



## **RESOLUTION OIV/OENO 390/2010**

### **GUIDELINES ON INFRARED ANALYSERS IN OENOLOGY**

The GENERAL ASSEMBLY

In view of article 2 paragraph 2 iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

On the proposal of the Sub-commission of Methods of Analysis,

CONSIDERING the Resolution oeno 10/2005 "Practical guide for the validation, the quality control, and the uncertainty assessment of an alternative oenological analysis method" which makes it possible for the laboratories to ensure the traceability of the internal automated method to the OIV reference method,

CONSIDERING that this guide also includes quality control procedures for the results obtained by these automated methods, enabling secure use.

CONSIDERING that only the methods published in the book of international wine and must analysis methods of the OIV or in the book of international grape-based spirits' analysis methods of the OIV act as official reference guides and are to be used to settle any disputes that may arise.

DECIDES to adopt and to publish separately the appended guidelines for the use of infrared analysers in oenology

### **GUIDELINES ON INFRARED ANALYSERS IN OENOLOGY**

*Warning; this guide is not a reference document and is only provided for its informative value. Unlike the methods contained in the book of international wine and must analysis methods of the OIV or in the book of international grape-based spirits' analysis methods of the OIV, the methods contained herein cannot be considered as reference methods. Only the methods published in the book of international wine and must analysis methods of the OIV or in the book of international grape-based spirits' analysis methods of the OIV are official and can be used for the settlement of any possible dispute that may arise.*

These analysers exploit the characteristic absorptions of the organic compounds in wines and musts in the infrared range to carry out quantification. There are two main families used in oenology:

- Near-infrared analysers (NIR) ;

- Fourier transform infrared analysers (FTIR).

## 1. Near-infrared analysers

### 1.1. Principle

The compounds to be titrated are subjected to near-infrared radiation, in which they demonstrate characteristic absorption bands. The spectral data of the analysed sample are compared to those obtained with the reference standard wines used for the initial calibration of the instrument, and the concentration of the sought compound is calculated using a multiple linear regression. The instrument is computerised and can be paired with a sampler. The level of absorption must be high enough that the results are operable, and the method is applicable only for the macro-compounds of wine or must, i.e. mainly ethanol and sugars. The main advantages of this method are its simple implementation, its high analytical rate and the fact that no preparation of the sample is required, except a decarbonation of fermenting musts.

### 1.2. Apparatus

The apparatuses used in oenology work by reflection. The bottom of the flow cell containing the wine or must to be analysed is equipped with a reflector which reflects the incident infrared ray, thus crossing the sample a second time before being analysed by the detector. The apparatus includes the following pieces.

#### 1.2.1. Pumping system for the sample

Usually, a peristaltic pump is used to fill the measuring cell. The pumping system is generally complemented by a constant-temperature water bath enabling adjustment of the sample temperature to the value required for the measurement.

#### 1.2.2. Light source

A tungsten lamp producing polychromatic light with a spectrum ranging from 320 to 2500 Nm. The power supply must be perfectly stabilised in order to ensure constant intensity.

#### 1.2.3. Wavelength selector

The instrument used in oenology uses interferential filters of known wavelengths or array monochromators to select wavelengths characterising the sought compounds.

#### 1.2.4. Measuring cell

The part which the incident and reflected radiations pass through is made of quartz. The bottom of the cell can be made of a gold plated ceramic, which is reflectable. This cell is maintained at a constant measurement temperature, in general by means of a Peltier effect system.

#### 1.2.5. Detectors

Two lead sulphide photocells collect the reflected radiation.

#### 1.2.6. Computer

The computer carries out the mathematical and statistical processing ensuring the comparisons with the instrument calibration and the determination of the required concentration.

### 1.3. Procedure

The implementation of a near-infrared analyser comprises several steps.

#### 1.3.1. Initial calibration

In this step, a permanent calibration is carried out of the instrument which will be used as a reference. The step requires the use of the highest possible number (at least 50) of wines or musts of known concentrations in the analyte. The values of these concentrations must be evenly distributed over the entire desired measuring scale. The matrices must be as close as possible to those of the wines or musts which will later be analysed. For each calibration sample, a measurement is made for a maximum number of wavelengths covering the near-infrared spectrum. A multilinear regression is carried out from the recorded results, making it possible to establish the following relation:

$$C = K_0 + K_1R_1 + K_2R_2 + K_3R_3 + \dots + K_iR_i$$

Where:

- C is the sought concentration value
- $K_0$  is a typical constant of the instrument for a sought compound, whatever the wavelength used.

- $K_i$  is a constant for an instrument, a sought compound and a given wavelength.
- $R_i$  is the expression of the spectral measure for  $\lambda_i$  wavelength

For each analyte sought, 2 to 10 characteristic wavelengths are selected.

The quality of calibration is then tested by running a new series of samples of reference wine or musts of known concentrations.

### 1.3.2. Periodic calibrations

Periodic calibrations are necessary when routine checks show a drift in the results attributable to the equipment (ageing of the electronic components, repair, substitution of parts, etc.). This procedure does not call into question the wavelength selection, but provides a new calculation of the  $K_0$  et  $K_i$  constants.

### 1.3.3. Routine bias corrections

Before each use of the equipment, one (or more) control sample(s) of known concentration in analyte is/are analysed. If a bias is found in relation to the expected value, a correction can be made.

## 2. The Fourier transform infrared analysers (FTIR)

### 2.1. Material and principle of the method

The material implemented is an interferometer using Fourier transform infrared spectrophotometry, which scans the whole infrared spectrum in a spectral band of 2,000 to 10000  $\text{cm}^{-1}$  covering part of the near and middle infrared. After calibration of the instrument for various organic compounds, the spectrum analysis can simultaneously titrate such compounds in the analysed wine or must.

### 2.2. Interferometry and Fourier transform

Interferometry is an alternative to the traditional methods of acquiring a spectrum in a given wavelength scale. These techniques are cumbersome and time-consuming if a fine resolution is required. Interferometry makes it possible to simultaneously process all the wavelengths emitted by a single infrared source, without previous selection, allowing the acquisition of a complete spectrum within one second.

The first step consists in carrying out an interferogram of the sample to be analysed. The interferometer works by separating polychromatic infrared light on a slide (produced in this case by an incandescent filament). Before reaching the detector, the

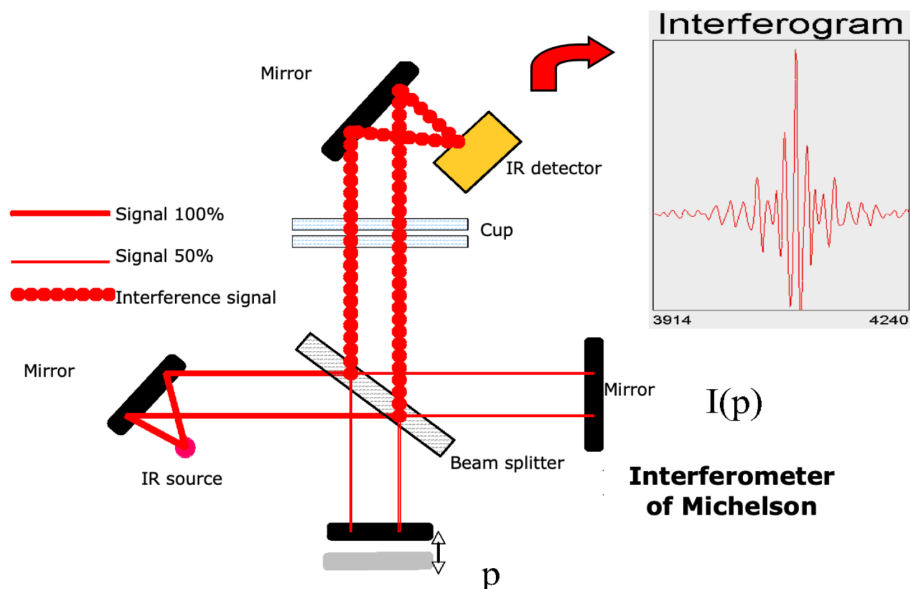
two parts of the signal will follow a different path: the first part directly crosses the sample, the second part is reflected onto a mobile mirror before returning to the sample.

Each elementary wavelength of this infrared emission will have a phase difference  $p$  when reaching the detector. Thanks to the mobile mirror, this phase difference  $p$  will continuously vary during the measurement.

The final signal obtained is thus an interference of two light signals of the same wavelength with a phase difference  $p$ .

Depending on the phase difference, the recombination will be either constructive or destructive, i.e. the interference signal will vary in intensity in relation to  $p$ . This intensity variation according to phase lag is called an interferogram. Its mathematical model is an integral.

The Fourier transform is a mathematical procedure which will determine, on the basis of the interferogram, the intensity of the signal according to the wavelength and then reconstitute the infrared spectrum. This sophisticated calculation can only be carried out using a powerful data-processing set-up.



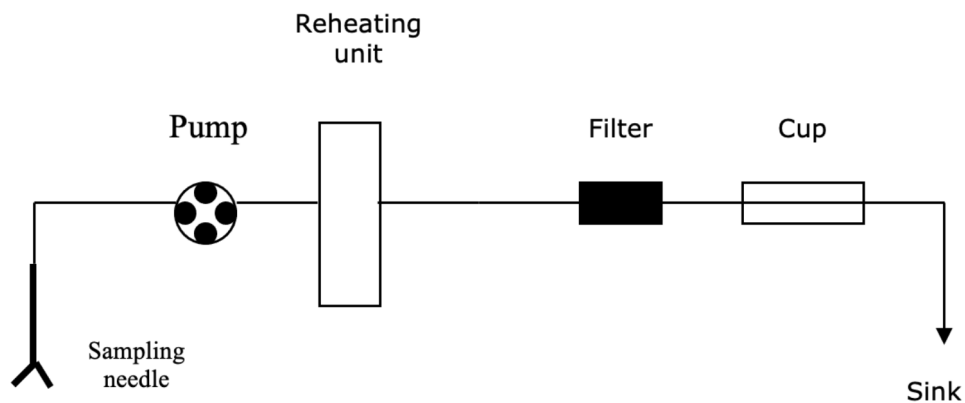
**Figure 1: Simplified diagram of a Michelson interferometer**

Now that calculated spectrum has been acquired, it is possible to turn to traditional spectrophotometry applications which use the specific absorption wavelengths of the various organic compounds whose concentration is being sought.

### 2.3. Implementation of the acquired data from the infrared spectrum

The sample to titrate does not require any specific preparation. However, in the case of musts or wines with a high level of sediment, clarification by centrifugation or filtration will be carried out in order to avoid clogging. For carbon dioxide contents greater than 750 mg/l, a preliminary partial removal is necessary to avoid degasification problems in the analysis circuit.

The circuit begins with a sample needle, which can be operated manually or controlled by a sample changer. A peristaltic pump transfers the sample into a heating chamber where it is heated to 40 °C. After passing through a filter, it crosses through the measuring cup. The latter is an essential part of the instrument. It is made in fluorosilicates since glass and quartz often absorb significant amounts of middle infrared. The sample is then be drained into the sink.



**Figure 2: Simplified diagram of the analytical circuit**

The complete cycle for a sample lasts 30 seconds. The automated version allows an effective rate of about 120 samples per hour.

### 2.4. Chemometrics

Generally, the middle infrared spectrum of a wine or a must contains information of

analytical interest which is not immediately extracted. In most cases, it requires very sophisticated mathematical processing methods. All the means which may be implemented to obtain this analytical information form part of a recent science: chemometrics. Chemometrics was defined in 1996 by GELADI as "the science of the use of mathematical, statistical and data processing methods to extract relevant information from chemical measurement data". Chemometrics therefore includes statistical methods appropriate to the processing of spectral data.

Analysts may seek to attain two main objectives:

The overall description of the analysed product (exploratory method), which involves classifying spectra according to predefined families. One application in oenology is to define the matrix of a product submitted to analysis, enabling determination of whether the product is a must, a fermenting must, a dry wine, a liquorous wine or a natural sweet wine.

This predictive approach identifies, from previously acquired bench-mark data (calibration), analytical values of compounds or indices characterising the composition of an unknown wine or must. This second approach is the most commonly used by laboratories at present, as it allows substitution of the FTIR for traditional analytical tools.

Numerous chemometric methods can be applied to obtain the results described above, but their implementation requires expert knowledge. They may be divided into two main groups according to whether they are based on a linear or non-linear model. They range from simple statistical tools such as principal component analysis (PCA) to highly sophisticated tools, both mathematically and in terms of difficulty of use, such as neural networks.

While all manufacturers offer optical measuring instruments which perform satisfactorily, the means of standardising such measurements, the tools used to process spectrum data and the quality of chemometrics tools available to users vary greatly. The difficulties encountered by laboratories are often related to this point. In addition, part of the controversy surrounding the method is rooted in this variability. The quality of the chemometric treatment used to read the spectral data of a wine or must is of major importance to the production of reliable and accurate analytical results.

## **2.5. Middle infrared spectrum analysis**

It is advisable to become informed about the very great complexity of information contained in the middle infrared spectrum of a wine or a must, which is due to the following:

1. the organic and mineral composition of these products is among the richest encountered in the agrifood sector.
2. the absorption zones of each organic molecule in the middle infrared are multiple because of considerable resonance phenomena.
3. the interactions between the various organic compounds result in significant matrix effects which cannot be modeled.

Therefore, contrary to other spectrophotometric analyses, theoretical knowledge about the absorption of a given compound can very seldom be applied to the FTIR analysis of musts and wines. Using an experimental approach is the only possible solution. This point is a fundamental characteristic of the method, which is based on a purely mathematical approach critical to the quality of the final results. The procedure of this experimental approach to calibration consists in multiplying the acquisition of spectra for a maximum number of sample varieties, the values of which are precisely known for the compound or the index to be analysed, before applying the mathematical statistical methods. It should be noted that this step is vital to obtaining an efficient analysis tool. Not only must the operator have as many representative samples as possible at their disposal, they must also implement the most appropriate chemometric methods and, if possible, carry out a critical comparative analysis of the results obtained with several of them. Such an approach must always be supplemented by a very broad statistical comparison of the results obtained by the selected model to results from reference analytical methods. Several thousands of determinations may be required for this last step. Calibration is therefore a cumbersome and delicate step which requires significant time and means.

To better understand the complexity of this approach, it is necessary to explain the following critical points:

#### 2.5.1. Behaviour of the analyte

All organic compounds in wine do not have the same analytical behaviour for several reasons:

- First, it is obvious that compounds with a high concentration (e.g. over 1 g/L) are likely to be easier to titrate because they engage more absorption phenomena.
- Secondly, the absorption capacities characterising a compound depend on its molecular constitution. Some molecules, such as carbon dioxide for instance, have a high absorption capacity in middle infrared whereas other molecules, although



more complex, absorb far less.

- Lastly, the influence of other compounds in the wine (matrix effect) is an essential detail. It is easy to see that if the absorption wavelengths characterising a compound overlap within the spectrum with those of water or ethanol, which are present in very high proportion, the measurement sensitivity for this compound will be greatly weakened. In the same way, interaction phenomena between components can move the absorption bands.

It must be conceded that, once again, theoretical knowledge is of little use and only the experimental approach can determine whether or not a given compound can be easily titrated by FTIR. For example, the titration of sugars is delicate for values lower than 1 g.L<sup>-1</sup>, whereas titration of ammonia nitrogen (NH<sub>3</sub>) can be performed from 10 mg.L<sup>-1</sup>.

These data on the behaviour of analytes indicate that it is easier to analyse a compound with high concentration, demonstrating very specific absorption characteristics in the middle infrared, outside the absorption zones of water and ethanol, and with sensitivity to matrix effects, than a compound demonstrating the opposite characteristics. Therefore, ethanol will be much easier to analyse than ethanal. In the same way, it has become evident that the more difficult it is to access a compound, the more difficult the calibration will be. In particular, the initial sampling will have to be sufficiently wide and perfectly selected, the chemometric tools more powerful and applicable, and the comparison with the reference methods carried out on a higher number of samples.

### 2.5.2. Extrapolation

Extrapolation is a vital component. During calibration, it is not possible to rely on the extrapolation capacity of the system. Therefore, if the initial sampling does not evenly cover the entire intended analysis field, the results obtained in the zones with the least initial information will contain gaps. In the same way, if a significant matrix effect exists, such as that often caused by phenolic compounds, it is essential that wines with different concentrations in phenolic compounds are present at all levels of the range. Failing this, the part of the range not covered by all the possible values of phenolic compounds will be more sensitive to matrix effects and will not be reliable.

### 2.5.3. Matrix effects

As noted above, the matrix effect is due to the other compounds present in the medium to be analysed, and their absorption in the middle infrared. It is possible,

however, to overcome the problem of the matrix effect. All calibrations can take into account multiple matrix effects. To do so, like expert systems, the tool must be provided with the necessary information to be able, through training, to measure the impact of the matrix effect on the titration of the sought compound. Once found in an unknown sample, the matrix effect can be removed by reference to the calibration value. At this stage, it is easy to see that the higher the number matrices are covered during a calibration, the more robust the calibration will be and the more it will escape matrix effects. This is one of the main advantages of the FTIR method. One limitation does exist, however. Increasing the capacity to manage the matrix effect introduced into a calibration proportionally reduces sensitivity and accuracy. The operator developing a calibration must therefore find a balance between robustness, sensitivity and precision. Once again, this approach quickly becomes very complex and implementation requires significant resources.

In oenology, it seems impractical to work with calibrations covering all the matrices likely to be encountered. This said, the state of the art allows, for the most common determinations, the division of wines and musts into five basic matrices:

- unfermented musts,
- fermenting musts,
- dry or mildly sweet wines,
- liquorous wines,
- natural sweet wines.

On the other hand, it should be noted that the matrix effects produced by different vine varieties, the colour of the wine or geographical origin are, in general, identified by well performed calibrations.

The complexity of the matrix effects has another important consequence: it is impossible to use synthetic or reloaded samples for calibration or control; the method is only practical for natural wines.

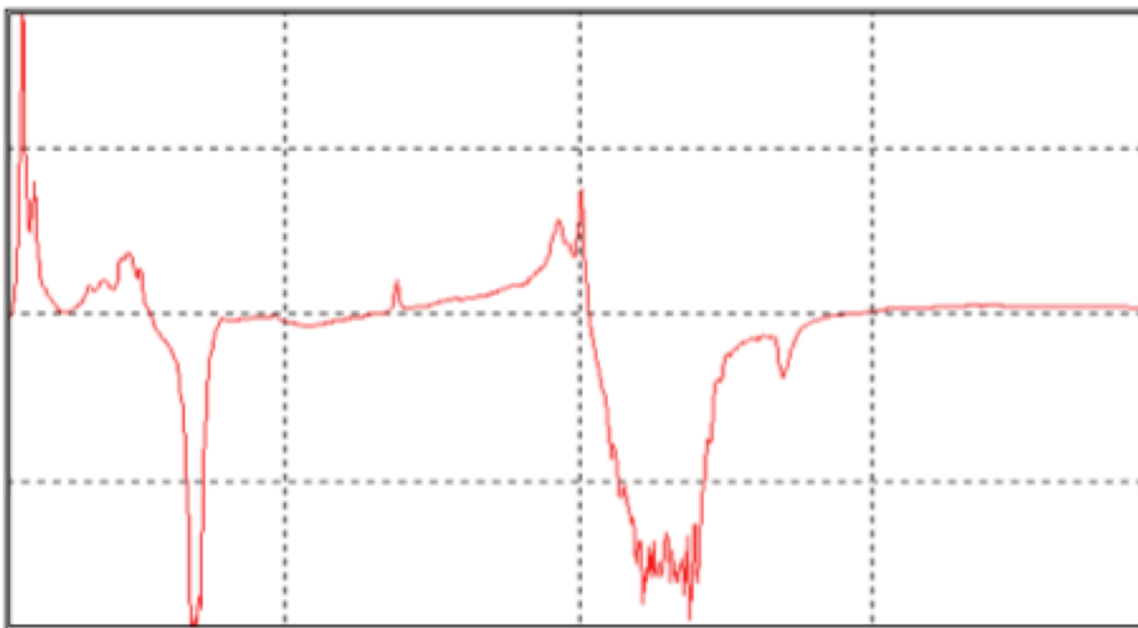
#### 2.5.4. Reference values

The quality of a calibration also depends on the quality of the reference values of the samples used. Gluconic acid is a good example. The enzyme titration method for gluconic acid is not reliable. Its lack of robustness is not mentioned in related literature and is probably due to unidentified matrix effects. The first FTIR calibrations

were carried out from results obtained with this method, and although gluconic acid demonstrates very qualitative absorption characteristics, the results obtained by FTIR turned out to be poor. New calibrations were later carried out, using more reliable techniques as reference values such as capillary electrophoresis, and high-quality results have now been obtained by FTIR.

## 2.6. Principal steps of spectral analysis

Figure 3 illustrates the infrared spectrum of a wine.



**Figure 3: Example of the middle infrared spectrum of a wine**

Using the data bank compiled from reference samples with a known value for the sought analyte, the implemented chemometric tools enable the selection of the most relevant spectral zones for such analyte (usually about ten zones), and then to calculate a regression model which will be used for prediction for any unknown sample.

## 2.7. The importance of controlling the quality of the results

Considering what has been stated above, it should be kept in mind that the use of FTIR requires that the laboratory implements a tool to control the quality of the results, which necessitates a strategy which is both targeted and original. The considerable

power of the system automation, combined with the ease of implementation of the machine, can indeed ensare users in serious traps in terms of drift in the results or unidentified outlying results. Only a very complete and strict system providing for the identification of matrices, the removal of results involving samples not in line with precise definitions, the introduction of a sufficient number of controls into sample series, and the perfect management of collected data can ensure quality results. Laboratories remain aware that with the FTIR, the time assigned to the quality control of the results represents a much larger share of the total time spent in analysis than with the other methods usually implemented in oenology laboratories.

2.7.1. A control strategy of result quality may include the following:

2.7.1.1. Work range

Validated work ranges must be established for each analytical parameter. All results other than the work range must be confirmed by validated methods which serve to support the FTIR performance.

2.7.1.2. Maximum spread admitted for two repetitions

This procedure must be introduced upon the implemntation of the new FTIR tool. The laboratory may then suspend the application if results obtained are satisfactory.

There are two readings are carried out on each sample. The readings are accepted if the spread is less than the maximum spread type established for each analytical parameter. The determination for each parameter is repeated if the reading spread goes over the established maximum standard deviation.

2.7.1.3. Reference material (RM)

Reference materials must present matrixes in accordance with wines analysed. They are used to verify the stability of the analytical system over time. The RM reference values are established based on analysis certificates (materials derived from interlaboratory circuits) or based on results obtained with methods used for the calibration of FTIR. Tolerances are associated with reference values to establish « alert limits » and « action limits ». The work serie must encompass several RM respresentative of amtrices analysed and the work range. The work sequence is validated if all the results of the MR are included in the alert limits. If the RM results are between the alert limit and the action limit, the analytical sequence is valid. the following items must nevertheless be verified including:

1. if the RM has evolved (for example due to bad storage)

2. the possible presence of systematic spreads which then require a calibration adjustment. In this case, the most recent results obtained by crossing over methods should be used (if possible daily results) (2.2.7.4). The situation must be assessed in accordance with all information available.

If one or several results of the RM analytical parameters are above the action limit, the analytical sequence is not validated. In that case, steps should be taken to:

1. Confirm abnormal result(s) using methods which serve to support IRTF performance
2. Normalise spectral conditions of FTIR apparatus.
3. Analyse other RM available to verify if the readers respect the established criteria.
4. To confirm whether the crossing of methods background (2.2.7.4) demonstrates that a calibration adjustment needs to be carried out (systematic spreads) for an abnormal parameter.

The situation must be weighed in accordance with all information available. In particular, the bias should not be corrected based solely on information brought by RM measurements.

#### 2.7.1.4. Crossing over methods

It is very important to systematically cross over results obtained by FTIR and by reference methods which serve to support performance. It is recommended to cross over methods daily for sensitive parameters.

Differences must be less than or equal to action limits (2.2.7.3). If the difference between the results obtained for an analytical parameter goes over the limit, the following items should be considered:

1. Find a probable explanation (for example: matrice not covered by calibration);
2. Carry out cross over of methods with other samples of the work series;
3. Confirm whether the cross over methods background demonstrates an adjustment of the calibration which needs to be carried out (systematic spreads) for an abnormal parameter.

### 2.7.2. Acceptable criteria of a new calibration

It is important to have criteria defined to replace a validated calibration and applied to a new calibration.

One of the possible strategies is to consider the following points:

1. The new calibration must include more observations than the number of calibrations used;
2. The “cross validation error” (CVR) of the new calibration must ideally be less than or equal to the previous calibration. A higher CVR may however be accepted if the least performing calibration zone is reinforced;
3. For a group of 15 wines representative of the work range, results obtained should be compared using a new calibration, with results obtained using a previous calibration and with methods which serve to support FTIR performance.
4. It is recommended to carry out a comparison of results obtained with a previous calibration and results obtained using a new calibration involving the introduction of external reference material. It is however important to ensure that these aforementioned results involve undoped natural wine and musts and not stabilised with compound which could alter the matrix.

## APPENDIX 1

### EXAMPLE OF NEAR INFRARED ANALYSER BASES TITRATION METHODS COMMONLY USED IN OENOLOGY (NIR)

The methods hereunder are used as examples

#### 1. Titration of the alcoholic strength of wines and fermenting wines and musts

##### 1.1. Principle

A wavelength range of 1150 Nm to 1200 Nm shows a specific absorption making it possible to determine the alcoholic strength of alcoholic beverages. In this interval, no other compound interferes with the titration. Two points at the ends of the range

make it possible to determine the base line. The calculation of the alcohol concentration is carried out using a simple algorithm. The measurement can be made for a scale ranging from 0 to 20%vol.

## **1.2. Reactive agents and reference samples**

1. Decarbonated distilled water.
2. Hydroalcoholic solutions with alcoholic strength determined following distillation by measuring mass density

## **1.3. Equipment**

A near-infrared spectrometer is used with a specific calibration to measure the alcoholic strength in fermenting wines and musts.

## **1.4. Sampling**

Samples must be stabilised beforehand at a temperature close to 20°C. The analysis must be carried out shortly after opening the samples.

It is advisable to eliminate surplus carbon dioxide by filtration on high porosity filter paper or by any other means.

## **1.5. Procedure**

Before any measure, the spectrometer must be adjusted by running distilled water and a hydroalcoholic solution of known strength.

Before any measure, the spectrometer must be adjusted by running distilled water and a hydroalcoholic solution of known strength.

## **1.6. Calculation**

The instruments are equipped with computation software which gives the result directly in % vol. to 2 decimals.

## **1.7. Validation of the method**

### **1.7.1. Interlaboratory comparison between titration by near-infrared method following the distillation in wines**

The main objective of this study was to compare the results obtained using the near-infrared method and those obtained using the OIV reference method by distillation. 14

laboratories listed in Table 1 took part in this comparison. Each laboratory was identified by a letter between A and N. 10 different wines coming from 5 European countries were submitted to laboratory examination. Their alcoholic strength covered a scale ranging from 9 to 14%vol. Each analysis, by the reference method or the near-infrared method was carried out 3 times by each laboratory. Table 2 gives the detail of the various wines including the dry or sweet white wines and dry red wines.

*Table 1: List of samples*

Country	Colour of wine	Alcoholic proof as indicated on label	Dry/sweet	Sample number
Portugal	white	9%vol	dry	1
France	white	11,5%vol	dry	2
Italy	white	12,5%vol	dry	3
Austria	white	13,0%vol	dry	4
Austria	white	10,0%vol	sweet	5
Hungary	white	13,0%vol	sweet	6
Italy	red	11,5%vol	dry	7
Austria	red	12,0%vol	dry	8
France	red	13,0%vol	dry	9
Austria	red	14,0%vol	dry	10

### 1.7.2. Results and validation of conclusions

The search for outlying values using the GRUBS test is negative for type 1 outliers.

The results of the measurement of the alcoholic strength by volume on a broad sampling of wines show that the values obtained by the near infrared spectrometer



are identical to those obtained by the reference method by distillation. The repeatability and reproducibility values are similar to the two methods. Results are given in Table 2.

*Table 2 : Summary of representativeness parameters*

parameter	All values		After removal of aberrant values	
	NIR:	Distillation:	NIR	Distillation:
r	0,0396	0,0638	0,0328	0,0639
sr	0,0142	0,0228	0,0117	0,0228
R	0,1705	0,1865	0,1136	0,1500
sR	0,0609	0,0666	0,0406	0,0536

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## APPENDIX 2

### NEAR-INFRARED ANALYSER-BASED TITRATION METHODS COMMONLY USED IN OENOLOGY

The methods described hereunder are given as examples. The number of methods continues to rise because new calibrations are continuously carried out. The most important methods currently being developed can be separated into three distinct categories.

#### 1. "Direct" methods

These methods involve the titration of organic compounds for which the correlation between molecular absorption and concentration can be considered as direct. The following table summarises the methods commonly used.

Analyte	Measuring scale	Intralaboratory reproductibility	Interlaboratory reproductibility
Glucose and fructose	0 to 400 g.L-1	1.3 g.L-1	
Malic acid	0.3 to 6 g.L-1	0.15 g.L-1	0.6 g.L-1

Lactic acid	0.3 to 6 g.L-1	0.15 g.L-1	0.6 g.L-1
Carbon dioxide	60 to 1300 mg.L-1	95 mg.L-1	230 mg.L-1
Glycerol	0.2 to 10 g.L-1	10%	
Tartaric acid	1 to 15 g.L-1	10%	
Gluconic acid	0.1 to 15 g.L-1	10%	

## 2. "Indirect" methods

In oenology two different cases are encountered.

1. The sought molecule does not have significant absorption in the near-infrared method either by nature, or because concentrations are too weak.
2. The research does not involve a specific molecule, but an index indicating a balance resulting from a complex set of organic compounds.

For the first case, we shall take the example of potassium titration. The potassium ion is not absorbed in the infrared range. Absorptions which are detected by chemometric methods and which are correlated with its concentration in the wine or analysed must be in fact those of organic compounds which are in balance with this ion.

For the second case, we shall take the example of pH or total acidity measurements, which are highly reliable, and are measured by taking a reading, in the infrared spectrum, of the complex balances of organic compounds responsible for these index values.

Method	Measuring scale	Intralaboratory reproductibility	Interlaboratory reproductibility
Density	0.0012 to 1.4000 g.cm <sup>-3</sup>	0.00015 g.cm <sup>-3</sup>	0.0010 g.cm <sup>-3</sup>
Alcoholic strength by volume	8 to 16%.vol.	0.10%.vol.	0.21%.vol.

Total acidity	3 to 33 g.L <sup>-1</sup> in tartaric acid	0.11 g.L <sup>-1</sup> in tartaric acid	0.15 g.L <sup>-1</sup> in tartaric acid
Volatile acidity	0.30 to 0.96 g.L <sup>-1</sup> in acetic acid	0.036 g.L <sup>-1</sup> in acetic acid	0.12 g.L <sup>-1</sup> in acetic acid
pH	2.6 to 4.7	0.06	0.12
Potassium	600 to 8000 mg.L <sup>-1</sup>	10%	
Index of total phenolic compounds	15 to 100	14	
Ammonia nitrogen	5 to 250 mg.L <sup>-1</sup>	16%	
$\alpha$ -amino nitrogen	5 to 250 mg.L <sup>-1</sup>	10%	

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