

An analysis of residue alkylphenols and bisphenol A using liquid chromatography-tandem mass spectrometry

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Abstract: Bisphenol A (BPA) and alkylphenols (APs) such as 4-*tert*-octylphenol (4-t-OP) and mixture of nonylphenol isomers (iso-NP) are frequent contaminants of various environmental analyses. Analyse of various blanks is a serious problem in the residue analysis. Secondary contamination of analysed samples can come from laboratory air, septa vials, solvents and other chemicals used. Plastic tubes and connections could be another important source of sample contamination. This can be a problem especially in determination of alkylphenols and bisphenol A at low environmentally relevant concentrations. The analytical method for determination of residues of pollutants in water samples using solid phase extraction (SPE) followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) is presented here. In principle, alkylphenols can be analysed without derivatization. However, pre-column derivatization with 5-(dimethylamino) naphthalene-1-sulfonyl chloride (dansylchloride, DNSC) improves the sensitivity and selectivity of LC-MS/MS analysis. The instrumental blank for all compounds is under the limit of detection (LOD). The system contamination was maintained about 0.5 ng (reagent blank), and below 0.05 µg/L for the glass SPE columns and below 0.1 µg/L for the plastic columns (procedure blank).

Key Words: blank, blank contamination, LC-MS, alkylphenols, bisphenol A

INTRODUCTION

Alkylphenols and bisphenol A as well known endocrine disrupting compounds (EDCs) are able to mimic or antagonize the female estrogens 17 β-estradiol (Salgueiro-González et al. 2012b). Nonylphenols, which form a mixture of 211 branched nonyl-chain isomers, and 4-*tert*-octylphenol are the most important alkylphenols due to their toxicological properties. Bisphenol A has been used as a material for the production of epoxy resins, phenol resins, polycarbonates, polyesters, lacquer coatings on food cans, and also in flame retardants, adhesives, and as a component of electronic circuits (Salgueiro-González et al. 2012a). The concentrations of BPA, APs and their parent compounds have been measured worldwide in all compartments of the environment and even in food products for human consumption (Xie et al. 2006).

The primary purpose of analysis of blanks is to trace sources of artificially and or randomly introduced contamination of real samples. The diagram (see Figure 1) shows how comparison of different blank sample results can be used to identify and isolate the source of contamination introduced in the field or the laboratory. The source of contamination introduced in the field or laboratory can be deduced by comparing blank results. An equipment blank could potentially be contaminated in the field, during transport to the lab or in the lab. The method blank, on the other hand, could only be contaminated in the lab. Using all the blanks described in this fact sheet will facilitate the identification of contamination sources (EPA 2009).

Nonylphenols can also be present in laboratory air. For example, concentrations around 100 ng/m³ were found in the air of a typical laboratory and it has also been reported that an LC-MS vial with methanol left in the autosampler of the instrument can absorb NP from the laboratory air within weeks. Septa vials and solvent used as mobile phases can also cause blank contamination.

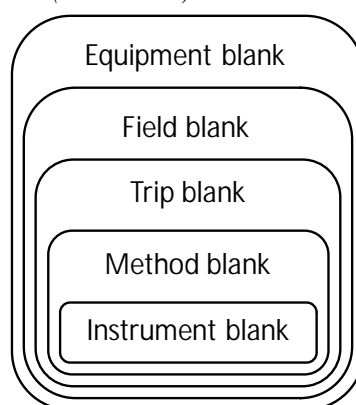
BPA contamination caused by the water purification system was observed in ultrapure water. Sampling, storage of samples, filtration and sample treatment are also important sources of blank contamination. APs and BPA from water samples are commonly extracted by liquid-liquid extraction (LLE) or solid-phase extraction (SPE). However, this procedure can also cause contamination due to the plastic materials of the SPE cartridges, the multiple steps involved and the use of relatively high volumes of solvent (Xie et al. 2006, Salgueiro-González et al. 2012a).

The terms such as ghost peaks, artifact (and artefact) peaks, system peaks, pseudo peaks, vacancy peaks, eigenpeaks, induced peaks and spurious peaks can be found in published papers. These terms are called peaks that are undesirable in the blank systems (Williams 2004).

It is important to fully characterise the analytical performance in order to understand their capability and limitations, and to ensure that they are “fit for purpose”. The terms such as LOB (limit of blank), LOD (limit of detection), and LOQ (limit of quantification) describe the smallest concentration of an analyte that can be reliably measured by an analytical procedure. LOB and LOD are important for tests used to distinguish between the presence or absence of an analyte and LOQ to reliably measure low levels of analyte and should be incorporated as part of any method evaluation (Armbruster and Pry 2008).

- Blank equipment (equipment blank) includes the total field and laboratory contamination sources, which are determined by sputtering the blank over the decontaminated field sampling device before sampling the environment. The objective of the assessment is to assess the total contamination of the sampling.
- Field blank includes overall ambient conditions during sampling and laboratory pollution sources. Blank determination is done as follows, a blank sample is transferred to the sampling container and then sent to a laboratory with a field sample. The aim is to assess contamination from field conditions during collection.
- Trip blank includes shipping and laboratory contamination sources, only for volatile substances. The transport blind sample is a sample which is transported from the laboratory to the point of collection and transported back to the laboratory without being subjected to a sampling procedure.
- The blank method (method blank) only shows laboratory sources of contamination. Blank methods are prepared and analyzed exactly in the same way as a field sample. The aim is to assess the contamination during sample preparation.
- The instrumental blank (instrument blank) only shows instrumental source of contamination. The blank itself is parsed with the field sample and the goal is to assess the presence or absence of device contamination (EPA 2009).

Figure 1 Comparison of different blank (EPA 2009)



The main purpose of our study was to compare individual types of blanks such as instrumental blank, reagent blank, method blank and also evaluated to effect use of glass and plastic SPE columns in residual analysis of alkylphenols and bisphenol A. Bisphenol A, 4-tert-octylphenol, 4-octylphenol, isomers of nonylphenol and 4-n-nonylphenol were selected for these purpose. The LC-ESI-MS/MS was used for intended study using pre-column derivatisation with dansyl chloride.

MATERIAL AND METHODS

Instruments and chemicals

Chromatographic separations were performed using an Agilent 1200 Infinity Series (Agilent Technologies, Santa Clara, CA, USA) with chromatographic column ACE 5 C18, 150 mm × 4.6 mm i.d., 5 µm particle size (ACE, Scotland, UK). The Agilent 6410 Triple Quadrupole (Agilent Technologies, Santa Clara, CA, USA) was used for MS/MS analysis. The glass and plastic SPE columns octadecyl C18, 500 mg (Supelco) was used for analysis. The analytical standards, derivatizing agent DNSC and water (ultrapure water for HPLC) were purchased from Sigma-Aldrich (Germany).

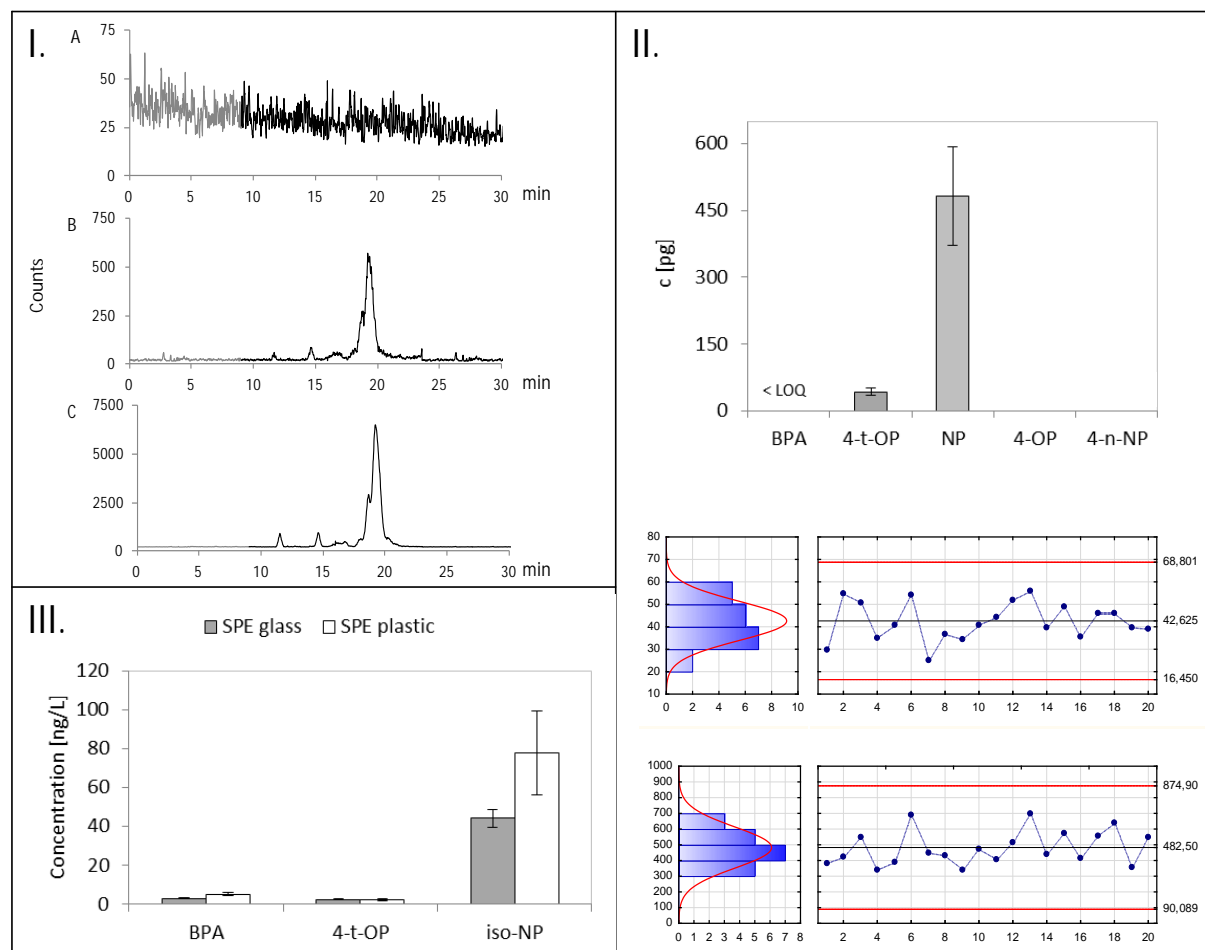
Alkylphenols measurements

Alkylphenols and bisphenol A were measured in 3 levels blank samples such as instrumental blank, reagent blank and method blank. Sample of ultrapure water for HPLC was used for determination of instrumental source contamination (the instrumental blank) during analyses by LC-MS/MS method, as described by Pernica et al. (2015). Analyse of *the reagent blank* was used in this study to assess relationship between the instrumental blank and the method blank. *The reagent blank* was prepared in ultrapure water for HPLC with all of chemicals used for derivatization of alkylphenols by dansyl chloride (DNSC). The derivatization reaction was carried out in a 2 mL amber glass sample vial with 200 µL ultrapure water for HPLC. Fifty microliters of 100 mmol/L NaHCO₃ (pH = 10.5) were added and the mixture was stirred (vortex) for 1 minute. Next 200 µL of DNSC in acetone (0.5 mg/mL) were added and stirred again. Subsequently the mixture was incubated for 60 minutes and 60 °C. The reaction mixtures were then cooled to the room temperature and evaporated to dryness under nitrogen atmosphere. The residue was redissolved in 1 mL of methanol and stirred (Pernica et al. 2015). The following procedure was used as the method blank for residue analyses of alkylphenols and bisphenols A. The glass and plastic SPE columns were conditioned with 2 mL of methanol and 2 mL of water (purity for LC/MS). 100 mL of ultrapure water for HPLC was passed through the SPE column using vacuum manifold flow rate 5 mL/min. After extraction, the columns were dried with air for 5 minutes. Elution was performed with 5 mL acetonitrile. The eluate was evaporated and converted into a mini-vial, derivatized with dansyl chloride and analysed by LC/ESI/MS/MS as described by Pernica et al. (2015). The method blank was measured 3 times for glass and plastic SPE columns.

RESULTS AND DISCUSSION

The instrumental blank was under the limit of detection (LOD) for selected compounds, such as bisphenol A (BPA), 4-tert-octylphenol (4-t-OP), mixture of nonylphenol isomers (iso-NP, technical mixture), 4-octylphenol (4-OP), and 4-n-nonylphenol (4-n-NP). Only BPA, 4-t-OP and iso-NP were detected and only 4-t-OP and iso-NP were quantified in the reagent blank. Three analytes were quantified in the method blank, which is about 10-times higher than the counts of chromatogram in the natural reagent blank. Repeatability of the reagent blank was measured once per day for a period of 20 days, when BPA was under of quantification (LOQ). The average concentration of 4-t-OP and iso-NP measured in the reagent blank was 42.6 pg/mL and 482.5 pg/mL, respectively. QC histograms for significant evaluation were used, the critical upper limit and lower limit was not exceeded in any way. Finally, a SPE glass column and SPE plastic column were compared in the method blank. The median concentration of BPA for the glass SPE column was 3.1 ng/L and 5.3 ng/L for the plastic SPE column. No significant differences were found in SPE columns for 4-t-OP. The median concentration of 4-t-OP was 2.45 ng/L for the glass SPE column and 2.42 ng/L for the plastic SPE column. The result of iso-NP was statistically significant with a concentration of 44.3 ng/L for the glass SPE column and 77.8 ng/L for the plastic SPE column (see Figure 2). The plastic SPE columns provided a higher method blank than the SPE glass columns. However, the SPE glass column had better repeatability than plastic materials. The SPE plastic columns provided relatively higher levels of alkylphenols and bisphenol A caused by common contamination of the prepared samples coming from laboratory air, septa vials, and solvents and chemicals used. These sources of contamination cannot be affected. The linear alkylphenols such as 4-OP and 4-n-NP were not present in any blank.

Figure 2 I. Chromatograms of blanks (A) instrumental blank (B) reagent blank (C) method blank. **II.** Intermittent repeatability ($n = 20$) of the reagent blank for selected alkylphenols and bisphenol A, include QC histograms for 4-t-OP and iso-NP. **III.** Comparison of SPE columns made of glass and plastic ($n = 3$).



CONCLUSIONS

Significant blank contamination is a notorious problem in ultratrace analyses of alkylphenols and bisphenol A, especially when we perform an analysis at concentrations close to the LOQ. A blank contamination may result in a significant increase in the detection limit. A systematic study and analysis of blanks together with analyses of a set of treated and analysed samples is necessary. In order to keep blank contamination as low as possible, the minimization of the number of potential contamination sources is required, such as plastic laboratory containers and plastic aids, water prepared in plastic devices etc., the work in a laboratory devoted solely to analyzes of alkylphenol is the strong requirement.

ACKNOWLEDGEMENTS

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