Analysis of Uncertainty in Calibration Curves

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Abstract – **The calculation of the measurements uncertainties is a subject that will occupy space in scientific articles for many years until some mastery over the subject is attained. The situation worsens in chemical metrology due to the huge amount of factors that can interfere with the results. The issue cannot be solved solely by the application of consensus methodologies, since the enormous amount of variables, even in simple measurements, makes the subject to be extremely complex. This article analyzes the results of a chemical laboratory in the calibration curves adjustment for chromatograph measurements of ethylene oxide residues in medical devices which have undergone a sterilization process. The methodology consisted in the analysis of the calibration curves that were used for two straight days in the laboratory measurements, adding curves adjusted to them without forcing through the zero (0,0) point. With the various curves plotted on the same graph it can be inferred solely uncertainties related to the calibration curves adjustment, which can serve as a reference for more complete studies of uncertainty in chemical measurements. Several problems were noted with the laboratory practice, as well as high uncertainty in the curves adjustment, especially for low concentrations of EO.**

Keywords— **Uncertainty in Calibration Curves, Chemical Metrology, Measurements of EO residues in medical devices.**

I. Introduction

According to the International Vocabulary of Metrology VIM (2012) uncertainty is the "parameter, associated with the result of a measurement which characterizes the dispersion of the values that can be reasonably attributed to the measurement object" [1]. As in chemical metrology things are not as well behaved as in physical metrology, which is the cradle of Guide to the expression of uncertainty in measurement (GUM) [2], several methods were created for chemical metrology to calculate uncertainties, trying to simplify the process and make it more easily applicable and comprehensible.

According to a study published by Oliveira and Aguiar [3], in chemical metrology there can be the so called top-down approaches that are based in interlaboratory studies of measurement methods performance. This technique derives from the 80's studies driven by Wernimont. There is also the classic methodology, recommended by EURACHEM which is proposed in the GUM, although the EURACHEM Guide [4] and IUPAC [5] also suggest using validation data in the estimate of uncertainty.

Limitations of the GUM approach have already been pointed out by Gläser M and Kochsiek M in 2010 [6] and refer to the Taylor first-order modeling adopted by the GUM, a model that can produce marked distortions for some situations. Inconsistencies in the GUM have also been found in the calculation of the effective degrees of freedom by using the Welch-Satterthwaite formula, which is the procedure indicated in the GUM.

Fuzzy methods were also searched by Zadeh in 1965 [7]. The fuzzy method can be used to evaluate vague statements or uncertain observations. If the variables of a process may be considered as fuzzy, it is possible to use this approach to evaluate the measurement uncertainty.

The Monte Carlo method (MMC) also constitutes an interesting alternative, mainly to work around inconsistencies from the traditional method proposed by the GUM, since the MMC is also an alternative proposed by the GUM. The MMC allows for the determination of uncertainty with asymmetric intervals, which is also a limitation of the traditional GUM method that always calculates the intervals as symmetrical.

The fact is that there is no method which is 100% applicable and secure for any situation in the calculation of uncertainties, mainly for chemical metrology. It always depends on the knowledge of the measurement method, common sense and metrological knowledge to assess which variables are significant, and which therefore deserve to be evaluated in the calculation of uncertainty. However despite the difficulties of trial and application of a well-proven methodology, it is important to assess the uncertainty in order to enhance the process of measurement, by the estimate of the sources of uncertainty and further refinement in an attempt to control them.

The present study addresses one of the variables that are part of the measurement uncertainty that is the setting of a Calibration Curve.

II. Method

This study makes a critical analysis of measures residues process of ethylene oxide EO in sterilized products, focusing on the uncertainties arising from the adjustment of the calibration curve from reference materials.

In order to determine the residues of ethylene oxide, ethylene chlorohydrin and ethylene glycol through gas chroma-

tography after sterilization, a validated extraction and measurement method must be used. There are two basic extraction methods used to determine sterilization residues by ethylene oxide in health products: extraction of simulated use (this being the reference method) and exhaustive extraction, that represents an acceptable alternative in certain situations. The choice of the extraction method should be based on the intended use for the product [8].

Aqueous extraction of simulated usage is the only method which produces results directly comparable to the recommended limits. A simulated usage extraction procedure should be validated to demonstrate the real level of exposure for patients. This method is recommended for products that feature limited and extended exposure time. However, exhaustive extraction produces results that would tend to represent a dose greater than or equal to that which the patient could receive. This method is recommended for products that have permanent contact with the patient [8]. The method used in this research was the aqueous extraction.

Samples for chromatographic analysis are forwarded to the measuring laboratory packed in polyethylene ziplock bag and polystyrene box, accompanied with an enclosed form that is duly filled out. In the measurement lab, the medical products to be measured as for the EO residues, are placed in suitable flasks, entirely covered with deionized water and held by 60 minutes or 24 hours according to the usage type (instant or prolonged). After the extraction period, an aliquot of the extract is transferred to a gas chromatograph bottle and placed to be analyzed. The gas chromatograph is calibrated daily with three residues patterns: ethylene oxide, ethylene chloridrine, and ethylene glycol.

A. Prediction Intervals

There are several methods that can be used in the evaluation of the uncertainties of the adjusted calibration curve, and consequently in the prediction of values from the adjusted curves. The analysis of uncertainties can be made by: Eurachem 2012; ISO GUM 2008; Kragten Method; Monte Carlo Numerical Simulation and ISO/TS 28037: 2010 regulation [9]. The study herein presents the evaluation of method uncertainty based on the adjustment of the curves by the method of Ordinary Least Squares, and their respective 95% prediction intervals. Experimental data obtained from the measurement process of an essay company in adjusting the calibration curves before making measurements in a gas chromatograph.

Prediction intervals tell you where you can expect to see the next data point sampled. Assume that the data really are randomly sampled from a Gaussian distribution. Collect a sample of data and calculate a prediction interval. Then sample one more value from the population. If you do this many times, you'd expect that next value to lie within that prediction interval in 95% of the samples. The key point is that the prediction interval tells you about the distribution of values, not the uncertainty in determining the population mean.

Prediction intervals must account for both the uncertainty in knowing the value of the population mean, plus data scatter. So a prediction interval is always wider than a confidence interval.

Prediction interval according to Johnson [10] for a future observation y when $x = x_0$ may be obtained through equation 1.

$$(a + bx_0) \pm t_{\propto/2} s_e \sqrt{1 + \frac{1}{n} + \frac{(x_0 - \bar{x})^2}{S_{xx}}} \qquad (1)$$

Where S_{xx} is equal to the sum of the squared differences of observations in x in relation to the average $\bar{x}$ and S_e = standard deviation of errors (or residues) or standard error of the estimate. In the present study the graphs and calculations were made using Minitab software [11].

B. Experimental part, adjustment of curves day 1 and day 2

From the data presented in Table 1, different calibration curves adjustments were made. It is observed that the point (0,0) was included, since the measurement company says there are no records obtained from the measured subjects when it was not present in the array. The various concentrations were obtained by means of ethylene oxide standard substance and its respective dilutions. Minitab software was used in the calculations and adjustments of the curves [11].

From the data in Table 1 one can get four curve adjustments, two forcing the passage of the curve at point zero (0,0) on days 1 and 2, and other two (days 1 and 2) without forcing the passage of the curve at point zero, although the zero point has been considered to be between the points. For the curves in which the passage at point zero was forced, which was the condition of the testing company, the prediction intervals were not considered, since there is only the equation of the curve. For the other two curves that forced passage through the zero point, all the points on the curve adjustment were considered, and thus the prediction intervals can be calculated. So we basically have 6 curves (calibration curves) that have been analyzed: 1) upper limit curve (UL) for the interval prediction (IP) day 1; 2) lower limit curve (LL) for the prediction interval day 1; 3) upper limit curve (UL) of the IP day 2; 4) lower limit curve (LL) of the IP day 2, 5) curve forcing the passage by zero day 1 and 6) curve forcing the passage by zero day 2. Their respective equations curves are shown in Table 2.

Table 1 Ethylene oxide concentrations and the respctive areas obtained in the cromatograph days 1 and 2

	Concen-tration µg/ml	Dilutions	Cromato-graph Area day 1	Cromato-graph Area day 2
1	0.000		0.000	0.000
2	0.226		0.921	1.477
3	0.566	2.5 x	2.139	2.638
4	1.131	2 x	3,245	4.274
5	2.263	2 x	7.096	9.359
6	11.313	5 x	31.633	34.046
7	22.626	2 x	73.759	66.923
8	56.566	2.5 x	178.552	168.181

With the six equations of curves available were all placed in a same graph, shown in Figure 1, where the same uncertainties based on calibration curves for the various concentrations can be obtained.

It is observed that replicated values were not obtained, thus deeper studies that could be made with respect the homoscedasticity are compromised and the prediction

Table 2 Six equations of the curves analized

Contions in which the curve was obtained	**Equation Curve**
Curve adjusted by the measurement company day 1	chomatograph area = 3.15823 x concentration
Curve adjusted by the measurement company day 2	chomatograph area = 2.97435 x concentration
Curve without forcing passage through (0,0) point day 1 UL	chomatograph area = 4.656 + 3.186 x concentration
Curve without forcing passage through (0,0) point day 1 LL	chomatograph area = -5.235 + 3.143 x concentration
Curve without forcing passage through (0,0) point day 2 UL	chomatograph area = 3.238 + 2.960 x concentration
Curve without forcing passage through (0,0) point day 2 LL	chomatograph area = 1.362 + 2.940 x concentration

intervals of the adjusted curves can be under-dimensioned.

By the analysis of the distribution of concentrations along the curve, there is a large number of values for the low concentrations and dispersed values to high concentrations. This can lead to errors, considering the fact that replicated items were not made as an aggravating factor. This fact can determine large variations in the adjusted curve compared with the case where the concentration values were equally distributed along the measuring range.

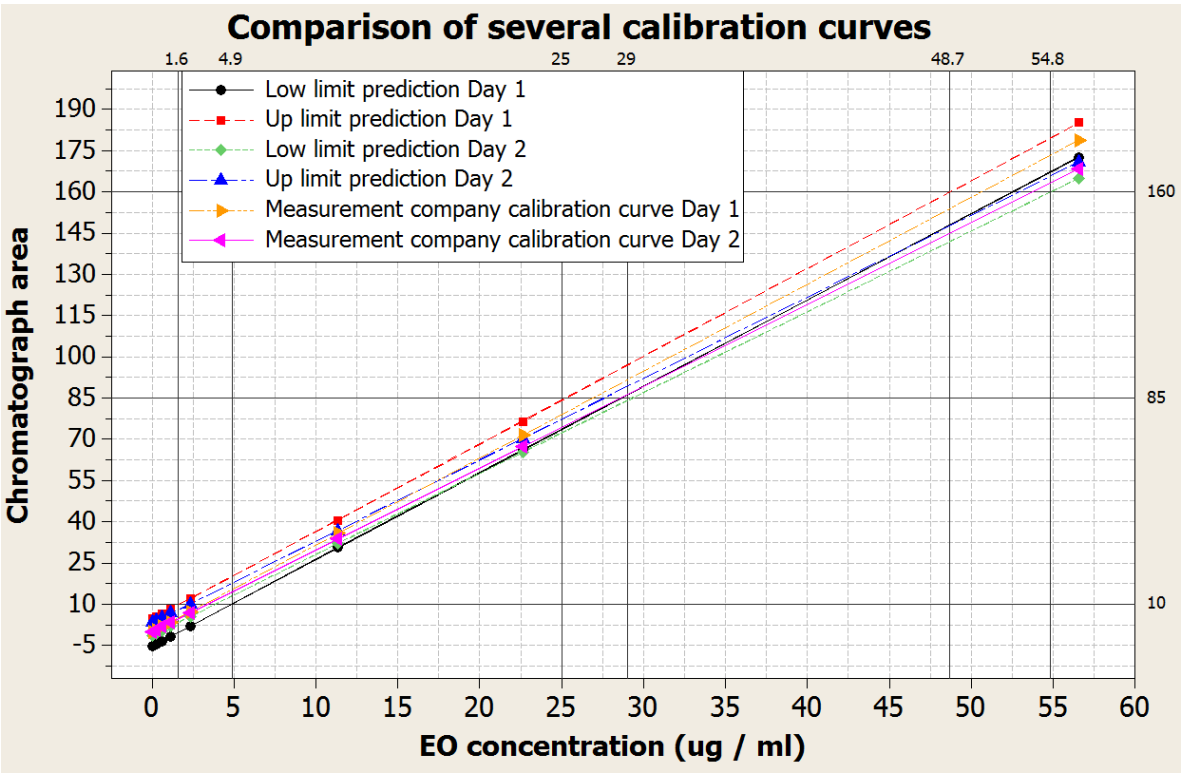

Figure 1 – The six calibration curves plotted in the same graph

III. Results

It is observed that for low concentrations up to 5 µg/ml, the uncertainty considering only the variability in the calibration curves adjustment provided values of ±1.7 µg/ml, namely uncertainty of approximately ± 34% of the base value. The uncertainty was obtained from the curves in Figure 1, considering a chromatograph area of 10, to which the concentrations obtained were around 4.9 µg/ml to 1.6 µg/ml.

For average concentrations around 25 µg/ml, the uncertainty considering only the variability in the calibration curves adjustment provided values of ± 2 µg/ml, namely uncertainty of approximately ± 8% of the base value. The uncertainty was obtained from Figure 1 curves considering a chromatograph area of 85, where it obtained concentrations around 29 µg/ml to 25 µg/ml.

For high concentrations around 50 µg/ml, the uncertainty considering only the variability in the calibration curves adjustment provided values of ± 3.1 µg/ml, namely uncertainty of approximately ± 6.2% of the base value. The uncertainty was obtained from Figure 1 curves, considering a chromatograph area of 160, for which concentrations around 48.7µg/ml to 54.8 µg/ml were obtained.

IV. Conclusions

The present study presents the limitation of metrological expertise on the part of laboratories that perform chemical tests, where indication of uncertainties is an exception.

This study comprises an analysis of uncertainty based solely on the adjustment of the calibration curve and its limits prediction for 95%, whereas the curves were obtained without the concentration replicated values. Still, very high uncertainties are observed.

The essay laboratory made serious mistakes that may compromise the reliability of the results, including: a) not replicating the measurements for the various concentrations; b) not distributing the concentrations equally along the measuring range; c) supposed correlation between the concentrations obtained for calibration (what can increase cumulative erros); d) not adapting the method for low concentrations measurements (5 ppm) due to the high uncertainty in the calibration curves adjustment (± 1.7 ppm).

A lack of knowledge of basic principles of metrology is observed in the execution of tests by the tests laboratory, in this sense a ISO Standard 17025 certification can force the laboratory to have a routine of tests to ensure the metrological reliability, although many do not seek certification due to the level of requirements and the costs that cannot be held.

Conflict of Interest

The authors declare that they have no conflict of interest.

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