Determining the LOD and LOQ in steel alloys analysis using NITON spectrometer

A Krummenauer¹, P R Dias² and H M Veit¹

¹ Programa de Pós-Graduação em Engenharia de Minas, Metalúrgica e de Materiais (PPGE3M), Universidade Federal do Rio Grande do Sul (UFRGS), Av. Bento Gonçalves, 9500, Porto Alegre, RS, Brazil.
² Faculty of Science and Engineering, Macquarie University, Sydney, NSW 2109, Australia

E-mail: alex_krum@yahoo.com.br

Abstract. Low concentrations standards spectrometric analysis using the NITON spectrometer require the LOD and LOQ to be determined from the analytical method along with identification of the sample matrix. The LOD (the smallest quantity of analyte that can be detected but not necessarily quantified) and LOQ (the smallest quantity of analyte that can be measured with acceptable accuracy) must be determined for each individual matrix because the energy dispersive X-ray fluorescence (EDXRF) method used by NITON spectrometer is strongly influenced by the matrix effect. This study determined LODs and LOQs for steel alloy samples using certified reference materials with low analyte concentration and compared the calculated LODs and LOQs to those specified by the manufacturer using a two-way ANOVA analysis for each chemical element.

1. Introduction
Magnusson and Örnemark [1] claim that measurements made in low concentrations needs to take into consideration three general concepts. The first concept is the decision limit, which indicates an analyte level value that is significantly different than zero. The second concept is the limit of detection (LOD), which quantifies the lowest analyte concentration that can be detected by the method with a given level of confidence. The third concept is the limit of quantification (LOQ), which establishes, for a given application, the lowest limit for which performance is acceptable. Therefore, LOD is the smallest analyte amount in a sample that can be detected, but not necessarily quantified under the conditions established for the test; while LOQ is the smallest analyte amount in a sample that can be quantitatively determined with acceptable precision and measurement trueness, i.e. measurement accuracy [1, 2, 3].

In steel alloys spectrometric analysis with the NITON spectrometer - EDXRF method - both LOD and LOQ are calculated from the standard deviation of the replicate measurements of test samples with low analyte concentration [1, 4, 5].

2. Materials and methods
2.1. Equipment
This study used a portable EDXRF spectrometer (Thermo Fisher Scientific, model NITON XL3t GOLDD +) coupled to its mobile test stand accessory. Certified reference materials (CRMs) were also used.
2.1.1. *Thermo Fisher Scientific NITON XL3t GOLDD+ spectrometer.* NITON is an energy dispersive X-ray fluorescence (EDXRF) analysis spectrometer (figure 1a) that can analyze light elements from magnesium to uranium. This equipment can measure samples ranging from 0.001% m/m (10 ppm) to 100% m/m of the analyte [4, 5].

2.1.2. *Mobile test stand.* Along with the NITON spectrometer, the mobile test stand was used (figure 1b). This device is used to avoid radiation scattering. Samples and certified reference materials (CRMs) are placed inside for analysis [4, 5].

![Figure 1](image-url)  
Figure 1. NITON XL3t GOLDD+ spectrometer and mobile test stand [4].

2.1.3. *Certified reference material (CRM).* Reference material, accompanied by documentation issued by an authoritative body and providing one or more specified property values with associated uncertainties and traceabilities, using valid procedures [6]. We used CRMs, with iron-based alloys matrix, which have low concentrations of the following analytes: molybdenum (Mo), niobium (Nb), tungsten (W), copper (Cu), nickel (Ni), cobalt (Co), manganese (Mn), chromium (Cr), and vanadium (V). Table 1 lists CRMs, their traceabilities, and applications in each chemical element [5].

| Identification number | Certificate date | Traceability  | Using to determine the LOD/LOQ |
|-----------------------|-----------------|---------------|-------------------------------|
| IARM 35KN             | 10/11/2011      | NISTb         | Cr               |
| SS 483/1              | Nov/2013        | BASc          | Co               |
| SS 486/1              | Nov/2013        | BAS           | Mn               |
| 2Q15S1                | 02/07/2015      | ASTMd         | Nb, Cu, Ni       |
| 2Q15S2                | 02/07/2015      | ASTM          | Mo               |
| 4Q15S1                | 31/12/2015      | ASTM          | V                |
| 4Q15S2                | 31/12/2015      | ASTM          | W                |

*a* Expiration of certification — valid indefinitely.  
*b* NIST: National Institute of Standards and Technology.  
*c* BAS: Bureau of Analyzed Samples Ltd.  
*d* ASTM: American Society for Testing & Materials.

2.2. *Procedures for determining LOD and LOQ*  
Both LOD and LOQ are normally calculated by multiplying a standard deviation ($σ_0^2$) by an appropriate factor. It is important that this standard deviation is representative of the precision obtained for typical test samples, and that enough replicate measurements are made to give a reliable estimate [1, 3]. Magnusson and Örnemark [1] state the following issues should be considered in determining LOD and LOQ from an experiment using simple replication.
2.2.1. **Suitable samples for estimating LOD and LOQ.** The samples used should preferably be either a) blank samples, i.e. matrices containing no detectable analyte, or b) test samples with concentrations of analyte close to or below the expected LOD [1], the second being employed in this study.

2.2.2. **Covering the scope of the method.** For methods with a scope covering very different matrices, it may be necessary to determine the standard deviation for each matrix separately [1]. For the EDXRF method this is valid. The matrix used was an iron-based alloy. Therefore, the LOD and LOQ results are only valid for this specific matrix.

2.2.3. **Ensuring representative replication.** Measurements were performed with NITON analyzer coupled to the Mobile Test Stand. NITON has been configured for the general metals analysis from the main menu using three filters – main range (20 s) and low range (20 s) and light range (20 s), disabled Al, and autoswitch ON [4, 5, 7]. All measurements were taken by using the following procedure quoted in the NITON manual:

1. sample preparation – cleaning using isopropyl alcohol and lint-free paper;
2. perform a system check once every working day;
3. warm up for ten minutes after start up [4, 5, 7].

2.2.4. **Conditions of measurement.** The standard deviation ($s_0$) is normally obtained under repeatability conditions [1].

2.2.5. **Number of observations.** The number of replicates ($n$) should be sufficient to obtain an adequate estimate of $s_0$. Between 6 and 15 replicates are considered necessary; 10 replicates are often recommended in validation procedures/protocols [1]. Seven CRMs, listed in table 1, were measured with 10 replicates, under repeatability conditions.

2.2.6. **Allowing for averaging.** In many measurement procedures the mean of replicates is reported in routine use of the method, where each replicate is obtained by following the entire measurement procedure. In this case the $s_0$ should be corrected by dividing with the square root of $n$, where $n$ is the number of replicates according to equation (1) [1]. Calculation of $s_0'$ arises from the application of the central limit theorem.

\[
s_0' = \frac{s_0}{\sqrt{n}} \quad (1)
\]

2.3. **Calculations for determining LOD and LOQ**

LOD can be estimated by equation (2) in case where test samples with low concentration of analyte are used [1].

\[
LOD = \left[ t_{(n-1,1-\alpha)} + t_{(n-1,1-\beta)} \right] \cdot s_0'
\]

Where:

- $t$: one-tailed Student $t$-value;
- $\alpha$: significance level, maximum probability of rejecting the null hypothesis when in fact it is true – type I error;
- $\beta$: failure to reject the null hypothesis when in fact the null hypothesis is not true – type II error.

In this work, where $s_0$ is obtained from 10 replicate measurements, the Student $t$-value at $\alpha = 0.05$ is 1.83 (9 degrees of freedom) and $t$-value at $\beta = 0.05$ is 1.83. This leads to an LOD calculated as equation (3).
\[ LOD = 3.66 \cdot s_0' \]  

(3)

LOQ from equation (4) is calculated by most conventions to be the analyte concentration corresponding to the obtained \( s_0' \) at low levels multiplied by a factor, \( k_Q \). The International Union of Pure and Applied Chemistry (IUPAC) default value for \( k_Q \) is 10 [1].

\[ LOQ = 10 \cdot s_0' \]  

(4)

3. Results and discussion

The measurements obtained from low concentration analyte steel CRMs (table 1) using the NITON spectrometer, via EDXRF method, are shown in table 2. The procedures described in item 2.2 of this article were used to carry out these measurements.

| Table 2. LODs and LOQs values for the iron-based alloys matrix. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Mo   | Nb   | W    | Cu   | Ni   | Co   | Mn   | Cr   | V    |
| \( n = 1 \)     | 0.055| 0.006| 0.037| 0.089| 0.312| 1.788| 0.256| 1.321| 0.055|
| \( n = 2 \)     | 0.055| 0.005| 0.051| 0.088| 0.299| 2.124| 0.215| 1.319| 0.054|
| \( n = 3 \)     | 0.052| 0.005| 0.037| 0.089| 0.331| 1.982| 0.267| 1.322| 0.057|
| \( n = 4 \)     | 0.055| 0.005| 0.035| 0.086| 0.306| 2.020| 0.239| 1.311| 0.053|
| \( n = 5 \)     | 0.055| 0.005| 0.038| 0.084| 0.312| 2.002| 0.218| 1.307| 0.053|
| \( n = 6 \)     | 0.054| 0.005| 0.037| 0.085| 0.295| 1.970| 0.239| 1.317| 0.053|
| \( n = 7 \)     | 0.055| 0.005| 0.043| 0.091| 0.320| 1.974| 0.220| 1.324| 0.055|
| \( n = 8 \)     | 0.053| 0.006| 0.045| 0.078| 0.312| 2.026| 0.218| 1.321| 0.056|
| \( n = 9 \)     | 0.054| 0.005| 0.042| 0.079| 0.297| 1.992| 0.225| 1.315| 0.049|
| \( n = 10 \)    | 0.055| 0.006| 0.037| 0.082| 0.307| 1.987| 0.195| 1.316| 0.056|

\[ s_0 \]  

\[ s_0' \]  

\[ s_0'' \]  

\[ LOD \]  

\[ LOQ \]  

*All values are expressed in % m/m.

The LOD and LOQ results from table 2 are only valid for an iron-based matrix and were calculated using equations (3) e (4). Table 3 shows a comparison among calculated LODs and LOQs, and the values specified by the manufacturer (Thermo Fisher Scientific) [8].

The data on table 3 shows that the calculated LODs values are equal to or less than those specified by the manufacturer for Mo, Nb, W and Cu. While for Ni, Co, Mn, Cr and V, they are larger. Figure 2 displays the comparison between calculated LODs and manufacturer’s LOD for each chemical element.
Table 3. Calculated and manufacturer’s LODs and LOQs for an iron-based alloy matrix for various chemical elements.

| Chemical element | Calculated LODs and LOQs | Manufacturer’s LODs and LOQs |
|------------------|--------------------------|-------------------------------|
|                  | LOD      | LOQ     | LOD      | LOQ*     |
| Mo              | 0.001    | 0.003   | 0.0015   | 0.0050   |
| Nb              | 0.001    | 0.002   | 0.0015   | 0.0050   |
| W               | 0.006    | 0.016   | 0.012    | 0.040    |
| Cu              | 0.005    | 0.014   | 0.007    | 0.023    |
| Ni              | 0.013    | 0.035   | 0.01     | 0.033    |
| Co              | 0.10     | 0.26    | 0.05     | 0.17     |
| Mn              | 0.025    | 0.067   | 0.016    | 0.054    |
| Cr              | 0.006    | 0.017   | 0.0035   | 0.012    |
| V               | 0.003    | 0.007   | 0.0025   | 0.008    |

*LOQs not informed by manufacturer and was calculated with 10·s₀. Where s₀ was calculated by dividing the manufacturer’s LODs by three, according to equation (2) and considering n > 100. All values are expressed in % m/m.

Figure 2. Comparison between calculated LODs and manufacturer’s LODs.

Since the LODs of W, Co and Mn appear to have significant differences between the calculated values and the values specified by the manufacturer (figure 2), a two-way ANOVA (factorial design) was performed to analyze whether these differences are statistically significant [4, 7, 9, 10].

The two-way ANOVA was used to verify if there is a significant difference between LODs: factor A – element – with nine levels (a = 9), factor B – LOD – with two levels (b = 2) and LOD value (n = 1). Table 4 presents these calculations in a structured way. As n = 1 the factor “interaction” was used in factor “error”, otherwise the degrees of freedom (Df) of the error would be zero. For more details see Ribeiro and Ten Caten or Montgomery [9, 10].

The studied factor is significant if Fcalc > Ftab [4, 7, 9, 10]. The analysis of the data on table 4 shows that the factor “element” was significant and the factor “LOD” was not significant. Therefore, these allow to affirm there is no significant difference between the calculated LODs and the ones specified by the manufacturer.
Figure 2 shows that the readings of cobalt (Co) are greater than the calculated LOD and the one specified by the manufacturer. Moreover, the biggest difference between LODs is observed for Co. This occurs because the detection of Co using the NITON spectrometer is more complex than that of other elements presented in this study, as verified by the variability of its reading displayed on table 2.

Table 4. Calculations for LOD with two-way ANOVA.

| Factor  | Sum of squares | Df | Mean square | Fcalc | Ftab |
|---------|----------------|----|-------------|-------|------|
| Element | 0.008784       | 8  | 0.001098    | 7.7   | 3.4  |
| LOD     | 0.000174       | 1  | 0.000174    | 1.2   | 5.3  |
| Error   | 0.001144       | 8  | 0.000143    |       |      |
| Total   | 0.010102       | 17 |             |       |      |

4. Conclusion
The LODs and LOQs calculated using ten replicates for each CRM resulted in values similar to those specified by the manufacturer on an iron-base matrix. This was statistically confirmed by a Two-Way ANOVA factorial design. The ANOVA results showed no statistic significant difference between the calculated LODs and the LODs specified by the manufacturer. Therefore, the differences among LODs occur because the calculated values of LOD and LOQ are susceptible to variations such as number of observations, imperfections in samples, instrumental errors, calibration curve, measurement conditions, instrument configuration, difference between test procedures and type of sample matrix, among others. Thus, the calculated values of LOD and LOQ of this study are valid only for the EDXRF method, for the measurement procedure described in this study and for the iron-based matrix.

Acknowledgments
The authors thank CAPES for the support with the doctoral program; UFRGS, PPGE3M and LACOR by technical and instrumental support; FINEP, for the resources obtained, through the SIBRATEC - Technological Services Provision Networks, for the acquisition, by LACOR, of the portable EDXRF spectrometer.

References
[1] Magnusson B and Örnemark U (Eds.) 2014 Eurachem Guide: the fitness for purpose of analytical methods – a laboratory guide to method validation and related topics (Available from http://www.eurachem.org)
[2] INMETRO 2018 DOQ-CGCRE-008 Orientação sobre Validação de Métodos Analíticos (Rio de Janeiro: INMETRO)
[3] JUNQUEIRAR G 2006 Limites dos Sistemas Analíticos (Rio de Janeiro: INMETRO)
[4] Thermo Fisher Scientific 2010 Niton XL3 Analyzer User’s Guide Revision C
[5] Krummenauer A 2017 Desenvolvimento e Validação de Metodologia Analítica para Análise de Aços por Espectrometria de Fluorescência de Raios X por Dispersão de Energia (EDXRF), Master Thesis (Porto Alegre: UFRGS)
[6] JCGM 2012 JCGM 200 International Vocabulary of Metrology – Basic and General Concepts and Associated Terms (VIM) (Available from http://www.bipm.org)
[7] Krummenauer A, Veit H M and Zoppas-Ferreira J 2018 J. Phys.: Conf. Ser. 975 012004
[8] Thermo Fisher Scientific 2010 Thermo Scientific Niton XL3 Goldl Alloy Analyzers: elemental limits of detection in aluminum / copper / iron / titanium - based alloys
[9] Ribeiro J L D and ten Caten C S 2014 Proyecto de Experimentos (Porto Alegre: UFRGS)
[10] Montgomery D C 1997 Design and Analysis of Experiments (New York: Wiley & Sons)