Method OIV-MA-BS-08 : R2009

Type IV method

ABV by near infrared spectroscopy in spirit drinks of viti-viniculture origin

(OENO 6/94;
OIV/OENO 382A/2009)

1. Introduction

This method of determining the real alcoholic strength by volume of alcoholic beverages and distillates is based on the physical principle of the spectral analysis of materials with absorption bands in the near infrared range.

Ethanol has this characteristic.

The spectral data of the sample being tested are compared with those obtained during an initial calibration covering the entire measurement range.

Spectrometers employing this principle are commercially available to perform this determination.

2. Object and scope of application

The purpose of this document is to describe a method for determining the real alcoholic strength of alcoholic beverages and distillates handled at atmospheric pressure.

The application of the method is restricted to products with a viscosity of less than around 15,000 mm^2/S (1 mm^2/S = 1 cSt) at the test temperature.

The analysis of alcoholic beverages whose composition is estimated to be close may nevertheless require a separate initial calibration for each of them.

With reference to the currently applicable regulations, the test temperature is set to 20°C.

3. Definition

Real alcoholic strength by volume (See pycnometry).

4. Principle

4.1. Physical principle

According to quantum theory, a molecule is capable of absorbing light energy according to Planck's formula:

$$\Delta = \frac{h.C}{E2 - E1}$$

Where:

- h = Planck constant
- C = speed of light
- E1 = fundamental energy state of the electron
- E2 = excited energy state of the electron,

From which it follows that the energy absorbed is proportional to the frequency of the incident light.

Near-infrared spectroscopy is a physical method of analysis based on the absorption of hv photons with very little energy that can be used to change the vibrational energy of molecules.

The number of v' waves is proportional to the v "frequencies" and hence to the hv energy of the photon.

The observed transitions correspond to vibrations of clearly identified groups of atoms.

They result in non-separable rays clustered into "bands" (band spectra), which can often be used for functional analysis.

In practice, only three transitions can be observed:

- transition from v = 0 to v = 1 with a high intensity,
- transition from v = 0 to v = 2 with a low intensity,
- transition from v = 0 to v = 3 with a negligible intensity,
- v = vibrational quantum number.

The corresponding three spectral bands are approximately at v (fundamental), 2v (first harmonic), 3v (second harmonic) frequencies.

The fundamental vibrations can be observed in the mid-infrared range, while the harmonics are only visible in the near infrared range (700 - 2500 nanometres).

In addition to the harmonics, combination bands can be observed, when several vibrations interact, resulting in bands whose frequency is the sum or difference of multiples of the fundamental frequencies.

$$v\,\text{comb.} = n1v1 \pm n2v2 \pm n3v3 \pm \ldots$$

Spectroscopy in the near infrared range spans the entire electromagnetic spectrum from 780 nm to 3000 nm. In this range, it is possible to study the transitions, harmonics and low-energy electronic combinations of the stretching and deformation vibrations of hydrogen bonds (C - H, N - H, O - H); the latter have high frequencies and are suitable for quantitative analysis applications.

Although it does not necessarily enable the characterisation of a complete structure, a near infrared spectrum provides useful information about the hydrogen clustering of a molecule.

Accordingly, near IR spectroscopy can be used first of all for the quantitative determination of components comprising clusters such as C - H, O - H or N - H, i.e. including water, alcohols, phenols , etc., preferably for the characterization of molecular structures.

Generally, the C - H cluster is characterized by stretching fundamental bands between: 3.0 and 3.6 microns, stretching bands of the first harmonics, between 1.6 and 1.8 microns and stretching bands of the second harmonics between 1.1 and 1.2 microns.

Because of the influence of other functional clusters of the molecule, these bands are liable to undergo shifts.

The vibrations of N - H secondary movements are characterized by stretching fundamental bands at 2.9 microns, the first harmonics at 1.5 microns and the second

harmonics at 1.0 microns. The N - H cluster has a highly characteristic band of combinations around 2.2 microns.
The O-H cluster has

- a fundamental stretching band at 2.8 microns,
- the first harmonics at 1.4 microns and
- the second harmonics at 1.0 microns;

in addition, there is

- a combination band around 2 microns.

Bands of this type are used for the quantitative determination of various organic components, both monomeric and polymeric.
Absorption bands in the near infrared range

2500 nm	_____ C-H	Combinations
2200 nm	_____ O-H N-H	Combinations
1800 nm	_____ C-H	First harmonics
1600 nm	_____ N-H H-O	First harmonics
1420 nm	_____ C-H	Combinations
1300 nm	_____ C-H	Combinations
1100 nm		

4.2. Principle de measurement

4.2.1. A sample of a few millilitres of liquid is introduced into a measuring cell thermostated at the test temperature.

4.2.2. The sample is exposed to infrared radiation whose wavelengths have been previously selected by a primary calibration specific to the analyte.

With regard to ethanol, these wavelengths are generally four to five in number.

4.2.3. The beam of the light source is located through a collimator and a "chopper" directly on a filter wheel, selected automatically by the microprocessor.

The monochromatic light is then directed either to the measuring cell or to the reference by a tilting mirror.

The infrared rays penetrate the sample, interact with its components and are then reflected to the detector(s).

4.2.4. The spectral data of the sample are processed by the microprocessor integrated with the spectroscope and compared with calibration curves that have been determined and stored beforehand.

The regression equation is of the type:

$$\%C = F_0 + F_1R_1 + F_2R_2 + \ldots + F_nR_n$$

Where

- % C = percentage strength by volume,
- F - Fn = constants corresponding to ethanol,
- R_1 - Rn = spectral reflection values measured at wavelengths from 1 to n.

After about a minute, the result is displayed by the apparatus. It is directly expressed as a percentage strength by volume.

5. Apparatus

5.1. NIR Spectroscopes

Two variants of apparatus suitable for measuring the real alcoholic strength of alcoholic beverages are available on the market.

They differ mainly by the type of measuring cell used.

Transflectance principle: the bottom of the measuring cell is fitted with a reflector which reflects the light beam having passed through the sample; the light can then interact again with the sample. This combination of transmission and reflection is called transflectance.

Transmission principle: the measuring cell is mounted vertically. The sample is directly crossed by the ray of light.

5.2. The apparatus consists of the following items:

- sample pumping system,
- light source,
- wavelength selection appliance,
- thermostated measuring cell,
- detectors converting light energy into an electrical signal,
- computer system to process the signals and display the results.

5.2.1. Sample pumping system.

The sample is injected into the measuring cell using a peristaltic pump. Some spectroscopes can be fitted with an automatic sample changer.

5.2.2. Light source.

Thermal light sources are mainly used. In tungsten filament lamps, the most commonly used, the filament is heated to 2100□C by the transformation of electrical energy. The polychromatic light obtained has a spectrum ranging between 320 nm and 2500 nm. It is necessary to control very precisely the intensity of the light in order to obtain repeatable measurements.

5.2.3. Wavelength selection appliance

Three basic principles are commonly applied: interference filters, tilting filters, and grating monochromators.

Interference filters are constituted by a layer of a semi-transparent material, such as magnesium fluoride, placed between two semi-reflecting layers. Under these conditions, the incident light interferes inside the filter and only certain wavelengths, depending on the thickness of the transparent layer, pass through the appliance.

Market-available appliances include three to twenty filters whose central wavelengths have been carefully selected.

The filters are attached onto a thermostated wheel, whose rotation is controlled by microprocessor.

Tilting filter systems take into account the fact that the wavelength selected by an interference filter depends on the angle of incidence between the ray of light and the filter. By varying this angle, it is possible to select different wavelengths around the central value. A large number of spectral measurements can thus be obtained with only a few filters. The correspondence between the position of the wheel carrying the filters and the measurement wavelength is, however, difficult to establish.

Grating monochromators are polished mirrors on which numerous parallel grooves are etched. The grooves diffract light and act as light sources that are phase-shifted in relation to each other. This causes light interference, as in the case of filters, and can be used to select wavelengths by turning the grate. Spectral measurements can be taken every 2 nm between 1100 and 2500 nm, providing 700 measurement points for each sample.

5.2.4. Thermostated measuring cell.

The optical walls are made of quartz.

Temperature control of the chamber comprising the cell is obtained by means of an integrated semiconductor element which uses the Peltier effect.

5.2.5. Detectors.

The detectors most commonly used are made of lead sulphide. These are semiconductors whose resistance decreases as the incident light intensity increases. They operate in the spectral range from 1000 nm to 2500 nm.

5.2.6. Data processing and display.

The spectral data are first of all collected in the form of continuous electrical signals. In general, the raw data includes successive measurements of the light intensity of the source (I0) and that of the sample (I). These data are converted analogously into absorbance values [log (I0/I)] and digitized.

On the simplest appliances, digitized spectral data are not stored but are used to predict the response variable, which is immediately displayed.

On other systems, the spectrometer is coupled to a microcomputer which can be used to store spectral information, manage data files, and carry out the mathematical and statistical analyses.

6. Products

6.1. Products to clean the measuring cell

Cell cleaning is recommended at the end of each series of measurements and can be performed using the following solutions:

- Aqueous solution of sodium hypochlorite (NaClO), at market-available concentration, diluted to one tenth,
- Laboratory glassware cleaning agent, suitably diluted.

Cleaning should be followed by prolonged rinsing with freshly prepared, distilled or demineralised water.

6.2. Calibration substances.

The calibration substances must be chosen such that:

- The values of their alcoholic strengths by volume cover those of the products to be measured,
- they are based on a common matrix, except for their alcohol content, with identical compositional characteristics. The nature and composition of the matrix are essential in ensuring the reliability of the NIR spectroscopy technique.

Given the large number of samples required for calibration, their alcoholic strength by volume may be determined beforehand using:

- the reference method,
- the areometric method using EC Class I alcoholmeters, - the electronic densimetry method.

(see the description of these methods).

Remark: The determination of alcoholic strength by volume according to one of these two methods is, where appropriate, performed after distilling the sample.

The analytical application in the near infrared range resulting from this calibration will have at best an accuracy equivalent to that of the method used.

7. Calibration of apparatus

7.1. The implementation of a quantitative analysis technique in the near infrared range involves several levels of calibration:

- initial calibration in order to select, according to statistical calculations, a variable number of significant wavelengths of the characteristic (ethanol) to be analyzed,
- periodic re-calibration to verify the reliability of the calibration equation,
- routine calibration to correct the bias of the reference curve. It should be performed before each series of measurements.

7.2. Initial calibration.

This operation requires the use of a spectroscope in the near IR capable of performing measurements in a series of twenty successive wavelengths.

The connection between the spectral data and the characteristics that are to be predicted by near NIR spectroscopy are often difficult to determine.

The spectral bands overlap to a considerable degree and it is usually impossible to

establish an analytical application of the single measurement of the height of a significant peak. On the contrary, a calibration procedure must be used that is quite complex to implement. The calibration is only valid for the determination of the alcoholic strength by volume of alcoholic beverages with strictly identical compositional characteristics. There are six steps in the process.

7.2.1. Establish a set of representative samples and analyze them using the reference method.

The collection of standard solutions must include thirty to fifty individual samples and cover the entire concentration range encountered in practice.

In addition, they must be divided into concentration classes of approximately the same size.

7.2.2. The collection is divided into two separate lots: one is used for calibration, the second for verification purposes.

7.2.3. Carry out the spectral measurement of the calibration collection: each standard solution must be analyzed twice in succession (double sampling).

7.2.4. The alcohol concentration values obtained by the reference method are entered on a microcomputer equipped with software for statistical computing.

The values are then correlated with the spectral measurements.

7.2.5. A multi-linear regression program is used to establish the following relation on the calibration samples:

$$C = a_0 + a_1 r_1 + a_2 r_2 + a_3 r_3 + \ldots$$

where

- C, is the characteristic being measured,
- a, a_1, a_2, ... : are the regression coefficients,
- r_1, r_2, r_3, ... : are the spectral reflectance measurements at wavelengths: L_1, L_2, L_3

...

Two to ten wavelengths are selected from among those that are available, based on statistical criteria. The residual error of calibration is calculated: it must be small compared with the standard deviation of the characteristic being studied.

$$\sqrt{\sum_{i=1} \frac{n(di-d)^2}{(n-k-1)}}$$

where

- d_i = difference between the concentrations obtained by the reference method and those obtained by NIR spectroscopy
- d = average di
- n = number of samples used for calibration
- k = number of calibration wavelengths.

7.2.6. Carry out the spectral measurement of the verification collection and apply the equation with the values obtained.

Compare the residual error of verification with that for the calibration: they should be close.
Standard deviation of prediction:

$$\sqrt{\sum_{i=1} \frac{n(di-d)^2}{(n-1)}}$$

where

- n = number of standard solutions used to verify the calibration,
- di = difference between the concentrations obtained by the reference method and those obtained by NIR spectroscopy,

- d = average di.

When the wavelengths have been selected and the calibration has been performed, and the results are recognized as being statistically consistent, the analytical method can be routinely applied.

7.3. Periodic recalibration.

Aging of the electronic components, repairs, parts replacement, or other abnormalities, require periodic recalibration of the equipment.

Similarly, the transfer of the calibration process from one appliance to another requires periodic recalibration.

Recalibration involves adjusting the bias and sometimes the slope of the initial calibration equation.

This procedure does not affect the selection of wavelengths.

In practice, in order to limit the sources of error, it is preferable to analyze the spectrum of ten representative samples covering the entire calibration range. These standard substances are recognized beforehand using the reference method.

In this case the multilinear calibration equation becomes a simple linear equation:

$$Y + K + mX = F_0 + m\,[F_1 log 1/R(\lambda 1) + F_2 log 1/r(\lambda 2) + \ ...]$$

where

- F, is the bias,
- m, is the slope.

7.4. Calibration of routine bias correction.

This correction must be made before any series of measurements, and at least once a day.

Using NIR spectroscopy, analyse a standard solution whose alcoholic strength by volume has been determined beforehand using the reference method.

The bias value is adjusted by assigning it the difference obtained between the measurement of the reference method and that of the spectroscopic method. The

difference can be negative or positive.

8. Procedure

8.1. Preparation of test apparatus.

Place the spectroscope:

- on a perfectly stable support, isolated from any vibrations.
- away from direct sunlight,
- free from corrosive vapours, magnetic fields, and large variations in temperature.

After connecting the apparatus to a power source, allow it to warm for at least thirty minutes.
Fill the thermostat unit of the measuring cell with a coolant in accordance with the manufacturer's instructions. Set the temperature in order to reach and maintain the requisite test temperature.

8.2. Measurement of alcoholic strength by volume.

8.2.1. If necessary, select the spectroscopic method corresponding to the alcoholic beverage to be analyzed.

8.2.2. Check the cleanliness of the measuring cell:

- no particles in the cell,
- if necessary, clean the window using a brush and a soft cloth dampened with ethyl alcohol.

8.2.3. Calibrate the bias correction in accordance with the method described in point: 7.40.

8.2.4. Filter the sample first, if necessary.

8.2.5. Rinse at length the measuring cell with the alcoholic beverage to be tested.

Filter if necessary.

8.2.6. Carry out the determination. After approximately one minute, the result is displayed on the easy-to-read display.

8.2.7. Carry out five determinations in a row for the same sample: (the use of this analytical technique allows measurements to be obtained in a very short period of time).

The value of the alcoholic strength by volume of the sample is based on the calculation of the average for the five determinations.

Note: The five determinations must result in homogeneous values, in all cases covering the range of accuracy of the reference method used.

In the opposite case, carry out a second complete series of measurements after checking the cleanliness of the measuring cell and if necessary re-calibrating the bias of the calibration curve.

8.2.8. Check the relevance of the measurement accuracy; determinations performed in series should include the periodic analysis of a standard solution recognized by the reference method.

The cycle is to be respected, under the conditions described above, involves the analysis of a standard after five determinations.

8.2.9. Clean and rinse the measuring cell at length, at the end of the analysis.

CALIBRATION OF AN INFRALYSER FOR THE DETERMINATION OF THE ALCOHOLIC STRENGTH BY VOLUME ON BRANDIES OR LIQUEUR WINES

1. Selection of filters

As an example, the filter selections listed below can be used to measure the alcoholic strength by volume in the following alcoholic beverages:

1.1. Wine brandies aged in wooden casks

1.1.1. First combination.

- 2310 nm
- 1778 nm
- 2100 nm
- 1680 nm

1.1.2. Second combination.

- 2310 nm
- 2230 nm
- 1769 nm
- 1940 nm
- 1680 nm

1.2. "Pastis" aniseed-flavoured alcoholic beverage.

- 2270 nm
- 2230 nm
- 1769 nm
- 1940 nm
- 1680 nm

Use of an infralyser for WINE BRANDIES
Select the following filters: 4, 13, 14 and 20.
Four levels of alcoholic strength by volume are calibrated on raw brandies:

1 : 37.5 to 43 % vol.

2 : 42.5 to 47.5 % vol.

3 : 57.5 to 62 % vol.

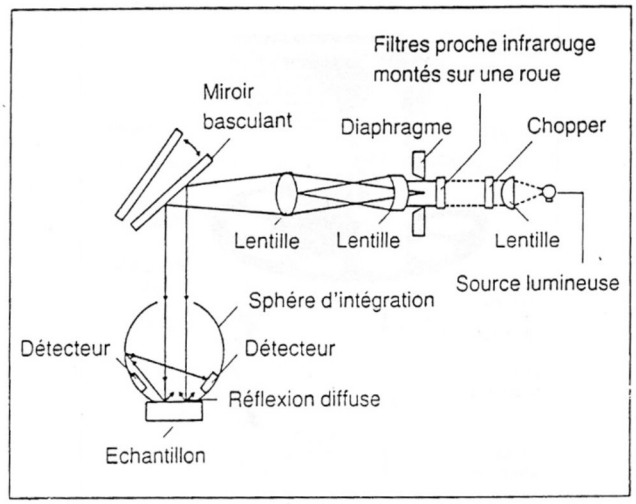
4 : 67.5 to 72.5 % vol.

For each level, and by increments of 0.5% vol., 10 to 11 determinations of the alcoholic strength by volume based on the pycnometric method are used to calibrate the infralyser

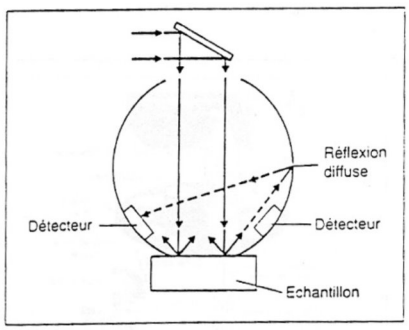
2. Bibliography

1. Calibration of an infralyser for the determination of the alcoholic strength by volume on brandies or liqueur wines.
2. Station viticole, Cognac National Interprofessionnel Bureau

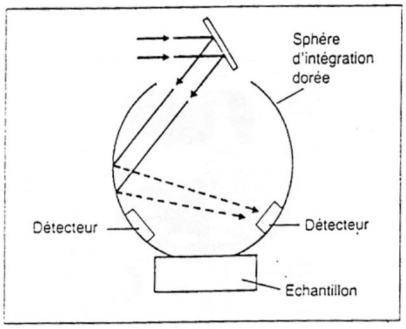
CONCEPTION DES SPECTROSCOPES DANS LE PROCHE I.R.
UTILISANT LE PRINCIPE DE MESURE PAR TRANSFLEXION



SCHEMA DE PRINCIPE



Mesure de l'échantillon



Mesure de la référence

CONCEPTION DES SPECTROSCOPES DANS LE PROCHE I.R.
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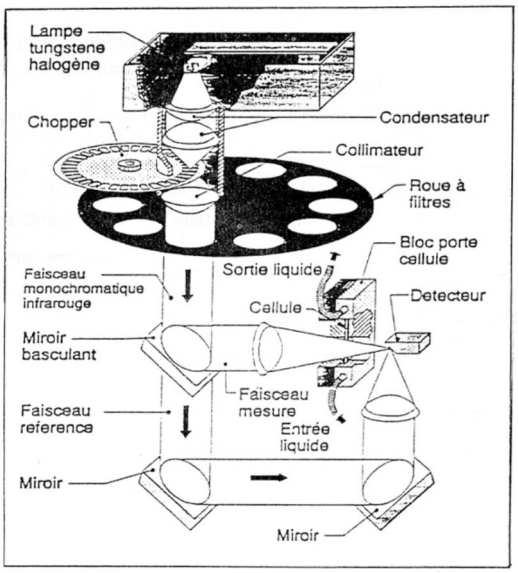

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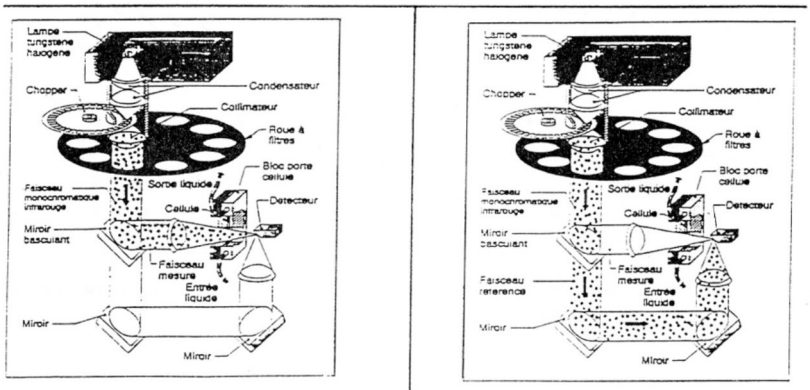

Mesure de l'échantillon

Mesure de référence

80