# DETERMINATION OF BISPHENOL-A, TRICLOSAN AND TETRABROMOBISPHENOL-A IN HUMAN SERUM USING SPE AND GG/ECNI-MS

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**Abstract**

The present study focussed on the optimization and validation of a sensitive method for the determination of bisphenol A (BPA), triclosan (TCS) and tetrabromobisphenol A (TBBPA) in human serum. The method implied protein denaturation with formic acid, followed by solid phase extraction on OASIS® HLB cartridge, the analytes being eluted with methanol (MeOH). A further clean-up on Florisil® was applied and the analytes were collected in a fraction of 9 mL MeOH-dichloromethane (DCM) (5/1, *v/v*) followed by derivatization with pentafluoropropionic acid (PFPA). To eliminate acid by-products formed during derivatization, a liquid-liquid extraction with hexane-DCM (4/1, *v/v*) and $K_2CO_3$ 3% solution was applied prior analysis of the final concentrated extract by GC–ECNI-MS using specific ions for the PFP-derivatives. The absolute recovery values of the analytes were ranged between 71 and 85% for two levels of concentration (0.16 and 14 ng/mL serum). Furthermore, the optimized method was applied for the determination of these compounds in 21 Belgian human serum samples and they were found at detection frequencies higher than 70%. The analytes were measured at concentrations up to 3.8 ng/mL for TCS and BPA, with TBBPA being found at lower concentrations, up to 0.18 ng/mL serum.

**Introduction**

Due to their potential health effects, the occurrence and fate of endocrine-disrupting phenolic compounds such bisphenol A (BPA), triclosan (TCS) and tetrabromobisphenol A (TBBPA), have frequently been discussed in recent literature. BPA is widely used as the monomer in the manufacture of polycarbonate plastic and epoxy and phenoxy-resins being well known as an endocrine-disruptor.[1] TCS has found widespread use in a variety of consumer products including toothpastes, deodorants, soaps, polymers and fibers due to its antibacterial properties and since it was found in various environmental compartments, it has attracted increasing interest as a potentially toxic contaminant.[2-4] TBBPA is mostly used as a reactive brominated flame retardant (BFR), primarily in epoxy resins used for circuit board laminates, but, to a lesser extent, it is also used as an additive BFR, e.g. in acrylonitrile-butadiene-styrene resins being found in a wide range of consumer products, such as telephones, refrigerators and packaging material.[5]

The acute toxicity of BPA, TCS and TBBPA is low, but a growing concern has arisen in relation to their potential long-term effects on human health and wildlife. Even if these compounds have previously been found in samples from different environmental compartments and also in humans, little attention was paid so far to the development of analytical procedures to determine simultaneously these compounds in human fluids.[4,6-11]

Hereby, the present study focussed on the optimization and validation of a sensitive method for the determination of BPA, TCS and TBBPA in human serum. Furthermore, the optimized method was applied for the determination of BPA, TCS and TBBPA in Belgian human serum samples and a comparison with other previously published data regarding the concentration of these compounds in human samples was also performed.

**Materials and Methods**

*Instrumentation*

The analysis of BPA, TCS and TBBPA derivatives was performed with an Agilent 6890 GC coupled to a HP 5973 MS operated in ECNI mode. The GC system was equipped with electronic pressure control and a programmable-temperature vaporizer (PTV). One μL extract was injected in cold pulsed splitless mode (injector temperature at 92 ˚C for 0.04 min, then increased at 700°C $min^{-1}$ to 290 °C, injection pulsed pressure 15 psi until 1.5 min and purge flow to split vent of 50 mL $min^{-1}$ after 1.5 min). The GC was used with a 15 m x 0.25 mm x 0.10 μm DB-5 column (J&W Scientific). Helium was used as carrier gas at constant flow (1.0 mL $min^{-1}$) with methane as moderating gas. The GC temperature program was 90 °C for 1.5 min, then 15 °C $min^{-1}$ to 250 °C and then with 40 °C $min^{-1}$ to 300 °C, kept for 3 min. Dwell times were set at 40 msec. Methane was used as moderating gas, while the ion source, quadrupole and interface temperatures were set at 170, 150 and 300 °C, respectively and the electron multiplier voltage was at 2100 V.

*Extraction and Cleanup*

An accurate volume of serum sample (typically 1.5 mL) was transferred to a test tube and fortified with 5 ng of the corresponding $^{13}C$-labeled analytes as internal standards (IS). The samples were thereafter mixed with 0.5 mL formic acid for protein denaturation and also diluted with 1.5 mL water and they were afterwards vortex and equilibrated by ultrasonic treatment for 20 min. Prior to the sample application, the Oasis® HLB extraction cartridges were prewashed with DCM and activated with MeOH and water. After sample loading, the SPE cartridges were rinsed with 1 mL water and the sorbent bed was afterwards dried by centrifugation (10 min, 3500 rpm). The SPE cartridges were eluted with 4 mL MeOH-DCM (1/1, *v/v*) and the eluate was concentrated under a nitrogen stream prior to be transferred to the clean-up cartridges.

A further clean-up procedure on Florisil® cartridge was applied for lipids separation. The cartridges were prewashed before use with a mixture of MeOH-DCM (5/1, *v/v*) and also with *n*-hexane. After applying the sample, a first fraction of 6 mL hexane was used to elute the lipids and the next fraction of 9 mL MeOH-DCM (5/1, *v/v*) was kept for analysis being concentrate until dryness before derivatization.

*Derivatization*

Samples (or calibration standards) were first evaporated and redissolved in 100 μL ethyl acetate and 50 μL PFPA were added afterwards together with 50 μL TEA 0.01M, used as acid scavenger, in a closed test tube. After vortex, the tube was kept at 70 °C for 30 min to allow the derivatization to be complete. A further liquid-liquid extraction was applied in order to reduce the acidity of the solution and the reagent excess. Two mL mixture Hex-DCM (4/1, *v/v*) and 1 mL $K_2CO_3$ 3% solution were added in the test tube and vortex for 3 min and the derivatives were extracted in the superior organic layer. The aqueous layer was afterwards extracted two more times for 2 min to ensure good recoveries for the derivatives. To avoid traces of water to reach the GC system, the combined organic extract was passed over a cartridge containing anhydrous $Na_2SO_4$ and eluted with 2.5 mL Hex-DCM (1/1, *v/v*) and further concentrated to approximately 50 μL. In the final extract, 80 μL PBB 80 (100 pg/μL) were added as recovery standard.

**Results and Discussion**

*Analytical characteristics*

A chromatogram of BPA, TCS and TBBPA derivatives from a standard mixture together with ECNI mass spectra is presented in Figure 1. The specific ions for each analyte which were acquired during all the analysis optimization steps are also enlarged in the Figure 1.

The instrumental LODs and LOQs of each analyte were calculated for a signal/noise (S/N) ratio equal to 3 and 10, respectively, at the chosen quantification ion(s) for each analyte. For this purpose, horse serum was used as matrix spiked with 0.15 ng/mL serum with standards being added before derivatization step. The method LOQs were calculated as 3 x SD of the procedural blanks above the blank mean values and taking into account the amount of sample used for analysis (typically 1.5 mL human serum). Calculated values of instrumental and method LODs and LOQs are presented in Table 1.

The relative recovery values of each analyte compared to PBB 80 were assessed for each step of the analysis by spiking 3 replicates at two different concentration levels of 14 and 0.16 ng/mL horse serum, used here to consider matrix effects. The recovery values for entire method for each analyte are presented in Table 1.

For $^{13}$C-labeled IS, the relative recovery values were also assessed compared to the same recovery standard, but at the concentration level similar with the amount which was used as internal standard in the samples (5 ng) and they were 75% for $^{13}$C-labeled TBBPA, 80% for $^{13}$C-labeled TCS and 95% for $^{13}$C-labeled BPA. No significant difference for relative recovery values were obtained between replicates and also between both levels for each analyte (3% difference for 3 replicates).

Assessing the recovery values for SPE and cleanup step individually, the main loss of analytes is believed to occur in the cleanup step when the first fraction of 6 mL hexane was used to remove lipids, but the use of this step was accepted as a compromise between a clean sample for the derivatization step and an acceptable recovery for the analytes (data not shown).

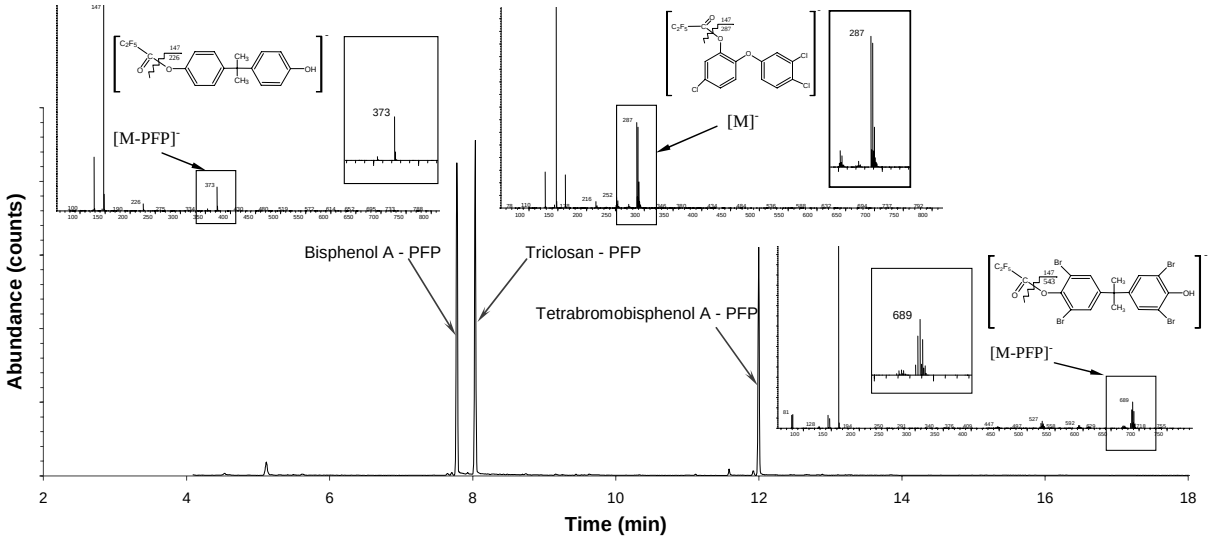


**Figure 1.** Typical mass-chromatogram for BPA, TCS and TBBPA derivatives together with ECNI mass spectrum and enlarged specific ions for each analyte

The repeatability of the measurements (% RSD) was tested for entire method using two concentration levels of analytes (14 and 0.16 ng/mL) added in horse serum matrix in four different days and 3 replicates for each concentration. The results for method precision within and also between days are presented in Table 1. The repeatability of the instrument response was also checked injecting the final extracts for two different concentration levels over a 6 days period, 4 injections daily. The within day precision for the instrument response was lower than 1.5 % and between days precision was 2.5 %.

**Table 1.** Validation parameters for determination of bisphenol A, triclosan and tetrabromobisphenol A in serum

| Analyte | Relative recovery value (%) | | Precision RSD (%) (within day) | | Precision RSD (%) (between day) | | LOD | LOQ | |
|---|---|---|---|---|---|---|---|---|---|
| | 14 ng/mL | 0.16 ng/mL | 14 ng/mL | 0.16 ng/mL | 14 ng/mL | 0.16 ng/mL | Instrumental (pg injected) | Instrumental (pg injected) | Method (pg/mL) |
| **BPA** | 83.1 | 81.3 | 1.6 | 5.1 | 2.4 | 14 | 0.1 | 0.3 | 280 |
| **TCS** | 86.0 | 85.9 | 1.6 | 5.9 | 3.3 | 15 | 0.1 | 0.4 | 90 |
| **TBBPA** | 71.5 | 77.7 | 1.8 | 13 | 2.6 | 11 | 0.1 | 0.2 | 50 |

*Method application*

To asses the feasibility of the developed method, BPA, TCS and TBBPA were measured in 21 human serum samples collected from Belgium. Only 7 from the analyzed samples were individual serum samples collected in 2007 and 14 were pooled from women of 55-60 years old collected in 1999.

A high detection frequency was recorded for each investigated analyte (> 70%) with TCS being measured in all samples. The analyte present at higher levels was BPA (median, 1st and 3rd quartile were 0.71, 0.2 and 1.77 ng/mL serum, respectively) and TBBPA was measured at lower levels (median, 1st and 3rd quartile of 0.08, 0.07 and 0.10 ng/mL serum, respectively) (Figure 2). Considering the age of individuals sampled for this study, no significant variations for the concentration of the contaminants was found and also no significant variation was found between concentrations of individuals and pooled serum samples.

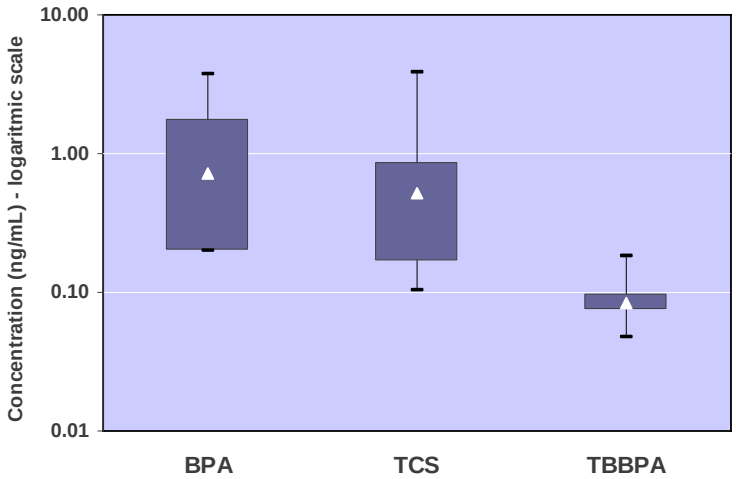


**Figure 2.** Distribution of BPA, TCS and TBBPA (ng/mL) in human serum samples (n=21) (median values, 1st and 3rd quartile and range)

A similar concentration range was found for BPA and TCS in Belgium human serum samples (present study) compared with previously reported data for phenolics in human fluids.[9,10] Slightly higher levels of TBBPA were found in Belgium samples compared to Norwegian serum.[11]

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**References**

1. Staples CA, Dorn PhB, Klecka GM, O'Block ST, Harris LR. *Chemosphere* 1998; 36:2149.
2. Bhargava HN, Leonard PA. *Am J Infect Control* 1996; 24:209.
3. Perencevich EN, Wong MT, Harris AD. *Am J Infect Control* 2001; 28:281.
4. Adolfsson-Erici M, Petterson M, Parkkonen J, Sturve J. *Chemosphere* 2002; 46:1485.
5. EHC-172. 1995, International Program on Chemical Safety, World Health Organization, Geneva, Switzerland.
6. Lee BH, Peart TE, Gris G, Chan J. *Water Qual Res J Canada* 2002; 37:459.
7. Canosa P, Rodriguez I, Rubi E, Cela R. *Anal Chem* 2007; 79:1675.
8. Allmyr M, McLachlan MS, Sandborgh-Englund G, Adolfsson-Erici M. *Anal Chem* 2006; 78:6542.
9. Ye X, Kuklenyik Z, Needham LL, Calafat AM. *J Chromatogr B* 2006; 831:110.
10. Allmyr M, Adolfsson-Erici M, McLachlan M, Sandborgh-Englund G. *Sci Total Environ* 2006; 372:87.
11. Thomsen C, Liane VH, Becher G. *J Chromatogr A* 2007; 846:252.