Detailed Validation in PCDD_F analysis - ISO17025 data from Brazil

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Introduction

When we define validation method we can use the ISO standard 8402, in reference to this, 'validation' is the 'confirmation by the examination and supplying of objective evidences that the particular requirements for a specific intended use are fulfilled'.[1] This concept is extremely important to guarantee the quality of results. Validation method is based on the combined use of different validation procedures, but in this selection we have to analyze the cost benefit conditions. We must focus on the critical elements, and these critical factors must be the essential elements for providing good properties and results.

If we have a solid validation methodology and a research of the source of uncertainty of our analytical method, we can generate results with confidence and veracity.[2]

When analyzing these two considerations, validation method and uncertainty calculations, we found out that there are very few articles and papers about these subjects, and it is even more difficult to find such materials on dioxins and furans.

This short paper describes a validation and uncertainty calculation methodology using traditional studies with a few adaptations, yet it shows a new idea of recovery study as a source of uncertainty.

Methods and Materials

In this study we use our standard operational procedure (SOP 4.9 – 102). Which is based on EPA 8290 and 1613, but it has a few changes. It was defined five different concentration values to study any properties, as: linearity range, equipment detection limit, method detection limit, method quantitation limit and sensibility. A sample of non-contaminated soil was spiked with labeled natural standards of seventeen congeners (tetra-octa polychlorinated dibenzo dioxins and polychlorinated dibenzo furans). Seven replicates of each concentration values were prepared and we used a Shaker (10 grams, 200 rpm, 40 minutes and 40 mL of CH_2Cl_2) and cleaned up with SiO_2:sulphuric acid and Florisil. After this preparation step the samples were analyzed using gas chromatography coupled mass spectrometry of high resolution (AutoSpec – Micromass).

We defined the linear range to work (0.2, 10.0, 50.0, 100.0, and 1,000.0 ng/Kg), and to establish this values we used information from laws, directives and clients' needs. After this, a statistical study was made using the results of the replicates. First, we calculated the linearity properties to check the linear profiles and systematic or random errors. For each group of seven samples of the same concentration value for each congener we calculated the response factor, the average of the response factor and the standard deviation. It was plotted a graphic with the concentration values versus response factor and the linear profile was evaluated in two steps: a) the quadratic correlation coefficient must be bigger than 0,999, and b) the standard deviation (SD) of residues for each group of replicates must be bigger than 25%. If the standard deviation of residues was smaller than 25% we conclude that there are systematic errors acting in this procedure. In this case, a second test is performed using all relative response factors (RRF) using all the concentration values.
This test with the relative response factors was performed by calculating the number of RRFs that was out of the range defined between RRF ± 2SD. There is a scale, and its function is to number the concentration values and to define the acceptability criteria. In this case, we used five points, the acceptability criteria is 10% of the RRF number out of the specific range. The following definition for residue was used here: is the difference between the experimental RRFs and the RRF value from linear regression line using these variables. The test using RRFs is more restrictive in relation to the Student test; because we used all values of RRF (the suspected accepted and rejected values).

After studying the linearity we evaluated the method detection limit (MDL), equipment detection limit (EDL) and method quantitation limit (MQL). MDL that was performed using the Student statistical factor (for 95% of acceptability and n – 1 replicates) and the standard deviation of concentration values in the first point of the linearity test, using MDL = t x SD. MQL is the first point evaluated in the linearity test, and EDL is performed using the ratio sign / noise.[3,5]

In our research, in relation to the uncertainty method the Figure-01 presents the fish diagram and our resources of error. We evaluated the main properties, as: original solution of standard compounds, dilution errors, aliquot of sample (mass or volume) error, calibration error (RRF), the recovery error (method tendency) and error of repeatability. This description about uncertainty is a consequence of the following mathematics expression:

$$Pop = \frac{I_{Op} \times M_{PI} \times F_{Rep}}{I_{PI} \times RRF \times F_{Rec} \times Q_{Am}}$$

where, P_{op} is the amount of compound of interest, I_{Op} is the compound of interest peak area, I_{PI} is the internal standard peak area, RRF is the relative response factor, F_{Rec} is the recovery factor, Q_{Am} is the sample amount, F_{Rep} is the repeatability factor and M_{PI} is the internal standard reference mass. All uncertainty sources were used to propagate the errors and the expression to calculate the relative uncertainty is the following[4]:

$$\frac{\mu P_{Op}}{P_{Op}} = \sqrt{\frac{\mu F_{Rep}^2}{F_{Rep}^2} + \frac{\mu F_{Rec}^2}{F_{Rec}^2} + \frac{\mu RRF^2}{RRF^2} + \frac{\mu M_{PI}^2}{M_{PI}^2} + \frac{\mu Q_{Am}^2}{Q_{Am}^2}}$$

The recovery factor of uncertainty was defined based on laboratory historical data. It was chosen 10% of the real results generated in the laboratory. After this, the average and standard deviation were calculated and the relative uncertainty of this term was evaluated.

Results and Discussion

In this methodology of validation the linearity is the main property to be evaluated, because it is the key to perform the other parameters. Triple checking in our methodology is very important to avoid systematic errors and mathematical coincidences. The Table-01 presents the results for three criteria in the linearity investigation for all PCDD/F congeners evaluated. The Figure-02 presents the RRF distribution profile for all replicates in the validation test.

Figure-01: Presents the fish diagram to PCDD/F analysis.

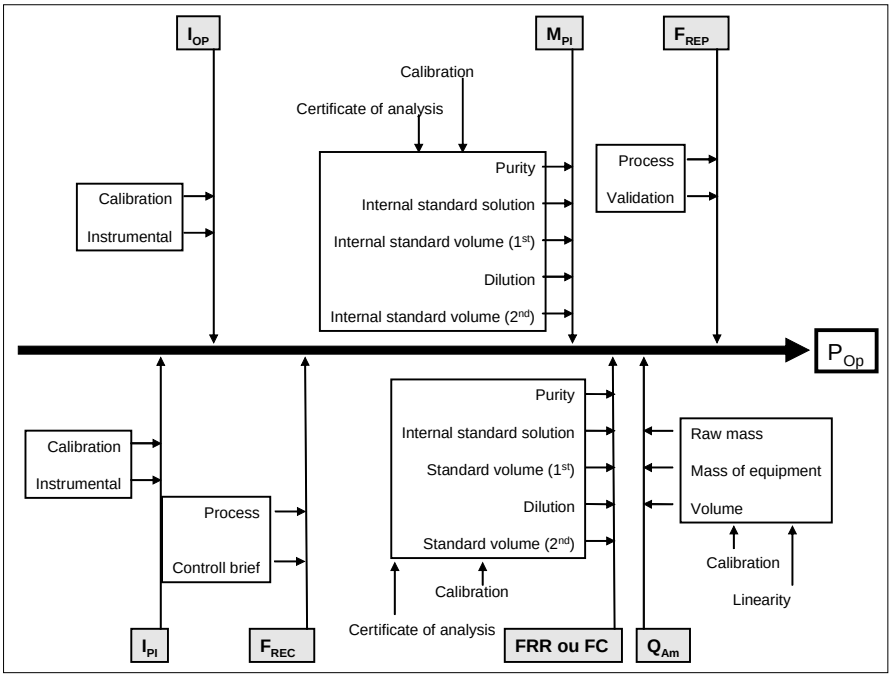
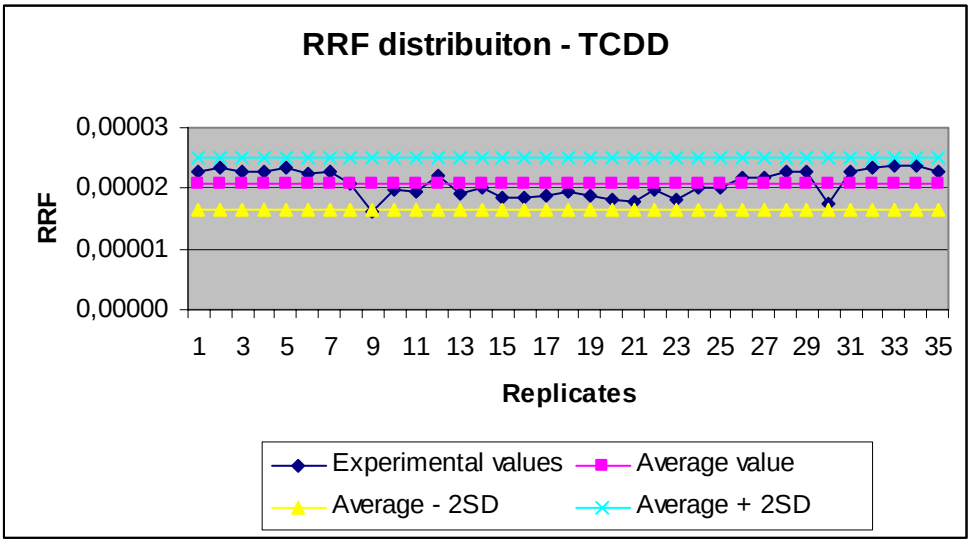


Figure-02: Presents the RRF distribution for TCDD for each replicate in validation test.



When we analyze the percentage standard deviation of residue values of 0.20 ng/Kg for each analyzed congener we noticed that these values were low. This situation can be explained by the evaluation of the coefficients from linear regression of plot RF versus concentration for all congeners. The linear coefficient is bigger than the product of angular coefficient and concentration value. It generates similar values of responses and it generates a SD% lower than the other concentration values. In this case, the RRF test is necessary to support the linearity profile.

Table-01: Shows the linearity criteria for each PCDD/F congener.

Congener	R^2	Residue test for each concentration value (SD%)					RRF out of range ± 2SD (%)
		0.20	10.00	50.00	100.00	1,000.00	
2,3,7,8 TCDD	0.9999	0.067	19.106	92.834	66.844	18.624	5.71
1,2,3,7,8 PeCDD	1.0000	0.133	62.096	37.006	26.147	87.754	0.00
1,2,3,4,7,8 HxCDD	0.9999	0.080	15.498	61.305	56.350	116.268	0.00
1,2,3,6,7,8 HxCDD	1.0000	0.225	47.137	62.386	91.019	107.747	2.86
1,2,3,7,8,9 HxCDD	1.0000	0.309	84.187	56.447	75.654	72.737	2.86
1,2,3,4,6,7,8 HpCDD	0.9999	0.055	35.633	33.368	68.282	80.330	2.86
OCDD	0.9998	0.223	36.006	44.596	41.366	81.447	8.57
2,3,7,8 TCDF	1.0000	9.368	65.985	57.419	51.851	82.134	0.00
1,2,3,7,8 PeCDF	1.0000	0.118	46.054	56.535	69.815	59.925	5.71
2,3,4,7,8 PeCDF	1.0000	5.238	62.801	85.336	73.491	75.924	5.71
1,2,3,4,7,8 HxCDF	0.9997	0.103	30.605	17.564	18.820	77.835	2.86
1,2,3,6,7,8 HxCDF	0.9999	0.216	81.594	14.914	26.352	82.554	5.71
1,2,3,7,8,9 HxCDF	0.9999	0.131	50.958	33.025	108.803	138.503	0.00
2,3,4,6,7,8 HxCDF	0.9998	0.102	38.832	62.468	81.037	57.439	2.86
1,2,3,4,6,7,8 HpCDF	0.9997	0.041	18.685	96.415	20.894	69.163	2.86
1,2,3,4,7,8,9 HpCDF	0.9998	0.215	37.123	46.854	28.683	52.204	0.00
OCDF	0.9999	0.243	39.830	13.897	23.898	60.173	8.57

Concentration values in ng/Kg.

Evaluating the residue test, in this methodology using a SD% for each concentration value, we may have a rigorous criterion, values may be approved by the Student test, but it presents %SD smaller than 25%. In this case, the dispersion of individual residue values in each group of replicates is better than the evaluated.

Table-02 presents the final results of validation parameters for each PCDD/F congener using this methodology. The parameter values are in accordance with laws and regulation criteria. This methodology uses a simple mathematic structure and a minimum replicate number in relation to statistical tests.

Figure-03 shows a comparison between the contribution values of each uncertainty resource for 2,3,7,8 - TCDD. The main contribution is the RRF and the $F_{Rec,}$ but this effect is very important, because it is the real effect and it presents the routine process contribution. There is an advantge in the usage of this methodology to calculate the uncertainty analysis value in relation to the calculations using all resources all the time, because de F_{Rec} can be evaluated from brief controls in a periodic test, the RRF's contribution must be evaluated after each calibration procedure and the other resources are neglected. For other congeners the results have the same profile.

Table-02: Presents the validation parameter values of PCDD/F analysis in soil.

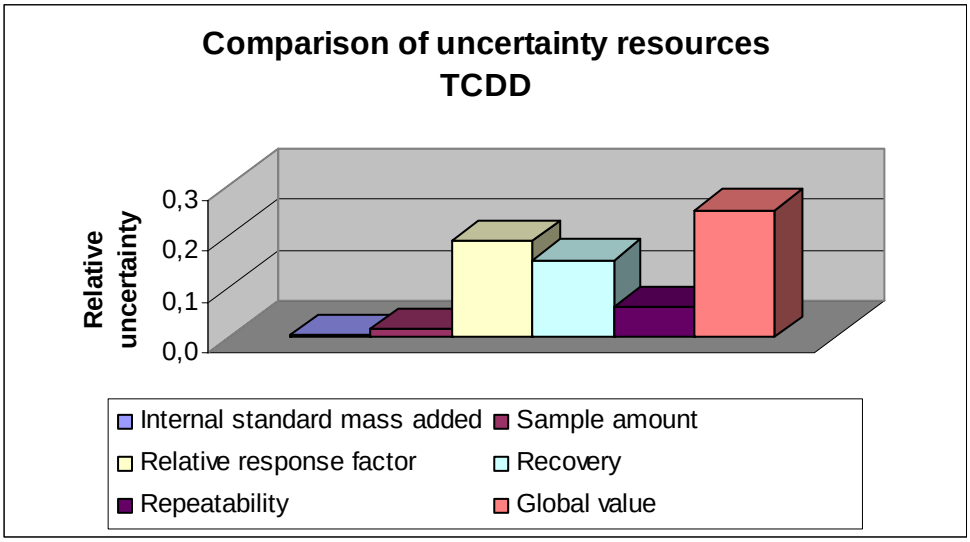
Congener	E.D.L.	M.D.L.	M.Q.L.	Work range
2,3,7,8 TCDD	0.002	0.0000	0.2000	0.20 – 1,000.00
1,2,3,7,8 PeCDD	0.002	0.0095	0.2000	0.20 – 1,000.00
1,2,3,4,7,8 HxCDD	0.002	0.0095	0.2000	0.20 – 1,000.00
1,2,3,6,7,8 HxCDD	0.002	0.0148	0.2000	0.20 – 1,000.00
1,2,3,7,8,9 HxCDD	0.002	0,0000	0.2000	0.20 – 1,000.00
1,2,3,4,6,7,8 HpCDD	0.002	0.0074	0.2000	0.20 – 1,000.00
OCDD	0.002	0.0190	0.2000	0.20 – 1,000.00
2,3,7,8 TCDF	0.002	0.0223	0.2000	0.20 – 1,000.00
1,2,3,7,8 PeCDF	0.002	0.0074	0.2000	0.20 – 1,000.00
2,3,4,7,8 PeCDF	0.002	0.0190	0.2000	0.20 – 1,000.00
1,2,3,4,7,8 HxCDF	0.002	0.0154	0.2000	0.20 – 1,000.00
1,2,3,6,7,8 HxCDF	0.002	0.0154	0.2000	0.20 – 1,000.00
1,2,3,7,8,9 HxCDF	0.002	0.0074	0.2000	0.20 – 1,000.00
2,3,4,6,7,8 HxCDF	0.002	0.0095	0.2000	0.20 – 1,000.00
1,2,3,4,6,7,8 HpCDF	0.002	0.0000	0.2000	0.20 – 1,000.00
1,2,3,4,7,8,9 HpCDF	0.002	0.0220	0.2000	0.20 – 1,000.00
OCDF	0.002	0.0103	0.2000	0.20 – 1,000.00

Concentration values in ng/kg.

Analysing the validation method proposed and the methodology for uncertainty of analysis calculations we can conclude that, this routine is useful for providing knowledge of analytical process and the variables of laboratory. In the client's point of view the uncertainty values reported have concrete values, because if the uncertainty calculation uses certificate of calibration values and / or propagation of error the relative uncertainty will have a low value.

The methodology to analyze and to validate the process used is useful and it evidences the requirements for a specific intended PCDD/F congeners analysis in soil using a Shaker.

Figure-03: Presents the uncertainty resources profile for 2,3,7,8-TCDD.



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