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Publisher’s version / Version de l'éditeur:
https://doi.org/10.1007/s00216-021-03383-8
Analytical and Bioanalytical Chemistry, 413, 16, pp. 4105-4107, 2021-06-24

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Analytical and Bioanalytical Chemistry manuscript No.
(will be inserted by the editor)

Bisphenol A measurement challenge

Enea Pagliano

Received: date / Accepted: date

1 Meet the challenge

Prof. Phenole, a renowned analytical chemist, is engaging into a *lectio magistralis* on polycarbonate materials obtained from bisphenol A (BPA). Prof. Phenole pointed out that, since the 1950s, the demand of BPA and related compounds has witnessed an extensive growth. In fact, already in the 1980s, the worldwide production of BPA exceeded million tons [1]. Prof. Phenole described the great mechanical and optical properties of polycarbonates and gave an overview of the risks related to these materials. She explained that food packaging, containers, and water bottles made of polycarbonates can leak BPA, and that this estrogen-like molecule can cause adverse health effects resulting in the disruption of the endocrine system [2].

As a part of the analytical chemistry class, Prof. Phenole has tasked the students to think about a surveillance program to monitor the BPA levels in the tap water. The first assignment was the development of an accurate method for measuring BPA and top marks were promised to those who can propose a method with a relative standard uncertainty below 3 %.

The students accepted the challenge and began looking for suitable approaches. They quickly found an official method from the ISO standard collection [3]: the ISO 18857-2:2009 describes determination of BPA by GC-MS with isotopically-labeled BPA-d_{16} as an internal standard. The students were enthusiastic to use GC-MS for the first time, but were skeptical about the fully-deuterated BPA-d_{16}. Many scholars have noted that deuterated materials are not ideal internal standards [4–6]. For example, the properties of deuterated compounds can be quite different from the natural compounds which leads to differences in extraction yields [6]. A good demonstration of the different chemical behavior between the BPA and fully-deuterated BPA can be seen from the GC-MS chromatogram of these two compounds - they can actually be resolved chromatographically [3]. In order to limit such unwanted isotopic effects, the students decided for a more robust ^{13}C-labelled internal standard with a small number of carbon-13 atoms. For this purpose, they selected the NRC BPAL-1 Certified Reference Material with five carbon-13 atoms (Fig. 1) as the internal standard.

Although quantitation using isotopic standards (isotope dilution) is often explained with complicated mathematical formulae [7], all students voted to keep things simple and use a calibration curve [8, 9]. Hence, a series of natural BPA standards (0-500 ng/g) was prepared gravimetrically. These standard solutions were then spiked with nearly-equal amounts of the $^{13}C_5$-BPA internal isotopic standard and the resulting solutions were analyzed by GC-MS. The response curve (*i.e.* the measured isotope ratio *vs* the mass fraction of BPA) was obtained in Excel using the ordinary linear regression.

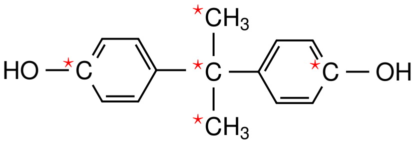

Fig. 1: NRC BPAL-1 CRM: the five ^{13}C labelled atoms are marked by red stars

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Table 1: Isotope dilution with $^{13}C_5$-BPA internal standard: calibration curve

w_A ng/g	m_A g	m_B g	$w_A(m_A/m_B)$ ng/g	r_{AB} V/V
0.000	0.8867	0.3844	0.000	0.0149 (23)
19.66	0.8693	0.3978	42.96	0.4338 (42)
85.80	0.8835	0.3981	190.4	1.907 (18)
126.7	0.8693	0.3965	277.8	2.753 (32)
210.5	0.8789	0.3965	466.6	4.644 (60)
304.5	0.8629	0.3963	663.0	6.592 (75)
392.5	0.8662	0.3971	856.2	8.415 (39)
477.4	0.8693	0.3948	1051	10.193 (33)

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 standard deviation of triplicate measurements (applicable to the last two digits of the value) is given in the parenthesis.

2 The challenge

The students set up the GC-MS acquisition method by following the experimental conditions found in the ISO 18857-2:2009. At this point, a series of BPA standards (0-500 ng/g) were prepared in acetone. The NRC BPAL-1 standard was also prepared in acetone and used as internal standard (94.4 ng/g $^{13}C_5$-BPA). The blends for calibration were prepared gravimetrically by mixing approx. 1.1 mL (0.86 g) of natural BPA standards with approx. 0.5 mL (0.39 g) of the internal standard. The blends were carefully mixed and 25 µL of N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA, Fig. 2) was added to form the volatile trimethylsilyl derivative which was analyzed by GC-MS.

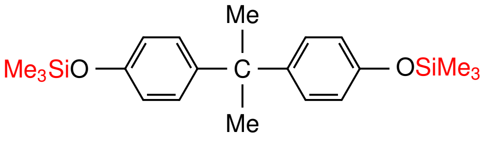

Fig. 2: BPA derivative after reaction with MSTFA

During the standard electron impact ionization the trimethylsilyl derivative of the BPA produces an intense fragment at $[M\text{-}15]^+$ (loss of a methyl group). Therefore, signal acquisition was carried out in a selected ion monitoring mode by monitoring m/z 357 (the most abundant signal in the natural BPA) and m/z 361 (the most abundant in the isotopically-labelled internal standard). The isotope ratio in the analyzed BPA samples was calculated by dividing the chromatographic peak area at m/z 357 with the peak at m/z 361. All experimental data acquired by the students is reported in Table 1 and Fig. 3 shows the calibration curve along with the best linear fit. The students were proud of the calibration curve and the high value of their correlation coefficient ($R^2 = 0.9998$). Things were looking very promising.

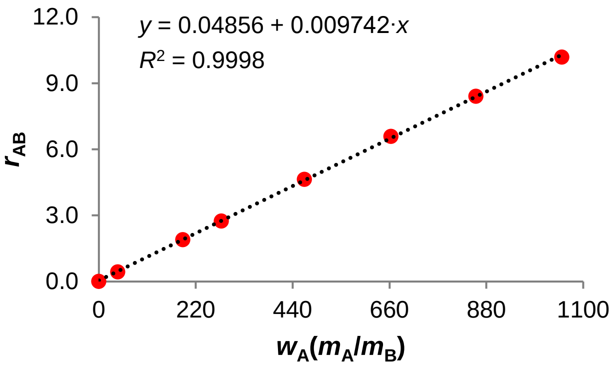

Fig. 3: Isotope dilution calibration curve of BPA with $^{13}C_5$-BPA internal standard

For quality control, the students tried to perform a calculation to verify if the equation obtained from the fitting was suitable to back calculate the mass fraction of the calibration standards. For example, if a_0 and a_1 are intercept and slope of the calibration curve in Fig. 3, the concentration of the lowest standard could be calculated as follow:

$$\begin{aligned} w_A &= \frac{m_B}{m_A}\cdot\frac{r_{AB}-a_0}{a_1} \\ &= \frac{0.3978}{0.8693}\cdot\frac{0.4338-0.04856}{0.009742} = \qquad (1) \\ &= 18.10 \text{ ng/g} \end{aligned}$$

When the calculated BPA result for the lowest standard (18.10 ng/g, Eq 1) was compared with the gravimetric preparation data (19.66 ng/g, Table 1), a deviation of –8.0 % was found. Since the calibration curve looks so good, relative errors on the r_{AB} was <1 % for data points above blank, and the uncertainty on $w_A(m_A/m_B)$ even smaller, students could not figure out why this standard shows such a big bias.

Is it too late for students to get top marks in the analytical chemistry class? Can you suggest a reason for this bias and provide a practical solution?

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