# A sensitive determination of bisphenol A and its 11 analogs and triclosan in urine by UPLC-MS/MS

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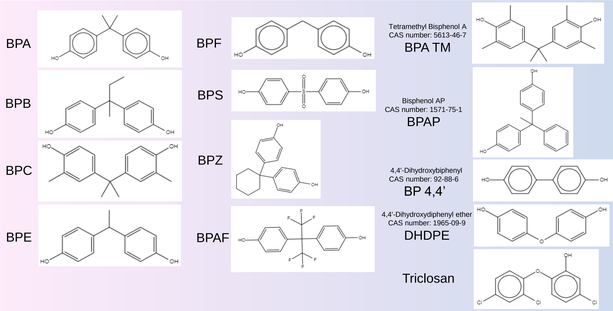
## Introduction

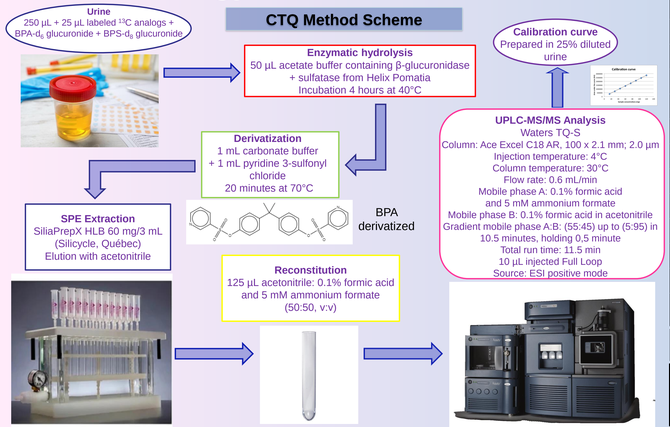
Bisphenol A (BPA) is used mainly as a monomer in the plasticizers with polycarbonate base or in epoxy resins. BPA is found in many consumer products (canned food, thermal paper, etc.). BPA is considered as an endocrine disruptor and because of regulatory actions and public health concerns, it was replaced by similar structure analog compounds by the industry. The effect of these alternate compounds on human health is still largely unknown. Triclosan is used as an antibacterial and antifungal agent in different consumer products (anti-bacterial soaps, toothpaste and deodorants). It is also considered as a suspected endocrine disruptor.




## Objectives

- To measure a large panel of BPA analogs and triclosan in a single run with good sensitivity, precision and accuracy.
- To present factors to consider to control the contamination and the hydrolysis step.
- To observe the rate of detection for BPA analogs in urine of the general population.

## Methodology




## Chromatograms

Figure 1. Chromatograms of BPA and its analogs and triclosan and their respective internal standards in spiked diluted urine.

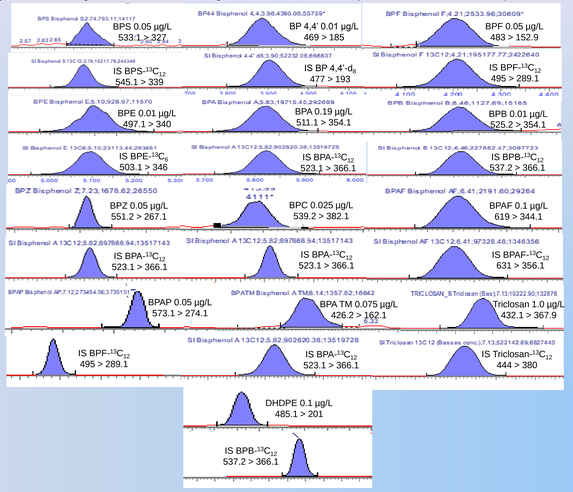



Table 1. Performance parameters for BPA, its analogs and triclosan analysis by UPLC-MS/MS.

## Method performance

| Analyte | Limit of detection (LOD)* (µg/L) | Limit of quantification (LOQ) (µg/L) | Linearity (µg/L) | Matrix effect (%) | Intra-day precision (%) | Intra-day precision Concentration (µg/L) | Inter-day precision (%) |
|---|---|---|---|---|---|---|---|
| BPA | 0.021 | 0.071 | LOQ to 25 | 100 ± 1 | 1.4 | 1.3 | 4.6 |
| BPB | 0.0068 | 0.023 | LOQ to 1.0 | 97 ± 3 | 6.2 | 0.036 | 6.1 |
| BPC | 0.082 | 0.27 | LOQ to 2.5 | 91 ± 8 | 8.1 | 0.085 | 14 |
| BPE | 0.0079 | 0.027 | LOQ to 1.0 | 95 ± 2 | 3.2 | 0.061 | 4.6 |
| BPF | 0.028 | 0.094 | LOQ to 50 | 100 ± 3 | 3.3 | 0.65 | 5.4 |
| BPS | 0.016 | 0.054 | LOQ to 25 | 89 ± 10 | 3.6 | 0.78 | 4.3 |
| BPZ | 0.026 | 0.085 | LOQ to 5.0 | 91 ± 2 | 5.7 | 0.18 | 5.2 |
| BPAF | 0.067 | 0.22 | LOQ to 10 | 97 ± 3 | 5.5 | 0.37 | 8.6 |
| BPAP | 0.017 | 0.056 | LOQ to 1.0 | 82 ± 16 | 5.0 | 0.035 | 5.1 |
| BPA TM | 0.069 | 0.23 | LOQ to 7.5 | 93 ± 6 | 8.1 | 0.26 | 12 |
| BP 4,4' | 0.046 | 0.15 | LOQ to 5.0 | 108 ± 4 | 1.8 | 0.36 | 4.8 |
| DHDPE | 0.050 | 0.17 | LOQ to 25 | 108 ± 3 | 3.6 | 1.6 | 8.0 |
| Triclosan | 1.0 | 3.3 | LOQ to 1250 | 102 ± 5 | 1.9 | 3.5 | 9.3 |

*Methodological limit of detection (LOD) = 3 times the SD of 30 determinations of human urine measured in 30 different days. Not possible to evaluate recovery because of the derivatization step at the beginning of the process.

- Adequate LODs in human urines according to expected reference levels.
- Good levels of precision and linearity obtained.
- Matrix effects well corrected by the internal standards.

## Method accuracy

Table 2. Method accuracy for BPA, BPF, BPS, BPZ and triclosan based on participation in External Quality Assessment Schemes.

| Analyte | Program 2023 | Measured value (µg/L) | Reference value (µg/L) | z'-score | Tolerance range (µg/L) |
|---|---|---|---|---|---|
| BPA | G-EQUAS (RV 71A) | 1.24 | 1.21 | - | 0.82 - 1.60 |
| | G-EQUAS (RV 71B) | 14.61 | 14.62 | - | 11.47 - 17.77 |
| | G-EQUAS (RV 72A) | 0.95 | 0.81 | - | 0.54 - 1.08 |
| | G-EQUAS (RV 72B) | 3.48 | 3.21 | - | 2.31 - 4.11 |
| BPF | G-EQUAS (RV 71A) | 1.66 | 1.68 | - | 1.32 -2.04 |
| | G-EQUAS (RV 71B) | 4.49 | 4.59 | - | 3.78 - 5.40 |
| | G-EQUAS (RV 72A) | 0.60 | 0.66 | - | 0.48 - 0.84 |
| | G-EQUAS (RV 72B) | 2.24 | 2.42 | - | 1.97 - 2.87 |
| BPS | G-EQUAS (RV 71A) | 0.62 | 0.63 | - | 0.45 - 0.81 |
| | G-EQUAS (RV 71B) | 3.12 | 3.30 | - | 2.67 - 3.93 |
| | G-EQUAS (RV 72A) | 0.38 | 0.35 | - | 0.26 -0.44 |
| | G-EQUAS (RV 72B) | 0.91 | 0.92 | - | 0.71 - 1.13 |
| Triclosan | G-EQUAS (RV 71A) | 11.2 | 11.0 | - | 7.1 - 14.9 |
| | G-EQUAS (RV 71B) | 53.5 | 54.7 | - | 39.4 - 70.0 |
| | G-EQUAS (RV 72A) | 2.3 | 2.5 | - | 1.6 - 3.4 |
| | G-EQUAS (RV 72B) | 25.1 | 25.5 | - | 18.9 - 32.1 |
| BPA[1] | OSEQAS OS-U-E2303 | 1.77 | 1.74 | 0.08 | 1.01 - 2.47 |
| | OSEQAS OS-U-E2304 | 1.23 | 1.31 | -0.29 | 0.722 - 1.90 |
| | OSEQAS OS-U-E2305 | 0.523 | 0.645 | -0.58 | 0.221 - 1.07 |
| | OSEQAS OS-U-E2306 | 1.58 | 1.70 | -0.33 | 0.985 - 2.42 |
| BPF[1] | OSEQAS OS-U-E2303 | 4.39 | 3.31 | 1.32 | 1.67 - 4.95 |
| | OSEQAS OS-U-E2304 | 0.662 | 0.641 | 0.16 | 0.378 - 0.904 |
| | OSEQAS OS-U-E2305 | 0.170 | 0.174 | -0.06 | 0.0486 - 0.299 |
| | OSEQAS OS-U-E2306 | 0.858 | 0.803 | 0.32 | 0.459 - 1.15 |
| BPS[1] | OSEQAS OS-U-E2303 | 0.642 | 0.519 | 1.08 | 0.292 - 0.746 |
| | OSEQAS OS-U-E2304 | 1.56 | 1.53 | 0.08 | 0.913 - 2.15 |
| | OSEQAS OS-U-E2305 | 0.327 | 0.320 | 0.11 | 0.190 - 0.450 |
| | OSEQAS OS-U-E2306 | 0.988 | 0.964 | 0.12 | 0.578 - 1.35 |
| BPZ[1] | OSEQAS OS-U-E2303 | 0.939 | 0.829 | 0.61 | 0.470 - 1.19 |
| | OSEQAS OS-U-E2304 | 1.01 | 0.845 | 0.94 | 0.497 - 1.19 |
| | OSEQAS OS-U-E2305 | < LOD | - | - | - |
| | OSEQAS OS-U-E2306 | 0.541 | 0.498 | 0.43 | 0.296 - 0.700 |
| Triclosan[1] | OSEQAS OS-U-E2303 | 211 | 195 | 0.40 | 116 - 274 |
| | OSEQAS OS-U-E2304 | 666 | 647 | 0.12 | 341 - 953 |
| | OSEQAS OS-U-E2305 | <LOD | - | - | - |
| | OSEQAS OS-U-E2306 | 390 | 395 | -0.06 | 217 - 573 |

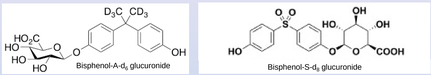
1 Data not shown for OSEQAS OS-U-E2301 and OSEQAS OS-U-E2302.

- Excellent comparability with other laboratories participating to German External Quality Assessment Scheme (G-EQUAS; Erlangen, Germany) and External Quality Assessment Scheme for Organic Substances (OSEQAS/CTQ; Québec, Canada)
- Most of the results for G-EQUAS are below 10% of deviation from the reference values.
- Most of the z'-scores are below 1.0 for BPA, BPF, BPS, BPZ and triclosan. Z'-score ≤ 1.0 is considered perfect and ≤ 2.0 satisfactory by the program OSEQAS.

## Contamination precautions

- All glass tubes must be heated at 500°C.
- All glassware and pipette tips must be washed with dichloromethane to reduce external contamination from dust.
- All reagents used (acetate buffer, carbonate buffer, β-glucuronidase and pyridine 3-sulfonyl chloride) must be monitored to control contamination.
- Pyridine 3-sulfonyl chloride may be a possible source of BPF while acetate buffer and β-glucuronidase could be possible sources of BPA, BP 4,4' and BPS.

## Monitoring deconjugation step




- Two labeled glucuronide standards (BPA-$d_6$ glucuronide and BPS-$d_8$ glucuronide) were added to each sample to measure the enzymatic hydrolysis efficiency.
- The added concentration for these glucuronide standards corresponded to the concentration of a calibration curve point (C-7) for BPA and BPS.
- An hydrolysis yield > 80% for both analytes must be obtained to validate the efficiency of the enzyme and to validate the analysis.

## Method application

Table 3. Proportion of analytes detected and geometric mean from a reference population recruited in our organization in 2018.

| Analyte | LOD (µg/L) | N | % > LOD | Min (µg/L) | Max (µg/L) | Median (µg/L) | Geometric mean (µg/L) | Geometric mean CHMS[1] (µg/L) |
|---|---|---|---|---|---|---|---|---|
| BPA | 0.021 | 65 | 100 | 0.099 | 4.3 | 0.88 | 0.78 | 0.68 |
| BPE | 0.0079 | 65 | 95.4 | 0.008 | 0.30 | 0.033 | 0.032 | 0.023 |
| BPF | 0.028 | 65 | 100 | 0.072 | 94 | 0.35 | 0.60 | 0.12 |
| BPS | 0.016 | 65 | 100 | 0.050 | 3.0 | 0.37 | 0.37 | 0.37 |
| BPZ | 0.026 | 65 | 0.2 | <LOD | 0.036 | - | - | - |
| BP 4,4' | 0.046 | 65 | 98.5 | 0.069 | 3.5 | 0.47 | 0.43 | 0.35 |
| DHDPE | 0.050 | 65 | 100 | 0.056 | 11 | 1.1 | 1.1 | -[2] |
| Triclosan | 1.0 | 50 | 64 | <LOD | 1325 | 2.0 | 86 | - |
| BPB | 0.068 | 65 | 0 | <LOD | <LOD | - | - | - |
| BPC | 0.082 | 65 | 0 | <LOD | <LOD | - | - | - |
| BPAF | 0.067 | 65 | 0 | <LOD | <LOD | - | - | - |
| BPAP | 0.017 | 65 | 0 | <LOD | <LOD | - | - | - |
| BPA TM | 0.069 | 65 | 0 | <LOD | <LOD | - | - | - |

1 Levels of environmental chemicals in the Canadian population from the Canadian Health Measures Survey (CHMS), Cycle 6, 2018-2019, The Canadian Biomonitoring Dashboard, https://infobase-dev.com/chms/.
2 Not quantified in CHMS.

- BPA, BPE, BPF, BPS, BP 4,4', DHDPE and triclosan are the most frequently detected analytes in the general population.
- Geometric means obtained from our sampled population are comparable with the geometric means obtained from the Canadian Health Measures Survey (CHMS) Cycle 6 2018-2019, except for BPF.
- Triclosan is most frequently detected in our sampled population (64%) compared to CHMS Cycle 6 2018-2019 (47,5%).

## Conclusion

- The current method has a good sensitivity, precision and accuracy to measure a large panel of BPA analogs and triclosan in a single run.
- Heating the glass tubes and washing the glassware and pipette tips are a good way to control contamination.
- Adding glucuronide conjugated standards is useful to evaluate the efficiency of the enzymatic hydrolysis step.
- BPA, BPE, BPF, BPS, BP 4,4' and DHDPE are detected between 95-100% of samples in the general population.
- This method is suitable for human biomonitoring studies.

**This method is currently used for the Canadian Health Measures Survey (CHMS) Cycle 7: 2023-2025 (4800 samples).**