Simultaneous determination of bisphenol A and its chlorinated derivatives in human plasma

Using the SCIEX QTRAP 6500+ system

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Bisphenol A (BPA) is a well-known chemical found in polycarbonate, polysulfone and epoxy resins, used in mass production for many consumer products. BPA exhibits endocrine disruptor properties that can potentially induce adverse health effects. In aquatic environments, it can react with chlorine to produce chlorinated derivatives (ClxBPAs). ClxBPAs are believed to exhibit estrogenic activity many times higher than BPA itself. Assessing human exposure to endocrine disrupting chemicals (EDCs) such as these is therefore of great importance when considering health risks associated with their use. Due to its ability to separate compounds of similar chemical structure, the specificity associated with direct detection of analytes and the ability to monitor large panels of analytes in a single analysis, LC-MS/MS is a powerful quantitative tool for this application.

Here, a method has been developed for detection and quantification of BPA and multiple ClxBPAs from human plasma in a single analysis using LC-MRM on the QTRAP 6500+ system. Specific MRM transitions were developed to measure BPA, as well as monochlorobisphenol A (MCBPA), dichlorobisphenol A (DCBPA), trichlorobisphenol A (TCBPA) and tetrachlorobisphenol A (TTCBPA) in human blood plasma. Figure 1 shows the structurally similar compounds involved.

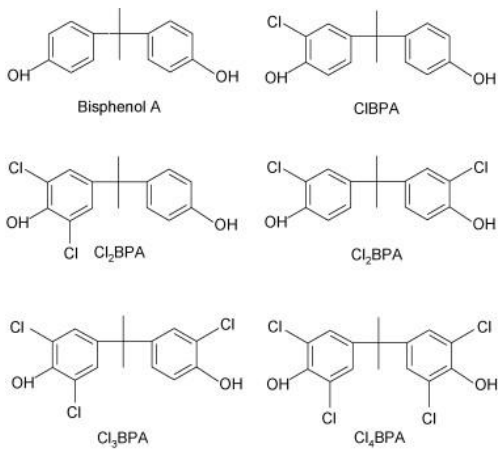

Figure 1: Bisphenol A and its chlorinated derivatives. Structural similarity of the compounds analyzed is very high.

Key features of the BPA and ClxBPA method

- Simple solid phase extraction to isolate target analytes from human plasma
- Eight minute run time using the Scheduled MRM algorithm to analyze 5 target analytes
- QTRAP 6500+ system for high sensitivity assays in bioanalysis
- IonDrive Turbo V source for improved ionization efficiency at high flows and more robustness
- Up to 6 orders of magnitude linear dynamic range
- Detection limits in the low ng/mL range from human plasma

Methods

Sample preparation: Plasma samples (500 µL) were extracted by solid phase extraction (SPE), a process which briefly consisted of these tasks:

- Condition cartridges with methanol and water
- Load samples onto wet cartridges
- Wash with methanol:water and dry under vacuum

- Elute with methanol
- Evaporate eluates to dryness
- Reconstitute in methanol:water and transfer to vials for injection

Liquid chromatography: Chromatographic separation was accomplished using an ExionLC AD system, with a Kinetex C18 column (100x2.1mm, 1.7µm, Phenomenex), at a flow rate of 0.35 mL/min. Water (A) and methanol (B) were used as mobile phase solvents. 20 µL of extract was injected into the UHPLC system.

Mass spectrometry: MS/MS detection was performed using the SCIEX QTRAP 6500+ system equipped with IonDrive Turbo V source and operated with the electrospray ionization (ESI) interface, operating in negative mode. Multiple reaction monitoring (MRM) mode was employed, using two specific transitions of each precursor ion. The first transition was used for quantification while the second was used for confirmation. Dwell and cycle times were optimized using the Scheduled MRM algorithm. Mass spectrometry parameters are given in Table 1.

Data processing: Data were acquired using Analyst software and processed using MultiQuant software.

Chromatographic separation

Chromatographic conditions were optimized and good separation between the target analytes was demonstrated, as seen in Figure 2. In blank plasma samples, no significant peak

Table 1: Mass spectrometry parameters of compounds

Compound	Q1 Ion	Q3 Ion	DP	CE	CXP
BPA	227.0	211.8	-65	-24	-19
BPA 2	227.0	*132.8*	-65	-34	-11
MCBPA 1	260.9	*181.7*	-120	-38	-19
MCBPA 2	260.9	*243.7*	-120	-30	-21
DCBPA	294.9	*243.7*	-100	-32	-25
DCBPA 2	294.9	*215.8*	-100	-42	-23
TCBPA	328.7	*249.7*	-85	-42	-15
TCBPA 2	328.7	*277.7*	-85	-34	-29
TTCBPA	364.8	*364.8*	-110	-36	-33
TTCBPA 2	364.8	*313.7*	-110	-44	-29
BPA-d16 (IS) 1	241.1	*222.9*	-90	-28	-25
BPA-d16 (IS) 2	241.1	*141.9*	-90	-34	-15
DCBPA-d12 (IS) 1	*307.0*	*224.9*	-120	-42	-23
DCBPA-d12 (IS) 2	*307.0*	*252.8*	-120	-34	-25

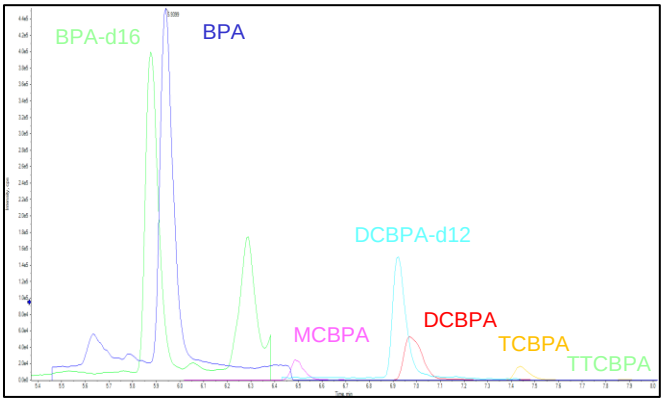

Figure 2: Chromatogram showing the separation of the BPA and other analytes.

(below LLOQs) of any of the ClxBPAs was detected at the corresponding retention time, in accordance with the high selectivity of the method. Indeed, BPA was detected in plasma from anonymous donors used for standards and quality controls (Figure 3), however it was always at a level markedly lower than the limit 0.1 ng/mL and has been taken into account in the calculation method.

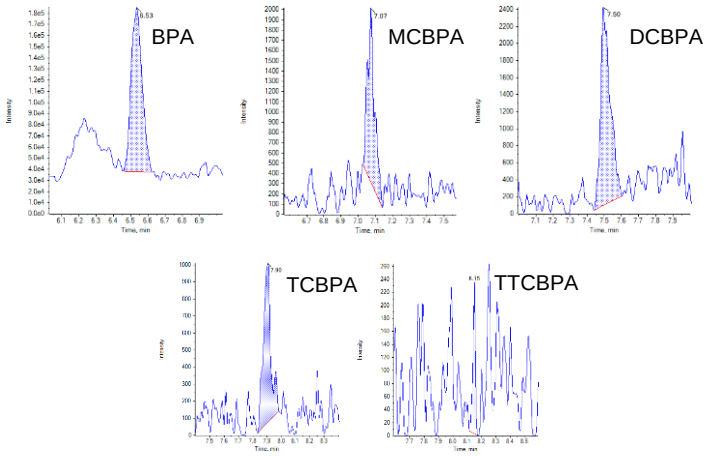

Figure 3. Examples of chromatograms obtained for the various analytes in blank plasma.

Analytical performance

The accuracy of the method was fully demonstrated. The variance did not exceed 17% at the lower limit of quantification (LLOQ) and was 12% at the other concentration levels for intra and inter-day analysis. Trueness or accuracy of the measured peak area ranged from 83 to 115 at the LLOQ, and from 85 to 107 % at the other concentration levels for intra and inter-day analysis. BPA (from 0.1 to 3.2 ng/mL) and ClxBPAs (from 0.005-0.02 to 0.64 ng/mL) calibration curves showed appropriate linearity (r^2>0,99) as in the example for BPA (Figure 4).

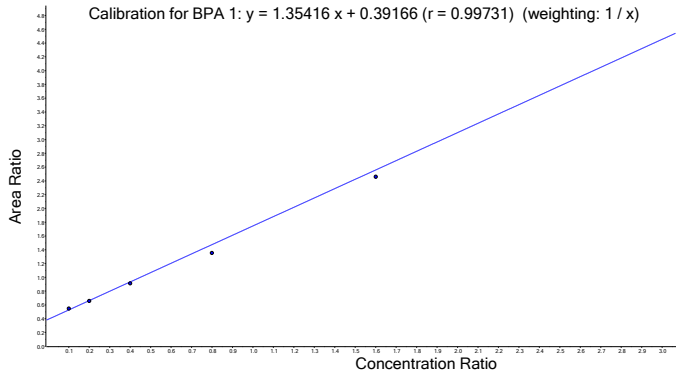

Figure 4. Calibration curve example for BPA.

Furthermore, due to contamination of human plasma, an intercept value of 0.320 ± 0.113 was obtained for BPA calibration curves. Indeed, BPA was detected in plasma from anonymous donors used for standards and quality controls (Figure 5), however it was always at a level markedly lower than the LLOQ (0.1 ng/mL for BPA). This was taken into account in the calculation method by subtracting the BPA/IS peak area ratio of blank plasma from the BPA/IS ratio of each standard used to construct the calibration curve.

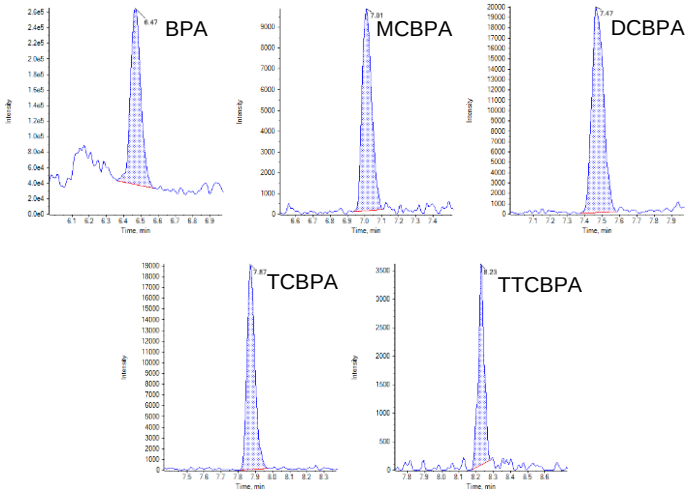

Figure 5. Examples of chromatograms obtained for a quality control sample. The concentration of the measured analytes was found to be 0.2 ng/mL for bisphenol A and 0.04 ng/mL for chlorinated derivatives.

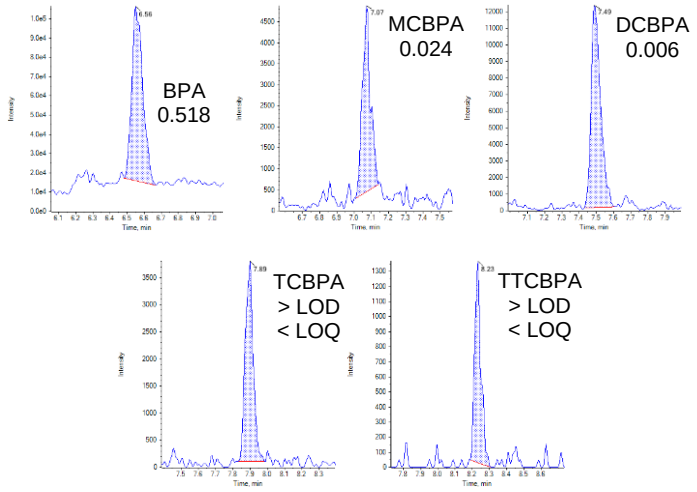

Figure 6. Detection of BPA and ClxBPAs in plasma samples positive for end stage renal disease. Examples of chromatograms obtained for BPA and ClxBPAs in human plasma sample. The detected concentration in these samples is marked on the XIC in ng/mL. TCBPA and TTCBPA were detected at a concentration below the LLOQ but above the LLOD.

The method was applied to anonymous human plasma samples previously identified as positive for end stage renal disease. Figure 6 shows a chromatogram obtained from a human plasma sample in which BPA and ClxBPAs were found. BPA was quantified in all ten samples with concentrations ranging from 0.266 to 86.8 ng/mL while MCBPA, DCBPA and TCBPA were quantified in two, one and one sample, respectively. TTCBPA was detected in three out of the ten samples. It is clear therefore that some samples show evidence of exposure to both BPA and ClxBPAs while others are only exposed to BPA.

Conclusions

Presented here is a method for simultaneous quantification of BPA and ClxBPAs in human plasma using LC-MS/MS analysis. The method is sensitive and robust, showing LLOQs of 0.1 ng/mL for BPA, 0.02 ng/mL for TTCBPA and 0.005 ng/mL for MCBPA, DCBPA, and TCBPA. This workflow is potentially suited to perform large-scale biomonitoring of these EDCs thereby heightening the statistical power of epidemiologic studies.

References

1. Cambien G *et al.* , (2020) Simultaneous determination of bisphenol A and its chlorinated derivatives in human plasma: development, validation and application of a UHPLC–MS/MS method, *Chemosphere* **242**, 125236.

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