REFRACTOMETRY - R 124

Uncertainty budgets and mpes in refractometry: A project study

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Introduction

Refractometers are instruments which measure the refractive index of grape must before fermentation, using the phenomenon of light refraction or of total internal reflection of light. These instruments may also be used to measure the refractive index of sugar solutions and, possibly, of concentrated must. A means of predicting the alcoholic strength of the wine made from the must is thus available.

Refractometers may ensure an appropriate level of credibility of measurement results on the condition that they are subject to legal metrological control. This can be achieved by complying with the requirements of OIML R 124 *Refractometers for the measurement of the sugar content of grape must* [1].

However, to improve the accuracy of measurement results, the relationship between uncertainty budgets and maximum permissible errors (mpe's) should be evaluated.

This paper contributes to improving the understanding of uncertainty components when metrological control of refractometers is applied, and presents the methods used to prepare and certify the reference solutions employed as working standards. Evidence is also given of the traceability chain and of the links to national measurement standards.

Based on a preparation and measurement process, an evaluation of uncertainty sources was carried out in accordance with the Eurochem Guide [2], which builds on the *Guide to the Expression of Uncertainty in Measurement* (GUM) [3]. The influence of repeatability and reproducibility were estimated and seem not to deviate from confidence intervals. The homogeneity and stability testing between (and within) bottles was evaluated by statistical analysis using an adequate, fully nested design. The relationship between the mass fraction and the refractive index of sugar has been addressed by several authors, in particular by Jaulmes (see OIML R 124, clause 13, page 7). According to his work the correlation between the sugar content and the refractive index can be defined as follows:

$c = 6844\ (n - 1.3358)$ for $n \leq 1.3706$ and

$c = 6712\ (n - 1.3351)$ for $n > 1.3706$

where:

c is the concentration of sugar; and
n the refractive index of the sample.

When used for determining the sugar content of grape must, refractometers are provided with an additional scale where the mass fraction is related to the expected alcoholic content by:

$T = C/K$

where:

C is the mass fraction of the sucrose solution;
T is the expected alcoholic content that a must can produce after fermentation; and
K is the chemical equivalence factor.

The basic principle governing the scale of refractometers for the wine industry is the stoichiometric transformation of the sugar in the grape into ethanol. Available information indicates that the chemical equivalence factor K is between 16.5 % and 17.7 %. Community legislation was put into force by a tabulation of the values of the refractive index of sucrose-water solutions and the alcoholic strength at 20 °C. These values were adopted from ICUMSA [4].

In the wine industry the sugar content of grape is determined by measuring the refractive index of the solution and referring the result to a standard curve. It is based on the principle of Abee refractometers where the adjustment was in agreement with ICUMSA and the relation between the concentration of sucrose in sucrose-water solutions and the refractive index n was established at a wavelength of 589 nm at a temperature of 20 °C.

As a consequence, the refractometry method of measurement has resulted in the widespread use of the units of mass fraction, i.e. sugar (sucrose) content and % vol., i.e. ethanol content. Invariably, these units are used in routine industrial measurements.

Metrological control

Refractometry has been designated as the reference method to measure the sugar content of grape must in Portugal. A survey of refractometer use for grape must was introduced in 1992. Since then all measuring systems used in the trade of grape for the wine industry must fulfil the performance requirements laid down by law; as a consequence, reliability of measurements is ensured. Therefore such instruments are traceable to national primary measurement standards. The refractometers used for this purpose must obtain pattern approval at IPQ and must pass initial and subsequent verifications (carried out by verification bodies). Refractometers located at wine cellars are checked once a year before the grapes are picked, the objective being to maintain the errors of the instruments as close as possible to the initial range. Within the framework of the Portuguese Metrological System, IPQ is also responsible for maintaining, disseminating and guaranteeing international traceability to SI units.

For this purpose IPQ prepares national standards, from which secondary standards are derived and certified, which are disseminated for calibration in industry and government laboratories. The performance of this framework is assessed annually. Commercial laboratories which are accredited under the National Measurement System and which use nationally traceable reference solutions for instruments are subject to regular accuracy checks using nationally traceable reference standards.

Traceability is assured by means of standard solutions which are produced gravimetrically from pure reagents and then disseminated. Sucrose is the indicated sugar, although hydrolyse of polysaccharides to hexose sugars (fructose and glucose) is very fast. Their disadvantage is that it is impossible to store these solutions, even for a few hours. As a consequence the sucrose-water solutions can be used neither as transfer standards, nor as reference solutions for checking the performance of the measuring system.

Recently, legal metrology has addressed these needs through OIML Recommendation R 124. The OIML suggests the use of glucose solutions as transfer standards; these solutions are stabilized by adding allyl isothiocyanate and tartaric acid. The mass fraction of the glucose solution is determined using a standard refractometer calibrated at 20 °C with solutions of chemically pure saccharose prepared gravimetrically. In the case of glucose solutions, the values of temperature corrections are read from tables of saccharose, multiplied by 1.3. The standard uncertainty should not exceed 0.06 %.

Preparation and certification of reference materials

Due to the absence of international measurement standards, the solutions are prepared gravimetrically using sucrose from ultra pure water of 18 MΩ resistance. The weighing is performed on a calibrated balance; these calibrations are carried out regularly in accordance with the protocol validated by the mass department, ensuring traceability to the national mass standards. The temperature is monitored by thermometers calibrated by the Portuguese Primary Temperature Laboratory. The volumetric equipment used is calibrated by the National Metrology Laboratory and is used to express the uncertainty in the reference materials, either in mass fraction or in volumetric concentration units.

The preparation of the solution is performed in steady ambient conditions under normal atmospheric pressure and at a temperature close to 20 °C. In order to avoid temperature corrections, all the reagents and glassware are placed inside the room some time beforehand so that they attain ambient laboratory conditions. The humidity conditions are set at 50 % ± 10 % in order to prevent systematic errors during the weighing. For humidity values higher than 60 %, some sugar hydration can occur. The concentration of glucose can be expressed as:

$$C_{C_6H_{12}O_6} = \frac{1\,000 \times m_{C_6H_{12}O_6} \times P_{C_6H_{12}O_6}}{V_{\mathrm{f}} \times M_{C_6H_{12}O_6}}$$

The uncertainty evaluation of a typical solution is shown in Tables 1 through 4 (see page 10).

Calibration of the measuring system

The glucose solutions are used as transfer standards. The same facilities are used to prepare glucose and sucrose-water solutions. The refractive index of the glucose-water solution is determined in a measuring system calibrated by means of a sucrose-water solution.

As calibration is defined as a set of operations that establish, under specified conditions, the relationship between values of quantities indicated by a measuring instrument and values represented by a material measure or a reference material, this is the key step in obtaining metrological control in order to assure traceable links [5]. The calibration model chosen can then be inverted to be used to convert response data from the solutions(s) to be certified into the same units as the standards used for calibration [6]. For the application as described, rigorous statistical control is vital, and therefore indirect determination is not recommended.

A typical calibration curve is shown in Fig. 1. Based on these data, which are inverted prior to fitting, a quadratic equation was chosen as a calibration function whose coefficients are $b_2 = 7.41 \times 10^{-6}$, $b_1 = 1.369 \times 10^{-3}$ and

Table 1 Calculation of combined standard uncertainty of m

Variable X_i	**Estimate** x_i	**Uncertainty**	**Distribution**	**Standard uncertainty** $u(x_i)$	**Sensitivity coefficient** c_i	**Contribution** $u_i(y)$
Repeatability	460.001	3.00×10^{-4}	Normal	3.00×10^{-4}	1	3.00×10^{-4}
Resolution	0	1.00×10^{-4}	Rectangular	5.77×10^{-5}	1	5.77×10^{-5}
m	460.001					3.11×10^{-4}

Table 2 Calculation of combined standard uncertainty of M

Variable X_i	**Estimate** x_i	**Uncertainty**	**Distribution**	**Standard uncertainty** $u(x_i)$	**Sensitivity coefficient** c_i	**Contribution** $u_i(y)$
C	72.0642	0.0008	Rectangular	4.62×10^{-4}	6	2.77×10^{-3}
H	12.096	0.00007	Rectangular	4.04×10^{-5}	12	4.85×10^{-4}
O	95.9964	0.0003	Rectangular	1.73×10^{-4}	6	1.04×10^{-3}
$C_6H_{12}O_6$	180.1566					3.00×10^{-3}

Table 3 Calculation of combined standard uncertainty of V_f

Variable X_i	**Estimate** x_i	**Uncertainty**	**Distribution**	**Standard uncertainty** $u(x_i)$	**Sensitivity coefficient** c_i	**Contribution** $u_i(y)$
Specification	2 000 ml	0.6	Triangular	0.24	1	0.24
Repeatability	0	0.0062	Normal	0.0062	1	0.0062
Temperature	0	0.00525	Rectangular	0.0030	1	0.0030
Volume	2 000 ml					0.24

Table 4 Calculation of combined standard uncertainty of C_{stock}

Variable X_i	**Estimate** x_i	**Uncertainty**	**Distribution**	**Standard uncertainty** $u(x_i)$	**Sensitivity coefficient** c_i	**Contribution** $u_i(y)$
P	0.999	0.001	Rectangular	5.77×10^{-4}	1.28	7.37×10^{-4}
m	460.001	3.11×10^{-4}	Normal	3.11×10^{-4}	$2.77\ 10^{-3}$	8.62×10^{-4}
V	2 000	0.24	Normal	0.24	$-6.38\ 10^{-4}$	1.56×10^{-4}
M	180.1566	3.00×10^{-3}	Normal	3.00×10^{-4}	$-7.08\ 10^{-3}$	2.12×10^{-2}
C	1.275×10^{-3}					0.021×10^{-3}

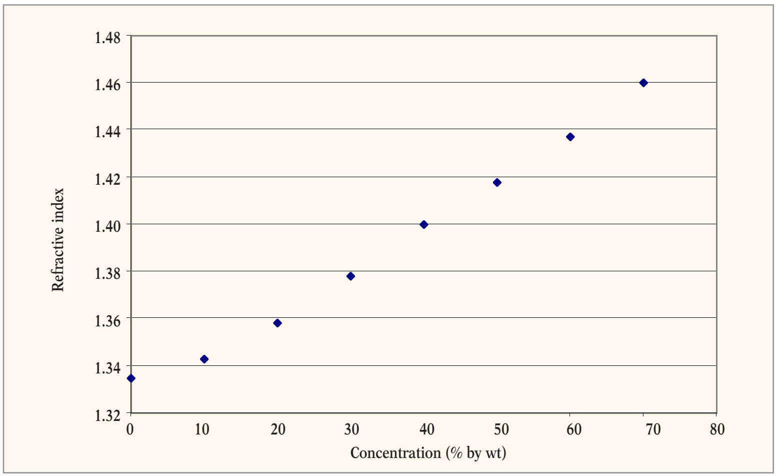

Fig. 1 Typical calibration curve

b_0 = 1.333356. The uncertainty from calibration for the solution(s) to be certified is computed from [7]:

$$(x_c) = G\,(y_c)$$

$$u(x_c)^2 = (\partial G / \partial y_c)^2\, u(y_c)^2 + \sum_{k=0}^{M} (\partial G / \partial b_k)^2 \operatorname{var}(b_k) +$$

$$+ 2 \times \sum_{k=0}^{M-1} \sum_{l=k+1}^{M} (\partial G / \partial b_k)(\partial G / \partial b_l) \operatorname{cov}(b_k, b_l)$$

Stability studies

The stability and homogeneity studies for glucose solutions were carried out in a reference refractometer calibrated daily using fresh sucrose solutions. For stability testing, two kinds of experiments were carried out.

First, a short-term stability study was undertaken in order to establish their validity for OIML pattern approval drift tests. Second, a long-term stability study was conducted to assess the shelf life and the effect of transport under normal conditions during metrological verifications in a wine cellar.

The protocol that specifies the storage and transport conditions was elaborated. The sampling was carried out using a nested design. For long-term stability studies the flasks were stored at room temperature for periods of 1, 3 and 5 months. The storage temperature limit condition was evaluated by thermal degradation. In this case the flasks were stored for ten days at 30 °C, 45 °C and 60 °C.

The performance of the glucose-water solution was tested using two different grades (high purity and normal glucose) and the stability protocol outlined above was applied. All the solutions prepared from the batch using normal glucose showed statistically significant degradation, in contrast to the high-grade solutions. Most high-grade solutions stored under the test conditions remained stable for over one year; only solutions stored at 60 °C were degraded, showing a lower refractive index value. Figure 2 shows the stability data for high-grade glucose solutions. The lower and upper limits of uncertainty (U = 0.02 %) have been estimated using the formula described by Pauwels [8]:

$$u_{\text{exp}}^2 = u_{\text{meas}}^2 = u_{\text{betw}}^2$$

where:

$$u_{\text{meas}} = \frac{s_{\text{method}}}{\sqrt{n}}$$

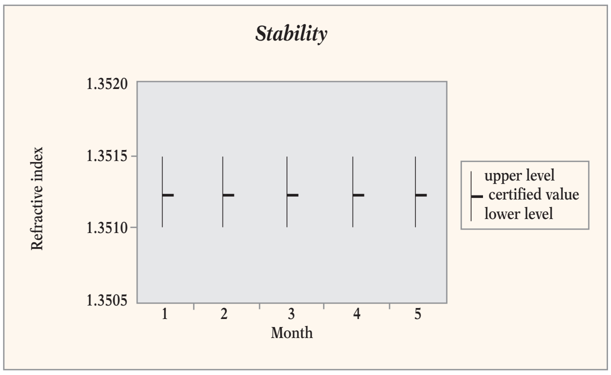

Fig. 2 Stability data for high-grade glucose solutions

The effect of instability for these solutions is so small that it can be concluded that this uncertainty component can be ignored, taking into consideration the repeatability of measurements.

Homogeneity tests

The homogeneity tests were carried out using a fully nested design. The assessment of the homogeneity was performed after filling, to permit all random process variations to be estimated. Five flasks were taken; from each of them, samples of 1 ml were withdrawn and their refractive index was determined. One flask was kept under normal conditions and divided into five layers. An aliquot was withdrawn from each and the refractive index determined. The overall protocol was applied to three different batches.

The refractometer was calibrated before use and checked at a refractive index of 1.3333. Three measurements were made against the refractive index of water. A statistical evaluation of the data was carried out by means of an F-Test. The variance of 5 results obtained on measurements in the 5 flasks was compared to the variance of 5 results obtained on the pooled aliquots from the flasks; no significant inhomogeneity was found.

The results of the homogeneity tests are presented in Figs. 3 and 4. No trends due to the filling sequences were detected. This good concordance between the sampling modes further confirms the reliability and feasibility of the method proposed by the OIML.

On-site measurements

The glucose solutions have been distributed to reference laboratories as working standards and are used on initial verification, verification after repair, periodic verification and supervision. The main tests are repeatability, drift and zero setting, using three different concentrations at the reference temperature of 20 °C. All the tests are repeated at least 5 times and the mean considered as being the reference value.

A database was constructed in which the test result conditions are stored, including the uncertainty from solutions and tests. This led to the conclusion that the uncertainty arising from solutions is lower compared to that arising from other sources such as instrument drift, temperature control, or zeroing.

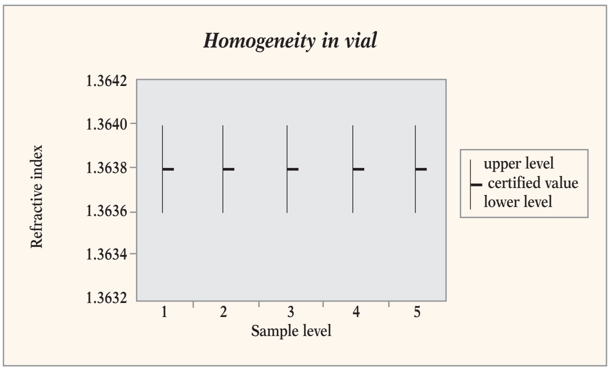

Fig. 3 Within-vial homogeneity test

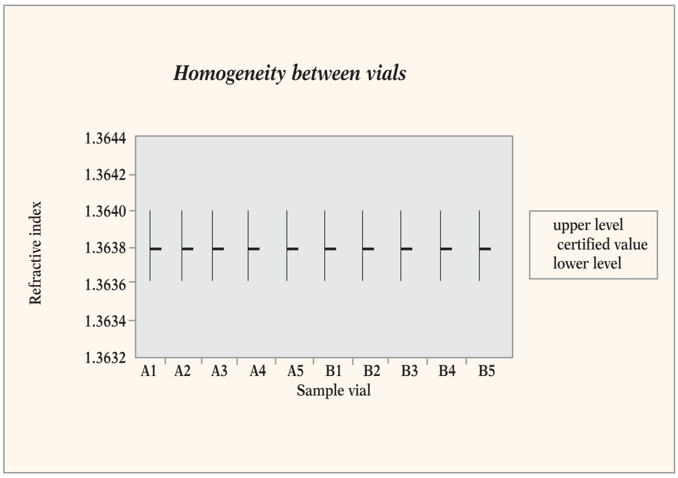

Fig. 4 Between-vial homogeneity test

Conclusions

The nature of wine and its commercial significance indicates the importance of regulations for its control. An EC Directive specifies that correspondence values for ethanol strength through the grape must be correct. In addition, Council Regulations lay down EC procedures for determining these values. OIML R 124 prohibits the placing on the market of refractometers with errors larger than one scale division.

In Portugal, regulations implemented for refractometers have led to better guarantees in trade. All the factors involved can be well tested using a glucose solution as the reference. Glucose solutions prepared according to the method indicated by OIML R 124 ensure the availability of accurate, repeatable and reproducible analytical procedures. The solutions allow the correct implementation of national regulations. International comparability of the results and their traceability according to the GUM concept provide a tool for harmonization of test results, support to laboratory accreditation, and last but not least, allow compliance limits to be set up with uncertainty contribution. ■

References

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