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EVALUATION OF ROBUSTNESS IN THE VALIDATION OF TOTAL ORGANIC CARBON (TOC) METHODOLOGY

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ABSTRACT

Water is used in many steps of production and quality control as raw material for reagent preparation or dilution of solutions and for cleaning apparatus and room areas in the pharmaceutical industry, including radiopharmaceutical plants. Regulatory requirements establish specifications of purified water for different purposes. The quality of water is essential to guarantee the safe utilization of radiopharmaceuticals. A variety of methods and systems can be used to produce purified water and water for injection and all of them must fulfill the requirements for their specific use, which include TOC (total organic carbon) analysis, an indirect measurement of organic molecules present in water. The principle of TOC method is the oxidation of organic molecules to carbon dioxide, related to the carbon concentration. The aim of this study was to evaluate the parameters of robustness in TOC method in water used in the production and quality control procedures in the Radiopharmacy Directory (DIRF), according to Resolution 899 from ANVISA (National Sanitary Agency). Purified water were obtained from Milli-RX45 system. TOC standard solutions in the range of 100-1000 ppb were prepared with potassium hydrogen phthalate anhydride, transferred to vials and sequentially analyzed by a catalytic photo-oxidation reaction with a TOC model Vwp equipment from Shimadzu Corporation (Japan). The evaluated parameters were: oxidizing volume from 0.5 to 2.5 mL, acidifying volume from 1 to 5%, integration time for TC (total carbon) and IC (inorganic carbon) curves from 2 to 10 minutes.

1. INTRODUCTION

Water is an item of major importance in the pharmaceutical industry. It is used in many steps of the production and the quality control as raw material, from reagent solution preparation to area and equipment cleaning purposes [1-3].

Water must be properly treated for reaching adequate purity. The quality of water is essential to guarantee the safe utilization of the pharmaceuticals, including radiopharmaceuticals.

Purified water and water for injection can be obtained by filtration, distillation, deionization and reverse osmosis methods and the storage must be adequate, to avoid external and internal contaminations that come mainly from the source of water, purification and distribution system [4, 5].

Regardless the production method, water must fulfill the requirements established by the pharmacopeias for its specific use, which include TOC (total organic carbon) analysis, an indirect measurement of organic molecules expressed as carbon [5-9].

TOC analysis evaluates the efficiency of the purification method in removing organic molecules from water. The quantitative method for measuring TOC was included in the United States Pharmacopeia (USP) in 1999 with a maximum limit of 500 ppb (parts per billion) for purified water and water for injection [6-8].

Nowadays, there are many technologies for TOC determination, but the two most commonly used are catalytic photo-oxidation reaction and catalytic combustion. The catalytic photo-oxidation measures two types of carbon: total carbon (TC) and inorganic carbon (IC). In TC determination, the organic compound, as potassium hydrogen phthalate, is oxidized to CO_2, at 80 ºC, as described by the Eq. 1 [10]:

$$\mathbf{HOOC\text{-}C_6H_4\text{-}COOK + Na_2S_2O_8 \xrightarrow{\Delta,\ UV} CO_2 + subproducts} \quad (1)$$

Inorganic Carbon (IC) reacts with ortho-phosphoric acid and generates CO_2, as expressed by the Eq. 2 and 3. [10]:

$$\mathbf{3Na_2CO_3 + 2H_3PO_4 \rightarrow 2Na_3PO_4 + 3CO_{2\,(g)} + 3H_2O} \quad (2)$$

$$\mathbf{2NaHCO_3 + H_3PO_4 \rightarrow Na_2HPO_4 + 2CO_2 + 2H_2O} \quad (3)$$

TOC concentration is calculated by the Eq. 4 [4, 10]:

$$\mathbf{TOC = TC - IC} \quad (4)$$

To assure and demonstrate that a method is scientifically accurate under the applying conditions of the laboratory, there are parameters that must be evaluated to certify the method efficiency known as validation. The parameters are dependent on the analytical method finality. The validation parameters are: robustness, specificity, precision, accuracy, linearity (range of measurement), detection limit and quantification limit [11]. All the parameters must fulfill the current regulament, in this case, the Resolution 899 from ANVISA (National Sanitary Agency).

The aim of this study was to evaluate the robustness parameter in the validation of total organic carbon (TOC) methodology for water quality control used in the Radiopharmacy Directory (DIRF). The analyzed parameters were oxidizing volume, acidifying volume and integration time for TC (total carbon) and IC (inorganic carbon) analyses.

2. MATERIAL AND METHODS

2.1. Materials

40 mL vials were washed with 20% HNO_3 and rinsed with purified water. 0.5 mol L^{-1} $Na_2S_2O_8$ and 3.0 mol L^{-1} H_3PO_4 were prepared with P.A. reagents (Merck). A 50 ppm stock solution of potassium hydrogen phthalate ($C_8H_5O_4K$) and sodium carbonate (Na_2CO_3) and sodium bicarbonate ($NaHCO_3$) were prepared to obtain TC and IC calibration curves,

respectively. 100, 250, 500 and 1000 ppb standard solutions were prepared by dilutions of the stock solutions. Water for standard solution dilution and water samples were taken ten minutes after the purification system was turned on.

2.2. TOC measurement system

TOC equipment model Vwp from Shimadzu Corporation with an auto sampler (ASI-V) was used for TOC measurement. Each standard solution was analyzed in triplicate. The data acquisition and TC and IC area integration were made by TOC-V control software. TOC was calculated by the Eq. 4.

TC oxidizing reagent volume, IC acidifying reagent volume, TC curve integration time and IC curve integration time were evaluated. The oxidizing volume range was 0.5-2.5 mL, the acidifying volume range was 1.0-5.0 mL, the TC integration curve time was 2-10 minutes and the IC integration curve time was 2-10 minutes.

2.3. Assays

One sample of 250 ppb standard solution was used for each parameter variation (TC oxidizing reagent volume, IC acidifying reagent volume, TC curve integration time and IC curve integration time) in one day analysis.

For evaluation of the correlation coefficient (R^2) and coefficient variation (CV) of the calibration curve, each standard solution was analyzed in triplicate for each parameter variation. Oxidizing reagent and acidifying reagent volumes were evaluated by using five standards TOC solutions (0, 100, 250, 500 and 1000 ppb) while TC integration curve time and IC integration curve time were assessed with three standard TOC solutions (0, 250 and 1000 ppb).

3. RESULTS AND DISCUSSION

For robustness evaluation of the methodology for TOC determination, TC oxidizing reagent volume, IC acidifying reagent volume, TC curve integration time and IC curve integration time were evaluated to choose the best condition of analysis.

The calibration curve in triplicate allowed the evaluation of each parameter variation. It was necessary to take the IC concentration into account as it is daily variable and subtract its value from the TOC result to obtain the real concentration.

3.1. Oxidizing reagent volume

Fig. 1 shows the oxidizing reagent volume effect on a 250 ppb TOC standard solution analysis carried out in one day.

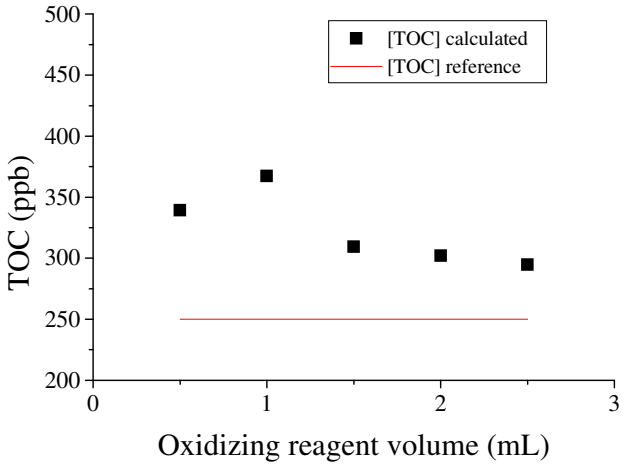

Figure 1. Effect of oxidizing reagent volume variation on a 250 ppb TOC standard solution

Fig. 1 shows that the calculated TOC concentration was near the reference value when volumes of 1.5, 2.0 and 2.5 mL were used.

Fig. 2 shows the calibration curves when oxidizing reagent volume was varied.

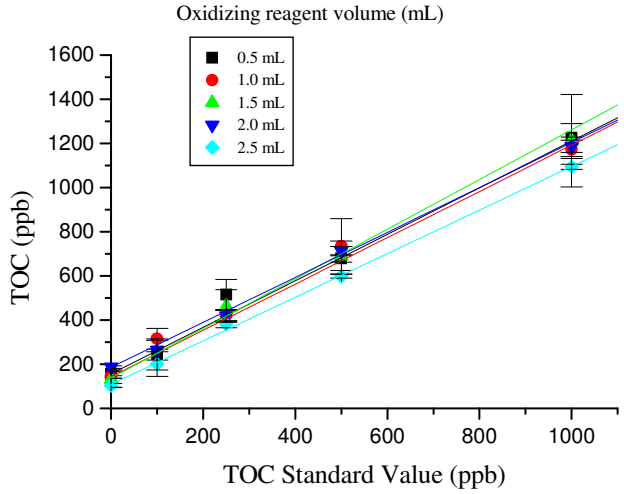

Figure 2. TOC calibration curve with oxidizing reagent volume variation.

The highest CV was 17.1 % in the triplicate results of TOC with 0.5 mL oxidizing reagent volume; the lowest was 0.5% for 2.0 mL.

The manufacturer recommended 1.5 mL oxidizing reagent volume but Fig. 1 and Fig. 2 show that the calculated TOC concentration had less variation and was near the reference value when 2.0 mL was used.

When an inadequate oxidizing reagent volume is used, the reaction described in the Eq. 1 does not occur properly as indicated by the deviation of the TOC final concentration when lower volumes were used.

Higher deviation was observed when all the TOC standard solution analyses were carried out. Correlation coefficient was above 0.99 for all analytical curves. It was observed the lowest variations in the replicates when oxidizing reagent volume 2.0 mL was used.

3.2. Acidifying reagent volume

Fig. 3 shows the results of a 250 ppb TOC standard solution analysis varying the acidifying reagent volume.

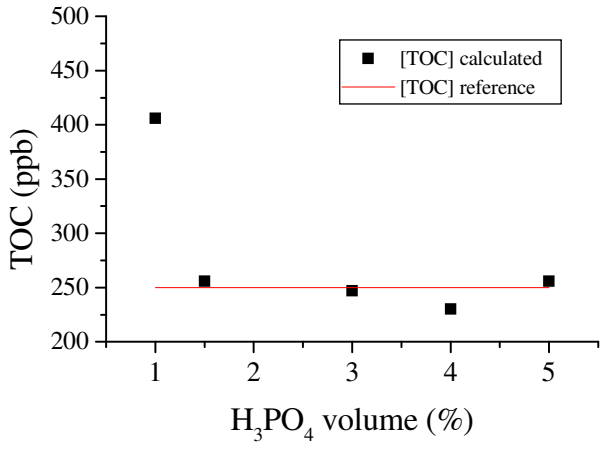

Figure 3. Effect of the acidifying reagent volume variation on a 250 ppb TOC standard solution

The manufacturer recommended acidifying reagent volume of 3.0 %, but Fig. 3 shows that the calculated TOC concentration was near to reference value when volumes of 1.5, 3.0 and 5.0 % were used.

Fig. 4 shows the calibration curves when acidifying reagent volume was varied.

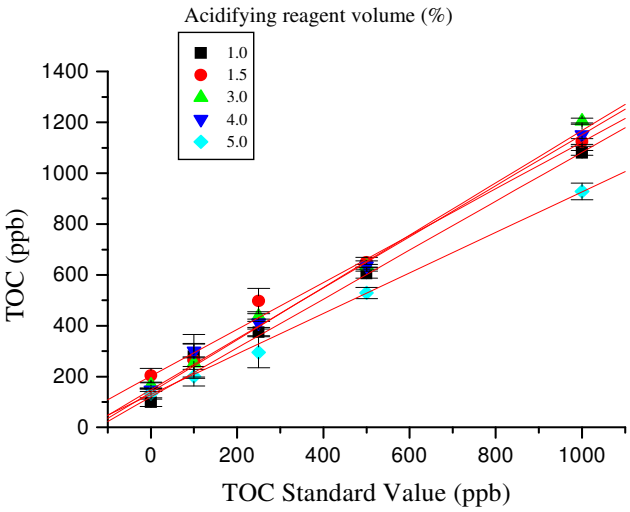

Figure 4. TOC calibration curve with acidifying reagent volume variation.

Correlation coefficients were above 0.99 for all analytical curves. The CV in the TOC analysis came from IC and TC results in the acidifying reagent volume variation. It was observed in Fig. 4 that the lowest CV (0.9 %) between triplicate was found when acidifying reagent volumes of 3.0 % was used, the highest CV was 30.8 % with 1.0 %.

3.3. TC Integration Curve time

The variation of the TC integration curve time makes difference in the results, indicating that in a wrong integration time the curve should not be reading the curve area properly.

Fig. 5 shows the 250 ppb TOC standard solution results with the TC integration curve time.

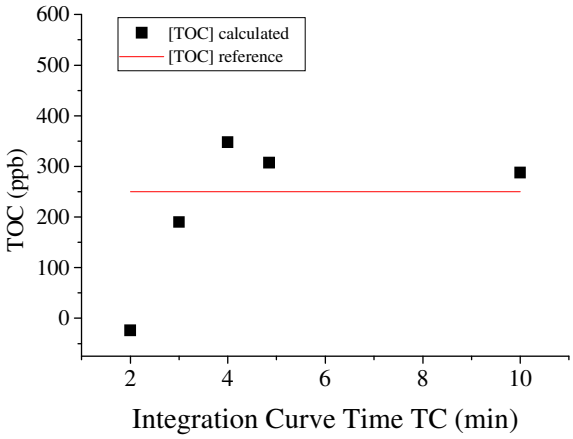

Figure 5. Effect of TC integration curve time variation on a 250 ppb TOC standard solution

Fig. 5 shows that TOC results near to reference value were found when times of 4.30 and 10 minutes integration curve time were used.

Fig. 6 shows the calibration curves when TC integration curve time was modified.

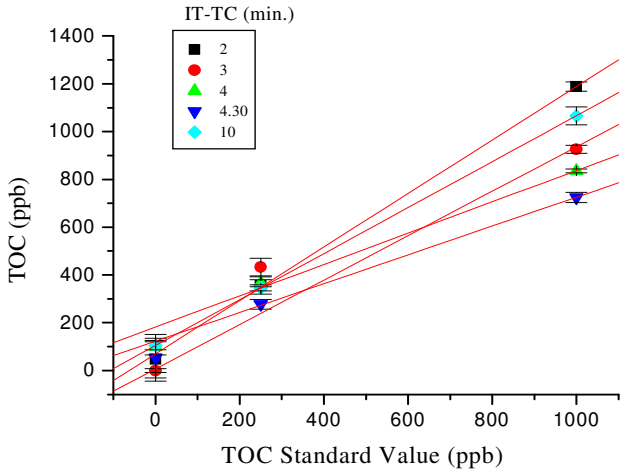

Figure 6. TOC calibration curve with TC integration curve time variation.

The CV of TOC calculated value came from TC and TOC results in the triplicate analysis. The CV of 2253% was observed during analysis with 3 minutes of TC integration curve time. Fig. 5 and Fig. 6 show that the best results were found with 10 minutes TC integration curve

time, with the lowest CV (3.5 %) and results near to reference value. The organic molecule oxidation to CO_2 is slow compared with inorganic reaction and the absorbance curve is broad and it is needed a larger reading time for integration. Correlation coefficients were above 0.99 in all analytical curves.

3.4. IC Integration Curve Time

Fig. 7 shows the 250 ppb TOC standard solution results with the IC integration curve time.

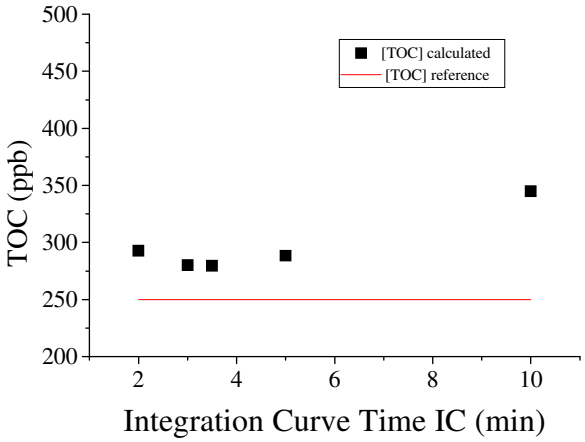

Figure 7. Effect of the IC integration curve time variation on a 250 ppb TOC standard solution

Fig. 7 shows that TOC results near to the reference value were found when times of 3 and 3.30 minutes were used.

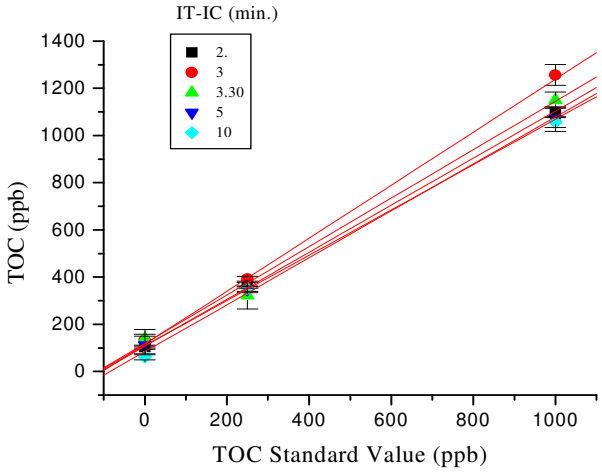

Figure 8. TOC calibration curve with IC integration curve time variation.

Fig. 8 shows the calibration curves when IC integration curve time was varied. The CV of TOC calculated value came from IC and TOC results in the triplicate analysis.

Correlation coefficients were above 0.99 for all analytical curves. Fig. 7 and Fig. 8 show that the best results were found with 3 minutes IC integration curve time, with the lowest CV (2.6%) and results near to TOC reference value.

4. CONCLUSIONS

Evaluation of the results showed that the best conditions for TOC determination were: 2.0 mL oxidizing reagent volume, 3.0 % acidifying reagent volume, 10 minutes for the TC integration curve time and 3 minutes for IC integration curve time. TC curve and IC integration curve time were the most critical parameters in the TOC determination. These conditions are going to be used in validation of TOC methodology for analysis of water used in the Radiopharmaceutical Directory production and quality control processes.

ACKNOWLEDGMENTS

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