



## Estimation of the minimum detectable value for the determination of PCBs in fatty food samples by GC–ECD: a curvilinear calibration case

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### Abstract

Electron capture detectors (ECDs) are known to have a limited linear calibration range. It was demonstrated that for ECDs, curvilinear calibration models yield more accurate results. To estimate the minimum detectable value (MDV), the ISO 11843-2 procedure was modified for curvilinear calibration graphs. At first, the graphs were linearized. The MDV was assessed with formulas based on the estimation of the MDV in the linear calibration case. Alternatively, the MDV was determined by a method based on confidence intervals for a second-order calibration plot. There was a close correspondence between the MDVs calculated by both methods. Weighted regression models were used to correct heteroscedasticity. It was found that applying weighted regression decreased the MDV with a factor of  $\geq 10$ . It was concluded that the presented modifications to ISO 11843-2 are valid alternatives to estimate the MDV when dealing with curvilinear calibration graphs, but that heteroscedasticity has a greater impact on the estimated MDV than the non-linearity. Therefore, weighted regression must be used when heteroscedasticity is suspected.

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### 1. Introduction

Concern about the prevalence of polychlorinated biphenyls (PCBs) in the food chain has grown recently. The European Community has published a regulation about the release of PCBs in the environment [1]. Since PCBs are known as endocrine disruptors, even in small quantities [2], any analytical method used to trace PCBs requires a low minimum detectable

value (MDV). This MDV should be determined during method validation. Several authors have proposed methodologies to determine the MDV (also called detection capability, detection limit or decision limit, depending on the author) of an analytical method [3–8]. In the ISO 11843-2 standard [9] a methodology for the calculation of the MDV via a linear calibration graph has been published. This methodology was first described by Hubaux and Vos [10]. The EU decision 2002/657/EC uses the same approach to calculate the detection capability (CC $\beta$ ) [11].

It is generally known that electron capture detectors (ECDs) have a limited linear calibration range [12].

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In particular, curvilinear regression models usually leads to a better fit of the calibration data. Therefore, the linear calibration model should not be used for the calculation of the analytical results, and hence the Hubaux and Vos approach is not appropriate to assess the MDV. In this paper, two methodologies for estimating the MDV based on curvilinear calibration graphs will be elaborated.

## 2. Methods and materials

### 2.1. Sample preparation

At each level of concentration (0, 7.5, 25, 62.5, 100, 137.5 and 175 ng g<sup>-1</sup> for each congener), 0.4 g of blank pork fat was spiked in triplicates with a stock solution containing the seven PCB markers: PCBs 28, 52, 101, 118, 138, 153, 180 (Dr. Ehrensthofer mixture (mix 3) from Alltech, Belgium). The 21 samples of fat were subsequently analyzed.

### 2.2. Analysis

The method is based on the prEN 1528-1:1996E method [13] and the recommendations of the Belgian Bureau of Accreditation Beltest [14]. Spiked fat (0.4 g) is dissolved in 2 ml of hexane grade “Suprasolv” (Merck, VWR, Belgium) and 50 μl of a 1 mg l<sup>-1</sup> PCB 143 solution is added to control the purification step. The resulting solution is loaded on an acidified silica column composed of 6 g of acidified silica (60/40 silica/H<sub>2</sub>SO<sub>4</sub>, both from Merck), 1 g of Al<sub>2</sub>O<sub>3</sub> (Fluka, Aldrich, Belgium) containing 10% of water and on the top, 0.5 g of anhydrous Na<sub>2</sub>SO<sub>4</sub> (Merck). The tube containing the fat solution is rinsed twice with 2.5 ml of hexane, and the column is further eluted with 20 ml of hexane. After concentration under a gentle dried-air stream to ca. 1 ml, 50 μl of a 1 mg l<sup>-1</sup> mirex solution (Merck) is added as internal standard and 3 μl is injected into the gas chromatography (GC) system.

### 2.3. Apparatus

All injections were done in a split/splitless mode at 220 °C on a GC Trace 2000 equipped with an AS2000 auto sampler (from Thermo-Finnigan, Interscience,

Belgium), a HT-8 column (25 m × 0.22 mm × 0.25 μm, from SGE, Achrom, Belgium) and an ECD at 300 °C. The capacity factor of the column is assessed by the resolution between PCBs 28 and 31. The seven PCBs under investigation are fully resolved. The oven temperature program used is 2.0 min at 80 °C, 25 °C min<sup>-1</sup> to 180 °C, 2.2 °C min<sup>-1</sup> to 250 °C, 10 °C min<sup>-1</sup> to 265 °C (6 min).

### 2.4. MDV models

#### 2.4.1. Linear regression models ( $y = a + bx$ )

The methodology for MDV determination in the case of a linear regression model (LRM) has been extensively described in ISO 11843-2 [9]. Under the assumption of linearity, normality, independence and homoscedasticity, the MDV (=x<sub>d</sub>) is given by:

$$\text{MDV} = \delta \frac{S_y}{b} \sqrt{\frac{1}{K} + \frac{1}{IJ} + \frac{\bar{x}^2}{J \sum (x_i - \bar{x})^2}} \quad (1)$$

In the case of a weighted linear regression model (WLRM), the MDV is given by:

$$\text{MDV} = \frac{\delta}{b} \sqrt{\frac{S_{x_d}^2}{K} + \left[ \left( \frac{1}{J \sum w_i} \right) + \frac{\bar{x}_w^2}{J \sum w_i (x_i - \bar{x}_w)^2} \right] S_y^2} \quad (2)$$

with  $b$  as the estimate of the slope,  $\delta$  the non-centrality parameter,  $I$  the number of reference states (equal to the number of replicates per concentration for the spiked or reference samples),  $i = 1, 2, \dots, I$ ,  $J$  the number of preparations for the reference states (equal to the number of concentrations for the spiked or reference samples),  $K$  the number of preparations of the actual state (equal to the number of replicates for the unknown samples),  $n = I \times J$ ,  $S_y$  the standard error of the estimate,  $S_{x_d}$  the residual standard deviation at  $x = x_d$ ,  $w_i$  the applied weights ( $w_i = 1$  in the case of unweighted regression),  $x_i$  the spiked concentration,  $\bar{x}$  the mean of the concentrations

$$\bar{x}_w = \frac{\sum w_i x_i}{\sum w_i}$$

### 2.4.2. Quadratic regression models ( $y = a + bx + cx^2$ )

The equation for the calculation of the MDV can be extended for a quadratic regression model (QRM) and is given by [15,16]:

$$\text{MDV} = \delta \frac{S_y}{b + 2cx_d} \sqrt{\frac{1}{K} + (X_h^T (X^T X)^{-1} X_h)} \quad (3)$$

with  $c$  as the second-order regression coefficient.

$$X_h = \begin{bmatrix} 1 \\ x_d \\ x_d^2 \end{bmatrix}; \quad X = \begin{bmatrix} 1 & x_1 & x_1^2 \\ 1 & x_2 & x_2^2 \\ \vdots & \vdots & \vdots \\ 1 & x_n & x_n^2 \end{bmatrix}$$

In the case of weighted quadratic regression model (WQRM), the MDV is given by:

$$\text{MDV} = \frac{\delta}{b + 2cx_d} \sqrt{\frac{S_{x_d}^2}{K} + (X_h^T (X^T W X)^{-1} X_h) S_y^2} \quad (4)$$

with:

$$W = \begin{bmatrix} w_1 & 0 & \dots & 0 \\ 0 & w_2 & \dots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \dots & w_n \end{bmatrix}$$

### 2.5. Linearized regression model (LZM)

The calibration graph was linearized according to Wang et al. [17]. Curved lines are straightened using a transformation of the response variable  $y^* = y^p$  and  $y^* = a + bx$ , in which  $y$  represents the original variable and  $y^*$  the transformed variable. The parameter  $p$  is determined iteratively, minimizing the standard error. Assuming that the parameter  $p$  is close to 1, the confidence intervals of the LRM can be used, and Eq. (1), as proposed in ISO 11843-2 is valid for estimating the MDV. Eq. (2) can be used in case of weighted linearized models (WLZM).

## 3. Results and discussion

### 3.1. Testing the regression model for the calibration graphs

Twenty-one spiked fat samples were analyzed under repeatability conditions. The relative peak height (response = peak height/peak height internal standard) obtained after analyzing the samples are plotted versus the concentration ( $\text{ng g}^{-1}$  fat). Linear calibrations were regressed through the data points.

Residual plots in combination with statistical tests were used to check whether or not the chosen regression model adequately fitted the experimental data [18].

Firstly, the plot is evaluated for non-constancy of the variance, also called heteroscedasticity. Fig. 1 represents the plot of the residuals for the LRM of PCB 180. In this figure, a horizontal V-shape is observed, indicating an increase in the variance at higher concentration. The heteroscedasticity of the PCBs 28- and 180-plots has been confirmed with the Breusch–Pagan (BP) test [15]. The BP test verifies the concentration dependency of the variance (the curves whose homoscedasticity was rejected with the BP test at the 95% confidence level, are labeled with the letter ‘a’ in Table 2). A weighted linear regression model (WLRM) was used to correct heteroscedasticity. The weights were estimated either from the triplicates ( $w_i = 1/S_i$ )

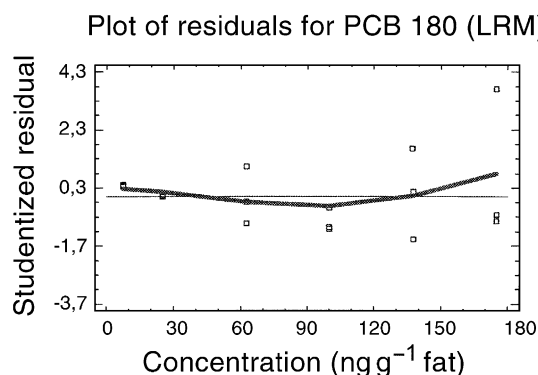


Fig. 1. Plot of the residuals for the linear regression model for PCB 180 (LRM). The horizontal V-shape is typical for non-constancy of the variance or heteroscedasticity. The slight U-shape of the residuals (visualized by the bold line) can be an indication that a curvilinear model should be preferred over a linear model.

Table 1  
Evaluation of the linearity of the LRM via Mandel's fitting test and the test for significance of the second-order calibration coefficient

PCB	<i>P</i> (Mandel's test value)	<i>P</i> (significance of <i>c</i> )
28	0.065	0.065
52	0.003 <sup>a</sup>	0.002 <sup>a</sup>
101	0.002 <sup>a</sup>	<0.001 <sup>a</sup>
118	0.635	0.317
138	<0.001 <sup>a</sup>	<0.001 <sup>a</sup>
153	<0.001 <sup>a</sup>	<0.001 <sup>a</sup>
180	0.034 <sup>a</sup>	0.033 <sup>a</sup>

<sup>a</sup> Probability values for the two tests are given. Significant probabilities are marked with the letter 'a' (at the 95% confidence level).

or with the variance function:  $S_i^2 = 1/w_i = f(\text{concentration})$ . The weights of the WLRM were estimated with an iterative procedure as described in the ISO 11843-2 standard [9]. This procedure assumes that the variance is linearly dependent on the concentration, and the weights were estimated according to:  $w_i = 1/(mx_i + q)^2$ .

Secondly, the linearity of the calibration plots was assessed with Mandel's fitting test [19] and with the significance test for the second-order regression coefficient [18]. The results are summarized in Table 1 (linearity was evaluated on the weighted models for PCBs 28 and 180 and on the unweighted models for PCBs 52, 101, 118, 138, 153 and 180). Calibration linearity for PCBs 52, 101, 138, 153 and 180 was rejected with the Mandel's fitting test and with the test for significance of the second-order regression coefficient at the 95% confidence level. This non-linearity effect was confirmed by examining the plots of residuals. The slightly U-shape of the residuals (see bold line in Fig. 1) also suggests that a curvilinear regression model would be more appropriate than a LRM. Linearity of the graphs for PCBs 28 and 101 was not rejected at the 95% confidence level. The plots of the residuals were also used to detect possible outliers. On the plots of PCBs 118 and 153, outliers were detected and replaced by their group mean.

A QRM [18] and a LZM [19] were proposed as alternatives when linearity was rejected.

The validity of the quadratic calibration graphs was tested with the plots of residuals. For several PCB calibration plots, an increase in the error variance with the concentration was observed. However,

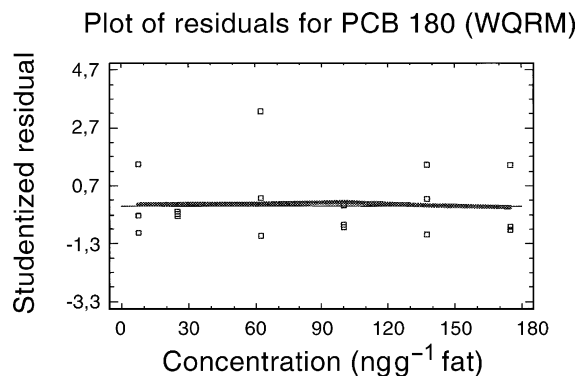


Fig. 2. Plot of the residuals of a weighted quadratic regression plot for PCB 180 (WQRM). Compared to Fig. 1, the horizontal V-shape and the slight U-shape (visualized by the dotted line) of the residuals has disappeared and the data points are now scattered around the center line.

homoscedasticity was rejected by the BP test at the 95% confidence level only for the 101 and 180 congeners. In agreement with LRMs, heteroscedasticity was corrected using weighing regression. The same weights as for WLRMs were applied. The plot of the residuals, upon application of a WQRM on the PCB 180 data, is given in Fig. 2. In this figure, neither the horizontal V-shape nor the U-shaped patterns of the residuals, as shown in Fig. 1, were observed. The data cloud is now scattered around the center line indicating the validity of the chosen regression model.

A general methodology to linearize curvilinear calibration graphs has been proposed by Wang et al. [17]. This methodology gives acceptable results for predicting concentrations of unknown samples when there is only a slight curvature of the calibration graph. The parameter *p* is close to 1 for such curves. The values of *p* obtained in the experiment for the various calibration graphs ranged from 0.96 to 1.12.

### 3.2. Assessing the MDV

The MDV for the PCB congeners ranged between 5.5 and 14.3 ng g<sup>-1</sup>, when estimated from the LRM (Table 2). The MDVs found upon application of both curvilinear models (QRM and LZM) were in close agreement, and significantly less than those determined from the LRM, except for PCB 180. Since the prediction interval is smaller when improving the

Table 2

Estimation of the minimum detectable value (MDV) for the seven PCB congeners based on different regression models

MDV (ng g <sup>-1</sup> fat)	PCB 28	PCB 52	PCB 101	PCB 118	PCB 138	PCB 153	PCB 180
LRM	12.9 <sup>a</sup>	14.1	14.3	5.5	7.6	8.9	7.9 <sup>a</sup>
WLRM	0.89	–	–	–	–	–	0.40
LZM	–	10.0	10.4	–	5.5	4.6	7.1
QRM	–	10.4	10.1 <sup>a</sup>	–	5.4	4.7	8.3 <sup>a</sup>
WLZM	–	–	0.51	–	–	–	0.38
WQRM	–	–	0.70	–	–	–	0.48

<sup>a</sup> Homoscedasticity was rejected with the Breusch–Pagan test for the curves marked with the letter ‘a’ at the 95% confidence level.

goodness of fit, it is expected that the MDV estimated with the curvilinear models are decreased. On the other hand, the non-linearity effect for PCB 180 was less pronounced, hereby indicating that the MDV derived from QRM and LZM did not significantly differ from that of the LRM.

Moreover, the equivalence between the MDV estimates from QRM and LZM and WQRM and WLZM (Table 2) demonstrates that linearized models may be used to estimate the MDV. The approach outlined in the ISO 11843-2 standard is valid for LZM, on the assumption that the calibration graph is close to linear, a condition that is met when the parameter  $p$  is close to 1.

Heteroscedasticity was demonstrated for several LR and QRMs. For these curves, weighted regression was applied. Table 2 shows a 10-fold decrease of the MDV values in cases of weighted regression. When applying weights, the confidence interval of the calibration curve changes, becoming narrower at low concentrations and broader at higher concentrations. This explains why the MDV estimates from weighted regressions were lower than those derived from unweighted regressions. Despite the fact that the BP test was significant for two LRM and two QRMs only, all the plots of residuals had a horizontal V-shape, indicating a larger variance at higher concentrations. This reveals a heteroscedasticity trend in all the calibration graphs (PCBs 28–180), raising the question about the power of the Breusch–Pagan test. It is also noted that the  $P$ -values found with Cochran’s  $C$ -test were even less significant. Finally, it is likely that the MDVs derived from unweighted regressions are seriously overestimated, as the signal to noise (S/N) ratio for the 7.5 ng g<sup>-1</sup> spiked samples ranged between 20 and 30 for the individual PCB congeners. From these results,

it can be stated that the heteroscedasticity of the calibration graph has to be tested at least by examining the plots of residuals and performing significance testing. Whenever heteroscedasticity is suspected, the MDV must be calculated with a weighted regression model.

As aforementioned, weighted regression modify the shape of the confidence interval around the calibration plot. If more weight is assigned to the data points in the low concentration range, the estimated MDV is lowered and vice versa. Hence, the applied weights play a leading role in the estimation of the MDV. A sufficient number of replicates is required for a reasonable estimation of the weighing factors.

#### 4. Conclusions

It is generally known that ECDs have a non-linear range. Therefore, the MDV estimated with a linear model is overestimated. We have shown that the MDV estimated with a linear regression model is significantly larger than the MDV estimated with a curvilinear model when dealing with homoscedastic calibration curves. The MDV can be estimated after linearization or with a quadratic regression model. Both models yield equivalent results.

The determination of the MDV with a linearized regression model is a valid alternative when dealing with curvilinear calibration graphs, on the assumption that the calibration graphs are almost completely linear.

Heteroscedasticity was present for the PCBs 28- and 180-calibration graphs. Correction for heteroscedasticity was performed with weighted regression models. The weights are estimated with a variance function. A correct estimation of the weights requires a large number of replicates.

It was demonstrated that heteroscedasticity has a larger impact on calculation of the MDV than non-linearity. The MDV calculated with a weighted regression model decreased by an order of magnitude compared to the unweighted model.

Heteroscedasticity of calibration graphs has a major influence on the estimation of the MDV and weighted regression must be used when heteroscedasticity is suspected. However, further research is needed to identify possible interferences when estimating the MDV via weighted regression models.

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