

The Application of Fundamental Analytical Principles to UV-Visible Spectrophotometry

Key Words

- Precision
- Accuracy
- Method Validation
- Chromogenic Compound
- UV-Vis Methods
- Criteria for Generation of UV-Vis Methods

Introduction

Any data manipulation procedure, no matter how simple or sophisticated, is only as good as the data supplied to it – in other words, “Garbage in, garbage out.”

This note provides the ground-rules. They are easily overlooked when you are excited by a new instrument, but all experimental measurements are subject to the same fundamental limitations.

This is the first in a series of notes and should be read in conjunction with the others.

Theory

Accuracy and Precision in Absorbance Measurement

Once you have measured your sample on the instrument and obtained the absorbance reading how valid is this number?

Any measuring system has two criteria of performance:

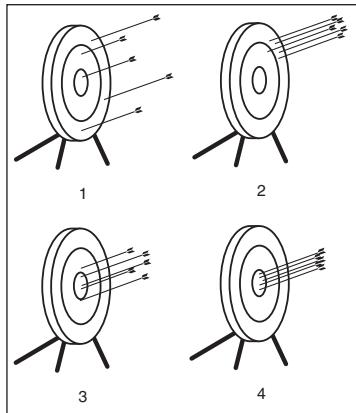
- Precision
- Accuracy

The precision and accuracy that can be achieved depend on:

- The environment
- The apparatus

This can be illustrated by thinking of the performance of an archer when shooting at a target. Factors such as the skill of the archer and the wind conditions form the environment, and the apparatus is the bow. In the laboratory the skill of the analyst and the quality of the equipment used in sample preparation form the environment, and the instrument itself is the apparatus.

In picture (1) the arrows are widely distributed over the target, and none have hit the bullseye. There is both lack of accuracy and poor precision. This may be due to poor performance by the archer (analyst), or not a very good bow (instrument), or both.



In picture (2) the arrows are closely grouped, i.e. the results are precise, but are not in the bullseye, i.e. the results are not accurate. The archer (analyst) is good, but the bow (instrument) is shooting wide (the wrong absorbance value is recorded).

In picture (3) some arrows have hit the bullseye, so the bow (instrument) is accurate (the results include the correct absorbance value), but the archer is not shooting consistently so the precision is poor (the analyst lacks skill in sample preparation)

In picture (4) the results are both accurate and precise. A skillful archer is shooting under good conditions, using a good bow.

Thermo spectrophotometers have been designed to give consistently accurate, precise absorbance measurements, enabling the analyst to concentrate on using his/her own skills to the best effect.

Choice of Procedure

A few analytes have specific absorbances that are sufficiently high to allow them to be quantified by direct measurement. However, in the majority of cases the analyte must be reacted with a spectrophotometric reagent (chromogenic compound) to form a highly absorbing derivative. Not all chromogenic compounds are suitable for quantitative measurements, and the choice of reagent is principally influenced by the chemistry of the species to be determined. When selecting or developing a procedure, the following factors should be investigated, either by direct experiment or by reference to the literature.

1. Color development

The colored derivative must be sufficiently stable to allow a reliable measurement to be made. The absorbance should be stable for at least 30 minutes after preparation of the derivative, and should not be affected by minor variations in pH, ionic strength or temperature.

2. Reagent stability

The storage requirements and lifetimes of the reagents must be established. If, for example, a reagent must be stored in the dark or at low temperature, the necessary storage facilities and procedures must be used. Each reagent must be replaced at the end of its life, and a new calibration should be prepared for each new batch of reagent.

3. Reagent selectivity

The degree of selectivity of a complexing agent should be understood. The effect of other species likely to be present must be considered, and also the effect of an excess of the reagent. If the reaction goes to completion,

and the product and reagent absorb at different wavelengths, it is often satisfactory, and very convenient, to use an excess of the reagent.

4. Conformity to the Beer-Lambert law (and the value of the molar absorptivity)

The product of the reaction should obey the Beer-Lambert law over a wide range of concentrations, thus producing a linear calibration graph.

5. Interfering substances

One of the major problems lies in the extent of interference from other constituents of a sample. A variety of techniques are available to minimize this interference, ranging from the use of masking agents, or pH control, to a whole diversity of computer based mathematical solutions. If no solution is available using these techniques, the interfering substances must be removed during sample preparation, for example using chromatography or solvent extraction.

Calculation of Effective Concentration Range

One approach to this problem was proposed by Ayres¹, based on an earlier proposal by Ringbom. Rather than plotting absorbance against known concentrations, T is plotted against the logarithm (base 10) of the concentration. The linear region of maximum slope indicates the range of concentration that will give maximum sensitivity and linearity for the determination. This method assumes constant instrumental error across the whole transmission range, which is in fact an invalid assumption.

A more rigorous method for estimating the region of precision for a selected method is given by Youmans & Brown², where statistical data from a series of measurements is used to generate an absorbance versus error curve.

Validation

Having established the methodology, and the range over which the calibration is applicable, the final task in any good analytical process must be validation of the procedure.

Essentially three processes are available to the user, which may be selected as required.

1. Check using Standard Reference Materials (S.R.M.).

S.R.M.s are complex matrices e.g. foods, sea water, etc, in which all the analytes of interest have been determined. The quoted concentrations are validated by various statistical processes, and are usually expressed as a definitive figure with an attached \pm tolerance.

This is usually the preferred procedure, but it is often limited by the availability of a suitable material.

Useful addresses for obtaining S.R.M.s

NIST

U.S. Department of Commerce
National Institute of Standards and Technology
Standard Reference Materials Program
100 Bureau Drive, Stop 2320
Gaithersburg, MD 20899-2320
Phone: (301) 975 6776
E-mail: srminfo@nist.gov
<http://www.nist.gov>

BCR

Community Bureau of Reference
Commission of the European Communities
303, Rue de la Loi 200-B-1049
Brussels
Fax: 322 235 80 72
<http://www.irmm.jrc.be/>

LGC

Office of Reference Materials
Laboratory of the Government Chemist
Queens Road
Teddington, Middlesex
TW11 0LY UK
Phone: +44 (0)20 8943 7000
Fax: +44 (0)20 8943 2767
<http://www.lgc.co.uk>

2. Check using values achieved by an unrelated technique.

The analyte in question is determined by another technique, the fundamental principle of which is totally different to UV-Visible spectrophotometry. Chromatography (HPLC or TLC) is often the chosen technique. This is useful when no SRM. is available, but of course this procedure relies upon the essential validation of the alternative technique.

3. Addition of known amounts of the analyte of interest at the start of the procedure, and then determination of the actual amounts at the end.

These 'recoveries' are usually expressed as a percentage of the original concentration added, and will, of course, reflect interference on the determination by the matrix. Ideally recoveries of about 100% should be achieved.

References

1. G. Ayres, *Anal. Chem.*, **21**, 652, 1949.
2. H.L. Youmans and V.H. Brown, *Anal. Chem.*, **48**, 1152, 1976.

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