MULTI-LEVELS INTERNAL QUALITY CONTROL FOR MONITORING OF PCDD/F AND DL-PCB IN SERUM SAMPLES AS A TOOL FOR TRENDS ANALYSIS

Eppe G., Focant J.-F., De Pauw E.

CART Mass Spectrometry Laboratory, Chemistry Department, University of Liège, Allée de la Chimie 3, B-6c Sart-Tilman, B-4000 Liège, Belgium. E-mail : G. Eppe@ulg.ac.be

Introduction

As defined in the Harmonized Guidelines for Internal Quality Control in Analytical Chemistry Laboratories[1]: '*internal quality control (IQC) is a set of procedures undertaken by laboratory staff for the continuous monitoring of operations and the results of measurements in order to decide whether results are reliable enough to be released*.' Above all, IQC is a control of precision of your analytical process with the aim of assuring a long term constancy of the results. It can also be a control of trueness depending of the control material (CM) used (e.g. Certified Reference Material). The main objective is to ensure the constancy of the results day-to-day and their conformity with defined criteria. Nowadays, laboratories must be able to produce reliable data when performing analytical tests for a customer or for regulatory purposes and this, regardless the type of method and application areas. The aim of the IQC undertaken in a laboratory is, once again, to monitor the analytical process in order of ensuring that data released by the laboratory is fit for purpose.
An analytical result is characterized by a systematic error and a random error. The faster their detection, the better the method of control. The conventional Shewhart chart is characterized with a certain lack of sensitivity to detect systematic errors. The EWMA (exponentially weighted moving average) control chart is better suited for this purpose. It is a specific method for improved bias detection and it is defined as *'a statistic for monitoring the process that averages the data in a way that gives less and less weight to data as they are further removed in time from the current measurement'*. It has been introduced for the first time in clinical chemistry by Neubauer in 1997. He demonstrated the superiority of this technique compared to Westgard mutli-rules[2]. A parameter λ, called the smoothing factor, determines the rate at which 'older' data enters into the calculation of the EWMA statistic. A value of λ= 1 implies that only the most recent measurement influences the EWMA (degrades to Shewhart chart). Thus, a large value of λ gives more weight to recent data and less weight to older data; a small value of λ gives more weight to older data. The value of λ is usually set between 0.2 and 0.3 although this choice is somewhat arbitrary; 0.15 is also a popular choice.
The EWMA statistic that is calculated is based on the recurrence formula:

$$EWMA_t = \lambda Y_t + (1 - \lambda)\, EWMA_{t-1} \quad \text{for } t = 1, 2, \ldots, n. \qquad (1)$$

where

- $EWMA_0$ is the mean of historical data (target)
- Y_t is the observation at time *t*
- *n* is the number of observations to be monitored including $EWMA_0$

$0 < \lambda \leq 1$ is a constant that determines the depth of memory of the EWMA
Figure 1 shows, with a value of λ = 0.25, that the EWMA takes into account around ten previous points with a high predominance of the five last points, whereas a λ = 0.05 goes back much further in time, beyond twenty previous points.

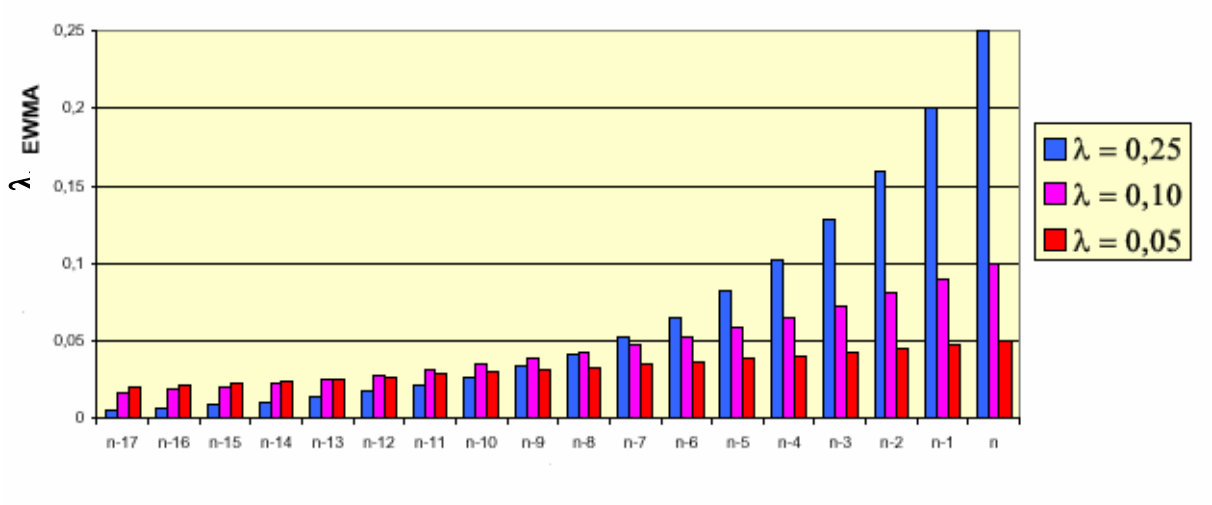

Figure 1: Weighting of EWMA for different λ values

Although the multi-levels IQC is frequently used in clinical chemistry, its application in classical chemistry analysis is rare. The main reasons are the cost and the time consuming aspect, especially for sophisticated analysis like dioxin. The classical approach of multi-levels IQC is characterized by one parameter controlled at three different levels: high, medium and low. The correlation between the levels has been introduced and developed by Hotelling[3]. He defined an index that combines dispersion information, means and correlation of several variables. This scalar, known as T^2, generalizes at p dimensions the student t test. This concept and the underlying statistics were used and adapted in the field of dioxin analysis. Instead of measuring each congener at 3 IQC levels with a series of samples, which is not economically feasible, we propose an adapted approach. One IQC containing the 29 toxics congeners is divided into 3 sub-groups. Three parameters (levels) are monitored: the sum of PCDD/Fs, the sum of NO-PCBs and the sum of MO-PCBs, all expressed in TEQ units. According to our analytical procedure and the clean-up fractionation approach on the Power-Prep system[4], the PCDD/Fs and the NO-PCBs are in the same fraction while the MO-PCBs are analyzed separately.

Materials and methods

All information on solvents, sorbents, labeled standards, equipments, IQC and analytical procedures can be found in a previous report[5,6]. The matrix IQC samples consisted in foetal calf bovine serum fortified with PCDD/Fs and dioxin-like PCBs (DL-PCBs). The mean level was 280 pg TEQ/l (157±15 pg TEQ/L for PCDD/Fs, 106±14 pg TEQ/L for NO-PCBs, 16±2.5 pg TEQ/L for MO-PCBs). IQC and unknown serum samples were stored at -20°C prior to analysis. The sample sizes were 20 g. The IQC was characterized by a sufficient homogeneity and stability for long-term control of the laboratory performances. A batch of three litres of serum was prepared to cover the seven months of the study. A complete series consisted of one blank procedure, one IQC and eighteen unknown's serum. More than ninety tests were performed during this period including homogeneity tests and technicians evaluations (IQC used as blind samples).

Results and discussion

Figure 2 illustrates the IQC charts. In Figure 2-A, B, and C, the central tangent line defines the mean value with the upper and lower control limits drawn in plain. The control limits are set at m±3 σ_M, where σ_M is the standard deviation recalculated each time a new data point is added in the dataset (floating chart). The tick curve with its control limits (dashed lines, m±3 σ_{EMWA}) represents the Exponentially Weighted Moving Average (EWMA) with a smoothing factor of 0.2. The relationship between σ_M and σ_{EMWA} is expressed by the following equation:

$$\sigma_{EMWA} = \sigma_M \sqrt{\frac{\lambda}{2-\lambda}} \qquad (2)$$

By setting $\lambda = 0.2$, equation (2) becomes:

$$\sigma_{EMWA} = \frac{\sigma_M}{3} \qquad (3)$$

Hence, the dashed lines represent m± σ_M.
The results are summarized on Figure 2. No data points were in an out-of-control situation during the period covered by the study. An intra-laboratory reproducibility expressed in RSD (%) of 5.9% (n=91) for the sum of PCDD/Fs was obtained at 156,2 pgTEQ/l (i.e. 26 pgTEQ/g lipids when assuming 0.6% of lipid). RSDs of 6.8% (n=91) and 9.8% (n=89) were obtained for the sum of NO-PCBs at 106.7 pgTEQ/l (17.8 pgTEQ/g lipids) and for the sum of MO-PCBs at 16.1 pg-TEQ/l (2.7 pgTEQ/g lipids) respectively. The trends or the drift of the analytical method was evaluated by the EWMA curves. The EWMA curves were randomly distributed above and below the mean values indicating that no systematic bias were observed during this period. In addition, the EWMA curves lay between the dashes lines demonstrating that the bias did not exceed 5.9% for the sum of PCDD/Fs; 6.7% for the sum of NO-PCBs and 9.8% for the sum of MO-PCBs.

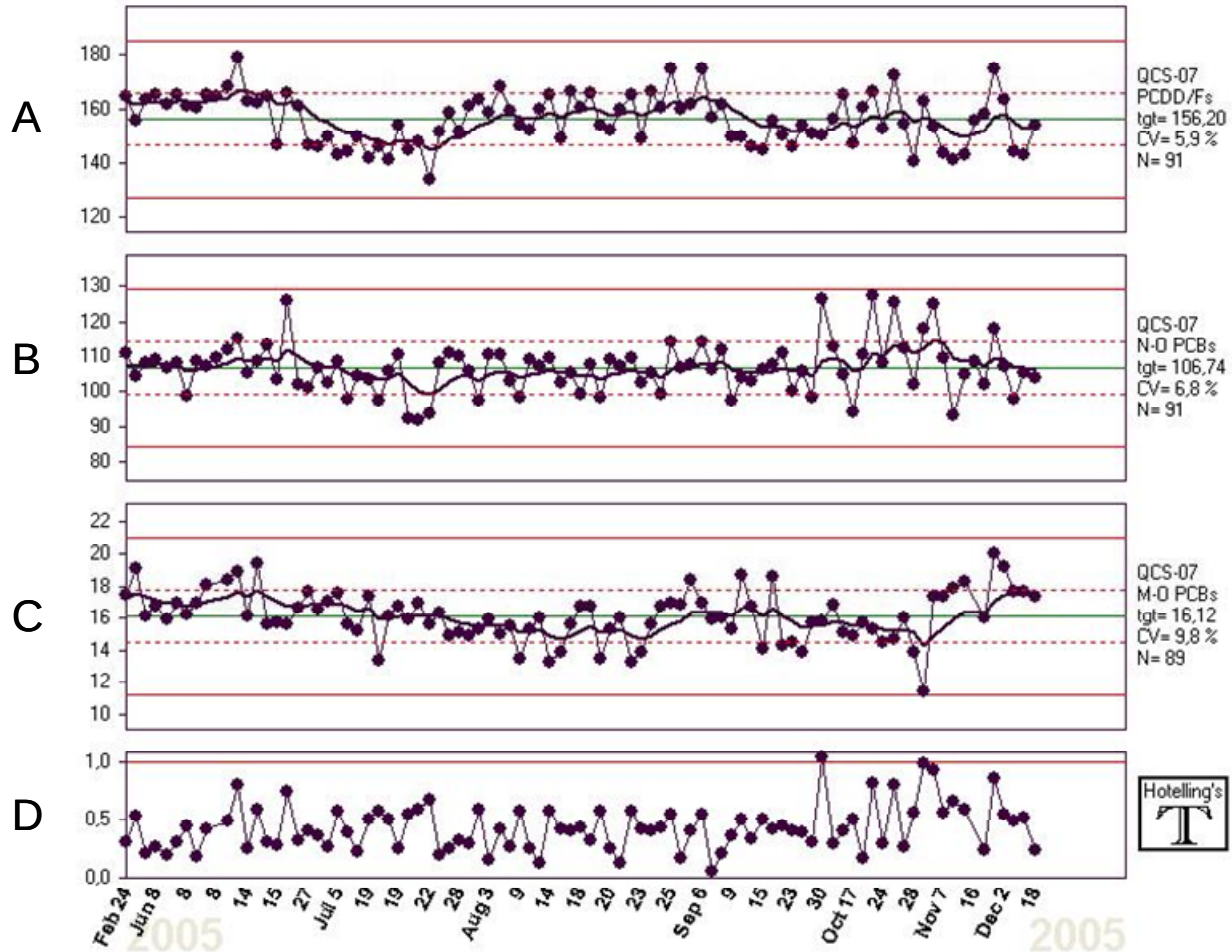

Figure 2: Internal Quality Control (IQC) charts for PCDD/Fs (A), NO-PCBs (B), and MO-PCBs (C) present in QC serum analyzed over time (concentrations in pg WHO-TEQ/L). Part D represents the Hotelling index.

The selection of the 3 sub-groups is related to our analytical procedure for clean-up, even if PCDD/Fs and NO-PCBs are collected in the same fraction. The distinction between PCDD/Fs and NO-PCBs should provide additional information if levels are not correlated. Once injected in parallel into the two GC-IDHRMS, the three monitored parameters were quantified and used to build up mutli-levels IQC charts (Figure 2). As the three

variables came from the same analytical procedure, a degree of correlation between control levels should be observed. In other words, when a level (e.g. PCDD/Fs) increases, the corresponding levels (e.g. NO-PCBs and MO-PCBs) should follow the same trend. In particular between levels A and B, as they are simultaneously analyzed on the same GC-HRMS and quantified according to the same calibration. The degree of correlation is quantified by the Hostelling's T^2 test. A $T^2 > 1$ gives a warning value. It indicates that an anomaly is detected even if each individual control material is in-control. As an example, Figure 2-D showed on September 30th a T^2 value of 1,04. Indeed, the PCDD/Fs and MO-PCBs values were close to the target mean value while the NO-PCBs value was close to the upper maximum limit (upper limit). Individually, values were accepted as they lay between the maximum limits. However, actions can be triggered in order to find out is there any reason explaining such discrepancy. As the three selected parameters gave a general overview of the analytical performances in TEQ unit, one obvious action undertaken was to check individually the congeners that contribute to the TEQ. In this particular case, PCB-126 was higher than expected. Further investigations have been carried out by checking blank level, recovery, integration of the peak, peak shape, retention time, isotopic ration or relative response factor.
The mutli-levels IQC approach proposed here gives an original and an interesting tool for analyst. As the general tendency for halogenated POPs analysis is to increase the number of target compounds to monitor, analysts have to face up to more and more data handling resulting in hard labour. The multi-levels IQC using appropriate statistics allows to record data in such a way that trends and warnings can be easily detected.

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