Toxicology Letters 210 (2012) 141–154

Contents lists available at SciVerse ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Mini review

Recent trends in biomonitoring of bisphenol A, 4-t-octylphenol, and 4-nonylphenol

Alexandros G. Asimakopoulos, Nikolaos S. Thomaidis*, Michael A. Koupparis**

Laboratory of Analytical Chemistry, Department of Chemistry, University of Athens, Panepistimiopolis-Zografou, 15771 Athens, Greece

ARTICLE INFO

Article history:
Available online 22 August 2011

Keywords:
Human biomonitoring
BPA
4-t-OP
4-NP

ABSTRACT

Bisphenol A (BPA), 4-t-octylphenol (4-t-OP), and 4-nonylphenol (4-NP) are man-made alkylphenolic environmental contaminants possessing controversial endocrine disruption properties. Nowadays, an increased interest is raised for their accurate determination in biological media in order to estimate the exposure to these compounds and the associated health risk. The aim of this review is to present the available analytical methodologies for biomonitoring these three EDCs in human population. In non-occupational human exposure, they are detected in human matrices in trace level concentrations, commonly lower than 1 ng/mL. The use of mass spectrometry based methods is particularly emphasized due to their well known superiority over sensitivity, selectivity and precision, even in difficult matrices, such as blood plasma and serum. Recent and most applicable sample preparation techniques are thoroughly presented. The benefits of solid phase extraction (SPE) and expected developments are demonstrated. Recent results from exposure assessment and epidemiologic studies for BPA, 4-t-OP and 4-NP are summarized and future trends are discussed.

Contents

Abbreviations: BPA, bisphenol A; 4-t-OP, 4-t-octylphenol; 4-OP, 4-octylphenol (mixture of isomers); 4-NP, 4-nonylphenol (mixture of isomers); APs, alkyl phenols; APEOs, alkyl phenol ethoxylates; EDCs, endocrine disrupting compounds; LOD, limit of detection; LOQ, limit of quantification.

* Corresponding author. Tel.: +30 210 7274317; fax: +30 210 7274750.
** Corresponding author. Tel.: +30 210 7274559; fax: +30 210 7274750.
E-mail addresses: ntho@chem.uoa.gr (N.S. Thomaidis), koupparis@chem.uoa.gr (M.A. Koupparis).

0378-4274/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.toxlet.2011.07.032

1. Introduction

Bisphenol A (BPA), 4-t-octylphenol (4-t-OP), and 4-nonylphenol (4-NP) (mixture of isomers) are man-made alkyl phenols (APs), well known in the scientific world as xenoestrogen compounds (Fig. 1) (CERHR, 2008; Van Miller and Staples, 2005; Vazquez-Duhalt et al., 2005). BPA is a high production volume chemical mainly used in the industry as an important intermediate in the production of the following resins and polymers: polycarbonate, epoxy, polysulphone, polyacrylate, polyetherimide, unsaturated polyester and phenolic. It can be found in a wide variety of materials and products (e.g. bottles, coatings, pipes, dental sealants, food packaging, nail polishes and flame-retardant materials) that human population can easily come across on a daily basis (Ballesteros-Gomez et al., 2009; CERHR, 2008; Maragou et al., 2006, 2008a). Very recently, European Union (E.U.) banned the use of BPA in plastic infant feeding bottles, making a landmark move to safeguard infants and general population's health (Commission Directive 2011/8/EC, 2011). The other two APs, 4-t-OP and 4-NP were extensively used until recently in the E.U. and in the United States (U.S.) as intermediates in the production of phenolic resins and alkyl phenol ethoxylates (non-ionic detergents) (Van Miller and Staples, 2005; Vazquez-Duhalt et al., 2005). Alkyl phenol ethoxylates (APEOs) were used in industrial formulations (paper, leather, tannery, textile, oil industries and metal working fluids), antifoamers, detergents, dispersants, emulsifiers, paint ingredients, pesticide adjuvants and personal care products (Cox, 1996; DEFRA, 2008; Vazquez-Duhalt et al., 2005). Biodegradation of APEOs was proven to be an important source of 4-OP and 4-NP environmental contamination (David et al., 2009). Since 2000, 4-OP and 4-NP were included in the list of priority hazardous substances by Directive 2000/60/EC (Commission Directive 2000/60/EC, 2000). Since 2003, a reduction policy has been implemented in the E.U. for 4-NP (Commission Directive 2003/53/EC, 2003). Nowadays only a few countries (e.g. Asian countries) continue to use APEOs (David et al., 2009). Nevertheless, 4-t-OP and 4-NP are still widespread and detected world widely in environmental media, such as wastewaters, potable water, rivers and biota (David et al., 2009; Hawker et al., 2011; Gatidou et al., 2010; Stasinakis et al., 2008).

As xenoestrogen compounds, BPA, 4-t-OP and 4-NP present multiple modes of endocrine disruption activity; with the most emphasized being the binding to the estrogen receptors (estrogen receptors α and β) (Mueller and Korach, 2001) and acting competitively towards natural hormones (e.g. 17β-estradiol). Although the affinity of these APs towards the receptors are much weaker (weak estrogen activity) than the affinity naturally induced from the natural hormones of a living organism (CERHR, 2008; Sun et al., 2008; Van Miller and Staples, 2005; Vazquez-Duhalt et al., 2005), reproductive and developmental toxicity studies have shown effects to aquatic organisms and animals (Diamanti-Kandarakis et al., 2009; Krishnan et al., 2010; Staples et al., 2004; Van Miller and Staples, 2005; Vazquez-Duhalt et al., 2005). In the case of 4-NP, that occurs as a mixture of many isomeric compounds, it was clearly pointed out that estrogen activity may differ between isomers and was directly related to the form of the obtained alkyl chain structure (isomer-specific activity) (Reinscheid, 2009). Due to the large number of 4-NP isomers (293 isomers in total), research was focused on their separation, tandem mass detection and toxicity evaluation (Kammann et al., 2009; Lalah et al., 2007; Moeder et al., 2006; Preuss et al., 2008; Zenkevich et al., 2009). As presented in Moeder et al. (2006), tandem mass detection is important since the possibility to identify an isomer individually can be attempted only by its reported fragmentation pattern.

The exact endocrine disruption properties of BPA are controversial on human population, because, firstly and foremost, conflicting data can be found across exposure assessment studies and secondly, correlating findings on animals with potential effects on humans contains a degree of uncertainty (Vandenberg et al., 2010a,b). As far as 4-t-OP and 4-NP are concerned, a limited number of human toxicity studies have been carried out (Section 4.2), a fact that highlights the urgent need to investigate the toxicity and biotransformation pathways of these compounds in the human body. Therefore, biomonitoring of 4-t-OP and 4-NP in human fluids and tissues is deemed necessary in order to assist in elucidation of potential correlations between exposure and adverse health effects.

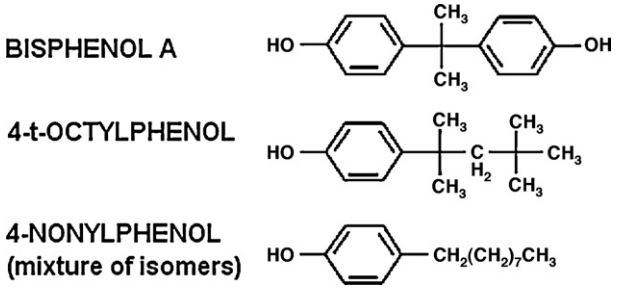

Fig. 1. Molecular structures of bisphenol A (BPA), 4-t-octylphenol (4-t-OP) and 4-nonylphenol (4-NP).

2. Biotransformation-biomarkers

When the three APs enter the blood circulation of the human body, biotransformation follows. At this point, the compounds are subject to glucuronidation and sulfation, mainly localized in the liver. Since they are converted in large extent into glucuronides and sulfates, their potential estrogenicity is deactivated. Then, the conjugates are rapidly cleared away from blood through the kidneys and end up in urine for excretion (Shangari et al., 2005). Due to biotransformation and rapid clearance, only low levels (trace levels) of the analytes are likely to be detected in blood after a specified time-period has elapsed from exposure. For instance, it was reported that BPA and 4-NP obtain approximately an elimination half-life in the human body of less than 6 (Völkel et al., 2002) and 3 h (Müller et al., 1998), respectively.

Therefore, biomonitoring should be performed through highly sensitive analytical methods and exposure assessment should be based, apart from the free species of the compounds, on the glucuronidated or/and sulfated conjugates. Glucuronide and sulfate species are present in urine and blood and can be used as biomarkers of exposure. When free plus conjugated species are quantified, then total concentration of the APs is determined and a more appropriate and integrated exposure evaluation is performed. Treatment of biological samples with glucuronidase and/or sulfatase enzyme during sample preparation prior to instrumental analysis is very common in order to cleave conjugate species and assess total concentration (Inoue et al., 2003a; Ye et al., 2005).

Due to the complexity of the alkyl phenol's metabolic pathways, biomarkers of exposure are an area of systematic research. Ye et al. (2007) identified 4-(3′,6′-dimethyl-3′-heptyl) catechol (P363-NC) in human liver microsomes as a potential biomarker for the main metabolite of one 4-t-NP isomer, 4-(3′,6′-dimethyl-3′-heptyl)phenol (P363-NP). 2-(3′,4′-dihydroxyphenyl)-2-(4′-hydroxyphenyl)propane (3-OH-BPA) (Nakagawa and Suzuki, 2001) and BPA catechol (Ye et al., 2011) may serve in the near future as potential biomarkers of human exposure to BPA. Suzuki et al. (2004) reported the detection of 3-OH-BPA even in river waters.

3. Analytical methodologies used for biomonitoring

3.1. Sample preparation

In general, trace analysis in biological media often requires laborious sample pre-treatment, as centrifugation, extraction, sample cleanup and pre-concentration. These steps of sample preparation should not be avoided, because direct determination is usually not possible due to interferences from endogenous compounds (e.g. proteins and phospholipids) that exist in large amounts in matrix. For extracting BPA, 4-t-OP and 4-NP or/and for further sample cleanup, the majority of methods involved liquid–liquid extraction (LLE) or/and solid phase extraction (SPE). Method protocols, like that of Ye et al. (2008), were developed to extract sample quantities even as small as 100 μL.

In liquid–liquid extraction, acetonitrile (Kuruto-Niwa et al., 2007) was the most preferred solvent since apart from extraction, it simultaneously precipitated effectively the endogenous proteins of matrix. Ethyl acetate (Schöringhumer and Cichna-Markl, 2007), chloroform (Kuroda et al., 2003), diethyl ether (Ouchi and Watanabe, 2002), isopropanol (Atkinson et al., 2002) and ammonium hydroxide (Kaddar et al., 2009) were also reported for analyte(s) extraction or/and protein precipitation purposes. *N*-Hexane, ethanol and petroleum ether were particularly used for lipid removal from matrix (Lin et al., 2009; Sajiki, 2003).

Solid phase extraction (SPE) is a technique that fractions the target analytes based on their affinity to the stationary phase (sorbent). The advantages of this technique are simplicity, reproducibility, and applicability. The type of SPE packing material, solvents, pH and the flow rates need to be correctly selected in order to retain analytes effectively within the cartridge or column. The interfering substances should be retained very strongly or not retained at all. The sorbents used in solid phase extraction may be specific or non-specific towards the analyte(s). The specific sorbents reported for human bio analysis of BPA, 4-t-OP and 4-NP were immunoaffinity columns (Schöringhumer and Cichna-Markl, 2007) and molecularly imprinted polymers (MIPs) (Alexiadou et al., 2008; Tan et al., 2009). The term non-specific sorbent concerns mostly silica based sorbents that are modified with hydrophilic or/and hydrophobic chemical moieties. In this category, a number of common SPE cartridges are available, such as OASIS HLB and Varian Bond-Elut C18 (Table 1). Recently, a new, rapid extraction method was developed for the simultaneous determination of BPA, 4-NP, and 4-t-OP in human blood serum by hybrid solid phase extraction—protein precipitation technique (Hybrid SPE-PPT), a new sample pretreatment technique that is considered a breakthrough in the field of solid phase extraction and generally speaking in bioanalysis (Asimakopoulos and Thomaidis, 2010; Lignos et al., 2010).

A number of other extraction techniques have been also reported, including automated solid phase extraction, on-line solid phase extraction, stir bar sorptive extraction (SBSE) and solid phase microextraction (SPME) (Table 1). These techniques present various advantages, like less contamination between samples, higher sample throughput, minimized consumption of solvents and less labour work. Ultimately, the choice of a certain sample preparation technique, as witnessed in the following section (Section 3.2), is often totally correlated to the instrumental analysis applied and the required speed and accuracy.

3.2. Instrumental analysis

So far, BPA, 4-t-OP and 4-NP were determined individually or simultaneously with other xenobiotic compounds by gas chromatography (GC) coupled with mass spectrometric detection (MS or MS/MS) and by liquid chromatography (LC) coupled with mass spectrometric (MS or MS/MS), electrochemical (ECD), fluorescence (FLD) and ultra-violet (UV) detection. Enzyme-linked immunosorbent assays (ELISAs) and a radioimmunoassay (RIA), were reported only for the simple and rapid determination of BPA in biological samples (Table 1). To the best of our knowledge, ELISA determination of 4-t-OP and 4-NP (simultaneously with their ethoxylates) was only applied with success in environmental matrices (Goda et al., 2004; Mart'ianov et al., 2005). In Table 1, a selection of methods is presented, mainly based on detailed reference to analytical figures of merit, highlighting their performance characteristics (sample preparation and instrumental parameters). More than 50 methods are published for the bioanalysis of BPA in human population. On the contrary, published methods for biomonitoring 4-t-OP and 4-NP in humans are by far fewer.

3.2.1. Gas chromatography coupled with mass spectrometric detection

Determination by GC–MS is the most commonly applied analytical technique due to its simplicity and robustness. However, a derivatization step is usually needed (Table 1) due to insufficient volatility of the three APs. Derivatization reagents such as pentafluorobenzyl bromide (PFBBr) (Brock et al., 2001; Kuklenyik et al., 2003; Yoshimura et al., 2002) and bis-trimethylsilyl trifluoroacetamide (BSTFA) (Arakawa et al., 2004; Kawaguchi et al., 2007; Otaka et al., 2003; Tan and Mohd, 2003; Yang et al., 2006a,b) were extensively used for all target APs. N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide (MtBSTFA) (Moors et al., 2007) and acetic acid anhydride (Kawaguchi et al., 2004a, 2008) reagents were reported for the derivatization of BPA only. In the absence of the derivatization step, sensitivity was low, rendering trace analysis in biological media almost impossible. This fact was clearly demonstrated through the comparison of the sensitivity of BPA, 4-t-OP and 4-NP obtained in urine (in a concentration of 10 ng/mL) with and without derivatization (Kawaguchi et al., 2005).

Despite the important advantage (higher sensitivity) offered by this step, two major drawbacks put into question the use of GC–MS technique in biomonitoring of a large number of subjects. Not only the derivatization step augments the risk of contamination of the analyzed samples from the environmental background but it is also time consuming. The contamination risk can be dealt with the development of method protocols that make use of in situ (Kawaguchi et al., 2004a, 2006, 2007, 2008), SPE on-column (Kuklenyik et al., 2003) or headspace derivatization (Yang et al., 2006a,b). On the other hand, the time consuming protocol issue cannot be overcome but at least time can be perceptibly decreased by using automated protocols as in Calafat et al. (2005). Few gas chromatographic methods were reported without a derivatization step; and even though acceptable limits of quantification were presented, laborious sample preparation protocol was needed in order to reduce matrix interferences (Table 1).

A variety of internal standards were reported to "correct" recoveries from extraction or/and signal fluctuations during instrumental analysis. The ideal internal standard should present the same physicochemical properties as the target analyte, rendering the ^{2}D- or ^{13}C-labeled compound the best choice. $^{13}C_{12}$-BPA (Brock et al., 2001; Kawaguchi et al., 2004a, 2005, 2008; Kuklenyik et al., 2003; Moors et al., 2007; Tsukioka et al., 2003; Yoshimura et al., 2002) and $^{2}D_{16}$-BPA (Arakawa et al., 2004; Atkinson et al., 2002) were the most popular internal standards reported for BPA. Zafra et al. (2002) determined BPA in saliva using a less common surrogate, bisphenol-F (BPF). In a multi-residue method that determined APs (and pesticides) in cord blood, $^{2}D_{10}$-pyrene internal standard was used for BPA and $^{2}D_{10}$-phenanthrene internal standard was used for 4-t-OP and 4-NP (Tan and Mohd, 2003). Kawaguchi et al. (2004b) reported a mixture in which the hydrogen of 4-t-OP was replaced with (1 to 12) deuterium ($^{2}D_{(1-12)}$-4-t-OP) and was

Table 1
Biomonitoring methods for bisphenol A, 4-t-octylphenol and 4-nonylphenol.

Analytical technique(s)	Determined analytes	Biological media	Sample pretreatment technique(s)/column(s) – syringe(s) – SPE cartridge(s)/solvents	Column(s)/mobile phase-electrolyte(s)	Analytical parameters	Ref.
ELISA	BPA	Blood serum	SPE/Oasis HLB/MeOH:ACN (3:1, v/v) (elution)	-/-	$RSD\%_{inter\text{-}day}$: 9.7%	Hiroi et al. (2004)
ELISA	BPA	Blood serum	-/-/-	-/-	Linear range: 0.5–5.000 ng/mL	Kodaira et al. (2000)
ELISA	BPA	Colostrum	LLE, SPE/Oasis HLB (30 mg, 1 mL)/ACN (LLE), MeOH:ACN (3:1, v/v) (elution, SPE)	-/-	LOD: 0.3 ng/mL *R*%: 103% (RSD%: 19%) Linear range: 1.56–100 ng/mL	Kuruto-Niwa et al. (2007)
RIA	BPA	Plasma, ovarian follicular and seminal fluids	LLE/-/NH_4OH and ETAC	-/-	LOD: 0.08 ng/mL *R*%: 96% (RSD: 4%) $RSD\%_{inter\text{-}day}$: < 8.6% Range of detection: 0.08–5 ng/mL	Kaddar et al. (2009)
LC-FLD	4-t-OP, 4-NP and one other AP	Plasma and urine	Enzymatic deconjugation, SPE/Varian PH SPE cartridge/MeOH (elution)	Luna C_{18}-A (150 mm × 4.6 mm, 5 μm)/ACN–H_2O (75:25, v/v)	$LOD_{4\text{-}NP,plasma}$: 0.36 ng/mL $LOD_{4\text{-}NP,urine}$: 1.60 ng/mL $LOD_{4\text{-}t\text{-}OP,plasma}$: 0.48 ng/mL $LOD_{4\text{-}t\text{-}OP,urine}$: 1.16 ng/mL $R\%_{4\text{-}t\text{-}OP,plasma}$: 108% $R\%_{4\text{-}t\text{-}OP,urine}$: 83.6% $R\%_{4\text{-}NP,plasma}$: 115% $R\%_{4\text{-}NP,urine}$: 109% RSD%: <10% Linear range: 5–385 ng/mL $R^2 \geq 0.9995$	Chen et al. (2005)
LC-FLD (derivatization with 4-(4′,5′-diphenyl-1′H-imidazol-2′-yl)benzoyl chloride reagent)	BPA	Blood serum and ascitic fluid	LLE/-/Chloroform	Precolumn, Wakosil-II 5C18 (1.0 mm × 150 mm, Wako) and Protein & Peptide C18 (4.6 mm × 150 mm, Vydac)/ACN–H_2O–MeOH–0.1 M acetate buffer	LOD: 0.04 ng/mL $R\%_{BLOOD\ SERUM}$: 78.6% $R\%_{ASCITIC\ FLUID}$: 77.7% RSD_{METHOD}: 8% Linear range: 0.1–7.0 ng/mL $R^2 \geq 0.998$	Kuroda et al. (2003)
LC-ECD	4-NP and 4-t-OP	Plasma	SPE/Varian Bond-Elut C18, C2 and Phenyl-base (PH) (500 mg/3 mL)/MeOH (elution)	Capcell-Pak UG 120 C_{18} (4.6 mm ×150 mm)/Phosphoric acid in H_2O–ACN (40:60, v/v)	$LOD_{4\text{-}NP}$: 1.0 ng/mL $LOD_{4\text{-}OP}$: 0.5 ng/mL *R*%* > 70% (*exception when C2 phase used) RSD% (of *R*%) < 15.5 $RSD\%_{4\text{-}NP}$: 1.79% $RSD\%_{4\text{-}t\text{-}OP}$: 1.86%	Inoue et al. (2000)
LC-ECD	BPA	Urine	Enzymatic deconjugation, LLE/-/DE	YMC basic (150 mm × 4.6 mm, 5 μm) and MCM (250 mm × 4.6 mm, 3 μm)/50 mM sodium acetate buffer (pH = 4.8):ACN (69:31, v/v)	LOD: 0.2 ng/mL *R*%: 96–103% $RSD\%_{METHOD}$: 3% Linear range: 50 pg to 50 ng R^2: 0.9998	Ouchi and Watanabe (2002)

Table 1 (*Continued*)

Analytical technique(s)	Determined analytes	Biological media	Sample pretreatment technique(s)/column(s) – syringe(s) – SPE cartridge(s)/solvents	Column(s)/mobile phase-electrolyte(s)	Analytical parameters	Ref.
LC–ESI(-)MS	BPA and BADGE-4OH(BPA diglicidyl ether metabolite)	Blood serum and plasma	Enzymatic deconjugation, SPE/Oasis HLB (20 mm × 2.1 mm)/H_2O–ACN (elution)	CAPCELL PAK MG C_{18} (2.0 mm × 250 mm) /0.01% acetic acid in H_2O–ACN	LOD_{BPA} : 0.1 ng/mL $LOD_{BADGE-4OH}$: 0.5 ng/mL $R\%_{BPA}$: 74.5% (±3.5% (SD)) $R\%_{BADGE-4OH}$: 97.4% (±4.6% (SD)) $RSD\%_{BPA}$: 0.30% $RSD\%_{BADGE-4OH}$: 0.77% Linear range: 1.0–100 ng/mL R^2: 0.999	Inoue et al. (2001)
LC–ESI(-)MS	BPA	Semen	SPE/Shodex SPEC EDS-1/MeOH (elution)	Senshu Pak PEGASIL ODS (2.0 mm × 150 mm, 3 μm)/0.01% Acetic acid in H_2O–ACN	$LOQ_{BPA,LC/MS}$: 0.5 ng/mL $LOQ_{BPA,ELISA}$: 2 ng/mL $R\%$: 100% RSD%: 5% (at 1.0 ng/mL) $R^2 \geq 0.999$	Inoue et al. (2002)
LC–ESI(-)MS	4-NP and 4-t-OP	Urine	Enzymatic deconjugation, on-line extraction/TSK-Precolumn BSA-ODS/S (10 mm × 4.6 mm)/-	Mightysil RP-18 GP pre-column (2.0 mm × 5 mm, 5 μm), Mightysil RP-18 (2.0 mm × 100 mm, 5 μm)/0.1 mM ammonium acetate in H_2O–ACN	LOD_{4-t-OP}: 0.05 ng/mL LOD_{4-NP}: 0.1 ng/mL LOQ_{4-t-OP}: 0.3 ng/mL LOQ_{4-NP}: 0.3 ng/mL Linear range: 0.2–100 ng/mL $R^2 \geq 0.999$	Inoue et al. (2003a)
LC–ESI(-)MS	BPA	Urine	Enzymatic deconjugation, size-exclusion flow extraction/TSK-Precolumn BSA-ODS/S (10 mm × 4.6 mm, 5 μm), CLNpak PAE-800 (300 mm × 8.0 mm)/-	Mightysil RP-18 GP pre-column (20 mm × 2.0 mm, 5 μm), Mightysil RP-18 GP Aqua (150 mm × 2.0 mm, 5 μm)/0.01% acetic acid in H_2O:ACN (65:35, v/v)/-	LOQ_{BPA}: 0.1 ng/mL $R\%$ (at 1.0 ng/mL): 107.0% (RSD: 7.4%) $R\%$ (at 5.0 ng/mL): 98.2% (RSD: 4.1%) Linear range: 0.45–90 ng/mL $R^2 \geq 0.999$	Inoue et al. (2003b)
LC–APCI (-)MS/MS	BPA, 4-t-OP, and seven other xenobiotic compounds	Urine	Enzymatic deconjugation, isotope-dilution on-line SPE/LiChrosphere RP-18 ADS (25 mm × 4.0 mm, 25 μm)/MeOH–H_2O	Two Chromolith Performance RP-18 (100 mm × 4.6 mm) in tandem/MeOH–H_2O	LOD_{BPA}: 0.4 ng/mL LOD_{4-t-OP}: 0.2 ng/mL $R\%_{BPA}$: 98–113% $R\%_{4-t-OP}$: 99–125% $RSD\%_{BPA,METHOD}$: 8–17% $RSD\%_{4-t-OP,METHOD}$: 13–24%	Ye et al. (2005)
LC–APCI (-)MS/MS	BPA, 4-t-OP, and seven other xenobiotic compounds	Breast milk	Enzymatic deconjugation, LLE, on-line SPE/LiChrosphere RP-18 ADS (25 mm × 4.0 mm, 25 μm)/IPA(LLE)	Two Chromolith Performance RP-18 (100 mm × 4.6 mm) in tandem/MeOH–H_2O	LOD_{BPA}: 0.28 ng/mL LOD_{4-t-OP}: 2.55 ng/mL $R\%_{BPA}$: 93.7% $R\%_{4-t-OP}$: 56% $RSD\%_{4-t-OP,METHOD}$: 18.9% (QC LOW) $RSD\%_{BPA,METHOD}$: 4.8% (QC LOW) Linear range: 0.1–100 ng/mL	Ye et al. (2006)

LC–APCI (-)MS/MS	BPA and chlorinated derivatives	Placental tissue	ETAC (LLE)	Gemini C18 (100 mm × 2 mm, 3 μm)/0.1% (v/v) NH_3 aqueous solution–0.1% (v/v) NH_3 in methanol	LOD_{BPA}: 0.2 ng/g LOQ_{BPA}: 0.5 ng/g *R*%: 97–105% RSD% < 8.1% Linear range: 0.5–50.0 ng/g R^2 = 0.9998	Jiménez-Díaz et al. (2010)
LC–ESI (-)MS/MS	BPA, 4-t-OP and 4-NP	Blood serum	Hybrid SPE-PPT technique/Hybrid SPE cartridge/MeOH (precipitating agent)	Ascentis C18 (75 mm × 2.1 mm, 2.7 μm)/MeOH–H_2O	LOD_{BPA}: 0.80 ng/mL $LOD_{4\text{-t-OP}}$: 1.26 ng/mL $LOD_{4\text{-NP}}$: 1.39 ng/mL LOQ_{BPA}: 2.70 ng/mL $LOQ_{4\text{-t-OP}}$: 4.20 ng/mL $LOQ_{4\text{-NP}}$: 4.65 ng/mL $R\%_{BPA}$: 106% $R\%_{4\text{-t-OP}}$:111% $R\%_{4\text{-NP}}$: 97% $RSD\%_{BPA}$: 12.8% $RSD\%_{4\text{-t-OP}}$: 8.3% $RSD\%_{4\text{-NP}}$: 7.1% Linear $range_{BPA}$: 1–100 ng/mL Linear $range_{4\text{-t-OP}}$: 1–100 ng/mL Linear $range_{4\text{-NP}}$: 2–400 ng/mL R^2: 0.991–0.998	Asimakopoulos (2010)
GC–MS LC-ECD LC-UV	BPA and two other compounds	Saliva	LLE/-/H_2O, N-hexane, ETAC, ACN	Zorbax SB phenyl column (7.5 mm × 4.6 mm, 3 μm) (LC), Metaguard Inertsil phenyl column (4.3 mm, 5 μm) (LC) and Capillary column (30 m × 0.25 mm, 0.25 μm) (GC)	LOQ: 1.58 ng/mL (±0.84 (SD)) (GC–MS)	Atkinson et al. (2002)
GC–CI(-)MS (derivatization with pentafluoro benzyl bromide reagent) compared with GC–HRMS	BPA	Urine	Enzymatic deconjugation, SPE/octyldecyl SPE (500 mg)/MeOH (elution)	Capillary column DB-5 (30 m × 0.25 mm, 0.25 μm)	$LOD_{GC\text{–}CI(-)MS}$: 0.12 ng/mL $LOD_{GC\text{–}HRMS}$: 0.11 ng/mL $R\%_{BPA}$: 102% RSD%: 6% Linear range: 0.26–10.6 ng/mL	Brock et al. (2001)

Table 1 (*Continued*)

Analytical technique(s)	Determined analytes	Biological media	Sample pretreatment technique(s)/column(s) – syringe(s) – SPE cartridge(s)/solvents	Column(s)/mobile phase-electrolyte(s)	Analytical parameters	Ref.
GC–EI(-)MS (derivatization in situ with acetic acid anhydride reagent)	BPA	Urine	Enzymatic deconjugation, miniaturized hollow fiber assisted liquid-phase microextraction (HF-LPME)/10 μL microsyringe (SGE 10F-HP-0.63), with a needle of 42 mm length (0.63 mm O.D.)/Toluene	DB-5MS fused silica column (30 m × 0.25 mm, 0.25 μm film thickness)	LOD_{BPA}: 0.02 ng/mL LOQ_{BPA}: 0.1 ng/mL $R\%_{BPA}$ (at 1 ng/mL): 101% (RSD: 6.7%) $R\%_{BPA}$ (at 5 ng/mL): 98.8% (RSD: 1.8%) Linear range: 0.1–50 ng/mL $R^2 > 0.999$	Kawaguchi et al. (2008)
Thermal desorption (TD)-GC–EI(-)MS (derivatization in situ with acetic acid anhydride reagent)	BPA	Urine, plasma and saliva	Enzymatic deconjugation, Stir bar (coated with polydimethylsiloxane) sorptive extraction (SBSE)/-/-	DB-5MS fused silica column (30 m × 0.25 mm, 0.25 μm film thickness)	LOD_{URINE}: 20 pg/mL LOD_{PLASMA}: 100 pg/mL LOD_{SALIVA}: 20 pg/mL LOQ_{URINE}: 100 pg/mL LOQ_{PLASMA}: 500 pg/mL LOQ_{SALIVA}: 100 pg/mL $R\%$: 95% (RSD% <10%) Linear range: 0.02–10 ng/mL $R^2 > 0.99$	Kawaguchi et al. (2004a,b)
Isotope dilution GC–CI(-)MS (derivatization with pentafluorobenzyl bromide reagent)	BPA, 4-NP, 4-n-NP, 4-t-OP and other three APs	Urine	Enzymatic deconjugation, automated-SPE/styrene–divinylbenzene copolymer-based cartridge/ACN and then ETAC (elution)	DB-5MS column (30 m × 0.25 mm , 0.25 μm film thickness)	LOD_{BPA}: 0.1 ng/mL $LOD_{4\text{-}NP}$: 5 ng/mL $LOD_{4\text{-}n\text{-}NP}$: 0.1 ng/mL $LOD_{4\text{-}t\text{-}OP}$: 0.7 ng/mL $R\%_{BPA}$: 95–116% $R\%_{4\text{-}NP}$: 91–109% $R\%_{4\text{-}n\text{-}NP}$: 99–105% $R\%_{4\text{-}t\text{-}OP}$: 94–109% $RSD\%_{4\text{-}t\text{-}OP,SLOPE}$: 15% $CV\%_{BPA,SLOPE}$: 6% $CV\%_{4\text{-}NP,SLOPE}$: 10% $CV\%_{4\text{-}n\text{-}NP,SLOPE}$: 15% $RSD\%_{4\text{-}t\text{-}OP,METHOD}$: 16% (QC LOW) $RSD\%_{BPA,METHOD}$: 7% (QC LOW) $RSD\%_{4\text{-}n\text{-}NP,METHOD}$: 5% (QC LOW) Linear $range_{4\text{-}NP}$: 1–200 ng/mL	Kuklenyik et al. (2003)

MD-GC–EI(-)MS (derivatization with acetic acid anhydride reagent)	BPA and BPB	Urine	Enzymatic deconjugation, dispersive liquid–liquid microextraction (DLLME)/-/ACN, T4CE	DB-5HT (5 m × 0.32 mm × 0.10 μm) as primary column, and DB-5MS (20 m × 0.18 mm × 0.18 μm) as secondary column	LOD_{BPA}: 0.03 μg/L LOD_{BPB}: 0.05 μg/L LOQ_{BPA}: 0.1 μg/L % DLLME yield for BPA: 68% % DLLME yield for BPB: 56% $RSD\%_{METHOD}$ < 20% Linear range: 0.1–5 μg/L $R^2 \geq 0.996$	Cunha and Fernandes (2010)
GC–MS	4-t-OP, 4-NP	Breast milk	LLE, SPE and further cleanup/HLB SPE cartridge (3 mL, 60 mg, 810 $m^2\ g^{-1}$ surf. area), alumina and anhydrous sodium sulfate column (10 cm × 0.5 cm I.D.) for cleanup/n-hexane (LLE), MeOH (elution) (SPE)	DB-5MS capillary column (15 m × 0.25 mm × 0.1 μm)/-/-	$LOQ_{4\text{-t-OP}}$: 0.2 ng/g $LOQ_{4\text{-NP}}$: 1.2 ng/g $R\%_{4\text{-t-OP}}$:81% $R\%_{4\text{-NP}}$: 89% RSD%: <10% $R^2 > 0.997$	Lin et al. (2009)

Abbreviations: LLE, liquid–liquid extraction; SPE, solid phase extraction; *R*%, recovery%; RSD, relative standard deviation; SD, standard deviation; LOD, limit of detection; LOQ, limit of quantification; CV, coefficient of variation; ACN, acetonitrile; MeOH, methanol; H_2O, water; T4CE, tetrachloroethylene; DE, diethylether; IPA, isopropanol; NH_4OH, ammonium hydroxide; ETAC, ethyl acetate; NH_3, ammonia; QC, quality control; I.D., internal diameter; O.D., outer diameter.

used as internal standard for 4-t-OP. In the same article, $^2D_5$-4-(1-methyl)octylphenol ($^2D_5$-m-OP) was used as internal standard for 4-NP. Kuklenyik et al. (2003) made use of $^2D_{17}$-4-n-OP surrogate for 4-t-OP and $^{13}C_6$-4-n-NP surrogate for 4-n-NP and 4-NP. Finally, Lin et al. (2009) stated that cumylphenol can be used as internal standard for both 4-t-OP and 4-NP.

Otaka et al. (2003) reported a laborious sample preparation protocol for the determination of BPA and 4-NP in human milk. The sample preparation involved an alkaline digestion step for degradation of proteins and lipids, followed by LLE, twice cleaning with SPE and finally, derivatization prior analysis. Kawaguchi et al. (2007) reported the determination of 4-NP-glucuronide and 4-t-OP-glucuronide in urine using SBSE with an in situ enzymatic deconjugation and in-tube silylation. Cunha and Fernandes (2010) reported a method with an extremely low LOD (0.03 μg/L) in which dispersive liquid–liquid microextraction (DLLME), in situ derivatization and multidimensional GC–MS was used for the determination of free and total bisphenol A (BPA) in urine.

In contrast to the development of GC–MS methods, the application of GC–MS/MS was extremely limited, counting to one reported method for the determination of BPA in urine (Arakawa et al., 2004). The detection limit (LOD) of this method was 0.38 ng/mL and even though it presented generally slightly higher detection limit than some GC–MS methods (Table 1), higher selectivity was definitely obtained.

3.2.2. *Liquid chromatography coupled with electrochemical, ultra-violet and fluorescence detection*

LC methods usually include a simpler sample preparation protocol than GC methods since derivatization is not essential. Individual cases of derivatization are only met in LC-FLD methods that implicate fluorophore reagents (Table 1).

Coupling an ECD detector to LC, great sensitivity and selectivity could be achieved for electroactive compounds, such as APs. Best ECD detector operation demands isocratic elution. Fine tuning of the detector's potential and a constant mobile phase composition was the key for achieving low LODs (Inoue et al., 2000; Sajiki, 2001, 2003). A low LOD was reported in urine for BPA (0.2 ng/mL) clearly demonstrating the potential of the detector (Ouchi and Watanabe, 2002). Nevertheless, isocratic elution leads to reduced separation efficiency. Thus, when a number of compounds needed to be determined simultaneously, injection run lasts longer in order to achieve adequate separation. Liu et al. (2005) reported a method in which a low steepness gradient was applied and LC run duration of 45 min was maintained with the aim to determine, apart from BPA, three phytoestrogens.

Liu et al. (2005) also referred to an attempt to determine BPA in urine with UV detection at 260 nm (using flavone as internal standard). This attempt was not successful due to high LOD. Endogenous matrix components interfered in LC-UV bioanalysis and selectivity was not adequate to trace analysis expectations. Thus, LC-UV is obsolete for this purpose and only two articles were reported for the determination of BPA in saliva (Olea et al., 1996; Schmalz et al., 1999).

Sensitive biomonitoring of BPA, 4-t-OP and 4-NP concentrations could be achieved by LC-FLD. Using this technique, Kuroda et al. (2003) reported a very low LOD for BPA (Table 1). Fluorescence of the three compounds was monitored at an excitation of 275 nm and an emission of 300 nm (Chen et al., 2005; Yang et al., 2003, 2006). Fluorophore derivatization, even though was considered time consuming, it was necessary in order to achieve high sensitivity and selectivity in difficult biological matrices. In literature, 4-(4,5-diphenyl-1H-imidazol-2-yl)benzoyl chloride (DIB-Cl) fluorophore reagent was reported for the determination of BPA in human blood serum, ascitic fluid and breast milk (Kuroda

et al., 2003; Sun et al., 2004); p-nitrobenzoyl chloride fluorophore reagent for the simultaneous determination of BPA and 4-NP in urine and serum (Mao et al., 2004, 2005); 2-(4-carboxyphenyl)-5,6-dimethylbenzimidazole (CDB) fluorophore reagent for the simultaneous determination of BPA and 4-NP in semen (Katayama et al., 2003).

Dekant and Völkel (2008) perfectly identified and noted an innovative HPLC-FLD method, in which an immunoaffinity column was used to extract BPA from urine and a column containing glucuronidase and arylsulfatase was used in order to determine total concentration of BPA (free plus conjugated species), achieving a method LOD of 0.2 ng/mL (Schöringhumer and Cichna-Markl, 2007). Finally, it should be reported that Katayama et al. (2003) determined BPA and 4-NP in human semen, not only by LC-FLD, but also with miceller electrokinetic chromatography (after proteinase K digestion), however, the achieved LODs were extremely high for biomonitoring purposes.

3.2.3. Liquid chromatography coupled with mass spectrometric detection

In contrast to GC, tandem mass spectrometry is predominantly used hyphenated to LC, with the majority of tandem mass instruments applied for bioanalysis of BPA, 4-t-OP and 4-NP in humans being triple quadrupole instruments. LC–MS or LC–MS/MS techniques combine superiority over sensitivity, selectivity and precision even in low concentration samples without the need of derivatization, establishing this technique as the best choice for biomonitoring APs. Still there are important issues to be solved, even in this powerful quantitative analytical technique. Making use of electrospray ionization interface (ESI), response is often greatly affected from co-eluting matrix components (matrix effect) decreasing (suppression) or increasing (enhancement) ionization efficiency and having a negative impact on signal reproducibility. On the contrary, using atmospheric pressure chemical ionization (APCI) interface, lower matrix effect is encountered, but limits of detection may be higher than ESI interface (Table 1). Nevertheless, it was clearly demonstrated that APCI was used extensively, especially for BPA bioanalysis (Jiménez-Díaz et al., 2010; Völkel et al., 2008; Ye et al., 2005, 2006). Turbo ion spray was reported as an optional electrospray ionization technique for simultaneous determination of BPA and BPA–glucuronide in plasma and urine (Völkel et al., 2005) and singularly determination of BPA (total and free concentration) in urine (Völkel et al., 2011). Turbo ion spray ionization works at higher flow rates using a heated probe that channels heated gas at the sample steam. Therefore high sensitivity was achieved by increasing the rate of droplet evaporation and desolvation. Besides the above mentioned interfaces, an approach was reported using atmospheric pressure photoionization (APPI) for the simultaneous determination of BPA (total and free) and twelve other xenobiotic compounds in human milk (Ye et al., 2008). In this publication, the sample was treated with on-line solid phase extraction reaching a low LOD of 0.1 ng/mL. The lowest recovery (56%) and higher LOD (2.55 ng/mL) was reported for 4-t-OP, when it was determined simultaneously with BPA and seven other compounds in human breast milk (Ye et al., 2006).

Internal standardization method is more common in LC–MS methods than in GC–MS ones, mainly due to the need to adequately compensated for the severe matrix effects. The appropriate choice of internal standard is of paramount importance for obtaining accurate results by LC–MS(MS) methods. As in gas chromatography (Section 3.2.1), the most common surrogates reported for BPA were $^{13}C_{12}$-BPA (Ye et al., 2005, 2006, 2008) and $^{2}D_{16}$-BPA (Jiménez-Díaz et al., 2010; Völkel et al., 2005, 2011). $^{13}C_{12}$-BPA is preferred, since ^{1}H and ^{2}D have higher differences in their physical properties than ^{12}C and ^{13}C (Van Eeckhaut et al., 2009), however, it is more expensive. The internal standards usually reported for 4-t-OP are $^{2}D_4$-4-t-OP (Ye et al., 2006) and $^{2}D_5$-4-(1-methyl) octylphenol ($^{2}D_5$-m-OP) (Inoue et al., 2003a). Inoue et al. (2003a) reported testing three labeled surrogates: $^{2}D_4$-4-t-OP and $^{2}D_5$-m-OP as surrogates for both 4-t-OP and 4-NP and $^{2}D_4$-4-n-NP as surrogate for 4-NP only. $^{2}D_4$-4-n-NP was found unsuitable and between $^{2}D_4$-4-t-OP and $^{2}D_5$-m-OP, the latter internal standard was found more appropriate.

Another important factor for obtaining suitable sensitivity in LC–MS or LC–MS/MS techniques is the appropriate choice of mobile phase composition. In literature, all methods reported use of methanol or acetonitrile in a binary mobile phase mixture with water (Table 1). Yet, from the two organic solvents, methanol solvent is more appropriate. From the authors' experience (Asimakopoulos, 2010), when using negative ionization mode, which is the case for APs determination, production of BPA, 4-t-OP and 4-NP anions are favored in the presence of methanol, augmenting signal intensity. A number of additives, such as ammonium acetate may be also used in the mobile phase composition (Table 1).

When enzyme deconjugation was used for assessing total concentrations (free plus conjugate species), the extent of the deconjugation reaction could be monitored in real-time during analysis with a deconjugation standard. The most common standard was a mix of $^{13}C_4$-4-methylumbelliferone, 4-methylumbelliferylsulfate, and 4-methylumbelliferyl glucuronide that were added to all samples. Monitoring the peak area ratio of 4-methylumbelliferone/$^{13}C_4$-4-methylumbelliferone, the success of deconjugation was evaluated for each analyzed sample (Ye et al., 2005). Direct monitoring of the biomarker BPA–monoglucuronide (m/z 403 → m/z 113) and its internal standard $^{2}D_{16}$-BPA–monoglucuronide (m/z 417 → m/z 113) was also being reported for the evaluation of the success of deconjugation (Völkel et al., 2005, 2008).

3.2.4. Immunoassays

The most important drawback of the ELISA determination of BPA is the reduced accuracy in low analyte concentrations, due to cross-reactivity with structurally related compounds (e.g. endogenous hormones) leading consequently to overestimation of BPA concentration (Fukata et al., 2006). Kodaira et al. (2000) compared BPA concentration of glucuronidase-treated urine obtained with HPLC-FLD technique with BPA concentration of the same but untreated urine sample obtained with ELISA technique. Dekant and Völkel (2008) noted that the good linear correlation between the two techniques (Kodaira et al., 2000) surely indicated the existence of cross reactivity of the antibodies that are intended for BPA, with BPA–glucuronide. Therefore, cross-reactivity made designation of the signal to bisphenol A difficult and the method was characterized unsuitable for exposure monitoring (Dekant and Völkel, 2008).

A radioimmunoassay was reported as a suitable and stable tool for the determination of BPA (Table 1). Kaddar et al. (2009) reported that the specificity of this single RIA was evaluated by checking cross-reactivity with a wide variety of other structurally similar compounds (e.g. 4-t-OP and 4-NP) and validated by obtaining good correlations with LC–MS (92%) and LC-FLD (80%) techniques.

3.3. Expected developments

Nowadays, the number of published LC–MS/MS methods, is constantly increasing simultaneously with the development of different approaches aiming to speed up LC (e.g. use of core–shell columns) (Carr et al., 2011). Thus, considerable improvements in analytical methodology of LC–MS/MS bioanalysis of BPA, 4-t-OP and 4-NP are expected, especially in terms of sensitivity.

As far as LC–MS and LC–MS/MS techniques are concerned, the authors' view is that when trace analysis is sought after, the

ionization parameters should always be selected by compromising the optimal parameters that are generated for each compound from a performed factorial experimental design. Recently, a systematic and detailed strategy was developed for the optimization and development of ESI and APCI MS and MS/MS methods (Maragou et al., 2008b, 2011) and the application of this strategy to the determination of BPA, 4-NP and 4-t-OP resulted in significant improvement in sensitivity (Asimakopoulos, 2010). Only after thorough optimization, appropriate values of the ionization parameters are obtained, and higher sensitivity, lower LODs and more stabilized signal intensities are reached, especially for difficult to ionize analytes, such as the discussed APs. This belief is being consistent to theory of interface operation (Kebarle and Tang, 1993; Sunner et al., 1988). So far, by studying all published methods concerning LC coupled to MS and MS/MS detection of BPA, 4-t-OP and 4-NP, there was not any published article on this aspect.

A new generation of SPE cartridges is presented through Hybrid-SPE technology, introducing a breakthrough in rapidness of biological sample extraction and clean-up. The Hybrid SPE cartridge is consisted of a zirconium packed-bed/low porosity filter-0.2 hydrophobic frit assembly that shortens three processes: removal of phospholipids, removal of proteins, and filtration of final eluant, in one. When a minimum quantity of 100–300 μL of the biological sample passes through the Hybrid SPE cartridge, phospholipids are retained specifically to the sorbent (that possesses zirconium functional groups) and proteins non-specifically to the low porosity filter. Thus, gross amounts of endogenous protein and phospholipid interferences are reduced simultaneously and target analytes are collected in the eluant (Pucci et al., 2009). As already mentioned, a hybrid SPE-PPT extraction method was developed and optimized for the simultaneous determination of BPA, 4-NP, and 4-t-OP in human blood serum, simplifying significantly the sample preparation step (Asimakopoulos and Thomaidis, 2010; Lignos et al., 2010). To the authors' best knowledge; there are no other reports on this topic for the trace bioanalysis of BPA, 4-t-OP and 4-NP.

4. Exposure assessment—epidemiology studies in humans

4.1. Bisphenol A

Since 2007, a number of very extensive reviews have been published for human exposure assessment and epidemiology studies on BPA (Vandenberg et al., 2007; Dekant and Völkel, 2008). Diamanti-Kandarakis et al. (2009) stated that clinical observations and epidemiological studies showed correlation between BPA and effects on human reproduction system, breast and prostate cancer, thyroid, and metabolic syndromes (e.g. obesity). Vandenberg et al. (2010a) cited more than 80 studies on BPA that were realized in the last decade. Thus, herein the most important studies are reported and discussed since 2009 and onwards.

In the U.S., the National Health and Nutrition Examination Survey (NHANES) performed a study with 1455 and 1493 subjects (ranging between 18 and 74 years of age), for the time periods of 2003–2004 and 2005–2006, respectively (Melzer et al., 2010). The geometric mean of BPA was found 2.49 ng/mL for the period 2003–2004 and 1.79 ng/mL for the period 2005–2006. The regression models were adjusted to a number of parameters (e.g. age, sex, race/ethnicity and education) and correlation was found between BPA and heart diseases, diabetes and elevation of levels of some serum liver enzymes. Another study performed by NHANES compared urinary BPA with serum cytomegalovirus (CMV) antibody levels, allergies and hay fever in subjects with more than 6 years of age (Clayton et al., 2011). BPA exhibited a negative effect in human immune function as measured by CMV antibody levels.

Galloway et al. (2010) conducted a study that included 715 adults. The subjects' age ranged between 20 and 74 years. The geometric mean urinary BPA concentration was established at 3.59 ng/mL. Higher excretion rates were found among men and were associated with higher total testosterone concentrations. Meeker et al. (2010a) measured urinary BPA, serum thyroid and reproductive hormone levels in 167 men. BPA was detected in 89% of urine samples with a median of 1.3 ng/mL. The results of these studies suggest that urinary BPA concentrations may be associated with altered hormone levels in men. Li et al. (2010) conducted a study in order to examine the effect of occupational exposure to BPA on the risk of male sexual dysfunction. 230 exposed and 404 non-exposed workers participated in the study. The results were adjusted to demographic characteristics, occupational and sexual history, alcohol use, presence of chronic diseases and exposure to other chemicals. Findings provided evidence that exposure to BPA could present adverse effects on male sexual dysfunction. Furthermore, three additional studies associated BPA with decreased sperm quality (Meeker et al., 2010b, 2011; Mendiola et al., 2010).

Mok-Lin et al. (2010) investigated the association between urinary BPA concentrations and ovarian response among women undergoing in vitro fertilization (IVF). Urinary BPA concentrations were measured in 84 women undergoing 112 IVF cycles and in 23 women that contributed more than one IVF cycle. BPA concentrations ranged from <0.4 to 25.5 ng/mL with a geometric mean of 2.52 ng/mL. BPA was detected in the urine of the majority of women undergoing IVF, and was inversely associated with the number of oocytes retrieved. Braun et al. (2011) measured BPA concentrations in urine samples during pregnancy and at birth from 389 women. The geometric mean creatinine-standardized concentrations were found 1.7 μg/g creatinine and 2.0 μg/g creatinine, after 16 and 26 weeks of pregnancy, respectively. At birth BPA concentration was found 2.0 μg/g. Higher concentrations (2.3 μg BPA/g creatinine) were assessed in subjects that consumed canned vegetables at least once a day. BPA concentrations were positively associated with urinary high-molecular-weight phthalates (e.g. DEHP) and serum tobacco smoke metabolite. Cantonwine et al. (2010) realized a study in Mexico City in which BPA concentrations were related with birth prematurity (≤37 weeks). 60 pregnant women participated in this study and BPA was detected in 80% of the urine samples. Total concentrations ranged from <0.4 ng/mL to 6.7 ng/mL and the uncorrected (with creatinine) geometric mean was 1.52 ng/mL.

A study evaluating the urinary concentrations of BPA and other compounds, in association with the use of medical devices was realized by Calafat et al. (2009), involving 42 premature infants. The percentage of BPA, present as its' conjugated species was more than 90% in the majority of the infants. The BPA geometric mean urinary concentration was 30.3 ng/mL. It was concluded that among infants undergoing intensive therapeutic medical interventions, BPA concentration was higher than that among the general population.

4.2. 4-tert-Octylphenol and 4-nonylphenol

In contrast to BPA, articles concerning 4-t-OP and 4-NP exposure assessment and epidemiology studies on human population are limited. Calafat et al. (2008) measured 4-t-OP (and BPA) in urine samples from a population of 2.517 subjects. The subjects were above the age of 6 years. 4-t-OP was detected in 57.4% of the participants with total (free plus conjugated species) concentrations ranging between 0.2 ng/mL and 20.6 ng/mL. The 95th percentile concentration was 2.8 ng/mL after creatinine correction and the median concentration was 0.3 ng/mL. Tan and Mohd (2003) measured 4-t-OP at 180 cord blood samples and 4-t-OP was detected in 31 samples in concentrations from <0.05 to 1.15 ng/mL.

A number of studies determined the technical mixture of 4-OP isomers and not specifically the 4-t-OP isomer. By assessing

the mixture of isomers does not necessary mean that conclusions are reached for 4-t-OP, since pharmacokinetic factors (e.g. absorption, distribution and elimination) may be isomer-specific. Bendsen et al. (2001) concluded that 4-OP exerts a sex-specific effect on male germ cells by culturing human gonads (five testes and five ovaries). Ademollo et al. (2008) studied the presence of 4-NP, and 4-OP in breast milk. 4-NP was found at the highest levels with mean concentrations of 32 ng/mL and 4-OP was found at a mean concentration of 0.08 ng/mL. Chen et al. (2010) determined 4-NP and 4-OP in 59 human milk samples and correlated findings with demographics and dietary factors. This study concluded that food pattern of cooking oil and processed meat products are strongly associated with 4-OP concentration in human milk. Müller et al. (1998) investigated levels of 4-NP and 4-OP in non-occupationally exposed persons by analyzing human autopsy adipose tissue samples. 4-NP concentrations ranged from 19 to 85 ng/g lipids and 4-OP concentrations from 0.58 to 4.07 ng/g lipids. In that study, it was stressed that these values were both in the range of the analytical background contamination. Lopez-Espinosa et al. (2009) also determined 4-NP and 4-OP concentrations in adipose tissue of 20 non-occupationally exposed women living in Southern Spain. 4-NP and 4-OP were detected in 100% and 23.5% of subjects, respectively. The median level of 4-NP was 57 ng/g and that of 4-OP was 4.5 ng/g of adipose tissue. Body mass index was associated with 4-NP levels. Finally, Calafat et al. (2005) measured only the isomer 4-n-NP (and BPA) in urine samples from a population of 394 adults. 4-n-NP was detected in 51% of the samples with a 95th percentile and a median concentration without creatinine correction of 1.57 ng/mL and <0.1 ng/mL, respectively.

4.3. Future trends

In the near future, additional studies should be done under established scientific regulatory criteria with the aim to investigate the toxicological conclusions that are reached so far in humans. The importance of regulatory criteria is stressed because, as Vandenberg et al. (2010b) reports, more than 80 biomonitoring studies on BPA were dismissed from European Food Safety Agency (EFSA) since they documented significant levels of BPA that did not match a model of BPA metabolism.

Furthermore, research is needed in order to determine more biomarkers to assess exposure to all isomers of 4-NP. Recently, in a pioneering work, a research group reported a number of metabolites of 4-NP in human liver microsomes that may eventually lead in the direction of discovering new biomarkers (Deng et al., 2010).

5. Discussion

GC still offers some advantages over LC, primarily higher separation efficiency and lower costs without the problems associated with matrix effects. It is known that a complete chromatographic separation improves detectability and reproducibility and thus lower LODs. GC–MS quantitative analysis provides, in general, better sensitivity and lower limits of detection (LODs) when dealing with complex matrices such as biological media (Table 1).

Comparison of concentration values obtained between different techniques has not been performed extensively. When comparison is realized, scientists tend to use LC–MS/MS as reference to other instrumental technique(s) (Sajiki et al., 1999, 2008). However, the question remains: how reliable is the LC–MS/MS technique? Yi et al. (2010) performed a comparison between LC-FLD and LC–MS/MS determination of BPA. This research group reported that low concentration levels may be overestimated with LC–MS/MS, undermining its reliability and proposing the issue for further consideration.

The best choice from the aforementioned analytical methods (Section 3) is eventually obtained by taking equally into consideration the analytical parameters (method performance) and the cited applications in literature for each method. The application of a method is crucial, since it can reveal carryover and contamination issues that may not be located and assessed during the validation step (Hughes et al., 2007). Therefore, methods that are particularly used in large volume biomonitoring studies prove extensively enough their trouble-free applicability and are considered a favorable choice, as in Kuklenyik et al. (2003) and Ye et al. (2005). In the final decision, the choice of a method depends on the resources of a laboratory (e.g. instrumental resources) and the demands of the analysis (fit for purpose). For instance, if the demand of analysis is the biomonitoring of occupational exposed subjects which they surely exhibit high concentrations of the target analytes in their biological media, then a method with a low LOQ or expensive instrumentation, standards and materials may not be needed.

The analytical performance of a method is often represented mainly by the LOD of the method (Table 1) without even reporting any information about how the LOD was calculated. If this information is not presented there can be up to a 10-fold difference in the LOD, in dependence to the calculations used for its assessment; compromising the validity of the methods' performance. LOQ must be stated for best presentation of method performance. The indication of LOQ is even more important than the LOD itself, since LOQ with adequate precision and accuracy determines perfectly the suitability of the analytical approaches intended for the biomonitoring purposes, e.g. Atkinson et al. (2002) presented the LOQ of the method and omits the LOD. On the other hand, a number of articles presented the linear range of the method, giving indirectly "clues" about the analytical performance of the presented method; e.g. Brock et al. (2001) omitted the LOQ and presented the linear range of the method. Nevertheless, for the reader it is not often obvious if the lowest concentration level of the linear range corresponds either to the LOQ of the method, or either to a higher or lower value than the LOQ. Therefore, it is the author's opinion that for a presented method it is best to report data concerning LOD, LOQ and linear range. For instance, Kawaguchi et al. (2004a,b), Kawaguchi (2008), Völkel et al. (2005) and Cunha and Fernandes (2010) presented their work in a conceivable manner by reporting all needed data.

The need of more strictly quality assurance from sampling to analysis and data interpretation was particularly emphasized by Dekant and Völkel (2008) and Calafat and Needham (2009). In literature, an in-depth study was reported assessing the impact of urine sample storage and shipping conditions on a variety of xenobiotic analytes, BPA included (Hoppin et al., 2006). The author's view is that studies like this should be extended to more biological media and research should be intensified particularly towards 4-t-OP and 4-NP, where data concerning preservation and contamination of biological samples is scarce. All findings should be incorporated to future quality assurance protocols for biomonitoring of BPA, 4-t-OP and 4-NP levels. Finally, the recommendations that were submitted by Calafat and Needham (2009), concerning the control of contamination during sampling and handling of specimens, including guidelines for collection of field blanks should be adopted in future practice.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

References

Ademollo, N., Ferrara, F., Delise, M., Fabietti, F., Funari, E., 2008. Nonylphenol and octylphenol in human breast milk. Environ. Int. 34, 984–987.

Alexiadou, D.K., Maragou, N.C., Thomaidis, N.S., Theodoridis, G.A., Koupparis, M.A., 2008. Molecularly imprinted polymers for bisphenol A for HPLC and SPE from water and milk. J. Sep. Sci. 31, 2272–2282.
Arakawa, C., Fujimaki, K., Yoshinaga, J., Imai, H., Serizawa, S., Shiraishi, H., 2004. Daily urinary excretion of bisphenol A. Environ. Health Prev. Med. 9, 22–26.
Asimakopoulos, A.G., 2010. Determination of bisphenol A, 4-t-octylphenol and 4-nonylphenol in human blood serum by liquid chromatography–electrospray tandem mass spectrometry (LC–MS/MS). M.Sc. Thesis submitted to the Department of Chemistry of the National and Kapodistrian University of Athens.
Asimakopoulos, A.G., Thomaidis, N.S., 2010. Quantitative detection of bisphenol A, 4-t-octylphenol and 4-nonylphenol in human blood serum by liquid chromatography–electrospray tandem mass spectrometry. In: Proceedings of the 7th Aegean Analytical Chemistry Days, pp. 3–28.
Atkinson, J.C., Diamond, F., Eichmiller, F., Selwitz, R., Jones, G., 2002. Stability of bisphenol A, triethylene-glycol dimethacrylate, and bisphenol A dimethacrylate in whole saliva. Dent. Mater. J. 18, 128–135.
Ballesteros-Gomez, A., Rubio, S., Perez-Bendito, D., 2009. Analytical methods for the determination of bisphenol A in food. J. Chromatogr. A 1216, 449–469.
Bendsen, E., Laursen, S.B., Olesen, C., Westergaard, L.G., Andersen, C.Y., Byskov, A.G., 2001. Effects of 4-octylphenol on germ cell number in cultured human fetal gonads. Hum. Reprod. 16, 236–243.
Braun, J.M., Kalkbrenner, A.E., Calafat, A.M., Bernert, J.T., Ye, X., Silva, M.J., Barr, D.B., Sathyanarayana, S., Lanphear, B.P., 2011. Variability and predictors of urinary bisphenol A concentrations during pregnancy. Environ. Health Perspect. 119, 131–137.
Brock, J.W., Yoshimura, Y., Barr, J.R., Maggio, V.L., Graiser, S.R., Nakazawa, H., Needham, L.L., 2001. Measurement of bisphenol A levels in human urine. J. Expo. Anal. Environ. Epidemiol. 11, 323–328.
Calafat, A.M., Kuklenyik, Z., Reidy, J.A., Caudill, S.P., Ekong, J., Needham, L.L., 2005. Urinary concentrations of bisphenol A and 4-nonylphenol in a human reference population. Environ. Health Perspect. 113, 391–395.
Calafat, A.M., Needham, L.L., 2009. What additional factors beyond state-of-the-art analytical methods are needed for optimal generation and interpretation of biomonitoring data? Environ. Health Perspect. 117, 1481–1485.
Calafat, A.M., Weuve, J., Ye, X., Jia, L.T., Hu, H., Ringer, S., Huttner, K., Hauser, R., 2009. Exposure to bisphenol A and other phenols in neonatal intensive care unit premature infants. Environ. Health Perspect. 117, 639–644.
Calafat, A.M., Ye, X., Wong, L.-Y., Reidy, J.A., Needham, L.L., 2008. Exposure of the U.S. population to bisphenol A and 4-tertiary-octylphenol: 2003–2004. Environ. Health Perspect. 116, 39–44.
Cantonwine, D., Meeker, J.D., Hu, H., Sánchez, B.N., Lamadrid-Figueroa, H., Mercado García, A., Fortenberry, G.Z., Calafat, A.M., Téllez-Rojo, M.M., 2010. Bisphenol A exposure in Mexico City and risk of prematurity: a pilot nested case control study. Environ. Health 9, 1–7.
Carr, P.W., Stoll, D.R., Wang, X., 2011. Perspectives on recent advances in the speed of high-performance liquid chromatography. Anal. Chem. 83, 1890–1900.
Center for the evaluation of risks to human reproduction (CERHR), 2008. NTP-CERHR monograph on the potential human reproductive and developmental effects of bisphenol A. NIH Publication No. 08-5994.
Chen, M.L., Lee, W.P., Chung, H.Y., Guo, B.R., Mao, I.F., 2005. Biomonitoring of alkylphenols exposure for textile and housekeeping workers. Int. J. Environ. Anal. Chem. 85, 335–347.
Chen, G.-W., Ding, W.-H., Ku, H.-Y., Chao, H.-R., Chen, H.-Y., Huang, M.-C., Wang, S.-L., 2010. Alkylphenols in human milk and their relations to dietary habits in central Taiwan. Food Chem. Toxicol. 48, 1939–1944.
Clayton, E.M.R., Todd, M., Dowd, J.B., Aiello, A.E., 2011. The impact of bisphenol A and triclosan on immune parameters in the U.S. population, NHANES 2003–2006. Environ. Health Perspect. 119, 390–396.
Cox, C., 1996. Nonylphenol and related chemicals. J. Pest. Reform. 16, 15–20.
Cunha, S.C., Fernandes, J.O., 2010. Quantification of free and total bisphenol A and bisphenol B in human urine by dispersive liquid–liquid microextraction (DLLME) and heart-cutting multidimensional gas chromatography–mass spectrometry (MD–GC/MS). Talanta 83, 117–125.
David, A., Fenet, H., Gomez, E., 2009. Alkylphenols in marine environments: distribution monitoring strategies and detection considerations. Mar. Pollut. Bull. 58, 953–960.
DEFRA, 2008. 4-*tert*-Octylphenol: Risk Reduction Strategy and Analysis of Advantages and Drawbacks. Department for Environment, Food and Rural Affairs, London, UK.
Dekant, W., Völkel, W., 2008. Human exposure to bisphenol A by biomonitoring: methods, results and assessment of environmental exposures. Toxicol. Appl. Pharmacol. 228, 114–134.
Deng, P., Zhong, D., Nan, F., Liu, S., Li, D., Yuan, T., Chen, X., Zheng, J., 2010. Evidence for the bioactivation of 4-nonylphenol to quinone methide and ortho-benzoquinone metabolites in human liver microsomes. Chem. Res. Toxicol. 23, 1617–1628.
Diamanti-Kandarakis, E., Bourguignon, J.-P., Giudice, L.C., Hauser, R., Prins, G.S., Soto, A.M., Zoeller, R.T., Gore, A.C., 2009. Endocrine-disrupting chemicals: an endocrine society scientific statement. Endocr. Rev. 30, 293–342.
Commission Directive 2000/60/EC, 2000. Establishment and framework for community action in the field of water policy. Off. J. Eur. Union L327, 1–73 (22.12.2000).
Commission Directive 2003/53/EC, 2003. Amending for the 26th time the Council directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (nonylphenol, nonylphenol ethoxylate and cement). Off. J. Eur. Union L178, 24–27 (17.7.2003).
Commission Directive 2011/8/EU, 2011. Amending Directive 2002/72/EC as regards the restriction of use of bisphenol A in plastic infant feeding bottles. Off. J. Eur. Union L26, 11–14 (29.1.2011).
Fukata, H., Miyagawa, H., Yamazaki, N., Mori, C., 2006. Comparison of ELISA and LC–MS-based methodologies for the exposure assessment of bisphenol A. Toxicol. Mech. Methods 16, 427–430.
Galloway, T., Cipelli, R., Guralnik, J., Ferrucci, L., Bandinelli, S., Corsi, A.M., Money, C., McCormack, P., Melzer, D., 2010. Daily bisphenol A excretion and associations with sex hormone concentrations: results from the InCHIANTI adult population study. Environ. Health Perspect. 118, 1603–1608.
Gatidou, G., Vassalou, E., Thomaidis, N.S., 2010. Bioconcentration of selected endocrine disrupting compounds in the Mediterranean mussel, *Mytilus galloprovincialis*. Mar. Pollut. Bull. 60, 2111–2116.
Goda, Y., Kobayashi, A., Fujimoto, S., Toyoda, Y., Miyagawa, K.-I., Ike, M., Fujita, M., 2004. Development of enzyme-linked immunosorbent assay for detection of alkylphenol polyethoxylates and their biodegradation products. Water Res. 38, 4323–4330.
Hawker, D.W., Cumming, J.L., Neale, P.A., Bartkow, M.E., Escher, B.I., 2011. A screening level fate model of organic contaminants from advanced water treatment in a potable water supply reservoir. Water Res. 45, 768–780.
Hoppin, J.A., Ulmer, R., Calafat, A.M., Barr, D.B., Baker, S.V., Meltzer, H.M., Rønningen, K.S., 2006. Impact of urine preservation methods and duration of storage on measured levels of environmental contaminants. J. Expo. Sci. Environ. Epid. 16, 39–48.
Hiroi, H., Tsutsumi, O., Takeuchi, T., Momoeda, M., Ikezuki, Y., Okamura, A., Yokota, H., Taketani, Y., 2004. Differences in serum bisphenol A concentrations in premenopausal normal women and women with endometrial hyperplasia. Endocr. J. 51, 595–600.
Hughes, N.C., Wong, E.Y.K., Fan, J., Bajaj, N., 2007. Determination of carryover and contamination for mass spectrometry-based chromatographic assays. AAPS J. 9, E353–E360.
Inoue, K., Yoshimura, Y., Makino, T., Nakazawa, H., 2000. Determination of 4-nonylphenol and 4-octylphenol in human blood samples by high-performance liquid chromatography with multi-electrode electrochemical coulometric-array detection. Analyst 125, 1959–1961.
Inoue, K., Yamaguchi, A., Wada, M., Yoshimura, Y., Makino, T., Nakazawa, H., 2001. Quantitative detection of bisphenol A and bisphenol A diglycidyl ether metabolites in human plasma by liquid chromatography–electrospray mass spectrometry. J. Chromatogr. B 765, 121–126.
Inoue, K., Wada, M., Higuchi, T., Oshio, S., Umeda, T., Yoshimura, Y., Nakazawa, H., 2002. Application of liquid chromatography–mass spectrometry to the quantification of bisphenol A in human semen. J. Chromatogr. B 773, 97–102.
Inoue, K., Kawaguchi, M., Okada, F., Takai, N., Yoshimura, Y., Horie, M., Izumi, S., Makino, T., Nakazawa, H., 2003a. Measurement of 4-nonylphenol and 4-tert-octylphenol in human urine by column-switching liquid chromatography–mass spectrometry. Anal. Chim. Acta 486, 41–50.
Inoue, K., Kawaguchi, M., Funakoshi, Y., Nakazawa, H., 2003b. Size-exclusion flow extraction of bisphenol A in human urine for liquid chromatography–mass spectrometry. J. Chromatogr. B 798, 17–23.
Jiménez-Díaz, I., Zafra-Gómez, A., Ballesteros, O., Navea, N., Navalón, A., Fernández, M.F., Olea, N., Vílchez, J.L., 2010. Determination of bisphenol A and its chlorinated derivatives in placental tissue samples by liquid chromatography–tandem mass spectrometry. J. Chromatogr. B 878, 3363–3369.
Kaddar, N., Bendridi, N., Harthé, C., De Ravel, M.R., Bienvenu, A.-L., Cuilleron, C.-Y., Mappus, E., Pugeat, M., Déchaud, H., 2009. Development of a radioimmunoassay for the measurement of bisphenol A in biological samples. Anal. Chim. Acta 645, 1–4.
Kammann, U., Vobach, M., Wosniok, W., Schäffer, A., Telscher, A., 2009. Acute toxicity of 353-nonylphenol and its metabolites for zebrafish embryos. Environ. Sci. Pollut. Res. 16, 227–231.
Katayama, M., Matsuda, Y., Shimokawa, K.-I., Ishikawa, H., Kaneko, S., 2003. Preliminary monitoring of bisphenol A and nonylphenol in human semen by sensitive high performance liquid chromatography and capillary electrophoresis after proteinase K digestion. Anal. Lett. 36, 2659–2667.
Kawaguchi, M., Inoue, K., Yoshimura, M., Ito, R., Sakui, N., Okanouchi, N., Nakazawa, H., 2004a. Determination of bisphenol A in river water and body fluid samples by stir bar sorptive extraction with in situ derivatization and thermal desorption–gas chromatography–mass spectrometry. J. Chromatogr. B 805, 41–48.
Kawaguchi, M., Inoue, K., Sakui, N., Ito, R., Izumi, S., Makino, T., Okanouchi, N., Nakazawa, H., 2004b. Stir bar sorptive extraction and thermal desorption–gas chromatography–mass spectrometry for the measurement of 4-nonylphenol and 4-tert-octylphenol in human biological samples. J. Chromatogr. B 799, 119–125.
Kawaguchi, M., Sakui, N., Okanouchi, N., Ito, R., Saito, K., Izumi, S., Makinoc, T., Nakazawa, H., 2005. Stir bar sorptive extraction with in situ derivatization and thermal desorption–gas chromatography–mass spectrometry for measurement of phenolic xenoestrogens in human urine. J. Chromatogr. B 820, 49–57.
Kawaguchi, M., Ito, R., Hayatsu, Y., Nakata, H., Sakui, N., Okanouchi, N., Saito, K., Yokota, H., Izumi, S., Makino, T., Nakazawa, H., 2006. Stir bar sorptive extraction with in situ de-conjugation and thermal desorption gas chromatography–mass spectrometry for measurement of 4-nonylphenol glucuronide in human urine sample. J. Pharm. Biomed. 40, 82–87.
Kawaguchi, M., Ito, R., Sakui, N., Okanouchi, N., Saito, K., Seto, Y., Nakazawa, H., 2007. Stir-bar-sorptive extraction, with in-situ deconjugation, and thermal desorption with in-tube silylation, followed by gas chromatography–mass spectrometry for

measurement of urinary 4-nonylphenol and 4-tert-octylphenol glucuronides. Anal. Bioanal. Chem. 388, 391–398.
Kawaguchi, M., Ito, R., Okanouchi, N., Saito, K., Nakazawa, H., 2008. Miniaturized hollow fiber assisted liquid-phase microextraction with in situ derivatization and gas chromatography–mass spectrometry for analysis of bisphenol A in human urine sample. J. Chromatogr. B 870, 98–102.
Kawaguchi, M., 2008. Stir bar sorptive extraction/thermal desorption/gas chromatography/mass spectrometry for trace analysis of phenolic xenoestrogens. Jpn. Soc. Anal. Chem. 57, 911–912.
Kebarle, P., Tang, L., 1993. The mechanism of electrospray mass spectrometry. Anal. Chem. 65, 972–986.
Kodaira, T., Kato, I., Li, J., Mochizuki, T., Hoshino, M., Usuki, Y., Oguri, H., Yanaihara, N., 2000. Novel ELISA for the measurement of immunoreactive bisphenol A. Biomed. Res. Tokyo 21, 117–121.
Krishnan, K., Gagné, M., Nong, A., Aylward, L.L., Hays, S.M., 2010. Biomonitoring equivalents for bisphenol A (BPA). Regul. Toxicol. Pharm. 58, 18–24.
Kuklenyik, Z., Ekong, J., Cutchins, C.D., Needham, L.L., Calafat, A.M., 2003. Simultaneous measurement of urinary bisphenol A and alkylphenols by automated solid-phase extractive derivatization gas chromatography/mass spectrometry. Anal. Chem. 75, 6820–6825.
Kuroda, N., Kinoshita, Y., Sun, Y., Wada, M., Kishikawa, N., Nakashima, K., Makino, T., Nakazawa, H., 2003. Measurement of bisphenol A levels in human blood serum and ascitic fluid by HPLC using a fluorescent labeling reagent. J. Pharm. Biomed. 30, 1743–1749.
Kuruto-Niwa, R., Tateoka, Y., Usuki, Y., Nozawa, R., 2007. Measurement of bisphenol A concentrations in human colostrums. Chemosphere 66, 1160–1164.
Lalah, J.O., Severin, G.F., Schramm, K.-W., Lenoir, D., Behechti, A., Guenther, K., Kettrup, A., 2007. Effects of a branched p-nonylphenol isomer (4(3(,6(-dimethyl-3(-heptyl)-phenol) on embryogenesis in *Lymnae stagnalis* L. Arch. Environ. Contam. Toxicol. 52, 104–112.
Li, D., Zhou, Z., Qing, D., He, Y., Wu, T., Miao, M., Wang, J., Weng, X., Ferber, J.R., Herrinton, L.J., Zhu, Q., Gao, E., Checkoway, H., Yuan, W., 2010. Occupational exposure to bisphenol-A (BPA) and the risk of self-reported male sexual dysfunction. Hum. Reprod. 25, 519–527.
Lignos, J.S., Moschos, E.G., Asimakopoulos, A.G., Thomaidis, N.S., 2010. Optimization of bisphenol A, 4-t-octylphenol and 4-nonylphenol extraction from human blood serum with hybrid solid phase extraction-protein precipitation technique (HYBRID SPE-PPT). In: Proceedings of the 7th Aegean Analytical Chemistry Days, pp. 3–29.
Lin, W.C., Wang, S.L., Cheng, C.Y., Ding, W.H., 2009. Determination of alkylphenol residues in breast and commercial milk by solid-phase extraction and gas chromatography–mass spectrometry. Food Chem. 114, 753–757.
Liu, Z., Wolff, M.S., Moline, J., 2005. Analysis of environmental biomarkers in urine using an electrochemical detector. J. Chromatogr. B 819, 155–159.
Lopez-Espinosa, M.J., Freire, C., Arrebola, J.P., Navea, N., Taoufiki, J., Fernandez, M.F., Ballesteros, O., Prada, R., Olea, N., 2009. Nonylphenol and octylphenol in adipose tissue of women in Southern Spain. Chemosphere 76, 847–852.
Mao, L., Sun, C., Zhang, H., Li, Y., Wu, D., 2004. Determination of environmental estrogens in human urine by high performance liquid chromatography after fluorescent derivatization with p-nitrobenzoyl chloride. Anal. Chim. Acta 522, 241–246.
Mao, L.S., Sun, C.J., Li, Y.X., Wu, D.S., Zhao, J.H., 2005. Determination of environmental estrogens in human urine and serum by high performance liquid chromatography with pre-column fluorescent derivatization. Chin. J. Anal. Chem. 33, 33–36.
Maragou, N.C., Lampi, E.N., Thomaidis, N.S., Koupparis, M.A., 2006. Determination of bisphenol A in milk by solid phase extraction and liquid chromatography–mass spectrometry. J. Chromatogr. A 1129, 165–173.
Maragou, N.C., Makri, A., Lampi, E.N., Thomaidis, N.S., Koupparis, M.A., 2008a. Migration of bisphenol A from polycarbonate baby bottles under real use conditions. Food Addit. Contam. 25, 373–383.
Maragou, N.C., Rosenberg, E., Thomaidis, N.S., Koupparis, M.A., 2008b. Direct determination of the estrogenic compounds 8-prenylnaringenin, zearalenone, α- and β-zearalenol in beer by liquid chromatography–mass spectrometry. J. Chromatogr. A 1202, 47–57.
Maragou, N.C., Thomaidis, N.S., Koupparis, M.A., 2011. Optimization and comparison of ESI and APCI LC–MS/MS methods: A Case Study of Irgarol 1051, Diuron and their degradation products in environmental samples. J. Am. Soc. Mass Spectrom., in press, doi:10.1007/s13361-011-0191-z.
Mart'ianov, A.A., Dzantiev, B.B., Zherdev, A.V., Eremin, S.A., Cespedes, R., Petrovic, M., Barcelo, D., 2005. Immunoenzyme assay of nonylphenol: study of selectivity and detection of alkylphenolic non-ionic surfactants in water samples. Talanta 65, 367–374.
Meeker, J.D., Ehrlich, S., Toth, T.L., Wright, D.L., Calafat, A.M., Trisini, A.T., Ye, X., Hauser, R., 2010b. Semen quality and sperm DNA damage in relation to urinary bisphenol A among men from an infertility clinic. Reprod. Toxicol. 30, 532–539.
Meeker, J.D., Yang, T., Ye, X., Calafat, A.M., Hauser, R., 2011. Urinary concentrations of parabens and serum hormone levels, semen quality parameters, and sperm DNA damage. Environ. Health Perspect. 119, 252–257.
Meeker, J.D., Calafat, A.M., Hauser, R., 2010a. Urinary bisphenol A concentrations in relation to serum thyroid and reproductive hormone levels in men from an infertility clinic. Environ. Sci. Technol. 44, 1458–1463.
Melzer, D., Rice, N.E., Lewis, C., Henley, W.E., Galloway, T.S., 2010. Association of urinary bisphenol A concentration with heart disease: evidence from NHANES 2003/06. PLoS One 5, e8673.
Mendiola, J., Jørgensen, N., Andersson, A.-M., Calafat, A.M., Ye, X., Redmon, J.B., Drobnis, E.Z., Wang, C., Sparks, A., Thurston, S.W., Liu, F., Swan, S.H., 2010. Are environmental levels of bisphenol A associated with reproductive function in fertile men? Environ. Health Perspect. 118, 1286–1291.
Moeder, M., Martin, C., Harynuk, J., Górecki, T., Vinken, R., Corvini, P.F.X., 2006. Identification of isomeric 4-nonylphenol structures by gas chromatography–tandem mass spectrometry combined with cluster analysis. J. Chromatogr. A 1102, 245–255.
Mok-Lin, E., Ehrlich, S., Williams, P.L., Petrozza, J., Wright, D.L., Calafat, A.M., Ye, X., Hauser, R., 2010. Urinary bisphenol A concentrations and ovarian response among women undergoing IVF. Int. J. Androl. 33, 385–393.
Moors, S., Blaszkewicz, M., Bolt, H.M., Degen, G.H., 2007. Simultaneous determination of daidzein, equol, genistein and bisphenol A in human urine by a fast and simple method using SPE and GC–MS. Mol. Nutr. Food Res. 51, 787–798.
Mueller, S.O., Korach, K.S., 2001. Mechanisms of estrogen receptor-mediated agonistic and antagonistic effects. In: Metzler, M. (Ed.), The handbook of environmental chemistry. Springer-Verlag, Berlin Heidelberg, pp. 3–11.
Müller, S., Schmid, P., Schlatter, C., 1998. Pharmacokinetic behavior of 4-nonylphenol in humans. Environ. Toxicol. Pharmacol. 5, 257–265.
Nakagawa, Y., Suzuki, T., 2001. Metabolism of bisphenol A in isolated rat hepatocytes and oestrogenic activity of a hydroxylated metabolite in MCF-7 human breast cancer cells. Xenobiotica 31, 113–123.
Olea, N., Pulgar, R., Pérez, P., Olea-Serrano, F., Rivas, A., Novillo-Fertrell, A., Pedraza, V., Soto, A.M., Sonnenschein, C., 1996. Estrogenicity of resin-based composites and sealants used in dentistry. Environ. Health Perspect. 104, 298–305.
Otaka, H., Yasuhara, A., Morita, M., 2003. Determination of bisphenol A and 4-nonylphenol in human milk using alkaline digestion and cleanup by solid-phase extraction. Anal. Sci. 19, 1663–1666.
Ouchi, K., Watanabe, S., 2002. Measurement of bisphenol A in human urine using liquid chromatography with multi-channel coulometric electrochemical detection. J. Chromatogr. B 780, 365–370.
Preuss, T.G., Telscher, M., Ratte, H.T., 2008. Life stage–dependent bioconcentration of a nonylphenol isomer in *Daphnia magna*. Environ. Pollut. 156, 1211–1217.
Pucci, V., Di Palma, S., Alfieri, A., Bonelli, F., Monteagudo, E., 2009. A novel strategy for reducing phospholipids-based matrix effect in LC–ESI-MS bioanalysis by means of HybridSPE. J. Pharm. Biomed. 50, 867–871.
Reinscheid, U.M., 2009. Determination of the absolute configuration of two estrogenic nonylphenols insolution by chiroptical methods. J. Molec. Struct. 918, 14–18.
Sajiki, J., Takahashi, K., Yonekubo, J., 1999. Sensitive method for the determination of bisphenol-A in serum using two systems of high-performance liquid chromatography. J. Chromatogr. B 736, 255–261.
Sajiki, J., 2001. Determination of bisphenol A in blood using high-performance liquid chromatography-electrochemical detection with solid-phase extraction. J. Chromatogr. B 755, 9–15.
Sajiki, J., 2003. Simple and accurate determination of bisphenol A in red blood cells prepared with basic glycine buffer using liquid chromatography-electrochemical detection. J. Chromatogr. B 783, 367–375.
Sajiki, J., Hasegawa, Y., Hashimoto, H., Makabe, Y., Miyamoto, F., Yanagibori, R., Shin, J., Shimidzu, Y., Morigami, T., 2008. Determination of bisphenol A (BPA) in plasma of hemodialysis patients using three methods: LC/ECD, LC/MS, and ELISA. Toxicol. Mech. Methods 18, 733–738.
Schöringhumer, K., Cichna-Markl, M., 2007. Sample clean-up with sol–gel enzyme and immunoaffinity columns for the determination of bisphenol A in human urine. J. Chromatogr. B 850, 361–369.
Shangari, N., Chan, T.S., O'Brien, P.J., 2005. Sulfation and glucuronidation of phenols: implications in coenzyme Q metabolism. Methods Enzymol. 400, 342–359.
Schmalz, G., Preiss, A., Arenholt-Bindslev, D., 1999. Bisphenol-A content of resin monomers and related degradation products. Clin. Oral Investig. 3, 114–119.
Staples, C., Mihaich, E., Carbone, J., Woodburn, K., Klecka, G., 2004. A weight of evidence analysis of the chronic ecotoxicity of nonylphenol ethoxylates, nonylphenol ether carboxylates, and nonylphenol. Hum. Ecol. Risk Assess. 10, 999–1017.
Stasinakis, A.S., Gatidou, G., Mamais, D., Thomaidis, N.S., Lekkas, T.D., 2008. Occurrence and fate of endocrine disrupters in Greek sewage treatment plants. Water Res. 42, 1796–1804.
Suzuki, T., Nakagawa, Y., Takano, I., Yaguchi, K., Yasuda, K., 2004. Environmental fate of bisphenol A and its biological metabolites in river water and their xenoestrogenic activity. Environ. Sci. Technol. 38, 2389–2396.
Sun, Y., Irie, M., Kishikawa, N., Wada, M., Kuroda, N., Nakashima, K., 2004. Determination of bisphenol A in human breast milk by HPLC with column-switching and fluorescence detection. Biomed. Chromatogr. 18, 501–507.
Sun, H., Xu, X.L., Qu, J.H., Hong, X., Wang, Y.B., Xu, L.C., Wang, X.R., 2008. 4-Alkylphenols and related chemicals show similar effect on the function of human and rat estrogen receptor a in reporter gene assay. Chemosphere 71, 582–588.
Sunner, J., Ikonomou, M.G., Kebarle, P., 1988. Sensitivity enhancements obtained at high temperatures in atmospheric pressure ionization mass spectrometry. Anal. Chem. 60, 1308–1313.
Tan, B.L.L., Mohd, M.A., 2003. Analysis of selected pesticides and alkylphenols in human cord blood by gas chromatograph–mass spectrometer. Talanta 61, 385–391.
Tan, F., Zhao, H., Li, X., Quan, X., Chen, J., Xiang, X., Zhang, X., 2009. Preparation and evaluation of molecularly imprinted solid-phase microextraction fibers for selective extraction of bisphenol A in complex samples. J. Chromatogr. A 1216, 5647–5654.

Tsukioka, T., Brock, J., Graiser, S., Nguyen, J., Nakazawa, H., Makino, T., 2003. Determination of trace amounts of bisphenol A in urine by negative-ion chemical ionization-gas chromatography/mass spectrometry. Anal. Sci. 19, 151–153.
Van Eeckhaut, A., Lanckmans, K., Sarre, S., Smolders, I., Michotte, Y., 2009. Validation of bioanalytical LC–MS/MS assays: evaluation of matrix effects. J. Chromatogr. B 877, 2198–2207.
Van Miller, J.P., Staples, C.A., 2005. Review of the potential environmental and human health-related hazards and risks from long-term exposure to p-tert-octylphenol. Hum. Ecol. Risk Assess. 11, 319–351.
Vandenberg, L.N., Hauser, R., Marcus, M., Olea, N., Welshons, W.V., 2007. Human exposure to bisphenol A (BPA). Reprod. Toxicol. 24, 139–177.
Vandenberg, L.N., Chahoud, I., Heindel, J.J., Padmanabhan, V., Paumgartten, F.J.R., Schoenfelder, G., 2010a. Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. Environ. Health Perspect. 118, 1055–1070.
Vandenberg, L.N., Chahoud, I., Padmanabhan, V., Paumgartten, F.J.R., Schoenfelder, G., 2010b. Biomonitoring studies should be used by regulatory agencies to assess human exposure levels and safety of bisphenol A. Environ. Health Perspect. 118, 1051–1054.
Vazquez-Duhalt, R., Marquez-Rocha, F., Ponce, E., Licea, A.F., Viana, M.T., 2005. Nonyphenol, an integrated vision of a pollutant. Appl. Ecol. Environ. Res. 4, 1–25.
Völkel, W., Colnot, T., Csanády, G.A., Filser, J.G., Dekant, W., 2002. Metabolism and kinetics of bisphenol A in humans at low doses following oral administration. Chem. Res. Toxicol. 15, 1281–1287.
Völkel, W., Bittner, N., Dekant, W., 2005. Quantitation of bisphenol A and bisphenol A glucuronide in biological samples by high performance liquid chromatography–tandem mass spectrometry. Drug Metab. Dispos. 33, 1748–1757.
Völkel, W., Kiranoglu, M., Fromme, H., 2008. Determination of free and total bisphenol A in human urine to assess daily uptake as a basis for a valid risk assessment. Toxicol. Lett. 179, 155–162.
Völkel, W., Kiranoglu, M., Fromme, H., 2011. Determination of free and total bisphenol A in urine of infants. Environ. Res. 111, 143–148.
Yang, L., Luan, T., Lan, C., 2006a. Solid-phase microextraction with on-fiber silylation for simultaneous determinations of endocrine disrupting chemicals and steroid hormones by gas chromatography–mass spectrometry. J. Chromatogr. A 1104, 23–32.
Yang, L., Lan, C., Liu, H., Dong, J., Luan, T., 2006b. Full automation of solid-phase microextraction/on-fiber derivatization for simultaneous determination of endocrine-disrupting chemicals and steroid hormones by gas chromatography–mass spectrometry. Anal. Bioanal. Chem. 386, 391–397.
Yang, M., Kim, S.-Y., Lee, S.-M., Chang, S.-S., Kawamoto, T., Jang, J.-Y., Ahn, Y.-O., 2003. Biological monitoring of bisphenol A in a Korean population. Arch. Environ. Contam. Toxicol. 44, 546–551.
Yang, M., Kim, S.-Y., Chang, S.-S., Lee, I.-S., Kawamoto, T., 2006. Urinary concentrations of bisphenol A in relation to biomarkers of sensitivity and effect and endocrine-related health effects. Environ. Mol. Mutagen. 47, 571–578.
Ye, X., Kuklenyik, Z., Needham, L.L., Calafat, A.M., 2005. Quantification of urinary conjugates of bisphenol A, 2,5-dichlorophenol, and 2-hydroxy-4-methoxybenzophenone in humans by online solid phase extraction–high performance liquid chromatography–tandem mass spectrometry. Anal. Bioanal. Chem. 383, 638–644.
Ye, X., Kuklenyik, Z., Needham, L.L., Calafat, A.M., 2006. Measuring environmental phenols and chlorinated organic chemicals in breast milk using automated on-line column-switching–high performance liquid chromatography–isotope dilution tandem mass spectrometry. J. Chromatogr. B 831, 110–115.
Ye, X., Bishop, A.M., Needham, L.L., Calafat, A.M., 2007. Identification of metabolites of 4-nonylphenol isomer 4-(3′,6′-dimethyl-3′-heptyl) phenol by rat and human liver microsomes. Drug Metab. Dispos. 35, 1269–1274.
Ye, X., Bishop, A.M., Needham, L.L., Calafat, A.M., 2008. Automated on-line column-switching HPLC–MS/MS method with peak focusing for measuring parabens, triclosan, and other environmental phenols in human milk. Anal. Chim. Acta 622, 150–156.
Ye, X., Zhou, X., Needham, L.L., Calafat, A.M., 2011. In-vitro oxidation of bisphenol A: is bisphenol A catechol a suitable biomarker for human exposure to bisphenol A? Anal. Bioanal. Chem. 399, 1071–1079.
Yi, B., Kimb, C., Yang, M., 2010. Biological monitoring of bisphenol A with HLPC/FLD and LC/MS/MS assays. J. Chromatogr. B 878, 2606–2610.
Yoshimura, Y., Brock, J.W., Makinoc, T., Nakazawa, H., 2002. Measurement of bisphenol A in human serum by gas chromatography/mass spectrometry. Anal. Chim. Acta 458, 331–333.
Zafra, A., Del Olmo, M., Pulgar, R., Navalón, A., Vílchez, J.L., 2002. Determination of bisphenol-A and related compounds in human saliva by gas chromatography–mass spectrometry. Chromatographia 56, 213–218.
Zenkevich, I.G., Makarov, A.A., Schrader, S., Moeder, M., 2009. A new version of an additive scheme for the prediction of gas chromatographic retention indices of the 211 structural isomers of 4-nonylphenol. J. Chromatogr. A 1216, 4097–4106.