

VALIDATION PROCEDURE FOR ASSESSING THE TOTAL ORGANIC CARBON IN WATER SAMPLES*

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The *Total Organic Carbon* (TOC) is an index of the total amount of organic substances in water, playing a key role in assessing the efficiency of a wastewater purification process. It is used in a wide array of applications from management of wastewater treatment plant influent and effluent, to drinking water supply management, and monitoring of impurities in process and surface waters.

In this respect, a key issue is to find the most efficient analytical method to determine the amount of TOC in the wastewater, both in terms of time, cost and accuracy of results.

Validation of the analytical method is relevant in choosing those parameters and performance requirements that demonstrate the suitability for the intended purpose. In our case, different validation parameters were measured using samples with known amounts of TOC. The method has provided good validation parameters for linearity, the correlation coefficient being 0.996 while precision and accuracy showed coefficients of variation lower than 1.6% respectively lower than 1.2%. LOD and LOQ were also good.

Key words: total organic carbon, validation, wastewater.

1. INTRODUCTION

The occurrence of organic contaminants in water usually occurs during heavy rains. Domestic and industrial activity also contributes to the organic contaminants amounts in various wastewaters. As a result of accidental spills or leaks, industrial organic wastes may not be completely removed by treatments processes and enter in waters sources. Therefore, it is important to know the organic content in a water sample.

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The determination of *Total Carbon Organic* (TOC) content in water is useful as a measure of pollution. Also, it is becoming increasingly important to have a reliable and rapid technique for determination of total carbon organic in wastewater used for better purification procedures by knowing the amount of TOC in the wastewater [1].

1.1. ENVIRONMENTAL IMPACT OF TOTAL ORGANIC CARBON

Capable of providing information regarding the organic character of water, the knowledge of carbon content in wastewater samples becomes an important element in water monitoring programs. Using TOC measurements, a number of compounds with carbon content could be determined. This is the sum of organically bound carbon present in water connected to dissolved or suspended matter. If the oxygen consumption is high, then the organic carbon content increases. An increased organic content is marked by a growth in microorganisms and thus more oxygen is consumed. That is why, to have efficiency in purification process of wastewater, information related to the total carbon organic content are determinant [2].

1.2. PRINCIPLE OF TOTAL ORGANIC CARBON ANALYSIS

The measurements were performed using a Total Organic Carbon Analyzer TOC – Apollo 9000 made by Teledyne Tekmar Instruments. Two types of carbon are present in water: *total organic carbon* (TOC) and *inorganic carbon* (IC). Organic carbon binds 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:

$$\text{TOC} = \text{TC} - \text{IC}$$

After acidifying the sample to pH 2 to 3, pure gas is bubbled through the sample to eliminate the IC component. The remaining carbon is measured to determine total organic carbon, and the result is generally referred to as TOC. TOC represent non-purgeable organic carbon and refers to organic carbon that is present in a sample in a non-volatile form [1].

Sample is introduced in the combustion tube, which is filled with an oxidation catalyst and heated to 680⁰C. In the samples, carbon is first converted to CO₂ by the combustion furnace for TOC and TC analysis or by the IC sparger for IC analysis. 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.

A carrier gas then sweeps the derived CO₂ through a non-dispersive infrared (NDIR) detector. Sensitive to the absorption frequency of CO₂, the NDIR generates a non-linear signal that is proportional to the instantaneous concentration of CO₂ in carrier gas. That signal is then plotted *versus* the samples analysis time. The peak area is proportional to the TC concentration of the sample. Calibration curve equation that mathematically expresses the relationship between the peak area and the TC concentration can be generated by analyzing various concentrations of a TC standard solution. The TC concentration in a sample can be determined by analyzing the sample to obtain the peak area and then using the peak area in the calibration curve equation.

The resulting area is then compared to the stored calibration data of a sample with concentration in parts per million.

2. MATERIALS AND METHODS

The standard measurement procedure described in Water quality – guidelines for the determination of *total organic carbon* (TOC) and *dissolved organic carbon* (DOC) (ISO 8245:2000) was followed [6], which provides guidelines for the determination of TOC in wastewater.

The procedure described in this international standard applies to wastewater samples containing concentrations of organic carbon of up to 1000 ppm, while higher concentrations can be determined after the appropriate dilution.

We used levels of TOC range between 5 ÷ 200 ppm for the present investigation.

2.1. EXPERIMENTAL

The principle is to oxidize the organic carbon (C) in wastewater to CO₂ by combustion at 720⁰C. There are two methods for TOC determination: a differential and a direct method. In addition to organic carbon, the wastewater sample may contain CO₂ and ions of carbonic acid. Prior to the TOC determination, it is essential to remove *total inorganic carbon* (TIC) by purging the acidified sample with a gas that is free of CO₂ and organic compounds. In the presence of these substances, the TOC concentration is determined separately or the differential method may be applied.

$$\text{TOC} = \text{TC} - \text{TIC} \quad (1)$$

The remaining TC is then measured to determine TOC, and the result is generally referred to as TOC. This value is referred to as a non-purgeable organic carbon that is present in a sample in a non-volatile form (NPOC) to distinguish it

from the TOC value obtained by calculating the difference between TC and TIC. We can assume that purgeable organic compounds are not present in our samples, thus,

$$\text{TOC} = \text{NPOC} = \text{TOC} \quad (2)$$

and the direct method can be applied.

The determination of CO₂ can be carried out by a number of different methods. In our case, a non-dispersive infrared detection (NDIR) was applied [2], where the carrier gas delivers the sample combustion products to the cell of a non-dispersive infrared gas analyzer, where the CO₂ is detected. The detector is filled with sample gas component (CO₂) to a specified concentration (8% CO₂ in N₂) and divided into two connected chambers. A microflow sensor is fitted in the connection tube of the chambers. The incident radiation is absorbed selectively only in the specific absorption bands of the CO₂ gas in the detector. The absorbed energy is instantaneously transformed to thermal energy through molecular collision. Due to the selectivity of the detector, variations in the absorbed energy directly associated with the CO₂ concentration in the sample cell alone causes temperature and pressure differences between the two detector chambers. This results in a gas flow between the two chambers that is detected by the microflow sensor situated between the chambers. Since the beam is interrupted at a specified frequency by a rotating sector measurement signal, the variation is also periodic and generates an AC voltage in the millivoltage range, which is then processed on the NDIR board (peak area). The amount of rays absorbed is proportional to the density of the gas (Lambert–Beer's Law).

The concentration of the TOC in the wastewater samples was calculated according to relation (2).

Potassium hydrogen phthalate (KHP) was manufactured by Teledyne Tekmar, USA and it was used as Certified Reference Material (CRM). This stock reference solution (C_{TOC}) has a concentration of 0.9994 ppm.

From the stock reference solution, the working reference solutions were prepared by further dilutions using volumetric flask and deionized water.

Most important step consisted of preparing six different working reference solutions C_{TOC}, calculated using equation (3).

$$C_{\text{TOC}} = \frac{c_{\text{TOC}}}{f_d} = \frac{c_{\text{TOC}} V_p}{V_{100}} \quad (3)$$

By subsequent dilutions working solutions were prepared in order to develop a best possible C_{TOC} curve.

Where:

- f_d is the dilution factor of working reference solutions and working solutions;
- V_{100} is the volume of one mark volumetric flask (L);
- V_p is the volume of the pipettes (L).

The same preparation procedure was followed for all the working solutions. Depending on the concentration range of TOC in the wastewater samples, the calibration curves were established as described in the manufacture using procedure.

A new calibration curve was established whenever new range was used. Prior to the measurement of the TOC in the sample and also in the reference solution, they are poured into a 40-mL vial. The concentrations of each working reference solution as well as the sample concentrations were measured in five replicates [2].

2.2. RESULTS AND DISCUSSIONS

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

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 being determined on the basis of analytical experience or by using statistical methods (trend analysis).

Validation procedures are part of the new method development process. According to the implementation of this analytical process, a series of validation parameters are determined, such as accuracy, precision, repeatability, intermediate precision, reproducibility, linearity, selectivity and robustness.

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 in analyzed mode.

To determine **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.

To determine the **linearity**, six equidistant experimental points were chosen in a determined experimental range. For this purpose, six standard solutions were prepared, in the same day.

The **limit of detection, LOD**, is the lowest quantity of a substance that can be distinguished from the absence of that substance (a blank value) within a stated confidence limit.

The **limit of quantification, LOQ**, is the limit which can reasonably tell the difference between two different values.

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.

Robustness shows the reliability of an analysis with respect to deliberate variations in method parameters such as stability of analytical solutions.

In this paper we describe the linearity, limit of detection, limit of quantification, precision and accuracy.

1. Determination of linearity

Six equidistant experimental points were chosen in a determined experimental range. The calibration curve and the regression equation were determined, and the correlation coefficient and residual standard deviations were also calculated.

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

$$y = 1422x + 302985$$

The correlation coefficient of 0.996 proved a good linearity of the analytical method.

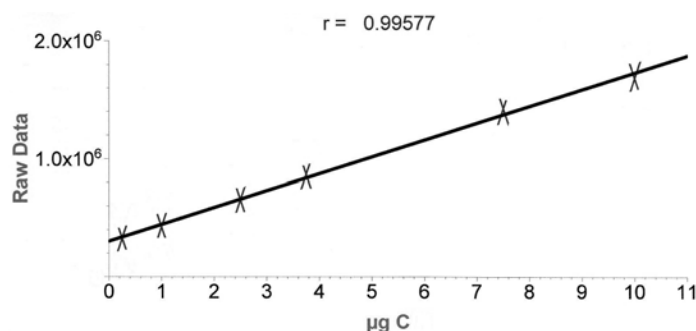


Fig. 1 – Calibration curve.

2. Determination of LOD (limit of detection) and (LOQ) limit of quantification

The method described in the above mentioned standard applies to those water samples containing carbon content ranging from 5 to 200 ppm.

The determination of LOD (limit of detection) for our experiments was done by mean of 10 samples with standard addition of 2 ppm, measured one time per sample, the resulted value being 1.2 ppm.

$$\text{LOD} = 0 + 3 s = 0 + 3 \times 0.405 \text{ ppm} = 1.215 \text{ ppm}$$

The obtained value for LOQ (limit of quantification) regarding carbonate was 4 ppm. LOQ is also calculated as mean of 10 fortified samples at lower acceptable concentration, measured one time per sample.

$$\text{LOQ} = 0 + 10 s = 0 + 10 \times 0.405 \text{ ppm} = 4.05 \text{ ppm}$$

$$\text{RSD} = (s \times 100) / x_p = 15.9 \%$$

where s – standard deviation; RSD – relative standard deviation; x_p – the mean value of concentration measured.

The obtained RSD value (15.9 %) was considered to be acceptable for our experiment.

A range between 5 and 200 ppm was applied for TOC determination. Higher concentration of TOC can be determined by appropriate dilution.

3. Determination of precision

Precision was calculated by multiple measurements of the same sample. Repeatability expresses the precision under the same operating conditions over a short interval of time.

Three 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 analyzed 10 times under the same conditions (Table 1):

Table 1

Data for determination of method repeatability

<i>i</i>	<i>x</i> (ppm)	<i>c</i> (ppm)	<i>s</i>	<i>RSD</i> (%)
1	25	25.73	0.40	1.55
2	50	48.89	0.78	1.60
3	75	76.22	0.45	0.59

i – serial number of concentration step (experimental point);

x – expected concentration of prepared standard sample;

c – average value for concentration (analyzed values) of ten analyses of the same sample;

s – standard deviation;

RSD – relative standard deviation.

The average value for standard deviation of all measurements was 0.54 and the average value for relative standard deviation of all measurements gave 1.25 %. That means that this method is repeatable.

4. Determination of accuracy

Two experimental samples with different concentrations covering the whole determined experiment range were prepared. For every experimental point ten replicate samples were prepared. Every prepared sample was analyzed under the strictly defined conditions and the obtained data are presented in Table 2:

Table 2

Data for determination of method accuracy

<i>i</i>	<i>x</i> (ppm)	<i>c</i> (ppm)	<i>s</i>	<i>RSD</i> (%)	<i>R_f</i> (%)
1	10	9.88	0.06	1.2	98.8
2	100	99.12	0.55	0.88	99.12

i – serial number of concentration step (experimental point);

x – expected concentration of standard sample;

c – average value for concentration (analyzed values) of ten replicate samples;

s – standard deviation;

RSD – relative standard deviation;

R_f – recovery factor calculated as a percentage which includes ratio between standard addition values and analyzed values.

The average value of relative standard deviation for measurements was 1.04%. The average value of recovery factor was 98,96 % and it is a satisfactory value for this method. The calculated parameters proved that the method is accurate.

3. CONCLUSIONS

Our analytical method showed very good validation parameters and proved to be acceptable for the intended purpose.

Table 3

Validation parameters and acceptance of obtained data

<i>Analytical principle</i>	<i>Validation parameters</i>	<i>Laboratory Requirements</i>	<i>Realized parameters</i>	<i>Decision</i>
Carbon from sample is converted to CO ₂ by combustion and determination of total organic carbon by non dispersiv infrared detector	Linearity	R > 0.995	R = 0.996	Accepted
	Limit of detection	2 ppm	1.215 ppm	Accepted
	Limit of quantification	4 ppm	4 ppm	Accepted
	Precision	1 ppm	1.25 ppm	Accepted
	Accurancy	1 ppm	1.04 ppm	Accepted

The purpose of this paper was to evaluate the validation parameters obtained by a combustion procedure and measured by an infrared detector for identifying the steps in the analytical procedure that contribute significantly to the overall budget.

The method gave good validation parameters for linearity, the correlation coefficient being 0.996 and for precision and accuracy showed coefficients of variation lower than 1.6 % respectively lower than 1.2 %. LOD and LOQ were also good.

The measurement of TOC was evaluated in a range between 5÷200 ppm. The input variables chosen were peak areas of the standards and the concentrations of TOC in the samples for the development of curves, dilution of sample, repeatability and sampling procedure. The last is associated with the water quality in terms of a high concentration of suspended solids.

These validation procedures have proved that this analytical process is applicable to determination of TOC content in water by Apollo 9000 Total Organic Carbon Analyzer, and that results of TOC content are valuable.

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