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Standard additions

USES AND LIMITATIONS IN SPECTROPHOTOMETRIC ANALYSIS

THE METHOD of standard additions (known additions or spiking) has long been used as a powerful tool for indicating accuracy of analyses in real-world samples. However, the analytical literature defining uses and limitations of this technique in spectrophotometry is minimal, and some erroneous conclusions have been made concerning what this technique can and cannot achieve. The purpose of this paper is to 1) mention the uses of standard additions, 2) describe some problems encountered in spectrophotometric analyses, and 3) present some limitations of this technique that analysts should be aware of.

Standard additions is performed by adding a small amount of standard solution to a portion of a previously analyzed sample and repeating the analysis using the same reagents, instrument, and technique. The amount of increase in the test result should exactly equal the amount of standard added to the sample. This procedure should not be confused with an internal standard method that has been recently reported in the literature.[1] An internal standard is used in ir, emission spectroscopy, and gas chromatography analyses to give a standard with which an unknown can be compared. The standard is a known constant quantity of some stable substance with an easily measured response. It is *not* the same element or compound being tested for. For maximum effectiveness, standard additions requires that the substance added be the same as the analyte and, if possible, the same specie.

Use with analytical methods

There are many examples of the normal use of the standard additions technique as a standardization method. Standard additions has long been used in electrochemistry because it gives more realistic values than calibration curves prepared in aqueous solution. Since the unknown and standard are measured under the same conditions, modern matrix-sensitive voltammetric techniques such as anodic stripping voltammetry (ASV) rely almost exclusively on standard additions for quantitation.[2,3] Orion[4] has described the use of known additions in potentiometric titrations with ion-selective electrodes via Gran's plot.

Atomic absorption spectrophotometry uses this method with complex sample matrices when the sample viscosity, surface tension, flame effect, and other components cannot be accurately reproduced with standard solutions.[5,6] By application of a modified standard addition procedure to colorimetric analysis, Kloster and Hach[7] were able to determine simultaneously the sample concentration and reagent blank. Weand[8] reports successful use of standard additions in colorimetric phosphate analyses in lake water where environmental conditions did not permit accurate calibration curves.

Advantages

The advantage of standard additions over normal standardization techniques is that the performance of a specific procedure can be checked under actual operating conditions and used to detect flaws and biases in a method. Certain pitfalls, however, must be avoided. A shortcut method of a single addition is not advisable. Higher precision will be obtained if multiple additions of increasing concentration are added to the sample rather than replicates of a single addition. A single addition, too, should never be used in place of a calibration curve. But when handled properly, the use of standard additions with a "decision tree," such as the one shown in *Figure 1,* can provide a systematic way to identify error sources in analyses.[9]

Decision tree

A decision tree serves as a physical check on the procedure. It also examines the condition of the

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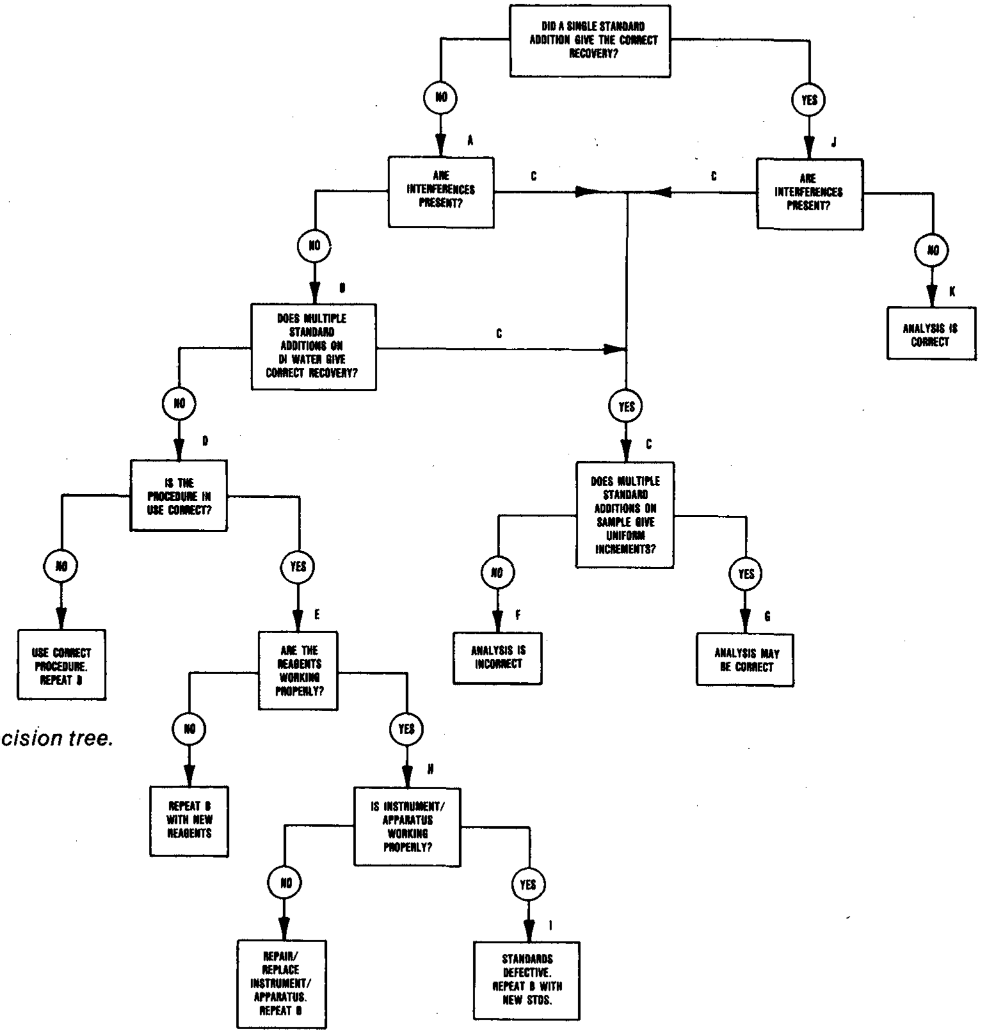


Figure 1 *Standard additions decision tree.*

instrument, reagents, and standards; verifies the analyst's technique; and provides a possible investigation of the absence or presence of interferences within the sample. Explanation of the various steps in the process follows.

Suppose a single standard addition on a sample shows incorrect recovery. A possible cause may be interferences present in the sample. If they are known or assumed to be absent, proceed to Branch B. If interferences are known to be present, proceed to Branch C.

If multiple standard additions on deionized water give correct increments between additions, also proceed to Branch C. If not, proceed to Branch D.

At Branch D, possible errors in the procedure are investigated. If the procedure is found to be in error, Branch B is repeated using the correct procedure. If the procedure is found to be correct, proceed to Branch E, where the performance of the reagents is checked.

If it is found that the reagents are defective, Branch B is repeated with new reagents. If the reagents are proved to be in good condition, proceed with Branch H.

At this point, the operation of the instrument and/or apparatus used in the test is checked. If a defect is found in the instrument or apparatus, repeat Branch B upon correction of the defect. If none is found, proceed to Branch I.

After demonstrating that the procedure and reagents are correct and the instrument and/or apparatus is operating properly, there is only one possible cause for standard additions not functioning properly in deionized water. The standards used in performing the standard additions or the standards used to prepare the calibration curve must be defective. A new set of standards is obtained, and Branch B is repeated.

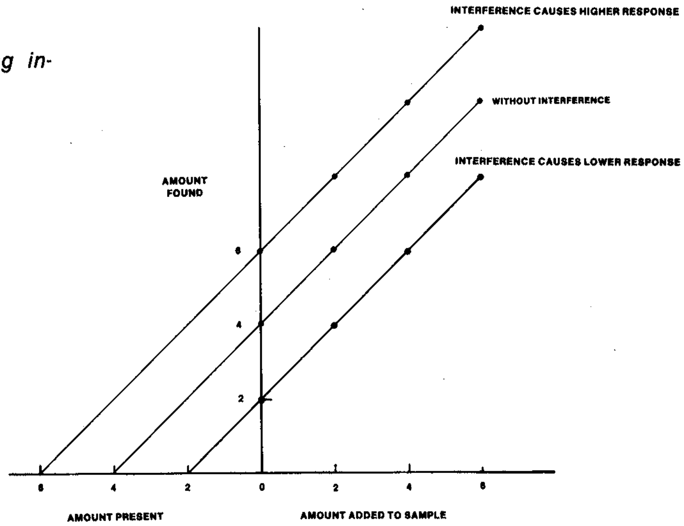
With completion of the troubleshooting steps, the analyst can be reasonably certain of the mechanical aspects of the instrument, procedure, and technique. Further use of standard additions will help point out possible interference problems. By eliminating interferences or switching to a new procedure, analytical accuracy will be further enhanced.

Question of interferences

If the standard addition gave the correct result initially, the analyst must then determine if interfering substances are present. If interfering substances are not present, the result of the analysis prior to the standard addition is correct (Branch K).

If interfering ions are present, it may be concluded that the initial analysis of the original sample is incorrect. However, a close approximation of the correct result may be arrived at through the use of

Figure 2 *Standard additions plots showing interferences.*



multiple standard additions (Branch C). If the steps between each addition are roughly uniform, the analysis may be correct (Branch G). If the increments are not uniform, the analysis is incorrect (Branch F) and a different procedure should be used.

Technique applications

A summary of interference problems that may be encountered in analyses of real-world samples is given in *Table 1.* The effectiveness of standard additions in enhancing the accuracy of these analyses is discussed.

Depressed and elevated instrument response due to sample interferences

Standard additions will *not* be effective if the interference gives a uniform depressed response for the analyte in the original sample and for the standard addition. This is the case for the formation of a hexacyanoferrate (III) complex in the 1,10-phenanthroline method for iron. Any cyanide ligands present in the sample will complex the analyte, reducing the amount of iron that can be analyzed, and therefore reduce the response. The same is true for an interference that gives a uniform elevated response, such as the presence of cadmium in the Zincon method for zinc. When cadmium is present, it will react in the same manner as zinc, giving a higher and incorrect amount of analyte. Graphical plots of standard additions with these types of interferences are shown in *Figure 2.* Note

Table 1

Standard additions: Summary of problems

Problem: General category	Specific example	Will standard additions be effective?
Interference:		
Causes constant decrease in instrument response	$Fe(CN)_6^{-3}$ in the 1,10-phenanthroline method for iron	No
Causes constant increase in instrument response	Cadmium in the Zincon method for zinc	No
Interference prevents test working	Cobalt blocks end point in the Calmagite method for hardness	Yes
Interference changes molar absorptivity of analyte	Substituted phenols in 4-aminoantipyrine	Yes
Interference depletes reagent	Colorless copper, cadmium 1,10-phenanthroline complexes in the method for iron	Yes
Reagent shows loss of efficacy	Loss of reducing power in the 1,10-phenanthroline method for iron	Yes
Matrix effects:		
Kinetics shift	Analysis of silicon dioxide in salt water	Yes
pH, buffer capacity inadequate		Yes
Temperature	Cr (III) in the EDTA back-titration method	Yes
Incorrectly prepared standards		Yes
Mechanical aspects of procedure:		
Incorrect wavelength		Yes
Unmatched cells (dirty, scratched, etc.)		Yes
Forgotten reagent		Yes
Incorrect timing		Yes

that although these interferences are present, uniform increments between standard additions for the amount found are observed, giving a linear plot. However, extension of the plot through the x-axis will not give a correct sample value.

No analyte response

When the interference concentration exactly equals the analyte concentration, standard additions again will not be effective because the existence of the interference cannot be determined. The test result will be incorrectly determined as 0, as shown by the top linear plot in *Figure 3.*

Standard additions will be effective, though, if the interference concentration is slightly higher than the analyte concentration. In this case, Figure 3c, there is sufficient interference to cause no response from the original sample and the first addition. The second addition shows a correct uniform response. Although the analyte concentration cannot be determined, the interference concentration can be.

If each standard addition gives no response and a linear plot along the x-axis is observed, either an incorrect procedure is being used or a very large interference concentration is present. Standard additions will be helpful by warning the analyst of an interference problem because of the unusual slope and lack of analyte found in the original sample.

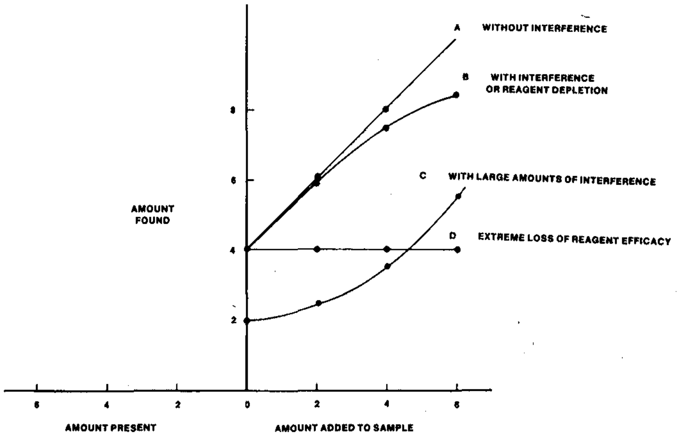


Figure 4 *Standard additions plot showing reagent depletion.*

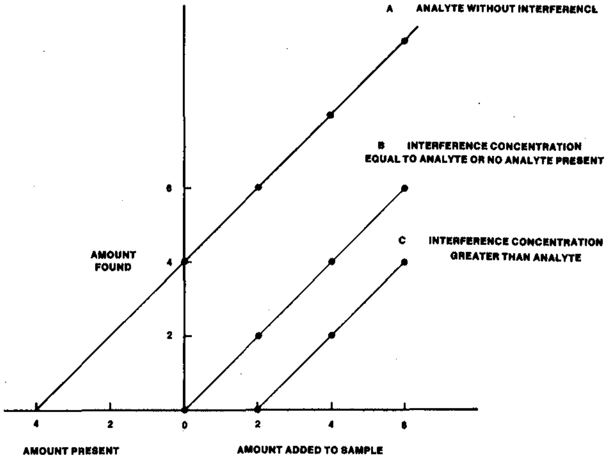


Figure 3 *Standard additions plots showing no analyte response.*

Test not working

Standard additions can serve to indicate when an interference prevents a test from working. Since, for example, cobalt has a higher formation constant than calcium with Calmagite and Eriochrome Black T indicators, it blocks the end point in water hardness titrations. The calcium concentration determined from multiple standard additions will be quite low and not reproducible.

Depletion of reagents

Reagent depletion will also show up on a standard additions plot. For example, copper and cadmium interfere with 1,10-phenanthroline in iron determinations by forming colorless complexes with the indicator, making it unavailable to the analyte. Multiple standard additions will show proper incremental response until all the indicator is complexed. Then the plot begins to curve downward (*Figure 4b*). The amount of iron found is decreased since it now must compete with the copper or cadmium for the indicator. Standard additions will not determine the proper sample result, but it will indicate the presence of such interferences.

Large amounts of interference pose a different problem in this system, as shown in Figure 4*c*. When the concentration of copper in the sample is equal to or greater than the indicator, multiple additions of an iron solution will initially show low incremental responses. As more iron is added, the responses increase and approach a linear plot. This can be attributed to the nearly equal formation complexes of Fe-phenanthroline and Cu-phenanthroline. As more iron is added, it begins to compete with the copper to form a Fe-phenanthroline complex, which gives a more positive response. Sample results, though, will still be incorrect.

In extreme cases, the concentration of interference is so high that there is only enough reagent to react with the analyte in the sample. Any additional analyte is beyond the reagent capability, and a straight line occurs (Figure 4*d*). Standard additions will be effective to show interferences are present, and the original analysis is probably not correct.

We see a similar plot for impaired reagent efficacy as the one for interferences. For example, in the phenanthroline method for iron, if the reducing agent used in the test (hydroxylamine hydrochloride or sodium sulfite) has lost its efficacy, the standard additions plot will look like Figure 4*b*. The analyst will not be able to know if the problem is one of efficacy of the reagents or reagent starvation, but sometimes an educated guess can be made. If the upper concentration limit of the test has been surpassed, deviations from the linear plot are most likely due to reagent starvation.

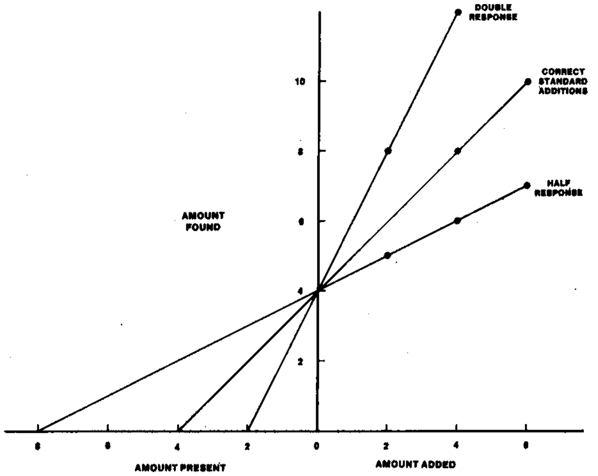


Figure 5 *Standard additions plot showing incorrectly prepared standards.*

Matrix effects

Many times, analyses are inaccurate because of matrix effects in the test system. Kinetic shifts in reagent response due to interfering substances in the sample can cause irreproducible color development times, and test accuracy is impaired. This problem occurs in sea water, where kinetic shifts due to salinity will adversely affect silicon dioxide analyses. Other matrix effects include altered pH or impaired buffer capacity of the test system due to excessive acidity or basicity and temperature effects. The EDTA back-titration of chromium (III), for example, is adversely affected by cold samples. When using multiple standard additions with these types of samples, uniform increments will be observed, but the amount of each addition will be less than expected.

Incorrectly prepared standards

Standard additions can also serve as a self-check. In conjunction with a correctly prepared calibration curve, standard additions can be used to detect incorrectly prepared standard addition solutions. If the standards are twice the concentration they are thought to be, then the amount found at the *x*-intercept will be double the expected value. If the standards are half the correct concentration, half the amount will be found (see *Figure 5*). It should be noted that if the units of the axes are both in terms of concentration and have equal increments, any deviation from a 45° slope will indicate an error in the original value (Figure 5). However, this method must be used in conjunction with a calibration curve to be true.

Mechanical problems

A final group of problems where standard additions is helpful involves the mechanical aspects of the procedure such as incorrect wavelength, unmatched cells, dirty or scratched cells, forgotten reagent, and incorrect timing. If any of the above should occur, standard additions will show a nonlinear plot, a slope different from 45°, or a straight line superimposed on the *x*-axis.

Conclusion

Although standard additions will not be effective if part of the analyte is tied up by an interference or an interference reacts in a similar manner as the analyte, this technique is a useful analytical tool. By determining whether analytical problems are present, standard additions can help the analyst arrive at a more accurate analysis. When applied to a decision tree, it, is particularly valuable for systematically checking the performance of the reagent, instrument or apparatus, and procedure.

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