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**DETERMINATION OF TRIBUTYL TIN AT PARTS-PER-
TRILLION LEVELS IN NATURAL WATERS BY SECOND-
ORDER MULTIVARIATE CALIBRATION AND
FLUORESCENCE SPECTROSCOPY**

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30 **ABSTRACT**

31 This work presents a non-sophisticated approach for the trace determination of tributyltin,
32 the most toxic organotin species, in very interfering environments, combining fluorescence
33 measurements of its morin complex and the selectivity of second-order chemometric
34 algorithms. The power of MCR–ALS (multivariate curve resolution/alternating least-
35 squares) to quantify tributyltin through fluorescence excitation-emission matrices in the
36 presence of its main degradation products and of a pool of additional twenty-three metal
37 ions is demonstrated. The applied algorithm successfully faces the challenge of solving the
38 strong overlapping among the spectra of the several sample components. The proposed
39 methodology was applied to tap, river, lagoon and seawater spiked samples, obtaining
40 satisfactory results at ng L^{-1} levels, after a pre-concentration step on a C18 membrane,
41 demonstrating the analytical potential of the proposed methodology.

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46 *Keywords:* Tributyltin; Natural samples; Multivariate calibration; Fluorescence
47 spectroscopy

48

49 **1. Introduction**

50 Due to its widespread use as an antifouling agent in boat paints, highly toxic tributyltin
51 (TBT) is a common contaminant of marine and freshwater ecosystems (Hoch, 2001; Fent,
52 2004). Exposure to water and sediments contaminated with TBT induces its accumulation
53 on marine biota, and leads to biological effects such as shell malformation in oysters
54 (Alzieu , 1998), mortality of mussel larvae (Barrosoi et al., 2005), and imposex of
55 gastropods (Toste et al., 2011). Potential harmful effects on human health may also result
56 from consumption of contaminated seafood or drinking water (Cao et al., 2009). For these
57 reasons, several constrains have been imposed to TBT industrial applications, and the
58 European Union has decided to specifically include TBT compounds in its list of priority
59 compounds in water (Antizar Ladislao, 2008). Unfortunately, present and future restrictions
60 will not immediately remove TBT and its degradation products, monobutyltin (MBT) and
61 dibutyltin (DBT) from aquatic environments since these compounds are retained in the
62 sediments where they persist (Antizar Ladislao, 2008; Díez et al., 2002).

63 Several analytical methodologies have been proposed to quantify organotin
64 compounds, most of them requiring hyphenated techniques, involving a combination of
65 extraction, separation and detection steps (de Carvalho Oliveira and Erthal Santelli, 2010).
66 Various pre-concentration procedures have been proposed based on liquid-liquid extraction
67 (Bancon Montigny et al., 2002), solid-phase extraction (SPE), solid-phase micro-extraction
68 (Aguerre et al., 2002; Bravo et al., 2005) and liquid-phase micro-extraction (Colombini et
69 al., 2004; Lambropoulou et al., 2007; Shioji et al., 2004). Following this analytical phase,
70 most reported methods combine a separation technique such as gas chromatography (GC)
71 with detection including atomic absorption spectrometry, flame photometry, pulsed flame

72 photometry or inductively coupled plasma mass spectrometry (Antizar Ladislao, 2008). In
73 the case of GC, an additional derivatization step must be included, in order to transform
74 organotins into volatile and thermally stable compounds. Although the analytical
75 performance of these methodologies is widely recognized, allowing to analyze complex
76 samples containing several unknown components and interferences, they are complex,
77 require a substantial experimental work and skilled analysts, and are difficult to implement
78 for routine analysis.

79 Modern multivariate calibration methods, especially those based on second-order
80 calibration, constitute an attractive alternative to cope with these situations, even when the
81 processed instrumental data arise from analytical techniques which are intrinsically less
82 selective than chromatography (Escandar et al., 2007). Certain second-order multivariate
83 algorithms have the property of predicting the concentration of an individual component in
84 the presence of any number of unsuspected constituents, a property commonly named as
85 ‘second-order advantage’ (Smilde et al., 1999; Olivieri, 2008). Usual algorithms employed
86 to analyze second-order data achieving this property are parallel factor analysis
87 (PARAFAC) (Bro, 1997), multivariate curve resolution-alternating least squares (MCR–
88 ALS) (de Juan and Tauler, 2001; de Juan and Tauler, 2006) and some latent-structured
89 methods, such as unfolded partial least-squares (U-PLS) (Borraccetti et al., 2009) and
90 multiway PLS (Gurden et al., 2001), both combined with residual bilinearization (Bohoyo
91 Gil et al., 2006; Lozano et al., 2009A). These chemometric methods have been scarcely
92 used for organotin speciation analysis in environmental samples. Only a single work
93 devoted to the quantitation of triphenyltin in seawaters has been reported (Saurina et al.,
94 2000). However, this latter method does not include TBT as analyte, and only seawater
95 matrices were evaluated.

96 In the present report, a new analytical method is proposed for quantitation of TBT,
97 which is the most toxic organotin (Kungolos et al., 2004; Solé, 2000; Fent, 1996; Fent et
98 al., 1998), based on the measurement of excitation-emission fluorescence matrices
99 (EEFMs) processed by second-order multivariate calibration based on MCR-ALS.
100 Fluorescent detection is possible thanks to the reaction between tributyltin and 3,5,7,2',4'-
101 pentahydroxyflavone (morin) in a triton X-100 micellar medium, which yields a fluorescent
102 complex. The feasibility of determining TBT in real matrices is demonstrated by applying
103 the proposed methodology to tap, river, lagoon and sea water samples.

104

105 **2. EXPERIMENTAL**

106

107 *2.1. Apparatus*

108

109 Fluorescence measurements were performed on an Aminco Bowman (Rochester, NY, USA)
110 Series 2 luminescence spectrometer equipped with a 150 W xenon lamp and using 1.0 cm
111 path length quartz microcells and slit widths of 4 nm for both monochromators. All
112 measurements were performed at 20°C with a thermostated cell.

113 The excitation-emission fluorescence matrices were collected exciting samples in
114 the range 380-460 nm (each 5 nm) and obtaining the corresponding emission spectra in the
115 range 510-600 nm (each 5 nm), resulting in a data matrix size 19×17 for sample.

116 All glassware was rinsed with deionized water, decontaminated overnight in a 20%
117 (v/v) nitric acid solution (Merck, Darmstadt, Germany) and then rinsed again with
118 deionized water.

119

120 **2.2. Reagents and standards**

121

122 High quality water (18 M Ω) obtained from a Barnstead Easypure II (Thermo, Dubuque,
123 MA USA) was used to prepare the solutions. The tributyltin standards were prepared from
124 tributyltin chloride (TBT, 96%) obtained from Sigma-Aldrich (St. Louis, MO, USA). Stock
125 solutions of these reagents (1000 mg L⁻¹ of Sn) were prepared in methanol and stored at
126 -20°C in the dark. Working standards were obtained by dilution with water. This was done
127 on a weekly basis for solutions containing Sn at 5 mg L⁻¹ and daily for solutions containing
128 Sn at 10–100 μ g L⁻¹.

129 An ethanolic solution 4.2 \times 10⁻³ M of morin (Sigma-Aldrich, Munich, Germany) was
130 prepared every day, while a stock solution 8.3% (w/v) of triton X-100 (Fluka Chemika,
131 Buchs, Switzerland) and a buffer solution pH 4.7 of succinic acid (Merck, Darmstadt,
132 Germany) 0.5 M were prepared weekly.

133 For metal additions, a Certipur® ICP multi-element standard solution IV was
134 purchased from Merck (Darmstadt, Germany). This standard includes 23 elements (Ag(I),
135 Al(III), B(III), Ba(II), Bi(III), Ca(II), Cd(II), Co(II), Cr(III), Cu(II), Fe(III), Ga(III), In(III),
136 K(I), Li(I), Mg(II), Mn(II), Na(I), Ni(II), Pb(II), Sr(II), Tl(I), Zn(II)) at 1000 mg L⁻¹
137 dissolved in diluted nitric acid.

138

139 **2.3. Synthetic samples**

140

141 A set of nine TBT calibration solutions with analyte concentrations was built: eight of them
142 contained equally spaced levels between 0 and 350 $\mu\text{g L}^{-1}$ (based on Sn content). They
143 were prepared adding adequate volumes of the standard solution (5 mg L^{-1}) in a calibrated
144 10.00 mL vessel. Subsequently, 200 μL of morin solution, 1.0 mL of buffer and 0.84 mL of
145 triton X-100 solution were added. Finally, completion to the mark was achieved with
146 deionized water and the EEFMs were registered.

147 For validation, two different sets of solutions were prepared. The first set involved
148 eight solutions containing random concentrations of TBT, DBT and MBT, all in the range
149 30–110 $\mu\text{g L}^{-1}$ of Sn. The second set consisted of seven solutions with random
150 concentrations of TBT and metals in the range of 32–90 and 38–120 $\mu\text{g L}^{-1}$, respectively.

151

152 ***2.4. Real samples***

153

154 Tap and river samples were collected from the Rosario city drinking water system and
155 Paraná River (Santa Fe, Argentina), respectively, while the remaining samples were
156 collected from Curauma lagoon and Baron harbor, both placed in the Province of
157 Valparaiso (Valparaiso, Chile). All samples were filtered using a nylon membrane (0.22
158 μm) and stored at 4 °C until analysis. TBT concentration was determined by GC with
159 pulsed flame photometric detection (Mzoughi et al., 2005), and was found to be below the
160 detection limit. Therefore, aliquots of these samples were spiked with known amounts of
161 TBT, reaching TBT concentrations ranging between 20 and 120 ng L^{-1} . Solid-phase
162 extraction (SPE) using a C18 extraction membrane (Empore, Supelco, Bellefonte, P.A.,
163 USA) was applied before sample analysis. The disks were loaded into a 13 mm stainless

164 steel filter syringe kit (Alltech, Deerfield, IL, USA) and placed into a syringe. Prior to
165 sample analysis, the disk was conditioned with methanol. Aliquots of either 100 or 200 mL
166 of aqueous samples were passed through the membrane under vacuum pump, with a flow
167 rate of about 10 mL min^{-1} . After elution of the retained organic compounds with 500 μL of
168 methanol, the solvent was evaporated by using dry nitrogen and reconstituted with 400 μL
169 of the fluorogenic solution. Finally, the EEFM was measured for each sample and the
170 concentration was estimated using second-order multivariate calibration.

171

172 ***2.5. Theory***

173

174 ***2.5.1. PARAFAC***

175

176 The theory of PARAFAC is well-known (Bro, 1997). In some of the presently studied
177 systems, this method was employed to successfully decompose the three-way arrays built
178 with the fluorescence data matrices. However, PARAFAC could not be applied with equal
179 success to samples containing uncalibrated interferences having excitation spectra which are
180 strongly overlapped with those of the calibrated components. This has been previously
181 shown to be a strong challenge to PARAFAC (Culzoni et al., 2008; Lozano et al. 2010).
182 The general problem of second-order calibration under strong profile overlapping in one of
183 the data dimensions can be solved using MCR-ALS, which is thus described in detail in
184 Section 2.4.2.

185

186 ***2.5.2. MCR-ALS***

187

188 In this second-order multivariate method, an augmented data matrix is created from the test
 189 and calibration data matrices. The matrices are all of size $J \times K$, where J is the number of
 190 excitation wavelengths and K the number of emission wavelengths. Augmentation can be
 191 performed either direction, depending on the type of experiment being analyzed and also on
 192 the presence of severe overlapping in one of the data modes (Smilde et al., 1999; Tauler,
 193 1995). In the presently studied case, the excitation spectra of some of the various sample
 194 components are very similar, and hence it is useful to implement augmentation in this
 195 direction, creating a row-wise augmented matrix \mathbf{D} by placing the different matrices
 196 adjacent to each other. Matrix augmentation in this mode helps to destroy the linear
 197 dependency caused by strong profile overlapping, as has been previously described
 198 (Culzoni et al., 2008; Lozano et al. 2010).

199 The bilinear decomposition of the augmented matrix is then performed according to
 200 the expression:

$$201 \quad \mathbf{D} = \mathbf{C} \mathbf{S}^T + \mathbf{E} \quad (3)$$

202 where the columns of \mathbf{C} contain the excitation profiles of the intervening species, the rows
 203 of \mathbf{S} the emission spectra in the different samples, and \mathbf{E} is a matrix of residuals not fitted
 204 by the model. Appropriate dimensions of \mathbf{D} , \mathbf{C} , \mathbf{S} and \mathbf{E} are $J \times (IK)$, $J \times N$, $N \times (KI)$ and
 205 $J \times (IK)$ respectively (I is the total number of samples in matrix \mathbf{D} , and N the number of
 206 responsive components). Decomposition of \mathbf{D} is achieved by iterative least-squares
 207 minimization of the Frobenius norm of \mathbf{E} . The minimization is started by supplying
 208 estimated emission spectra for the various components, which are employed to estimate $\hat{\mathbf{S}}$
 209 (with the 'hat' implying an estimated matrix) from equation (3):

$$210 \quad \hat{\mathbf{S}} = \mathbf{D}^T (\mathbf{C}^T)^+ \quad (4)$$

211 where the superscript '+' indicates the generalized inverse. With matrix $\hat{\mathbf{S}}$ from equation (4)
212 and the original data matrix \mathbf{D} , the matrix \mathbf{C} is re-estimated by least-squares:

$$213 \quad \hat{\mathbf{C}} = \mathbf{D}(\hat{\mathbf{S}}^T)^+ \quad (5)$$

214 and finally \mathbf{E} is calculated from equation (3) using \mathbf{D} and the estimated $\hat{\mathbf{C}}$ and $\hat{\mathbf{S}}$ matrices.
215 These steps are repeated until convergence, under suitable constraining conditions during
216 the ALS process, for example, nonnegativity in spectral and time profiles. It is important to
217 point out that MCR-ALS requires initialization with spectral profiles in the emission mode.
218 Several alternatives were evaluated, and the finally selected one depended on the type of
219 analyzed samples. For a set composed of only calibration samples, two chemical
220 components were considered: free morin and the TBT-morin complex, whose spectra were
221 estimated from the corresponding PARAFAC decomposition of the three-way calibration
222 data array. When additional components (unexpected interferences) occurred in the samples,
223 their spectral emission profiles were estimated by PARAFAC decomposition of a three-
224 way array composed of calibration and also from data for the test sample.

225 After MCR-ALS decomposition of \mathbf{D} , concentration information contained in \mathbf{S} can
226 be used for quantitative predictions, by first defining the analyte concentration score as the
227 area under the profile for the i th sample:

$$228 \quad a(i, n) = \sum_{k=1+(i-1)K}^{iK} S(n, k) \quad (6)$$

229 where $a(i, n)$ is the score for the component n in the sample i . In this way, the scores are
230 employed to build a pseudo-univariate calibration graph against the analyte concentrations,
231 predicting the concentration in the test samples in the usual univariate manner:

$$232 \quad [a(2, n) \mid a(3, n) \mid \dots \mid a(I, n)] = m_2 \mathbf{y}^T + n_2 \quad (7)$$

233
$$y_u = [a(1,n) - n_2] / m_2 \quad (8)$$

234 where n indicates the analyte, y_u is the predicted concentration, and y the vector [size
235 $(I-1) \times 1$] of nominal concentrations in the calibration samples.

236

237 **2.6. Software**

238

239 All calculations were carried out using MATLAB 7.0 routines (The Mathworks Inc., 2003).
240 The codes available on the internet for MCR-ALS (Jaumot et al., 2005;
241 http://www.ub.edu/mcr/web_mcr/download.html) and PARAFAC
242 (www.models.kvl.dk/algorithms) were employed for multivariate analysis. PARAFAC was
243 applied through a MATLAB graphical user interface which is also available on the Web
244 (Olivieri et al., 2009; [http://www.chemometry.com/Index/ Links%20and%20downloads/
245 Programs.html](http://www.chemometry.com/Index/Links%20and%20downloads/Programs.html)).

246

247 **3. Results and discussion**

248

249 **3.1. Optimization of the fluorescence signal**

250

251 TBT forms stable complexes with several flavones, such as morin and fisetin (Leal et al.,
252 1995), with morine giving the most intense fluorescence signal. The presently proposed
253 method is based on the reaction between TBT and morin in a triton X-100 micellar medium
254 to yield a fluorimetrically active complex. The fluorescence emission of the complex is
255 affected by several experimental variables, which were evaluated with a Plackett-Burman

256 design, in accordance with the factor levels presented in Table 1. The evaluated response
257 was the emission of the TBT complex at 550 nm. After statistical analysis of the
258 significance of effects, it was concluded that the pH and the type of acid employed
259 significantly affected the fluorescence emission of the TBT-morin complex (see Table 1).
260 Thus succinic acid was selected. Concerning the pH, a univariate optimization was carried
261 out, and the maximum response was found for pH 4.7, retaining this condition for all
262 experiments. For the non significant factors, the low levels were retained for all
263 experiments.

264 The overlapping between the fluorescence spectra of free morin and its TBT
265 complex hinders the direct spectrofluorimetric determination of the analyte, and the
266 situation becomes more serious if other potential interferents are present. Therefore, in
267 order to overcome this problem, a chemometric analysis was proposed, testing different
268 second-order algorithms. In a first stage, samples only containing TBT were processed, and
269 more complex samples were subsequently studied.

270

271 ***3.2. Set number 1***

272

273 With the purpose of building a second-order calibration model, EEFMs were recorded for
274 the calibration samples. This calibration set was first analyzed using PARAFAC, which is
275 one of the most frequently applied second-order algorithm, building a three-way array with
276 data corresponding to the calibration samples only. The analysis revealed the presence of
277 two components, which gave a reasonably low residual error to the PARAFAC model, as
278 well as a reasonable value for the so-called core consistency parameter (Bro and Kiers,
279 2003). The analysis of the scores (relative component concentrations) allowed to establish

280 that these two species correspond to free morin and to the TBT-morin complex, because:
281 (1) an excellent linear correlation between scores and nominal calibration concentrations
282 was obtained for one of the components, ascribed to the TBT-morin complex, and (2) the
283 constancy of the scores for the remaining component, which was thus identified as free
284 morin.

285 As expected, two components were also detected with the MCR-ALS approach and
286 similar prediction results were obtained for the calibration set.

287

288 **3.3. Set number 2**

289

290 TBT degradation products, such as MBT and DBT, can be present in environmental
291 samples. These products do also react with morin, forming fluorescent complexes which
292 may in principle constitute potential interferences (Leal et al., 1995). Therefore, a set of
293 solutions including TBT, MBT and DBT was prepared and evaluated with both studied
294 algorithms.

295 The number of PARAFAC responsive components was selected using the same
296 procedures applied to set No. 1 (calibration samples without unexpected components),
297 allowing to assess that three components were required for samples of set No. 2. Figs. 1A
298 and 1B show the excitation and emission loadings retrieved by PARAFAC for a typical
299 sample of this set. In addition to the spectra corresponding to morin and TBT-morin
300 complex observed in the calibration samples, a new profile is clearly detected. This profile
301 is ascribed to a combination of the spectra of the uncalibrated species (i.e., MBT- and
302 DBT-morin complexes), a usual phenomenon when interferent profiles with similar spectra
303 occur (Bortolato et al., 2008). Table 2 shows the prediction results corresponding to the

304 application of PARAFAC to the samples of set No. 2. The root mean square error of
305 prediction (RMSEP) and the relative error of prediction (REP) values indicate rather poor
306 results, suggesting that the trilinear model is not adequate for this data. This phenomenon
307 may occur for a number of reasons, such as: (1) lack of profile reproducibility in
308 chromatography, (2) linear dependency among profiles due to closure, or (3) identical
309 profiles for sample components (Escandar et al., 2007; Lozano et al., 2010, Olivieri et al.
310 2011). As can be appreciated in Fig. 1A, the excitation profile corresponding to the
311 interference signal strongly overlaps with that of free morin. It may be noticed that this
312 problem cannot be solved by employing any of the PLS/RBL algorithms, as has been
313 shown for kinetic (Culzoni et al. 2008) and lanthanide-sensitized excitation-time decay
314 (Lozano et al., 2009B) data.

315 The best alternative for coping with this situation is to apply MCR–ALS in the
316 proper augmentation mode, as explained above. Figs. 1C and 1D show the results of the
317 MCR–ALS resolution of excitation-wise augmented data matrix for a typical sample of the
318 same set No. 2, and Table 2 displays the corresponding prediction results of TBT
319 concentration. As can be seen, the results are close to the nominal ones, reaching a REP of
320 11%. Although it is difficult to assess the limit of detection using MCR–ALS, the results
321 suggest that this figure of merit is around $5 \mu\text{g L}^{-1}$, based on the RMSEP values quoted in
322 Table 2. In view of the complexity of the samples and of the analytical problem at hand,
323 the present results are deemed to be reasonably good.

324

325 ***3.4. Set number 3***

326

327 In coastal impacted sites, seawater samples can contain high levels of metals, such as
328 aluminium, cadmium, lead or zinc (Barba Brioso et al., 2010). These metal ions, and other
329 potentially present in natural waters, are able to form complexes with morine, which have
330 fluorescence signals overlapped with that of the studied analyte. Therefore, seven test
331 samples containing TBT and 23 inorganic elements other than Sn (see Experimental) were
332 prepared and evaluated with the PARAFAC and MCR-ALS algorithms. The prediction
333 results for this set are shown in Table 2, and they also indicate a poor performance of
334 PARAFAC. On the other hand, the results given by MCR-ALS are encouraging: the
335 RMSEP and REP values are comparable to those obtained for set No. 2. Based on these
336 values, the limit of detection can presumably be estimated as $5 \mu\text{g L}^{-1}$.

337 The MCR-ALS algorithm retrieved spectra for all sample components which are
338 shown in Fig. 2. In the excitation dimension (Fig. 2A) strong overlapping occurs, which
339 was successfully taken into account by matrix augmentation in this particular dimension.
340 Notice the presence of the interferent in the test sample, and their absence in the calibration
341 samples. This is essential to achieve the second-order advantage.

342

343 ***3.5. Analysis of real aqueous samples***

344

345 With the purpose of evaluating the application of the present method and the potential
346 interference from background matrices, a recovery study by spiking waters of different
347 origins with TBT was carried out.

348 Before the restrictions on TBT use in antifouling paints, concentrations higher than
349 500 ng L^{-1} have been detected in North American and European marinas (Antizar Ladislao,

350 2008). However, recent investigations have reported that TBT concentrations in water have
351 generally declined, and maximum concentrations in seawater rarely exceed 100 ng L^{-1}
352 (Antizar Ladislao, 2008). Some countries have set an environmental quality standard for
353 TBT of 20 ng L^{-1} for fresh water (Bermejo Barrera, 2002). The US Environmental
354 Protection Agency (US-EPA) has developed acute and chronic criteria recommendations
355 for TBT designed to protect aquatic life (EPA, 2011). US-EPA indicates that aquatic life
356 would not be significantly affected if the one-hour average TBT concentration does not
357 exceed 460 and 420 ng L^{-1} in freshwater and saltwater, respectively, more than once every
358 three years on the average (acute criterion), and if the four-day average TBT concentration
359 does not exceed 72 and 7.4 ng L^{-1} in freshwater and saltwater, respectively, more than once
360 every three years on the average (chronic criterion).

361 As a conclusion, the quantification of TBT in natural waters requires highly
362 sensitive techniques, able to detect concentrations in the order of ng L^{-1} , and therefore these
363 methods usually require pre-concentration steps. The sensitivity of the present method was
364 improved applying solid-phase extraction by employing C18 membranes. The use of these
365 membranes allows us to develop a sensitive, robust and fast method for real matrices.

366 In view of the above results obtained with synthetic samples, MCR-ALS was the
367 algorithm selected for the present analysis. Figs. 3A and 3B show the MCR-ALS
368 decomposition obtained by processing the data matrices of a typical spiked river sample
369 and some standards, and Fig. 3C displays emission profiles corresponding to a seawater
370 sample (the corresponding excitation spectra are very similar to those shown in Fig. 3A). In
371 both of these samples, three chemical species are clearly identified. Two of them
372 correspond to TBT and free morin, whose spectral profiles are reasonably similar to those
373 of the corresponding standards. Interestingly, the remaining spectral profiles may be

374 ascribed to a completely unknown interferent component, absent in the calibration set, but
375 detected by the multivariate calibration method. This demonstrates the high potential of the
376 presently applied chemometric strategy.

377 The obtained results for different real samples are presented in Table 3. Taking into
378 account the simple sample treatment, the analytical results are reasonably good, with
379 recovery percentages ranging from 85 to 120%. This conclusion is also reflected in Fig. 4,
380 which shows the elliptical joint confidence region (González et al, 1999) for the slope and
381 intercept of the found vs. nominal TBT concentrations plot. The ellipse includes the
382 theoretically expected values of (1,0), indicating accuracy of the developed methodology.

383 Based on the average concentration uncertainty which can be measured by the
384 RMSEP in Table 3, the limit of detection for the pre-concentrated water samples can be
385 estimated as ca. 9 ng L⁻¹, which matches the requirements of most official agencies.

386 The obtained results suggest that interference from the background was successfully
387 removed in the investigated waters by the applied chemometric methodology. Additionally,
388 in the specific case of the seawater samples, the high salt content did not cause difficulties
389 neither in the accuracy or in the repeatability of the TBT determinations.

390

391 **4. Conclusions**

392

393 It has been demonstrated that complexation of tributyltin with morin to form a fluorescent
394 complex, measurement of excitation-emission fluorescence matrix and data processing
395 using multivariate curve resolution/alternating least-squares produces a simple, fast and
396 sensitive method for the determination of tributyltin in aqueous matrices. Through a very

397 simple pre-concentration step with a C18 membrane, it was possible to successfully
398 quantify TBT at part-per trillion levels in environmental water samples. The method
399 represents a valuable alternative for the determination of tributyltin in contaminated water
400 samples.

401

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403

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407

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Figure captions

561 **Figure 1. PARAFAC excitation (A) and emission (B) loadings of free morine (long**
562 **dashed-blue line), TBT-morin complex (solid-red line) and a combined contribution**
563 **attributed to both MBT and DBT interferents (short dashed-green line), as obtained**
564 **for a typical sample from set 2. MCR-ALS excitation (C) and emission (D) loadings of**
565 **the same components as obtained for the same sample from set 2. Loadings have been**
566 **normalized to unit length.**

567

568 **Figure 2. (A) Excitation spectral profiles of free morine (long dashed-blue line), TBT-**
569 **morin complex (solid-red line) and a combined contribution attributed to metal-**
570 **morine complex interferents (short dashed-dark yellow line) obtained after applying**
571 **MCR-ALS to a typical sample from set 3, and those corresponding to two calibration**
572 **samples containing analyte concentrations of 25 and 50 $\mu\text{g L}^{-1}$ (as indicated). The**
573 **vertical lines separate the three samples. (B) Emission profiles obtained after applying**
574 **MCR-ALS to the same sample of set 3. Loadings have been normalized to unit length.**

575

576 **Figure 3. (A) Excitation spectral profiles of free morine (long dashed-blue line), TBT-**
577 **morin complex (solid-red line) and unknown interferent (short dashed-pink line)**
578 **obtained after applying MCR-ALS to a spiked river sample, and those corresponding**
579 **to three calibration samples containing analyte concentrations of 0, 25 and 50 $\mu\text{g L}^{-1}$**
580 **(as indicated). The vertical lines separate the four samples. (B) and (C) Emission**
581 **profiles obtained after applying MCR-ALS to river and seawater samples,**

582 respectively. Short dashed-light green line in (C) corresponds to a unknown
583 intereferent in the seawater sample. Loadings have been normalized to unit length.

584

585 **Figure 4. Plot for TBT predicted concentrations by MCR-ALS in real samples, as a**
586 **function of the nominal values (the solid line is the perfect fit), and the elliptical joint**
587 **region (at 95% confidence level) for the slope and intercept of the regression of the**
588 **data. The black point marks the theoretical (intercept = 0, slope = 1) point.**