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VALIDATION PROCEDURES FOR DETERMINATION OF TOTAL ORGANIC CARBON IN WATER

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Abstract – The determination of total organic carbon (TOC) content in water is useful as a measure of pollution. It is an analytical process which is validated and it demonstrates that it is suitable for its intended purpose. Several validation parameters are done to conduct validation procedures.

Keywords: validation, total organic carbon.

1. INTRODUCTION

1.1. Environmental impact of total organic carbon

Total organic carbon (TOC) is the sum of organically bound carbon present in water, bonded to dissolved or suspended matter, including cyanate, elemental carbon and thiocyanate [1].

By using TOC measurements, a number of carbon--containing compounds in a source can be determined. This is important because knowing the amount of carbon in a freshwater stream is an indicator of the organic character of the stream. The larger the carbon or organic content, the more oxygen is consumed. A high organic content means an increase in the growth of microorganisms which contributes to the depletion of oxygen supplies. It affects biogeochemical processes, nutrient cycling, biological availability, chemical transport and interactions. It also has direct implications in the planning of wastewater treatment and drinking water treatment. Organic matter content is typically measured as TOC and it is essential component of the carbon cycle. Organic matter in water consists of thousands of components, including macroscopic particles, colloids, dissolved macromolecules and specific compounds.

1.2. Principles of TOC Analysis

Determination of TOC is done by "Total Organic Carbon Analyser TOC-V_{CPN}" (made by Shimadzu Corporation) [2].

Two types of carbon are present in water: total organic carbon (TOC) and inorganic carbon (IC). Organic carbon bonds with hydrogen or oxygen to form organic compounds. Collectively, the two forms of carbon are referred to as total carbon (TC) and the relationship between them is expressed as:

$$TOC = TC - IC \quad (1)$$

After acidifying the sample to pH 2 to 3, sparge gas is bubbled through the sample to eliminate the IC component. The remaining TC is measured to determine total organic carbon, and the result is generally referred to as TOC. TOC stands for non-purgeable organic carbon and refers to organic carbon that is present in a sample in a non-volatile form.

Sample is introduced into the TC combustion tube, which is filled with an oxidation catalyst and heated to 953 K. The sample is burned in the combustion tube and, as a result, the TC components in the sample are converted to carbon dioxide. Carrier gas flows to the combustion tube and carries the sample combustion products from the combustion tube to an electronic dehumidifier, where the gas is cooled and dehydrated. The gas then carries the sample combustion products through a halogen scrubber to remove chlorine and other halogens. Finally, the carrier gas delivers the sample combustion products to the cell of a non-dispersive infrared (NDIR) gas analyser, where the carbon dioxide is detected. The NDIR outputs an analogue detection signal that forms a peak; the peak area is measured by the "TOC-Control V" software.

The peak area is proportional to the TC concetration of the sample. A calibration curve equation that mathematically expresses the relationship between the peak area and the TC concentration can be generated by analysing various concentrations of a TC standard solution. The TC concentration in a sample can be determined by analysing the sample to obtain the peak area and then using the peak area in the calibration curve equation.

2. VALIDATION

2.1. Validation procedures in general

Validation is the process of proving that an analytical method is acceptable for its intended purpose [3].

Validation needs to be conducted:

- as a part of the development of the new method process
- after the changes made in any part of the analytical method which has been validated before
- after the major repairs or services of instruments used for analytical process
- after the periods of long duration (usually two years); the time period can be determined on the basis of analytical experience or by using statistical methods (trend analysis).

2.2. Validation parameters

Validation procedures are done as a part of the development of the new method process. According to the application of this analytical process the next validation parameters are conducted:

- accuracy
- precision: repeatability, intermediate precision, reproducibility
- linearity
- selectivity
- robustness.

3. EXPERIMENT

3.1. Determination of accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found (analysed value) [4].

Five experimental samples with different concentrations covering the whole determined experiment range were prepared. For every experimental point five replicate samples were prepared. Every prepared sample was analysed under the strictly defined conditions and here are the obtained data (Table I):

TABLE I. Data for determination of method accuracy

i	*x* (*mg/l*)	*c* (*mg/l*)	SD	RSD %	R_f %
1	1	1,05	0,03	2,86	95,2
2	10	9,88	0,06	0,61	101,2
3	20	19,11	0,15	0,78	104,7
4	50	48,05	0,40	0,83	104,1
5	100	99,12	0,55	0,55	100,9

i serial number of concentration step (experimental point)
x expected concentration of standard sample (standard addition values)
c average value for concentration (analysed values) of five replicate samples
SD standard deviation
RSD relative standard deviation
R_f recovery factor calculated as a percentage which includes ratio between standard addition values and analysed values

The average value of relative standard deviation for all measurements is 1,13 %. The average value of recovery factor is 101,2 % and it is satisfactory value for this method. This method is accurate.

3.2. Determination of precision

The precision expresses the closeness of agreement between series of measurements obtained from multiple sampling of the same homogeneous sample under the same conditions. Precision is considered at three levels: repeatability, intermediate precision and reproducibility [4].

Repeatability expresses the precision under the same operating conditions over a short interval of time. Five experimental points covering whole linear range were chosen. For each experimental point one sample was prepared following the exact sample preparation procedure. Each sample was analysed 10 times under the same conditions. Here are the obtained data (Table II):

TABLE II. Data for determination of method repeatability

i	*x* (*mg/l*)	*c* (*mg/l*)	SD	RSD %
1	10	9,89	0,12	1,21
2	20	19,32	0,12	0,62
3	50	48,75	0,58	1,19
4	70	68,07	0,70	1,03
5	90	91,23	0,91	1,00

i serial number of concentration step (experimental point)
x expected concentration of prepared standard sample
c average value for concentration (analysed values) of ten analyses of the same sample
SD standard deviation
RSD relative standard deviation

The average value for standard deviation of all measurements is 0,49 and the average value for relative standard deviation of all measurements is 1,01 %. That means that this method is repeatable.

Intermediate precision expresses the precision within laboratories variations. Samples were analysed at different days and by different analysts. Five experimental points covering whole linear range were selected. For each experimental point one sample was prepared following the exact sample preparation procedure. Each sample was analysed 10 times by different analysts over a longer period of time under the same conditions. The obtained data are (Table III):

TABLE III. Data for determination of method intermediate precision

i	*x* (*mg/l*)	*c* (*mg/l*)	SD	RSD %
1	10	9,62	0,15	1,56
2	20	19,68	0,16	0,81
3	50	49,11	0,85	1,73
4	70	68,79	1,01	1,47
5	90	92,15	0,94	1,02

i serial number of concentration step (experimental point)
x expected concentration of prepared standard sample
c average value for concentration (analysed values) of ten analyses of the same sample
SD standard deviation
RSD relative standard deviation

The average value for standard deviation of all measurements is 0,62. The average value for relative standard deviation of all measurements is 1,32 %. The obtained data are satisfactory and therefore this method fulfils intermediate precision.

Reproducibility expresses the precision between laboratories. Five experimental points were chosen covering the entire linear range. For each experimental point one sample was prepared following exact sample preparation procedure. Each sample was analysed 10 times in different laboratories (different analysts, different solutions, long period of time) under the same conditions. The obtained data are in Table IV:

TABLE IV. Data for determination of method reproducibility

i	*x* (*mg/l*)	*c* (*mg/l*)	SD	RSD %
1	10	9,84	0,15	1,52
2	20	19,88	0,44	2,21
3	50	50,19	1,58	3,15
4	70	70,07	1,98	2,83
5	90	92,93	2,58	2,78

i serial number of concentration step (experimental point)
x expected concentration of prepared standard sample
c average value for concentration (analysed values) of ten analyses of the same sample
SD standard deviation
RSD relative standard deviation

The average value for standard deviation of all measurements is 1,35 and the average value for relative standard deviation of all measurements is 2,50 %. The obtained data are satisfied and show that this method is reproducible.

3.3. Determination of linearity

Five equidistant experimental points were chosen in a determined experimental range. For every experimental point 3 standard solutions were prepared. Graphical presentation of calibration curve was made and a mathematical equation which defines the calibration curve, and a correlation coefficient and residual standard deviations were calculated [5]. Table V contains the obtained data which are used for calibration curve (Fig. 1) and calculations.

TABLE V. Data for determination of method linearity

i	*x* (*mg/l*)	*y*
1	10	23,90
2	30	73,18
3	50	120,20
4	70	171,90
5	90	221,26

i serial number of concentration step (experimental point)
x expected concentration of prepared standard sample
y average value for instrument response (Area) of 3 standard solutions with the same concentration

Mathematical equation which defines the calibration curve (calibration function) is:

$$y = a + bx = -1{,}260 + 2{,}467x \quad (2)$$

a line segment cut off from the *y*-axis (-1,260)
b slope of the calibration curve (2,467)

The correlation coefficient is 0,9998.

The residual standard deviation is 1,219.

All this data are satisfied and the calibration curve is linear.

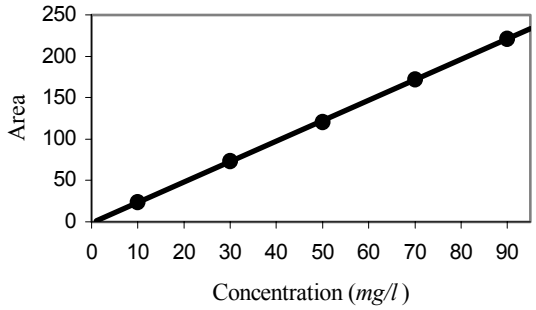

Fig. 1. Calibration curve

3.4. Determination of selectivity

Selectivity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present such as impurities, degradants, matrix, etc.

One standard sample was chosen and analysed first. Then, three different samples which contain matrix component were chosen and were analysed, too. After that the same amount of all of these three samples were added in the standard and were analysed again. All samples, their concentration and instrument response are shown in Table VI:

TABLE VI. Data for determination of method selectivity

i	*Sample*	*c* (*mg/l*)	*y*
1	Standard	29,89	73,29
2	Sample 1	18,00	42,11
3	Sample 1 + Standard	47,98	118,30
4	Sample 2	35,67	87,72
5	Sample 2 + Standard	75,02	185,00
6	Sample 3	42,30	101,50
7	Sample 3 + Standard	72,22	177,10

i serial number of experimental point
c concentration of prepared sample
y instrument response (Area) for prepared samples

All measured values from Table VI are inserted on the same calibration curve (Fig. 1) and are shown on Fig. 2:

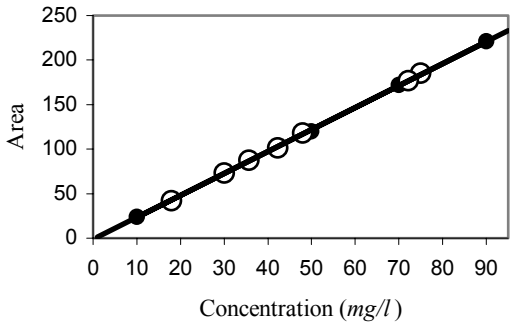

Fig. 2. Calibration curve and obtained points

Obtained points from Table VI are marked on Fig. 2 as○ and show that they all lay on the calibration curve. That proves that matrix components have not influence on the determination and that this method is selective.

3.5. Determination of robustness

Robustness shows the reability of an analysis with respect to deliberate variations in method parameters such as stability of analytical solutions [4]. Each sample was analysed 5 number of times under the same conditions over the defined time periods: immediately after the preparation, after the 24 hrs and after the 48 hrs. The obtained data are shown in Table VII:

TABLE VII. Data for determination of robustness method

i	*x* (*mg/l*)	*c* (*mg/l*)	SD	RSD %
1	1	1,20	0,10	8,33
2	10	9,93	0,05	0,50
3	20	20,20	0,05	0,25
4	50	51,20	0,07	0,16

i serial number of concentration step (experimental point)
x expected concentration of prepared standard sample
c average value of measured concentrations of standard samples, measured 5 number of times under the same conditions and immediately after the preparation, after the 24 hrs and after the 48 hrs
SD standard deviation
RSD relative standard deviation

The obtained data show that standard solution with lower concentration (such as 1 mg/l standard) has RSD 8,33 % what proves that it is less stable than standard solutions with higher concentrations. Therefore, the average value for standard deviation of all measurements is 0,07 and the average value for relative standard deviation of all measurements is 2,31 %. Robustness of the method is satisfactory.

4. CONCLUSIONS

According to the application of this analytical method the next validation parameters were conducted and obtained data were acceptable (Table VIII):

TABLE VIII. Validation parameters and acceptance of obtained data

VALIDATION PARAMETER	*OBTAINED DATA*		*Acceptable*
ACCURACY	Average value: RSD Recovery factor	 1,13 % 101,2 %	Yes
PRECISION: REPEATABILITY INTERM. PRECIS. REPRODUCIBILITY	Average value: RSD RSD RSD	 1,01 % 1,32 % 2,50 %	Yes
LINEARITY	Corelation coef. Residual stand. deviation	0,9998 1,219	Yes
SELECTIVITY	Shown at Fig. 2		Yes
ROBUSTNESS	Average value: RSD	 2,31 %	Yes

These validation procedures have proved that this analytical process is applicable to determination of TOC content in water by "Total Organic Carbon Analyser TOC--V_{CPN}" and that results of TOC content are valuable.

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