# PO-MA-03: SCREENING OF ESTROGENIC COMPOUNDS IN CONSUMER-ELECTRONICS PLASTICS BY LIQUID CHROMATOGRAPHY NANOFRACTIONATION-BIOACTIVITY DETECTION AND MASS SPECTROMETRY

**Ana Ballesteros-Gómez[1] , Willem Jonker[2] , Timo Hamers[3] , Govert W. Somsen[2] , Marja H. Lamoree[3] and Jeroen Kool[2]**

[1]Department of Analytical Chemistry, Institute of Fine Chemistry and Nanochemistry, Marie Curie Building (Annex), Campus of Rabanales, University of Córdoba, 14071 Córdoba, Spain

[2]Vrije Universiteit Amsterdam, Institute for Environmental Studies, De Boelelaan 1087, 1081. HV Amsterdam, the Netherlands

[3]Vrije Universiteit Amsterdam, Division of Bioanalytical Chemistry, De Boelelaan 1085, 1081. HV Amsterdam, the Netherlands

## Introduction

The chemical safety of consumer products is an issue of emerging concern. Plastics are widely used, e.g. as casings of consumer electronics (TVs, computers, routers, etc.), which are present in houses and offices in continuously increasing numbers. In this study, we investigate the estrogenic activity of components of plastics coming from electronics' casings. A recently developed fractionation platform for effect-directed analysis (EDA) was used.[1] This platform combines reversed-phase liquid chromatography in parallel with bioassay detection via nanofractionation and with on-line high-resolution time-of-flight mass spectrometry (TOFMS) for the identification of bioactives.

## Sample collection, preparation and analysis

Sample preparation and extraction was adapted from Ballesteros-Gómez et al.[2] Plastic samples from the hard plastics casings of electronic/electrical devices were taken using a surgical cutter. Samples (approximately 50 mg) were extracted with 10 mL of a mixture THF-MeOH (50:50, v/v) by sonicating (30 min) and stirring (200 rpm) for 12 h. Extracts were evaporated (40 0C, N2) and reconstituted in 1 mL of MeOH, ultra-centrifuged (10.000 rpm, 5 min) and further filtrated if required for removal of remaining particles in suspension (with 0.2 µm micro-centrifuge filters). Aliquots of 7 µL were analyzed by LC-MS/nanofractionation. In order to cover a wide range of compounds, no further clean-up was performed before analysis.

The LC flow rate was 0.25 mL/min and the injection volume was 7 µL. The column eluate was split 9:1 towards, respectively, the fraction collector and the mass spectrometer. All samples were analyzed in positive and negative mode with both ESI and APCI sources.

The fraction collection time was set at 10 s/fraction and was started 1 min after injection by turning the switch valve from fraction collector waste to fraction collector tip. A total of 108 fractions were collected per sample in 2 well plates. After fraction collection with parallel MS detection, well plates were dried prior to bioassay analysis. Detection of estrogenic compounds was performed with a reporter gene assay using human VM7Luc4E2 cells and analysed with a plate reader. A bioassay chromatogram was reconstructed by plotting the bioassay response of each fraction against the corresponding fraction time for comparison with the MS signal.

## Identification of estrogenic compounds in plastic casings from electronics

Extracts of the plastic were analysed by LC and the column eluate was split towards the mass spectrometer and fraction collector. Subsequently, fractions were subjected to bioassay testing and a reconstructed chromatogram was created for pinpointing bioactive peaks in the MS base-peak chromatogram. Molecular formulas were generated with high resolution MS software and ranked on their ppm error and isotope pattern fit. Next, a database and literature search was performed to retrieve the identity of the estrogenic compounds for the given formulas. Those matches that were most frequently cited in the literature (e.g. Chemspider database, number of references) and/or those with reported estrogenic activity were selected as the most suitable candidates. MS/MS experiments were carried out for structure confirmation on the basis of fragmentation. Finally, an authentic standard of the potential estrogenic compound candidate was injected for confirmation if available.

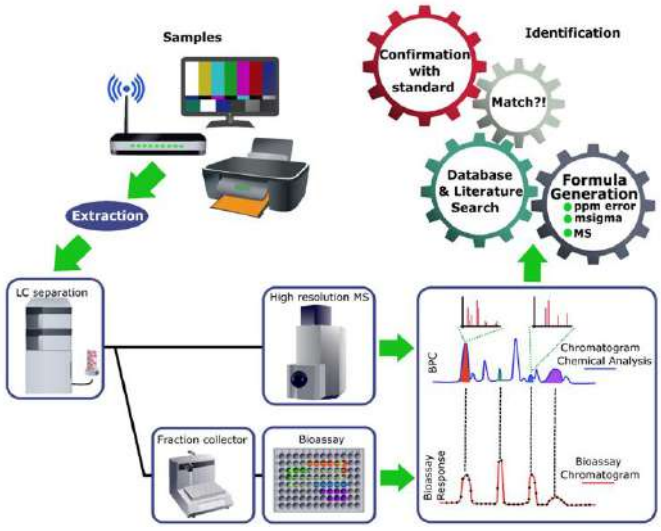


***Figure 1.*** *LC-nanofractionation-MS workflow to screen for the presence of estrogenic compounds in plastics from electronic products*

The reconstructed chromatograms obtained after EDA on the eight samples tested showed bioactive peaks in four of them. As an example, Figure 2 shows the results obtained for sample 4 (A) and 8 (B) depicting the base peak MS chromatogram, the bioactivity chromatogram, the extracted-ion chromatogram of the observed estrogenic compounds and their corresponding mass spectra.

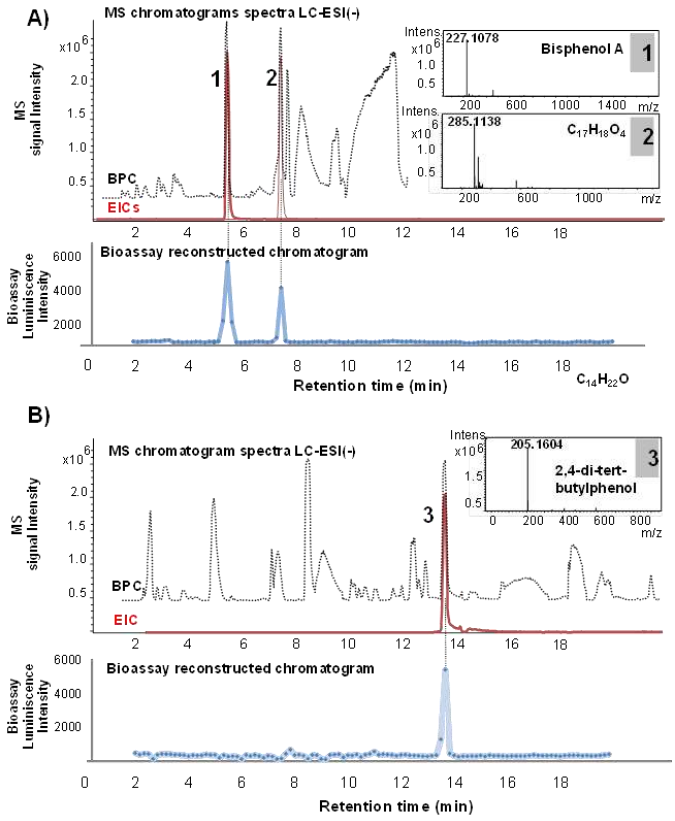


***Figure 2.*** *Reconstructed bioassay chromatograms aligned with the MS chromatograms of (A) sample 4 and (B) sample 8. MS base-peak chromatograms (BPCs), extracted-ion chromatograms (EICs), and mass spectra of the most abundant ion in the bioactive peak are shown*

The LC-MS/nanofractionation platform allowed to detect three estrogenic compounds in 4 of the 8 plastic casings analyzed, namely, BPA, 2,4-di-tert-butylphenol and a suspected BPA analog. 2,4-DTBP is used as an intermediate in a variety of chemical syntheses, such as the manufacture of UV stabilizers and antioxidants, that are common additives in plastics. This compound is classified as very toxic to aquatic life with long lasting effects.[3] Alkylphenols are known to be endocrine disruptors and 2,4-DTBP has been recently described as estrogenic compound.[4,5] To the best of our knowledge the presence of 2,4-DTBP in plastics has not been reported yet and could be a source of introduction of this compound into the environment. For the compound with formula C17H18O4, an unequivocal chemical match could not be proposed but fragmentation spectra showed similarities with BPA. The third identified compound, BPA, is a well-known weak estrogenic compound used as monomer (unpolymerized residue) and additive in a variety of plastics. Th plastics analysed were made of HIPS, so that BPA was not related to the plastic polymer itself, but most probably as degradation product or impurity of TBBPA flame retardants. While its presence in food contact materials has been widely reported, plastic casings from electronics have not been reported as potential sources of BPA contamination thus far.

Flame retardants that were present in the analyzed samples, did not show any estrogenic response in the human cell-based bioassay. However, bisphenol A and the suspected analog could be present as an impurity of BDP, TBBPA and TBBPA-based polymers. In general, we could conclude that plastic casings from consumer electronics contained estrogenic compounds. Consequently, these common consumer products could constitute a source of estrogenic contamination for human exposure indoors but also at primitive electronic waste recycling sites.

## References

**1.** Jonker, W.; Lamoree, M.H.; Houtman, C.J.; Hamers, T.; Somsen G.W.; Kool. . J. Chrom. A 2015, 1406, 165-174

**2.** Ballesteros-Gómez, A.; Jonkers, T.; Covaci, A.; de Boer, J. Anal Bioanal Chem. 2016, 408, 2945-53.

**3.** ECHA European database

**4.** Tollefsen, K-E.; Nilsen, A.J. Ecotoxicol. Environ. Saf. 2008 69, 163-172

**5.** Creusot, N.; Budzinski, H.; Balaguer, P.; Kinani, S.; Porcher, J.-M.; Aït-Aïssa, S. Anal. Bioanal. Chem. 2013, 405, 2553–2566.

## ACKNOWLEDGEMENTS

Ana Ballesteros-Gómez acknowledges the funding by the Netherlands Organization for Scientific Research (NWO) (VENI2014-722.014.003). Willem Jonker acknowledges the Dutch Technology Foundation STW for financial support, which is part of NWO, and which is partly funded by the Ministry of Economic Affairs. Project number: 12936.