ORIGINAL ARTICLE

A C G publications *J. Chem. Metrol.* 17:2 (2023) 225-237

journal of chemical metrology

Comparison between top-down and bottom-up approaches in the estimation of measurement uncertainty in Bisphenol A analysis by HPLC-FLD

Mehmet Kılınçer and Mustafa Özyürek*

Istanbul University-Cerrahpasa, Faculty of Engineering, Department of Chemistry, Division of Analytical Chemistry, Avcilar 34320, Istanbul, Turkey

(Received August 04, 2023; Revised October 18, 2023; Accepted October 30, 2023)

Abstract: Measurement uncertainty is a metrological concept that characterizes distributions/variations that can make the measurement results logical and is linked to the result of the measurement. The measurement uncertainty of the method was estimated using two basic approaches (top-down and bottom-up approach). The top-down approach covers in-house validation data (trueness, repeatability and intra-lab reproducibility), while the bottom-up approach involves individual contributions to all uncertainty at each stage of the analysis/process. We estimated measurement uncertainty of BPA analysis by HPLC-FLD test according to JCGM GUM and EURACHEM-CITAC guidelines. The relative expanded uncertainties at the BPA concentration by the bottom-up approach and top-down approaches were ±3.2% and ±4.8% respectively (95% confidence interval, k=2). Thus, although it is seen that the results of the two approaches are different in chromatographic BPA analysis, it is concluded that the measurement uncertainty related to BPA analysis, especially in food analysis laboratories, can be determined by a simpler top-down approach.

Keywords: Bisphenol A; measurement uncertainty; bottom-up approach; top-down approach; HPLC-FLD.

1. Introduction

Bisphenol A (BPA) is a synthetic chemical used in all packaging materials, especially in PET bottles, baby bottles, sports equipment, lining the insides of food cans, etc. On the other hand, although BPA interacts with estrogen receptors in the human body as an endocrine disrupting chemical, BPA is the main ingredient in polycarbonate plastics in making baby bottles and similar food containers [1]. Therefore, BPA, even at low concentrations, causes some cancer formations, fat cell production and negative changes in the nervous systems [2]. In 2015, European Food Safety Authority (EFSA) recommended a temporary tolerable daily intake (t-TDI) for BPA of 4 μg/kg bw/day. In 2021, a draft scientific opinion was published in which the t-TDI value of BPA was revised to 0.04 ng/kg/day, taking into account the effects of BPA on the immune system [3]. In accordance with Regulation 10/2011/EU on plastic materials and objects intended to come into contact with foodstuffs, BPA is permitted for use in food contact materials in the European Union (EU). The usage of BPA in plastic bottles and other

* Corresponding author: E-Mail: mozyurek@iuc.edu.tr

The article was published by ACG Publications
http://www.acgpubs.org/journal/journal-of-chemical-metrology July-December 2023 EISSN:1307-6183
DOI:http://doi.org/10.25135/jcm.95.2308.2862
Available online: November 09, 2023

packaging containing food for children and babies under the age of three is banned from September 2018. Currently, BPA is authorized for use in plastics, coatings and varnishes with a certain migration limit (SML) of 0.05 mg/kg of food, with the exception of FCMs for infants, which are not allowed to be used.

Analytical techniques for BPA analysis in various matrices include radioimmunology, competitive ELISA (enzyme-linked immunosorbent assays), electrochemistry, chromatography (HPLC-UV, HPLC-FLD, HPLC-ED, GC, GC-MS, LC-MS, LC-MS/MS), fluorescence and electrochemical sensor in recent years. On the other hand, among the existing techniques, the HPLC-FLD method is frequently used in high-sensitivity BPA analysis in different matrices.

Measurement uncertainty is a parameter that characterizes distributions that can rationalize the values obtained as a result of the measurement and is linked to the measurement result [4, 5]. Measurement uncertainty is also a basic requirement for accredited laboratories and is also considered as a quantitative indicator of the quality of an analysis/test result in terms of showing the extent to which a test result represents the true value. The contribution of each step in an analysis or process to the overall uncertainty is estimated using a "bottom-up" approach [6]. In this approach, a clear definition of what is being measured is made by clearly revealing the relationship between the quantity and the parameters to which it depends, and as a result, a systematic evaluation of all sources of uncertainty is carried out. Within the framework of statistical propagation rules, the compound uncertainty of the process/analysis is obtained by combining the uncertainties identified for each parameter [7]. In this approach, it can be time-consuming to identify and perform experiments to generate some additional data in the estimation. On the other hand, this approach is considered to be useful in terms of determining the stages encountered in method optimization and eliminating the deficiencies in these stages [6]. Measurement uncertainty is also estimated by evaluating method validation experimental data or quality control (QC) data by a "top-down" approach [8]. Compared to the bottom-up approach, this method is more practical and at the same time more cost-effective. It is possible to update this approach with additional data using proficiency test (PT), routine internal quality control (IQC) and method verification data.

In the literature, there is no study about the assessment of top down and bottom-up approaches uncertainty for BPA. In this study, the quantity intended to be measured [9] is µg/kg BPA in plastic baby bottles by method BS EN 14372:2004 [10]. The measurement uncertainty parameters of BPA analysis were identified and quantified as suggested by the EURACHEM-CITAC Guide [11] and JCGM GUM-6:2008 [9] standards. Experimental studies were also performed using bottom-up and top-down approaches according to the ISO/IEC 17025:2017 standards [12] metrology.

2. Experimental

2.1. Chemicals and Instruments

All chemicals used in this study were of analytical reagent grade. Bisphenol A (CAS 80-05-7) (2,2-Bis (4- hydroxyphenyl) propane, 99%) was purchased from Sigma Aldrich (Steinheim, Germany). All chromatographic experiments were performed using an Agilent 1100 High Performance Liquid Chromatograpy-Fluorescence Detector (HPLC-FLD) system (Agilent Technologies, US). Other instruments were Sartorius CP 224 S balance and oven as a thermostate (Bınder GmbH, Germany). Statistical analyses were realized using Microsoft Excel 2016 for all calculations.

2.2. Chromatographic Conditions

BPA was analyzed with an high-performance liquid chromatography-fluorescence detector (HPLC-FLD). LC was performed with an ACE 5 C18 liquid chromatography column (250 mm×4.6 mm, 5 µm) maintained at 25 °C. Flow rate constant and 1.0 mL/min all the analysis time. Chromatographic separation was performed with %70:30 methanol and water isocratic system. FLD offers time-programmable excitation and emission wavelength switching. BPA was detected using FLD with excitation wavelength at 275 nm and emission wavelength 313 nm. The related compound was identified by comparing the retention time and emission spectra with the standard.

2.3. Method Procedure

The method procedure was performed with reference to BS EN 14372:2004 [10]. BPA analysis in plastic materials and materials that come into contact with food, included in the scope of use and care items for children, plastic cutlery, knives and baby food items (baby bottles, pacifiers, etc.). As a food simulant, 100 mL of 50% ethyl alcohol containing solution was transferred to the baby bottle sample. This sample filled with solution was stored under static conditions for 24 h at 40 °C in a drying oven and transferred 1 mL of the solution into a vial suitable for HPLC injection.

3. Results and discussion

3.1. Uncertainty Measurement of BPA

Uncertainty is a term generally used to make analysis/measurement results more meaningful and easier to understand. The measurement uncertainty allows to see how much variability there is in the measurement results under repeatable conditions. To visualize how different sources of uncertainty contribute to the overall measurement result in chromatographic BPA analysis, all sources/parameters of uncertainty that contribute to the overall uncertainty budget are given in the cause effect diagram in Figure 1.

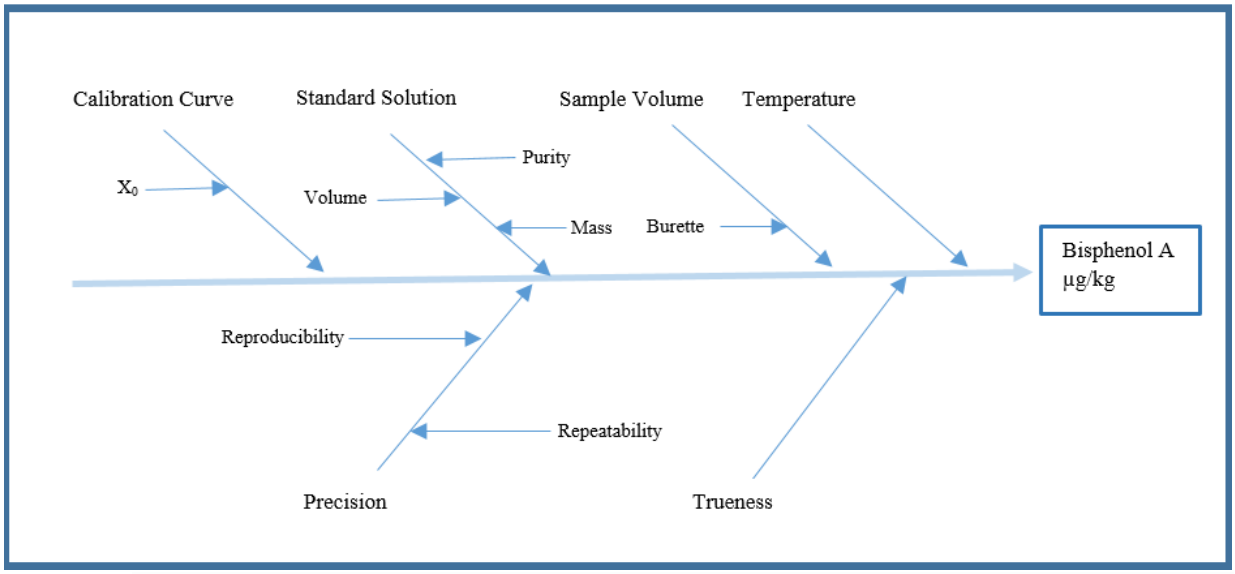

Figure 1. Cause and effect diagram of the potential sources of uncertainty in measurement the BPA analysis by HPLC-FLD.

$BPA_{Uncertainty}$ value was calculated using the Eq. 1 [8]:

$$BPA_{Uncertaint\,y} = f_{calibration\ curve} \cdot f_{precision} \cdot f_{trueness} \cdot f_{temperature} \cdot f_{c_{BPA}} \cdot f_{volume} \qquad \text{(Eq. 1)}$$

3.1.1. Bottom-up Approach

In the bottom-up approach, the important factors that contribute to the overall uncertainty are individually identified and quantified. Even with a relatively simple method, identifying and quantifying all the individual uncertainty components can be difficult and time-consuming. Type A and Type B uncertainty evaluations were used in the bottom-up measurement uncertainty budget. Type A uncertainty involve the method of evaluation of uncertainty by the statistical analysis of series of observations. Type

B uncertainty involves the evaluation by methods other than statistical analysis of a set of observations, such as manufacturer specifications and data contained in calibration and other certifications.

3.1.1.1. Uncertainty of Standard Solution of BPA, $u_r(C_{BPA})$

The uncertainty of the C_{BPA} of standard/reference solution was estimated by combining the measurement uncertainties of stock and working solutions of BPA. The mass, volume, and purity uncertainties affecting the final concentration of the stock BPA solution were combined (Table 1) and the related uncertainty of measurement was calculated.

Table 1. The relative contributions of individual input to the uncertainty of stock BPA solution

Components	Estimate	Uncertainty	Distribution factor	u(x)	u(x)/x
mass (M)/g	0.08	-	-	$4.2\ 10^{-5}$ [a]	0.00052
purity (P)	0.99	0.01	rectangular $\sqrt{3}$	0.00577	0.0058
volume (V)/mL	100	0.0136	triangular $\sqrt{6}$	-	0.0055

[a] mass balances standard uncertainty

The mass concentration of BPA in MeOH (1000 µg/kg) was given as follows (Eq. 2):

$$C_{BPA} = \frac{m_{BPA} \cdot P}{V \cdot d} = 1000\ \mu g/kg \qquad \text{(Eq. 2)}$$

The measurement uncertainty of the C_{BPA} solution was calculated according to the Eq. 3.

$$u_r(C_{stock}) = \frac{u(C_{BPA})}{C_{BPA}} = \sqrt{\left(u_r(m)^2 + u_r(P)^2 + u_r(V)^2\right)} \qquad \text{(Eq. 3)}$$

$$u_r(C_{stock}) = \sqrt{(0.00052)^2 + (0.0058)^2 + (0.0055)^2} = 0.0080$$

According to the standard preparation steps specified in the BS EN 14372:2004 [10]; 1 mL of the main stock BPA solution is taken and 1 mL of this level II stock solution is taken and 100 mL of the level III standard is prepared. Calibration working standards are also prepared from a level III standard solution.

3.1.1.1.1. Repeatability

The repeatability of a series of fill-and-empty test results for a 100 mL balloon was found to be 0.05 standard deviation. The repeatability of a series of fill-and-empty test results for a 1 mL automatic pipette was found to be 0.0015 standard deviation. The repeatability of a series of fill-and-empty test results for the 0.1 mL automatic pipette was found to be 0.0005 standard deviation.

3.1.1.1.2. Calibration

The calibration certificate value for the 100 mL balloon is given as ±0.0136 mL at the 95% confidence level. It is calculated as 0.0136/2 = 0.0068 mL with a rectangular distribution. The calibration certificate value for a 1 mL pipette is given as ±0.00186 mL at the 95% confidence level. It is calculated as 0.00186/2 = 0.00093 mL with a rectangular distribution. The calibration certificate value for the 0.1

mL pipette is given as ±0.00103 mL at the 95% confidence level. It is calculated as 0.00103/2 = 0.000515 mL with a rectangular distribution. Each calibration working standard was calculated separately in the volume uncertainty calculation.

3.1.1.1.3. Temperature

The temperature change in the laboratory is given as ±5 °C from 22 °C. The volumetric expansion coefficient of water with temperature is 2.1×10^{-4}/°C.

Standard uncertainty according to rectangular distribution

100 mL balloon $\frac{100 \times 2.1 \cdot 10^{-4} \times 5}{\sqrt{3}} = 0.06$ mL

1 mL pipette $\frac{1 \times 2.1 \cdot 10^{-4} \times 5}{\sqrt{3}} = 0.0006$ mL

0.1 mL pipette $\frac{0.1 \times 2.1 \cdot 10^{-4} \times 5}{\sqrt{3}} = 0.00006$ mL

The uncertainty of volume for working standards preparation in 1 mL HPLC vials:

- Std 1 = 1 mL pipette was used once, 0.1 mL pipette was used once.

(from 1 mL pipette) = $\sqrt{0.0015^2 + 0.00093^2 + 0.0006^2} = 0.001864$

(from 0.1 mL pipette) = $\sqrt{0.0005^2 + 0.000515^2 + 0.0006^2} = 0.00072$

- Std 2 = 1 mL pipette was used 1 times, 0.1 mL pipette was used 2 times.

(from 1 mL pipette) = $\sqrt{0.0015^2 + 0.00093^2 + 0.0006^2} = 0.001864$

(from 0.1 mL pipette) = $\sqrt{0.0005^2 + 0.000515^2 + 0.0006^2} = 0.00072 \rightarrow 0.00072 \times 2 = 0.00144$

- Std 3 = 1 mL pipette was used 2 times, 0.1 mL pipette was used 3 times.

(from 1 mL l pipette) = $\sqrt{0.0015^2 + 0.00093^2 + 0.0006^2} = 0.001864$

(from 0.1 mL pipette) = $\sqrt{0.0005^2 + 0.000515^2 + 0.0006^2} = 0.00072 \rightarrow 0.00072 \times 3 = 0.00216$

- Std 4 = 1 mL pipette was used 2 times.

(from 1 mL pipette) = $\sqrt{0.0015^2 + 0.00093^2 + 0.0006^2} = 0.001864 \rightarrow 0.001864 \times 2 = 0.003728$

- Std 5 = 1 mL pipette was used 2 times.

(from 1 mL pipette) = $\sqrt{0.0015^2 + 0.00093^2 + 0.0006^2} = 0.001864 \rightarrow 0.001864 \times 2 = 0.003728$

- III. level stock solution = 1 mL pipette was used once, 100 mL balloon was used once.

(from 100 mL balloon) = $\sqrt{0.05^2 + 0.0068^2 + 0.06^2} = 0.0784$

(from 1 mL pipette) = $\sqrt{0.0015^2 + 0.00093^2 + 0.0006^2} = 0.001864$

Table 2. Total working standard volume uncertainty components

Component		Value	Standard Dev.	Rel. Std.Dev.	Combined Dev.
std 1	1 mL pipette	1	0.001864	0.001864	0.001865
	0.1 mL pipette	0.1	0.00072	0.000072	
std 2	1 mL pipette	1	0.001864	0.001864	0.001865
	0.1 mL pipette	0.2	0.00144	0.000072	
std 3	1 mL pipette	1	0.001864	0.001864	0.001865
	0.1 mL pipette	0.3	0.00216	0.000072	
std 4	1 mL pipette	2	0.003728	0.001864	0.001864
std 5	1 mL pipette	2	0.003728	0.001864	0.001864
III level	100 mL balloon	100	0.0784	0.000784	0.002022
	1 mL pipette	1	0.001864	0.001864	
u_r					0.004634

$$u_r\left(C_{BPA}\right)=\frac{u\left(C_{BPA}\right)}{C_{BPA}}=\sqrt{\left(u_r\left(C_{stock}\right)^2+u_r\left(C_{working}\right)^2\right)} \quad \text{(Eq. 4)}$$

$$\Rightarrow u_r\left(C_{BPA}\right)=\sqrt{\left(0.0080\right)^2+\left(0.0046\right)^2}=0.0092$$

3.1.1.2. Uncertainty from Sample Volume

100 mL automatic burette was used in the experimental studies for baby bottles. Individual parameters:

3.1.1.2.1. Repeatability

The repeatability of a series of fill-and-empty test results for a 100 mL automatic burette was found to be 0.009 mL as standard deviation.

3.1.1.2.2. Calibration

For the 100 mL automatic burette is given as ±0.01238 mL at a 95% confidence level. It is calculated as 0.01238/2= 0.00619 mL with a rectangular distribution.

3.1.1.2.3. Temperature

Laboratory temperature set as 22±5 ºC. Coefficient of volumetric expansion of water with temperature $2.1\text{x}10^{-4}$/ ºC is.

Standard uncertainty according to rectangular distribution 100 mL automatic burette;
$\left(100\times 2.1\cdot 10^{-4}\times 5\right)/\sqrt{3}=0.06$ mL is calculated as.

By combining these uncertainty components, the total volume uncertainty is given in Table 3.

$$\Rightarrow u_r\left(V\right)=\sqrt{\left(0.009\right)^2+\left(0.00619\right)^2+\left(0.0606\right)^2}=0.0625$$

Table 3. Uncertainty table from sample volume

Component	Value	Standard Dev.	Relative Std. Dev.
Sample volume (mL) (auto.burette)	100	0.0625	0.000625
Total uncertainty(u_{vol})			0.000625

3.1.1.3. Uncertainty Related to the Calibration Curve, $u_r(X_0)$

The BPA calibration standards at five different concentrations were analyzed and calibration curve measurement uncertainty was calculated using Eq.s 5 and 6 according to the the guidelines of EURACHEM/CITAC [11].

$$\mathrm{var}(X_0) = \frac{\mathrm{var}(y_{obs})}{b^2} + \frac{S^2}{b^2}\left[\frac{1}{\sum w_i} + \frac{(X_0 - \overline{X})^2}{\sum(w_i X_i^2) - \frac{\sum(w_i X_i)^2}{\sum w_i}}\right] \quad \text{(Eq. 5)}$$

var(X_0) and var(y_{obs}): the variances related to X_0 and of the observed variable, respectively; S: RMSD; b: the slope; X_o and X_i: the concentration obtained from calibration curve and reference solutions, respectively; $\overline{X}$: the mean of the concentrations used in the formation of calibration curve; w_i : the weight of y_i.

$u_r(X_0)$ was calculated according to the Eq. 6:

$$u(X_0) = \sqrt{\mathrm{var}(X_0)} = 0.3652 \text{ µg/kg}$$

$$u_r(X_0) = \frac{u(X_0)}{X_0} = \frac{0.3652}{30} = 0.0122 \quad \text{(Eq. 6)}$$

3.1.1.4. Uncertainty Related to the Temperature

BPA analyses were performed under the condition of 40 ± 0.5 ℃ and 24 h in oven. The uncertainty associated with the temperature ($u_r(T)$) was calculated using Eq. 7.

$$u(T) = \frac{\Delta T}{\sqrt{6}} = 0.204$$

$$u_r(T) = \frac{u(T)}{T} = 0.0051 \quad \text{(Eq. 7)}$$

The combined uncertainty obtained by the bottom-up approach was calculated according to the Eq.8.

$$\frac{u(BPA)}{BPA} = \sqrt{(u_r(X_0))^2 + (u_r(C_{BPA}))^2 + (u_r(T))^2 + (u_r(V))^2} \quad \text{(Eq. 8)}$$

$$u_r(BPA) = \sqrt{(0.0122)^2 + (0.0092)^2 + (0.0051)^2 + (0.000625)^2} = 0.016$$

3.1.2. Top-down Approach

Top-down approaches are based on both random and systematic error components of each analytical step involved in a process/analysis. The uncertainty of the mean caused by random errors is

given by the repeatability of measurement results (RSD_R) in BPA analysis. Type A uncertainty evulation was used in the top-down measurement uncertainty budget. Type A uncertainty evaluation involves the evaluation of uncertainty by the statistical analysis of series of observations. Statistical evaluations of top-down approach measurement uncertainty calculations based on verification data and proficiency test results were made according to Barwick and Ellison [13].

3.1.2.1. Uncertainty Related to Precision, $u_r(prec)$

3.1.2.1.1 Repeatability

The repeatability has also been considered for the determination of Bisphenol A in baby bottles. Repeatabiliy studies were carried out on baby bottle sample. BPA free baby bottle were spiked at the level of 20 µg/kg BPA for low concentration and 50 µg/kg BPA for high concentration, and the data obtained are given in Table 4.

Table 4. Low level and high level repeatability studies on baby bottle sample

	Low level (20 µg/kg)		**High level (50 µg/kg)**	
The number of repetitions	**X_1**	**X_2**	**X_1**	**X_2**
1	18.821	19.264	47.379	48.798
2	18.956	19.701	47.883	49.332
3	18.615	19.176	47.844	48.981
4	18.946	19.861	48.348	49.564
5	19.112	19.698	47.232	47.595
6	19.373	20.465	47.455	47.717
7	19.249	19.727	49.196	49.645
8	19.578	19.825	49.198	49.691
9	19.125	19.593	48.065	48.367
10	20.602	20.721	48.426	50.071
Average	19.2400	19.8000	48.1030	48.9760
SDr	0.4028	0.3860	0.6975	0.8515
RSD_R	0.0201	0.0193	0.0145	0.0174
% RSD_R	2.01%	1.93%	1.45%	1.74%

Calculation of uncertainty from repeatability studies were carried out [13] Eq.9:

$$RSD_{pool} = \sqrt{\frac{(n_1 - 1)\cdot RSD_1^2 + (n_2 - 1)\cdot RSD_2^2 ....}{(n_1 - 1) + (n_2 - 1) ....}} \qquad \text{(Eq. 9)}$$

$$\Rightarrow RSD_{pool} = \sqrt{\frac{(9\times 0.0201^2) + (9\times 0.0193^2) + (9\times 0.0145^2) + (9\times 0.0174^2)}{9+9+9+9}}$$

$$\frac{u_r(rep)}{rep} = 0.0179$$

3.1.2.1.2. Uncertainty Related to Reproducibility, u_r(repr)

In order to calculate the uncertainty from reproducibility, the recovery spiked BPA standard solution at the level of 20 µg/kg in the BPA free bottle sample was studied 2 times by 2 repeat on 5 different days. Study results were listed in Table 5. Reproducibility RSD_R and the uncertanity values were found by dividing it the working average.

Table 5. Reproducibility studies on baby bottle sample

The number of repetitions	X_1	X_2
1	18.801	19.211
2	18.910	19.717
3	18.510	19.276
4	18.776	19.113
5	19.101	19.224
6	19.573	20.465
7	19.549	19.447
8	19.021	19.574
9	19.125	19.593
10	20.602	20.012
Average	19.197	19.563
SD_R	0.537	0.379
RSD_R	0.0279	0.0194

Calculation of uncertainty from repeatability studies were carried out [13] Eq.10:

$$\mathrm{RSD}_{\mathrm{pool}} = \sqrt{\frac{(n_1 - 1)\cdot \mathrm{RSD}_1^2 + (n_2 - 1)\cdot \mathrm{RSD}_2^2}{(n_1 - 1) + (n_2 - 1)}} \qquad \text{(Eq. 10)}$$

$$\Rightarrow \mathrm{RSD}_{\mathrm{pool}} = \sqrt{\frac{\left(9 \times 0.0279^2\right) + \left(9 \times 0.0194^2\right)}{9 + 9}} = 0.0104$$

$$\Rightarrow \frac{u_r(repr)}{repr} = 0.0104$$

$$\Rightarrow \frac{u(prec)}{(prec)} = \sqrt{\left(u_r(repr)\right)^2 + \left(u_r(rep)\right)^2} \qquad \text{(Eq.11)}$$

$$\Rightarrow u_r(prec) = \sqrt{(0.0104)^2 + (0.0179)^2} = 0.021$$

3.1.2.2. Uncertainty related to Trueness u_r(true)

Measurement trueness: ‘Closeness of agreement between a reference quantity value and the average of an infinite number of replicate measured quantity values [9]. The contribution of trueness to

the measurement uncertainty of BPA analysis has been demonstrated by studying the international proficiency test FAPAS T 12111.

Table 6. Trueness studies on FAPAS T 12111

FAPAS T 12111		
BPA amount = 56.4 µg/kg		
The number of repetitions	**X_1**	**X_2**
1	55.642	56.120
2	54.778	56.005
3	56.086	54.321
4	57.740	56.114
5	53.698	57.233
6	54.815	55.873
7	54.415	54.128
8	53.699	55.412
9	52.521	55.478
10	56.586	55.719
Average	55.320	
Std . Dev.	1.245	
RSD_{pool}%	2.247	

Table 7. Results from the analysis of FAPAS T 12111

	Declared value			**Observed value**	
Analyte	**Concentration Fapas T 12111 (µg kg^{-1})**	**Quoted uncertainty (µg kg^{-1})***	**Standard uncertainty $u(C_{Fapas})$ (µg kg^{-1})****	**Mean C_{obs} (µg kg^{-1})**	**Standard deviation s_{obs} (µg kg^{-1})**
Bisphenol A	56.4	1.10	0.56	55.3	1.24

*The quoted uncertainty is an expanded uncertainty given at the 95% confidence level.
** The standard uncertainty is obtained by dividing the expanded uncertainty by 1.96.

The calculation of measurement uncertainty from the trueness studies was carried out [13] Eq.s 12-14.

$$\overline{R}_m = \frac{\overline{C}_{obs}}{C_{Fapas}} \qquad \text{(Eq. 12)}$$

$$\Rightarrow \overline{R}_m = \frac{55.3}{56.4} = 0.981$$

$\overline{R}_m$, taking into account u($\overline{R}_m$), is not significantly different from 1, so results are not corrected for recovery.

$$u\left(\overline{R}_m\right)=\overline{R}_m \times \sqrt{\frac{S_{obs}^2}{n \times \overline{C}_{obs}^2}+\left(\frac{u\left(C_{Fapas}\right)}{C_{Fapas}}\right)^2} \quad \text{(Eq. 13)}$$

$$\Rightarrow 0.981 \times \sqrt{\frac{(1.24)^2}{20 \times (55.3)^2}+\left(\frac{0.56}{56.4}\right)^2}=0.0111$$

$$t=\frac{\left|1-\overline{R}_m\right|}{u\left(\overline{R}_m\right)} \quad \text{(Eq. 14)}$$

$$\Rightarrow t=\frac{1-0.981}{0.0111}=1.70$$

If the degrees of freedom associated with u($\overline{R}_m$) are known, *t* value is compared with the 2-tailed critical value (t_{crit}) for the appropriate number of degrees of freedom at 95% confidence. If *t* is less than $t_{crit,}$ then $\overline{R}_m$ is not significantly different from 1 [13]. In this case, *t* was compared with the coverage factor (k = 2). When *t* value (1.70) value compared with the t_{crit} (1.73) for 19 degrees of freedom at 95 % confidence, there is no evidence to suggest that $\overline{R}_m$ is significantly different from 1. Therefore, $\overline{R}_m$ was assumed to equal 1 with an uncertainty, u ($\overline{R}_m$) of 0.011.

The combined uncertainty obtained by the top-down approach was calculated according to the Eq.15.

$$\frac{u(BPA)}{BPA}=\sqrt{\left(u_r\left(prec\right)\right)^2+\left(u_r\left(true\right)\right)^2} \quad \text{(Eq. 15)}$$

$$u_r\left(BPA\right)=\sqrt{(0.021)^2+(0.011)^2}=0.024$$

4. Conclusions

As a result of the uncertainty measurement studies in BPA analyses, the repeatability and reproducibility %RSD values were calculated according to the Horwitz equation (Eq.16).

$$\%RSD_R=2^{(1-0.5\log C)} \quad \text{(Eq.16)}$$

where C: the concentration (ppb, µg/kg)= 10^{-9}.

$$\%RSD_R=2^{\left(1-0.5\log 10^{-9}\right)}=\%45.25$$

$$\%RSD_r=0.66\times\%RSD_R=0.66\times 45.25=29.87$$

The $\%RSD_R$ = 2.65% and $\%RSD_r$ = 2.57% value obtained in this study were evaluated as appropriate because they were less than the target values ($\%RSD_R$ = 45.25% and $\%RSD_r$ = 29.87). The adjusted expected $\%RSD_R$= 44 for the ppb level given in the article (the item of acceptability criteria for precision) was calculated and the acceptability of the precision values obtained in this study was ensured [14]. The accuracy error obtained in this study was found as 5.30% with k=2 expansion, and the laboratory recovery values were obtained as 97% at low level (20 µg/kg) and 97% at high level (50 µg/kg) and acceptability was achieved. In another respect, in annex-B of the original method document, EN

14372:2004, where the BPA certainty data is located, the RSD_R is declared as ≤ 4.5%, typically ≤2.0%. The validity of this study was confirmed by the RSD_R= 2.28% value obtained.

The combined measurement uncertainties obtained by the top down and bottom-up approaches were as follows. The extended measurement uncertainties were calculated according to Eq. 17 (95% confidence interval, k=2).

$u_r(BPA)_{top\ down}$=0.024 and $u_r(BPA)_{bottom\ up}$=0.016

$U(BPA)=k*u_r(BPA)$ Eq.17

$U(BPA)= k*u_r(BPA)_{top\ down}=2*u_r(BPA)_{top\ down}= 0.048$

$U(BPA)= k*u_r(BPA)_{bottom\ up} =2*u_r(BPA)_{bottom\ up}= 0.032$

The comparison of the uncertainties obtained by the two approaches was made on the basis of the results of the FAPAS T 12111 proficiency test. The assigned value for proficiency test BPA was declared as 56.4 µg/kg and the standard deviation of proficiency σ_P =0.0124 [15]. The measurement uncertainties obtained as a result of both approaches are consistent with the mentioned reference. In summary, in this study we showed practical and detailed examples to estimate uncertainty using both bottom-up and top-down approaches. We revealed that the top down measurement uncertainty value for BPA is higher than the bottom up approach. We think that this is because the uncertainty components of the top down measurement uncertainty have many random uncertainty components compared to the bottom up approach. In addition, this situation is compatible with literature studies [16,17]. However, since the measurement uncertainties obtained with both approaches are similar to each other, a cost- and time-effective top-down approach can be preferred for the calculation of measurement uncertainty in BPA analysis in plastic materials in contact with food, materials included in the scope of use and care for children, plastic cutlery, and infant formula products.

ORCID
Mehmet Kılınçer: 0000-0002-4699-9222
Mustafa Özyürek: 0000-0001-5426-9775

References

[1] S. Jitka, G. Valeriya, B. Sara, K. Therese and B.Vito (2022). A call to Action: Free children from BPA's toxic legacy International Pollutants Elimination Network (IPEN).

[2] Y. Ma, H. Liu, J. Wu, L. Yuan, Y. Wang and X. Du (2019). The adverse health effects of bisphenol A and related toxicity mechanisms, *Environ. Res*. **176**,108575.

[3] EFSA CEP Panel (2023). Re-evaluation of the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs, EFSA Journal.

[4] I. Farrance and R. Frenkel (2012). Uncertainty of measurement: a review of the rules for calculating uncertainty components through functional relationships, *Clin. Biochem. Rev.* **33**(**2**), 49-75.

[5] M. Kılınçer, G. Çiçek, M. Özyürek and R. Apak (2022). Uncertainty estimation for total antioxidant capacity measurement of apple juice using main CUPRAC method, *J. Chem. Metrol.* **16**(**1**), 28-37.

[6] I.M. Dabalus, T.M. Schweikert and A Cannavan (2008). Comparison of methods for the estimation of measurement uncertainty for an analytical method for sulphonamides, *Food Addit. Contam. Part A Chem. Anal. Control Expo Risk Asses.* **25**, 1439-50.

[7] R. Rigo-Bonnin, A. Blanco-Font and F. Canalias (2018). Different topdown approaches to estimate measurement uncertainty of whole blood tacrolimus mass concentration values, *Clin Biochem.* **57**, 56-61.

[8] J.H. Lee, J.H. Choi, J.S. Youn, Y.J. Cha, W. Song and A.J. Park (2015). Comparison between bottom-up and top-down approaches in the estimation of measurement uncertainty, *Clin. Chem. Lab. Med*. **53**,1025-32.

[9] Joint Committee for Guides in Metrology (BIPM, IEC, IFCC, ILAC, ISO, IUPAC, IUPAP and OIML) Evaluation of measurement data – Guide to the expression of uncertainty in measurement (Sevres: International Bureau of Weights and Measures (BIPM)) JCGM 100:2008.

[10] BS EN 14372:2004. Child use and care articles. Cutlery and feeding utensils. Safety requirements and tests.

[11] EURACHEM/CITAC guide quantifying uncertainty in analytical measurement, Third Edition (2012).

[12] ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories, Geneva 2017.

[13] V.J. Barwick and S.L.R Ellison (2000). VAM Project 3.2.1 Development and Harmonisation of Measurement Uncertainty Principles Part (d): Protocol for uncertainty evaluation from validation data. LGC/VAM/1998/088.

[14] S. Bratinova, B. Raffael and C. Simoneau (2009). Guidelines for performance criteria and validation procedures of analytical methods used in controls of food contact materials. EUR 24105 EN. Luxembourg (Luxembourg): Publications Office of the European Union; JRC53034.

[15] FAPAS-Food chemistry proficiency test report 12111 (2022).

[16] T. Burr, S. Croft, A. Favalli, T. Krieger and B. Weaver (2021). Bottom-up and top-down uncertainty quantification for measurements, *Chemom. Intell. Lab. Syst.* **211**, 104224.

[17] R.R. Bonnin, P. Alía and F. Canalias (2018) Measurement uncertainty and metrological traceability of whole blood cyclosporin A mass concentration results obtained by UHPLC-MS/MS, *Clin. Chem. Lab. Med.* **56**, 1458-1468.