

Australian Standard™

**Recommended practice for chemical
analysis by ultraviolet/visible
spectrophotometry**



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STANDARDS AUSTRALIA

RECONFIRMATION

OF

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**Recommended practice for chemical analysis by ultraviolet/visible
spectrophotometry**

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NOTES

Australian Standard™

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PREFACE

This Standard was prepared by the Standards Committee CH-016, Spectroscopy to supersede AS 3753—1990.

The objective of this Standard is to describe recommended procedures, principles and techniques involved in the setting up and operation of UV/visible spectrophotometer for use in chemical methods for analysis.

In preparing this revised Standard, the opportunity was taken to update the description of the components of UV/visible spectrophotometers in line with modern practice. Recognisance was also made of the variability of performance among the different instruments available in the market and in consequence, the manufacturer's specifications for performance characteristics have been specified as the preferred criteria to be followed. The performance criteria specified in the superseded Standard have been retained as default criteria in cases where the manufacturer's specifications are unavailable.

The term 'informative' has been used in this Standard to define the application of the appendix to which it applies. An 'informative' appendix is only for information and guidance.

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STANDARDS AUSTRALIA

Australian Standard

Recommended practice for chemical analysis by ultraviolet/visible spectrophotometry

SECTION 1 SCOPE AND GENERAL

1.1 SCOPE

This Standard sets out recommendations for instrumentation and operating techniques suitable for chemical analysis of solutions by ultraviolet/visible spectrophotometry, and includes a summary of testing procedures, calibration procedures and recommendations for safe operation. Although most sections of this Standard refer to the analysis of solutions, sections on instrumentation and instrument optimization are relevant to instruments used for gas analysis.

1.2 APPLICATION

This Standard is intended to be read in conjunction with the instrument manufacturer's recommendations.

1.3 PRINCIPLE

Ultraviolet/visible spectrophotometry relies upon the following:

- (a) The absorption of radiation by a substance where the wavelength of the radiation corresponds to the energy required to raise the substance to a state of higher energy (excited state) in an allowed electronic transition. Absorption may be highly wavelength specific or may occur over a broad wavelength range.
- (b) The excited state of the substance being unstable and reverting rapidly to the normal state without emission of radiation.
- (c) The absorbance by the substance being a function of the number of absorbing molecules in the light path (see Appendix A, Bouguer's (Lambert's) Law and Beer's Law).

The technique described in this Standard involves passing radiation of known wavelength through a solution of a sample, and determining the absorbance at that wavelength.

1.4 REFERENCED DOCUMENTS

The following documents are referred to in this Standard:

AS	
2162	Verification and use of volumetric apparatus
2162.1	Part 1: General—Volumetric glassware
2162.2	Part 2: Guide to the use of piston-operated volumetric apparatus (POVA)
2164	Laboratory glassware—One-mark volumetric flasks
2166	One-mark pipettes
2167	Graduate straight pipettes

- AS
2850 Chemical analysis—Interlaboratory test programs—For determining precision of analytical method(s)—Guide to the planning and conduct
- AS/NZS
1338 Filters for eye protectors
1338.2 Part 2: Filters for protection against ultraviolet radiation
- ISO
3696 Water for analytical laboratory use—Specification and test methods

1.5 DEFINITIONS

For the purpose of this Standard, the definitions given in Appendix A apply.

1.6 APPARATUS

1.6.1 Volumetric glassware

Grade A volumetric glassware should be used throughout. Volumetric flasks should comply with AS 2164 and pipettes should comply with AS 2166 and AS 2167. The use of volumetric glassware should conform with AS 2162, Parts 1 and 2.

1.7 SAFETY PRECAUTIONS

1.7.1 UV sources

WARNING: UV SOURCES, SUCH AS DEUTERIUM LAMPS, CAN CAUSE EYE DAMAGE IF OBSERVED DIRECTLY. WHERE EXPOSURE TO UV SOURCES IS UNAVOIDABLE, SUITABLE UV PROTECTIVE GLASSES IN ACCORDANCE WITH AS/NZS 1338.2 SHOULD BE WORN.

1.7.2 Hazardous chemicals

This Standard specifies the handling of hazardous chemicals. Material Safety Data Sheets (MSDS) for all chemicals to be used should be consulted. The information on safe handling of such chemicals should be strictly followed.

1.7.3 Strong acids

In some cases, analyses may involve the use of strong acids for the development of colour in the sample. A typical example is the determination of boron with carminic acid in a medium of concentrated sulfuric acid. Care should be exercised to avoid skin contact and spillage of acids within the sample cell compartment, and capping of cells is recommended to minimize acid fume damage in the cell compartment.

1.7.4 Flammable solvents

In view of the possibility of accidental ignition, it is recommended that cells be capped after filling with solutions of materials in flammable solvents.

1.7.5 Flow-through cells

Some instruments are equipped with flow-through cells, which effectively reduce the time required to change sample solutions. Attached to the flow-through cell are flexible plastic connecting hoses to deliver reagents to, and drain them from the cell. The connecting hoses are susceptible to attack by strong oxidizing agents (e.g. KMnO_4 , $\text{K}_2\text{Cr}_2\text{O}_7$) and aggressive solvents (e.g. halogenated hydrocarbons, acetone). Therefore, it is recommended that such reagents not be used in flow-through cells unless these cells are fitted with tubing resistant to these reagents.

SECTION 2 INSTRUMENTATION

2.1 GENERAL

Instruments used for measuring absorbance at ultraviolet (UV) or visible wavelengths can be classified as either photometers or spectrophotometers.

A photometer incorporates filters to pass discrete bands of wavelengths. It cannot produce any continuous variation in wavelength.

A spectrophotometer is a specific type of spectrometer (See Appendix A.)

A spectrometer incorporates a wavelength dispersing mechanism, typically either a grating or a prism. The wavelength dispersing mechanism can be driven mechanically to provide continuous variation of the wavelength of the detected light. The scanning performance will be governed by variables of the mechanical drive system, such as the range of scanning speeds available and the repeatability of the system. Alternatively, the simultaneous measurement of intensity at a range of wavelengths may be carried out by the use of a diode array, in which the diodes can serve both as detectors and exit slits. (See Clause 2.4.4.)

A typical UV visible spectrophotometer furnishes the ratio (transmittance) or a function of the ratio (absorbance) of two beams as a function of wavelength.

The two beams may be separated in time or space, or both.

UV/visible spectrophotometers and photometers consist essentially of the following components:

- (a) A source (or sources) of radiation covering the visible and ultraviolet wavelength regions (Clause 2.2).
- (b) A device for isolating a suitably narrow wavelength band of radiation (Clause 2.3).
- (c) An optical system for shaping and directing the radiation beam (Clause 2.4).
- (d) A measurement system which usually consists of a radiation detector, an electronic amplifier and a suitable display (Clause 2.5).
- (e) Sample cells or vessels of fixed or known path length which can be reproducibly positioned in the radiation path (Clause 2.6).

2.2 SOURCES OF RADIATION

UV/Visible spectrophotometers and photometers are usually equipped with continuum radiation sources. For measurements in the wavelength range from about 320 nm to beyond 1000 nm a quartz-halogen lamp is suitable and from about 190 nm to 360 nm a deuterium (or hydrogen) discharge lamp is appropriate. Some instruments use other forms of continuum lamp such as a xenon flash-lamp.

Lamps which emit band spectra, such as light-emitting-diodes (LEDs), are also used occasionally, especially in simple photometers.

Metal-vapour lamps, which emit line spectra, can also be used as the radiation source in photometers or for wavelength calibration of spectrophotometers.

2.3 WAVELENGTH SELECTION

With continuum radiation sources it is necessary to have some means of restricting the wavelength region measured to that in which the analyte absorbs. The most common method is the use of a monochromator incorporating a grating or prism but absorbing filters and interference filters are also used, especially in small photometers.

The resolution provided by a monochromator is a function of the groove density of the grating, the focal length of the monochromator and the width of the entrance and exit slits. Higher groove density, longer focal lengths and narrower slit widths all provide increased resolution. The groove density and the focal length are determined by the instrument design, except for some monochromators which allow for exchange of gratings. Where monochromators provide for variable slit widths, the setting chosen should provide a spectral bandwidth which is narrow compared with the width of the absorption band being measured. The spectral bandwidth is an important performance criterion in many applications.

Fourier transform spectrophotometers, which are based on interferometer techniques, are also available, and are commonly used in the infra-red spectral region. However, Fourier transform spectrometers capable of measuring in the UV and visible spectral regions are more difficult to construct and are not generally available other than as research instruments.

2.4 OPTICAL SYSTEM

2.4.1 General

Photometers and spectrophotometers include a variety of optical components such as collimators, lenses, fixed and/or rotating mirrors, diaphragms and slits. These are required to give the radiation beam the appropriate spatial definition, i.e. cross-section, parallelism, focus and path-type (single or double beam).

2.4.2 Single-beam instrument

In a single-beam instrument there is only a single optical cell position. The measurement result is obtained from sequential comparison of the transmissions of the sample and reference solutions.

2.4.3 Double-beam instrument

In a double-beam instrument, the measurement result is obtained from a simultaneous comparison of the sample transmission with the reference transmission; hence two optical cells are placed in two separate light paths. Measurement is effected either by simultaneous direction of the light through the two paths onto two detectors, or by alternate transmission of light from the two paths to a single detector, at a frequency sufficiently high to effectively simulate simultaneous comparison.

2.4.4 Diode-array instrument

A diode-array spectrophotometer uses an array of photodiodes to simultaneously measure selected wavelengths within the ultraviolet/visible spectrum. These instruments differ from conventional spectrophotometers in that the entire spectral emission of the source is incident on the sample, and dispersion into component wavelengths occurs after the radiation has passed through the sample.

In instruments such as the above, where the entire radiation from the source is incident on the sample prior to its dispersion into component wavelengths, there is an increased possibility of changes occurring in the sample during absorbance measurement. This factor needs to be considered carefully when performing measurements on samples that might be sensitive to photolysis.

2.5 MEASUREMENT SYSTEM

2.5.1 General

The electronic measurement system typically consists of a detector, amplification circuitry and suitable display device.

2.5.2 Detectors

Different types of detector may be used together in the same instrument. The most common form of UV/visible detectors, photoelectric detectors, generate an electric current which is directly proportional to the intensity of the radiant energy incident upon them. They may take the form of either photosensitive semiconductor devices, phototubes or photomultipliers as follows:

- (a) Photosensitive semiconductor devices include solid-state photodiodes and phototransistors. The most common semiconductor material is silicon, which is sensitive to wavelengths in the range 400 nm to 800 nm but some silicon devices have extended sensitivity from 190 nm to 1000 nm. Diode arrays are typically made of silicon and hence have a similar wavelength response. Other semiconductor materials can provide wavelength response to several micrometers.
- (b) Phototubes consist of an evacuated glass envelope enclosing a coated cathode and a central anode. Their useful wavelength range is typically from 200 nm to 900 nm and is determined by both the transmission of the window material and the type of material forming the photocathode. Phototubes are rarely used in modern instruments.
- (c) Photomultipliers have similar cathodes to phototubes but have additional electron-sensitive plates, known as dynodes, which amplify the electrons released initially by the absorption of the incident radiation. Photomultipliers have typical wavelength response from 160 nm to 900 nm, although special photocathode materials can provide response to higher wavelengths. These devices are used in most double-beam instruments and are up to 10^6 times more sensitive than phototubes.

2.5.3 Amplifier

It is generally necessary to amplify the very weak electrical signals produced by the detector. Apart from amplifying the signal, the amplifier and associated circuitry should perform the following functions:

- (a) Removal of any residual signal from the detector (dark current).
- (b) Filtering out of any unwanted high-frequency signals and noise.
- (c) Scaling of the signal to a convenient level.

2.5.4 Output device

The measured signal may be displayed in either digital or analog form, as a printout or as a video screen display. The signal can be displayed in a number of modes, which include absorbance, transmission and direct concentration. Some readouts may also include provision for derivative calculations or measurement of reaction kinetics.

Spectrophotometers may incorporate inbuilt microprocessors, or may be controlled entirely by an external computer. The storage or re-entry of calibration graph data is also possible, with the advantage that recalibration may be carried out using a reslope facility. (See Clause 4.4.1.3.)

The output may be expressed as absorbance, transmittance, percent transmission, log absorbance and, in some instruments (after suitable calibration), directly as concentration, enzyme activity or reaction rate. Other instruments, using microprocessors, may also present derivatives of the absorbance versus wavelength function.

Whatever the form of output presentation, the accuracy of the result will depend on the degree of uncertainty of the measurement. The most common form of output is absorbance and for most practical purposes, a good working range is 0.2 to 0.8 absorbance.

The analyst should be aware of the number of figures which are significant in the readout, particularly where concentration mode and scale expansion are available. It should be noted that scale expansion always expands the noise level as well as the signal level.