INSTRUMENTS

DETERMINING THE CORRECT CONCENTRATIONS OF TOC IN PURIFIED WATER AND WFI

The need for increased sensitivity in the pharmaceutical industry has lead to many stricter requirements for analyzing samples for total organic carbon (TOC). The United States Pharmacopeial Convention Inc. (USP) (1) is regarded as one of the foremost authorities on standards for Water For Injection (WFI) and Purified Water (PW). The USP method that details analyzing WFI and PW for TOC is *United States Pharmacopeia 23 (USP 23)*.

The pharmaceutical industry uses PW for preparation of drugs in liquid and solid form and WFI for preparing drugs for intravenous injection. Instead of having to go through the various biochemical and microscopic tests, pharmaceutical companies have the option to test the PW and WFI for the concentration of TOC. In 1990, the USP first released suggestions for using TOC for determining acceptable PW and WFI (*USP 22*) (2). The *USP 23* took effect on Jan. 1, 1995 (2) and on Nov. 15, 1996, the TOC testing of PW and WFI became official (3). The Water and Parenterals Subcommittee (W&P), however, allowed the continuing use of the Oxidizable Substances test to provide time for familiarization with the test and the apparatus for TOC. Effective May 15, 1998, the Eighth Supplemental will delete the Oxidizable Substances test making TOC the exclusive test for PW and WFI (4).

It can be assumed that the concentration of TOC in the PW or WFI is either

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proportional to the concentration of bacteria or some other organic contaminant in the sample. The most common contributor to contaminants is elution of TOC from the water system's components (5). Some compounds that could cause contamination are trihalomethanes, haloacetic acids, haloacetonitriles, and haloketones. If the concentration of TOC is less than an established amount, it can be inferred that the contamination is not significant. The significant amount as established by USP is approximately 500 parts per billion (ppb) (3). The term "approximately" must be used because the significant amount is actually determined by a calculation. The sample is acceptable for PW or WFI if Equation 1 is true.

$$(\text{WFI or PW}) \, \pounds \, r_s - r_w \qquad \text{Eq. 1}$$

Where r_s is the concentration or area counts of a 500 ppb sucrose standard and r_w is the reagent water (blank). Reagent water refers to the water that was used to prepare the standards.

The USP also has a guideline for determining system suitability prior to analyzing PW or WFI. System suitability refers to the ability of an instrument to efficiently oxidize a compound that is not easily oxidized. The USP selected compound is 1,4-Benzoquinone (p-Benzoquinone) at a concentration of 500 ppb. The system suitability is determined by Equation 2. Table A identifies the abbreviations used in the calculation.

$$\%R = (r_{ss} - r_w) \div (r_s - r_w) \times 100 \qquad \text{Eq. 2}$$

These requirements alone allow for several other sources of error, depending on the instrument and the software used to do the calculations. Some instruments have the capability to eliminate these sources of error by using two functions: shifting through zero and blank check.

Shifting through Zero

Since it is impossible to obtain reagent water that has no carbon, a correction in the calibration curve must be made when measuring samples. Since the standards are prepared with the same water that is used as the reagent water, the same correction must be made for the standards as the reagent water. This correction is called shifting through zero. To shift through zero, the entire curve is moved down proportional to the area counts of the reagent water. Figure 1 is an example of a curve that is not shifted through zero. In this example, the reagent water area count is 10,000 and the 500 ppb standard area count is 43,000. Figure 2 is the same curve shifted through zero.

As can be seen from comparing Figure 1 and Figure 2, the area counts for the reagent water are subtracted from the reagent water and the standard. Notice that the slope of the line remains the same when shifting through zero. When a sample is analyzed against the shifted curve, the actual concentration of carbon will be displayed.

Shifting through zero should not be confused with forcing the intercept through zero. When a curve is forced through zero, the entire curve does not move down. The curve is "pivoted" to run through the origin, thus changing the slope of the curve. This method will not account for the carbon in the reagent water and the analyst will get false low results for samples.

If an analyst is attempting to analyze a standard as an "unknown sample" using a curve that is shifted through zero, the reagent water concentration must be subtracted from the result. The reason is simply that the same principle has been applied to the standards in the calibration curve. The concentration of a standard that has been spiked with an amount of stock solution appropriate to give a concentration of 500 ppb will actually have a concentration of 500

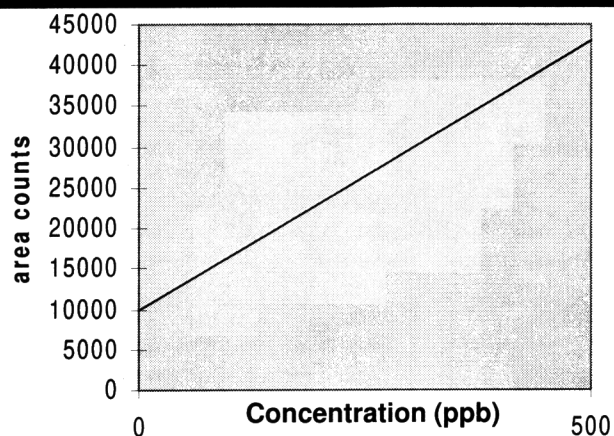| TABLE A |
|---|
| **System Suitability Abbreviations** |
| AbbreviationDefinition |
| r_{ss}1,4 Benzoquinone (area counts) |
| $r_s$sucrose (area counts) |
| $r_w$reagent water (area counts) |
| %Rpercent recovery (%) |

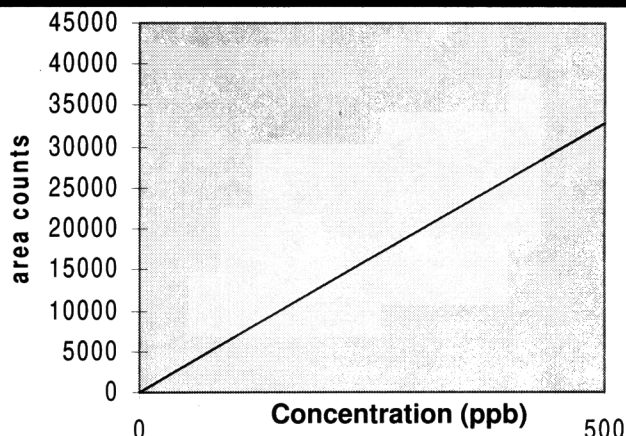Figure 1. An example of a curve that is not shifted through zero.

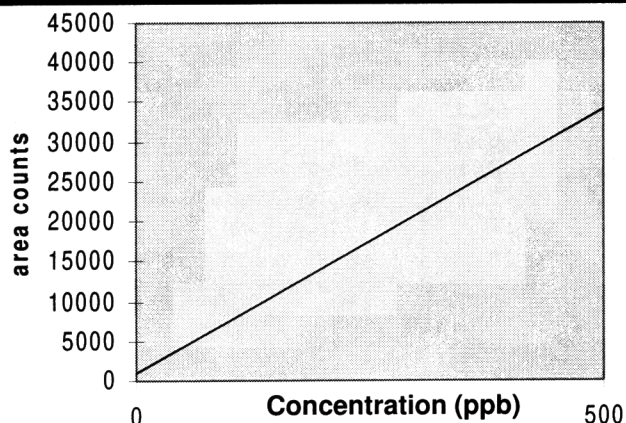Figure 2. The same curve in Figure 1 shifted through zero.

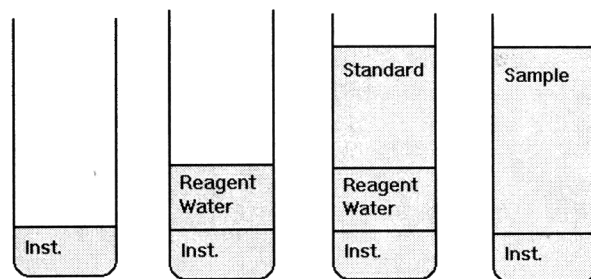Figure 3. Linear regression with shift through zero.

Figure 4. The effect of the blank check in conjunction with shifting through zero.

ppb plus the concentration of the reagent water. When the curve is shifted through zero, the reagent water is removed from the calibration. Suppose that the reagent water had a concentration of 25 ppb (which could be measured from the shifted through zero curve). If the standard is measured with this curve, the concentration will be 525 ppb. Since the reagent water was removed from the calibration curve, it must be removed from any sample or standard prepared with that reagent water to achieve the correct result except in the following case. If an analyst has created a calibration curve using PW or WFI as the reagent water, then obviously the reagent water concentration would not be subtracted to get the true concentration.

Although shifting through zero accounts for the amount of carbon in the reagent water, it does not account for instrument background. This is what differentiates some TOC instruments[a] from others. If the instrument does not determine the amount of the area that is due to the instrument background, then the correct TOC concentration is unattainable. To account for the instrument background, the instrument must have some kind of blank check. This blank check must utilize high-purity water that contains no carbon to obtain the area counts that are solely due to the instrument background.

Blank Check
The blank check function available with some instruments[a] allows the user to accurately determine the carbon area counts due to instrument background. Instruments that can create high-purity water and store it without exposure to the atmosphere can have an advantage over those that cannot. As soon as high-purity water is exposed to the atmosphere, it will start to absorb carbon from atmospheric carbon dioxide (CO_2). The carbon will continue to be absorbed as long as the water container is not isolated from atmospheric carbon. To accomplish this, one instrument[a] injects samples of reagent water into a total carbon (TC) combustion tube that contains a platinum catalyst. This combus-

tion tube is also used to measure the TC in unknown samples. The reagent water is evaporated by the heat of the combustion tube (680 °C) and all carbon in the sample is converted to CO_2 gas. The CO_2 passes through the flow path of the instrument and is collected by a CO_2 scrubber. The reagent water is condensed and collected in a purified water trap. All carbon in the reagent water has now been removed. The purified water is then reinjected into the TC combustion tube and the area of the combustion profile is measured. This is repeated several times and the lowest value is used in the shift through zero calculations. The area for the blank check should be set to equal zero. The actual calculation for shifting through zero is shown in Equation 3.

Reagent Water Area = $r_w - r_w + b$ Eq. 3

Where b is the area recorded for the blank check. Similarly, the equation for calculating a standard area when shifting through zero would be as shown in Equation 4.
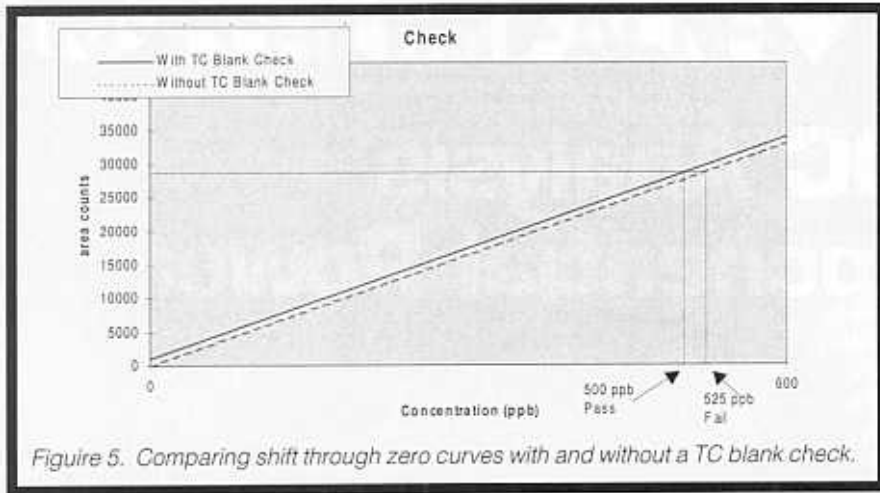
Figuire 5. Comparing shift through zero curves with and without a TC blank check.

Standard Area = $r_s - r_w + b$ Eq. 4

The graph would now appear as shown in Figure 3 if the blank check area count was 1,000.

Figure 4 is provided to further explain the effect of the blank check in conjunction with shifting through zero. As can be seen from the Figure 4 (on the far left), the only source of area counts in the blank check is from the instrument background. Figure 4 (middle left) also shows that the reagent water has two sources of area counts, the reagent water and the instrument background. The figure also shows (middle right) that the standards have three sources of area counts: the standard solution, the reagent water and the instrument background. Finally, the figure (far right) shows that the samples do not contain area counts due to the reagent water or the standard solution, but do contain the instrument background. The only constant is the instrument background that is found by performing the blank check. Since this is found in every analysis it must be either added to the calibration curve or subtracted from the samples.

If this is not done, the results are incorrect proportional to the instrument background. Thus, the reported concentration will be higher than the actual concentration. For example, Figure 5 compares the shifted through zero curves with and without the blank check. As can be seen from the graph, the PW or WFI sample could PASS *USP 23* when the correct calculation using the blank check is used. When the blank check is not used, the sample could have the same area counts and FAIL *USP 23*. Many TOC manufactures and analysts fail to realize the effect of a feature like the blank check. Of course, other simi-lar type instruments may have higher instrument background and the effect will be more pronounced.

The other acceptable methods for oxidizing carbon compounds into CO_2 incorporates ultraviolet light (UV) and UV/persulfate. These methods could use similar means of preparing and collecting high-purity water. While either method is acceptable, the instrument must have a system of isolating the high-purity water from atmospheric carbon. However, if the sample contains solid or particulate matter, contaminants could be introduced into the high-purity water container. High temperature combustion is the only technique that can oxidize carbon containing compounds in solid or particulate matter (6). If a technique does not oxidize all the carbon containing components in the sample, then carry over from the high-purity water trap could occur. When the water is reanalyzed to determine the instrument background, the area counts may reflect some of this carryover. If this occurs, the analyst will incur false low results. Therefore, since all organic compounds are completely oxidized by high temperature, catalyst aided combustion (680 °C), this would be the most suitable method for determining the TOC concentration in PW and WFI.

Conclusion

When analyzing WFI and PW, to get the true TOC concentration the analyst must include a function that establishes the instrument background. The blank check accomplishes this function. The blank check in conjunction with shift through zero provides a way for high temperature, catalyst aided combustion instruments to determine the exact concentration of TOC in WFI and PW. By eliminating false high results due to instrument background, pharmaceuticals will increase productivity and limit product loss.■

References

1. For more information on WFI and PW requirements, contact United States Pharmacopeial Convention Inc., 12601 Twinbrook Parkway, Rockville, MD 20852; 301/881-0666.

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