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VALIDITY OF THE SPECTROPHOTOMETRIC DETERMINATIONS AND THE NECESSITY TO APPLY THE 17025:2005 STANDARD IN ORDER TO OBTAIN CERTAIN RESEARCH RESULTS

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Abstract. *The implementation of the SR EN ISO 17025:2005 Standard –"Competence of the trial and calibration laboratories" has become a necessity for the laboratories that wish to generate valid results from a technical point of view. No issued result can be reliable without the compliance with the SR EN ISO 17025:2005. Thus, this study presents, by means of a concrete example, the main technical activities that need to be carried out before the spectrophotometric findings proper. The study aims to contribute to the training of the young researchers or to the consolidation of certain skills that are specific of the field of spectrophotometry.*

Keywords: *reliable results, spectrophotometry, validation, measurement uncertainty.*

1 Introduction

The SR EN ISO 17025:2005 is an international standard that applies for standardised methods, non-standardised methods and lab developed methods [1]. The need to apply this standard should be understood by any organisation or analyst who makes trials and/or calibrations [1]. Applying these standard ensure customer satisfaction [2].

Starting from a concrete example (the spectrophotometric determination of the content of nitrates in soluble water), this study presents the preliminary actions that need to be taken to obtain reliable results.

2 Preliminary activities required to obtain reliable spectrophotometric results

For reliable spectrophotometric results, a multitude of requirements need to be met in accordance with the 17025 Standard, as follows:

2.1 The use of qualified personnel

The persons who implement the working method in the laboratory need to be skilled, to understand the principle of the method and to possess instrumental chemical analysis knowledge, as the applied method is a spectrophotometric one.

2.2 The use of reagents, glassware and high quality consumables

Only high quality reagents will be used: those with a higher purity than 98%. They have to be within their guarantee period and they need to have quality certificates.

The glassware should be class A, measuring pipettes, burettes (class AS recommended) accompanied by quality certificates to allow knowledge of the tolerance and of the measuring precision for that respective lot. Moreover, the glassware used should be recalibrated internally for the safety of the volumes under work and to allow establish the filling variation. If the burettes are key equipment in a determination, then they need to undergo extra calibration performed by an acknowledged entity.

2.3 The use of certified reference materials

The verification using certified reference materials (CRM) and NIST (National Institute of Standards and Technology) traceability is mandatory. In this case, the reference material used was a standard 1000 mg/L NO_3^- nitrate solution, Merck Germany, CertiPur, lot HC388517, within its term of guarantee.

2.4 The exclusive use of calibrated, checked and benchmarked equipment

The spectrophotometer is the key equipment in this case. The spectrophotometer has to be

calibrated and checked by acknowledged organisations. It is of utmost importance for the calibration to be performed at the wave lengths at which the determinations are performed.

2.5 Ensuring the optimal ambient temperature

The ambient temperature should be within the 20 ± 2°C temperature range, which is the temperature at which laboratory glassware is calibrated.

2.6 Making the calibration curve

The calibration curve has to be drawn in a field that best matches the needs of the laboratory, in accordance with the concentration of the samples that are to be subjected to the trial process.

In order to determine the content of nitrates in the water, a calibration curve was plotted in the 1÷5 µg N range [4].

In order to plot the calibration curve, 9 calibration samples of increasing concentrations and with constant concentration increments in the working field were prepared. The processing of the standard samples was done according to the working manner described by the SR ISO 7890-3/2000 Standard, and the absorbance values corresponding to the standards are presented in Table 1.

2.7 Plotting the calibration curve and the evaluation of the calibration function

Based on the previously obtained data, the calibration curve is plotted (fig. 1).

Table 1. Absorbance of the standard solutions

Standard no.	Mass N (µg) (X_i)	Absorbance of the (Y_i) standard for λ = 415 nm, vat with optical path 40mm
1.	1	0,151
2.	1,5	0,222
3.	2	0,295
4.	2,5	0,362
5.	3	0,429
6.	3,5	0,492
7.	4	0,557
8.	4,5	0,627
9.	5	0,690
Average	**X_m=3**	**Y_m=0.425**

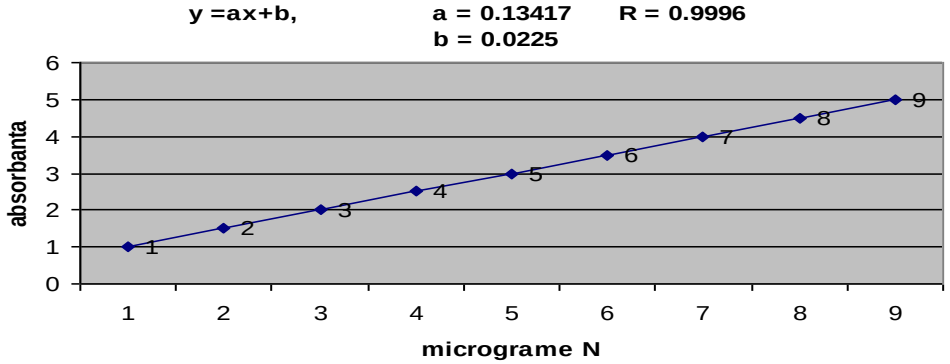

Fig. 1 The plotting of the calibration curve

Even if the calibration curve has a correlation coefficient of approximately 1, the calibration linear function needs to be evaluated statistically [3] as follows:

• *establishing the equation of the regression line*

$$y = ax + b$$
$$y = 0{,}13417x + 0.0225 \quad (1)$$

• *establishing the sensitivity of the method*

$$a = \frac{\sum_{1}^{10}(x_i - x_m)(y_i - y_m)}{\sum_{1}^{10}(x_i - x_m)^2} = 0{,}13417 \quad (2)$$

• *the control sample - calculated or ordinatelineat the origin*

$$b = y_m - ax_m = 0{,}022 \quad (3)$$

• *residual standard deviation, S_y*

The estimation accuracy of the method is given by the residual standard deviation – S_y, as a measure of the dispersion of the absorbance values around the calibration line:

$$S_y = \sqrt{\frac{\sum_{1}^{10}[y_i - (b + ax_i)]^2}{N-2}} = 0{,}0001 \text{ µg N} \quad (4)$$

where: N= the number of dots on the curve

• *standard deviation of the method, S_{x0}*

$$S_{x0} = \frac{S_y}{a} = 0{,}0007 \ \mu g \ N \qquad (5)$$

• *standard deviation of the intersection with theordinate line, S_b*

$$S_b = S_y \sqrt{\frac{1}{N} + \frac{x_m^2}{\sum_1^{10}(x_i - x_m)^2}} = 0{,}003 \qquad (6)$$

• *standard deviation of the slope, S_a*

$$S_a = \sqrt{\frac{S_y^2}{\sum_1^{10}(x_i - x_m)^2}} = 0{,}001 \qquad (7)$$

• *variation coefficient of the method, V_{x0}*

$$V_{x0} = \frac{S_{x0}}{x_m} x100 = 0{,}023\% \qquad (8)$$

• *dispersion homogeneity*

Repeated determinations are performed for the lowest concentration and for the highest concentration (table 2), their standard deviations (s) are calculated and the trial value is determined as follows:

$$PG = \frac{s_9^2}{s_1^2} \text{ pentru } s_9^2 > s_1^2 \qquad (9)$$

$$PG = \frac{s_1^2}{s_9^2} \text{ pentru } s_1^2 > s_9^2 \qquad (10)$$

PG is compared to the tabular values of the *F*distribution *(5.35 in this case).* If PG<F, the deviation between the s_1^2 and s_9^2 dispersions is not significant, but if PG>F, the deviation between the dispersions is significant.

PG = 1,56 → 1,56 < F (5,35)

• *the recovery process, R*

It is determined for different levels of concentrations (at least 3 levels) comprised in the working range.

3 solution samples of different concentrations are prepared and the concentration of the analyte (CI) in each of them is measured. Then each sample is enriched by adding a known amount of analyte (CA) and the concentration of the enriched samples (CF) is measured at the end.

The recovery percentage is determined as the ratio between the amount of analyte measured in the enriched sample and the theoretically added amount:

$$R = \frac{CF - CI}{CA} X100(\%) \qquad (11)$$

• *establishing the measuring accuracy,p*

$$p = s_r = \sqrt{\frac{\sum_1^n (x_i - x_m)^2}{n-1}} \qquad (12)$$

• *Repeatability limit,r*

It is calculated according to the equation:

$$r = t_{5\%}^{n-1} \text{ x } s_r \qquad (13)$$

where $t_{5\%}^{n-1}$: Student factor for the confidence level 95%, corresponding to the number of replicated trials.

• *Confidence range, I*

For the individual values, with a probability of 95%, the confidence range is calculated with the aid of the relation:

$$I = x_m \pm r \qquad (14)$$

• *establishing the detection limit, L_d*

In order to establish L_d, a series of 10 independent blanck samples are measured under repeatability conditions.

The detection limit is expressed as:

$$L_d = C_{mPM} + 3 \text{ x } s_r \qquad (15)$$

where: C_{mPM} = average concentration of the measured control sample series

• *establishing the quantification limit, L_c*

The quantification limit strictly corresponds to the lowest analyte concentration that can be determined with an acceptable amount of precision under repeatability conditions.

The quantification limit is established for each calibration curve by performing measurements on independent series of 10 samples of solutions with a low analyte content (strengthened control samples), situated in the range between L_d and the concentration of the first calibration point, under repeatability conditions:

$$L_c = 10 \text{ x } s_r \qquad (16)$$

where: s_r=standard deviation of the solution with low analyte content

• *standard uncertainty associated to the calibration curve*

$$S_x = S_{x0} \sqrt{1/N + 1/n + \frac{(y - y_m)^2}{a^2 \sum (x_i - x_m)^2}}$$

2.8 Data centralization

The verification using All the statistical data obtained are centralized under the form of tables (Table 2, Table3).

Table 2. Statistical data obtained

ple	Results (µg N)	Average, xm (µg N)	Standard deviation sr
Blanck sample	Ab: 0.016; 0.012; 0.013; 0.018; 0.016; 0.024; 0.022; 0.02	Abm = 0.0176	0,004
Solutions with a low analyte content	Ab: 0.034, 0.042, 0.034, 0.033, 0.034, 0.039, 0.029, 0.030, 0.039, 0.039 C: 0.153, 0.211, 0.153, 0.146, 0.153, 0.190, 0.190, 0.116, 0.124, 0.190	Abm = 0.0353 Cm = 0.1626	0.004 (Ab) 0.031 (C)
Standard 1µg N	Ab: 0.152, 0.150, 0.148, 0.150, 0.158, 0.155, 0.164, 0.153, 0.158, 0.155 C: 1.015, 1.001, 0.986, 1.001, 1.059, 1.037, 1.103, 1.023, 1.059, 1.037	Abm = 0.1543 Cm = 1.0321	0.004 (Ab) 0.034 (C)
Standard 2µg N	Ab: 0.291, 0.282, 0.292, 0.291, 0.290, 0.291, 0.292, 0.291, 0.288 C: 2.031, 1.965, 2.039, 2.031, 2.024, 2.031, 2.039, 2.031, 2.009	Abm = 0.2897 Cm = 2.0222	0.003 (Ab) 0.023 (C)
Standard 3µg N	C: 3,018; 3,025; 3,077; 3,096; 3,012; 3,022; 3,000; 3,025; 3,111; 3,012	Cm = 3,0398	0,039401
Standard 5µg N	Ab: 0.685; 0.683; 0.683; 0.689; 0.677; 0.681; 0.681; 0.672 C: 4,937; 4,922; 4,922; 4,967; 4,878; 4,907; 4,907; 4,840	Abm = 0.681 Cm = 4,91	0,005(Ab) 0,038(C)
Water sample	Ab: 0.413; 0.420; 0.414; 0.419; 0.414; 0.426; 0.413; 0.418; 0.419; 0.419 C: 2.923; 2,974; 0.930; 2.967; 2.930; 3.018; 2.923; 2.959; 2.967; 2.967	Abm = 0.417 Cm = 2.9558	0,004(Ab) 0,0299(C)

Table 3. Date statisice si concluzii

Sample	Repeatability limit r	Relative standardde viation RSD	Confidence range 95%	Conclusion
Blanck sample	0,009	0,227	0.0176±0.009 (Ab)	Range: 0.008÷0.026 (Ab)
Solutions with a low analyte content	0.009 (Ab) 0.070 (C)	0.19065	0.035±0.009 (Ab) 0.162±0.070 (C)	Range of detection: 0.026÷0.044 (Ab) 0.092÷0.232 (C) µg N
Standard 1µg N	0.009 (Ab) 0.076 (C)	0.03294	0.154±0.009 (Ab) 1.032±0.076 (C)	Range of cuantification: 0.145÷0.163 (Ab) 0.956÷1.108 (C) µg N
Standard 2µg N	0.006 (Ab) 0.046 (C)	0.01137	0.290±0.006 (Ab) 2.022±0.046 (C)	Precision 0.023
Standard 3µg N	0,089 (C)	0,01296	3,0398±0,089(C)	Precision 0,040
Standard 5µg N	0,076 (C)	0,00774	4,91±0.076 (C)	Precision 0.038
Water sample	0,009 (Ab) 0,067 (C)	0,01011	2,9558 ± 0.067 (C)	Precision 0.029

Table 4. Validation report

<table>
<tr><td>NAME OF TEST</td><td>Determination of the content of nitrates in soluble water - spectrophotometric method with sulfosalicylic acid – SR ISO 7890-3:2000</td><td rowspan="10">Specifications:
Law 458/02 republished, Law 311/04, Table 3, HG 925/05, SR ISO 7890-3:2000

1. Maximum allowable concentration = 50 mg/L NO_3^-
2. The sensitivity of the method: for ρ_N=0,2 mg/ L N we obtain an absorbance 0,68 unit., for a sample volume of 25 ml
3. The limit of detection: 10% of the maximum allowable concentration→5 mg NO_3^- / L
4.Precision: 10% of the maximum allowable concentration→ 5 mg NO_3^- / L
5. Accuracy: 10% of the maximum allowable concentration →5 mg NO_3^- / L</td></tr>
<tr><td>RESPONSIBLE FOR TEST</td><td>Chemist Nicoleta Damian</td></tr>
<tr><td>PERFORMER</td><td>Name</td></tr>
<tr><td>THE PLACE</td><td>Laboratory Transilvania University</td></tr>
<tr><td>WORKING PROCEDURE</td><td>The paper Laboratory, code xxx</td></tr>
<tr><td>ANALYTE</td><td>Nitrogen on NO_3</td></tr>
<tr><td>MEASUREMENT EQUIPMENT</td><td>Spectrophotometer UV-VIS T60 New Century PG Instruments Ltd., series 04-1650-18-0189
Measuring range: 190-1100 nm
Record sheet code xxx</td></tr>
<tr><td>REAGENTS USED</td><td>Preparation of working solutions: The paper Laboratory, code xxx
Identification documents:
Quality Certificate / batch / shelf life
Specification for acquisition
Note reception
Data Storage</td></tr>
<tr><td>REFERENCE MATERIALS</td><td>Nitrate standard solution 1000 mg/ l NO_3 / l, Merck Germania, CertiPur, batch HC388517, available until 31.05.2016</td></tr>
<tr><td colspan="2">Performance parameters evaluated in order to validate the method</td><td rowspan="6">CONCLUSIONS
– Declaration of validation of the method –

The results are in the areas of values imposed by legislation and standard.

Method for measuring the parameter nitrate, SR EN 7890-3/2000, satisfies the conditions of reference documents.
The method is suitable for the purpose for which it is used.

THE METHOD IS CONSIDERED VALID FOR APPLICATION IN THE LABORATORY</td></tr>
<tr><td>CHARACTERISTICS CALIBRATION FUNCTION</td><td>Range: - 1÷5 µg N – NO_3^-
Variation coefficient of the method, V_{x0} = 0,023%
Residual standard deviation, S_y = 0,0001
Standard deviation of the method, S_{x0} = 0,0007
Percent recovery media: 100%
Calibration: SR ISO 8466-1/98, SR EN 7890-3/2000
Evaluation of the calibration graph: SR ISO 8466-1:98</td></tr>
<tr><td>RANGE OF DETECTION, RD</td><td>RD: 0.092÷0.232 µg N</td></tr>
<tr><td>QUATIFICATION RANGE, QR</td><td>QR: 0.956÷1.108 µg N</td></tr>
<tr><td>PRECISION</td><td>p = 0,013÷0,038 µg N</td></tr>
<tr><td>MEASUREMENT UNCERTAINTY</td><td>Associated expanded uncertainty:
U_e = 0,179÷0,91µg N
Uncertainty of the result is calculated for each concentration level</td></tr>
<tr><td>ACCURACY OF MEASUREMENT</td><td>Accuracy of measurement = 0 µg N (from standard control chart)</td></tr>
</table>

2.9 Drawing-up the validation report

All the results of the previous evaluations are centralized under the form of a validation report and they are compared to the specifications imposed by standards and the law (Table 4)

2.10 Establishing a continuous plan to secure the quality of results

Only after all the above described activities are completed, the determination of the samples proper may start [5].

3 Conclusions

In the field of spectrophotometry, as in other fields, the preparation of the working method entails a considerable amount of work as compared to the determination proper of the sample. In this study, only the beginning part has been presented, i.e. the implementation of a spectrophotometric method. The application of the 17025 standard represents an uninterrupted series of activities thatrequired time, dedication, additional costs, but also permanent consultation of the specialty literature. The application of the standard ensures the certainty of the obtained results. The reporting or publication of spectrophotometric results without taking the above exposed criteria into account contravenes the values of ethics and scientific research in the field.

Acknowledgement

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VALIDITATEA DETERMINARILOR SPECTROFOTOMETRICE SI NECESITATEA APLICARII STANDARDULUI 17025:2005 PENTRU OBTINEREA DE REZULTATE SIGURE IN CERCETARE

Rezumat:

Aplicarea standardului SR EN ISO 17025:2005, Competenta laboratoarelor de incercari si etalonari, se impune ca o necessitate pentru laboratoarele care doresc sa genereze rezultate valide din punct de vedere tehnic. Niciun rezultat emis nu poate fi credibil fara respectarea cerintelor SR EN ISO 17025:2005. Astfel, lucrarea de fata prezinta, printr-un exemplu concret, principalele activitati tehnice care trebuie intreprinse inaintea determinarilor spectrofotometrice propriu-zise. Lucrarea isi propune sa contribuie la formarea tinerilor cercetatori sau la consolidarea unor competente specifice domeniului spectrofotometriei.