INTERNAL VALIDATION METHOD OF PCDD/Fs BY HRGC/LRMS/MS

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Introduction

For ultra-trace analysis, method validation is a key element in the assessment of a laboratory's competence in producing reliable analytical data[1]. To ensure high quality of results a specific analytical method used for PCDD/Fs analysis should be demonstrated in laboratory experiments[2]. In the PCDD/PCDF analysis, validation might include instrumental step and also preparation of the laboratory sample, extraction and clean-up of the samples[1]. Which criteria apply to the validation of a particular method depends on the purpose and the nature of the method[1,2]. The validation protocol includes information about the accuracy (trueness and precision), ruggedness, detection capability and selectivity of the method[3,4]. The use of adequate reference materials is of paramount importance in the evaluation and validation of analytical methods[1]. We found out that it is difficult to obtain a standard material for dioxins and furans in gaseous emission samples. Also, there are few reports about method validation for PCDD/Fs determination by high resolution gas chromatography coupled to ion-trap low resolution mass spectrometry HRGC/LRMS/MS in this matrix. The validation usually requires a study design involving a minimum of five test materials[1]. These materials are submitted to the laboratory's routine as a real sample and the possible influence of the conditions during the analysis (robustness)[1,4]. This work presents details of the analytical methodology to determine Method Detection Limit (MDL), limit of quantification, recovery, linearity, accuracy and precision (reproducibility) for PCDD/F quantification from stack gas emissions. Traditional methods of extraction, clean-up by liquid-solid adsorption chromatography at atmospheric pressure, and quantification by ion trap HRGC/LRMS/MS were used.

Materials and Methods

XAD-2 resin (Supelco) was selected as a matrix for the experiments. 30 g of XAD-2 resin was spiked with a native PCDD/Fs solution (EPA 1613 PAR) from Wellington. The first round of experiments was focused on the fit of the calibration curve (linearity). 2 µL of EN-1948:1996 standard solutions in nonane (CS1 to CS6, Wellington Labs., Guelph, Ontario, Canada) were injected. Relative Response Factors (RRFs) were determined using ions identification and comparison with retention time of ^{13}C labeled internal standards. In round 2 the detection and quantification limit were assessed. For the detection limit the material was prepared so that the levels of PCDD/F in the final material give clearly defined peaks with a S/N ratio between three and five. Noise was determined by Varian Saturn Workstation software using baseline peak-to-valley height ratio. Seven replicates of selected concentration values were prepared. The accuracy, precision and recovery were evaluated in round 3. For accuracy and precision, six and nine samples were spiked respectively, so that the levels of PCDD/F were based on the concentration nearby the level of interest and in the range of calibration curve. The recovery of the ^{13}C spikes standards was found respect to the injection ^{13}C standards in all the samples. The selectivity of the method is checked by comparing the ions and the retention time of ^{13}C labeled internal standards. The global uncertainty due to sampling on stack gas emissions is very difficult to evaluate. For this reason the measurement is focused on the analytical methodology. Therefore, limits of detection are just expressed in pg.

All samples were extracted with toluene. Then, extracts were rotary concentrated and clean-up was performed by liquid-solid adsorption chromatography at atmospheric pressure using glass columns filled with silica, florisil and alumina as adsorbents. Standard solution mixtures of labelled PCDD/Fs EN-1948-SS and EN-1948-ES were added during extraction and EN-1948-IS during injection from Wellington. Purified extracts were analyzed by high resolution gas chromatography coupled to ion-trap low resolution mass spectrometry (HRGC/LRMS/MS) in a CP-3800 GC equipped with an 8400 autosampler coupled to a Saturn 2000 ion-trap spectrometer and a DB-5MS column (60 m x 0.25 mm I.D., 0.25 µm film thickness). The equipment was previously calibrated with EN-1948:1996 standard solutions in nonane (CS1 to CS6, Wellington Labs., Guelph, Ontario, Canada). Quantification of PCDDs/PCDFs was performed using the isotope dilution method. Relative Response Factors

(RRFs) were determined using CS1 to CS6 injections and area comparison with ^{13}C labeled internal standards. Congener identification was carried out by comparison of retention times between labeled and native compounds based on co-elusion concept.

Results and Discussion

Linearity

Range linearity was evaluated by calculating %RSD (Relative Standard Deviation) of RRF. The RRFs are a correlation between the concentration and response of the analyte and ^{13}C standards. The particular analyte is considered linear in the calibration range if the variation (%RSD) $\leq$ 30 % for the labeled and $\leq$ 20 % for the native PDCC/Fs[5]. For the data set the relative standard deviations were between 3% and 26 %.

Method Detection and Quantification Limit

MDL was calculated using the Student *(t-value)* statistical factor (for 99% confidence and n – 1 degrees of freedom), *MDL = (t-value)*(s)* where *(s)* is sample standard deviation. MDL is evaluated using coefficients of variation (CV) for individual PCDD/Fs. This is a measure of data variability and is calculated as the estimated standard deviation divided by the arithmetic mean of the observed values, $CV\ (\%) = (100) * s/\overline{X}$. Overall, the results are satisfactory as CVs are lower than 30%[1, 6]. Table 1 presents obtained results of Method Detection Limit for each PCDD/F congener and sample coefficient of variation. In general, parameter values are within acceptability criterion (CV < 30%). Values obtained by the MDL with 99 of confidence are low. MDL values reported for PCDD/F analysis by HRGC/HRMS in food[7] ranged from 0.005 to 0.4 ng/kg and in soils[8] from 0.0074 to 0.0223 ng/kg with 95% confidence. In this work we found values ranging from 0.0082 ng for TCDD to 0.1364 ng for OCDD, with 99 % confidence. Higher MDL were found for more chlorinated compounds, hexa to octa. MDL was calculated since standard deviation value represents not only a reliable estimation of the minimum amount that can be distinguished from the average amount of background present, but also the variability of the background throughout a study[9]. Lower limit of quantification (LOQ) is defined[6] as the level above which quantitative results may be obtained with a specified degree of confidence. The LOQ is mathematically defined as equal to 10 times the standard deviation of the results for a series of replicates used to determine a justifiable limit of detection, *LOQ = 10 * (s)*, table 1. Limits of quantification are matrix, method, and analyte specific[5].

Table 1. MDL, %CV and LOQ parameter values of PCDD/F analysis in stack gas emissions.

Congener	% CV	MDL (pg)	LOQ (pg)
2378-TCDF	21	19.3	61.3
2378-TCDD	10	8.2	27.4
2378-PCDF	18	64.7	216.0
23478-PCDF	19	84.0	280.1
12378-PCDD	14	45.3	144.2
123478-HxCDF	19	92.7	275.4
123678-HxCDF	13	51.7	172.5
234678-HxCDF	21	85.8	273.0
123789-HxCDF	25	117.7	374.4
123478-HxCDD	24	129.8	385.7
123678-HxCDD	23	101.6	323.2
123789-HxCDD	8	39.7	126.3
1234678-HpCDF	16	63.8	203.0
1234789-HpCDF	20	64.3	204.7
1234678-HpCDD	32	116.0	344.7
OCDD	22	136.4	434.0
OCDF	12	86.3	256.5

Analytical considerations are of primary importance to establish the precision criteria, a pre-established CV of 20% to 30% is considered as an acceptable criterion for the ability to determine dioxin congeners in different matrixes[4-7]. In spite of a relative large variation it is noticed for the HpCDD (table 1) around 32%, it is near the acceptability criterion. In general, the values of CV were lower in most all the cases, showing a good correlation of the data.

Accuracy

The accuracy reflects the closeness of a measured value to a true value[6] (reference material). This was evaluated with the Student *(t-value)* statistical factor (for 99.5% confidence and n – 1 degrees of freedom). The experimental *t-values* (t_{exp}) were calculated following the equation:

$$t_{\exp} = \left[\left(X_{real} \right)_{ave} - \left(X_{spike} \right)_{ave} \right] \frac{\sqrt{n}}{s}$$

Where X_{ave} is the average concentration of the measured samples and spike level with the initial spike concentration and *(s)* is measured sample standard deviation for n=6. The values are compared with the tabulated factor (t_{tab}) (99.5% and n-1=5 degrees of freedom). The calculated values are accepted if $t_{\exp} \le t_{tab}$. Figure 1 presents obtained results. In general, the congeners were concerned with value of t_{tab} =. 4.032. A very small deviation was observed by OCDF, t_{exp} = 4.19. Considering the complex of the analytical methodology these results are very satisfactory.

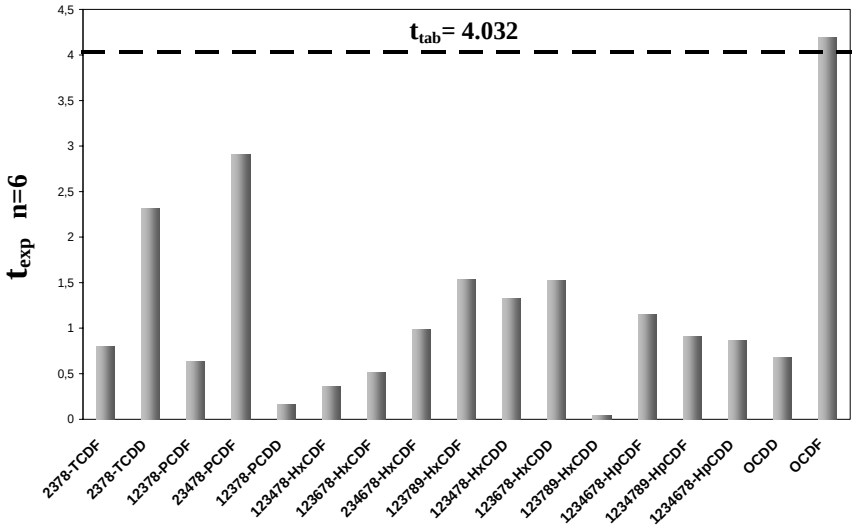

Figure 1. Values of experimental and tabulated, t_{exp} and t_{tab}, by PCDD/F congener with n=6.

Precision

Precision is a measure of the random error associated with a series of repeated measurements of the same parameter within a sample[6]. This is determined by standard deviation *(s)* of a series of measurements and it is evaluated using oefficients of variation (CV) for individual PCDD/Fs. Precision describes the closeness with which multiple analyses of a given sample agree with each other[6]. When the analyses are performed by different personnel is known as reproducibility. In this case, it was evaluated by determining the reproducibility standard deviation and coefficients of variation in nine samples. Table 2 presents obtained results. The criterion[3] that CV < 30% were complied by all individual PCDD/Fs.

Table 2. X_{ave}, standard deviation *(s)* and % CV by nine samples analyzed.

Congener	X_{ave} I-TEQ pg	*s*	%CV
2378-TCDF	5.2	0.64	12
2378-TCDD	55.8	7.64	14
2378-PCDF	14.3	3.22	22
23478-PCDF	116.2	25.77	22
12378-PCDD	139.6	33.77	24
123478-HxCDF	27.2	2.93	11
123678-HxCDF	29.6	5.81	20
234678-HxCDF	28.7	7.18	25
123789-HxCDF	24.2	5.46	23
123478-HxCDD	33.1	2.24	7
123678-HxCDD	30.4	4.36	14
123789-HxCDD	32.8	7.86	24
1234678-HpCDF	3.1	0.50	16
1234789-HpCDF	3.0	0.63	21
1234678-HpCDD	2.5	0.29	12
OCDD	0.5	0.09	18
OCDF	0.5	0.08	17

Recovery

Recovery of the ^{13}C spikes standards were evaluated in all the samples as is defined in the international protocol[5]. The recovery of the samples generally varied between 50% and 110% by individual PCDD/Fs. The data set ranges complied with the general variation accepted by the international methodology[3-5].

Acknowledgements

Authors are grateful to Universidad de Antioquia for supporting this work through sustainability project 2005-2006 and to COLCIENCIAS-CSIC for project 2004CO0006.

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