Supporting Information

Urinary Concentrations of Bisphenol A and Three Other Bisphenols in Convenience Samples of U.S. Adults during 2000–2014

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Analytical Method for the Quantification of Urinary Concentrations of Bisphenols

1. Preparation of Standard Stock Solutions and Quality Control (QC) Materials

We prepared the stock solutions of individual analytical standards and stable isotope-labeled internal standards in MeOH. Ten stock solutions containing BPA, BPS, BPF, and BPAF were generated by serial dilution of the individual stocks with MeOH; the final concentrations ranged from 0.01 to 100 ng/mL for all four analytes. The internal standard solution containing the stable isotope-labeled analogs of BPA, BPS and BPF was prepared so that a 50 μL spike would result in a concentration of each analyte of 25 ng/mL; we used $^{13}C_{12}$- BPA as the internal standard for BPAF. The stock solutions and internal standard solution containing all four analytes, dispensed into 1.5 mL glass vials and 10 mL glass vials respectively, were stored at -70 °C until used.

QC materials were prepared from blank urine pre-screened to confirm that it did not contain detectable concentrations of the target analytes. The blank urine was divided into two aliquots to create QC low (QCL) and QC high (QCH) concentration pools. The QCL and the QCH pools were enriched with different levels of native target compounds, and all QC materials were stored in 1.5 mL glass vials at -70 °C until used.

2. Limits of Detection (LODs)

Pre-screened blank urines spiked with the three lowest standards (0.01 μg/L, 0.1 μg/L, 1.0 μg/L) and isotope-labeled internal standard solutions were analyzed repeatedly to determine the LODs. LODs were calculated as $3S_0$, where S_0 is the standard deviation as the concentration approaches zero (*1*). The calculated LODs were 0.1 μg/L for all four bisphenols.

3. Method Validation

We obtained calibration curves after spiking water or blank urine with the analytical standards. The slopes of the calibration curves in H_2O or urine were quite similar (i.e., percentage of the difference <3%, calculated from the mean slope of three sets of standards prepared in H_2O and in urine), suggesting that the accuracy of the method was not compromised by the matrix. We used H_2O-based curves for quantification. The calibration curves included ten standard points with concentrations ranging from 0.01 μg/L to 100 μg/L; constructed using 1/x weighing linear regression, the calibration curves showed adequate linearity with correlation coefficients >0.99 for all four analytes.

The method accuracy was assessed by analyses of blank urine spiked at four spiking levels (0.5, 1.0, 5.0, 10.0 μg/L), and expressed as the percentage of the recovery. The method accuracy of the four analytes varied from 91 % to 107 % (Supporting Information Table 1). We determined the method precision from 20 repeated measurements of the two QC materials over one month. The relative standard deviations (RSDs) which reflect the intra- and inter-day variability of the method ranged from 5.0 % to 11 % for all four analytes (Supporting Information Table 2).

Reference List

[1] Taylor, J.K. 1987. Quality Assurance of Chemical Measurements. Chelsea, MI:Lewis Publishers

Supporting Information Table 1. Accuracy obtained from the spike recovery (%) of select analytical standards. The number in parenthesis is the spiking standard concentration (in μg/L).

Analyte	Spike Recovery (%)			
	(0.5)	(1.0)	(5.0)	(10.0)
BPA	106	104	102	99
BPS	107	106	94	104
BPF	104	95	91	102
BPAF	104	91	107	99

Supporting Information Table 2. Precision obtained from 20 repeated measurements of two QC materials (QC low and QC High) over one month.

Analyte	QC High		QC Low	
	Mean Concentration (μg/L)	RSD (%)	Mean Concentration (μg/L)	RSD (%)
BPA	9.9	5.0	2.1	5.4
BPS	4.9	6.4	0.50	6.1
BPF	5.0	6.7	0.50	9.6
BPAF	4.8	8.5	0.49	11