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# Solution to bisphenol A measurement challenge

Enea Pagliano

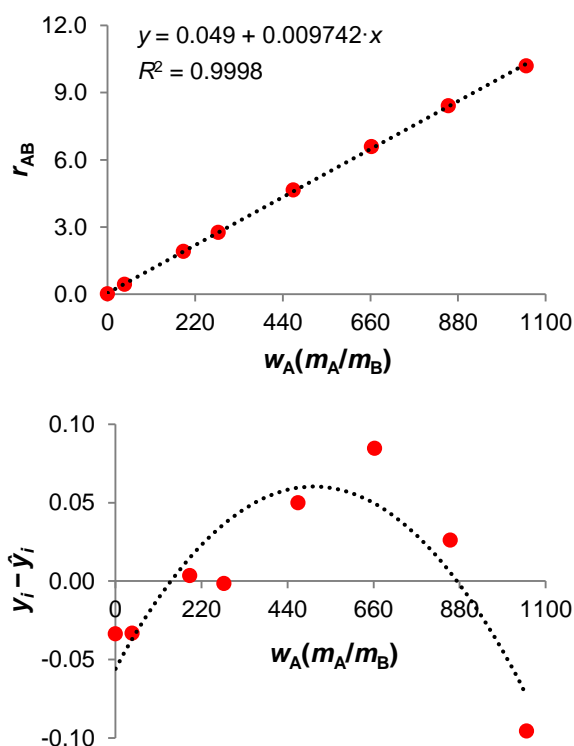
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In the *Bisphenol A measurement challenge* [1] Prof. Phenole proposed to her class to develop a high-precision analytical procedure for the determination of bisphenol A in water. The students promptly embraced the challenge and developed an isotope dilution gas chromatography mass spectrometry (ID GC-MS) method using the isotopically labeled  $^{13}\text{C}_5$ -BPA internal standard [2]. The students did all due diligence to set up the method but, during the quality control check, they found a  $-8.0\%$  bias at the low-end of the calibration curve.

When looking for a possible explanation, the students came across two interesting studies: in 1976 Pickup and McPherson showed that the isotope dilution mass spectrometry calibration (IDMS) curve can be nonlinear [3], and in 2016 Raposo noted that the coefficient of determination ( $R^2$ ) is not at all a suitable to establish whether a calibration curve is linear or not [4].

With this information at hand, the students decided to spend some time investigating the linearity of their calibration curve. As expected, the graph of the residuals showed a trend typical of a nonlinear response (Fig. 1) [5].

The students were reassured by this finding and were confident that this was a data analysis problem with no additional experiments needed. They decided to use a nonlinear mathematical model for the calibration curve. At first, they used a generic quadratic function of the form  $y = a_0 + a_1 \cdot x + a_2 \cdot x^2$ . When the quadratic model was applied to the data in Table 1, the fitting coefficients were calculated using the ordinary least squares



**Fig. 1:** Top: isotope dilution calibration curve of BPA with  $^{13}\text{C}_5$ -BPA internal standard from Ref. [1]. Bottom: graph of the residuals.

(OLS), as reported in Table 2. The quadratic function was reasonably adequate to account for the nonlinear effects, also in the low end of the calibration. Since the adoption of the quadratic model was not motivated by any theoretical reason, the students decided to further investigate the origin of the nonlinear trend. Using some

**Table 1:** Isotope dilution with  $^{13}\text{C}_5$ -BPA internal standard: calibration curve and errors using linear, quadratic and rational model equations

$w_A$ ng/g	$m_A$ g	Experimental data			Linear model		Quadratic model		Rational model	
		$m_B$ g	$w_A(m_A/m_B)$ ng/g	$r_{AB}$ V/V	$\hat{w}_A$ ng/g	error %	$\hat{w}_A$ ng/g	error %	$\hat{w}_A$ ng/g	error %
0.000	0.8867	0.3844	0.000	0.0149 (23)						
19.66	0.8693	0.3978	42.96	0.4338 (42)	18.10	-8.0 %	19.83	0.9 %	19.83	0.8 %
85.80	0.8835	0.3981	190.4	1.907 (18)	85.96	0.2 %	85.29	-0.6 %	85.26	-0.6 %
126.7	0.8693	0.3965	277.8	2.753 (32)	126.6	-0.1 %	125.0	-1.4 %	124.9	-1.4 %
210.5	0.8789	0.3965	466.6	4.644 (60)	212.8	1.1 %	210.1	-0.2 %	210.1	-0.2 %
304.5	0.8629	0.3963	663.0	6.592 (75)	308.5	1.3 %	306.2	0.6 %	306.2	0.6 %
392.5	0.8662	0.3971	856.2	8.415 (39)	393.7	0.3 %	393.5	0.3 %	393.5	0.3 %
477.4	0.8693	0.3948	1051	10.193 (33)	472.9	-0.9 %	476.3	-0.2 %	476.2	-0.2 %

The standard deviation of triplicate measurements (applicable to the last two digits of the value) is given in the parenthesis.  $w_A$ , mass fraction of natural BPA;  $m_A$ , mass of the solution having  $w_A$  mass fraction;  $m_B$ , mass of the internal standard solution having  $w_B = 94.4$  ng/g;  $w_A(m_A/m_B)$  = corrected mass fraction ( $x$ -axis);  $r_{AB}$  = measured isotope ratio of the blend ( $y$ -axis). The error associated to the model was calculated as the relative difference between the mass fraction predicted using the model ( $\hat{w}_A$ ) and the gravimetric one ( $w_A$ ):  $(\hat{w}_A - w_A)/w_A$

**Table 2:** Mathematical models for the interpretation of the isotope dilution calibration curve in Table 1

Model equation	$a_0$	$a_1$	$a_2$	Excel code
$y = a_0 + a_1 \cdot x$	0.049 <i>0.034</i>	0.009742 <i>0.000059</i>		=LINEST( $y,x$ ,TRUE,TRUE)
$y = a_0 + a_1 \cdot x + a_2 \cdot x^2$	-0.007 <i>0.021</i>	0.01020 <i>0.00011</i>	$-4.51 \cdot 10^{-7}$ <i><math>1.01 \cdot 10^{-7}</math></i>	=LINEST( $y,x^{\{1,2\}}$ ,TRUE,TRUE)
$y = (a_0 + a_1 \cdot x) / (1 + a_2 \cdot x)$	-0.008 <i>0.022</i>	0.01021 <i>0.00012</i>	$4.71 \cdot 10^{-5}$ <i><math>1.12 \cdot 10^{-5}</math></i>	=LINEST( $y,x^{\{1,1\}} \cdot y^{\{0,1\}}$ ,TRUE,TRUE)

Microsoft Excel 2013 was used to perform the ordinary least squares fitting with the LINEST function. The value of the fitting parameters is reported on the first line whereas the standard uncertainty is reported in italic on the second line. Here,  $y$  is the isotope ratio ( $r_{AB}$ ) and  $x$  is the analyte mass fraction normalized by the gravimetric composition ( $w_A \cdot m_A/m_B$ )

**Table 3:** Isotope dilution with  $^{13}\text{C}_5$ -BPA internal standard: more internal standard, more calibration data, and shorter dynamic range

$w_A$ ng/g	$m_A$ g	Experimental data			Linear function	
		$m_B$ g	$w_A(m_A/m_B)$ ng/g	$r_{AB}$ V/V	$\hat{w}_A$ ng/g	error %
0.000	0.8723	0.3857	0.000	0.00098 (52)		
44.41	0.8555	0.3867	98.24	0.18877 (56)	44.73	0.7 %
92.01	0.8687	0.3941	202.8	0.3888 (31)	92.60	0.6 %
131.9	0.8760	0.3892	296.8	0.5675 (20)	132.4	0.4 %
180.7	0.8655	0.3902	400.8	0.76696 (92)	181.6	0.5 %
225.2	0.8735	0.3888	505.9	0.959 (12)	224.2	-0.4 %
275.8	0.8622	0.3909	608.3	1.159 (10)	276.1	0.1 %
317.1	0.8661	0.3893	705.5	1.3308 (65)	314.3	-0.9 %
363.4	0.8677	0.3920	804.3	1.518 (19)	360.4	-0.8 %
457.9	0.8636	0.3915	1010	1.9386 (65)	461.9	0.9 %

The absolute difference of duplicate measurements (applicable to the last two digits of the value) is given in the parenthesis. All other conventions are the same as reported in Table 1 with the exception of  $w_B = 512.0$  ng/g

algebra, the general isotope dilution equation could be reworked into the following expression [6]:

$$y = \frac{a_0 + a_1 \cdot x}{1 + a_2 \cdot x} \quad (1)$$

where  $y$  is the isotope ratio ( $r_{AB}$ ) and  $x$  is the mass fraction corrected by the gravimetric composition of the

blends ( $w_A \cdot m_A/m_B$ ). Since Eq. 1 was obtained from first principles, the theoreticians tried fitting it to the isotope dilution data. As shown in Table 1, the performance of both quadratic and rational model were similar with relative errors <1.5 % across the calibration range - well within the target requested by Prof. Phenole.

Although the theoreticians were satisfied about the results obtained, another group of students was more resistant to take all this mathematics and decided to go back to the laboratory and find a suitable practical solution that could tolerate the use of a linear model. This is possible because in IDMS the level of nonlinearity can be tuned by changing the experimental conditions [7]. For example, the linearity improves when the amount of internal standard increases. With this in mind, the experimental folks returned to the laboratory and decided to use 5.4 times more internal standard. Just to be sure, they also reduced the dynamic range and increased the number of working standards. Under these conditions, they observed that the linear model could be used to build the calibration curve with good performance even at low concentration: with an error  $<1\%$  - the solution proposed by the experimentalists exceeded the expectation of Prof. Phenole (Table 3).

Prof. Phenole was impressed by both approaches. Although the students did not provide a full bottom-up evaluation of uncertainty [8], which should have been done to properly qualify the method, Prof. Phenole was delighted to see the students' commitment to deal with nonlinear issues in isotope dilution and full marks were awarded to the class.

Notably, the theoreticians revealed that when the isotopes of the analyte overlap with those of the internal standard (which happens almost all the time) the IDMS response is *always* nonlinear, and, therefore, a nonlinear model should be used for calibration [6]. On the other hand, the experimentalists sidestepped the nonlinearity issue by adopting better experimental conditions aimed to minimize the nonlinear deviations [7]; this way, the linear model could still be considered a viable option for IDMS calibration.

Before dismissing the class, Prof. Phenole commented that measurements in bioanalytical chemistry always benefit from the synergy between a well designed measurement methods and proper data analysis approaches.

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