Designing valid and optimised standard addition calibrations:

Application to the determination of anions in seawater

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Abstract

A strategy for designing valid standard addition calibrations and for optimising their uncertainty is presented. The design of calibrations involves the development of models of the sensitivity and precision of the instrumental signal, in a wide range of analyte concentration (or any other studied quantity), and the definition of sample dilution and standard addition procedures that allow fulfilling the assumptions of the linear unweighted regression model in, typically, a smaller range of standard addition calibrations. Calibrators are prepared by diluting the sample and adding analyte with negligible uncertainty to fit in a concentration range where signals are homoscedastic. The minimisation of the uncertainty is supported on detailed measurement uncertainty models function of calibrators preparation procedure and of analytical instrumentation performance. The number of collected signals replicates is defined by balancing their impact on the estimated expanded uncertainty, the resources needed and the target (maximum) uncertainty for the intended use of measurements.

The calibration design strategy was successfully applied to the determination of the mass concentration (mg L^{-1}) of Cl^- , Br^- , NO_3^- and SO_4^{2-} in seawater by ion chromatography. A target

expanded uncertainty of 20 % was defined for the determination of Cl^- , NO_3^- and SO_4^{2-} , or 40 % for the determination of the smaller mass concentration of Br^- . The developed measurement model produced reliable predictions of the measurement uncertainty from approximate concentration of the analyte in the sample, before its accurate quantification, thus proving optimisation is effective. Predictions are more prone to the variability of the measurement uncertainty estimation if based on low number of calibrators signals. The reported relative expanded uncertainty ranged from 7.1 to 49 %.

1. Introduction

Instrumental methods of analysis are widely used to characterise different analytes in various types of matrices. These methods involve collecting the instrumental signals of calibrators, i.e. items with known quantity value or increments, to establish a response function between measured signal and the known quantity, i.e. the calibration curve. In this section the term quantity is preferred to specific examples such as concentration of analyte.

The flexibility to determine various analytes in different matrices with high selectivity and sensitivity makes the use of these analytical tools extremely attractive. However, the more complex the matrix of the studied item becomes, the more the interferences tend to affect the measurement performance. Interferences that affect the sensitivity of the determination of the analyte and do not produce a signal equivalent to that of the analyte, are designated matrix interferences [1]. Matrix effects can be overcome by adding the interfering species to calibrators or, if interferences do not significantly affect the measurement performance, by adding an excess of these species both to calibrators and samples. Sample dilution can also be used to mitigate interfering effects to the extent that increase of the limit of quantification does not become an issue. Nevertheless, if it is not possible to *a priori* know the nature and the quantity of the interferences, or if these vary with the analysed item, no procedure based on adding interferences to calibrators is feasible.

1 In samples where it is hard to predict the magnitude of matrix effects, calibrations are
2 performed in the sample matrix using the standard addition method.
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4 The main disadvantage of standard addition calibrations is the large number of resources
5 required to prepare calibrators, namely the amount of sample, chemicals and time needed for
6 preparation. Another disadvantage of these calibrations is the fact that quantifications by
7 extrapolation are more uncertain than by interpolation in a valid calibration curve [1].
8 Therefore, when standard addition calibrations have to be performed, optimisation is frequently
9 required.
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11 Some authors studied the variables that affect the quality of the extrapolation of standard
12 addition calibration curves [1-7]. However they did not discuss the use of models of the
13 variation of the instrumental signal with the studied quantity to guarantee the validity of
14 calibrations, namely the applicability of the regression model, and to optimise the uncertainty of
15 these quantifications. Use of the linear unweighted regression model requires homoscedastic
16 signals, calibrators of adequate quality given signal precision and sensitivity equivalent to that
17 observed next to the analytical zero. This work proposes to go further in designing and
18 optimising standard addition calibrations.
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20 The strategy developed for designing and optimising standard addition calibrations is applied
21 to the determination of anions in seawater, a matrix with high complexity due to both the variety
22 of species in solution and the different concentration ranges in which they are present.
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24 The quantification of major ions in seawater, such as chloride or sodium, does not usually
25 represent a problem. However, the determination of other ionic species can be affected by the
26 lack of selectivity [8] and matrix effects [9].
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28 The selectivity of these analyses can be improved by using efficient separation techniques
29 prior to a selective detection. Capillary electrophoresis (CE) [10] and ion chromatography (IC)
30 [11-13] have been used for the determination of the ionic composition of seawater. Ion
31 chromatography with conductometric detection is the most popular technique for this
32

determination due to its selectivity and sensitivity. The standard addition method has been used to compensate matrix effects [9].

The developed methodology guided successfully the analyst to produce valid determinations of Cl^- , NO_3^- , SO_4^{2-} and Br^- in seawater, by ion chromatography in isocratic conditions and conductometric detection, with the minimum resources needed to achieve a relative expanded uncertainty smaller than target values of 20 %, for larger components, or 40 % for Br^- .

2. Experimental

2.1. Apparatus, chemicals and samples

Dionex DX-500 ion chromatograph equipped with an isocratic pump IP20, a 25 μL loop, an IonPac AS9-HC separation column with an AG9-HC pre-column, and a conductivity detector CD20 with an ASRS Ultra II 4 mm suppressor. Peak Net *software* was used for instrument control, data acquisition and processing. Chromatographic and detection conditions were optimized in order to get an adequate resolution of peaks and sensitivity. The instrumental conditions were 3.5 mmol L^{-1} Na_2CO_3 and 1 mmol L^{-1} Na_2HCO_3 buffer eluent, flow rate of 1.2 mL min^{-1} , and detector current and output range of 50 mA and 1 μS respectively.

All chemicals used to prepare standard solutions were of analytical-reagent grade, namely NaCl ($\geq 99.8\%$) from Riedel-de Haën, NaBr ($\geq 99.7\%$) from BDH Prolabo, NaNO_3 ($\geq 99.0\%$) and Na_2SO_4 ($\geq 99.0\%$) from Merck. $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$ ($\geq 99.0\%$) and NaHCO_3 ($\geq 99.7\%$) from Merck were used in eluent preparation. All solutions were prepared with ultrapure water obtained from a Milli-Q Academic system (Millipore). The impurities of the various salts did not affect the content of the other analytes significantly [14].

Four seawater samples were collected from two tanks with marine species at Vasco da Gama Aquarium, a Portuguese scientific and pedagogic institution established in 1898. Duplicate samples (A1 and A2) and (B1 and B2) from tanks number 35 and 36, respectively, were collected and analysed.

1 Additionally, a reference artificial seawater was prepared, by weighing and dissolving salts,
2 and analysed to test measurement reliability. The composition of the reference seawater [15]
3 was $(19750 \pm 23) \text{ mg L}^{-1}$ in chloride, $(67.51 \pm 0.82) \text{ mg L}^{-1}$ in bromide, $(207.2 \pm 1.9) \text{ mg L}^{-1}$ in
4 nitrate, $(501.1 \pm 4.1) \text{ mg L}^{-1}$ in sulphate and $(13023 \pm 15) \text{ mg L}^{-1}$ in sodium. The uncertainty
5 associated with the reference values was estimated from the combination of the uncertainty
6 associated with salts purity, and the performed weighing and volumetric operations.
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15 2.2. Measurement procedure

16 The measurement procedure begins with the preparation of five calibrators. Calibrators are
17 five dilutions, using the same dilution factor, F_{Dil} , of the sample to be quantified, to which four
18 of them known and equidistant increasing mass concentrations of analyte (γ_{+1} to γ_{+4}) are
19 added to. If possible, diluted sample with no standard addition ($\gamma_{+0}=0$) should have a mass
20 concentration larger than the Limit of Quantification. Calibrators signals should be univocal; i.e.
21 the confidence interval of signals ($\bar{y} \pm t_{99}s_y$) should not overlap each other (where $\bar{y}$ and s_y
22 are the mean and standard deviation of signals respectively and t_{99} the Student's t for a
23 confidence level of 99 % and the degrees of freedom of $\bar{y}$ and s_y).
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40 In order to avoid the impact of instrumental signal drift on the calibration, the signals of all
41 calibrators are collected before collecting the subsequent replicate.
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43 In this work the instrumental signal is the area of analyte peak.
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45 The mass concentration of the ion in the sample, γ , ($\gamma = F_{Dil}\gamma_E$) is calculated by
46 multiplying the mass concentration of the diluted sample, γ_E , by the dilution factor, F_{Dil} . γ_E is
47 estimated by extrapolating the regression line to zero signal [1] by calculating the ratio
48 ($\gamma_E = a/b$) between the intercept, a , and slope, b , of the regression line ($y = a + b\gamma_+$) that
49 describes the relation between peak area (y) and added mass concentration of ion (γ_+).
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3. Methodology

The design of valid standard addition calibrations and the optimisation of the uncertainty of anion measurement in seawater is based on models of the variation of IC signals with analyte concentration. The IC signal was studied in a wide concentration range, through the replicate collection of signals of calibrators of anions prepared in purified water. Typically, standard addition calibrations are performed in a range narrower than the studied one. The matrix effects to be compensated in standard addition calibrations are not severe enough to force studying IC signals with calibrators prepared in a complex matrix.

The validation of the measurement procedure consists in the comparison of the estimated measurement uncertainty with the target measurement uncertainty. The measurement quality is subsequently controlled through the analysis of an artificial seawater with known composition.

3.1. Assessment of the performance of the instrumental signal

Six replicate signals of eleven standard solutions with equidistant mass concentrations enabled assessment of: i) signal's precision, ii) homoscedasticity of signals (homogeneity of the variance of signals), iii) linearity of the variation of signal with the mass concentration and iv) constancy of the sensitivity. The number of signals collected at each mass concentration seems to be adequate for the performed statistical tests if standard addition calibrations are not performed with a significantly larger number of signals. The statistical representativeness of the six replicate signals seems to be adequate for a standard addition calibration with 10 calibrator signals. The adequacy of this option is subsequently tested through the assessment of measurement repeatability and the analysis of an artificial seawater with known composition.

Although performance-check iv) is somewhat redundant to response linearity check iii), it was performed due to its relevance for standard addition calibrations and due to the fact that heteroscedasticity can mask sensitivity variations relevant for narrower homoscedastic ranges. The standard additions must be performed in a concentration range where signal increases

linearly with the mass concentration and sensitivity (quantified by the slope of regression line) is equivalent to the value estimated next to the analytical zero (Figure 1).

The homoscedasticity was tested, in a specific concentration range, by assessing if the variance at the highest concentration is statistically different from the one at the lowest concentration, using a F-test for a confidence level of 99 % (i.e. $P=0.01$) [16, 17].

The linearity of the variation of the signal in the studied range (the term “studied range” is used to describe the range between the lowest and highest studied level of analyte), where signal is heteroscedastic, is tested with a lack-of-fit χ^2 test [18]. If signal is proved to vary linearly in the studied range, this condition is extrapolated to a narrower range regardless signal homoscedasticity or heteroscedasticity.

The constancy of the sensitivity was tested by the statistical equivalence of the slopes estimated in the highest and widest (b_2) and lowest and widest (b_1) homoscedastic ranges (Figure 2.a). This test involves assessing whether or not the absolute difference of slopes is smaller than the expanded standard deviation of their difference for a 99 % confidence level. Homogeneity of the sensitivity can also be tested by assessing the statistical equivalence of the confidence interval of the intercept (a_2) of the highest and widest homoscedastic calibration range, to the expected mean signal of a blank solution, in this case zero value (Figure 2.b). Signals of higher concentrations are expected to produce worst case intercept due to possible signal saturation.

In the determination of the anionic composition of seawater by ion chromatography with conductometric detection, the instrumental signal linearity and homoscedasticity, should not be significantly affected by matrix effects. Therefore, the developed performance models can be used to guide standard addition calibrations. These calibrations are used to accurately compensate matrix effects on sample analysis.

3.2. Measurement uncertainty evaluation

The evaluation of the measurement uncertainty was based in the principles and procedure described in the “Guide to the expression of uncertainty in measurement”, GUM [28]. The developed measurement uncertainty model was subsequently tested through the analysis of an artificial seawater with known composition. The evaluation of the measurement uncertainty was performed using the bottom approach [19], also designated sub-analytical [20] or modelling [21] approach, which involves dividing the measurement procedure into individual uncertainty components responsible for random or systematic effects. These are quantified and further combined using adequate algorithms, thus allowing estimation of their respective relevance for the measurements quality.

3.2.1. Definition of the measurand and measurement procedure

The studied measurand is the mass concentration of a specific ion in a particular seawater sample. The measurement procedure is described in section 2.2.

3.2.2. Identification of the sources of uncertainty

Figure 3 presents the cause-effect diagram representing the identified sources of uncertainty. F_{Dil} is affected by both the repeatability of the manipulation and the tolerance of the nominal value (Bias) of the volumetric material used for sample dilution. γ_E is affected by the statistical extrapolation of the regression line and by the uncertainty of the mass concentration added to diluted samples, represented by the unitary factor f_+ . The f_+ is affected by the repeatability and bias of the weighing and volumetric operations involved in mass concentration additions. No additional uncertainty components need to be considered if the assumptions of the used regression model are proved valid.

3.2.3. Quantification of uncertainty components

This section describes the quantification of the uncertainty components, subsequently used for estimating the combined standard uncertainty, and for checking the assumptions of the used least square regression model (also known as linear unweighted regression model) [1].

The uncertainty associated with weighing and volumetric operations was estimated as described in the Eurachem/CITAC Guide [22].

3.2.3.1. Dilution factor

3.2.3.1.i) Standard uncertainty

The standard uncertainty, u_{Dil} , associated with F_{Dil} is estimated by combining the standard uncertainties of volumes measured to dilute sample [22]. If sample is diluted, by the same dilution factor, using different volumetric material, the largest relative standard uncertainty of F_{Dil} (u_{Dil} / F_{Dil}) should be considered for estimating the combined uncertainty and for checking assumptions of the regression model.

3.2.3.1.ii) Requirements of the regression model

The relative standard uncertainty, $u'_{R_{Dil(i;j)}}$, (where the apostrophe stands for a relative quantity) of the unitary ratio $R_{Dil(i;j)}$ of the various dilution factors [$R_{Dil(i;j)} = (F_{Dil(i)} / F_{Dil(j)}) = 1$, where i and j vary between the 1st and the 5th sample dilution and $i \neq j$] must be negligible given the minimum relative standard deviation of signal repeatability, $s'_{y(Min)}$. This assumption is tested by assessing whether or not $u'_{R_{Dil(i;j)}}$ is statistically larger than $s'_{y(Min)} / 3$ through a one-tailed F -test [1]. This condition must be valid since regression is performed by minimizing the distance between the experimental points and the regression line in terms of signal value. Therefore, the relative positioning of added mass concentrations of analyte must be affected by negligible uncertainty given instrumental signal

precision [23]. The uncertainty associated with sample dilution indirectly affects the axis of added concentration of analyte.

Since typically the same pipet is used to dilute sample for various and equivalent volumetric flasks, the tolerance of its nominal value does not contribute to $u'_{R_{Dil}(i;j)}$. Nevertheless, this tolerance will affect u_{Dil} required to estimate the combined standard uncertainty of sample measurement (see 3.2.3.1.i).

3.2.3.2. *Standard addition*

3.2.3.2.i) *Standard uncertainty*

The relative standard uncertainty, u'_+ , associated with f_+ is equal to the relative standard uncertainty of the mass concentration of analyte in the stock solution from where volumes are taken to add analyte to diluted samples.

3.2.3.2.ii) *Requirements of the regression model*

The standard addition procedure must guarantee a negligible relative standard uncertainty, $u'_{R_{+(k;l)}}$, associated with the ratio, $R_{+(k;l)}$, of the added mass concentrations (where $R_{+(k;l)} = \gamma_{+k} / \gamma_{+l}$; with k and l varying between 1st and 4th standard addition and $k \neq l$), given $s'_{y(Min)}$. This assumption is tested by proving that $u'_{R_{+(k;l)}}$ is not statistically larger than $s'_{y(Min)} / 3$ through a one-tailed F -test [1]. The justification for this assessment is equivalent to the one presented for quality requirements of sample dilution (3.2.3.1.ii). The $u'_{R_{+(k;l)}}$ is independent of u'_+ if the same stock solution is used for standard additions. Nevertheless, u'_+ affects the quality of the estimated mass concentration of samples (see 3.2.4).

3.2.3.3. *Extrapolation of the regression line*

3.2.3.3.i) *Standard uncertainty*

The standard uncertainty, u_E , of the extrapolation of the regression line is estimated by Eq.(1)

[1]:

$$u_E = \frac{s_{y/\gamma}}{b} \sqrt{\frac{1}{np} + \frac{\bar{y}^2}{b^2 p \sum_i (\gamma_{+i} - \bar{\gamma}_+)^2}} \quad (1)$$

where $s_{y/\gamma}$ is the residual standard deviation [1], b is the slope of the regression line, n the number of calibrators (number of standard additions plus 1 for addition zero), p the number of replicate readings of calibrators, $\bar{y}$ the mean signal of calibrators, γ_{+i} the added mass concentration of analyte in calibrator i (i varies from 1 to 5) and $\bar{\gamma}_+$ is the mean of γ_{+i} values. This equation can only be applied if the assumptions of the linear unweighted regression models are valid (3.2.3.3.ii).

3.2.3.3.ii) Requirements of the regression model

Sections 3.2.3.1.ii) and 3.2.3.2.ii) describe the assessment of the adequacy of sample dilution and standard additions procedures for the application of the linear unweighted regression model. Additionally, calibrators signals must be homoscedastic and the linearity of the variation of the instrumental signal with the mass concentration of analyte must be proved.

The homoscedasticity of signals is checked by comparing the variance of the signals at the lowest and at the highest calibration levels through an F-test for a confidence level of 99 % [16, 17, 24].

The linearity of the instrumental response, in a homoscedastic range, can be tested by an ANOVA lack-of-fit test [23, 25].

Although the studied range to be made available for standard addition calibrations can be heteroscedastic, the specific standard addition must be performed in a narrower range where signal's variance is homoscedastic.

3.2.4. Combination of the uncertainty components

The uncertainty components are combined following the uncertainty propagation law (Eq. 2):

$$u_{\gamma} = \gamma \sqrt{\left(\frac{u_{Dil}}{F_{Dil}}\right)^2 + \left(\frac{u_E}{\gamma_E}\right)^2 + \left(\frac{u_+}{1}\right)^2} \quad (2)$$

The linearity of the variation of γ with the variation of the input quantities, namely F_{Dil} , γ_E and f_+ , within their uncertainty, was tested by comparing u_{γ} estimated by the numerical Kragten method using either positive or negative increments of the input quantities [26, 27]. Relative differences of estimated u_{γ} smaller than 1 % are considered an evidence of linearity. In those cases, the uncertainty propagation law can be used without restrictions. Deviations to γ linearity can be observed if sample aliquot volume of sample dilution (denominator of F_{Dil}) is affected by a relevant uncertainty.

3.2.5. Expansion of the combined standard uncertainty

The confidence level of the combined standard uncertainty, u_{γ} , is expanded by multiplying it by a coverage factor, k , equivalent to the Student's t [$k = t_{0,05}^{v_{eff}}$] for the number of effective degrees of freedom, v_{eff} , of u_{γ} and a confidence level of 95 %. The resulting expanded uncertainty, U_{γ} ($U_{\gamma} = k u_{\gamma}$), is used for reporting measurement result ($\gamma \pm U_{\gamma}$). The v_{eff} is estimated by the Welch-Satterthwaite equation that defines the combination of the degrees of freedom of the input quantities [28].

3.3. Measurement design and optimization

The design of the measurement must take the impact of uncertainty components in the assumptions of the regression model into account. The optimisation of the measurement uncertainty must take the impact of uncertainty components in the combined standard uncertainty, u_{γ} , and its coverage factor, k , into account.

u_E is expected to be the major uncertainty component since low relative uncertainty associated with sample dilution and calibrators preparation can be easily achieved. u_E is also expected to have a major impact on the coverage factor since their degrees of freedom ($np-2$) will only be high if many experimental signals are collected for calibration. On the other hand, the number of degrees of freedom associated with u_{Dil} and u_+ are easily high due to the involved type B uncertainty evaluations [28].

The following sections describe the optimization of the identified sources of uncertainty having in mind the restrictions of the design of standard addition calibrations.

3.3.1. Optimization of the u'_E

It can be observed from Eq.(1) that u_E increases with larger $s_{y/\gamma}$ and $\bar{y}$ values, and decreases with larger b , n , p and range of concentrations of calibrators described by term $\sum(\gamma_{+i} - \bar{\gamma}_+)^2$. The $s_{y/\gamma}$ quantifies the precision of the instrumental signal.

Since the uncertainty associated with the regression line extrapolation is combined with the other components as a relative quantity, the optimization of this component must be focused on the ratio $u'_E = u_E/\gamma_E$ instead of the absolute u_E value. The γ_E can be partially managed by selecting the dilution factor of sample.

The optimization of parameters of Eq.(1) rely on empirical models of the variation of signal value (y) and precision (s) with analyte concentration (γ).

The model y vs. γ is used to estimate $\bar{y}$ and b . The b value is not expected to vary significantly in the analytical range for the determination of the same analyte in a specific instrument. Although b value vary with matrix effects, this is unlikely to affect the optimization strategy of u'_E significantly. Nevertheless, if major matrix effects are observed in sample analysis, performance data used for u'_E optimization (i.e. y vs. γ) must be based in signals of matrix-matched calibrators. Even if matrix adjustment is not accurate, it should be adequate to

define the optimum standard addition interval that will, afterwards, compensate this effect in an accurate way.

The model of s vs. γ is used to estimate $s_{y/\gamma}$.

In this study, n is 5 and p vary as the number of replicate measurements of calibrators.

Since u'_E decreases as p increases, it was selected a p value that produces fit for the intended use measurement uncertainty with an acceptable outlay of resources. The impact of the p value on the coverage factor, k , (Section 3.2.5) is also considered in this optimisation.

The range of concentrations of calibrators, that defines term $\sum(\gamma_{+i} - \bar{\gamma}_+)^2$ of Eq.(1), is set after selecting the maximum standard addition to the diluted sample. The concentration range starts in the concentration of the diluted sample, γ_1 , and ends in the concentration of the diluted sample plus maximum analyte addition, γ_2 . The term $\sum(\gamma_{+i} - \bar{\gamma}_+)^2$ can be calculated either considering added analyte concentrations or these values plus estimated diluted sample concentration. Figure 4 and Eq.(3) present the calculation of $\sum(\gamma_{+i} - \bar{\gamma}_+)^2$ given that three equidistant calibrators between γ_1 and γ_2 are prepared. This term is a quadratic function of γ_1 and γ_2 .

$$\begin{aligned} \sum_i^n (\gamma_{+i} - \bar{\gamma}_+)^2 &= \\ &= \left(\frac{\gamma_1 - \gamma_2}{2}\right)^2 + \left(\frac{\gamma_1 - \gamma_2}{4}\right)^2 + \left(\frac{\gamma_2 - \gamma_1}{4}\right)^2 + \left(\frac{\gamma_2 - \gamma_1}{2}\right)^2 = \\ &= \frac{5}{8}(\gamma_2 - \gamma_1)^2 \end{aligned} \quad (3)$$

Since u'_E is smaller for larger ranges of calibrators concentrations, the γ_2 will be the largest concentration which present a signal variance, s_2^2 , not statistically larger than the variance, s_1^2 , observed for γ_1 . Assuming s_2^2 is larger than s_1^2 , the homoscedasticity is tested using Eq.(4):

$$\frac{s_2^2}{s_1^2} \leq F_{0.01}^{v_2; v_1} \quad (4)$$

where $F_{0.01}^{v_2;v_1}$ is the critical value of an F-distribution for the degrees of freedom of s_2 and s_1 , v_2 and v_1 respectively, and a confidence level of 99 %. Therefore, the maximum γ_2 is the concentration producing s_2 estimated by Eq.(5):

$$s_2 = s_1 \sqrt{F_{0.01}^{v_2;v_1}} \quad (5)$$

The model s vs. γ is used together with Eq.(5) to calculate γ_2 given the estimated concentration of the diluted sample, γ_1 . For instance, if s increases linearly with γ , where $s=C+D\gamma$ (C and D are the intercept and slope of the linear regression), and precision was estimated with 5 degrees of freedom, γ_2 is calculated using Eq.(6):

$$\begin{aligned} C + D\gamma_2 &= (C + D\gamma_1)\sqrt{14.94} \Leftrightarrow \\ \Leftrightarrow \gamma_2 &= \frac{2.86C}{D} + 3.86\gamma_1 \end{aligned} \quad (6)$$

The highest standard addition is the difference $(\gamma_2-\gamma_1)$.

The linearity of the variation of signal and the constancy of the sensitivity in the studied range is extrapolated to the narrow range $[\gamma_1; \gamma_2]$ (see section 3.1).

The graphic representation of the variation of the relative expanded extrapolation uncertainty, U'_E , with γ_1 (where $U'_E = u'_E t_{0.05}^{pn-2}$ and $t_{0.05}^{pn-2}$ is the Student's-t for $pn-2$ degrees of freedom and 95 % confidence level) can be used to select the sample dilution factor, analyte additions and the number of replicate readings to achieve a minimum U'_E with acceptable outlay of resources. If the other uncertainty components are relevant for the combined uncertainty and coverage factor, the variation of measurement relative expanded uncertainty, U'_γ , with γ_1 should be considered (section 3.3.4).

3.3.1.1. Model of the variation of the signal with analyte concentration

Analyte peak areas of replicate injections of standard solutions are collected to develop models of the variation of the mean, $\bar{y}$, and standard deviation, s , of signals with the concentration (γ). For ion chromatography, peak area is expected to increase, at least

approximately, linearly with the concentration. The standard deviation (s) and the relative standard deviation (s') of signal are expected to increase and decrease, respectively, with γ . These trends are observed in many other instrumental methods of analysis [27, 29, 30].

The collected peak areas are used to check the linearity and the homoscedasticity of signals, the constancy of the sensitivity and to estimate $s'_{y(Min)}$ required to define sample dilution and standard addition procedures. This information is subsequently used to develop models of the variation of measurement uncertainty with γ_I required to optimised measurement uncertainty.

3.3.1.2. Approximate estimation of the mass concentration of the analyte in the sample

Once the concentration of the analyte in the sample is approximately estimated, $\gamma_{\sim 0}$, the possible γ_I values are defined as fractions of $\gamma_{\sim 0}$ given possible F_{Dil} values. The $\gamma_{\sim 0}$ can be estimated by comparing sample signal with the ones of calibrators prepared in a simple matrix or, in case matrix effects are severe, after performing a single standard addition and a two points standard addition calibration.

If the sample concentration, γ , estimated by the optimised standard addition is significantly different from $\gamma_{\sim 0}$, the optimisation must be reviewed, in particular, for the assumptions of the regression model.

Sample concentration can be performed to ensure standard addition calibration at larger concentration ranges. In these cases, concentration efficiency should be accessed.

3.3.2. Optimization of u'_{Dil} and u'_+

The minimisation of u'_{Dil} and u'_+ must be supported in the algorithms used for their calculation.

Sample dilution can be performed either volumetrically or gravimetrically. The dilution of a sample by taking the mass and density of the aliquot and diluted sample can be less uncertain than by using volumetric equipment. However, gravimetric dilutions are more time consuming

1 than volumetric ones and should only be performed if required to ensure uncertainty is smaller
2 than the target value. If an adequate volume of sample is available, volumetric sample dilution
3 with adequately low uncertainty can be easily performed. This work discuss the volumetric
4 dilution of samples and calibrators.
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8 Since the relative standard uncertainty of measured volume tends to decrease with the
9 volume, u'_{Dil} tends to be smaller if larger volumes are measured for sample dilution. If sample
10 amount is small, u'_{Dil} optimisation is less flexible. Different dilution strategies can be used to
11 reach the same F_{Dil} : sample solution aliquot can be diluted in a volumetric flask or by the
12 addition of another volume of the same solvent. Dilution by solvent addition is particularly
13 useful if a small sample amount is available and is not necessarily more uncertain than
14 conventional dilution in a volumetric flask [31]. Solvent addition also allows more flexible F_{Dil} .
15 If sample dilution becomes too uncertain, it can either affect the assumption of the linear
16 unweighted regression model (3.2.3.1.ii) or become a relevant component of u_y . It is expected
17 that before u_{Dil} becomes relevant, it will be inadequate for the use of the regression model.
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33 The u'_+ is estimated by combining the relative standard uncertainties associated with stock
34 solution concentration and their volumes added to the diluted sample. The uncertainty of stock
35 solution concentration is estimated from the uncertainty of solid reference material mass and
36 purity, and stock solution volume. The relative standard uncertainty of reference material mass
37 decreases as mass value increases and can be easily kept negligible if not too small mass is
38 measured. The optimisation of measured volumes should be managed as previously described
39 for F_{Dil} . The analyte purity can only be optimised by selecting the reference material with
40 smaller relative uncertainty of purity value. Typically, a limited number of reference materials
41 will be available and the uncertainty of their purity will be a negligible uncertainty component.
42 If purity is reported without uncertainty, it should be estimated by a pragmatic type B
43 evaluation. The standard addition procedure must be checked for the ability to fulfil regression
44 model requirements (3.2.3.2.ii). Standard addition procedure can be adequate and still constitute
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1 a major component of u_γ since only the final dilution of the stock solution affects the
2 applicability of the regression model [23].
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7 3.3.3. Optimization of u_γ 8 9

10 The optimisation of the combined standard uncertainty, u_γ , involves the optimisation of its
11 components. It should be estimated the impact of the optimisation of a specific component in
12 the global uncertainty before deciding to spend time and resources in this process.
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18 3.3.4. Optimization of U_γ 19 20 21

22 The impact of the calibration design in the coverage factor used to expand measurement
23 uncertainty must be taken into account in U_γ optimisation. If u'_{Dil} and u'_+ are kept negligible in
24 comparison with u'_E , the optimisation of U_γ can be based on its variation with γ_I and p for
25 maximum u'_{Dil} and u'_+ values. Lines of U_γ vs. γ_I for different p values are particularly useful to
26 define optimum measurement conditions.
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38 3.4. Quality control 39

40 The quality of the optimisation strategy was assessed by comparing the uncertainty predicted
41 by the developed measurement model with the actually estimated after experimental calibration.
42 The quality of the estimated measurement uncertainty is assessed through the analysis of an
43 artificial seawater and the evaluation of the metrological compatibility of estimated and known
44 mass concentrations of the ionic species. This assessment was complemented with the
45 evaluation of possible analyte contaminations, through the analysis of blank samples, and the
46 evaluation of measurement precision due to its relevance for measurement uncertainty. The
47 linearity of a specific standard addition calibration curve is confirmed, after the detailed
48 linearity check of instrumental response, by calculating Pearson's linear correlation coefficient.
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Relevant deviations of the linear correlation coefficient to the ideal 1 value are attributed to the occurrence of outliers of calibrators readings.

Blank samples signals (i.e. the analyte peak area) should be smaller than three times the residual standard deviation ($s_{y/\gamma}$) of the regression line that quantifies signal's precision. $3s_{y/\gamma}$ is the estimated signal at the limit of detection of the chromatographic determination.

Duplicate standard additions of samples were performed to check extrapolation repeatability. Since sample and stock solution dilution uncertainties are negligible given signal repeatability, the extrapolation repeatability is checked by comparing the absolute difference of duplicate results, $|d_E|$, $[|d_E|=(|\gamma_{E1} - \gamma_{E2}|)]$ with the repeatability limit [28], expanded to 99 % confidence level, of extrapolated concentrations, r_E ($r_E=t\sqrt{2} u_E$) (Eq. 1) (where t is the Student's t for the degrees of freedom of u_E and a confidence level of 99 %). If $|d_E|\leq r_E$, the difference of duplicate results is justified by the extrapolation uncertainty.

The linear correlation of standard addition calibration curves is assessed by comparing Pearson's coefficient with a minimum value defined for 99 % confidence level and the number of calibrators signals [1]. For instance, for ten calibrators signals the Pearson's correlation coefficient should be not smaller than 0.765.

The compatibility between known ($\gamma_{\text{Ref}} \pm k_{\text{Ref}} \cdot u_{\text{Ref}}$) and estimated ($\gamma \pm k \cdot u_\gamma$) mass concentrations of analyte in the artificial seawater (where γ_{Ref} and γ are both measured quantity values, k_{Ref} and k are both coverage factors for defined confidence level and u_{Ref} and u_γ are both standard uncertainties respectively) is assessed by comparing the absolute difference ($|d|=|\gamma - \gamma_{\text{Ref}}|$) of measured quantities with the expanded uncertainty of the difference, U_d ($U_d=k_d \cdot u_d$; where k_d is the coverage factor for typically 95 or 99 % and u_d the standard uncertainty of the difference) (Eq. 7). If $|d|\leq U_d$, measurements are compatible and therefore metrologically equivalent. In this case, measurement model is confirmed to be adequate to report the analytical information.

$$|d|=|\gamma - \gamma_{\text{Ref}}| \leq k_d \sqrt{u_\gamma^2 + u_{\text{Ref}}^2} = k_d u_d = U_d \quad (7)$$

4. Results and discussion

4.1. Models of the variation of the signal with the mass concentration of analyte

The variation of $\bar{y}$ and s with the γ of analyte (Cl^- , Br^- , NO_3^- or SO_4^{2-}) was assessed in a range from the expected limit of detection to 100 times larger this value, namely: 0.04-4 mg L⁻¹ for Cl^- , 0.1-10 mg L⁻¹ for Br^- , 0.1-10 mg L⁻¹ for NO_3^- and 0.2-20 mg L⁻¹ for SO_4^{2-} . These mass concentration ranges were characterised from 6 replicate signals of 11 equidistant calibrators. It was observed a linear increase of signals and their standard deviations with the mass concentration of analyte. The variance of the instrumental signal (s^2) at the highest γ of the studied range is statistically different from that observed at the lowest γ , as proved by an F -test for a confidence level of 99 %. The instrumental signal (y) varies linearly with γ in the studied ranges as proved by a χ^2 lack-of-fit test for a confidence level of 99 %. The instrumental response sensitivity is constant in the studied ranges as proved by the equivalence of slopes of the highest and lowest linear unweighted calibrations.

Table 1 presents the equations used to describe the variation $\bar{y}$ vs. γ and s vs. γ . Figure 5 presents the graph s vs. γ for the determination of bromide. The low Pearson's linear correlation coefficient, r_p , ($r_p=0.95$) observed in this graph results from the large dispersion of s values given the small increase of s with γ . Nevertheless, the slight increase of s is evident and well described by a straight line.

4.2. Optimisation of standard addition determinations of seawater samples

4.2.1. Selection of optimum calibration conditions

After the detailed study of the variation of the instrumental signal with γ , it was developed a model to define the maximum standard addition ($\gamma_2 - \gamma_1$) for a given mass concentration of the diluted sample (γ_I). The standard additions should ensure a maximum range of mass concentrations of calibrators [i.e. a maximum $\Sigma(\gamma_i - \bar{\gamma})^2$] producing signals within the studied range and with homoscedastic variance. Table 2 presents the algorithms and conditions used to

define γ_2 given γ_1 . The equation of the second column of Table 2 results from the application of regression parameters of the linear relation s vs. γ (third columns of Table 1) to Eq.(6).

The equation used to define γ_2 , the models $\bar{y}$ vs. γ and s vs. γ , and the algorithm used to calculate U'_γ were used to define models of the variation of U'_γ with γ_1 in optimised standard addition conditions for specific p , u'_{Dil} and u'_+ values. Figure 6 presents lines U'_γ vs. γ_1 , for various p values, for the determination of NO_3^- in seawater after diluting sample 50 times using a class A 1 mL pipette and a 50 mL flask, and a specific stock solution of nitrate. The uncertainty associated with sample dilution and stock solution concentration are minor uncertainty components. These lines define how many times sample should be diluted with negligible uncertainty and how many calibrators signals replicates should be collected to reach a specific U'_γ value.

It can be observed in Figure 6 that U'_γ decreases from values above 100 % to much lower ones as γ_1 increases in the lower portion of the analytical range and, in the larger half of this range U'_γ increases exponentially with γ_1 . The reduction is explained by the increase of the signal-to-noise ratio as concentration increases. The increase of U'_γ is explained by the restriction of the increase of γ_2 to ensure all signals are within the studied range. When γ_2 defined by the equation of the second column of Table 2 is larger than the maximum of the studied analytical range, this maximum is used instead of the calculated γ_2 forcing the reduction of $(\gamma_2 - \gamma_1)$ and consequently of the term $[\Sigma(\gamma_i - \bar{\gamma})^2]$ of Eq. (1). This restriction drives to extremely large U'_γ as γ_1 tends to the maximum of the studied range and $(\gamma_2 - \gamma_1)$ tends to zero. It can also be observed from Figure 6 that U'_γ decreases significantly if duplicate collections of signals are performed instead of single ones. The improvement of measurement quality is not so relevant for triplicate or more replicate readings. Another advantage of collecting some replicate signals of calibrators is the fact that a wider range of γ_1 values produce an almost constant U'_γ value making calibrators preparation more flexible.

Equivalent trends are observed for all the other analytes.

These models were used to define optimum standard additions calibrations for the determination of the anionic composition of three seawater samples: two waters from marine species tanks of Vasco da Gama Aquarium, and one Artificial seawater.

In this work, it was defined a target relative expanded uncertainty of 20 % for the determination of Cl^- , NO_3^- and SO_4^{2-} in seawater, likewise in some determinations of chemical parameters in environmental samples [32-34]. For the determination of Br^- in seawater, the target expanded uncertainty was increased to 40 % due to the lower concentration of this ion.

In order to use the lines U'_γ vs. γ_I in measurement uncertainty optimisation, approximate concentration of analyte in sample (γ_{-0}) is estimated by comparing the signal of the sample solution, or its dilution, with the one of a standard solution prepared in purified water. Matrix effects are not expected to affect the optimisation process significantly.

The selection of sample dilution allows positioning the γ_I in the curve U'_γ vs. γ_I , for a specific p value, where predicted U'_γ is fit for the intended use.

It was decided to be performed the quantification of Cl^- , Br^- , NO_3^- and SO_4^{2-} , where SO_4^{2-} was only quantified in the artificial water, with the minimum number of solutions and the minimum number of collected signals required to reach the target measurement uncertainty. Sample dilution and standard additions were planned to guarantee quantifications in the minimum plateau of the curves U'_γ vs. γ_I for $p=2$ (i.e. for duplicate signals).

Table 3 presents the estimated approximate concentration of samples (γ_{-0}), the selected sample dilution, the diluted sample expected concentration and the maximum admissible (MAA) and maximum performed (MPA) standard additions (γ_2 - γ_I) [MAA►MPA]. This table also presents the predicted $s_{y/\gamma}$, the proportion of predicted $s_{y/\gamma}$ given the expected mean signal of calibrators ($s_{y/\gamma}/\bar{y}$), and the predicted relative expanded and standard uncertainties of measurements. The predicted measurement uncertainties are smaller than the defined target value.

Standard additions for the determination of Br^- , NO_3^- and SO_4^{2-} in samples, were performed in the same dilution of 1 mL of sample to 50 mL of final volume to which all analytes were added to. Standard additions for the determinations of Cl^- in samples were performed after diluting sample 10,000 times by performing two consecutive 100 times dilutions.

The samples of Aquarium Vasco da Gama were analysed twice, in repeatability conditions, by performing independent sample dilutions and standard additions of the same stock solution. Each group of calibrators were used to build separate calibrations curves and to perform independent quantification.

Each calibration was based on duplicate signals of five calibrators with equidistant concentrations (diluted samples plus four standard additions).

The expected mean value and dispersion of signals of the five calibrators, estimated from the model for s vs. γ and y vs. γ , were assessed to ensure signals are univocal for a confidence level of 99 %. The intervals of the expected dispersion of calibrators signals ($\bar{y} \pm s \cdot t_{99}$) do not overlap each other (where $\bar{y}$ is the expected mean signal, s the standard deviation of signals and t_{99} the Student's-t for 99 % confidence level and the degrees of freedom of $\bar{y}$ and s).

Subsequently, it was defined a procedure and selected equipment for sample dilution and standard addition that allows fulfilling the assumptions of the used linear unweighted regression model regarding calibrators preparation quality. The model for s vs. γ was used to estimate $s'_{y(\text{Min})}$ required to assess the magnitude of the $u'_{R_{Di}}$ and $u'_{R_{+(k,l)}}$. The defined preparation procedure of the calibrators ensured $u'_{R_{Di}}$ and $u'_{R_{+(k,l)}}$ values not statistically larger than one third of the estimated $s'_{y(\text{Min})}$.

4.2.2 Standard addition determinations

After all details have been defined, calibrators were prepared and calibrations performed. Table 4 presents the most relevant performance parameters of the calibrations and Table 5 the estimated anionic composition of seawater samples. Table 6 and 7 present the assessment of the

repeatability of the standard additions calibrations and of the metrological compatibility of known and estimated concentrations of the artificial seawater respectively.

The observed Pearson's correlation coefficients are much larger than its minimum admissible value defined for 10 signals (i.e. 0.765) [1]. The smallest observed r_p is 0.988. The analysed blank samples present an instrumental signal smaller than $3s_{y/\gamma}$ of the calibration curves.

The comparison of $(s_{y/\gamma}/\bar{y})$ and (U'_γ) values of Table 3 and 4 allows concluding that the data of Table 3 accurately predicts the value of these parameters observed in experimental calibrations (Table 4). The $(s_{y/\gamma}/\bar{y})$ of Table 3 are randomly above and below the ones of Table 4 and both are statistically equivalent as proved by an F-test for 99 % confidence level. The U'_γ estimated from models prior calibrations are also equivalent to the observed experimentally, except for the determination of nitrate in A2 sample, as proved by F-tests where effective degrees of freedom of estimations are considered. In the determination of nitrate in sample A2, the high agreement of experimental signals, confirmed in the r_p of 1.000 and low u'_E of 2.4 %, made the u'_+ a relevant uncertainty component that increased the v_{eff} to 38. The high number of degrees of freedom of experimentally estimated u'_γ allowed distinguishing a deviation to the predicted u'_γ . This mismatch is attributed to weaknesses of the used convention to approximately define the degrees of freedom of type B evaluations dominant in the calculation of u'_+ . This convention tends to overestimate the reliability of type B evaluations. In three other occasions, predictions match exactly the observed experimentally and in the other 13 comparisons, 7 and 6 experimentally estimated uncertainties are above and below the predicted ones respectively. Therefore, models for producing U'_γ are proved valid.

In two occasions (determination of Br^- in Art. and determination of NO_3^- in A1) measurement uncertainty from experimental calibration is larger than the target value. This situation is explained by the variability of estimations based on a small number of replicate

signals of calibrators. This conclusion is somehow confirmed by the difference between U'_γ of duplicate measurements of ions in seawaters. In cases where U'_γ is larger than the target measurement uncertainty, this difference is not statistically relevant as proved by an χ^2 test for 99 % confidence level. The ratios $(\nu_{eff} u_{\gamma'}'^2 / (u_{\gamma'}'^{tg})^2)$ (where $u_{\gamma'}'^{tg}$ is the relative target standard uncertainty, 0.2 or 0.1 for the determinations of Br^- or NO_3^- respectively) are smaller than the target maximum χ_2^2 value (20.09 for 8 degrees of freedom). Since this test is not robust to the normality assumption of the distribution of the variable γ [35], it was only performed after confirming the approximate linearity of the model equation ($\gamma = F_{DiI} \cdot \gamma_E$) given the standard uncertainty of the input quantities by the Kragten method (section 3.2.4.).

The collection of more replicate signals of calibrators is expected to reduce the variability of the quality of results.

Therefore, uncertainty predicted from developed models, prior to experimental calibration, is only indicative particularly for calibrations performed with a low number of degrees of freedom. Nevertheless, independently of the final value of U'_γ , the determination is optimised by the developed strategy since it is support on a significant amount of experimental data.

Results of Table 6 prove extrapolation repeatability is adequately controlled by u_E value as described in the theory.

Known and estimated concentrations of the artificial seawater are metrologically compatible for a confidence level of 99 %, proving the adequacy of the developed measurement uncertainty models.

The approximate and accurate estimations of the analyte concentrations are also compatible assuming approximate estimation is two times more uncertain than the accurate one. This agreement suggests that there is no need to revise performed standard additions for regression model assumptions.

5. Conclusions

1 The strategy developed for designing standard addition calibrations is extremely useful to
2 guide analysts in performing valid calibrations (i.e. calibrations fulfilling regression model
3 assumptions in ranges where sensitivity is equivalent to that observed in the analytical zero) and
4 in producing measurements with fit for the intended use quality.
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8 After an approximate estimation of sample concentration, the model suggests the extent to
9 which sample should be diluted and the amount of analyte that should be added to diluted
10 samples in order to reach a certain measurement expanded uncertainty. When selecting
11 optimum calibration parameters, the analyst should be aware of the variability of the calibration
12 performance and of the estimated expanded uncertainty. If a small number of calibration signals
13 is collected, this variability is more relevant and should be accounted for whenever the
14 quantified result has to be reported with an uncertainty smaller than the target value.
15 Alternatively, the estimated uncertainty can be compared with its target value, allowing for
16 estimation variability, using a χ^2 test.
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19 The developed quantification strategy was successfully applied to the determination of some
20 anions in seawater. Predicted and experimentally estimated uncertainties of quantifications are
21 statistically equivalent for 99 % confidence level. Experimentally estimated expanded
22 uncertainty is not statistically larger than the target value for a confidence level of 99 %.
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25 Estimated and known mass concentrations of the artificial seawater are metrologically
26 compatible for 99 % confidence level, proving the validity of the developed measurement
27 model.
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30 Therefore, the developed work appears to have produced a tool that can be readily used by
31 laboratories that perform standard addition calibrations for regulation purposes or with strict
32 quality requirements. This strategy is particularly useful to extract more information from
33 studied systems with a minimal outlay.
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36 **Acknowledgements**

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Abbreviations

A1 and A2 – Duplicate samples of aquarium 35 of Vasco da Gama Aquarium;

Art. – Artificial seawater sample (see section 2.1 for composition);

B1 and B2 – Duplicate samples of aquarium 36 of Vasco da Gama Aquarium;

CE – Capillary electrophoresis;

IC – Ion chromatography;

MAA – Maximum admissible standard addition;

MPA – Maximum performed standard addition.

Symbols

a – intercept of the linear unweighted regression of standard addition calibrations (y vs. γ_+);

a_1 – intercept of the linear unweighted regression of the lowest homoscedastic range of the studied analytical range;

a_2 – intercept of the linear unweighted regression of the highest homoscedastic range of the studied analytical range;

b – slope of the linear unweighted regression of the standard addition calibration (y vs. γ_+);

b_1 – slope of the linear unweighted regression of the lowest homoscedastic range of the studied analytical range;

b_2 – slope of the linear unweighted regression of the highest homoscedastic range of the studied analytical range;

C – intercept of the linear regression of s vs. γ ;

d – difference between reference and estimated (γ) mass concentration of the artificial seawater;

D – slope of the linear regression of s vs. γ ;

d_E – difference between γ_{E1} and γ_{E2} ;

f_+ – unitary factor representing the impact of the quality of mass concentration additions in the quantification of γ ;

$F_{0.01}^{v_1;v_2}$ – critical F value for the ratio (s_2^2 / s_1^2) for 99 % confidence level;

F_{Dil} – dilution factor;

k – coverage factor to expand the measurement uncertainty;

k_d – coverage factor used to expand u_d to U_d ;

k_{Ref} – coverage factor used to expand u_{Ref} to U_{Ref} ;

n – number of calibrators of the standard addition calibration ($n=5$);

p – number of replicate collection of calibrators signals;

$R_{+(k; l)}$ – ratio of a pair of added analyte mass concentrations γ_+ ($\gamma_{+k} / \gamma_{+l}$) to the diluted samples

(where $k \neq l$ and k and l varies between the 1st and 4th standard addition);

$R_{\text{Dil}(i;j)}$ – ratio of a pair of F_{Dil} ($F_{\text{Dil}_i} / F_{\text{Dil}_j}$) describing the dilution of sample analysed by

standard addition calibrations (where $i \neq j$ and i and j varies between the 1st and 5th sample dilution);

r_E – expanded repeatability limit for 99 % confidence level;

r_P – Pearson's linear correlation coefficient;

s – standard deviation of areas of analyte peaks;

s' – relative standard deviation of areas of analyte peaks;

s_1 – standard deviation of areas of analyte peaks at γ_1 ;

s_2 – standard deviation of areas of analyte peaks at γ_2 ;

s_y – standard deviation of the instrumental signal;

$s'_{y(\text{Min})}$ – minimum relative standard deviation of replicate areas of analyte peaks in a specific mass concentration range;

$s_{y/\gamma}$ – residual standard deviation of the linear unweighted regression line;

t – Student's t ;

t_{99} – Student's t for 99 % confidence level and corresponding degrees of freedom;

$t_{0.05}^{pn-2}$ – Student's t for $np-2$ degrees of freedom and 95 % confidence level;

$t_{0.05}^{v_{\text{eff}}}$ – Student's t for v_{eff} degrees of freedom and 95 % confidence level;

u_{+} – standard uncertainty associated with f_{+} ;

u'_{+} – relative standard uncertainty associated with f_{+} ;

u_d – standard uncertainty associated with d_E ;

U_d – expanded uncertainty associated with d_E ;

u_{Dil} – standard uncertainty associated with F_{Dil} ;

u'_{Dil} – relative standard uncertainty associated with F_{Dil} ;

u_E – standard uncertainty associated with γ_E due to the extrapolation of the regression line;

u'_E – relative standard uncertainty associated with γ_E ;

U'_E – relative expanded uncertainty associated with γ_E ;

$u'_{R_{\text{Dil}}(i;j)}$ – relative standard uncertainty associated with $R_{\text{Dil}}(i;j)$ values;

$u'_{R_{+}(k;l)}$ – relative standard uncertainty associated with $R_{+}(k;l)$ values;

u_{Ref} – standard uncertainty associated with γ_{Ref} ;

u_{γ}^{tg} – target standard uncertainty associated with γ ;

u_{γ} – standard uncertainty associated with γ ;

U_{γ} – expanded uncertainty associated with γ ;

U'_{γ} – relative expanded uncertainty associated with γ ;

y – instrumental signal (area of analyte peak);

$\bar{y}$ – mean instrumental signal;

$\bar{y}_{Bk}$ – mean signal of blank solutions;

γ – mass concentration of the ion in seawater sample estimated by standard addition calibration;

γ_{+i} ($i=0$ to 4) – added mass concentration of analyte to the diluted sample (for $i=0$, γ_{+0} , no standard addition is performed);

$\bar{\gamma}_{+}$ – mean of γ_{+} values;

$\gamma_{\sim 0}$ – approximate mass concentration of the ion in sample estimated by comparing signal of sample with the one of a standard solution prepared in ultrapure water;

1 γ_1 – mass concentration of analyte in the diluted sample;

2 γ_2 – mass concentration of analyte in the diluted sample with larger added analyte mass
3
4 concentration;

5
6 γ_E – mass concentration of the ion in the diluted seawater sample estimated by standard addition
7
8 calibration (extrapolated mass concentration);

9
10
11 γ_{E1} and $\gamma_{E2} - \gamma_E$ values of duplicate standard additions;

12
13
14 γ_{Ref} – reference mass concentration of the artificial seawater;

15
16 ν_1 – degrees of freedom associated with s_1 ;

17
18 ν_2 – degrees of freedom associated with s_2 ;

19
20
21 ν_{eff} – number of effective degrees of freedom of γ ;

22
23 ρ_1 to ρ_5 – mass concentration of analyte in calibrators prepared for standard addition
24
25 calibrations. This concentration is the sum of analyte in the diluted sample and added for the
26
27 calibration ($\rho_1=\gamma_1$ and $\rho_5=\gamma_5$).

Fig. 1: Standard addition calibration (y vs. γ_+), dashed line (---), in a linear range where sensitivity is smaller than observed next to analytical zero. This curves overestimates sample concentration. The solid line (—) represents signal (y) in a range where sensitivity decreases as mass concentration of analyte (γ) increases.

Fig. 2: Assessment of the constancy of the sensitivity in the studied concentration range. Sensitivity is proved constant if (a) linear regressions in the lowest and highest portions of the studied range present statistically equivalent slopes (b_1 and b_2 respectively); or (b) the linear regression in the highest portion of the studied range presents and intercept (a_2) statistically equivalent to the mean signal of blank samples ($\bar{y}_{Bk}$). The solid line (—) represents the instrumental signal and the dashed lines (---) linear regressions in different homoscedastic ranges within the studied range.

Fig.3. Cause-effect diagram representing the sources of uncertainty associated with the measurement of the mass concentration of a specific ion in a seawater sample, by ion chromatography.

	γ_i	$\bar{\gamma}$	$(\gamma_i - \bar{\gamma})$
ρ_1	γ_1		$\left(\frac{\gamma_1 - \gamma_2}{2}\right)$
ρ_2	$\gamma_1 + \left(\frac{\gamma_2 - \gamma_1}{4}\right)$		$\left(\frac{\gamma_1 - \gamma_2}{4}\right)$
ρ_3	$\gamma_1 + \left(\frac{\gamma_2 - \gamma_1}{4}\right) \times 2$	$\frac{\gamma_2 + \gamma_1}{2}$	0
ρ_4	$\gamma_1 + \left(\frac{\gamma_2 - \gamma_1}{4}\right) \times 3$		$\left(\frac{\gamma_2 - \gamma_1}{4}\right)$
ρ_5	γ_2		$\left(\frac{\gamma_2 - \gamma_1}{2}\right)$

Fig.4. Standard addition curve for 5 equidistant points and equations for the difference between the added and the mean concentrations.

Fig. 5. Variation of the standard deviation (s_i) of instrumental signal replicates with the mass concentration of bromide ion (γ) in studied instrumental conditions.

Fig.6. Variation of U'_γ with γ_I value for the determination of nitrate in seawater by ion chromatography following a specific procedure for the preparation of calibrators. Five lines with different numbers, p , of replicate signals of calibrators are presented.

Table 1 Linear relation between mean signal ($\bar{y}$) or standard deviation of signals (s) and the mass concentration of the ion (γ).

Analyte	$\bar{y}$ vs. γ	s vs. γ
Cl ⁻	$\bar{y}_i = 106157\gamma_i + 3489$	$s_i = 3493.2\gamma_i + 548.6$
Br ⁻	$\bar{y}_i = 43836\gamma_i - 3724$	$s_i = 1175.1\gamma_i + 832.5$
NO ₃ ⁻	$\bar{y}_i = 57336\gamma_i - 6177$	$s_i = 1253.3\gamma_i + 2480.2$
SO ₄ ²⁻	$\bar{y}_i = 75765\gamma_i - 21380$	$s_i = 1687.9\gamma_i + 8238.6$

Table 2 Conditions for defining sample dilution to γ_1 and maximum added analyte ($\gamma_2 - \gamma_1$) in calibrators.

Conditions for homoscedasticity		
Analyte	of signals	Range for γ_1 and γ_2 values
Cl ⁻	$\gamma_2 = 0.449 + 3.86\gamma_1$	[0.04-4]
Br ⁻	$\gamma_2 = 2.03 + 3.86\gamma_1$	[0.1-10]
NO ₃ ⁻	$\gamma_2 = 5.66 + 3.86\gamma_1$	[0.1-10]
SO ₄ ²⁻	$\gamma_2 = 14.0 + 3.86\gamma_1$	[0.2-20]

Table 3 Estimated approximate mass concentration of analyte in seawater samples, $\gamma_{\sim o}$, (Sample A and B, and artificial seawater, Art.; samples A and B were quantified twice, A1 and A2, and B1 and B2 respectively), selected dilution factor (F_{Dil}) for samples, expected mass concentration of the diluted sample, γ_I , ($\gamma_{\sim o}/F_{\text{Dil}}$), maximum admissible (MAA) and performed (MPA) standard addition ($\gamma_2 - \gamma_I$) [MAA ► MPA]§, predicted residual standard deviation ($s_{y/\gamma}$) and relative $s_{y/\gamma}$ ($s_{y/\gamma}/\bar{y}$; where $\bar{y}$ is the expected mean signal of calibrators), and predicted relative expanded, U'_γ , and standard, u'_γ , uncertainties of measurements.

Sample	Parameter	Analyte			
		Cl ⁻	Br ⁻	NO ₃ ⁻	SO ₄ ⁻²
A1	$\gamma_{\sim o}$ (mg L ⁻¹) [F_{Dil}]	17×10 ³ [1×10 ⁴]	55 [50]	90.0 [50]	-
	γ_I [$\gamma_2 - \gamma_I$]§ (mg L ⁻¹)	1.7 [2.3 ► 1.6]	1.1 [5.2 ► 5.1]	1.8 [8.2 ► 8.1]	-
	$s_{y/\gamma}$ [$s_{y/\gamma}/\bar{y}$] (%)	9282 [3.4]	5122 [3.3]	9812 [3.0]	-
	U'_γ [u'_γ] (%)	18 [7.9]	18 [7.9]	16 [7.2]	-
A2	$\gamma_{\sim o}$ (mg L ⁻¹) [F_{Dil}]	18×10 ³ [1×10 ⁴]	58 [50]	92 [50]	-
	γ_I [$\gamma_2 - \gamma_I$]§ (mg L ⁻¹)	1.8 [2.2 ► 1.6]	1.2 [5.5 ► 5.1]	1.8 [8.2 ► 8.1]	-
	$s_{y/\gamma}$ [$s_{y/\gamma}/\bar{y}$] (%)	9631 [3.4]	5239 [3.3]	9875 [3.0]	-
	U'_γ [u'_γ] (%)	18 [8.0]	17 [7.6]	16 [7.1]	-
B1	$\gamma_{\sim o}$ (mg L ⁻¹) [F_{Dil}]	17×10 ³ [1×10 ⁴]	40 [50]	285 [50]	-
	γ_I [$\gamma_2 - \gamma_I$]§ (mg L ⁻¹)	1.7 [2.3 ► 1.6]	0.80 [4.3 ► 4.1]	5.7 [4.3 ► 4.1]	-
	$s_{y/\gamma}$ [$s_{y/\gamma}/\bar{y}$] (%)	9282 [3.4]	4182 [3.4]	12193 [2.8]	-
	U'_γ [u'_γ] (%)	18 [7.9]	19 [8.5]	15 [6.8]	-
B2	$\gamma_{\sim o}$ (mg L ⁻¹) [F_{Dil}]	17×10 ³ [1×10 ⁴]	38 [50]	195 [50]	-
	γ_I [$\gamma_2 - \gamma_I$]§ (mg L ⁻¹)	1.7 [2.3 ► 1.6]	0.75 [4.2 ► 4.1]	3.9 [6.1 ► 6.1]	-
	$s_{y/\gamma}$ [$s_{y/\gamma}/\bar{y}$] (%)	9282 [3.4]	4123 [3.5]	11191 [2.8]	-
	U'_γ [u'_γ] (%)	18 [7.9]	20 [8.8]	13 [5.9]	-
Art.	$\gamma_{\sim o}$ (mg L ⁻¹) [F_{Dil}]	20×10 ³ [1×10 ⁴]	40 [50]	190 [50]	450 [50]
	γ_I [$\gamma_2 - \gamma_I$]§ (mg L ⁻¹)	2.0 [2.0 ► 1.7]	0.80 [4.3 ► 4.1]	3.8 [6.2 ► 6.0]	9 [11 ► 9.0]
	$s_{y/\gamma}$ [$s_{y/\gamma}/\bar{y}$] (%)	10504 [3.4]	4182 [3.4]	11003 [2.9]	31025 [3.1]
	U'_γ [u'_γ] (%)	18 [8.1]	19 [8.5]	13 [5.9]	14 [6.3]

Table 4 Performance parameters of standard addition calibration curves and measurement uncertainty of the characterisation of seawater samples (see glossary for symbols).

Sample	Parameter	Analyte			
		Cl ⁻	Br ⁻	NO ₃ ⁻	SO ₄ ⁻²
A1	r_P^a	0.995	0.999	0.993	-
	$s_{y/\gamma} [s_{y/\gamma}/\bar{y}_0 (\%)]$	7141 [2.8]	3437 [2.3]	25482 [6.0]	-
	$u'_\gamma [u'_E (\%)]$	6.2 [5.7]	5.7 [5.1]	13 [13]	-
	$k [v_{\text{eff}}]$	2.201 [11]	2.179 [12]	2.306 [8]	-
	$U'_\gamma (\%)$	14	12	30	-
A2	r_P^a	0.996	0.998	1.000	-
	$s_{y/\gamma} [s_{y/\gamma}/\bar{y}_0 (\%)]$	5925 [2.2]	4674 [3.2]	3440 [1.1]	-
	$u'_\gamma [u'_E (\%)]$	5.3 [4.7]	7.7 [7.2]	3.5 [2.4]	-
	$k [v_{\text{eff}}]$	2.160 [13]	2.228 [10]	2.024 [38]	-
	$U'_\gamma (\%)$	12	17	7.1	-
B1	r_P^a	0.993	0.992	0.992	-
	$s_{y/\gamma} [s_{y/\gamma}/\bar{y}_0 (\%)]$	7111 [2.7]	8252 [6.6]	11655 [3.2]	-
	$u'_\gamma [u'_E (\%)]$	6.4 [5.9]	14 [14]	7.1 [6.7]	-
	$k [v_{\text{eff}}]$	2.201 [11]	2.306 [8]	2.228 [10]	-
	$U'_\gamma (\%)$	14	33	16	-
B2	r_P^a	0.989	0.997	0.998	-
	$s_{y/\gamma} [s_{y/\gamma}/\bar{y}_0 (\%)]$	9799 [3.7]	4536 [3.7]	7669 [1.8]	-
	$u'_\gamma [u'_E (\%)]$	8.1 [7.8]	8.6 [8.2]	4.3 [3.5]	-
	$k [v_{\text{eff}}]$	2.262 [9]	2.262 [9]	2.093 [19]	-
	$U'_\gamma (\%)$	19	20	9.1	-
Art.	r_P^a	0.988	0.997	0.994	0.992
	$s_{y/\gamma} [s_{y/\gamma}/\bar{y}_0 (\%)]$	10045 [3.3]	11818 [5.5]	15319 [4.0]	36298 [3.4]
	$u'_\gamma [u'_E (\%)]$	8.1 [7.7]	21 [21]	8.0 [7.6]	7.3 [7.2]
	$k [v_{\text{eff}}]$	2.262 [9]	2.306 [8]	2.228 [10]	2.306 [8]
	$U'_\gamma (\%)$	18	49	18	17

^a – The minimum admissible r_P for 10 calibration points is 0.765 [1]

Table 5 Estimated mass concentration of studied ions in seawater samples A and B (duplicate results #1 and #2), and artificial seawater (Art.).

Sample	Mass concentration of analyte/ ($\gamma \pm U_\gamma$) ^a			
	Cl ⁻ (g L ⁻¹)	Br ⁻ (mg L ⁻¹)	NO ₃ ⁻ (mg L ⁻¹)	SO ₄ ⁻² (mg L ⁻¹)
A1	15.3 ± 2.1	64.7 ± 8.0	119 ± 35	-
A2	18.4 ± 2.1	60 ± 10	100.7 ± 7.2	-
B1	18.9 ± 2.7	56 ± 18	215 ± 34	-
B2	17.2 ± 3.2	50.4 ± 9.8	219 ± 20	-
Art.	22.9±4.2	40±19	183 ± 33	445±75
^a – confidence level: 95 %				

Table 6 Parameters of the assessment of the agreement between replicate measurements of samples A and B obtained under repeatability conditions.

Analyte	Sample A		Sample B	
	$ d_E $ (mg L ⁻¹)	r_E (mg L ⁻¹)	$ d_E $ (mg L ⁻¹)	r_E (mg L ⁻¹)
Cl ⁻	3.1*	4.1*	1.7*	5.7*
Br ⁻	5	18	6	29
NO ₃ ⁻	18	44	4	51
*g L ⁻¹				

Table 7 Parameters involved in the assessment of the compatibility between the reference and the estimated mass concentration of studied ions in the artificial seawater.

Ion	γ_{Ref} (mg L ⁻¹)	γ (mg L ⁻¹)	$ \gamma_{\text{Ref}} - \gamma $ (mg L ⁻¹)	U_d
Cl ⁻	19.75*	22.9*	3.1*	6.0*
Br ⁻	67.5	40	28	28
NO ₃ ⁻	207	183	24	46
SO ₄ ²⁻	501	445	56	109
*g L ⁻¹				

Table 1 Linear relation between mean signal ($\bar{y}$) or standard deviation of signals (s) and the mass concentration of the ion (γ).

Analyte	$\bar{y}$ vs. γ	s vs. γ
Cl ⁻	$\bar{y}_i = 106157\gamma_i + 3489$	$s_i = 3493.2\gamma_i + 548.6$
Br ⁻	$\bar{y}_i = 43836\gamma_i - 3724$	$s_i = 1175.1\gamma_i + 832.5$
NO ₃ ⁻	$\bar{y}_i = 57336\gamma_i - 6177$	$s_i = 1253.3\gamma_i + 2480.2$
SO ₄ ²⁻	$\bar{y}_i = 75765\gamma_i - 21380$	$s_i = 1687.9\gamma_i + 8238.6$

Table 2 Conditions for defining sample dilution to γ_1 and maximum added analyte (γ_2 - γ_1) in calibrators.

Conditions for homoscedasticity		
Analyte	of signals	Range for γ_1 and γ_2 values
Cl ⁻	$\gamma_2=0.449+3.86\gamma_1$	[0.04-4]
Br ⁻	$\gamma_2=2.03+3.86\gamma_1$	[0.1-10]
NO ₃ ⁻	$\gamma_2=5.66+3.86\gamma_1$	[0.1-10]
SO ₄ ²⁻	$\gamma_2=14.0+3.86\gamma_1$	[0.2-20]

Table 3 Estimated approximate mass concentration of analyte in seawater samples, $\gamma_{\sim o}$, (Sample A and B, and artificial seawater, Art.; samples A and B were quantified twice, A1 and A2, and B1 and B2 respectively), selected dilution factor (F_{Dil}) for samples, expected mass concentration of the diluted sample, γ_I , ($\gamma_{\sim o}/F_{\text{Dil}}$), maximum admissible (MAA) and performed (MPA) standard addition ($\gamma_2 - \gamma_I$) [MAA ► MPA]§, predicted residual standard deviation ($s_{y/y}$) and relative $s_{y/y}$ ($s_{y/y}/\bar{y}$; where $\bar{y}$ is the expected mean signal of calibrators), and predicted relative expanded, U'_γ , and standard, u'_γ , uncertainties of measurements.

Sample	Parameter	Analyte			
		Cl ⁻	Br ⁻	NO ₃ ⁻	SO ₄ ⁻²
A1	$\gamma_{\sim o}$ (mg L ⁻¹) [F_{Dil}]	17×10 ³ [1×10 ⁴]	55 [50]	90.0 [50]	-
	γ_I [$\gamma_2 - \gamma_I$]§ (mg L ⁻¹)	1.7 [2.3 ► 1.6]	1.1 [5.2 ► 5.1]	1.8 [8.2 ► 8.1]	-
	$s_{y/y}$ [$s_{y/y}/\bar{y}$] (%)	9282 [3.4]	5122 [3.3]	9812 [3.0]	-
	U'_γ [u'_γ] (%)	18 [7.9]	18 [7.9]	16 [7.2]	-
A2	$\gamma_{\sim o}$ (mg L ⁻¹) [F_{Dil}]	18×10 ³ [1×10 ⁴]	58 [50]	92 [50]	-
	γ_I [$\gamma_2 - \gamma_I$]§ (mg L ⁻¹)	1.8 [2.2 ► 1.6]	1.2 [5.5 ► 5.1]	1.8 [8.2 ► 8.1]	-
	$s_{y/y}$ [$s_{y/y}/\bar{y}$] (%)	9631 [3.4]	5239 [3.3]	9875 [3.0]	-
	U'_γ [u'_γ] (%)	18 [8.0]	17 [7.6]	16 [7.1]	-
B1	$\gamma_{\sim o}$ (mg L ⁻¹) [F_{Dil}]	17×10 ³ [1×10 ⁴]	40 [50]	285 [50]	-
	γ_I [$\gamma_2 - \gamma_I$]§ (mg L ⁻¹)	1.7 [2.3 ► 1.6]	0.80 [4.3 ► 4.1]	5.7 [4.3 ► 4.1]	-
	$s_{y/y}$ [$s_{y/y}/\bar{y}$] (%)	9282 [3.4]	4182 [3.4]	12193 [2.8]	-
	U'_γ [u'_γ] (%)	18 [7.9]	19 [8.5]	15 [6.8]	-
B2	$\gamma_{\sim o}$ (mg L ⁻¹) [F_{Dil}]	17×10 ³ [1×10 ⁴]	38 [50]	195 [50]	-
	γ_I [$\gamma_2 - \gamma_I$]§ (mg L ⁻¹)	1.7 [2.3 ► 1.6]	0.75 [4.2 ► 4.1]	3.9 [6.1 ► 6.1]	-
	$s_{y/y}$ [$s_{y/y}/\bar{y}$] (%)	9282 [3.4]	4123 [3.5]	11191 [2.8]	-
	U'_γ [u'_γ] (%)	18 [7.9]	20 [8.8]	13 [5.9]	-
Art.	$\gamma_{\sim o}$ (mg L ⁻¹) [F_{Dil}]	20×10 ³ [1×10 ⁴]	40 [50]	190 [50]	450 [50]
	γ_I [$\gamma_2 - \gamma_I$]§ (mg L ⁻¹)	2.0 [2.0 ► 1.7]	0.80 [4.3 ► 4.1]	3.8 [6.2 ► 6.0]	9 [11 ► 9.0]
	$s_{y/y}$ [$s_{y/y}/\bar{y}$] (%)	10504 [3.4]	4182 [3.4]	11003 [2.9]	31025 [3.1]
	U'_γ [u'_γ] (%)	18 [8.1]	19 [8.5]	13 [5.9]	14 [6.3]

Table 4 Performance parameters of standard addition calibration curves and measurement uncertainty of the characterisation of seawater samples (see glossary for symbols).

Sample	Parameter	Analyte			
		Cl ⁻	Br ⁻	NO ₃ ⁻	SO ₄ ⁻²
A1	r_P^a	0.995	0.999	0.993	-
	$s_{y/\gamma} [s_{y/\gamma}/\bar{y}_0 (\%)]$	7141 [2.8]	3437 [2.3]	25482 [6.0]	-
	$u'_\gamma [u'_E (\%)]$	6.2 [5.7]	5.7 [5.1]	13 [13]	-
	$k [v_{\text{eff}}]$	2.201 [11]	2.179 [12]	2.306 [8]	-
	$U'_\gamma (\%)$	14	12	30	-
A2	r_P^a	0.996	0.998	1.000	-
	$s_{y/\gamma} [s_{y/\gamma}/\bar{y}_0 (\%)]$	5925 [2.2]	4674 [3.2]	3440 [1.1]	-
	$u'_\gamma [u'_E (\%)]$	5.3 [4.7]	7.7 [7.2]	3.5 [2.4]	-
	$k [v_{\text{eff}}]$	2.160 [13]	2.228 [10]	2.024 [38]	-
	$U'_\gamma (\%)$	12	17	7.1	-
B1	r_P^a	0.993	0.992	0.992	-
	$s_{y/\gamma} [s_{y/\gamma}/\bar{y}_0 (\%)]$	7111 [2.7]	8252 [6.6]	11655 [3.2]	-
	$u'_\gamma [u'_E (\%)]$	6.4 [5.9]	14 [14]	7.1 [6.7]	-
	$k [v_{\text{eff}}]$	2.201 [11]	2.306 [8]	2.228 [10]	-
	$U'_\gamma (\%)$	14	33	16	-
B2	r_P^a	0.989	0.997	0.998	-
	$s_{y/\gamma} [s_{y/\gamma}/\bar{y}_0 (\%)]$	9799 [3.7]	4536 [3.7]	7669 [1.8]	-
	$u'_\gamma [u'_E (\%)]$	8.1 [7.8]	8.6 [8.2]	4.3 [3.5]	-
	$k [v_{\text{eff}}]$	2.262 [9]	2.262 [9]	2.093 [19]	-
	$U'_\gamma (\%)$	19	20	9.1	-
Art.	r_P^a	0.988	0.997	0.994	0.992
	$s_{y/\gamma} [s_{y/\gamma}/\bar{y}_0 (\%)]$	10045 [3.3]	11818 [5.5]	15319 [4.0]	36298 [3.4]
	$u'_\gamma [u'_E (\%)]$	8.1 [7.7]	21 [21]	8.0 [7.6]	7.3 [7.2]
	$k [v_{\text{eff}}]$	2.262 [9]	2.306 [8]	2.228 [10]	2.306 [8]
	$U'_\gamma (\%)$	18	49	18	17

^a – The minimum admissible r_P for 10 calibration points is 0.765 [1]

Table 5 Estimated mass concentration of studied ions in seawater samples A and B (duplicate results #1 and #2), and artificial seawater (Art.).

Sample	Mass concentration of analyte/ ($\gamma \pm U_\gamma$) ^a			
	Cl ⁻ (g L ⁻¹)	Br ⁻ (mg L ⁻¹)	NO ₃ ⁻ (mg L ⁻¹)	SO ₄ ⁻² (mg L ⁻¹)
A1	15.3 ± 2.1	64.7 ± 8.0	119 ± 35	-
A2	18.4 ± 2.1	60 ± 10	100.7 ± 7.2	-
B1	18.9 ± 2.7	56 ± 18	215 ± 34	-
B2	17.2 ± 3.2	50.4 ± 9.8	219 ± 20	-
Art.	22.9±4.2	40±19	183 ± 33	445±75
^a – confidence level: 95%				

Table 6 Parameters of the assessment of the agreement between replicate measurements of samples A and B obtained under repeatability conditions.

Analyte	Sample A		Sample B	
	$ d_E $ (mg L ⁻¹)	r_E (mg L ⁻¹)	$ d_E $ (mg L ⁻¹)	r_E (mg L ⁻¹)
Cl ⁻	3.1*	4.1*	1.7*	5.7*
Br ⁻	5	18	6	29
NO ₃ ⁻	18	44	4	51
*g L ⁻¹				

Table 7 Parameters involved in the assessment of the compatibility between the reference and the estimated mass concentration of studied ions in the artificial seawater.

Ion	γ_{Ref} (mg L ⁻¹)	γ (mg L ⁻¹)	$ \gamma_{\text{Ref}} - \gamma $ (mg L ⁻¹)	U_d
Cl ⁻	19.75*	22.9*	3.1*	6.0*
Br ⁻	67.5	40	28	28
NO ₃ ⁻	207	183	24	46
SO ₄ ²⁻	501	445	56	109
*g L ⁻¹				

Abbreviations

A1 and A2 – Duplicate samples of aquarium 35 of Vasco da Gama Aquarium;

Art. – Artificial seawater sample (see section 2.1 for composition);

B1 and B2 – Duplicate samples of aquarium 36 of Vasco da Gama Aquarium;

CE – Capillary electrophoresis;

IC – Ion chromatography;

MAA – Maximum admissible standard addition;

MPA – Maximum performed standard addition.

Symbols

a – intercept of the linear unweighted regression of standard addition calibrations (y vs. γ_+);

a_1 – intercept of the linear unweighted regression of the lowest homoscedastic range of the studied analytical range;

a_2 – intercept of the linear unweighted regression of the highest homoscedastic range of the studied analytical range;

b – slope of the linear unweighted regression of the standard addition calibration (y vs. γ_+);

b_1 – slope of the linear unweighted regression of the lowest homoscedastic range of the studied analytical range;

b_2 – slope of the linear unweighted regression of the highest homoscedastic range of the studied analytical range;

C – intercept of the linear regression of s vs. γ ;

d – difference between reference and estimated (γ) mass concentration of the artificial seawater;

D – slope of the linear regression of s vs. γ ;

d_E – difference between γ_{E1} and γ_{E2} ;

f_+ – unitary factor representing the impact of the quality of mass concentration additions in the quantification of γ ;

$F_{0.01}^{\nu_1; \nu_2}$ – critical F value for the ratio (s_2^2 / s_1^2) for 99% confidence level;

F_{Dil} – dilution factor;

k – coverage factor to expand the measurement uncertainty;

k_d – coverage factor used to expand u_d to U_d ;

k_{Ref} – coverage factor used to expand u_{Ref} to U_{Ref} ;

n – number of calibrators of the standard addition calibration ($n=5$);

p – number of replicate collection of calibrators signals;

$R_{+(k;l)}$ – ratio of a pair of added analyte mass concentrations γ_+ (γ_{+k}/γ_{+l}) to the diluted samples

(where $k \neq l$ and k and l varies between the 1st and 4th standard addition);

$R_{\text{Dil}(i;j)}$ – ratio of a pair of F_{Dil} ($F_{\text{Dil}_i}/F_{\text{Dil}_j}$) describing the dilution of sample analysed by

standard addition calibrations (where $i \neq j$ and i and j varies between the 1st and 5th sample dilution);

r_E – expanded repeatability limit for 99% confidence level;

r_P – Pearson's linear correlation coefficient;

s – standard deviation of areas of analyte peaks;

s' – relative standard deviation of areas of analyte peaks;

s_1 – standard deviation of areas of analyte peaks at γ_1 ;

s_2 – standard deviation of areas of analyte peaks at γ_2 ;

s_y – standard deviation of the instrumental signal;

$s'_{y(\text{Min})}$ – minimum relative standard deviation of replicate areas of analyte peaks in a specific mass concentration range;

$s_{y/\gamma}$ – residual standard deviation of the linear unweighted regression line;

t – Student's t ;

t_{99} – Student's t for 99% confidence level and corresponding degrees of freedom;

$t_{0.05}^{pn-2}$ – Student's t for $np-2$ degrees of freedom and 95% confidence level;

$t_{0.05}^{v_{\text{eff}}}$ – Student's t for v_{eff} degrees of freedom and 95% confidence level;

u_+ – standard uncertainty associated with f_+ ;

u'_+ – relative standard uncertainty associated with f_+ ;

u_d – standard uncertainty associated with d_E ;

U_d – expanded uncertainty associated with d_E ;

u_{Dil} – standard uncertainty associated with F_{Dil} ;

u'_{Dil} – relative standard uncertainty associated with F_{Dil} ;

u_E – standard uncertainty associated with γ_E due to the extrapolation of the regression line;

u'_E – relative standard uncertainty associated with γ_E ;

U'_E – relative expanded uncertainty associated with γ_E ;

$u'_{R_{\text{Dil}(i;j)}}$ – relative standard uncertainty associated with $R_{\text{Dil}(i;j)}$ values;

$u'_{R_{+(k;l)}}$ – relative standard uncertainty associated with $R_{+(k;l)}$ values;

u_{Ref} – standard uncertainty associated with γ_{Ref} ;

u_{γ}^{tg} – target standard uncertainty associated with γ ;

u_{γ} – standard uncertainty associated with γ ;

U_{γ} – expanded uncertainty associated with γ ;

U'_{γ} – relative expanded uncertainty associated with γ ;

y – instrumental signal (area of analyte peak);

$\bar{y}$ – mean instrumental signal;

$\bar{y}_{Bk}$ – mean signal of blank solutions;

γ – mass concentration of the ion in seawater sample estimated by standard addition calibration;

γ_{+i} ($i=0$ to 4) – added mass concentration of analyte to the diluted sample (for $i=0$, γ_{+0} , no standard addition is performed);

$\bar{\gamma}_+$ – mean of γ_+ values;

γ_{-0} – approximate mass concentration of the ion in sample estimated by comparing signal of sample with the one of a standard solution prepared in ultrapure water;

γ_1 – mass concentration of analyte in the diluted sample;

γ_2 – mass concentration of analyte in the diluted sample with larger added analyte mass concentration;

γ_E – mass concentration of the ion in the diluted seawater sample estimated by standard addition calibration (extrapolated mass concentration);

γ_{E1} and $\gamma_{E2} - \gamma_E$ values of duplicate standard additions;

γ_{Ref} – reference mass concentration of the artificial seawater;

ν_1 – degrees of freedom associated with s_1 ;

ν_2 – degrees of freedom associated with s_2 ;

ν_{eff} – number of effective degrees of freedom of γ ;

ρ_1 to ρ_5 – mass concentration of analyte in calibrators prepared for standard addition calibrations. This concentration is the sum of analyte in the diluted sample and added for the calibration ($\rho_1=\gamma_1$ and $\rho_5=\gamma_5$).