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ICI REPORT Bisphenols/round 1

Bisphenols in urine

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1 Summary

Within the framework of the HBM4EU project, an Inter-Laboratory Comparison Investigation (ICI) was organized and conducted for the analysis of Bisphenol A (BPA), Bisphenol S (BPS) and Bisphenol F (BPF) in human urine. The study was conducted from June 2018 to July 2018.

In total, 24 laboratories from 16 countries participated in this ICI. The participation in the ICI was satisfactory, as 24 out of 24 laboratories submitted their results.

Five different test samples consisting of 10 mL urine each were prepared, corresponding to different concentration levels of the targeted biomarkers (incurred and fortified), and sent to the participating laboratories for analysis. These samples were defined as follows:

- 1 sample at very low level (VL), corresponding to a non-fortified pool of individual human urine samples for which the concentration of the targeted markers were expected to be below or as close as possible to expected instrumental detection limits,

- 1 sample at low level (L), corresponding to an incurred pool of individual human urine samples for which the concentration of the targeted markers were expected to be near the p25 of the concentration distribution of a European general population,

- 2 samples (blind replicates) at medium level (Ma and Mb), corresponding to a fortified pool of individual human urine samples for which the concentration of the targeted markers were expected to be near the p50 value of a European general population,

- 1 samples at high level (H), corresponding to a fortified pool of individual human urine samples for which the concentration of the targeted markers were expected to be near the p95 value of a European general population.

Homogeneity and stability assessment of the control materials confirmed that the medium and high concentration levels samples were adequately homogeneous and stable for BPA, BPS and BPF. Stability results for low concentration level were not found all satisfying; it has been taken into account in the score calculations. As expected, the concentrations determined by the organizer for the very low concentration level sample did not permit to correctly assess the homogeneity and the stability of this material.

Laboratory results were rated using z-scores in accordance with ISO 13528 and ISO 17043. The default standard deviation applied for proficiency assessment (i.e. target standard deviation) was set to FFP = 25 %, as described in 5.3.

Assessment scores were calculated for BPA and BPS for low, medium and high level samples. As a global overview, the proportion of satisfying results (-2 < Z-score < 2) was from 76% to 88% for BPA and from 68% to 76% for BPS.

The calculation of the score was not achievable for BPF because of unacceptable high variability of the concentrations as well as the limited number of laboratories involved in the reporting of the results. As expected, scores associated to the very low concentration level sample (VL) were not

calculated due to the high number of non quantified samples ("<LOQ") and to the high variability between laboratories.

The results of the blinded replicate analysis, assessed on two identical medium level samples (a and b) globally demonstrated a satisfying repeatability except for 4 and 2 participating laboratories for BPA and BPS respectively (see Youden plot in Appendix 10).

2 Introduction

Interlaboratory Comparison Investigations (ICI) and External Quality Assurance Schemes (EQUAS) are efficient tools to assess the proficiency of laboratories, and the comparability and reliability of the analytical methods used. Participation in ICI/EQUAS is full part of Laboratory Quality Assurance system together with initial and on-going in-house method validation.

This ICI study was organised within the frame of HBM4EU as part of the Quality Assurance program for biomonitoring analyses. Within HBM4EU, participation in ICI/EQUAS exercises is mandatory for laboratories that will be further involved in the analytical characterization of the HBM4EU samples.

This report describes the 1st round for Bisphenols in urine and was organised by the LABoratoire d'Etude des Résidus et Contaminants dans les Aliments (LABERCA), a Research Laboratory located in Nantes (France) and affiliated both to Oniris (Nantes-Atlantic National College of Veterinary Medecine, Food Science and Engineering) and to INRA (French National Institute for Agricultural Research).

Bisphenols A, S and F were included in the scope of this ICI. Concentration levels were decided so that to assessing the laboratory performances at different concentration levels. Two samples amongst the five were strictly identical (medium level). Thus, it permitted to assess the accuracy and the repeatability of the laboratories.

2.1 Confidentiality

In this report the identity of the participants and the information provided by them is treated as confidential. However, lab codes of the participants will be disclosed to the HBM-QAU for performance assessments.

3 Control material

3.1 Preparation of control material

First, several litres of human urine were collected from several volunteers individuals at different times of the day (morning, afternoon, evening). These samples were split into four different pools that were then separately filtered in a 2 L Erlenmeyer flask using a vacuum pump and pre-washed pleated filters. The filtered urine pools were transferred to a 4 L glass bottle, placed under magnetic stirring for at least 30 min and then analysed to determine the concentration levels of each target biomarker.

Two pools were directly used to prepare very low level (VL) and low level (L) samples. Ten millilitres aliquots were distributed into 15 mL tubes immediately closed with a suitable cap (polypropylene, Falcon). The concentration of the targeted markers were expected to be below or as close as possible to expected instrumental detection limits for VL sample, and to be near the p25 of the concentration distribution of a European general population for L sample.

Two other pools were fortified with glucuronide-BPA, glucuronide-BPS and glucuronide-BPF at two different expected concentration levels for coming close to the p50 and p95 values typically expected for European general population. They were identified as medium (M) and high (H) concentration levels, respectively. After being agitated 30 min on a magnetic stir, 10-mL of the two spiked materials were introduced in 15 mL tubes equipped with suitable caps (polypropylene, Falcon). For medium level material, the two tubes were filled as it was sent in duplicate.

The tubes were stored in the freezer (≤ -18 °C) until transportation. The four different concentrations (VL, L, M, H) were measured by GC-MS/MS (see analysis method in Appendix 6).

3.2 Homogeneity of control material

Ten tubes of each concentration of the control material (VL, L, M, H) were randomly selected from the freezer (≤ -18 °C). The thawed samples were re-homogenised by vortex shaking and analysed in duplicate using GC-MS/MS (analysis method see Appendix 6). The homogeneity was evaluated according to HBM4EU-SOP-QA-002 “Preparation of control materials for Interlaboratory Comparison Investigations (ICI) and External Quality Assurance Schemes (EQUAS)”, ISO 13528:2015, Fearn et al [2001] and Thompson [2000]. The full data are presented in Appendix 2.

The results are summarized in the table 1 below.

Table 1: conclusions associated to the homogeneity test

		HOMOGENEITY CRITERIA		
	Concentration Level	**s_s < 0.3*σH**	**s_w< 0.5*σ_H**	***Outliers ?***
BPA	Low Level (L)	ACCEPT	ACCEPT	NO
	Medium Level (M)	ACCEPT	ACCEPT	NO
	High Level (H)	ACCEPT	ACCEPT	NO
BPS	Low Level (L)	ACCEPT	NOT ACCEPTED	YES
	Medium Level (M)	ACCEPT	ACCEPT	YES
	High Level (H)	ACCEPT	ACCEPT	NO
BPF	Low Level (L)	ACCEPT	ACCEPT	NO
	Medium Level (M)	ACCEPT	ACCEPT	NO
	High Level (H)	ACCEPT	ACCEPT	NO

As expected, the concentrations determined by the organizer for the very low concentration level sample did not permit to correctly assess the homogeneity and the stability of this material.

Homogeneity assessment of the control materials confirmed that the medium and high concentration levels samples were adequately homogeneous and stable for BPA, BPS and BPF.

Note: Due to a technical reason, a batch of analyses did not meet the required quality control criteria for being included in the calculation of homogeneity results for BPS at L and M level. Nevertheless, based on the satisfactory results obtained for BPS in H sample and for BPA, BPF in L and M samples, by extrapolation the materials L and M were considered homogeneous for BPS.

3.3 Stability of control material

In accordance with HBM4EU-SOP-QA-002 “Preparation of control materials for Interlaboratory Comparison Investigations (ICI) and External Quality Assurance Schemes (EQUAS)” and with ISO 13528:2015, three randomly selected test samples from each concentration (VL, L, M, H) were analysed in duplicate by GC-MS/MS (see analysis method in Appendix 6). The randomly selected control materials were stored at ≤ -18 °C.

The stability was evaluated using the Excel-sheet “HBM4EU ICI-EQUAS stability test CM v1”. The results are presented in Appendix 3. The conclusions are summarized in table 2. Non satisfying stability results were observed for VL (as expected) and L samples. Indeed, at very low (VL) and low (L) concentration levels, the stability criteria were not met. This factor was included in the score calculation as foreseen in the procedure.

Table 2: conclusions associated to the stability test

		STABILITY CRITERIA	
	Concentration Level	**X-Y < 0.3*σH Fit-for-purpose (25%)**	**Fischer's test**
BPA	Low Level (L)	NOT ACCEPTED	NOT ACCEPTED
	Medium Level (M)	ACCEPT	ACCEPT
	High Level (H)	ACCEPT	ACCEPT
BPS	Low Level (L)	NOT ACCEPTED	ACCEPT
	Medium Level (M)	ACCEPT	ACCEPT
	High Level (H)	ACCEPT	ACCEPT
BPF	Low Level (L)	NOT ACCEPTED	NOT ACCEPTED
	Medium Level (M)	ACCEPT	ACCEPT
	High Level (H)	ACCEPT	ACCEPT

As expected, the concentrations determined by the organizer for the very low concentration level sample did not permit to correctly assess the stability of this material. Stability results for low concentration level were not found all satisfying, in that case this observation has been taken into account in the score calculations. Stability assessment of the control materials confirmed that the medium and high concentration levels samples were adequately stable for BPA, BPS and BPF.

4 Organisational details

4.1 Participants

A list of 33 candidate laboratories from different countries previously identified as potential candidates for the analysis of Bisphenols had been compiled by the Work Package (WP) Task 9.2 leaders and made available to the institution organizing the respective ICI.

Invitation letters were sent by e-mail to all those 33 candidate laboratories on 28th March 2018 (see Appendix 4), indicating that participation would be free of charge.

Twenty-four laboratories from 16 countries indicated their interest in participating in this ICI and sent their registration form to LABERCA, with their agreement to abide by the conditions for participation. These laboratories received an individual laboratory code to report their measurement results.

The deadline to submit the test results was initially fixed to 29th June 2018 but was further extended to 20th July 2018. Of the 24 participants, 24 performed the assays and submitted results. The names of the participating laboratories are available in Appendix 1.

4.2 Dispatch and instructions

Five test materials consisting of 10 mL urine tube each were shipped to participants under frozen conditions (package shipping from LABERCA, 5th June 2018).

The characteristics of the five test samples are described in the section 3.1.

Moreover, a letter with instructions related to sample handling (instruction letter, see Appendix 5), an acknowledgment of receipt, as well as a result submission/method information form were sent to the participants by e-mail the 5th June 2018. Information related to the analytical method used for quantification was compiled in Appendix 6. Participants were asked to perform for each sample a single analysis using the same procedure they will routinely use in HMB4EU. Participants were asked to report results according to the instructions given.

5 Data evaluation

5.1 False negatives and false positives

Classification of results as false negatives or false positives was done as described in HBM4EU-SOP-QA-003.

A result was assigned as false negative when the following conditions all apply:

1) the biomarker was present in the control material (as established during the homogeneity/ stability assessment) and reported by the majority of the participants.

2) the biomarker was measured by the participant, but reported as below the specified LOQ value.

3) the participant's LOQ for the biomarker was below [assigned value - 3*target standard deviation].

A result was assigned as false positive when the following conditions all apply:

1) the biomarker was not present in the control material, i.e. below the LOQ value as used by the organiser during the homogeneity/ stability assessment, and not reported by the majority of the participants.

2) the biomarker was reported by the participant.

5.2 Assigned value

For ICI studies, the consensus value is used as assigned value and calculated as described in SOP HBM4EU-SOP-QA-003. In brief, the consensus values and its uncertainty were calculated from the results submitted by the participants using robust statistics to minimize the influence of outliers.

5.3 Target standard deviation

For calculation of the Z-scores, Fit-for-purpose (FFP = 25 %) target standard deviation was used as a default value for this first round, in lack of prior information on interlaboratory performance within the HBM4EU laboratories.

5.4 ICI / EQUAS standard deviation

To gain insight in the actual variability of the biomarker analysis in this study, the robust relative standard deviation was calculated as described in HBM4EU-SOP-QA-003.

5.5 Z-scores

Z-scores were calculated according to SOP HBM4EU-SOP-QA-003.

In accordance with ISO 13528 and ISO 17043 and the deliverable D 9.4 "*The Quality Assurance/Quality Control Scheme in the HBM4EU project*, Z-scores are classified as presented in Table 3.

Table 3: Classification of Z-scores

$\|Z\| \leq 2$	Satisfactory
$2 < \|Z\| < 3$	Questionable
$\|Z\| \geq 3$	Unsatisfactory

6 Results and discussion

6.1 Results submitted by participants

In total 24 laboratories from 16 countries agreed to participate in this study (see Appendix 1); all participants submitted results.

Laboratories were also asked to provide LOQs and details on the method used for analysis. This information is compiled in appendix 11 and 12. An overview of results submitted by the participants is included in appendix 7.

More precisely, and with regard to the total set of five test materials to be analysed, all the 24 participants reported results for BPA, while 19 and 21 reported results for BPS and BPF, respectively.

The LOQs were generally in the range 0.05-0.5 ng/mL, for few laboratories higher (see appendix 11). For the test materials of this ICI these LOQs were found adequate in most cases.

False positives: two false positives were reported in this ICI. Participant n°4 and n°4 reported quantitative results (14.16 and 19.60 ng/mL) for BPA in VL sample, while these biomarkers were not quantified by any of the other participants.

6.2 Assigned values and (target) standard deviations

The assigned value and its uncertainty, the relative standard deviation as derived from the participant's data, and the fit-for-purpose (FFP) target standard deviation for each of the analytes/control materials are included in appendix 7.

The robust relative standard deviation was calculated as described in HBM4EU-SOP-QA-003 and was compared to the FFP target standard deviation, in order to evaluate whether the FFP fitted well with the variability actually observed. All these observations are presented in the appendix 7.

6.3 Assessment of laboratory performance

Z-scores calculated for all target biomarkers and analysed sample are reported in appendix 7. Graphical representations of the Z-scores are provided in appendix 9. A summary of number of laboratories that reported results and the number of acceptable/questionable/unacceptable scores are presented in tables 4, 5 and 6.

Table 4: Summary of ICI results for BPA

	BPA			
	Low Level	**Medium Level A**	**Medium Level B**	**High Level**
Nb of reported quantitative results	21	23	23	24
Nb of reported <LOQ	3	1	1	0
Nb of acceptable score	16	19	18	21
Nb of questionnable score	0	0	1	1
Nb of unacceptable score	5	4	4	2

Table 5: Summary of ICI results for BPS

	BPS			
	Low Level	**Medium Level A**	**Medium Level B**	**High Level**
Nb of reported quantitative results	16	16	17	19
Nb of reported <LOQ	3	3	2	0
Nb of acceptable score	11	11	13	13
Nb of questionnable score	1	3	2	1
Nb of unacceptable score	4	2	2	5

Table 6: Summary of ICI results for BPF

	BPF			
	Low Level	**Medium Level A**	**Medium Level B**	**High Level**
Nb of reported quantitative results	12	15	14	17
Nb of reported <LOQ	9	6	7	4
Nb of acceptable score	No calculated scores			
Nb of questionnable score				
Nb of unacceptable score				

Assessment scores were calculated for BPA and BPS for low, medium and high level samples. As a global overview, the proportion of satisfying results (-2 < Z-score < 2) was from 76% to 88% for BPA and from 68% to 76% for BPS.

The calculation of the score was not achievable for BPF because of unacceptable high variability of the concentrations as well as the limited number of laboratories involved in the reporting of the results. As expected, scores associated to the very low concentration level sample (VL) were not calculated due to the high number of non quantified samples ("<LOQ") and to the high variability between laboratories.

The results of the blinded replicate analysis, assessed on two identical medium level samples (a and b) globally demonstrate a satisfying repeatability except for 4 and 2 participating laboratories for BPA and BPS respectively (see Youden plot in Appendix 10).

The summary of participant's scores are included in the Appendix 8.

6.4 Conclusions and recommendations

The participation was 24 out of 33 candidate labs. Twenty-four laboratories out of 24 registered candidate laboratories reported results, representing a participation rate of 100%.

For BPA, between 76% and 88% of satisfactory results were observed whereas for BPS, the satisfaction score ranged between 68% and 76%. Unsurprisingly the percentage of satisfactory results increases with the concentration level. Globally, these results indicate the reality of a quite significant core network of competent laboratories for BPA and BPS, but a still limited number of competent labs for BPF.

The presence of a relatively high variability in the observed results (in particular some overestimated values delivered by some labs, probably in relation with external contamination issue), together with

a non-satisfying repeatability for few labs, reveals the need for further discussions between HBM4EU partners regarding analytical methods and QA precautions. Critical issues such as external contamination (control and reporting) as well as internal calibration options for quantification merit to be deeply discussed in view of harmonization between collaborating laboratories.

7 References

HBM4EU-SOP-QA-001 "Organisation of Interlaboratory Comparison Investigations (ICI) and External Quality Assurance Schemes (EQUAS) of interlaboratory studies"

HBM4EU-SOP-QA-002 "Preparation of control materials for Interlaboratory Comparison Investigations (ICI) and External Quality Assurance Schemes (EQUAS)"

HBM4EU-SOP-QA-003 "Evaluation of results from Interlaboratory Comparison Investigations (ICI) and External Quality Assurance Schemes (EQUAS)"

HBM4EU-SOP-QA-004 "Reporting of results of Interlaboratory Comparison Investigations (ICI) and External Quality Assurance Schemes (EQUAS)"

ISO/IEC 17043:2010, Conformity assessment – General requirements for proficiency testing

ISO 13528, 2015, Statistical methods for use in proficiency testing by interlaboratory comparison.

Thompson, M., 2000, Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing, Analyst, 125, 385-386.

Thompson M., Ellison R. and Wood, R., 2006, The International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories, Pure Appl. Chem, 78(1), 145-196.

Analytical Methods Committee, 1989a, Robust statistics - How not to reject outliers Part 1. Basic concepts, Analyst, 114, 1693-1697.

Analytical Methods Committee, 1989b, Robust statistics - How not to reject outliers Part 2. Interlaboratory trials, Analyst, 114, 1699-1702.

Official Methods of Analysis Program Manual, 2002, Appendix D: Guidelines for Collaborative Study Procedures To Validate Characteristics of a Method of Analysis. Association Of Analytical Communities International. http://www.aoac.org/vmeth/Manual_Part_6.pdf.

Appendix 2. Homogeneity data

Homogeneity

Version HBM4EU v1

Control material: **Urine**

Analyte: **BPA**

Preparation of control material: **LOW LEVEL**

10 randomly chosen test samples, analysed in duplicate

Target standard deviation:
Fit-for-purpose RSD **25** (25% is default value)
if you want to use Horwitz/Thompson,
then delete FFP from cell H5

[1] ISO 13528:2005
[2] Fearn, T. and M. Thompson, 2001, A New Test for 'Sufficient homogeneity', Analyst, 126, 1414-1417
[3] Thompson M., 2000, Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing, Analyst, 125, 385-386

	replicate 1	replicate 2	x_t	w_t	w_t^2	$(x_t-\bar{x})^2$
1	0.623	0.647	0.6	0.0	0.0	0.0
2	0.563	0.698	0.6	-0.1	0.0	0.0
3	0.635	0.672	0.7	0.0	0.0	0.0
4	0.726	0.626	0.7	0.1	0.0	0.0
5	0.671	0.643	0.7	0.0	0.0	0.0
6	0.683	0.619	0.7	0.1	0.0	0.0
7	0.680	0.631	0.7	0.0	0.0	0.0
8	0.677	0.669	0.7	0.0	0.0	0.0
9	0.660	0.681	0.7	0.0	0.0	0.0
10	0.654	0.651	0.7	0.0	0.0	0.0

Lowest:	0.6 µg/kg	Σ =	0.0	0.0
Highest	0.7 µg/kg			
Grand mean ($\bar{x}$):	0.66 µg/kg			
Stdev:	0.03 µg/kg			
VC%:	5.3% µg/kg			

Outliers: Cochran's test

$C=w^2_{max}/\Sigma w_t^2$

--> C = 0.474
--> Ccrit= 0.602 **C < Ccrit → No outliers detected**

Horwitz [3]:

Mean > 120 ppb: CV=2(1-½ log c)		Mean < 120 ppb: σ = 0,22c		FFP (fit-for-purpose)	
RSD% =	48.23	RSD% =	22	RSD% =	25
σ_H =	0.32	σ_H =	0.14	σ_H =	0.16

σ_H used: 0.14

Homogeniteit [1]:

s_x =	0.02	
s_w =	0.04	(within sample standard deviation)
s_s =	0.00	(between sample standard deviation)
critical=	0.04	

s_s < critical? **→ ACCEPT: Homogeneity adequate**
s_w < 0.5*σ_H? **→ ACCEPT: Method suited**

Homogeneity

Version HBM4EU v1

Control material: **Urine**

Analyte: **BPS**

Preparation of control material: **LOW LEVEL**

10 randomly chosen test samples, analysed in duplicate

Target standard deviation:
Fit-for-purpose RSD **25** (25% is default value)
if you want to use Horwitz/Thompson,
then delete FFP from cell H5

[1] ISO 13528:2005
[2] Fearn, T. and M. Thompson, 2001, A New Test for 'Sufficient homogeneity', Analyst, 126, 1414-1417
[3] Thompson M., 2000, Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing, Analyst, 125, 385-386

	replicate 1	replicate 2	x_t	w_t	w_t^2	$(x_t-\bar{x})^2$
1	0.152					
2	0.116	0.212	0.2	-0.1	0.0	0.0
3	0.093	0.157	0.1	-0.1	0.0	0.0
4	0.140	0.295	0.2	-0.2	0.0	0.0
5	0.144	0.267	0.2	-0.1	0.0	0.0
6						
7	0.151					
8	0.151					
9	0.121	0.550	0.3	-0.4	0.2	0.0
10	0.133					
				Σ =	0.2	0.0

Lowest:	0.1	µg/kg
Highest	0.5	µg/kg
Grand mean ($\bar{x}$):	0.19	µg/kg
Stdev:	0.12	µg/kg
VC%:	61.4%	µg/kg

Outliers: Cochran's test

$C=w^2_{max}/\Sigma w_t^2$

--> C = 0.778
--> $Ccrit$= 0.638 **C > Ccrit → Outliers detected**

Horwitz [3]:

Mean > 120 ppb: CV=2(1-½ log c)		Mean < 120 ppb: σ = 0,22c		FFP (fit-for-purpose)	
$RSD\%$ =	58.03	$RSD\%$ =	22	$RSD\%$ =	25
σ_H =	0.11	σ_H =	0.04	σ_H =	0.05

σ_H used: 0.04

Homogeniteit [1]:

s_x =	0.06	
s_w =	0.11	(within sample standard deviation)
s_s =	0.00	(between sample standard deviation)
$critical$=	0.01	

s_s < critical? **→ ACCEPT: Homogeneity adequate**
s_w < 0.5*σ_H? **→ NOT ACCEPTED: Method not suited**

Homogeneity

Version HBM4EU v1

Control material: **Urine**

Analyte: **BPF**

Preparation of control material: **LOW LEVEL**

10 randomly chosen test samples, analysed in duplicate

Target standard deviation:
Fit-for-purpose RSD **25** (25% is default value)
if you want to use Horwitz/Thompson,
then delete FFP from cell H5

[1] ISO 13528:2005
[2] Fearn, T. and M. Thompson, 2001, A New Test for 'Sufficient homogeneity', Analyst, 126, 1414-1417
[3] Thompson M., 2000, Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing, Analyst, 125, 385-386

	replicate 1	replicate 2	x_t	w_t	w_t^2	$(x_t-\bar{x})^2$
1	0.060	0.056	0.1	0.0	0.0	0.0
2	0.048	0.060	0.1	0.0	0.0	0.0
3	0.054	0.060	0.1	0.0	0.0	0.0
4	0.063	0.055	0.1	0.0	0.0	0.0
5	0.054	0.058	0.1	0.0	0.0	0.0
6	0.057	0.056	0.1	0.0	0.0	0.0
7	0.058	0.055	0.1	0.0	0.0	0.0
8	0.060	0.059	0.1	0.0	0.0	0.0
9	0.059	0.060	0.1	0.0	0.0	0.0
10	0.062	0.060	0.1	0.0	0.0	0.0
				Σ =	0.0	0.0

Lowest:	0.0 µg/kg
Highest	0.1 µg/kg
Grand mean ($\bar{x}$):	0.06 µg/kg
Stdev:	0.00 µg/kg
VC%:	5.8% µg/kg

Outliers: Cochran's test

$C=w^2_{max}/\Sigma w_t^2$

--> C = 0.468
--> $Ccrit$= 0.602 **C < Ccrit → No outliers detected**

Horwitz [3]:

Mean > 120 ppb: CV=2(1-½ log c)		Mean < 120 ppb: σ = 0,22c		FFP (fit-for-purpose)	
$RSD\%$ =	69.53	$RSD\%$ =	22	$RSD\%$ =	25
σ_H =	0.04	σ_H =	0.01	σ_H =	0.01

σ_H used: 0.01

Homogeniteit [1]:

s_x =	0.00	
s_w =	0.00	(within sample standard deviation)
s_s =	0.00	(between sample standard deviation)
$critical$=	0.00	

s_s < critical? **→ ACCEPT: Homogeneity adequate**
s_w < 0.5*σ_H? **→ ACCEPT: Method suited**

Homogeneity

Version HBM4EU v1

Control material: **Urine**

Analyte: **BPA**

Preparation of control material: **MEDIUM LEVEL**

10 randomly chosen test samples, analysed in duplicate

Target standard deviation:
Fit-for-purpose RSD **25** (25% is default value)
if you want to use Horwitz/Thompson,
then delete FFP from cell H5

[1] ISO 13528:2005
[2] Fearn, T. and M. Thompson, 2001, A New Test for 'Sufficient homogeneity', Analyst, 126, 1414-1417
[3] Thompson M., 2000, Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing, Analyst, 125, 385-386

	replicate 1	replicate 2	x_t	w_t	w_t^2	$(x_t-\bar{x})^2$
1	2.213	2.076	2.1	0.1	0.0	0.0
2	2.196	2.043	2.1	0.2	0.0	0.0
3	2.100	2.062	2.1	0.0	0.0	0.0
4	1.871	2.012	1.9	-0.1	0.0	0.0
5	2.036	1.905	2.0	0.1	0.0	0.0
6	1.851	1.803	1.8	0.0	0.0	0.0
7	1.947	1.911	1.9	0.0	0.0	0.0
8	1.905	1.994	1.9	-0.1	0.0	0.0
9	1.885	2.128	2.0	-0.2	0.1	0.0
10	1.974	1.807	1.9	0.2	0.0	0.0
				Σ =	0.2	0.1

Lowest:	1.8 µg/kg
Highest	2.2 µg/kg
Grand mean ($\bar{x}$):	1.99 µg/kg
Stdev:	0.12 µg/kg
VC%:	6.1% µg/kg

Outliers: Cochran's test

$C=w^2_{max}/\Sigma w_t^2$

--> C = 0.332
--> $Ccrit$= 0.602 **C < Ccrit → No outliers detected**

Horwitz [3]:

Mean > 120 ppb: CV=2(1-½ log c)		Mean < 120 ppb: σ = 0,22c		FFP (fit-for-purpose)	
$RSD\%$ =	40.81	$RSD\%$ =	22	$RSD\%$ =	25
σ_H =	0.81	σ_H =	0.44	σ_H =	0.50

σ_H used: 0.44

Homogeniteit [1]:

s_x =	0.10	
s_w =	0.09	(within sample standard deviation)
s_s =	0.08	(between sample standard deviation)
$critical$=	0.13	

s_s < critical? **→ ACCEPT: Homogeneity adequate**
s_w < 0.5*σ_H? **→ ACCEPT: Method suited**

Homogeneity

Version HBM4EU v1

Control material: **Urine**

Analyte: **BPS**

Preparation of control material: **MEDIUM LEVEL**

10 randomly chosen test samples, analysed in duplicate

Target standard deviation:
Fit-for-purpose RSD **25** (25% is default value)
if you want to use Horwitz/Thompson,
then delete FFP from cell H5

[1] ISO 13528:2005
[2] Fearn, T. and M. Thompson, 2001, A New Test for 'Sufficient homogeneity', Analyst, 126, 1414-1417
[3] Thompson M., 2000, Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing, Analyst, 125, 385-386

	replicate 1	replicate 2	x_t	w_t	w_t^2	$(x_t-\bar{x})^2$
1		0.962				
2	0.761					
3	0.574					
4	0.681	0.845	0.8	-0.2	0.0	0.0
5	0.573					
6	0.843					
7	0.733					
8	0.809					
9	0.819					
10						

Lowest:	0.6 µg/kg	Σ =	0.0	0.0
Highest	1.0 µg/kg			
Grand mean ($\bar{x}$):	0.76 µg/kg			
Stdev:	0.12 µg/kg			
VC%:	16.3% µg/kg			

Outliers: Cochran's test

$C=w^2_{max}/\Sigma w_t^2$

--> C = 1.000
--> $Ccrit$= 0.679 **C > Ccrit → Outliers detected**

Horwitz [3]:

Mean > 120 ppb: CV=2(1-½ log c)		Mean < 120 ppb: σ = 0,22c		FFP (fit-for-purpose)	
$RSD\%$ =	47.16	$RSD\%$ =	22	$RSD\%$ =	25
σ_H =	0.36	σ_H =	0.17	σ_H =	0.19

σ_H used: 0.17

Homogeniteit [1]:

s_x =	0.00	
s_w =	0.04	(within sample standard deviation)
s_s =	0.00	(between sample standard deviation)
$critical$=	0.05	

s_s < critical? **→ ACCEPT: Homogeneity adequate**
s_w < 0.5*σ_H? **→ ACCEPT: Method suited**

Homogeneity

Version HBM4EU v1

Control material: **Urine**

Analyte: **BPF**

Preparation of control material: **MEDIUM LEVEL**

10 randomly chosen test samples, analysed in duplicate

Target standard deviation:
Fit-for-purpose RSD **25** (25% is default value)
if you want to use Horwitz/Thompson,
then delete FFP from cell H5

[1] ISO 13528:2005
[2] Fearn, T. and M. Thompson, 2001, A New Test for 'Sufficient homogeneity', Analyst, 126, 1414-1417
[3] Thompson M., 2000, Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing, Analyst, 125, 385-386

	replicate 1	replicate 2	x_t	w_t	w_t^2	$(x_t-\bar{x})^2$
1	0.432	0.487	0.5	-0.1	0.0	0.0
2	0.448	0.422	0.4	0.0	0.0	0.0
3	0.423	0.428	0.4	0.0	0.0	0.0
4	0.414	0.448	0.4	0.0	0.0	0.0
5	0.433	0.418	0.4	0.0	0.0	0.0
6	0.392	0.463	0.4	-0.1	0.0	0.0
7	0.401	0.431	0.4	0.0	0.0	0.0
8	0.403	0.418	0.4	0.0	0.0	0.0
9	0.414	0.417	0.4	0.0	0.0	0.0
10	0.407	0.449	0.4	0.0	0.0	0.0

Lowest:	0.4 µg/kg	Σ =	0.0	0.0
Highest	0.5 µg/kg			
Grand mean ($\bar{x}$):	0.43 µg/kg			
Stdev:	0.02 µg/kg			
VC%:	5.3% µg/kg			

Outliers: Cochran's test

$C=w^2_{max}/\Sigma w_t^2$

--> C = 0.391
--> $Ccrit$= 0.602 **C < Ccrit → No outliers detected**

Horwitz [3]:

Mean > 120 ppb: CV=2(1-½ log c)		Mean < 120 ppb: σ = 0,22c		FFP (fit-for-purpose)	
$RSD\%$ =	51.43	$RSD\%$ =	22	$RSD\%$ =	25
σ_H =	0.22	σ_H =	0.09	σ_H =	0.11

σ_H used: 0.09

Homogeniteit [1]:

s_x =	0.01	
s_w =	0.03	(within sample standard deviation)
s_s =	0.00	(between sample standard deviation)
$critical$=	0.03	

s_s < critical? **→ ACCEPT: Homogeneity adequate**
s_w < 0.5*σ_H? **→ ACCEPT: Method suited**

Homogeneity

Version HBM4EU v1

Control material: **Urine**

Analyte: **BPA**

Preparation of control material: **HIGH LEVEL**

10 randomly chosen test samples, analysed in duplicate

Target standard deviation:
Fit-for-purpose RSD **25** (25% is default value)
if you want to use Horwitz/Thompson,
then delete FFP from cell H5

[1] ISO 13528:2005
[2] Fearn, T. and M. Thompson, 2001, A New Test for 'Sufficient homogeneity', Analyst, 126, 1414-1417
[3] Thompson M., 2000, Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing, Analyst, 125, 385-386

	replicate 1	replicate 2	x_t	w_t	w_t^2	$(x_t-\bar{x})^2$
1	5.65	6.15	5.9	-0.5	0.2	0.0
2	5.83	6.31	6.1	-0.5	0.2	0.0
3	6.26	6.11	6.2	0.2	0.0	0.0
4	5.57	6.32	5.9	-0.7	0.6	0.0
5	5.69	6.17	5.9	-0.5	0.2	0.0
6	6.19	6.20	6.2	0.0	0.0	0.0
7		6.23				
8	5.28	6.01	5.6	-0.7	0.5	0.1
9	5.89	6.30	6.1	-0.4	0.2	0.0
10	5.97	6.09	6.0	-0.1	0.0	0.0
				Σ =	2.0	0.2

Lowest:	5.3 µg/kg
Highest	6.3 µg/kg
Grand mean ($\bar{x}$):	6.01 µg/kg
Stdev:	0.29 µg/kg
VC%:	4.8% µg/kg

Outliers: Cochran's test

$C=w^2_{max}/\Sigma w_t^2$

--> C = 0.277
--> $Ccrit$= 0.638 **C < Ccrit → No outliers detected**

Horwitz [3]:

Mean > 120 ppb: CV=2(1-½ log c)		Mean < 120 ppb: σ = 0,22c		FFP (fit-for-purpose)	
$RSD\%$ =	34.55	$RSD\%$ =	22	$RSD\%$ =	25
σ_H =	2.08	σ_H =	1.32	σ_H =	1.50

σ_H used: 1.32

Homogeniteit [1]:

s_x =	0.17	
s_w =	0.33	(within sample standard deviation)
s_s =	0.00	(between sample standard deviation)
$critical$=	0.40	

s_s < critical? **→ ACCEPT: Homogeneity adequate**
s_w < 0.5*σ_H? **→ ACCEPT: Method suited**

Homogeneity

Version HBM4EU v1

Control material: **Urine**

Analyte: **BPS**

Preparation of control material: **HIGH LEVEL**

10 randomly chosen test samples, analysed in duplicate

Target standard deviation:
Fit-for-purpose RSD **25** (25% is default value)
if you want to use Horwitz/Thompson,
then delete FFP from cell H5

[1] ISO 13528:2005
[2] Fearn, T. and M. Thompson, 2001, A New Test for 'Sufficient homogeneity', Analyst, 126, 1414-1417
[3] Thompson M., 2000, Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing, Analyst, 125, 385-386

	replicate 1	replicate 2	x_t	w_t	w_t^2	$(x_t-\bar{x})^2$
1	7.58	6.72	7.2	0.9	0.7	0.0
2	7.19	6.67	6.9	0.5	0.3	0.0
3	8.77	7.50	8.1	1.3	1.6	1.1
4	6.21	6.79	6.5	-0.6	0.3	0.3
5	7.26	5.86	6.6	1.4	2.0	0.3
6	7.14	7.56	7.4	-0.4	0.2	0.1
7	7.97	5.91	6.9	2.1	4.2	0.0
8	6.71	6.21	6.5	0.5	0.2	0.4
9	7.36	7.39	7.4	0.0	0.0	0.1
10	7.33	7.42	7.4	-0.1	0.0	0.1
				Σ =	9.6	2.4

Lowest:	5.9 µg/kg
Highest	8.8 µg/kg
Grand mean ($\bar{x}$):	7.08 µg/kg
Stdev:	0.71 µg/kg
VC%:	10.1% µg/kg

Outliers: Cochran's test

$C=w^2_{max}/\Sigma w_t^2$

--> C = 0.440
--> $Ccrit$= 0.602 **C < Ccrit → No outliers detected**

Horwitz [3]:

Mean > 120 ppb: CV=2(1-½ log c)		Mean < 120 ppb: σ = 0,22c		FFP (fit-for-purpose)	
$RSD\%$ =	33.71	$RSD\%$ =	22	$RSD\%$ =	25
σ_H =	2.39	σ_H =	1.56	σ_H =	1.77

σ_H used: 1.56

Homogeniteit [1]:

s_x =	0.52	
s_w =	0.69	(within sample standard deviation)
s_s =	0.16	(between sample standard deviation)
$critical$=	0.47	

s_s < critical? **→ ACCEPT: Homogeneity adequate**
s_w < 0.5*σ_H? **→ ACCEPT: Method suited**

Homogeneity

Version HBM4EU v1

Control material: **Urine**

Analyte: **BPF**

Preparation of control material: **HIGH LEVEL**

10 randomly chosen test samples, analysed in duplicate

Target standard deviation:
Fit-for-purpose RSD **25** (25% is default value)
if you want to use Horwitz/Thompson,
then delete FFP from cell H5

[1] ISO 13528:2005
[2] Fearn, T. and M. Thompson, 2001, A New Test for 'Sufficient homogeneity', Analyst, 126, 1414-1417
[3] Thompson M., 2000, Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing, Analyst, 125, 385-386

	replicate 1	replicate 2	x_t	w_t	w_t^2	$(x_t-\bar{x})^2$
1	0.93	1.01	1.0	-0.1	0.0	0.0
2	1.04	1.04	1.0	0.0	0.0	0.0
3	1.07	1.07	1.1	0.0	0.0	0.0
4	0.92	1.06	1.0	-0.1	0.0	0.0
5	0.96	1.10	1.0	-0.1	0.0	0.0
6	1.08	1.03	1.1	0.0	0.0	0.0
7		1.05				
8	0.90	1.00	1.0	-0.1	0.0	0.0
9	1.04	1.07	1.1	0.0	0.0	0.0
10	1.00	1.05	1.0	-0.1	0.0	0.0
				Σ =	0.1	0.0

Lowest:	0.9 µg/kg
Highest	1.1 µg/kg
Grand mean ($\bar{x}$):	1.02 µg/kg
Stdev:	0.06 µg/kg
VC%:	5.5% µg/kg

Outliers: Cochran's test

$C=w^2_{max}/\Sigma w_t^2$

--> C = 0.310
--> $Ccrit$= 0.638 **C < Ccrit → No outliers detected**

Horwitz [3]:

Mean > 120 ppb: CV=2(1-½ log c)		Mean < 120 ppb: σ = 0,22c		FFP (fit-for-purpose)	
$RSD\%$ =	45.10	$RSD\%$ =	22	$RSD\%$ =	25
σ_H =	0.46	σ_H =	0.22	σ_H =	0.26

σ_H used: 0.22

Homogeniteit [1]:

s_x =	0.04	
s_w =	0.06	(within sample standard deviation)
s_s =	0.00	(between sample standard deviation)
$critical$=	0.07	

s_s < critical? **→ ACCEPT: Homogeneity adequate**
s_w < 0.5*σ_H? **→ ACCEPT: Method suited**

Appendix 3. Stability data

HBM4EU ICI-EQUAS stability test CM v1

Stability test in frame of ICI-EQUAS

Material: **Urine**
Storage: Frozen conditions (-20°C)

Biomarker **BPA - VERY LOW LEVEL**

	t=0 (storage)	t=a (analysis)
dates:	31/05/2018	25/07/2018
values:	0.07	0.08
	0.07	0.10
	0.07	0.04
	0.08	0.05
	0.07	0.07
	0.08	0.06
	0.08	
	0.07	
	0.09	
	0.07	
	0.08	
	0.08	
	0.07	
	0.09	
	0.07	
	0.10	
	0.10	
	0.07	
	0.05	
	0.07	
number=	20	6
average=	0.1	0.1
std dev=	0.012	0.020

analysis date	time (days)	µg/L	n	std dev
31/05/2018	0	0.1	20	0.012
25/07/2018	55	0.1	6	0.020

x0-xa= **0.01**

test 'consequential instability': Horwitz/Thompson **Fit-for-purpose (FFP)**
xav-yav =<0,3σH

σH=	0.017	0.019044679
0,3*σH=	0.005	0.005713404
x0-xa<0,3*σH?	**Consequential instability detected**	**Consequential instability detected**

test 'significant difference':

F=	3.110
Fcrit=	2.740
Significant difference?	**Significant difference in std detected**
sed^2=	0.01
n=	24
std difference=	0.01
t=	1.65
t-crit=	2.06
Significant difference?	**No statistic instability detected**

HBM4EU ICI-EQUAS stability test CM v1

Stability test in frame of ICI-EQUAS

Material: **Urine**
Storage: Frozen conditions (-20°C)

Biomarker **BPS - VERY LOW LEVEL**

	t=0 (storage)	t=a (analysis)
dates:	31/05/2018	25/07/2018
values:	0.054	0.057
	0.055	0.071
	0.054	0.054
	0.060	0.055
	0.060	0.057
	0.056	0.058
	0.057	
	0.057	
	0.067	
	0.060	
	0.061	
	0.074	
number=	12	6
average=	0.060	0.059
std dev=	0.006	0.006

analysis date	time (days)	µg/L	n	std dev
31/05/2018	0	0.1	12	0.006
25/07/2018	55	0.1	6	0.006

x0-xa= **0.001**

test 'consequential instability': Horwitz/Thompson **Fit-for-purpose (FFP)**
xav-yav =<0,3σH

σH=	0.013	0.015
0,3*σH=	0.004	0.004
x0-xa<0,3*σH?	**No consequential instability detected**	**No consequential instability detected**

test 'significant difference':

F=	1.084
Fcrit=	3.204
Significant difference?	**No significant difference in std detected**
sed^2=	0.01
n=	16
std difference=	0.00
t=	0.34
t-crit=	2.12
Significant difference?	**No statistic instability detected**

HBM4EU ICI-EQUAS stability test CM v1

Stability test in frame of ICI-EQUAS

Material: **Urine**
Storage: Frozen conditions (-20°C)

Biomarker **BPF - VERY LOW LEVEL**

	t=0 (storage)	t=a (analysis)
dates:	31/05/2018	25/07/2018
values:	0.004	0.003
	0.005	0.008
	0.005	0.005
	0.005	0.005
	0.004	0.005
	0.005	0.004
	0.012	
	0.004	
	0.003	
	0.004	
	0.007	
	0.005	
	0.005	
	0.004	
	0.004	
	0.015	
	0.005	
	0.004	
number=	18	6
average=	0.005	0.005
std dev=	0.003	0.002

analysis date	time (days)	µg/L	n	std dev
31/05/2018	0	0.0	18	0.003
25/07/2018	55	0.0	6	0.002

x0-xa= **0.0003**

test 'consequential instability': Horwitz/Thompson **Fit-for-purpose (FFP)**
xav-yav =<0,3σH

σH=	0.0012	0.0014
0,3*σH=	0.0004	0.0004
x0-xa<0,3*σH?	**No consequential instability detected**	**No consequential instability detected**

test 'significant difference':

F=	3.720
Fcrit=	4.590
Significant difference?	**No significant difference in std detected**
sed^2=	0.00
n=	22
std difference=	0.00
t=	0.22
t-crit=	2.07
Significant difference?	**No statistic instability detected**

HBM4EU ICI-EQUAS stability test CM v1

Stability test in frame of ICI-EQUAS

Material: **Urine**
Storage: Frozen conditions (-20°C)

Biomarker **BPA - LOW LEVEL**

	t=0 (storage)	t=a (analysis)
dates:	31/05/2018	25/07/2018
values:	0.62	0.61
	0.56	0.56
	0.63	0.53
	0.73	0.55
	0.67	0.52
	0.68	0.52
	0.68	
	0.68	
	0.66	
	0.65	
	0.65	
	0.70	
	0.67	
	0.63	
	0.64	
	0.62	
	0.63	
	0.67	
	0.68	
	0.65	
number=	20	6
average=	0.66	0.55
std dev=	0.035	0.034

analysis date	time (days)	µg/L	n	std dev
31/05/2018	0	0.7	20	0.035
25/07/2018	55	0.5	6	0.034

x0-xa= 0.11

test 'consequential instability': Horwitz/Thompson **Fit-for-purpose (FFP)**

xav-yav =<0,3σH

σH=	0.14	0.16
0,3*σH=	0.04	0.05
x0-xa<0,3*σH?	**Consequential instability detected**	**Consequential instability detected**

test 'significant difference':

F=	1.034
Fcrit=	4.568
Significant difference?	**No significant difference in std detected**
sed^2=	0.03
n=	24
std difference=	0.02
t=	6.62
t-crit=	2.06
Significant difference?	**Statistic instability detected**

HBM4EU ICI-EQUAS stability test CM v1

Stability test in frame of ICI-EQUAS

Material: **Urine**
Storage: Frozen conditions (-20°C)

Biomarker **BPS - LOW LEVEL**

	t=0 (storage)	t=a (analysis)
dates:	31/05/2018	25/07/2018
values:	0.152	0.132
	0.116	0.137
	0.093	0.127
	0.140	0.136
	0.144	0.132
	0.151	0.130
	0.151	
	0.121	
	0.133	
	0.212	
	0.157	
	0.295	
	0.267	
	0.550	
number=	14	6
average=	0.192	0.132
std dev=	0.118	0.004

analysis date	time (days)	µg/L	n	std dev
31/05/2018	0	0.2	14	0.118
25/07/2018	55	0.1	6	0.004

x0-xa= 0.059

test 'consequential instability': Horwitz/Thompson **Fit-for-purpose (FFP)**

xav-yav =<0,3σH

σH=	0.042	0.048
0,3*σH=	0.013	0.014
x0-xa<0,3*σH?	**Consequential instability detected**	**Consequential instability detected**

test 'significant difference':

F=	1037.027
Fcrit=	4.655
Significant difference?	**Significant difference in std detected**
sed^2=	0.10
n=	18
std difference=	0.05
t=	1.21
t-crit=	2.10
Significant difference?	**No statistic instability detected**

HBM4EU ICI-EQUAS stability test CM v1

Stability test in frame of ICI-EQUAS

Material: **Urine**
Storage: Frozen conditions (-20°C)

Biomarker **BPF - LOW LEVEL**

	t=0 (storage)	t=a (analysis)
dates:	31/05/2018	25/07/2018
values:	0.060	0.06
	0.048	0.06
	0.054	0.06
	0.063	0.06
	0.054	0.07
	0.057	0.06
	0.058	
	0.060	
	0.059	
	0.062	
	0.056	
	0.060	
	0.060	
	0.055	
	0.058	
	0.056	
	0.055	
	0.059	
	0.060	
	0.060	
number=	20	6
average=	0.058	0.064
std dev=	0.003	0.003

analysis date	time (days)	µg/L	n	std dev
31/05/2018	0	0.1	20	0.003
25/07/2018	55	0.1	6	0.003

x0-xa= -0.006

test 'consequential instability': Horwitz/Thompson **Fit-for-purpose (FFP)**

xav-yav =<0,3σH

σH=	0.013	0.014
0,3*σH=	0.004	0.004
x0-xa<0,3*σH?	**Consequential instability detected**	**Consequential instability detected**

test 'significant difference':

F=	1.242
Fcrit=	4.568
Significant difference?	**No significant difference in std detected**
sed^2=	0.00
n=	24
std difference=	0.00
t=	3.81
t-crit=	2.06
Significant difference?	**Statistic instability detected**

HBM4EU ICI-EQUAS stability test CM v1

Stability test in frame of ICI-EQUAS

Material: **Urine**
Storage: Frozen conditions (-20°C)

Biomarker **BPA - MEDIUM LEVEL**

	t=0 (storage)	t=a (analysis)
dates:	31/05/2018	25/07/2018
values:	2.21	1.89
	2.20	1.80
	2.10	1.79
	1.87	1.92
	2.04	1.98
	1.85	
	1.95	
	1.91	
	1.88	
	1.97	
	2.08	
	2.04	
	2.06	
	2.01	
	1.90	
	1.80	
	1.91	
	1.99	
	2.13	
	1.81	
number=	20	5
average=	1.99	1.88
std dev=	0.121	0.081

analysis date	time (days)	µg/L	n	std dev
31/05/2018		0	2.0	20 0.121
25/07/2018		55	1.9	5 0.081
	x0-xa=	**0.11**		

test 'consequential instability':	Horwitz/Thompson	**Fit-for-purpose (FFP)**
xav-yav =<0,3σH		
σH=	0.44	0.50
0,3*σH=	0.13	0.15
x0-xa<0,3*σH?	**No consequential instability detected**	**No consequential instability detected**

test 'significant difference':	
F=	2.23
Fcrit=	5.81
Significant difference?	**No significant difference in std detected**
sed^2=	0.11
n=	23
std difference=	0.06
t=	1.92
t-crit=	2.07
Significant difference?	**No statistic instability detected**

HBM4EU ICI-EQUAS stability test CM v1

Stability test in frame of ICI-EQUAS

Material: **Urine**
Storage: Frozen conditions (-20°C)

Biomarker **BPS - MEDIUM LEVEL**

	t=0 (storage)	t=a (analysis)
dates:	31/05/2018	25/07/2018
values:	0.76	0.63
	0.57	0.62
	0.68	0.70
	0.57	0.76
	0.84	0.91
	0.73	
	0.81	
	0.82	
	0.96	
	0.84	
number=	10	5
average=	0.76	0.72
std dev=	0.124	0.119

analysis date	time (days)	µg/L	n	std dev
31/05/2018		0	0.8	10 0.124
25/07/2018		55	0.7	5 0.119
	x0-xa=	**0.04**		

test 'consequential instability':	Horwitz/Thompson	**Fit-for-purpose (FFP)**
xav-yav =<0,3σH		
σH=	0.17	0.19
0,3*σH=	0.05	0.06
x0-xa<0,3*σH?	**No consequential instability detected**	**No consequential instability detected**

test 'significant difference':	
F=	1.07
Fcrit=	6.00
Significant difference?	**No significant difference in std detected**
sed^2=	0.12
n=	13
std difference=	0.07
t=	0.55
t-crit=	2.16
Significant difference?	**No statistic instability detected**

HBM4EU ICI-EQUAS stability test CM v1

Stability test in frame of ICI-EQUAS

Material: **Urine**
Storage: Frozen conditions (-20°C)

Biomarker **BPF - MEDIUM LEVEL**

	t=0 (storage)	t=a (analysis)
dates:	31/05/2018	25/07/2018
values:	0.43	0.41
	0.45	0.49
	0.42	0.43
	0.41	0.45
	0.43	0.47
	0.39	
	0.40	
	0.40	
	0.41	
	0.41	
	0.49	
	0.42	
	0.43	
	0.45	
	0.42	
	0.46	
	0.43	
	0.42	
	0.42	
	0.45	
number=	20	5
average=	0.43	0.45
std dev=	0.023	0.032

analysis date	time (days)	µg/L	n	std dev
31/05/2018		0	0.4	20 0.023
25/07/2018		55	0.5	5 0.032
	x0-xa=	**-0.02**		

test 'consequential instability':	Horwitz/Thompson	**Fit-for-purpose (FFP)**
xav-yav =<0,3σH		
σH=	0.09	0.11
0,3*σH=	0.03	0.03
x0-xa<0,3*σH?	**No consequential instability detected**	**No consequential instability detected**

test 'significant difference':	
F=	1.94
Fcrit=	2.90
Significant difference?	**No significant difference in std detected**
sed^2=	0.02
n=	23
std difference=	0.01
t=	1.84
t-crit=	2.07
Significant difference?	**No statistic instability detected**

HBM4EU ICI-EQUAS stability test CM v1

Stability test in frame of ICI-EQUAS

Material: **Urine**
Storage: Frozen conditions (-20°C)

Biomarker **BPA - HIGH LEVEL**

	t=0 (storage)	t=a (analysis)
dates:	31/05/2018	25/07/2018
values:	5.65	5.99
	5.83	6.68
	6.26	5.94
	5.57	5.70
	5.69	5.77
	6.19	5.43
	5.28	
	5.89	
	5.97	
	6.15	
	6.31	
	6.11	
	6.32	
	6.17	
	6.20	
	6.23	
	6.01	
	6.30	
	6.09	
number=	19	6
average=	6.01	5.92
std dev=	0.291	0.423

analysis date	time (days)	µg/L	n	std dev
31/05/2018	0	6.0	19	0.291
25/07/2018	55	5.9	6	0.423

x0-xa= 0.09

test 'consequential instability': Horwitz/Thompson — **Fit-for-purpose (FFP)**
xav-yav =<0,3σH

σH=	1.32	1.50
0,3*σH=	0.40	0.45
x0-xa<0,3*σH?	**No consequential instability detected**	**No consequential instability detected**

test 'significant difference':

F=	2.12
Fcrit=	2.77
Significant difference?	**No significant difference in std detected**
sed^2=	0.32
n=	23
std difference=	0.15
t=	0.62
t-crit=	2.07
Significant difference?	**No statistic instability detected**

HBM4EU ICI-EQUAS stability test CM v1

Stability test in frame of ICI-EQUAS

Material: **Urine**
Storage: Frozen conditions (-20°C)

Biomarker **BPA - HIGH LEVEL**

	t=0 (storage)	t=a (analysis)
dates:	31/05/2018	25/07/2018
values:	7.58	7.29
	7.19	7.34
	8.77	7.08
	6.21	7.13
	7.26	7.31
	7.14	7.24
	7.97	
	6.71	
	7.36	
	7.33	
	6.72	
	6.67	
	7.50	
	6.79	
	5.86	
	7.56	
	5.91	
	6.21	
	7.39	
	7.42	
number=	20	6
average=	7.08	7.23
std dev=	0.711	0.104

analysis date	time (days)	µg/L	n	std dev
31/05/2018	0	7.1	20	0.711
25/07/2018	55	7.2	6	0.104

x0-xa= -0.15

test 'consequential instability': Horwitz/Thompson — **Fit-for-purpose (FFP)**
xav-yav =<0,3σH

σH=	1.56	1.77
0,3*σH=	0.47	0.53
x0-xa<0,3*σH?	**No consequential instability detected**	**No consequential instability detected**

test 'significant difference':

F=	46.89
Fcrit=	4.57
Significant difference?	**Significant difference in std detected**
sed^2=	0.63
n=	24
std difference=	0.30
t=	0.52
t-crit=	2.06
Significant difference?	**No statistic instability detected**

HBM4EU ICI-EQUAS stability test CM v1

Stability test in frame of ICI-EQUAS

Material: **Urine**
Storage: Frozen conditions (-20°C)

Biomarker **BPF - HIGH LEVEL**

	t=0 (storage)	t=a (analysis)
dates:	31/05/2018	25/07/2018
values:	0.93	1.00
	1.04	1.03
	1.07	1.08
	0.92	1.05
	0.96	1.03
	1.08	0.98
	0.90	
	1.04	
	1.00	
	1.01	
	1.04	
	1.07	
	1.06	
	1.10	
	1.03	
	1.05	
	1.00	
	1.07	
	1.05	
number=	19	6
average=	1.02	1.02
std dev=	0.056	0.036

analysis date	time (days)	µg/L	n	std dev
31/05/2018	0	1.0	19	0.056
25/07/2018	55	1.0	6	0.036

x0-xa= -0.002

test 'consequential instability': Horwitz/Thompson — **Fit-for-purpose (FFP)**
xav-yav =<0,3σH

σH=	0.22	0.26
0,3*σH=	0.07	0.08
x0-xa<0,3*σH?	**No consequential instability detected**	**No consequential instability detected**

test 'significant difference':

F=	2.52
Fcrit=	4.58
Significant difference?	**No significant difference in std detected**
sed^2=	0.05
n=	23
std difference=	0.02
t=	0.10
t-crit=	2.07
Significant difference?	**No statistic instability detected**

Appendix 4. Copy of letter of invitation

HBM4EU science and policy for a healthy future

HBM4EU: Announcement / invitation to participate in ICI / EQUAS study

Bisphenols/Round 1.

Title of ICI/EQUAS: Bisphenols in urine

Dear Colleague,

within the frame of HBM4EU, the

LABERCA (LABoratoire d'Etude des Résidus et des Contaminants dans les Aliments), UMR 1329 Oniris-INRA,

Route de Gachet – CS50707

44307 NANTES Cedex

France

announces the 1st round of ICI/EQUAS for the determination of Bisphenols in urine.
The aim of ICI/EQUAS exercises is to provide laboratories with an assessment of their analytical performance and reliability of their data in comparison with other laboratories and/or expert laboratories. This will aid in the quality improvement of analysis in human biomonitoring at each of the laboratories.

Participation is mandatory for laboratories analysing samples in the frame of HBM4EU.

Test samples
The matrix will be human urine. The participants will receive:

- 4 materials of urine (4 samples of 10 mL each) for determination of Bisphenols in urine

Target biomarkers
Please see registration form for Bisphenols/Round 1 for the biomarkers potentially present in the test samples. We would be pleased if your laboratory could participate with the analysis of as most as possible Bisphenols.
LOQs should allow the analysis of Bisphenols in samples of the general population

HBM4EU science and policy for a healthy future

Calendar:

Deadline registration	11-04-2018
Distribution of test samples (projected)	29-05-2018
Deadline for reporting the results (projected)	29-06-2018

Registration

For registration, please find attached a registration form for parameter Bisphenols in urine. Please send it back to us by mail in case you want to register.
Upon registration, the participant will receive a lab-code to be used for submission of results.

Fee

For partners and linked-third parties of HBM4EU, participation is free of charge. Please note that the participant is responsible for custom clearance and associated costs if applicable.

Confidentiality:

All laboratory specific information will be treated confidentially, and will never be disclosed to third parties (government, accreditation bodies) except the HBM4EU QAU, without permission of the laboratory

Contact information organiser:

Coordinator:
Vincent Vaccher

LABERCA (LABoratoire d'Etude des Résidus et des Contaminants dans les Aliments), UMR 1329 Oniris-INRA,
Route de Gachet – CS50707
44307 NANTES Cedex
France

Email: vincent.vaccher@oniris-nantes.fr

Tel: +33 2 40 68 78 80

Appendix 5. Copy of letter/instructions sent together with test samples

HBM4EU science and policy for a healthy future

HBM4EU: Instruction letter ICI / EQUAS study Bisphenols/Round1

Title of ICI/EQUAS: Bisphenols in urine

Dear participant,

Thank you for participation in HBM4EU ICI/EQUAS study Bisphenols in urine/Round 1 for the determination of Bisphenols in urine.

You will receive a parcel containing 5 test samples. Each sample consists of 10 mL urine.

The parcel will be shipped 05.06.2018 under frozen conditions.

Instructions:

- Upon receipt, please check the content for any damage/leaking of the containers, complete the sample receipt form and return it to the organiser.
- Store the test samples under frozen (-18°C) conditions until analysis.
- Analyse the samples for the biomarkers indicated in the invitation letter ref/ 28.03.2018.
- Thaw the samples and re-homogenise them according to your own procedure.
- Analyse the samples using the same procedure as will be used for analysis of samples in the frame of HBM4EU.
- Carry out a single analysis for each sample.
- For submission of results and method information use the forms provided by email.
- The deadline for submission of analysis results and method details is **06.07.2018**

If you have any questions or need any assistance, please contact:

Vincent Vaccher

Email: vincent.vaccher@oniris-nantes.fr

Tel: +33 2 40 68 78 80

LABERCA (LABoratoire d'Etude des Résidus et des Contaminants dans les Aliments), UMR 1329 Oniris-INRA,
Route de Gachet – CS50707
44307 NANTES Cedex
France

Appendix 6. GC-MS/MS method LABERCA

Laboratory	LABERCA - France
ISO17025 accredited	yes
Volume of urine used to perform the analysis	2 mL
Type of deconjugation	enzymatic deconjugation
Enzyme used (ref number)	Abalonase purified enzymatic formula Bglucu (beta gluc 10)
SPE offline (yes/no)	Yes
Type of column used for SPE offline	Chromabond HR-X / Affinimip
Derivatisation agent	MSTFA
Name and version of mass spectrometer	Agilent 7010
GC-MS/MS (yes/no)	Yes
Type of column (GC or LC)	GC (Optima 17 MS)
Response normalised to IS (yes/no)	Yes
Internal standard	BPA 13C; BPS 13C; BPF 13C
Calibration type	Isotopic dilution before extraction

Appendix 7. Assigned values and participant's performance

Control material		**VERY LOW LEVEL SAMPLE (VL)**					
Parameter		**BPA**		**BPS**		**BPF**	
Number of participants		24		19		21	
Number of quantitative results		16		9		4	
Target value estimated by ICI organizer ng/mL		0.08		0.06		0.01	
Assigned value ng/mL		-		-		-	
Uncertainty of assigned value ng/mL		-		-		-	
Study RSD_R (%)		-		-		-	
Relative target standard deviation (%)		-		-		-	
Laboratory code	**ID sample**	**Value**	**Z-score**	**Value**	**Z-score**	**Value**	**Z-score**
3	18-BPs-466	< 0.10	-	1.70	-	< 0.25	-
4	18-BPs-104	14.16	-	3.48	-	3.41	-
6	18-BPs-787	0.15	-	0.11	-	< 0.05	-
17	18-BPs-581	0.09	-	0.06	-	0.003	-
20	18-BPs-936	1.00	-	< 10	-	< 1.00	-
25	18-BPs-293	< 1.40	-	< 0.30	-	< 2.00	-
26	18-BPs-669	< 0.10	-	< 0.10	-	< 0.10	-
31	18-BPs-136	< 0.10	-	< 0.40	-	< 0.20	-
36	18-BPs-431	3.41	-	-	-	< 0.50	-
39	18-BPs-229	0.60	-	0.06	-	< 0.02	-
41	18-BPs-883	0.13	-	0.35	-	< 0.27	-
49	18-BPs-573	19.60	-	< 0.50	-	< 0.50	-
51	18-BPs-132	0.63	-	< 0.10	-	< 0.10	-
55	18-BPs-389	0.79	-	-	-	< 0.50	-
57	18-BPs-83	0.26	-	-	-	< 0.10	-
61	18-BPs-470	< 0.50	-	0.07	-	< 0.10	-
66	18-BPs-592	0.09	-	0.10	-	< 0.05	-
68	18-BPs-86	0.31	-	-	-	0.31	-
76	18-BPs-648	3.27	-	-	-	-	-
87	18-BPs-299	< 3.96	-	< 1.86	-	< 5.63	-
89	18-BPs-731	0.08	-	0.06	-	-	-
96	18-BPs-331	< 0.80	-	< 0.20	-	< 0.03	-
98	18-BPs-487	0.27	-	< 0.02	-	-	-
100	18-BPs-442	ND	-	< 0.03	-	0.85	-

Control material		**LOW LEVEL SAMPLE (L)**					
Parameter		**BPA**		**BPS**		**BPF**	
Number of participants		24		19		21	
Number of quantitative results		21		16		12	
Target value estimated by ICI organizer ng/mL		0.60		0.15		0.06	
Assigned value ng/mL		0.92		0.19		-	
Uncertainty of assigned value ng/mL		0.10		0.02		-	
Study RSD_R (%)		41		39		81	
Relative target standard deviation (%)		25		25		25	
Laboratory code	**ID sample**	**Value**	**Z'_i-score**	**Value**	**Z'_i-score**	**Value**	**Z-score**
3	18-BPs-492	0.64	-1.0	9.17	173	3.54	-
4	18-BPs-855	10.50	38	0.16	-0.4	0.44	-
6	18-BPs-620	0.84	-0.3	0.18	-0.1	0.43	-
17	18-BPs-218	0.67	-0.9	0.14	-0.5	0.06	-
20	18-BPs-126	1.70	3.1	< 10	-	< 1.00	-
25	18-BPs-160	< 1.40	-	< 0.30	-	< 2.00	-
26	18-BPs-983	0.61	-1.1	0.17	-0.2	< 0.10	-
31	18-BPs-171	0.83	-0.3	0.45	5.1	< 0.20	-
36	18-BPs-40	1.25	1.3	-	-	< 0.50	-
39	18-BPs-513	0.94	0.1	0.19	0.1	0.07	-
41	18-BPs-278	0.58	-1.2	0.21	0.4	< 0.27	-
49	18-BPs-154	2.51	6.3	1.26	20.6	2.30	-
51	18-BPs-353	2.31	5.5	0.11	-1.0	0.29	-
55	18-BPs-261	1.26	1.4	-	-	< 0.50	-
57	18-BPs-680	0.83	-0.3	-	-	0.12	-
61	18-BPs-257	0.59	-1.2	0.18	-0.1	< 0.10	-
66	18-BPs-976	0.51	-1.5	0.23	0.8	0.13	-
68	18-BPs-459	0.88	-0.1	-	-	0.42	-
76	18-BPs-32	2.72	7.1	-	-	-	-
87	18-BPs-19	< 3.96	-	< 1.86	-	< 5.63	-
89	18-BPs-891	0.64	-1.0	0.15	-0.4	-	-
96	18-BPs-75	< 0.80	-	0.40	4.1	0.09	-
98	18-BPs-701	0.84	-0.3	0.02	-2.1	-	-
100	18-BPs-812	0.52	-1.5	0.03	-2.0	0.28	-

Control material		**MEDIUM LEVEL SAMPLE A (Ma)**					
Parameter		**BPA**		**BPS**		**BPF**	
Number of participants		24		19		21	
Number of quantitative results		23		16		15	
Target value estimated by ICI organizer ng/mL		2.00		1.00		0.50	
Assigned value ng/mL		2.44		0.75		-	
Uncertainty of assigned value ng/mL		0.14		0.06		-	
Study RSD_R (%)		16		26		67	
Relative target standard deviation (%)		25		25		25	
Laboratory code	**ID sample**	**Value**	**Z-score**	**Value**	**Z'-score**	**Value**	**Z-score**
3	18-BPs-851	2.18	-0.4	2.65	9.6	2.02	-
4	18-BPs-748	11.00	14	0.25	-2.5	0.46	-
6	18-BPs-385	2.39	-0.1	0.65	-0.5	1.06	-
17	18-BPs-893	2.12	-0.5	0.73	-0.1	0.44	-
20	18-BPs-998	2.70	0.4	< 10	-	< 1.0	-
25	18-BPs-399	1.99	-0.7	0.85	0.5	< 2.0	-
26	18-BPs-880	2.06	-0.6	0.81	0.3	0.47	-
31	18-BPs-556	1.72	-1.2	1.24	2.5	< 0.20	-
36	18-BPs-925	3.03	1.0	-	-	3.16	-
39	18-BPs-897	2.64	0.3	0.87	0.6	0.40	-
41	18-BPs-164	1.35	-1.8	0.28	-2.4	< 0.27	-
49	18-BPs-545	12.39	16	< 0.50	-	< 0.50	-
51	18-BPs-705	6.19	6.1	0.67	-0.4	1.17	-
55	18-BPs-609	2.51	0.1	-	-	1.37	-
57	18-BPs-498	2.98	0.9	-	-	0.36	-
61	18-BPs-8	2.26	-0.3	0.95	1.0	0.18	-
66	18-BPs-972	2.06	-0.6	0.83	0.4	0.60	-
68	18-BPs-90	2.65	0.3	-	-	1.36	-
76	18-BPs-624	6.21	6.2	-	-	-	-
87	18-BPs-904	< 3.96	-	< 1.86	-	< 5.63	-
89	18-BPs-421	2.14	-0.5	0.87	0.6	-	-
96	18-BPs-538	2.10	-0.6	0.69	-0.3	0.31	-
98	18-BPs-517	2.70	0.4	0.07	-3.5	-	-
100	18-BPs-690	1.80	-1.0	0.36	-2.0	0.98	-

Control material		**MEDIUM LEVEL SAMPLE B (Mb)**					
Parameter		**BPA**		**BPS**		**BPF**	
Number of participants		24		19		21	
Number of quantitative results		23		17		14	
Target value estimated by ICI organizer ng/mL		2.00		1.00		0.50	
Assigned value ng/mL		2.58		0.80		-	
Uncertainty of assigned value ng/mL		0.19		0.07		-	
Study RSD_R (%)		15		29		66	
Relative target standard deviation (%)		25		25		25	
Laboratory code	**ID sample**	**Value**	**Z-score**	**Value**	**Z'-score**	**Value**	**Z-score**
3	18-BPs-679	2.56	0.0	2.11	6.2	2.11	-
4	18-BPs-636	12.30	15	0.43	-1.8	0.74	-
6	18-BPs-14	2.74	0.2	0.69	-0.5	1.17	-
17	18-BPs-166	2.10	-0.7	0.70	-0.5	0.43	-
20	18-BPs-982	1.90	-1.1	< 10	-	< 1.0	-
25	18-BPs-672	1.96	-1.0	0.84	0.2	< 2.0	-
26	18-BPs-747	2.17	-0.6	0.84	0.2	0.53	-
31	18-BPs-42	42.71	62	0.84	0.2	< 0.20	-
36	18-BPs-950	2.02	-0.9	-	-	< 0.50	-
39	18-BPs-330	2.67	0.1	0.98	0.8	0.40	-
41	18-BPs-625	1.71	-1.3	0.55	-1.2	< 0.27	-
49	18-BPs-536	5.08	3.9	1.28	2.3	< 0.50	-
51	18-BPs-971	5.89	5.1	0.71	-0.4	1.31	-
55	18-BPs-497	2.49	-0.1	-	-	2.99	-
57	18-BPs-198	3.00	0.7	-	-	0.30	-
61	18-BPs-619	2.10	-0.7	0.97	0.8	0.11	-
66	18-BPs-209	1.90	-1.1	1.00	0.9	0.53	-
68	18-BPs-316	2.68	0.2	-	-	1.08	-
76	18-BPs-732	4.00	2.2	-	-	-	-
87	18-BPs-653	< 3.96	-	< 1.86	-	< 5.63	-
89	18-BPs-476	2.47	-0.2	0.79	-0.1	-	-
96	18-BPs-779	2.80	0.3	0.85	0.2	0.40	-
98	18-BPs-106	2.58	0.0	0.07	-3.5	-	-
100	18-BPs-544	1.68	-1.4	0.32	-2.3	0.99	-

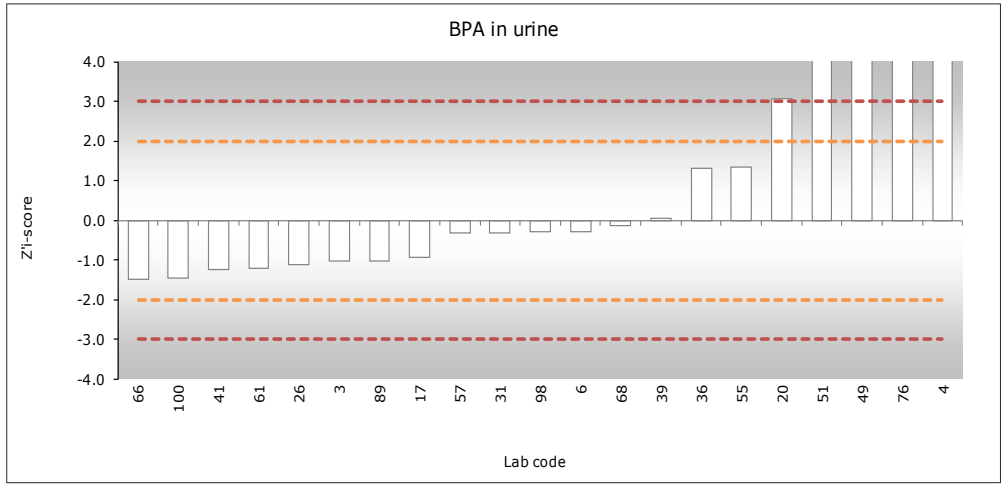
Control material		**HIGH LEVEL SAMPLE (H)**					
Parameter		**BPA**		**BPS**		**BPF**	
Number of participants		24		19		21	
Number of quantitative results		24		19		17	
Target value estimated by ICI organizer ng/mL		7.00		6.00		1.50	
Assigned value ng/mL		7.09		5.38		-	
Uncertainty of assigned value ng/mL		0.48		0.19		-	
Study RSD_R (%)		5		12		62	
Relative target standard deviation (%)		25		25		25	
Laboratory code	**ID sample**	**Value**	**Z-score**	**Value**	**Z-score**	**Value**	**Z-score**
3	18-BPs-716	5.86	-0.7	12.43	5.2	9.03	-
4	18-BPs-917	10.51	1.9	0.91	-3.3	< 0.32	-
6	18-BPs-526	7.81	0.4	5.45	0.1	2.33	-
17	18-BPs-833	6.69	-0.2	5.31	-0.1	1.04	-
20	18-BPs-765	5.10	-1.1	10.80	4.0	1.20	-
25	18-BPs-62	6.65	-0.2	5.77	0.3	< 2.0	-
26	18-BPs-289	6.54	-0.3	5.41	0.0	1.01	-
31	18-BPs-496	7.50	0.2	5.23	-0.1	< 0.20	-
36	18-BPs-994	9.46	1.3	-	-	1.80	-
39	18-BPs-376	16.69	5.4	5.74	0.3	0.88	-
41	18-BPs-637	6.49	-0.3	4.18	-0.9	2.26	-
49	18-BPs-77	12.30	2.9	8.80	2.5	2.60	-
51	18-BPs-861	15.27	4.6	5.12	-0.2	2.70	-
55	18-BPs-363	8.07	0.5	-	-	1.43	-
57	18-BPs-464	5.16	-1.1	-	-	0.46	-
61	18-BPs-434	8.20	0.6	6.41	0.8	0.51	-
66	18-BPs-84	6.85	-0.1	5.61	0.2	1.05	-
68	18-BPs-2	8.80	1.0	-	-	2.35	-
76	18-BPs-312	6.09	-0.6	-	-	-	-
87	18-BPs-367	5.30	-1.0	3.63	-1.3	< 5.63	-
89	18-BPs-127	5.46	-0.9	5.51	0.1	-	-
96	18-BPs-741	4.60	-1.4	5.20	-0.1	0.85	-
98	18-BPs-26	6.17	-0.5	0.59	-3.6	-	-
100	18-BPs-901	5.51	-0.9	0.90	-3.3	5.59	-

Appendix 8. Summary of participant's score

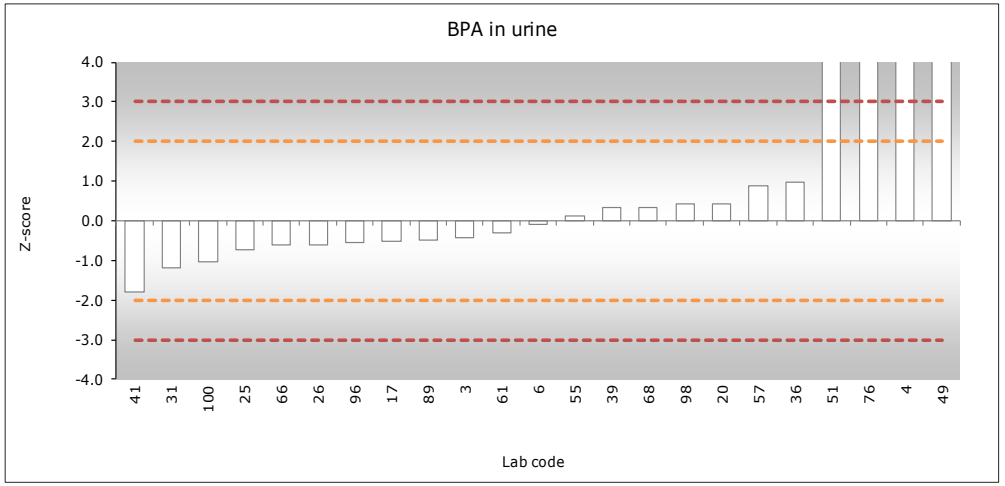
Lab code	BPA				BPS			
	Z'$_i$-score L sample	Z-score Ma sample	Z-score Mb sample	Z-score H sample	Z'$_i$-score L sample	Z'-score Ma sample	Z'-score Mb sample	Z-score H sample
3	-1.0	-0.4	0.0	-0.7	173	9.6	6.2	5.2
4	38	14	15	1.9	-0.4	-2.5	-1.8	-3.3
6	-0.3	-0.1	0.2	0.4	-0.1	-0.5	-0.5	0.1
17	-0.9	-0.5	-0.7	-0.2	-0.5	-0.1	-0.5	-0.1
20	3.1	0.4	-1.1	-1.1	-	-	-	4.0
25	-	-0.7	-1.0	-0.2	-	0.5	0.2	0.3
26	-1.1	-0.6	-0.6	-0.3	-0.2	0.3	0.2	0.0
31	-0.3	-1.2	62	0.2	5.1	2.5	0.2	-0.1
36	1.3	1.0	-0.9	1.3	-	-	-	-
39	0.1	0.3	0.1	5.4	0.1	0.6	0.8	0.3
41	-1.2	-1.8	-1.3	-0.3	0.4	-2.4	-1.2	-0.9
49	6.3	16	3.9	2.9	20.6	-	2.3	2.5
51	5.5	6.1	5.1	4.6	-1.0	-0.4	-0.4	-0.2
55	1.4	0.1	-0.1	0.5	-	-	-	-
57	-0.3	0.9	0.7	-1.1	-	-	-	-
61	-1.2	-0.3	-0.7	0.6	-0.1	1.0	0.8	0.8
66	-1.5	-0.6	-1.1	-0.1	0.8	0.4	0.9	0.2
68	-0.1	0.3	0.2	1.0	-	-	-	-
76	7.1	6.2	2.2	-0.6	-	-	-	-
87	-	-	-	-1.0	-	-	-	-1.3
89	-1.0	-0.5	-0.2	-0.9	-0.4	0.6	-0.1	0.1
96	-	-0.6	0.3	-1.4	4.1	-0.3	0.2	-0.1
98	-0.3	0.4	0.0	-0.5	-2.1	-3.5	-3.5	-3.6
100	-1.5	-1.0	-1.4	-0.9	-2.0	-2.0	-2.3	-3.3

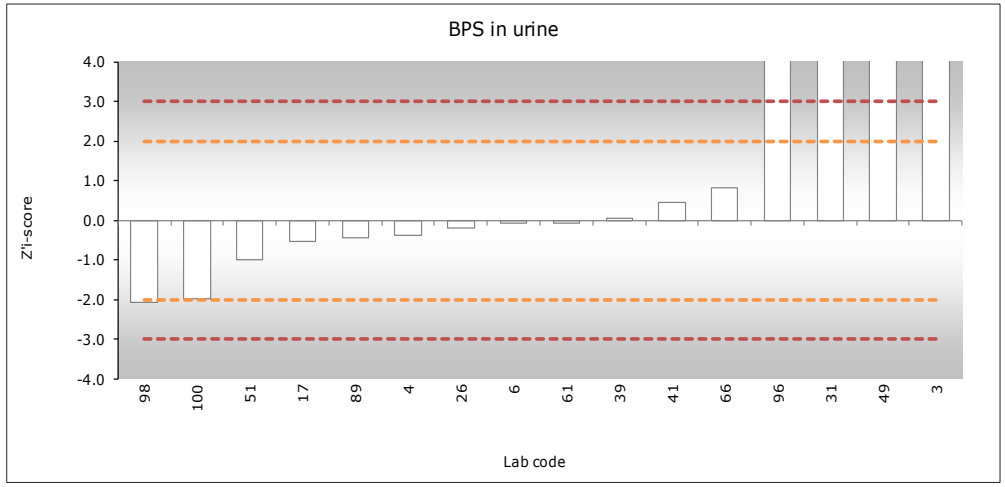
Appendix 9. Graphical representation of the Z'i-scores, the Z'-scores and the Z-scores

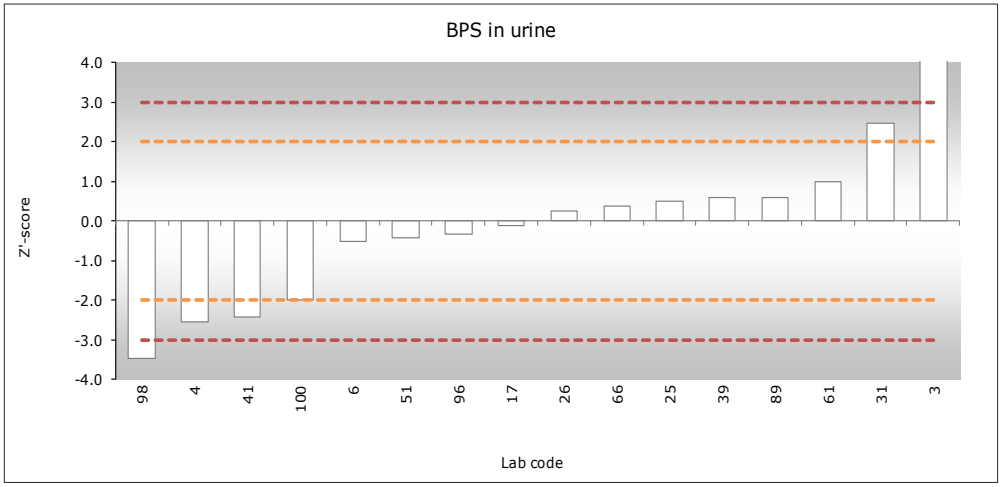
LOW LEVEL SAMPLE (L)



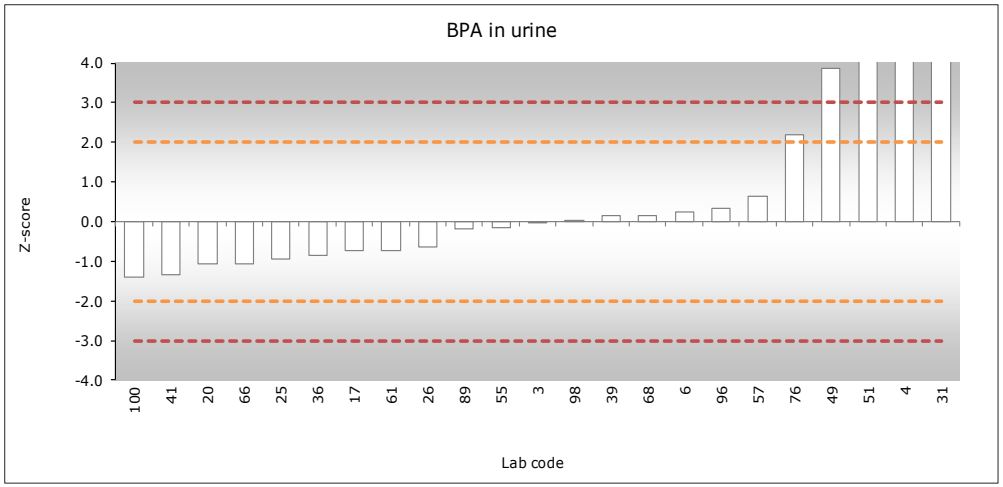
MEDIUM LEVEL SAMPLE A (Ma)



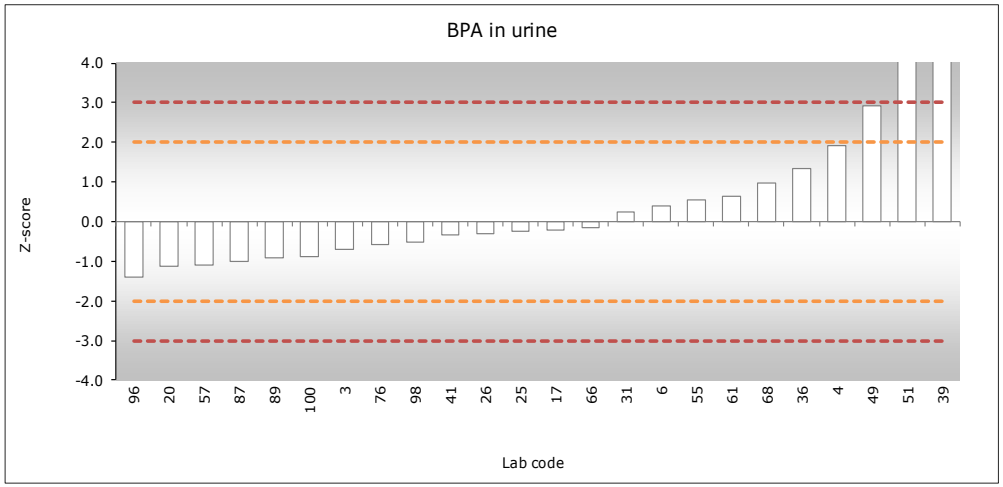


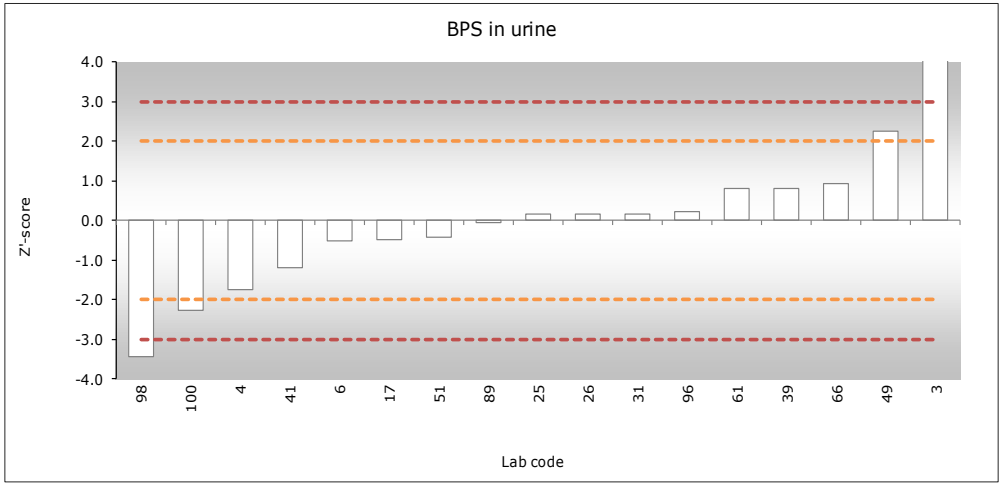


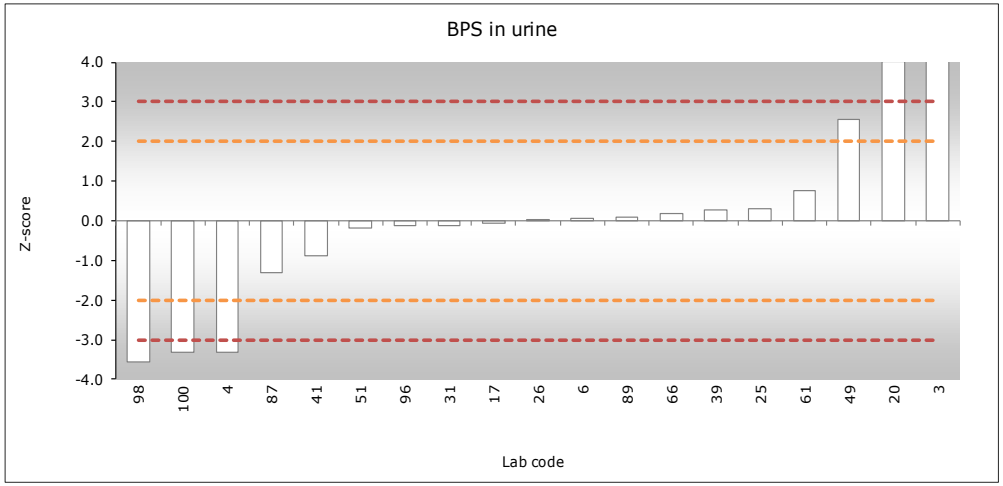
MEDIUM LEVEL SAMPLE B (Mb)



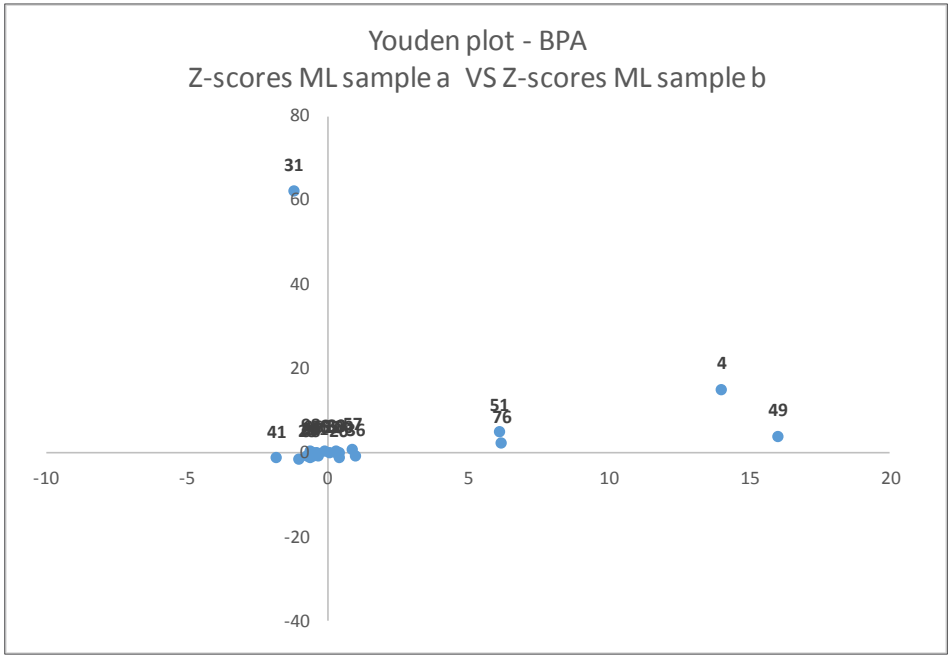
HIGH LEVEL SAMPLE

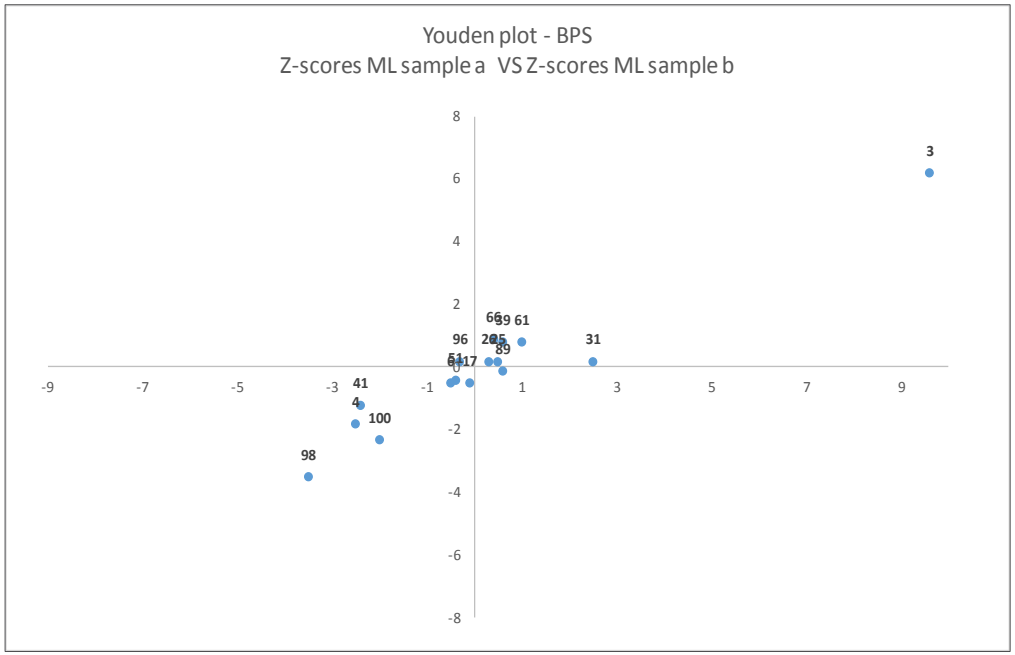






Appendix 10. Youden Plot – Z-scores associated to the medium level samples (a and b)





Appendix 11. Summary of participant's LOQ

	LOQ (ng/mL)		
Lab code	**BPA**	**BPS**	**BPF**
3	0.10	0.10	0.25
4	0.44	0.06	0.32
6	0.10	0.05	0.05
17	0.09	0.01	0.03
20	1.00	10.00	1.00
25	1.40	0.30	2.00
26	0.10	0.10	0.10
31	0.10	0.40	0.20
36	0.20	-	0.50
39	0.20	0.04	0.02
41	0.28	0.06	0.27
49	0.20	0.50	0.50
51	0.02	0.10	0.10
55	0.20	NA	0.50
57	0.03	NA	0.10
61	0.50	0.05	0.10
66	0.07	0.02	0.05
68	0.03	-	0.04
76	4.00	-	-
87	3.96	1.86	5.63
89	0.10	0.05	1.00
96	0.80	0.20	0.03
98	0.20	0.02	-
100	0.10	0.03	0.03

Appendix 12. Details of analysis methods used by the participants

	Extraction			Clean-up				
Lab Code	Volume of urine used (mL)	Type of deconjugation	Enzyme used (ref number)	SPE offline (yes/no)	Type of column used for SPE offline	Liquid/liquid (yes/no)	SPE online (yes/no)	Type of column used for SPE online
3	3	enzymatic	G7017 Sigma	yes	Oasis HLB	no	no	
4	0.5	enzymatic hydrolysis	9001-45-0	no		yes	no	
6	3	enzyme hydrolisis	beta-glucuronidase and sulfatase from Helix Pomatia	yes	Oasis HLB	Yes	no	-
17	2	enzymatic deconjugation	Abalonase purified enzymatic formula Bglucu (beta gluc 10)	yes	chromabond HR-X / Affinimip	no	no	-
20	1		B-Glucuronidase (from red abalone)	yes	Waters Oasis HLB (3ml, 60 mg)	no	no	
25	0.5	enzymatic	beta-glucuronidase E. coli (Roche), ref. No. 03707598001	no	--	no	yes	Waters X-Bridge BEH C8 Direct Connect 10 µm
26	0.2	glucuronidase	b-Glucuronidase from Roche 03707601001	no	no	no	no	no
31	0.2	enzymatic	beta-glucuronidase, type H-1from Helix pomatia (CAS number: 9001-45-0)	no	not applicable	No	Yes	Betasil C 18 (3 x 10 mm, 5 micrometer particle size)
36	0.1	Enzymatic deconjugation: acid hydrolysis	β- glucuronidase from Helix-pomatia (G7017 Sigma)	no		no	yes	polimer
39	15	enzymatic	ß-glucuronidase and sulphatase from H. Pomatia (Merck)	yes	AFFINIMIP® SPE Bisphenols	no	no	-
41	0.4	no deconjugaison quantification of BPs-glucuronide	no enzyme	yes	Chromabond HR-XAW	no	yes	Xbridge C8
49	0.5	enzymatic hidrolisis	BETA GLUCORONIDASE ARYLSULFATASE HT1	no	no	no	no	no
51	0.6	Enzymatic	B-Glucuronidase Sigma G7017	no		yes / SLE (Extrelut columns)	no	
55	3	emzymatic	β-Glucuronidase from Helix pomatia Type HP-2 (CAS 9001-45-0), Sigma cat. Number G7017-2ML	no	not applicable	yes +dSPE (dispersive SPE for cleanup)	no	not applicable
57	0.5	D-glucuronidase	Sigma-Aldrich G7017-2ML	no		yes	no	
61	1	enzymatic	glucuronidase/arylsulfatase (Roche, REF 10127698001)	no	-	yes	no	-
66	0.2	de-sulfatation and de-glucuronidation	aryl sulfatase (sulfatase from Aerobacter Aerogenes, cas no: 9016-17-5, Sigma, S1629-50UN), beta-glucuronidase (from E-coli K12, ref no: 03707580001, Roche)	no	no	no	yes	TurboFlow Cyclone P column (0.5 mm x 50 mm) from Thermo Scientific
68	2	deglucuronidation	b-Glucuronidase Helix pomatia (Calbiochem cat: 347420-1MU)	no	NA	yes	no	NA
76	0.5	β-glucuronidase	Helix Pomatia ≥ 100.000 units/mL (Type HP-2)	yes	ODS C18 Agilent	no	no	
87	0.5	Enzyme	Helix Pomatia	no	–	yes	no	–
89	0.3	enzymatic hydrolysis	ß-Glucuronidase from Helix pomatia Type HP-2 (Sigma G0876)	no		no	yes	Merck LiCrospher RP-8 ADS (RAM)
96	1	enzymatic	beta-glucuronidase from Helix Pomatia, type H-2, G0876	yes	Oasis HLB, Isolute SI	no	no	/
98	3	enzymatic	B-Glucuronidase Arylsulfatase (EC 3.2.1.31+3.16.1)	yes	C18	no	no	N/A
100	0.5	E. coli B-glucuronidase &Helix pomatia B-glucuronidase/Arylsulfatase	Roche 3707580001& 10127060001	no	-	no	yes	C18

	Measurement								Identification		
Lab Code	Derivatisation agent	Name and version of mass spectrometer	GC-MS (yes/no)	GC-MS/MS (yes/no)	LC-MS/MS (yes/no)	Type of column (GC or LC)	Response normalised to IS (yes/no)	Correction for recovery (yes/no)	Retention Time Tolerance (RRT)	Number of ions/transitions	Ion ratio tolerance
3	pyridine-3-sulfonyl chloride	Xevo TQ-XS Triple Quadrople Mass Spectrometer	no	no	yes	C18	Yes	no		2	
4	no	LTQ Discovery Orbitrap (Ion Trap)	no	no	yes	Fortis 2.6um SpeedCore C18-PFP, Size: 100x2.1mM	yes	yes	60 second	Quan/qual: 133/211(BPA) 107/155 (BPS), 93/123 (BPF)	10%(BPA), 40%(BPS), 75%(BPF)
6	MSTFA	7000A GC Triple Quad mass spectrometer	no	yes	no	HP5-MS	yes	no	0.05	2	0.2
17	MSTFA	Agilent 7010	no	yes	no	GC (Optima 17 MS)	yes	yes	0.005	2	0.2
20	NA	AB SCIEX API 3200	no	no	yes	LC	no	no	0.1min	1	
25	no	Waters Xevo TQ-S	no	no	yes	Macherey-Nagel Nucleoshell Bluebird RP18 100*2 mm, 2.7µm	yes	no	no formal criteria (comparison of RT with internal standard)	1MRM transition per analyte	no formal criteria
26	no	QTRAP5500	no	no	yes	LC	yes	no	yes	2	
31	Not applicable	Agilent Triple Quad MS/MS 6490	no	no	Yes	LC: Zorbax Eclipse Plus (2.1x50 mm, 1.8 micrometer paricle size)	Yes	no	0.05 min relative to Internal standard	MRM single transition	
36	no	Vantage MS/MS	no	no	yes	C18	yes	yes	±2,5%	2	±30%
39	BSTFA	Agilent 7000C	no	yes	no	Zebron semi-volatiles (20 m x 180 µm x 0.18 µm)	yes	no	<5%	5	25
41	Yes	Xevo TQD	no	no	yes	Phenyl-Hexyl	yes	no	0.01	2	0.4
49	no	TSQ-QUANTUM ULTRA	no	no	yes	LC	yes	no	0.025	2	0.2
51	Dansyl Chloride	Thermo TSQ Quantum Ultra	no	no	yes	LC Kinetex C18	yes	no	2 s	2	not used
55	BSTFA:TMCS (99:1)	ion trap Varian 220 MS	no	yes	no	GC: VF 5-ms 30m x0.25mm x0.25um +10m EZ-Guard	no	no	± 0.100 min	2	20
57		Qtrap 5500	no	no	yes	Phenomenex Luna C18(2)	yes	yes	0.1min	2	0.2
61	-	Waters Xevo TQ-S	no	no	yes	C8	yes	no	0.25 min	2	
66	no	TSQ Vantage MS/MS system from Thermo Fisher Scientific	no	no	yes	LC-column: Hypersil Gold aQ (0.4 mm x 50 mm, 3 um particle size) from Thermo Scientific	yes	no	0.05 min	2 for BPA, 1for BPF, 1for BPS	not calculated
68	TFAA (Trifluoroacetic anhydride)	Agilent 7000B GC-MS/MS triple quadrupole	no	yes	no	Phenomenex Zebron ZB-5MSi (30x0.25x0.25)	yes	no	0.05	4	0.2
76	N,O-Bis(trimethylsilyl)trifluoroacetamide with trimethylchlorosilane (BSTFA+1%TMCS)	Agilent 7890A coupled with Agilent 5975C	yes	no	no	Capillary column DB-5MS UI (30m X 0.25mm X 0.25µm)	yes	no	Integrated Retention Time-Locking software	2	
87	no	Agilent 6490	no	no	yes	Kinetex Phenyl-hexyl 100A 2.6 µm (150 x 2.1mm)	yes	yes	0.2min	2	0.2
89		AB Sciex 5500 Qtrap	no	no	yes	Waters Atlantis T3 (3.0x150; 3µm) for BPA Thermo Accucore Phenyl-X (3.0x150; 2.6µm) for BPS	yes	no	n.a.	2	0.2
96	MSTFA	Agilent 7000B	no	yes	no	DB 5 MS UI	yes	no	0.01	3	0.25
98	N/A	Thermo Quantiva	no	no	yes	C18	yes	no, calibration subie la SPE	0.1min	3	N/A
100	no	EVOQ elite MS/MS	no	no	yes	LC C18	no	no, isotope dilution	0.1min	2 or 3	0.2

	Internal Standards used to quantify			Calibration type						
Lab Code	Bisphenol A (BPA)	Bisphenol S (BPS)	Bisphenol F (BPF)	Isotopic dilution Addition before extraction (yes/no)	Isotopic dilution Addition to final extract (yes/no)	standard addition Addition before extraction (yes/no)	standard addition Addition to final extract (yes/no)	Matrix matched Addition to blank matrix before extr (yes/no)	Matrix matched Addition to blank extract (yes/no)	Solvent standards (yes/no)
3				yes	no	yes	no	no	no	no
4	Bisphenol A - d16	Bisphenol A - d16	Bisphenol A - d16	yes	no	no	no	no	yes	yes
6	BPA d14	BPS d8	BPA d14	yes	no	no	no	no	yes	no
17	BPA 13C	BPS 13C	BPF 13C	yes						yes
20				no	no	no	no	yes	no	no
25	13C12 (ring)	13C12 (ring)	13C12 (ring)	no	no	no	no	yes	no	no
26	2H14	13C12	2H10	no	yes	no	yes	no	yes	no
31	13C	13C	13C	yes	no	no	no	yes	no	no
36	13C12BPA	NA	13C12BPA	yes	no	no	no	yes (synthetic urine)	no	no
39	13C-BPA	13C-BPS	13C-BPF	yes	no	no	no	no	no	yes
41	BPA-Gluc 13C12	BPS-Gluc d8	BPA-Gluc 13C12	yes	no	no	no	yes	no	no
49	BPA-D16	BPS-D8	BPF-D10	no	no	no	no	yes	no	no
51	Bisphenol A 2H		Bisphenol A 2H	no	no	no	no	yes	no	no
55	BPA d16	NA	BPA d16	yes	no	no	no	no	no	no
57	13C12		13C12	yes	no	no	no	no	no	yes
61	d8-BPA	d8-BPS	d10-BPF	yes	no	no	no	no	no	yes
66	D8-BPA	13C12-BPS	13C12-BPF	yes	no	no	no	yes	no	yes
68	BPA-D16	NA	BPA-D16	yes	no	no	no	yes	no	yes
76	BPA D16			yes	no	yes	no	no	no	yes
87	BPA (D14)	BPA (D14)	BPA (D14)	yes	no	no	no	no	no	no
89	D16-BPA	13C12-BPS	D10-BPF	yes	no	no	no	no	no	yes
96	2d-BPA	13C-BPF	13C-BPS	yes	no	no	no	yes	no	no
98	BPA d6	no	N/A	yes	no	no	no	no	no	no
100	d16	13C12	13C12	yes	no	no	no	no	no	no