Determinations are made by comparison with reference solutions with known concentrations of the element to be determined either by the method of direct calibration (Method I) or the method of standard additions (Method II).

*METHOD I - DIRECT CALIBRATION*

For routine measurements 3 reference solutions of the element to be determined and a blank are prepared and examined.

Prepare the solution of the substance to be examined (test solution) as prescribed in the monograph. Prepare not fewer than 3 reference solutions of the element to be determined, the concentrations of which span the expected value in the test solution. For assay purposes, optimal calibration levels are between 0.7 and 1.3 times the expected content of the element to be determined or the limit prescribed in the monograph. For purity determination, calibration levels are between the limit of detection and 1.2 times the limit specified for the element to be determined. Any reagents used in the preparation of the test solution are added to the reference solutions and to the blank solution at the same concentration.

Introduce each of the solutions into the instrument using the same number of replicates for each solution, to obtain a steady reading.

**Calculation**. Prepare a calibration curve from the mean of the readings obtained with the reference solutions by plotting the means as a function of concentration. Determine the concentration of the element in the test solution from the curve obtained.

*METHOD II - STANDARD ADDITIONS*

Add to at least 3 similar volumetric flasks equal volumes of the solution of the substance to be examined (test solution) prepared as prescribed. Add to all but 1 of the flasks progressively larger volumes of a reference solution containing a known concentration of the element to be determined to produce a series of solutions containing steadily increasing concentrations of that element known to give responses in the linear part of the curve, if at all possible. Dilute the contents of each flask to volume with solvent.

Introduce each of the solutions into the instrument using the same number of replicates for each solution, to obtain a steady reading.

**Calculation**. Calculate the linear equation of the graph using a least-squares fit, and derive from it the concentration of the element to be determined in the test solution.

VALIDATION OF THE METHOD

Satisfactory performance of methods prescribed in monographs is verified at suitable time intervals.

*LINEARITY*

Prepare and analyse not fewer than 4 reference solutions over the calibration range and a blank solution. Perform not fewer than 5 replicates.

The calibration curve is calculated by least-square regression from all measured data. The regression curve, the means, the measured data and the confidence interval of the calibration curve are plotted. The operating method is valid when:

– the correlation coefficient is at least 0.99,
– the residuals of each calibration level are randomly distributed around the calibration curve.

Calculate the mean and relative standard deviation for the lowest and highest calibration level.

When the ratio of the estimated standard deviation of the lowest and the highest calibration level is less than 0.5 or greater than 2.0, a more precise estimation of the calibration curve may be obtained using weighted linear regression. Both linear and quadratic weighting functions are applied to the data to find the most appropriate weighting function to be employed. If the means compared to the calibration curve show a deviation from linearity, two-dimensional linear regression is used.

*ACCURACY*

Verify the accuracy preferably by using a certified reference material (CRM). Where this is not possible, perform a test for recovery.

**Recovery**. For assay determinations a recovery of 90 per cent to 110 per cent is to be obtained. For other determinations, for example for trace element determination, the test is not valid if recovery is outside of the range 80 per cent to 120 per cent at the theoretical value. Recovery may be determined on a suitable reference solution (matrix solution) which is spiked with a known quantity of analyte (middle concentration of the calibration range).

*REPEATABILITY*

The repeatability is not greater than 3 per cent for an assay and not greater than 5 per cent for an impurity test.

*LIMIT OF QUANTIFICATION*

Verify that the limit of quantification (for example, determined using the $10\ \sigma$ approach) is below the value to be measured.

**01/2008:20223**

## 2.2.23. ATOMIC ABSORPTION SPECTROMETRY

GENERAL PRINCIPLE

Atomic absorption is a process that occurs when a ground state-atom absorbs electromagnetic radiation of a specific wavelength and is elevated to an excited state. The atoms in the ground state absorb energy at their resonant frequency and the electromagnetic radiation is attenuated due to resonance absorption. The energy absorption is virtually a direct function of the number of atoms present.

This chapter provides general information and defines the procedures used in element determinations by atomic absorption spectrometry, either atomisation by flame, by electrothermal vaporisation in a graphite furnace, by hydride generation or by cold vapour technique for mercury.

Atomic absorption spectrometry is a technique for determining the concentration of an element in a sample by measuring the absorption of electromagnetic radiation by the atomic vapour of the element generated from the sample. The determination is carried out at the wavelength of one of the absorption (resonance) lines of the element concerned. The amount of radiation absorbed is, according to the Lambert-Beer law, proportional to the element concentration.

APPARATUS

This consists essentially of:

– a source of radiation;
– a sample introduction device;
– a sample atomiser;
– a monochromator or polychromator;
– a detector;
– a data-acquisition unit.

The apparatus is usually equipped with a background correction system. Hollow-cathode lamps and electrodeless discharge lamps (EDL) are used as radiation source. The emission of such lamps consists of a spectrum showing very narrow lines with half-width of about 0.002 nm of the element being determined.

There are 3 types of sample atomisers:

- Flame technique

  A flame atomiser is composed of a nebulisation system with a pneumatic aerosol production accessory, a gas-flow regulation and a burner. Fuel-oxidant mixtures are commonly used to produce a range of temperatures from about 2000 K to 3000 K. Fuel gases include propane, hydrogen and acetylene; air and nitrous oxide are used as oxidants. The configuration of the burner is adapted to the gases used and the gas flow is adjustable. Samples are nebulised, acidified water being the solvent of choice for preparing test and reference solutions. Organic solvents may also be used if precautions are taken to ensure that the solvent does not interfere with the stability of the flame.

- Electrothermal atomisation technique

  An electrothermal atomiser is generally composed of a graphite tube furnace and an electric power source. Electrothermal atomisation in a graphite tube furnace atomises the entire sample and retains the atomic vapour in the light path for an extended period. This improves the detection limit. Samples, liquid as well as solid, are introduced directly into the graphite tube furnace, which is heated in a programmed series of steps to dry the sample and remove major matrix components by pyrolysis and to then atomise all of the analyte. The furnace is cleaned using a final temperature higher than the atomisation temperature. The flow of an inert gas during the pyrolysis step in the graphite tube furnace allows a better performance of the subsequent atomisation process.

- Cold vapour and hydride technique

  The atomic vapour may also be generated outside the spectrometer. This is notably the case for the cold-vapour method for mercury or for certain hydride-forming elements such as arsenic, antimony, bismuth, selenium and tin. For mercury, atoms are generated by chemical reduction with stannous chloride or sodium borohydride and the atomic vapour is swept by a stream of an inert gas into a cold quartz cell mounted in the optical path of the instrument. Hydrides thus generated are swept by an inert gas into a heated cell in which they are dissociated into atoms.

## INTERFERENCES

Chemical, physical, ionisation and spectral interferences are encountered in atomic absorption measurements. Chemical interference is compensated by addition of matrix modifiers, of releasing agents or by using high temperature produced by a nitrous oxide-acetylene flame; the use of specific ionisation buffers (for example, lanthanum and caesium) compensates for ionisation interference; by dilution of the sample, through the method of standard additions or by matrix matching, physical interference due to high salt content or viscosity is eliminated. Spectral interference results from the overlapping of resonance lines and can be avoided by using a different resonance line. The use of Zeeman background correction also compensates for spectral interference and interferences from molecular absorption, especially when using the electrothermal atomisation technique. The use of multi-element hollow-cathode lamps may also cause spectral interference. Specific or non-specific absorption is measured in a spectral range defined by the band-width selected by the monochromator (0.2-2 nm).

## BACKGROUND CORRECTION

Scatter and background in the flame or the electrothermal atomisation technique increase the measured absorbance values. Background absorption covers a large range of wavelengths, whereas atomic absorption takes place in a very narrow wavelength range of about 0.005-0.02 nm. Background absorption can in principle be corrected by using a blank solution of exactly the same composition as the sample, but without the specific element to be determined, although this method is frequently impracticable. With the electrothermal atomisation technique the pyrolysis temperature is to be optimised to eliminate the matrix decomposition products causing background absorption. Background correction can also be made by using 2 different light sources, the hollow-cathode lamp that measures the total absorption (element + background) and a deuterium lamp with a continuum emission from which the background absorption is measured. Background is corrected by subtracting the deuterium lamp signal from the hollow-cathode lamp signal. This method is limited in the spectral range on account of the spectra emitted by a deuterium lamp from 190-400 nm. Background can also be measured by taking readings at a non-absorbing line near the resonance line and then subtracting the results from the measurement at the resonance line. Another method for the correction of background absorption is the Zeeman effect (based on the Zeeman splitting of the absorption line in a magnetic field). This is particularly useful when the background absorption shows fine structure. It permits an efficient background correction in the range of 185-900 nm.

## CHOICE OF THE OPERATING CONDITIONS

After selecting the suitable wavelength and slit width for the specific element, the need for the following has to be ascertained:

- correction for non-specific background absorption,
- chemical modifiers or ionisation buffers to be added to the sample as well as to blank and reference solutions,
- dilution of the sample to minimise, for example, physical interferences,
- details of the temperature programme, preheating, drying, pyrolysis, atomisation, post-atomisation with ramp and hold times,
- inert gas flow,
- matrix modifiers for electrothermal atomisation (furnace),
- chemical reducing reagents for measurements of mercury or other hydride-forming elements along with cold vapour cell or heating cell temperature,
- specification of furnace design (tank, L'vov platform, etc).

## METHOD

Use of plastic labware is recommended wherever possible. The preparation of the sample may require a dissolution, a digestion (mostly microwave-assisted), an ignition step or a combination thereof in order to clear up the sample matrix and/or to remove carbon-containing material. If operating in an open system, the ignition temperature should not exceed 600 °C, due to the volatility of some metals, unless otherwise stated in the monograph.

Operate an atomic absorption spectrometer in accordance with the manufacturer's instructions at the prescribed wavelength. Introduce a blank solution into the atomic generator and adjust the instrument reading so that it indicates maximum transmission. The blank value may be determined by using solvent to zero the apparatus. Introduce the most concentrated reference solution and adjust the sensitivity to obtain a maximum absorbance reading. Rinse in order to avoid contamination and memory effects. After completing the analysis, rinse with *water R* or acidified water.

If a solid sampling technique is applied, full details of the procedure are provided in the monograph.

Ensure that the concentrations to be determined fall preferably within the linear part of the calibration curve. If this is not possible, the calibration plots may also be curved and are then to be applied with appropriate calibration software.

Determinations are made by comparison with reference solutions with known concentrations of the element to be determined either by the method of direct calibration (Method I) or the method of standard additions (Method II).

*METHOD I - DIRECT CALIBRATION*

For routine measurements 3 reference solutions and a blank solution are prepared and examined.

Prepare the solution of the substance to be examined (test solution) as prescribed in the monograph. Prepare not fewer than 3 reference solutions of the element to be determined, the concentrations of which span the expected value in the test solution. For assay purposes, optimal calibration levels are between 0.7 and 1.3 times the expected content of the element to be determined or the limit prescribed in the monograph. For purity determination, calibration levels are the limit of detection and 1.2 times the limit specified for the element to be determined. Any reagents used in the preparation of the test solution are added to the reference and blank solutions at the same concentration.

Introduce each of the solutions into the instrument using the same number of replicates for each of the solutions to obtain a steady reading.

**Calculation**. Prepare a calibration curve from the mean of the readings obtained with the reference solutions by plotting the means as a function of concentration. Determine the concentration of the element in the test solution from the curve obtained.

*METHOD II - STANDARD ADDITIONS*

Add to at least 3 similar volumetric flasks equal volumes of the solution of the substance to be examined (test solution) prepared as prescribed. Add to all but 1 of the flasks progressively larger volumes of a reference solution containing a known concentration of the element to be determined to produce a series of solutions containing steadily increasing concentrations of that element known to give responses in the linear part of the curve, if possible. Dilute the contents of each flask to volume with solvent.

Introduce each of the solutions into the instrument, using the same number of replicates for each of the solutions, to obtain a steady reading.

**Calculation**. Calculate the linear equation of the graph using a least-squares fit and derive from it the concentration of the element to be determined in the test solution.

VALIDATION OF THE METHOD

Satisfactory performance of methods prescribed in monographs is verified at suitable time intervals.

*LINEARITY*

Prepare and analyse not fewer than 4 reference solutions over the calibration range and a blank solution. Perform not fewer than 5 replicates.

The calibration curve is calculated by least-square regression from all measured data. The regression curve, the means, the measured data and the confidence interval of the calibration curve are plotted. The operating method is valid when:

- the correlation coefficient is at least 0.99,
- the residuals of each calibration level are randomly distributed around the calibration curve.

Calculate the mean and relative standard deviation for the lowest and highest calibration level.

When the ratio of the estimated standard deviation of the lowest and the highest calibration level is less than 0.5 or greater than 2.0, a more precise estimation of the calibration curve may be obtained using weighted linear regression. Both linear and quadratic weighting functions are applied to the data to find the most appropriate weighting function to be employed. If the means compared to the calibration curve show a deviation from linearity, two-dimensional linear regression is used.

*ACCURACY*

Verify the accuracy preferably by using a certified reference material (CRM). Where this is not possible, perform a test for recovery.

**Recovery**. For assay determinations a recovery of 90 per cent to 110 per cent is to be obtained. For other determinations, for example, for trace element determination the test is not valid if recovery is outside of the range 80 per cent to 120 per cent at the theoretical value. Recovery may be determined on a suitable reference solution (matrix solution) which is spiked with a known quantity of analyte (middle concentration of the calibration range).

*REPEATABILITY*

The repeatability is not greater than 3 per cent for an assay and not greater than 5 per cent for an impurity test.

*LIMIT OF QUANTIFICATION*

Verify that the limit of quantification (for example, determined using the 10 $\sigma$ approach) is below the value to be measured.

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## 2.2.24. ABSORPTION SPECTROPHOTOMETRY, INFRARED

Infrared spectrophotometers are used for recording spectra in the region of 4000-650 $cm^{-1}$ (2.5-15.4 μm) or in some cases down to 200 $cm^{-1}$ (50 μm).

APPARATUS

Spectrophotometers for recording spectra consist of a suitable light source, monochromator or interferometer and detector.

Fourier transform spectrophotometers use polychromatic radiation and calculate the spectrum in the frequency domain from the original data by Fourier transformation. Spectrophotometers fitted with an optical system capable of producing monochromatic radiation in the measurement region may also be used. Normally the spectrum is given as a function of transmittance, the quotient of the intensity of the transmitted radiation and the incident radiation. It may also be given in absorbance.

The absorbance ($A$) is defined as the logarithm to base 10 of the reciprocal of the transmittance ($T$):

$$A = \log_{10}\left(\frac{1}{T}\right) = \log_{10}\left(\frac{I_0}{I}\right)$$

$T$ = $\frac{I}{I_0}$,

$I_0$ = intensity of incident radiation,

$I$ = intensity of transmitted radiation.