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

Experimental design for TBT quantification by isotope dilution SPE–GC–ICP–MS under the European water framework directive

Enrica Alasonati [a,*], Barbara Fabbri [a], Ina Fettig [b], Catherine Yardin [a], Maria Estela Del Castillo Busto [a], Janine Richter [b], Rosemarie Philipp [b], Paola Fisicaro [a]

[a] Department of Biomedical and Inorganic Chemistry, Laboratoire National de Métrologie et d'Essais (LNE), 1 Gaston Boissier, 75015 Paris, France
[b] Bundesanstalt fuer Materialforschung und -pruefung, Richard-Willstaetter-Str. 11, 12489 Berlin, Germany

ARTICLE INFO

Article history:
Received 6 August 2014
Received in revised form
26 November 2014
Accepted 28 November 2014
Available online 6 December 2014

Keywords:
Tributyltin
Organotin compounds
Solid-phase extraction
Experimental design
Water framework directive
Isotope dilution

ABSTRACT

In Europe the maximum allowable concentration for tributyltin (TBT) compounds in surface water has been regulated by the water framework directive (WFD) and daughter directive that impose a limit of 0.2 ng L^{-1} in whole water (as tributyltin cation). Despite the large number of different methodologies for the quantification of organotin species developed in the last two decades, standardised analytical methods at required concentration level do not exist. TBT quantification at picogram level requires efficient and accurate sample preparation and preconcentration, and maximum care to avoid blank contamination. To meet the WFD requirement, a method for the quantification of TBT in mineral water at environmental quality standard (EQS) level, based on solid phase extraction (SPE), was developed and optimised. The quantification was done using species-specific isotope dilution (SSID) followed by gas chromatography (GC) coupled to inductively coupled plasma mass spectrometry (ICP–MS). The analytical process was optimised using a design of experiment (DOE) based on a factorial fractionary plan. The DOE allowed to evaluate 3 qualitative factors (type of stationary phase and eluent, phase mass and eluent volume, pH and analyte ethylation procedure) for a total of 13 levels studied, and a sample volume in the range of 250–1000 mL. Four different models fitting the results were defined and evaluated with statistic tools: one of them was selected and optimised to find the best procedural conditions. C18 phase was found to be the best stationary phase for SPE experiments. The 4 solvents tested with C18, the pH and ethylation conditions, the mass of the phases, the volume of the eluents and the sample volume can all be optimal, but depending on their respective combination. For that reason, the equation of the model conceived in this work is a useful decisional tool for the planning of experiments, because it can be applied to predict the TBT mass fraction recovery when the experimental conditions are drawn. This work shows that SPE is a convenient technique for TBT pre-concentration at pico-trace levels and a robust approach: in fact (i) number of different experimental conditions led to satisfactory results and (ii) the participation of two institutes to the experimental work did not impact the developed model.

1. Introduction

Since tributyltin (TBT) was first used as a pesticide in 1925, and dibutyltin (DBT) as stabilizer for polyvinyl chloride polymers in the 1940s, an increasing number of organotin compounds (OTC) have been produced for commercial and industrial applications. Nowadays, OTC are present as global pollutants in the environment and known to be toxic for living organisms at extremely low levels [1–4]. Imposex in dogwhelk, oyster shell malformation and mussel larvae mortality have been observed at sub ng L^{-1} concentrations [5,6]. In mammals, OTC are considered endocrine disruptors, neurotoxic, hepatotoxic, immunotoxic and potential obesogens [7,8]. The European Union (EU) has included TBT and its compounds in the list of priority pollutants (Decision 2455/2001/EC amending the Water Framework Directive 2000/60/EC). Afterwards, the Directive 2008/105/EC laid down a strategy against pollution of water and defined specific measures for pollution control and environmental quality standards (EQS), expressed as an annual average values (AAV) and maximum allowable concentrations (MAC). The MAC–EQS of tributyltin compounds has been set up at 0.2 ng L^{-1} (as tributyltin cation).

* Corresponding author. Tel.: +33 1 40 43 39 71; fax: +33 1 40 43 37 37.
E-mail address: enrica.alasonati@lne.fr (E. Alasonati).

http://dx.doi.org/10.1016/j.talanta.2014.11.064
0039-9140/

Notwithstanding the number of different methodologies for the quantification of organotin species, developed in the last two decades by a combination of sensitive analytical techniques [9], standardised analytical methods for TBT in water at EQS level do not exist. The ISO standard 17353:2004 [10] allows quantification of TBT down to a limit of about 10 ng L^{-1} which is two orders of magnitude above the EQS. The big challenge of TBT quantification at such low concentration levels subsists in the sample pre-treatment procedure, more than in the instrumental measurement. In fact, quantification at sub-nanogram level is perturbed by the presence of high level of blanks and requires maximal care during all the sample preparation steps as well as an efficient preconcentration approach [11,12]. Among the different extraction techniques used to isolate and concentrate OTC from the matrix are liquid–liquid extraction (LLE) [13], supercritical fluid extraction (SFE) [14], solid-phase microextraction (SPME) [15], stir bar sorptive extraction (SBSE) [16], liquid-phase microextraction (LPME) and solid-phase extraction (SPE) [17]. All these techniques show some advantages and some disadvantages, as reviewed by Dietz and Oliveira [14,9]. This work aims to evaluate the relevance of the solid-phase extraction (SPE) as a preconcentration method for the quantification of TBT at EQS levels. Main advantages of SPE are the easy application during field sampling, the need of lower amount of toxic solvents than in other extraction techniques as LLE and Soxhlet extraction, and the possible integration of columns and cartridges in on-line injection systems [14]. SPE is a separation process which allows to separate specific compounds in a mixture, according to their physical and chemical properties and their interaction with the sorbent and the solvent [18]. SPE is used to select and concentrate the analytes and to remove the interfering matrix components. Two separation procedures are commonly used: in the first case the analytes are retained on the sorbent (preconcentration approach), while in the second case the interfering components are retained meanwhile the analytes are eluted (purification approach). The preconcentration approach, which is the method of interest for this work, is composed of several steps: (i) a first crucial step is the conditioning of the sorbent; (ii) then the sample is percolated through the solid phase. (iii) After optional washing and drying of the sorbent, (iv) the analytes of interest are finally eluted by an appropriate solvent. Different solid phases, eluents, preparative and instrumental conditions have been applied in literature for the quantification of OTC in water media using the SPE as a preconcentration method. Tropolone-loaded C-silica has been used for the retention of TBT, ethylated with Grignard reagent and separated by gas chromatography coupled to flame photometric detection (GC–FPD), yielding to a sensitivity of low ng L^{-1} in surface waters [19]. Amberlite XAD-2 impregnated with tropolone and addition of 0.8% sulfuric acid to the water sample are also been reported to selectively retain TBT. The limit of detection of TBT in water samples was about 14.4 ng L^{-1} by electrothermal atomic absorption spectrometry (ET-AAS) [20]. A method for the determination of TBT in spiked Mediterranean seawater was reported with a detection limit of 98 ng L^{-1}, using SPE and reversed-phase high-performance liquid chromatography (RP-HPLC) fluorimetry with post-column derivatization. Elution with a mixture of methanol, acetic acid, and water was found most suitable [21]. C18 column using a mobile phase containing 0.1% (v/v) HCOOH/5mMHCOONH$_4$ and methanol were used for on-line SPE-LC electrospray ionization mass spectrometry (ESI–MS) method for the determination of tributyltin (TBT) in spiked freshwater and seawater samples. The detection limit for TBT was 20 ng L^{-1} [17]. These applications, characterized by a large variety of solid phases, eluents, and operatives conditions, have in common a reported detection limit in the range of 10 to 40 ng L^{-1}, which is hundred times higher than the EQS level for TBT imposed by the water framework directive (WFD). Therefore, there is a need for analytical methods able to quantify TBT at much lower concentrations.

In this work, for the first time, a SPE method for the quantification of TBT in mineral water at the mass fraction of about 0.5 ng kg^{-1} was developed and optimized. The quantification was done using species-specific isotope dilution (SSID) as a calibration primary method, and gas chromatography coupled to inductively coupled plasma mass spectrometry (GC–ICP–MS) for TBT separation and detection. The parameters of the method were optimized with the use of a design of experiment (DOE), based on a factorial fractionary plan. For an extensive method development considering all variables, the number of experiments could rise to a high number. The DOE allowed to evaluate many experimental conditions, with less experimental work and with a structured approach. Experimental design methodology was successfully used for analysis of OTC to optimize the conditions affecting the headspace solid-phase microextraction in ultra-pure water [22], sediments [23], fish and natural waters [24,25]. This work first apply the chemometric approach to SPE optimization for OTC analyses. The experiments were shared between two institutes, the *Bundesanstalt fuer Materialforschung und -pruefung* (BAM) and the *Laboratoire National de Métrologie et d'Essais* (LNE). Different models representing the results were estimated and analysed with statistic tools. Finally, a linear model was assessed and it allowed to find the optimal solution for the set of factors.

2. Experimental

2.1. The choice of the influents factors for the DOE

SPE is influenced by the following factors in the case of TBT preconcentration: (1) the type of stationary phase; (2) the amount of stationary phase; (3) the pre-treatment of the stationary phase (conditioning, washing); (4) the sample volume; (5) the pH of the sample; (6) the type and volume of elution solvent; (7) the flow rate of elution step; (8) the ethylation of the analytes that can be performed before or after SPE. Information on SPE applications for TBT from literature [26,17,19,27] and preliminary experiments, allowed to select four influent factors and their associated levels (Table 1). It was essential to reduce as much as possible the number of factors and the number of levels for each factor, otherwise the experimental work would has been unmanageable and the model too complex.

(A) The *phase* and the *eluent* are the most relevant factors. We decided to couple them in one factor, in order to test only the interesting conditions and to avoid the phase/eluent combinations not desirable. In order to reduce the number of levels, the choice of phases was restricted to C18, strong cation exchange (SCX), and NH_2/C18 phases. C18 and SCX are the most commonly used for TBT and they are representative of the double TBT characteristic to be both ionic and soluble in non-polar organic solvents. Better performances are reported for TBT with strong cation exchange silica-based bonded phases (Bond-Elut SCX) than for strong cation exchange polymeric-based phase (Oasis-MCX), therefore we disregarded polymeric phases [21]. The aminopropyl phase NH_2/C18 is a phase used for the enrichment of polycyclic aromatic hydrocarbons (PAHs) from humic acids-rich water: the NH_2 phase allows removal of the interfering humic acids. This column has been chosen as a good candidate for future extension of the SPE method to whole water samples. The selection of eluents has been done as a function of their polarity and their coherence with the sorbent, according to preliminary experiments, literature and supplier recommendations (as an example [26,17,19,27]). In this regard, the following eluents were selected: ethylacetate, methanol (MeOH), tetrahydrofurane (THF), dichloromethane (CH_2Cl_2) and a polar mixture composed of 0.3 M NH_4Cl in MeOH/HAc/H_2O (60:2.5:37.5) [21].

Table 1
Factors of the DOE and their levels or range.

Factors	Symbols	Levels/range	Labels
(A) Phase and eluent; qualitative	φE	8 levels:	
		(1) C18+ethylacetate	C18E
		(2) C18+MeOH	C18M
		(3) C18+THF	C18T
		(4) C18+CH_2Cl_2	C18C
		(5) SCX+MeOH	SCXM
		(6) SCX+0.3 M NH4Cl in MeOH/HAc/H_2O (60:2.5:37.5)	SCXMN
		(7) NH_2/C18+CH_2Cl_2	Phase 3A
		(8) NH_2/C18+THF	Phase 3B
(B) Phase mass and eluent volume; qualitative	$m_\varphi V_E$	2 levels:	
		(1) 0.5 g and 6 mL	0.5/6
		(2) 1 g and 12 mL	1/12
(C) pH and ethylation; qualitative	*pHeth*	3 levels:	
		(1) pH5+ethylation before	pH5Before
		(2) pH5+ethylation after	pH5After
		(3) pH2+ethylation after	pH2After
(D) Sample volume; quantitative	V_s	Range: 250 mL $< V_s <$ 1000 mL	

(B) The *eluent volume* is usually selected as a function of the phase mass. Both phase mass and eluent volume have been coupled in one factor with two levels: 0.5 g of phase coupled to 6 mL of volume and 1 g coupled to 12 mL, based on supplier recommendations.
(C) The *sample volume* is function of the phase mass and the kind of sample. A range between 250 and 1000 mL has been chosen in this work in order to have a reasonable preconcentration factor for the quantification at EQS level.
(D) The pH is also a relevant factor, especially when it is related to the derivatization step. The derivatization has been tested before and after SPE using two levels of pH, acid (pH=2) and the optimal for ethylation (pH=5) [28]. The *pH of the sample* and the *ethylation* were coupled in one factor. The combination of pH 2 and ethylation before was disregarded as it is well known that pH 2 is not appropriate for the derivatization process [29].

The conditioning of SPE columns was not considered as a factor and was done following the supplier's recommendations. Also the SPE flow rate was not taken into account and was fitted according to the instrumental setup.

2.2. Reagents and standards

Tributyltin chloride (96.2%), *n*-Hexane CHROMASOLV (97%) and methanol were purchased from Sigma-Aldrich (USA). Glacial acetic acid (HAc), tetrahydrofuran, (THF; 99.8%) and sodium tetraethylborate ($NaBEt_4$) were purchased respectively from Merck (Germany), Carlo Erba (Italy) and Strem Chemicals (USA) (LNE), or from Merseburger Spezialchemikalien (Germany) (BAM). The sodium acetate (NaAc) was purchased from Merck (Germany). The 119Sn-enriched butyltin mix was purchased from ISC Science (Spain). All stock and intermediate solutions were prepared in disposable 12 mL amber glass vials with PTFE caps from Chromacol Limited (United of Kingdom). 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_{Sn} kg^{-1} are stable for three months. Solutions at lower concentrations were prepared daily. The last dilutions were prepared in purified water from a Milli-Q system Q-POD Element (Millipore, USA) to avoid the addition of any solvent to the reference solution.

2.3. In-house reference solution

To this day, there is no commercially available certified reference material for organotin in water. To validate the accuracy of the method, a reference water with a gravimetric reference value was used. The water was prepared by diluting the intermediate standard solution of TBT in Evian® mineral water (conditioned in glass bottles) at the mass fraction of 0.5 ng kg^{-1} (as organocation), that is about twice higher than the WFD EQS. Since compounds stability remains to be determined at low concentrations, the reference solution was prepared daily. For comparison aims, the average TBT mass fraction calculated for every combination of experimental conditions has been normalized to the reference solution daily prepared.

2.4. SPE analysis

A SPE 12-position vacuum manifold Phenomenex (USA) was used in LNE and an Autotrace Caliper Life Sciences (USA) in BAM. The SPE cartridges evaluated in this study were: Strata C18-E (55 μm, 70 Å) and Strata SCX (55 μm, 70 Å) purchased from Phenomenex, and the CROMABOND® aminopropyl phase (45 μm, 60 Å) purchased from Macherey-Nagel (Germany). Conditioning, washing and drying procedures are given in Table 2.

2.5. Derivatization procedure

TBT separation by gas chromatography requires the analyte derivatization, combined with a liquid–liquid extraction to a solvent compatible with injection in the GC system. Organotin derivatization occurred in an environment buffered at pH 5 by an acetate buffer solution (HAc–NaAc; 0.1 mol L^{-1} for LNE and 1 mol L^{-1} for BAM). Stock solutions of the derivatization agent, 10% sodium tetraethylborate ($NaBEt_4$) in tetrahydrofuran (THF) were prepared monthly. Solutions of 0.5% $NaBEt_4$ in milli-Q water were prepared right before being used. The derivatization step was adapted to the elution solvent used. MeOH was evaporated to dryness under nitrogen stream, then 2 mL of buffer were added. 1 mL of $NaBEt_4$ 0.5% was added to the solution and

Table 2
Procedures for conditioning, washing and drying of C18, SCX and NH_2/C18 phases.

Phase and eluent	Conditioning	Washing	Drying
C18+ethylacetate	6 mL MeOH, 6 mL water	3 mL MeOH/water (5/95)(v/v)	20 min (vacuum)
C18+MeOH	6 mL MeOH, 6 mL water	3 mL MeOH/water (5/95)(v/v)	20 min (vacuum)
C18+THF	6 mL MeOH, 6 mL water	3 mL MeOH/water (5/95)(v/v)	20 min (vacuum)
C18+CH_2Cl_2	6 mL MeOH, 6 mL water	3 mL MeOH/water (5/95)(v/v)	20 min (vacuum)
SCX+MeOH	6 mL MeOH, 6 mL water	3 mL Milli-Q water	20 min (vacuum)
SCX+0.3 M NH4Cl in MeOH/ HAc/H_2O (60/2.5/37.5) (v/v)	5 mL water, 5 mL MeOH	5 mL MeOH, 5 mL MeOH/HAc/H_2O (65/5/30)	20 min (vacuum)
NH_2/C18+CH_2Cl_2	10 mL CH_2Cl_2, 10 mL MeOH, 10 mL water/2-propanol (9/1) (v/v)	2 mL water/2-propanol (9/1)	20 min (vacuum)
NH_2/C18+THF	10 mL THF, 10 mL MeOH, 10 mL water/2-propanol (9/1) (v/v)	2 mL water/2-propanol (9/1)	20 min (vacuum)

Table 3
GC–ICP-MS parameters.

	Parameters	GC Agilent 7890A/ICP–MS Agilent 7700x—Institute 1 (LNE)	GC Agilent 7890A/ICP-MS Agilent 7500cx—Institute 2 (BAM)
GC	Column	HP-5 (30 m × 0.25 mm ID and 0.25 μm film thickness, 5% phenyl and 95% methylpolysiloxane)	DB-5 MS UI (30 m × 0.25 mm ID and 0.25 μm film thickness, 5% phenyl and 95% methylpolysiloxane)
	Carrier gas	Helium	Helium
	Carrier gas flow	2 mL min^{-1}	2 mL min^{-1}
	Injected volume	2 μL	2 μL
	Injector temperature	250 °C	250 °C
	Injection mode	Splitless	Splitless
	Furnace temperature	Initial temperature of 50 °C, 40 °C min^{-1} up to 250 °C (5 min). Then 0.9 min at 250 °C.	Initial temperature of 60 °C, 30 °C min^{-1} up to 300 °C (4 min).
Interface	Transfer line temperature	250 °C	290 °C
	Injector temperature	250 °C	290 °C
ICP-MS	RF Power	1550 W	500 W
	Plasma-forming gas flow	15 L min^{-1}	15 L min^{-1}
	Carrier gas flow	0.9 mL min^{-1}	1.1 mL min^{-1}
	Addition of oxygen	Yes	No
	Integration time	30 ms	18 ms
	Isotopes measured	^{119}Sn and ^{120}Sn	^{119}Sn and ^{120}Sn

LLE was performed by adding 1 mL of hexane and agitating the samples on a rotary table for 20 min. On the other side, when using ethylacetate, THF and CH_2Cl_2 eluents, the ethylation was performed directly in the solvent, using 1 mL of $NaBEt_4$ 0.5% and agitating. Then the solvent was preconcentrated to 1 mL under nitrogen stream. Samples were analyzed by GC–ICP-MS at once, otherwise they were stored at −20 °C for a maximum of 48 h before being analyzed.

2.6. GC–ICP–MS analyses

A 7890A Agilent Technologies (USA) gas chromatograph (LNE, BAM) coupled to a 7700x Agilent Technologies (USA) ICP–MS (LNE) or a 7500cx Agilent Technologies (USA) ICP–MS (BAM) were used for isotope measurements. Instrumentation parameters for GC, GC–ICP-MS interface and ICP–MS are given in Table 3. Pure argon and pure helium (N2) were purchased from Airliquide (respectively France and Germany). Data acquisition was performed using Agilent Technologies *MassHunter* software (version B.01.01).

2.7. Quantification by species-specific isotope dilution

Isotope dilution (ID) has been used for TBT quantification at EQS level. ID allows the development of highly accurate and precise quantification approaches in elemental speciation even when analysing complicated matrices [30]. The amount content of the labelled compounds in the spike solution was determined by reverse isotope dilution (RID). The isotope ratio measured was the ratio of the isotope 119 to the isotope 120. The selected ratios were $R^{119/120}=1$ and $R^{119/120}=0.4$ for LNE and BAM, respectively. The measurand of the DOE, the normalized TBT mass fraction, w_x^* is calculated using the ID equation, normalized to the gravimetric mass fraction of the reference solution:

$$w_x^* = w_{std} \times \frac{m_{std}}{m_{spike\mathrm{RID}}} \times \frac{m_{spike\mathrm{DID}}}{m_x} \times \frac{R_{\exp\mathrm{RID}} A_{nat}^{119} - A_{nat}^{120}}{A_{spike}^{120} - R_{\exp\mathrm{RID}} A_{spike}^{119}} \frac{R_{\exp\mathrm{DID}} A_{spike}^{119} - A_{spike}^{120}}{A_{nat}^{120} - R_{\exp\mathrm{DID}} A_{nat}^{119}} \times \frac{1}{w_x^{ref}} \quad (1)$$

The following parameters are measured:

w_{std}=mass fraction of the standard solution used for reverse isotope dilution (RID)
m_{std}=mass of the standard solution for RID
$m_{spike\mathrm{RID}}$=mass of the isotope-enriched standard for RID
$R_{exp\mathrm{RID}}$=isotope ratio of the mixture 120/119 for RID
$m_{spike\mathrm{DID}}$=mass of the isotope-enriched standard for direct isotope dilution (DID)
m_x=sample mass
$R_{exp\mathrm{DID}}$=isotope ratio of the mixture 120/119 for DID
$A_{spike119}$=abundance of the isotope 119 in the isotope-enriched standard
$A_{spike120}$=abundance of the isotope 120 in the isotope-enriched standard
w_x^{ref}=TBT mass fraction within the sample as calculated by gravimetry.

The following parameters are known from the IUPAC tables:

A_{nat120} = natural abundance of the isotope 120
A_{nat119} = natural abundance of the isotope 119.

All the variables in Eq. (1), except for R_{expDID}, are independent from the experimental SPE procedure. On the other side, the isotope ratio of the mixture 120/119 for the DID, R_{expDID}, and by consequence the normalized TBT mass fraction in the water sample, w_x^*, are directly related to the different factors appearing in the analytical procedure (phase type, eluent, phase mass, eluent volume, sample volume, pH, ethylation, *etc.*).

2.8. Assessment of the fractional factorial design

A fractional factorial design has been set up using the ;*Design-Expert*® software (version 7.1.4). The experiments were shared between the two partners. Fig. 1 shows the experimental space. To visualize the four-dimensional (4D) space generated by the 4 factors, a double 3D representation has been chosen, showing the volume corresponding to the 2 levels of the "Phase mass and eluent volume" factor. For each experimental condition, nine TBT mass fraction values were measured (3 samples replicates, 3 independent measurements per sample) and their average value was normalized to the gravimetrically daily prepared reference solution. The results have been processed altogether and by institute (BAM and LNE). *Design-Expert*® was used to model the data and for the optimization. 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%. Another software, *Statgraphics*® (Centurion XV, version 15.2.13), was also used to compare the estimated models.

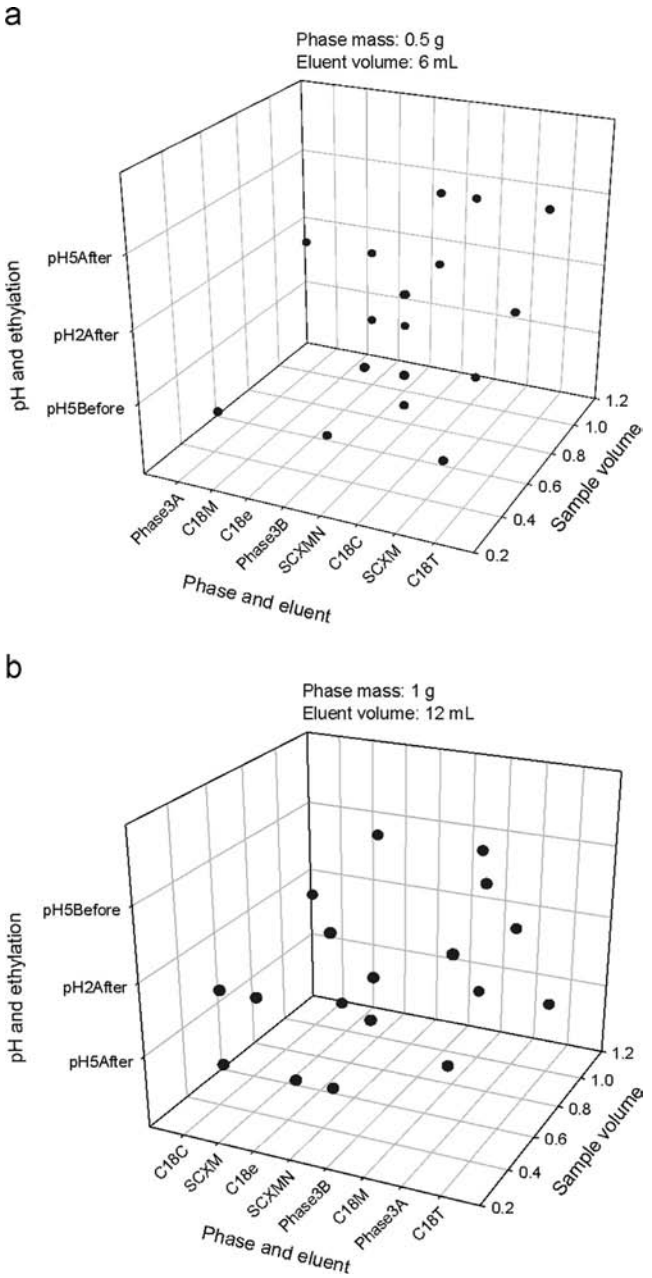

Fig. 1. Four-dimensional experimental space. The "Phase mass and eluent volume" factor has been set to allow a 3D display: (A) level 1, phase mass = 0.5 g and eluent volume = 6 mL; (B) level 2, phase mass = 1 g and eluent volume = 12 mL.

3. Results and discussion

3.1. The DOE for TBT quantification

The two institutes involved, BAM and LNE, compared their analytical performances realizing both the same experiment (n. 13) involving C18 phase, THF solvent, 1 g of phase and 12 mL of elution volume, pH5, ethylation after and 1 L of sample volume (Fig. 2). The normalized average values were 0.954 (x) and 0.924 (y) for LNE and BAM, respectively, with standard deviations s_x of 0.037 and s_y of 0.103, respectively. A test based on E_N score (ISO 13528) has been applied to the difference of the two average values showing that it is not significant in the confidence range of 95%:

$$E_N = \frac{|x-y|}{\sqrt{(s_x)^2+(s_y)^2}} = 0.544 < 2 \quad (2)$$

The two institutes performed 33 experiments between July and August 2013 (Fig. 3, Table 4). Twenty-one of them have been processed to define the model. All the individual results are represented in Fig. 3, that means 9 measurements for each experiment (3 samples replicates, 3 independent measurements per sample). The constraints (n=4, 18) correspond to particular conditions, proposed by the DOE, which are not suitable for testing. The 12 experiments that have not been included in data treatment gave non workable data. In fact, when the acquired isotopic signal is too low or if there is a blank contamination, the raw data cannot be processed as the application

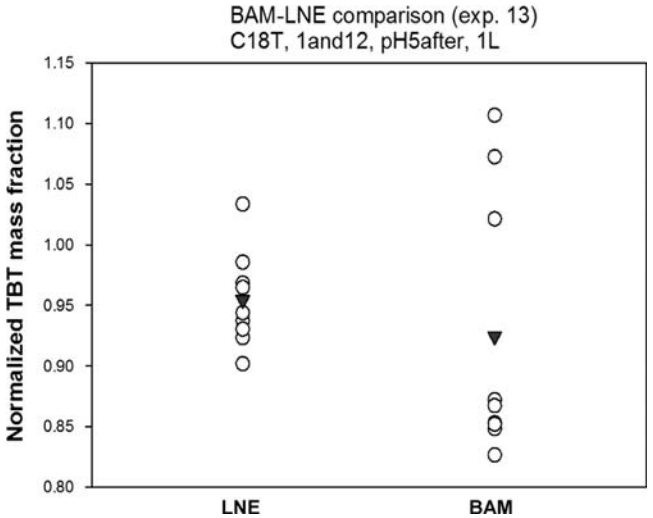

Fig. 2. Comparison of the two institutes (x-axis) on the same experiment: C18/THF, 1 g of phase, 12 mL eluents, pH5, ethylation after, V_s = 1 L. Triangles correspond to the average values.

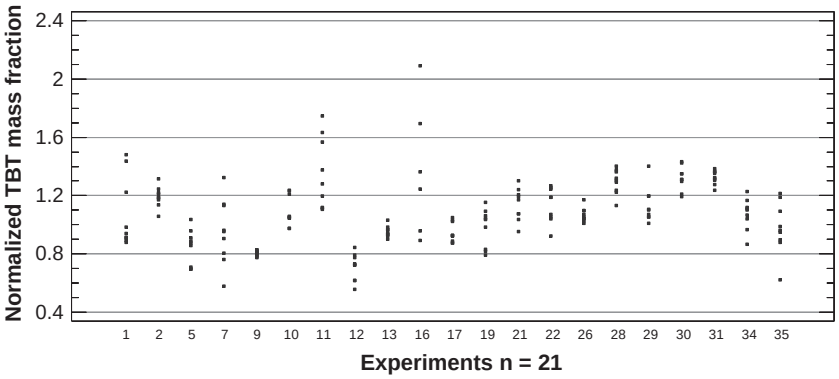

Fig. 3. Graphical representation of the experimental data used for model processing. Experiments 1 to 17 were performed by LNE and 19 to 35, by BAM. Only 6 measurements have been done for experiment $n=16$.

Table 4
List of the experiments performed by each partner derived by the *Design-Expert*® software. Factors: V_s=volume of the sample (L); φE=phase and eluent; $m_\varphi V_E$=phase mass (g) and eluent volume (mL); *pHeth*=pH and ethylation; levels are defined in Table 2. Blank and sample signals are the average signals of the isotope 120 (cps; $n=9$). S/N=signal to noise.

	n Experiment	V_s (L)	φE	$m_\varphi V_E$ (g; mL)	*pHeth*	*Blank signal* (cps)	*Sample signal* (cps)	*S/N*
Partner: LNE	1	0.25	C18T	1/12	pH5Before	198	1,632	8.3
	2	1	C18T	0.5/6	pH5After	266	2,223	8.4
	3	1	Phase3A	0.5/6	pH2After	14,504	13,998	1.0
	4	0.75	SCXM	1/12	pH5Before			
	5	0.75	C18T	1/12	pH2After	1,035	4,051	3.9
	6	1	C18C	1/12	pH5After	167	153	0.9
	7	0.25	C18M	0.5/6	pH2After	386	557	1.4
	8	0.5	Phase3B	0.5/6	pH2After	20,625	15,979	0.8
	9	1	C18M	0.5/6	pH5Before	243	4,408	18.1
	10	0.25	SCXM	1/12	pH5After	719	1,930	2.7
	11	0.25	C18T	0.5/6	pH5Before	145	1,529	10.6
	12	1	SCXMN	1/12	pH2After	276	855	3.1
	13	1	C18T	1/12	pH5After	222	2,268	10.2
	14	1	Phase3B	1/12	pH5Before	14,481	15,504	1.1
	15	0.25	Phase3B	0.5/6	pH5Before	21,664	10,362	0.5
	16	0.25	SCXMN	0.5/6	pH5After	139	1,780	12.8
	17	1	C18E	1/12	pH2After	149	2,826	18.9
Partner: BAM	18	0.25	SCXMN	1/12	pH5Before			
	19	0.75	Phase3A	0.5/6	pH5Before	993	2,687	2.7
	20	0.25	Phase3B	0.5/6	pH5After	15,780	14,950	0.9
	21	0.25	C18E	0.5/6	pH2After	177	992	5.6
	22	1	C18E	1/12	pH5Before	121	2,491	20.7
	23	0.25	SCXM	0.5/6	pH2After	141	153	1.1
	24	0.5	Phase3A	0.5/6	pH5After	1,909	8,832	4.6
	25	0.25	SCXM	0.5/6	pH5After	244	346	1.4
	26	1	C18C	0.5/6	pH2After	765	5,309	6.9
	27	0.25	SCXM	1/12	pH2After	276	241	0.9
	28	0.25	C18C	1/12	pH2After	659	2,470	3.7
	29	0.25	C18M	1/12	pH5Before	142	531	3.8
	30	0.5	C18C	1/12	pH5Before	271	2,639	9.8
	31	1	C18E	0.5/6	pH5Before	238	4,559	19.2
	32	1	C18M	0.5/6	pH5After	63	43	0.7
	33	1	Phase3A	1/12	pH2After	21,394	18,034	0.8
	34	0.75	C18E	0.5/6	pH5After	319	2,298	7.2
	35	1	SCXMN	1/12	pH5After	146	333	2.3
	High blanks							
	Low signals							
	Constraint							

of the isotope dilution equation results in negative or largely overestimated TBT mass fractions. The presence of considerable amounts of TBT in the analytical blanks, giving rise to average blank signals of about 15,700 cps on the isotope 120, was the reason for the following experiments giving false results: n. 3, 8, 14, 15, 20, 24, 33; Table 4—light grey). On the other hand, the following analyses showed a too low signal of approximately 200 cps: n. 6, 23, 25, 27, 32 (Table 4—dark grey). The average signal to noise (S/N) of these 12 experiments was 1.2. These experiments were repeated and the previous results were confirmed: they were finally excluded from data treatment. It has been observed that two phases were mostly responsible for these "false" results (high blanks/low signals): the phase NH_2/C18 (Phase 3) and the strong cation exchange phase (SCX). The phase NH_2/C18 with both elution solvents (CH_2Cl_2 and THF) was always associated with the presence of blanks significantly higher than the blank average. The SCX phase was involved in 7 experiments (10, 12, 16, 23, 25, 27, 35): the mixture 0.3 M NH_4Cl in MeOH/HAc/H_2O (60:2.5:37.5) worked better than pure MeOH as eluent (Fig. 4). Nevertheless, 3 out of the

7 SCX experiments presented very low signals and were excluded, and the four experiments giving workable results were mostly characterized by low S/N (experiments n. 10, 12, 35). It can be concluded that the SCX phase is responsible for poor TBT recoveries so that the measured signal is too close to the blank signal. Hence, these two phases, NH_2/C18 and SCX, were considered as not satisfactory and have been excluded from the final list of the optimized conditions.

In general, a S/N higher than 2 was necessary to process the data and to quantify the TBT content (exception: experiment n. 7). Average blank signals were about 250 cps on the isotope 120. An example of representative blanks and samples chromatograms at EQS level is given in Fig. 5.

3.2. Models to fit the experimental data

The workable results have been processed altogether and by institute (BAM and LNE). Three models have been tested and statistically evaluated. Model 1 (M1) is a linear model fitting the average values of the 9 replicates done for each set of experimental conditions, while Model 2 (M2) fits all the individual results. The Model 3 (M3) fits all the individual measurements, but removing the *pHeth* factor and taking into account the interaction between *pHeth* and V_s. The three models and their statistical evaluations will be described below.

3.3. M1

The first model has been established using the normalized average TBT mass fractions ($\overline{w}^*_{TBT}$) measured for each set of experimental conditions (average of nine mass fraction values: three sample replicates and three measurements per sample). The adjustment of the general linear model which links the measurand, $\overline{w}^*_{TBT}$, with the four factors was evaluated using an analysis of variance (ANOVA). The probability associated to the model, $p=0.0184$, was lower than 0.05 (Table 5), which means that the relationship between $\overline{w}^*_{TBT}$ and the factors is significant at the 0.05 significance level. The coefficient of determination, R^2, that indicates how well data fit a statistical model, was 80.3%. The statistic significance of every factor in the model has been evaluated (Table 6). The factors are statistically significant except for the "phase mass and eluent volume" factor.

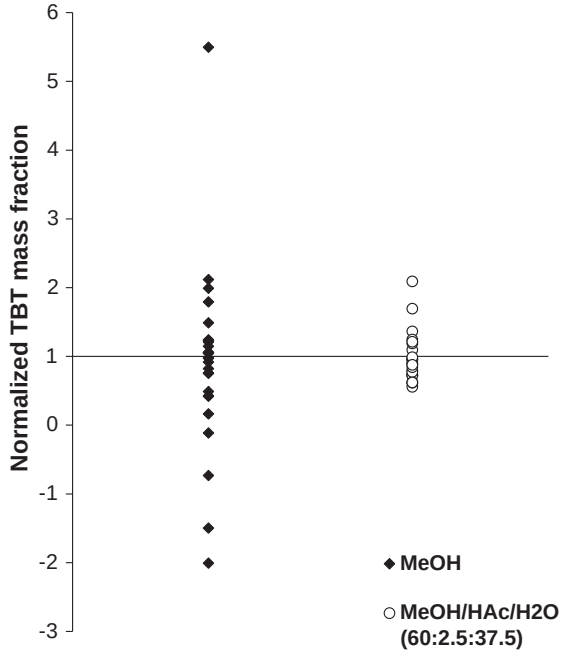

Fig. 4. Normalized TBT mass fractions obtained in the experiments n. 10, 12, 16, 23, 25, 27 and 35, using the SCX phase. The results are here represented as a function of the solvent used for elution: pure methanol and the mixture 0.3 M NH_4Cl in MeOH/HAc/H_2O (60:2.5:37.5).

3.4. M2

From a statistical point of view it is interesting to treat all the data obtained, that means all the individual measurements without averaging the 9 TBT mass fraction values measured for every experimental condition. This data treatment gave rise to the second linear model. This model presents a lower R^2 equal to 48.8%, and a higher standard deviation of the estimation, $s=0.168$, but the relationship between $\overline{w}^*_{TBT}$ and the factors is more significant and the four factors themselves are significant and all influence the final result.

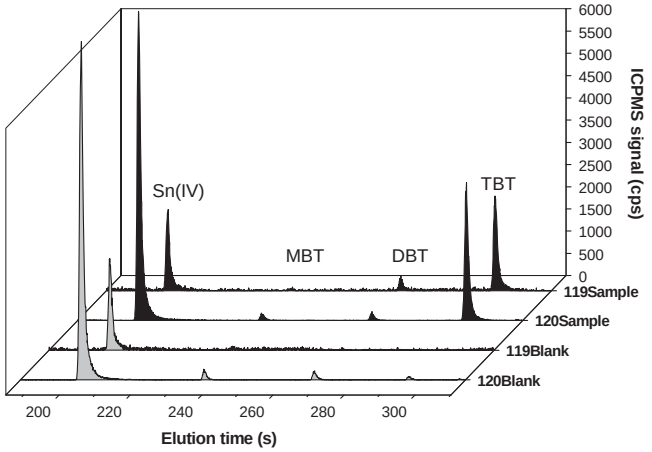

Fig. 5. Representative blanks and samples chromatograms corresponding to the isotopes 119 and 120. Experiment 17. Elution order and retention times (t_r): inorganic tin—Sn(IV) ($t_r=202$ s), monobutyltin—MBT ($t_r=237$ s), dibutyltin—DBT ($t_r=268$ s), and tributyltin—TBT ($t_r=294$ s).

Table 5
ANOVA of the linear model adjustment for M1, M2, M3 and for M2 and M3 applied to the data of the institutes, BAM and LNE, separately ($M2_{BAM}$, $M2_{LNE}$). Probability associated to the model, *P*; coefficient of determination, R^2; estimation standard deviation, *s*.

	M1	M2	$M2_{BAM}$	$M2_{LNE}$	M3	$M3_{BAM}$	$M3_{LNE}$
P	0.0184	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$
R^2	80.3%	48.8%	50.0%	57.5%	47.3%	58.7%	57.4%
s	0.116	0.168	0.120	0.178	0.170	0.110	0.179

Table 6
ANOVA of the DOE factors: phase and eluent (φE); phase mass and eluent volume ($m_\varphi V_E$); pH and ethylation (*pHeth*); sample volume (V_s). *P* is the probability associated to the factors and express the statistic relevance of each factor in the order as they are introduced in the model.

	M1	M2	$M2_{BAM}$	$M2_{LNE}$	M3	$M3_{BAM}$	$M3_{LNE}$
Factor	*P*						
φE	0.0364	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$
$m_\varphi V_E$	0.1177	0.0008	0.1498	$<10^{-4}$	0.0021	0.0016	$<10^{-4}$
pHeth	0.0156	$<10^{-4}$	$<10^{-4}$	0.0351	–	$<10^{-4}$	–
V_s	0.0049	$<10^{-4}$	0.0005	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$
pHeth × V_s	–	–	–	–	$<10^{-4}$	0.0001	0.0389

3.5. M3

Several tests were performed to check whether it was possible to improve M2, removing one of the factors and/or introducing one or more first-order interactions between the factors. Thereby the best combination was found with Model 3. M3 has been established using all the individual measurements, removing the *pHeth* factor and adding the interaction $pHeth \times V_s$. The coefficient of determination was lower than in M2, with a similar standard deviation and the less significant factor was again the $m_\varphi V_E$ factor.

The independent data processing of the two institutes, BAM and LNE led to similar models, with R^2 varying from 50% to 58.7% (Table 5). This statement is coherent with the fact that the two institutes show similar analytical performances when realizing the same experiment, as previously discussed (Fig. 2). The estimation standard deviation was lower for BAM than for LNE: This is in agreement with the dispersion of the 9 measurements that appears to be higher in many of the first 17 experiments (Fig. 3). The only factor that resulted non-significant was the $m_\varphi V_E$ factor in the case of BAM data, processed with M2. The fact that the $m_\varphi V_E$ factor appears often as a non-significant parameter is not surprising, because the chosen levels are coherent with the sample volume tested and the samples are prepared in mineral water at low analyte concentration, so there is no risk to saturate the phase, neither competition with other species.

3.6. A model for outliers data

A fourth linear model (M4) has been outlined in the aim to use all the experimental data, even the false results, defining a different measurand. In fact, the non workable results still represent an information about the experimental conditions associated, information that is lost when these data are discarded. Such approach was especially interesting to not lose the information concerning the two phases, NH_2/C18 and SCX, whose data were mostly unusable for model development. Therefore, to include in data treatment the information associated with the experiments previously excluded (n. 3, 6, 8, 14, 15, 20, 23, 24, 25, 27, 32, 33), we have defined another way to express the results, the "blank to signal" normalized ratio, $(B/S)_N$. $(B/S)_N$ is the average blank signal Sn_{Bk}^{120} divided by the average sample signal Sn_{sample}^{120} and normalized to the TBT mass fraction of the reference solution calculated by gravimetry, $C_{TBTgrav}$:

$$\left(\frac{B}{S}\right)_N = \frac{Sn_{Bk}^{120}}{Sn_{sample}^{120} C_{TBTgrav}} \quad (3)$$

This definition allows to use all the data (Fig. 6). The "blank to signal" normalized ratio is a kind of evaluation of the performance of the procedure. In the case of "good experiments", when the blank is very low (close to zero), the ratio is also close to zero. Inversely, when the blank and the sample have similar signals the ratio increases. This approach gave rise to a model with all the factors significant, a coefficient of determination, R^2 of 48.8% and an estimation standard deviation of 0.17. These values are in agreement with M2 and confirm the results obtained even when the aberrant results are included in data processing. Unfortunately, as a consequence of the high data dispersion (Fig. 6), *Design-Expert*® found a significant lack of fit, so the model could not be optimised.

3.7. The chosen model: M2

In conclusion, M2 was preferred, despite of the R^2 lower than in M1. The reasons of this choice are the following: (i) The factors are all significant and (ii) working with individual measurements allows to process all the information together. The model equation is the following:

$$\hat{w}^*_{TBT} = 1.237 + 0.241\varphi E(1) - 0.152\varphi E(2) + 0.018\varphi E(3) + 0.123\varphi E(4) - 0.166\varphi E(5) - 0.079\varphi E(6) + 0.048 m_\varphi V_E - 0.136 pHeth(1) + 0.057 pHeth(2) - 0.292 V_s \quad (4)$$

where the indicators for the qualitative factors are given in Table 7. In fact, the software needs to define a code for the qualitative factors, in order to combine the different qualitative levels in the final model equation. For a given set of experimental conditions,

Table 7
Indicators of the model corresponding to the qualitative factors φE, $m_\varphi V_E$ and *pHeth*.

If φE=	Indicator	If $m_\varphi V_E$=	Indicator
C18 C	$\varphi E(1)=1$	0.5 g; 6 mL	$m_\varphi V_E\ (1)=1$
SCXM N	$\varphi E(1)=-1$	1 g; 12 mL	$m_\varphi V_E\ (1)=-1$
otherwise	$\varphi E(1)=0$	otherwise	$m_\varphi V_E\ (1)=0$
C18 M	$\varphi E(2)=1$		
SCXM N	$\varphi E(2)=-1$		
otherwise	$\varphi E(2)=0$	If *pHeth*=	Indicator
C18 T	$\varphi E(3)=1$	pH2 After	*pHeth* (1)=1
SCXM N	$\varphi E(3)=-1$	pH5 Before	*pHeth* (1)=−1
otherwise	$\varphi E(3)=0$	otherwise	*pHeth* (1)=0
C18 e	$\varphi E(4)=1$	pH5 After	*pHeth* (2)=1
SCXM N	$\varphi E(4)=-1$	pH5 Before	*pHeth* (2)=−1
otherwise	$\varphi E(4)=0$	otherwise	*pHeth* (2)=0
Phase3 A	$\varphi E(5)=1$		
SCXM N	$\varphi E(5)=-1$		
otherwise	$\varphi E(5)=0$		
SCX M	$\varphi E(6)=1$		
SCXM N	$\varphi E(6)=-1$		
otherwise	$\varphi E(6)=0$		

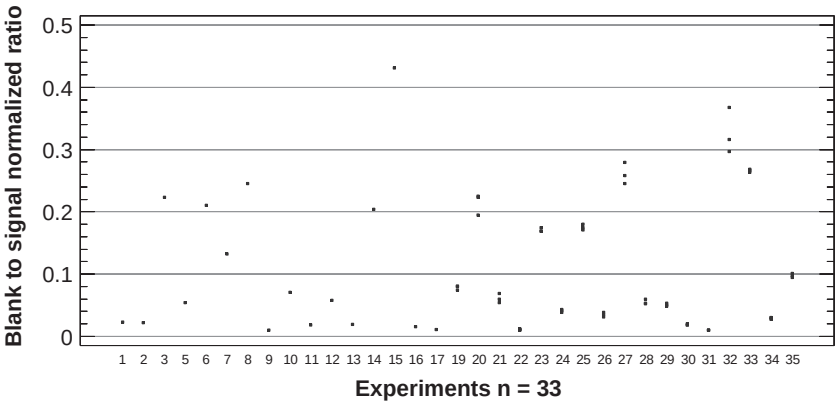

Fig. 6. Blank to signal normalized ratios for the 33 experiments realized in the experimental plan. Experiments 1 to 17 were performed by LNE and 19 to 35, by BAM.

the indicators are extrapolated from the table, then inserted in the model equation (Eq. (4)) to calculate the expected TBT mass fraction.

Graphical representations of the final model are not easy when there are numerous and mainly qualitative factors. The expected normalized TBT mass fraction was plotted as a function of the phase/eluent and the sample volume factors. This output was given 6 times, in function of the combinations of the other two factors, $m_\varphi V_E$ and *pHeth* (Fig. 7). The optimal region is in the range $0.9 < \hat{w}^*_{TBT} < 1.1$. As previously discussed (Table 4), the NH_2/C18 and SCX phases were considered as not satisfactory: the levels corresponding to these two phases have been disregarded, so that only the C18 phase is here represented coupled to 4 different eluents. It can be observed in the six experimental surfaces that the eluents increase the estimated TBT mass fraction in the following order: CH_2Cl_2 > ethylacetate > THF > MeOH. Depending on the other factors, this increase can result in a better TBT recovery (*e.g.* Fig. 7A and B), or in a overestimation of the final concentration (*e.g.* Fig. 7C and E).

The increase of the volume of the sample led to a decrease of the TBT recovery and this is true for every pH, ethylation, phase mass and eluent volume conditions chosen. However, depending on the phase type, the increase of the sample volume can be an advantage or a disadvantage.

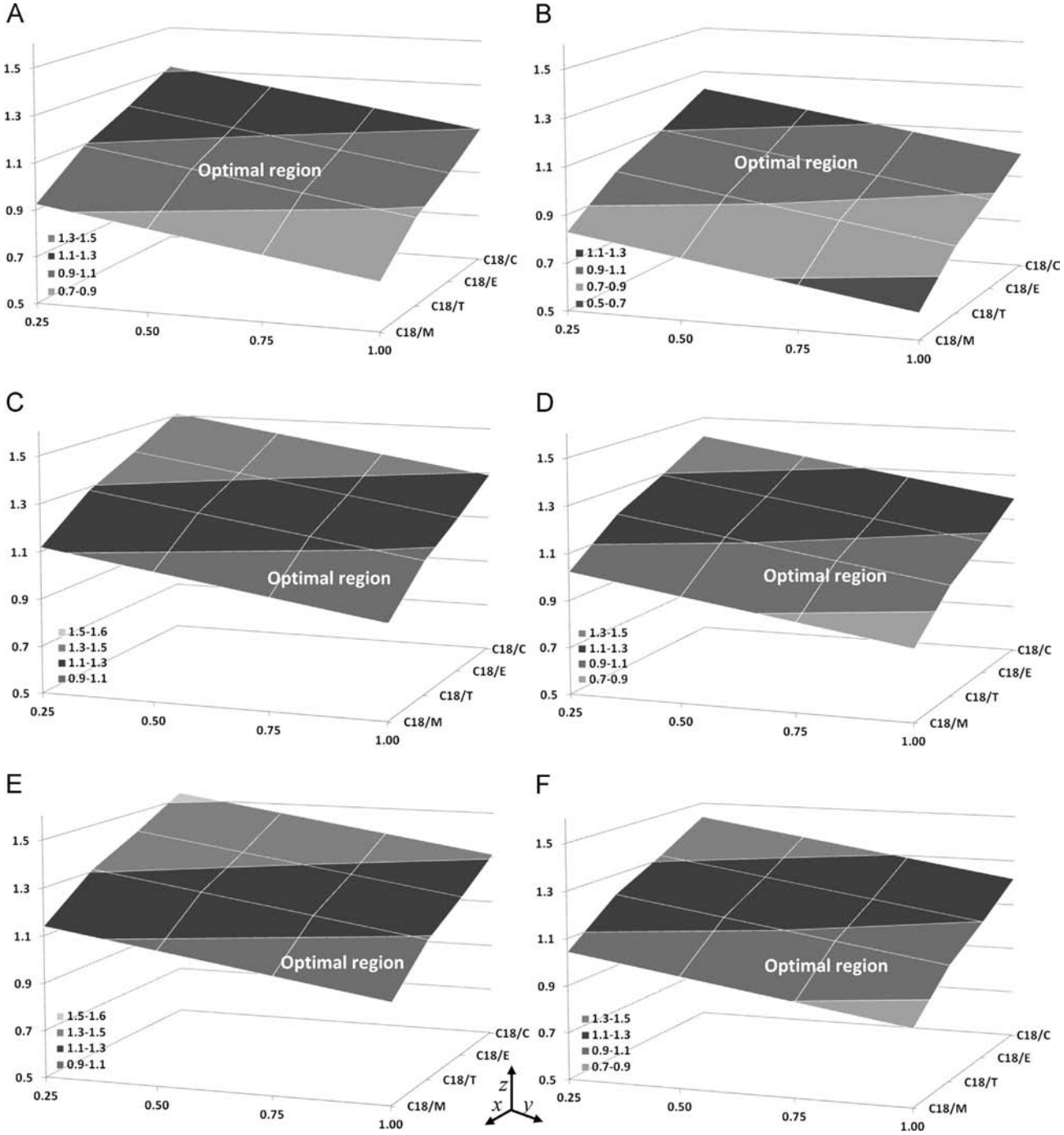

Fig. 7. Response surfaces of the studied experimental conditions based on M2. Modelled TBT mass fraction (*z*-axis) as a function of the phase/eluent (*x*-axis) and the sample volume (*y*-axis) factors. The surfaces are represented as a combination of the other two factors, $m_\varphi V_E$ (2 levels) and *pHeth* (3 levels). Different grey nuances indicate regions with similar normalized TBT mass fractions: the optimal region is in the range 0.9–1.1.

For the same *pHeth* conditions, the increase of the phase mass and the eluent volume from 0.5 to 1 g, and from 6 to 12 mL, respectively, results in a decrease of the estimated TBT mass fraction. Concerning the pH environment and the derivatization step, no effect is observed at pH=5, when the ethylation is performed before or after the SPE. Instead, when the pH of the sample is acid, more experimental conditions give an output in the "optimal region" between 0.9 and 1.1 (Fig. 7A and B). Moreover, acidifying the medium before the SPE would be useful in applications with more complex matrixes, as real water samples, to favour the desorption of TBT from the possible ligands present in the sample.

The model 2 was optimized by *Design-Expert*® with the criterion of a normalized TBT mass fraction close to 1, which is the value obtained when the recovery is 100%. The optimization gave rise to a list of combinations of factor's levels *versus* the predicted normalized TBT mass fraction (Fig. 8): Best combinations allow to reach a predicted TBT recovery between 90% to 110%. It is worth nothing that we discharged from the list the experimental conditions involving the phase NH_2/C18 and the strong cation exchange phase (SCX). The standard uncertainty associated to the normalized TBT mass fraction, $\hat{w}^*_{TBT}$ was obtained by propagating the variances and covariances of the coefficients associated to categoric variables of the model, following the *Guide to the expression of uncertainty in measurement* (GUM) [31]. The expanded measurement uncertainty was obtained using a coverage factor $k=1.96$ issued from the Gaussian distribution. The limit of quantification (LOQ) of the method was 0.06 ng L^{-1}. Methods employing SPE for the preconcentration/extraction of OTC in environmental water samples reported detection limits in the range of 10 to 40 ng L^{-1} [19–21,17]. A LOQ of about 2.5 ng L^{-1} has been found in seawater by stir bar sorptive extraction (SBSE) coupled to liquid chromatography tandem mass spectrometry (SBSE-LC–MS/MS) [32]. The same preconcentration method, SBSE, coupled to GC–MS gave rise to LOD of 0.8 ng L^{-1} [33]. Analytical performances for TBT have been evaluated by headspace single drop microextraction (SDME) coupled to GC–MS (LOD of 3 ng L^{-1}) [34], to GC–MS/MS (LOD of 0.36 ng L^{-1}) [35] and to GC–ICPMS (LOD of 0.8 ng L^{-1}) [36]. Solid phase microextraction (SPME) coupled to GC with flame photometric detection (FPD) or to ICP with time of flight mass spectrometry (TOFMS) gave rise to LODs respectively of 0.5 and 0.62 ng L^{-1} [37,38]. To our knowledge the lowest LOD existing in literature for TBT in water was obtained by dispersive liquid–liquid microextraction (DLLME) and GC-FPD and is 0.2 ng L^{-1} [39], that is more than 3 times higher than the LOQ of the method developed in this work. The model can be used to make prediction about the TBT mass fraction recovery. As an example, if someone wants to use the C18 phase with ethylacetate as an eluent, derivatization before the SPE at pH5 and wonders about the volume of sample to use, one can enter the chosen conditions in the model equation (Eq. (4)), using Table 7 to identify the indicators, and calculate the coefficients as follows:

$$\overline{C}_{TBTs} = 1.237 + 0.123 - 0.048 + 0.136 - 0.057 - 0.292V \quad (5)$$

For V_S equal to 0.25, 0.5 and 1 L, the following predicted normalized TBT mass fractions can be found respectively: 1.318, 1.245 and 1.099. It can be concluded that a large sample volume of 1 L is more appropriate to the chosen experimental conditions.

In conclusion, if we should advise a future user of the SPE method on the experimental conditions to select, we would suggest the following: (i) use a C18 phase; (ii) acidify the medium *before* the SPE; (iii) derivatize TBT *after* SPE; (iv) decide the other parameters (eluent type, phase mass, eluent volume and sample volume) predicting the TBT recovery thanks to the model equation.

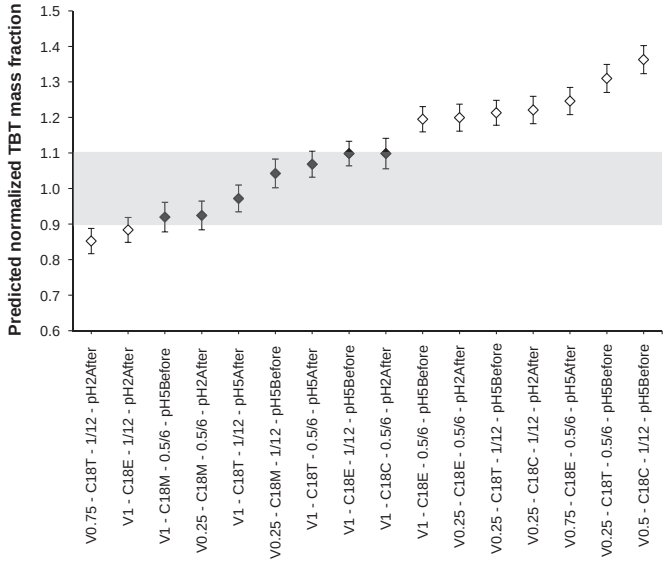

Fig. 8. Optimization of M2: predicted normalized TBT mass fraction *versus* different combinations of factor's levels. Black dots correspond to the experimental conditions giving a recovery factor between 90% to 110%.

4. Conclusions

A SPE method for the quantification of TBT in mineral water at the concentration level demanded by the water framework directive was developed and optimized. The quantification was done using SSID as a primary method, and the coupling GC–ICP-MS for TBT separation and detection. A chemometric approach based on the use of factorial fractionary plan has been defined. The plan has three qualitative factors (the phase and eluent, the phase mass and eluent volume, the pH and ethylation) and one quantitative factor (the sample volume). The experimental work has been shared between two institutes. A common experiment allowed to check that the two institutes have the same measurement capability. The results obtained have been processed altogether and by institute. Four linear models have been tested and statistically evaluated: the chosen one is a linear model describing the four studied factors, fitting all the individual results, without interactions between factors. The model was optimised imposing the closeness to the theoretical 100% recovery and led to a list of best conditions.

(i) The C18 phase was found to be the best stationary phase for SPE experiments.
(ii) All the studied solvents were optimal, depending on the experimental conditions. In fact the eluents increased the estimated TBT mass fraction in the following order: CH_2Cl_2 > ethylacetate > THF > MeOH, but the increase can result in a benefit for TBT recovery or in an overestimation of the final concentration, depending on the sample volume, the pH/ethylation conditions and the phase mass/eluent volume.
(iii) The increase of the volume of the sample led to a decrease of the TBT recovery, which can also result in an overestimation or underestimation of the TBT mass fraction.
(iv) For the same pH/ethylation conditions, the increase of the phase mass/eluent volume resulted in a decrease of the estimated TBT mass fraction.
(v) At pH 5, performing the ethylation before or after the SPE did not affect the TBT quantification, while the acidic environment in the sample seemed to favour a better recovery.

SPE appears to be a convenient technique for TBT pre-concentration at pico-trace levels. The model equation can be a tool for experimental planning, as it can be used to predict the TBT mass fraction recovery corresponding to a set of experimental conditions and help to take decisions when a SPE experiment will be outlined.

Acknowledgements

The research leading to these results has been performed within the scope of the EMRP Joint Research Project ENV08: "Traceable measurements for monitoring critical pollutants under the European Water Framework Directive (WFD-2000/60/EC)". The EMRP is jointly funded by the EMRP participating countries within EURAMET and the European Union.

References

[1] J.B. Graceli, G.C. Sena, P.F.I. Lopes, G.C. Zamprogno, M.B. da Costa, A.F.L. Godoi, et al., Reprod. Toxicol. 36 (2013) 40–52. http://dx.doi.org/10.1016/j.reprotox.2012.11.008.
[2] T. Bolam, J. Barry, R.J. Law, D. James, B. Thomas, S.G. Bolam, Mar. Pollut. Bull. 79 (2014) 326–332. http://dx.doi.org/10.1016/j.marpolbul.2013.12.003.
[3] M. Choi, Y.-R. An, K.J. Park, I.-S. Lee, D.-W. Hwang, J. Kim, et al., Mar. Pollut. Bull. 66 (2013) 78–83. http://dx.doi.org/10.1016/j.marpolbul.2012.11.007.
[4] L.-L. Liu, J.-T. Wang, K.-N. Chung, M.-Y. Leu, P.-J. Meng, Mar. Pollut. Bull. 63 (2011) 535–540. http://dx.doi.org/10.1016/j.marpolbul.2011.02.003.
[5] M.A. Fernandez, A. de L.R. Wagener, A.M. Limaverde, A.L. Scofield, F.M. Pinheiro, E. Rodrigues, Mar. Environ. Res. 59 (2005) 435–452. http://dx.doi.org/10.1016/j.marenvres.2004.07.001.
[6] A.C.A. Sousa, I.B. Oliveira, F. Laranjeiro, S. Takahashi, S. Tanabe, M.R. Cunha, et al., Mar. Pollut. Bull. 64 (2012) 422–426. http://dx.doi.org/10.1016/j.marpolbul.2011.11.013.
[7] A. Pagliarani, S. Nesci, V. Ventrella, Toxicol. In Vitro. 27 (2013) 978–990. http://dx.doi.org/10.1016/j.tiv.2012.12.002.
[8] R.V. García-Mayor, A. Larrañaga Vidal, M.F. Docet Caamaño, A. Lafuente Giménez, Endocrinol. Nutr. Engl. Ed 59 (2012) 261–267. http://dx.doi.org/10.1016/j.endoen.2012.05.001.
[9] R. de Carvalho Oliveira, R.E. Santelli, Talanta 82 (2010) 9–24. http://dx.doi.org/10.1016/j.talanta.2010.04.046.
[10] ISO 17353:2004(E), Water Quality – Determination of Selected Organotin Compounds – Gas Chromatographic Method, 2004.
[11] P. Lepom, B. Brown, G. Hanke, R. Loos, P. Quevauviller, J. Wollgast, J. Chromatogr. A 1216 (2009) 302–315. http://dx.doi.org/10.1016/j.chroma.2008.06.017.
[12] M. Monperrus, E. Krupp, D. Amouroux, O.F. Donard, R. Rodríguez Martín-Doimeadios, TrAC, Trends Anal. Chem. 23 (2004) 261–272. http://dx.doi.org/10.1016/S0165-9936(04)00313-9.
[13] G.A. Zachariadis, E. Rosenberg, J. Chromatogr. B 877 (2009) 1140–1144. http://dx.doi.org/10.1016/j.jchromb.2009.02.065.
[14] C. Dietz, J. Sanz, E. Sanz, R. Muñoz-Olivas, C. Cámara, J. Chromatogr. A 1153 (2007) 114–129. http://dx.doi.org/10.1016/j.chroma.2006.11.064.
[15] C. Devos, M. Vliegen, B. Willaert, F. David, L. Moens, P. Sandra, J. Chromatogr. A 1079 (2005) 408–414. http://dx.doi.org/10.1016/j.chroma.2004.12.020.
[16] N.R. Neng, R.P. Santalla, J.M.F. Nogueira, Talanta 126 (2014) 8–11. http://dx.doi.org/10.1016/j.talanta.2014.03.021.
[17] Q. Sun, Z. Chen, D. Yuan, M. Megharaj, R. Naidu, Rapid Commun. Mass Spectrom. 23 (2009) 3795–3802. http://dx.doi.org/10.1002/rcm.4321.
[18] V. Camel, Spectrochim. Acta 58 (2003) 1177–1233. http://dx.doi.org/10.1016/S0584-8547(03)00072-7.
[19] M.D. Mueller, Anal. Chem. 59 (1987) 617–623. http://dx.doi.org/10.1021/ac00131a017.
[20] P. Bermejo-Barrera, R. Anllo-Sendín, M. Cantelar-Barbazán, A. Bermejo-Barrera, Anal. Bioanal. Chem. 372 (2002) 837–839. http://dx.doi.org/10.1007/s00216-002-1275-1.
[21] E. González-Toledo, A. Ortuño, R. Compañó, M. Granados, M.D. Prat, Chromatographia 55 (2002) 19–24. http://dx.doi.org/10.1007/BF02492309.
[22] F. Bianchi, M. Careri, M. Maffini, A. Mangia, C. Mucchino, J. Anal. At. Spectrom. 21 (2006) 970–973. http://dx.doi.org/10.1039/B603310E.
[23] M. Bravo, G. Lespes, I. De Gregori, H. Pinochet, M. Gautier, Anal. Bioanal. Chem. 383 (2005) 1082–1089. http://dx.doi.org/10.1007/s00216-005-0131-5.
[24] M. Le Gac, G. Lespes, M. Potin-Gautier, J. Chromatogr. A 999 (2003) 123–134.
[25] C. Coscollà, S. Navarro-Olivares, P. Martí, V. Yusà, Talanta 119 (2014) 544–552. http://dx.doi.org/10.1016/j.talanta.2013.11.052.
[26] E. González-Toledo, R. Compañó, M.D. Prat, M. Granados, J. Chromatogr. A 946 (2002) 1–8.
[27] J. Szpunar-Łobińska, M. Ceulemans, R. Łobiński, F.C. Adams, Anal. Chim. Acta 278 (1993) 99–113. http://dx.doi.org/10.1016/0003-2670(93)80089-4.
[28] M. Vahčič, R. Milačič, J. Ščančar, Anal. Chim. Acta 694 (2011) 21–30. http://dx.doi.org/10.1016/j.aca.2011.03.061.
[29] R. Morabito, P. Massanisso, P. Quevauviller, TrAC, Trends Anal. Chem 19 (2000) 113–119. http://dx.doi.org/10.1016/S0165-9936(99)00196-X.
[30] P. Rodriguez-Gonzalez, J.I. Garcia Alonso, J. Anal. At. Spectrom. 25 (2010) 239–259. http://dx.doi.org/10.1039/B924261A.
[31] JCGM 100:2008, Evaluation of Measurement Data—Guide to the Expression of Uncertainty in Measurement, 2008.
[32] F.J. Camino-Sánchez, A. Zafra-Gómez, B. Oliver-Rodríguez, I. Ruiz-Naranjo, J. Ruiz-García, J.L. Vílchez, J. Chromatogr. A 1263 (2012) 14–20. http://dx.doi.org/10.1016/j.chroma.2012.09.018.
[33] A. Prieto, O. Zuloaga, A. Usobiaga, N. Etxebarria, L.A. Fernández, C. Marcic, et al., J. Chromatogr. A 1185 (2008) 130–138. http://dx.doi.org/10.1016/j.chroma.2008.01.046.
[34] V. Colombini, C. Bancon-Montigny, L. Yang, P. Maxwell, R.E. Sturgeon, Z. Mester, Talanta 63 (2004) 555–560. http://dx.doi.org/10.1016/j.talanta.2003.11.035.
[35] H. Shioji, S. Tsunoi, H. Harino, M. Tanaka, J. Chromatogr. A 1048 (2004) 81–88. http://dx.doi.org/10.1016/j.chroma.2004.07.016.
[36] Q. Xiao, B. Hu, M. He, J. Chromatogr. A 1211 (2008) 135–141. http://dx.doi.org/10.1016/j.chroma.2008.09.089.
[37] G. Jiang, J. Liu, K. Yang, Anal. Chim. Acta 421 (2000) 67–74. http://dx.doi.org/10.1016/S0003-2670(00)01034-5.
[38] P. Jitaru, H. Goenaga Infante, F.C. Adams, J. Anal. At. Spectrom. 19 (2004) 867–875. http://dx.doi.org/10.1039/B404106B.
[39] A.P. Birjandi, A. Bidari, F. Rezaei, M.R.M. Hosseini, Y. Assadi, J. Chromatogr. A 1193 (2008) 19–25. http://dx.doi.org/10.1016/j.chroma.2008.04.003.