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# Comparative study between EDXRF and ASTM E572 methods using two-way ANOVA

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**Abstract**: Comparison with reference method is one of the necessary requirements for the validation of non-standard methods. This validation was performed using the technique of planning of experiment with two-way ANOVA, where the results obtained using the EDXRF method, to be validated, are compared with the results obtained by ASTM E572-13 standard test method. Analyzing the results of the hypothesis tests, it can be observed that, for all chemical element analyzed, Fisher's *F*-test indicated that the methods no have significant differences. Therefore, according to this study, the method was approved in this requirement.

**Keywords**: Validation of analytical methods; Comparison with reference method; Energy dispersive X-ray fluorescence spectrometry – EDXRF; Spectrometric analysis of alloy steels; Analysis of variance – ANOVA.

## 1. INTRODUCTION

In Brazil, the laboratories of Brazilian Network of Testing Laboratories (RBLE), accredited by INMETRO, according to ABNT NBR ISO/IEC 17025:2005 standard [1] must comply, among other things, the requirements: non-standard methods (item 5.4.4), and validation of methods (item 5.4.5), described in this standard. To assist in this task, INMETRO provides document DOQ-CGCRE-08 - Guidance for validation of analytical methods [2] which guides laboratories how to validate a non-standard method, i.e., that is not described in a national or international standard.

The LACOR (Laboratório de Corrosão, Proteção e Reciclagem de Materiais), at UFRGS, is accredited by INMETRO (RBLE 1139), and it has the test for determination of metals by energy dispersive X-ray fluorescence spectrometry (EDXRF) for alloy steels in its scope of accreditation. However, the EDXRF method is non-standard method and therefore it must be validated. One of the requirements for validation, as determined by item 8.2.6.3.4 of document DOQ-CGCRE-08 [2], is the comparison with reference method. The purpose of this comparison is to evaluate the accuracy of method in validation by analyzing samples with concentrations through the entire range in which the method is to be validated. Samples were analyzed in replicate using the two methods, separately. [2]

There are several techniques that can be used to compare the results obtained by two test methods, document DOQ-CGCRE-08 [2]

suggests: hypothesis tests and planning of experiment. In this comparison, it was chose to use the two-way factorial experiment (two-way ANOVA) described in the technique of planning of experiment [3].

To obtain the results of the method to be validated, the Thermo Scientific NITON model XL3t GOLDD+ portable EDXRF analyzer was used to analyze the samples 2Q15S1 [4], 2Q15S2 [4], 4Q15S1 [5], and 4Q15S2 [5]. These samples were measured by ASTM E572-13 reference method [6], with the method wavelength dispersive X-ray fluorescence spectrometry (WDXRF), in the Inter-Laboratory Analysis Programs (ILAP), performed by ASTM International [4,5], in which LACOR laboratory participated. In this study, it was analyzed the results of only the following chemical elements: molybdenum (Mo), niobium (Nb), copper (Cu), nickel (Ni), manganese (Mn), chromium (Cr), and vanadium (V). Comparison of the results for tungsten (W) and cobalt (Co) was also obtained, but with other methods, outside this scope. Therefore, these results are not mentioned in this article.

## 2. MATERIALS AND METHODS

In this study, the four samples, used in interlaboratory comparisons [4,5], were measured with six replicates, under repeatability conditions, using the NITON analyzer along with the Mobile Test Stand accessory. NITON has been configured with: calibration curve (table 1), general metals analysis from the main menu, two filters – main range (20s) and low range (20s), enable Al, and autoswitch ON [7]. All measurements were taken using the following procedure quoted in the NITON manual [7]: 1 - preparation of the sample; 2 - clean with isopropyl alcohol and lint-free paper; 3 - perform a system check once every working day; 4 - warm up for ten minutes after start up.

Results with six replicates ($n$ = 6), in each sample, were compared with six randomly selected results from other laboratories that participated in interlaboratory comparisons [4,5]. Two-way ANOVA was used for each chemical element: factor A – concentration – with four levels ($a$ = 4) and factor B – method – with two levels ($b$ = 2).

**Table 1.** Parameters of the calibration curve and coefficient of determination ($R^2$).

| *Chemical element* | *Intercept (a)* | *Slope (b)* | *$R^2$* |
|---|---|---|---|
| Mo | -0.0054 | 1.0219 | 0.9999 |
| Nb | 0.0033 | 0.8916 | 0.9999 |
| Cu | -0.0148 | 1.1157 | 0.9993 |
| Ni | 0.0021 | 1.0096 | 1.0000 |
| Mn | -0.0258 | 0.9803 | 0.9997 |
| Cr | -0.0932 | 0.9758 | 0.9995 |
| V | 0.0019 | 0.9116 | 0.9996 |

### *2.1. Factorial Experiment (two-way ANOVA)*

According to Ribeiro and Ten Caten [3], the statistical model of two-way ANOVA is

$$y_{ijk} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \varepsilon_{ijk} \qquad (1)$$

Where, μ: is the population mean, $\tau_i$: is the effect of the $i$-th level of "concentration", $\beta_j$: is the effect of the $j$-th level of "method", $\tau\beta_{ij}$: is the effect of the "concentration·method" interaction, $\varepsilon_{ijk}$: is the random error, with $i$ = 1,$a$; $j$ = 1,$b$ and $k$ = 1,$n$. Assumption: $\varepsilon_{ijk} \rightarrow N(\mu=0,\sigma)$.

Thus, there are three hypothesis tests to be performed, according to Ribeiro and Ten Caten [3]:

For the factor "concentration": Ho: $\tau_i = 0$ and H1: $\tau_i \neq 0$ for some $i$, $i$ = 1,$a$.

For the factor "method": Ho: $\beta j = 0$ and H1: $\beta j \neq 0$ for some $j$, $j$ = 1,$b$.

For the factor "concentration·method": Ho: $\tau\beta_{ij} = 0$ and H1: $\tau\beta_{ij} \neq 0$ for some $ij$.

For each hypothesis tests it was compute $F$ tabulated of the Fisher distribution ($F$-distribution)

and compare it with the *Fcalc*. If *Fcalc* > *Ftab* then the null hypothesis is rejected and therefore the change in levels of the factor analyzed has a significant effect on the mean response [3].

The value of *Fcalc* is obtained from equation (2) and *Ftab* is calculated by equation (3). [3]

$$Fcalc = \frac{\text{Variance between groups}}{\text{Variance within the group}} = \frac{MQ(factor)}{MQR} \quad (2)$$

$$Ftab = F_{\alpha;GLnumerador;GLdenominador} \quad (3)$$

If *Fcalc* > *Ftab*, or *p-value* < 0.05 (α = 5%), the effect studied is significant for a confidence level of 95 percent [3]. According to Ribeiro and Ten Caten [3], the formulas for calculations are described below.

$$TC = \frac{(T...)^2}{abn} \quad (4)$$

$$SQA = \sum_{i=1}^{a} \frac{(T_i..)^2}{bn} - TC \quad (5)$$

$$SQB = \sum_{j=1}^{b} \frac{(T._j.)^2}{an} - TC \quad (6)$$

$$SQAB = \sum_{i=1}^{a}\sum_{j=1}^{b} \frac{(T_{ij}.)^2}{n} - TC - SQA - SQB \quad (7)$$

$$SQR = \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n} y_{ijk}^2 - \sum_{i=1}^{a}\sum_{j=1}^{b} \frac{(T_{ij}.)^2}{n} \quad (8)$$

$$SQT = \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n} y_{ijk}^2 - TC \quad (9)$$

The verification of equation (9) can be done by equation (10).

$$SQT = SQA + SQB + SQAB + SQR \quad (10)$$

Where *TC* is the correction term, *SQ*(*factor*) is the sum of square of each factor, *MQ*(*factor*) is the calculation of the square mean or variance, equation (11), and GDL are the degrees of freedom of each factor. Table 2 presents these calculations in a structured way.

$$MQ(factor) = \frac{SQ(factor)}{GDL} \quad (11)$$

**Table 2.** Calculations for two-way ANOVA.

| *Factor* | *Sum of squares* | *GDL* | *Square means* | *Fcalc* | *Ftab* |
|---|---|---|---|---|---|
| A | SQA | (*a*-1) | MQA | $\frac{MQA}{MQR}$ | $F_{\alpha;GLA;GLT}$ |
| B | SQB | (*b*-1) | MQB | $\frac{MQB}{MQR}$ | $F_{\alpha;GLB;GLT}$ |
| AB | SQAB | (*a*-1)* (*b*-1) | MQAB | $\frac{MQAB}{MQR}$ | $F_{\alpha;GLAB;GLT}$ |
| Error | SQR | *ab*(*n*-1) | MQR | | |
| Total | SQT | *abn*-1 | | | |

## 3. RESULTS AND DISCUSSION

The six results obtained with NITON, in the four samples for Mo, and also the six means of these samples from laboratories that participated in ILAP of the ASTM [4,5], method E572, are presented in table 3. Results of the other chemical elements were obtained using the same methodology. Using the methods and calculations (described in item 2 of this article), with the data from table 3, the following values were obtained for Mo: *a* = 4, *b* = 2, *n* = 6, *TC* = 53.129788, *SQA* = 45.504863, *SQB* = 0.000199, *SQAB* =0.000051, *SQR* = 0.003461 e *SQT* = 45.508574. Table 4 presents the values of two-way ANOVA calculations for Mo.

**Table 3.** Values obtained with NITON (EDXRF) and ASTM E572 (WDXRF) for Mo.

| *Concentration for Mo (A)* | *Values measured with NITON (%)* | | *Means obtained with E572 (%)[4,5]* | |
|---|---|---|---|---|
| 0.051 % 2Q15S2 | 0.049 | 0.050 | 0.0576 | 0.050 |
| | 0.052 | 0.051 | 0.052 | 0.0544 |
| | 0.050 | 0.049 | 0.055 | 0.051 |
| 0.102 % 2Q15S1 | 0.102 | 0.101 | 0.110 | 0.110 |
| | 0.104 | 0.104 | 0.1081 | 0.109 |
| | 0.101 | 0.105 | 0.106 | 0.107 |
| 2.003 % 4Q15S1 | 2.020 | 2.008 | 2.020 | 2.020 |
| | 2.003 | 2.011 | 2.008 | 2.001 |
| | 2.002 | 2.008 | 2.003 | 2.007 |
| 2.058 % 4Q15S2 | 2.044 | 2.011 | 2.046 | 2.033 |
| | 2.043 | 2.035 | 2.056 | 2.024 |
| | 2.052 | 2.046 | 2.076 | 2.0347 |

**Table 4.** ANOVA two-way calculations for Mo.

| *Factor* | *Sum of squares* | *GDL* | *Square means* | *Fcalc* | *Ftab* |
|---|---|---|---|---|---|
| Concen-tration (A) | 45.504863 | 3 | 15.168288 | 175298 | 2.8 |
| Method (B) | 0.000199 | 1 | 0.000199 | 2.3 | 4.1 |
| AB | 0.000051 | 3 | 0.000017 | 0.2 | 2.8 |
| Erro | 0.003461 | 40 | 0.000087 | | |
| Total | 45.508574 | 47 | | | |

As mentioned previously, if *Fcalc* > *Ftab* the studied effect is significant. Analyzing table 4 it can be verified that the factor "concentration" was significant and the factor "method" and the interaction were not significant. This shows that the concentrations had great variation between the samples, but this was expected, since each sample actually had a very different value of concentration of Mo in relation to the others. However, the most important, which is precisely the purpose of this study, the methods do not have significant differences and therefore for Mo the EDXRF method is approved in requirement 8.2.6.3.4 of document DOQ-CGCRE-08 [2], for a confidence level of 95 percent.

To the other elements that followed the same methodology, table 5 presents a summary of two-way ANOVA calculations of each of the chemical elements.

**Table 5.** Summary of two-way ANOVA calculations for the factor "method" of chemical elements.

| *Chemical element* | *Sum of squares* | *GDL* | *Square means* | *Fcalc* | *Ftab* |
|---|---|---|---|---|---|
| Mo | 0.000199 | 1 | 0.000199 | 2.3 | 4.1 |
| Nb | 0.000006 | 1 | 0.000006 | 4.0 | 4.1 |
| Cu | 0.000284 | 1 | 0.000284 | 4.0 | 4.1 |
| Ni | 0.001349 | 1 | 0.001349 | 1.0 | 4.1 |
| Mn | 0.000524 | 1 | 0.000524 | 1.0 | 4.1 |
| Cr | 0.003485 | 1 | 0.003485 | 1.0 | 4.1 |
| V | 0.000003 | 1 | 0.000003 | 0.2 | 4.1 |

Analyzing the data in table 5, it is concluded that all chemical elements, which have been analyzed and compared with the E572 method [6], are approved for requirement 8.2.6.3.4 of document DOQ-CGCRE-08 [2], for a confidence level of 95 percent. There was no significant difference between the methods because no *Fcalc* was greater than *Ftab* (see table 5).

## 4. CONCLUSION

The comparison of the EDXRF method with the ASTM E572 reference method (WDXRF), using the two-way ANOVA technique, resulted in the approval of the method for measuring Mo, Nb, Cu, Ni, Mn, Cr, and V, in the alloy steels analysis. However, it should be noted that this alone does not characterize the complete validation of the analytical method, since many other requirements have to be approved, as described, for example, in DOQ-CGCRE-08 [2], for final definitive approval of the EDXRF method.

## 5. REFERENCES

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