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Extraction of bisphenol-A and 17 β -estradiol from water samples via solid-phase extraction (SPE)

Abstract: Solid-phase extraction (SPE) is one of the most commonly applied methods for extracting endocrine-disrupting compounds (EDCs) from aqueous samples prior to chemical analysis. Here, we critically review the application of SPE to two particular EDCs, namely bisphenol-A (BPA) and 17 β -estradiol (E2). These two EDCs were selected because of their frequent occurrence, their environmental significance, and their chemical similarity to other important EDCs (e.g., steroids and alkylphenols). The review of previous literature considers factors such as which solid phase is best suited for extraction of the target analytes from an aqueous sample, which eluent should be employed for eluting the target analytes from the solid phase, and how the target analytes should be derivatized prior to analysis by gas chromatography and mass spectrometry (GC/MS). We also conducted original experiments in order to: (1) compare two types of SPE cartridges; (2) compare two common derivatization agents [*N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (MSTFA) and *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA)] identified during the critical review; and (3) assess the extent to which the range of linearity of GC/MS calibration curves can be extended by varying the sample volume applied during SPE prior to GC/MS analysis. Results of the experiments indicate that: (1) under the conditions tested, there was no significant difference in analyte recovery between C₁₈ and poly(divinylbenzene-co-*N*-vinylpyrrolidone) SPE cartridges; (2) MSTFA and BSTFA perform almost identically as silylation agents for BPA and E2 under the conditions tested; and (3) by varying the applied aqueous sample volume from 20 ml to 4000 ml, it is possible to obtain linear GC/MS calibration curves over a concentration range of 0.01 μ g/l–100 μ g/l for both BPA and E2.

Keywords: 17 β -estradiol; bisphenol-A; BSTFA; calibration; derivatization; linearity; MSTFA; solid-phase extraction.

Introduction

In recent years, the presence of endocrine-disrupting compounds (EDCs) in the environment has become a topic of significant concern. EDCs are chemicals that interfere with the normal operation of the endocrine (hormone) system in humans or other organisms (Colborn et al. 1993, Jobling et al. 1998, Iguchi et al. 2001). EDCs may be introduced into the environment through a variety of pathways. In particular, many EDCs are present in treated wastewater, and although the concentrations are typically low, the discharge of treated wastewater has led to measurable concentrations of EDCs in rivers across the US and elsewhere in the world (Routledge et al. 1998, Kolpin et al. 2002). Exposure of organisms (including humans) to EDCs in the environment has been implicated in several health issues, including the feminization of fish, reproductive problems in alligators, decreased sperm counts in human males, and childhood obesity (Colborn et al. 1993, Sharpe and Skakkebaek 1993, Purdom et al. 1994, Guillete et al. 1996, Jobling et al. 1998, Routledge et al. 1998, Safe 2000, Vos et al. 2000, Iguchi et al. 2001, Metcalfe et al. 2001, Verhulst et al. 2009).

Concern over the presence and health consequences of EDCs in the environment has led to the development of sensitive analytical methods for detecting and quantifying EDCs in water (river water, ground water, effluent from wastewater treatment plants, etc.). Analytical methods commonly employ gas chromatography with mass spectrometry (GC/MS) or liquid chromatography with MS (LC/MS) (Castillo and Barceló 1997, Rudel et al. 1998, Croley et al. 2000, Mol et al. 2000, Huang and Sedlak 2001, Jeannot et al. 2002, Ternes 2001, Ternes et al. 2001, 2002, Petrovic et al. 2002, Vanderford et al. 2003). Because the concentrations of EDCs are often very low (μ g/l or lower) in environmental samples, suitable methods for extracting and concentrating target EDCs must be applied prior to analysis by GC/MS or LC/MS. These include solid-phase extraction (SPE), liquid-liquid extraction, and solid-phase micro-extraction, each with its own advantages and disadvantages (Kuch and Ballschmiter 2000, Moeder et al. 2000, Mol et al. 2000, Ternes 2001, Ternes et al. 2001, Liu et al. 2004, López-Blanco et al. 2002, Petrovic et al. 2002,

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Braun et al. 2003, Basheer and Lee 2004, Carpinteiro et al. 2004, Soliman et al. 2004). In this paper, we focus on SPE because it is one of the most commonly applied methods for extracting EDCs from aqueous samples prior to analysis by GC/MS.

As will be discussed in more detail subsequently, SPE is a multi-step process, and a chemist must make a number of decisions for each step. Relevant factors include what medium will be used for the solid phase, how the solid phase will be prepared prior to extraction, how much aqueous sample volume is required, what eluent will be used to remove target analytes from the solid phase, what volume of eluent is required, whether the target analytes must be chemically derivatized prior to analysis, how the derivatization will be performed, etc. Because of this complexity, there are dozens, or perhaps hundreds or even thousands, of variations available for the SPE process when analyzing EDCs in water. For a chemist approaching this problem for the first time, it is a daunting task to figure out which variation is “best” or is most applicable for a particular problem. Hence, there is a need for a critical review of the existing literature on SPE for analysis of EDCs in water.

However, because “endocrine-disrupting compounds” is a broad category, it is not feasible to review SPE methods for all EDCs of concern. A method that is applicable to one individual EDC, or a particular suite or subset of EDCs, is not likely to be applicable to all. Therefore, in this paper, we focus on two particular EDCs: bisphenol-A (BPA) and 17 β -estradiol (E2). These two EDCs are selected for the following reasons: (1) they are commonly found in wastewater effluent and in receiving waters (Desbrow et al. 1998, Körner et al. 2000, Kuch and Ballschmiter 2001, Kolpin et al. 2002, Lagana et al. 2004, Nakada et al. 2006); (2) improper exposure to these compounds can result in potentially severe health consequences (Hess et al. 1997, Staples et al. 1998, Ho et al. 2006, Lang et al. 2008); (3) they have been widely studied in the literature; and (4) they share some chemical similarities to many other EDCs, including other steroid hormones (e.g., ethinyl estradiol) and other phenolic compounds (e.g., alkylphenols), so an understanding of how to analyze BPA and E2 may aid the development of methods for many common EDCs.

Therefore, the objectives of this paper are: (1) to review and critically analyze previously published SPE methods applied to the analysis of BPA and E2 in water samples; (2) to compare two types of SPE cartridges; (3) to compare two common derivatization agents (identified during the critical review) to see if one is preferable in conjunction with SPE of BPA and E2; and (4) to assess the extent to which the range of linearity of GC/MS calibration curves can be

extended by varying the sample volume applied during SPE prior to GC/MS analysis. Taken together, these aims should provide valuable information to chemists who desire to analyze BPA, E2, or chemically similar EDCs in aqueous matrices.

Overview of SPE method

The principle of the SPE method is that target analytes present in the aqueous phase at low concentrations can be adsorbed onto a solid phase, and then desorbed back into a small volume of solvent (e.g., methanol, acetone, dichloromethane). This allows for increased concentration of the analytes. For instance, the analytes in 1 l of aqueous sample might be transferred to 5 ml of methanol, increasing the concentration by a factor of 200. Evaporation of the methanol can then concentrate the sample even further. Coupling the SPE process with analysis by GC/MS can therefore allow the detection and quantification of analytes that were originally present in very low concentrations (typically $\mu\text{g/l}$ or ng/l) in the aqueous phase. Also, if impurities in the water are not adsorbed onto the solid phase (due to a lack of affinity for the particular solid phase employed), then SPE serves as a means to separate the target analytes from these impurities, which further aids in the analysis of the target analytes.

SPE cartridges designed specifically for use in this process are commercially available. These cartridges often are in the shape of glass or plastic (typically polypropylene) syringes packed with the solid-phase adsorbent. Typically, cartridges can be attached to a vacuum manifold, so that aqueous samples can be drawn through the cartridge under a slight vacuum. This allows for high volumes of aqueous samples to be processed relatively quickly.

Although many variations of the SPE method exist (as reviewed subsequently), most of these variations are based on the same basic sequence of steps. The general procedure is shown in Figure 1. The procedure involves conditioning of the sorbent, sample loading, washing of the sorbent, and sample elution. Following sample elution, the eluent may be evaporated and/or the target analytes may be derivatized prior to analysis.

Within this general framework, a chemist must make a number of decisions, all of which have the potential to affect the sensitivity or reliability of the analytical method. These decisions include the following:

- What type of solid phase or SPE cartridge should be selected?
- How much aqueous sample volume should be applied to the SPE cartridge?

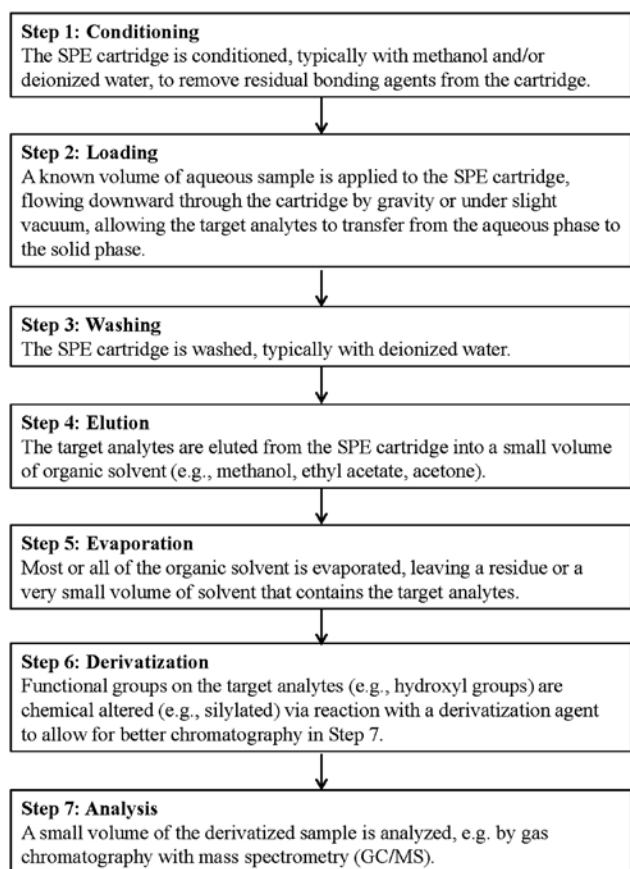


Figure 1 Sequence of steps involved in typical solid-phase extraction (SPE) of endocrine-disrupting compounds from aqueous samples.

- What eluent should be used to elute the target analytes from the cartridge?
- Should the eluted target analytes be derivatized prior to analysis, and, if so, how?

In the following sections, we critically review how these questions have been addressed by prior researchers using SPE to analyze for BPA and/or E2 in aqueous samples.

Critical review of SPE methods

Table 1 lists previous studies in which SPE was used in the analysis of BPA and/or E2. In many cases, these studies analyzed additional compounds as well, but this review focuses on BPA and E2 only. For each study, Table 1 indicates the following: which of the two target analytes were considered; the water source being analyzed (e.g., river water, wastewater); the concentration range of the target analyte in the water source; the volume of water sample loaded onto the SPE cartridge; what type of SPE medium was employed; which eluent was used; and what

derivatization agent was used to derivatize the target analytes prior to analysis.

Selection of solid phase (cartridge type)

Selection of a suitable SPE sorbent depends on the interactions between the sorbent and the analyte(s) of interest. The most frequently used sorbents are silica gel, polymer sorbents, and graphitized or porous carbon (Zwir-Ferenc and Biziuk 2006). Sorbents rely on different adsorption mechanisms, such as van der Waals forces (non-polar interactions), hydrogen bonding, dipole-dipole forces (polar interactions), and/or cation-anion interactions (ionic interactions). The chemistry of some common SPE sorbents has been summarized by Zwir-Ferenc and Biziuk (2006). The most common sorption mechanism is based on hydrophobic interactions, and thus reverse-phase alkyl-bonded silica is the most common SPE medium (Pacakova et al. 2009). Reverse-phase SPE separates compounds based on their polarity.

As can be seen from Table 1, several different types of SPE cartridges have been employed to extract BPA and/or E2 from water samples. Some studies have compared the relative effectiveness of different SPE media (Liu et al. 2004, D'Archivio et al. 2007). Overall, the two most common SPE media used for the analysis of BPA and E2 are C_{18} (typically octadecylsilane) and poly(divinylbenzene-co-*N*-vinylpyrrolidone), the latter of which is better known by the brand name Oasis HLB (Waters Corp., Milford, MA, USA). C_{18} is a hydrophobic silica-bonded phase, typically used to adsorb hydrophobic analytes (including weakly hydrophobic analytes) from aqueous solutions (Zwir-Ferenc and Biziuk 2006). Oasis HLB is made from two monomers, hydrophilic *N*-vinylpyrrolidone and lipophilic divinylbenzene; this chemistry is intended to allow the retention of both polar and non-polar analytes (Tachon et al. 2008). Oasis HLB cartridges have sometimes been reported to have a higher recovery than other SPE sorbents like C_{18} (OASIS HLB Sample Extraction Products 1998, Liu et al. 2004, D'Archivio et al. 2007).

The efficiency of extraction from water during SPE depends on the sample characteristics, the type of SPE sorbent or cartridge, the concentration of target material, and the eluent used to remove the target analytes from the solid phase (Liu et al. 2004, D'Archivio et al. 2007, Arditoglou and Voutsas 2008). SPE cartridges have a finite sorption capacity. If the mass of EDC loaded onto the cartridge during the loading step exceeds the sorption capacity, then some fraction of the target analyte will not be retained on the cartridge, and hence will not be recovered during elution.

Table 1 Studies in which solid-phase extraction (SPE) was used for the analysis of bisphenol-A (BPA) or 17 β -estradiol (E2) in water.

References	Compounds	Water source(s)	Concentration range ($\mu\text{g/l}$)	Sample volume (ml)	SPE medium	Eluent	Derivatization agent
Desbrow et al. 1998	E2	Treated wastewater	0.001–0.050	20,000	C ₁₈ (octadecylsilane)	5 ml or 2.5 ml methanol/water mixtures, then sequentially eluted with diethyl ether, 50/50 diethyl ether/hexane, and finally hexane	No derivatization
Routledge et al. 1998	E2	Synthetic river water	0.001–0.100	2000 or 20,000	C ₁₈	2 \times 10 ml dichloromethane	No derivatization
Ternes et al. 1999	E2	Raw sewage, treated wastewater, river water, spiked ground water	0.0005– \geq 0.064	1000	Polystyrene-divinylbenzene and C ₁₈	4 \times 1 ml acetone	MSTFA
Bolz et al. 2000, Körner et al. 2000	BPA	Raw sewage, treated wastewater	0.16–0.36	1000	C ₁₈ and polystyrene copolymer resin	2 \times 2.5 ml acetone or 2 \times 2.5 ml ethyl acetate	Phenyltrimethylammonium hydroxide in methanol
Mol et al. 2000	BPA	Surface water	0.05–0.12	500	C ₁₈ or styrene-divinylbenzene	2 \times 2.5 ml ethyl acetate	MTBSTFA
Brock et al. 2001	BPA	Human urine	0.27–10.6	0.25–10	C ₁₈	8 ml methanol	Pentafluorobenzyl bromide
Huang and Sedlak 2001	E2	Treated wastewater, surface water	0.00005–0.010	2000–6000 (surface water); 300–2000 (wastewater)	C ₁₈	15–30 ml methanol	Heptafluorobutyric anhydride
Nakamura et al. 2001	E2	Spiked river water	0.010–10	200	Not reported	3 ml acetone	Pentafluorobenzyl bromide followed by trimethylsilyl imidazole (TMSI) BSTFA
Jeannot et al. 2002	BPA	Treated wastewater, surface water	10.3–1030	1000 (surface water); 250 (wastewater)	Poly(divinylbenzene-co- <i>N</i> -vinylpyrrolidone)	10 ml methanol–diethylether (10:90, v/v)	
Yoshimura et al. 2002	BPA	Human serum	0.01–100	1 ml serum mixed w/10 ml water and 1 ml formic acid (32%)	C ₁₈	8 ml methanol	Pentafluorobenzyl bromide
Braun et al. 2003	BPA	Raw sewage, treated wastewater, spiked purified water	0.34–195	1000	Polystyrene-divinylbenzene and C ₁₈	8 \times 1 ml acetone	<i>N,O</i> -bis(trimethylsilyl) acetamide, trimethylchlorosilane, and TMSI (3:2:3 mixture) BSTFA with trimethylchlorosilane (TMCS), MSTFA with 1% <i>tert</i> -butyl-dimethylchlorosilane
Ding and Chiang 2003	E2	Spiked river water, spiked purified water	100–5000	1000	Poly(divinylbenzene-co- <i>N</i> -vinylpyrrolidone)	1 ml methanol plus 6 ml methanol/methyl <i>t</i> -butyl ether (1:9, v/v)	

(Table 1 Continued)

References	Compounds	Water source(s)	Concentration range (µg/l)	Sample volume (ml)	SPE medium	Eluent	Derivatization agent
Fine et al. 2003	E2	Ground water, swine lagoons	Ground water 0.1–10, swine lagoons 1–1000	500 (ground water); 25 (swine lagoons)	Poly(divinylbenzene-co- <i>N</i> -vinylpyrrolidone)	6 ml of 10% methanol in methyl <i>t</i> -butyl ether	Pentafluorobenzyl bromide and <i>N</i> -trimethylsilyl imidazole
Latorre et al. 2003	BPA	Surface water, ground water	10–1300	200	Poly(divinylbenzene-co- <i>N</i> -vinylpyrrolidone)	5 methyl acetate and 4 ml of ethyl acetate: methanol (1:1)	No derivatization
Fuh et al. 2004	E2	Spiked calibration standards	30–1000	Not reported	C ₁₈	20 ml acetonitrile	100 µL MSTFA with 5 µL trimethylsilylacetate and 5 µg dithiothreitol
Hernando et al. 2004	BPA	Waste water	Not reported	100	Poly(divinylbenzene-co- <i>N</i> -vinylpyrrolidone)	2×4 ml ethyl acetate	BSTFA
Liu et al. 2004	BPA	River water, sea water, spiked ultrapure water	0.01–0.5	500 (spiked ultrapure water); 1000 (river water or sea water)	Compared 9 different types of SPE cartridges	Compared 4 different eluents, (10 ml methanol, 10 ml ethyl acetate, 10 ml acetone, or 10 ml dichloromethane)	BSTFA with 1% TMCS
Quintana et al. 2004	E2	River water, sewage	Not reported	1000 (river); 2000 (sewage)	Poly(divinylbenzene-co- <i>N</i> -vinylpyrrolidone)	River: 3 ml ethyl acetate sewage: 10 ml ethyl acetate	MSTFA
Suzuki et al. 2004	BPA	River water	5–100	500	Styrene-divinylbenzene	5 ml dichloromethane	BSTFA
Lee et al. 2005	BPA	Sewage	Not reported	Not reported	Poly(divinylbenzene-co- <i>N</i> -vinylpyrrolidone)	5 ml methanol and 10 ml of 2% formic acid in methanol	MTBSTFA
Seo et al. 2005	E2	Beef cattle	Not reported	Not reported	C ₈ (octylsilane)	4 ml methanol	MSTFA/NH ₄ I/dithiothreitol (1000:4:2, v/w/w)
Stuart et al. 2005	BPA	Marine samples	2000–40,000 (underivatized) or 25,000–250,000 (derivatized)	100	Poly(styrene divinylbenzene)	3×2 ml acetone	Phenyltrimethylammonium hydroxide
Wang et al. 2005	BPA	Reclaimed water	1–100	1000	C ₁₈	2×5 ml ethyl acetate	MTBSTFA
Sarmah et al. 2006	E2	Sewage or animal waste	Not reported	1000	C ₁₈	2×10 ml acetone or dichloromethane	Trifluoroacetic anhydride
Yang et al. 2006	E2	River water, blood serum	0.1–100	50	C ₁₈	15 ml diethyl ether	BSTFA
Zhang et al. 2006	BPA	River water, sewage effluent, spiked ultrapure water	Not reported	500	Poly(divinylbenzene-co- <i>N</i> -vinylpyrrolidone)	10 ml ethyl acetate	BSTFA
Fernandez et al. 2007	BPA	Mill wastewater, domestic wastewater	Not reported	100–200	Poly(divinylbenzene-co- <i>N</i> -vinylpyrrolidone)	5 sequential elutions with different solvents to fractionate analytes by polarity	BSTFA

(Table 1 Continued)

References	Compounds	Water source(s)	Concentration range (µg/l)	Sample volume (ml)	SPE medium	Eluent	Derivatization agent
Gatidou et al. 2007	BPA	Wastewater, sewage sludge	100–10,000	100	C ₁₈	4×2 ml dichloromethane:hexane (4:1)	BSTFA
Möder et al. 2007	BPA	River water, wastewater	Not reported	Not reported	Poly(ethylvinylbenzene divinylbenzene) and C ₁₈	40 ml methanol	Pentafluorobenzyl bromide
Moors et al. 2007	BPA	Human urine	0.165–55	Not reported	Mixed C ₁₈ , C ₈ , and C ₂ (dimethylsilane)	0.3 ml acetonitrile: ethyl acetate (1:1)	MTBSTFA
Stanford and Weinberg 2007	E2	Septic, ground water	0.04–10	500	N-vinylpyrrolidone	5 ml methyl t-butyl ether: methanol (9:1)	BSTFA
Zhang et al. 2007a	BPA	River water	Not reported	1000	C ₁₈ or poly(styrene divinylbenzene)	Consecutively eluted with 10 ml methanol, 10 ml acetone, and 10 ml dichloromethane	BSTFA
Zhang et al. 2007b	E2	Spiked ultrapure water	Not reported	Not reported	Poly(divinylbenzene-co-N-vinylpyrrolidone)	15 ml ethyl acetate	BSTFA
Arditsoglou and Voutsas 2008	BPA	Spiked ultrapure water, artificial seawater, river water and seawater, total suspended solid, marine sediment	Not reported	1000	Poly(divinylbenzene-co-N-vinylpyrrolidone), C ₁₈ , florisil, silica, alumina-silica	Compared 3 different eluents, (10 ml acetone, 10 ml methanol, or 10 ml ethyl acetate)	BSTFA
Stasinakis et al. 2008	BPA	Water	Not reported	100	C ₁₈	4×2 ml dichloromethane/hexane	BSTFA
Vigano et al. 2008	BPA	River sediment extracted into 15 ml MeOH and reduced to 1 ml, then mixed with 9 ml MilliQ water	Not reported	10	Poly(divinylbenzene-co-N-vinylpyrrolidone)	10% methanol in methyl t-butyl ether (volume not reported)	No derivatization
Hibberd et al. 2009	BPA	Surface water, sediment	10–5000	2500	Poly(divinylbenzene-co-N-vinylpyrrolidone)	15 ml ethyl acetate	BSTFA
Ribeiro et al. 2009	BPA	River water	BPA: 0.9–135 E2: 0.8–20	2000	Poly(divinylbenzene-co-N-vinylpyrrolidone)	7 ml methanol: dichloromethane (50:50)	MSTFA
Zhao et al. 2009	BPA	River water	Not reported	1000	Poly(divinylbenzene-co-N-vinylpyrrolidone)	7 ml methanol/5 ml dichloromethane	Pentafluorobenzoyl chloride and pentafluorobenzyl bromide
Sodre et al. 2010	BPA	Drinking water	Not reported	3000–4000	N-vinylpyrrolidone	1 ml acetone, 1 ml methanol	MTBSTFA
Wang et al. 2010	BPA	Sewage	0.01–1	Not reported	Poly(divinylbenzene-co-N-vinylpyrrolidone)	10 ml methyl-t-butyl ether/ methanol (9:1)	BSTFA

(Table 1 Continued)

References	Compounds	Water source(s)	Concentration range (µg/l)	Sample volume (ml)	SPE medium	Eluent	Derivatization agent
Andrasi et al. 2011	E2	Waste water	0.000188–0.00281	250–500	Poly(divinylbenzene-co-N-vinylpyrrolidone)	5 ml hexane, 5 ml ethyl acetate, and 10 ml methanol	225 µl hexamethyldisilazane with 25 µl trifluoroacetic acid
Mead and Seaton 2011	BPA	BPA fortified water	Not reported	Not reported	C ₁₈	Methanol (volume not reported)	BSTFA
Rocha et al. 2011	BPA	River water, seawater	Not reported	500	Poly(divinylbenzene-co-N-vinylpyrrolidone)	10 ml ethyl acetate	BSTFA or MSTFA
Sanchez-Avila et al. 2011	BPA	River water, sea water, wastewater treatment plant effluent	Not reported	1000 (river or sea); 250 (waste water treatment plant effluent)	Poly(divinylbenzene-co-N-vinylpyrrolidone)	15 ml dichloromethane/hexane (1:1), and 15 ml dichloromethane/acetone (1:1)	No derivatization
Bizkarguenaga et al. 2012	BPA	Wastewater, river water, sea water, effluent wastewater, estuarine, coastal water, surface water	Not reported	50–100 (waste); 1000 (river, sea, effluent wastewater, estuarine and coastal); 500–2,500 (surface)	Poly(divinylbenzene-co-N-vinylpyrrolidone), C ₁₈	8 ml ethyl acetate, and 8 ml <i>n</i> -hexane	BSTFA
Liu et al. 2012	BPA	Biological samples	Not reported	Not reported	Poly(divinylbenzene-co-N-vinylpyrrolidone), C ₁₈ (octadecylsilane)	15 ml ethyl acetate	BSTFA
Rocha et al. 2012	BPA	River water, seawater	Not reported	500	Poly(divinylbenzene-co-N-vinylpyrrolidone)	10 ml ethyl acetate	BSTFA
Suri et al. 2012		Wastewater	Not reported	200, 1000, 3000	C ₁₈ (octadecylsilane)	3 ml methanol	BSTFA

BSTFA, *N,O*-bis(trimethylsilyl)-trifluoroacetamide; MSTFA, *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide; MTBSTFA, *N*-(*tert*-butyl-dimethylsilyl)-*N*-methyltrifluoroacetamide; TMSI, *N*-trimethylsilyl imidazole.

Therefore, to ensure analyte recoveries close to 100%, it is important to consider the capacity of the SPE cartridge and the concentration of target analytes in the water sample.

Selection of eluent

After target analytes are extracted from an aqueous sample onto an SPE medium, the analytes need to be extracted from the SPE medium by an eluent (see Figure 1). The eluent interrupts the interactions between the sorbent and the analytes of interest, allowing the analytes to desorb (Yang et al. 1998). Desirable characteristics of an eluent include high extraction efficiency of the target analytes, low volume required, weak (or no) toxicity, and compatibility with the chromatographic system used (Liu et al. 2004, Arditoglou and Voutsas 2008). Often, water-miscible organic solvents (e.g., acetone, acetonitrile, methanol, isopropanol) are chosen as eluents; however, as can be seen from Table 1, other organic solvents (dichloromethane, diethyl ether, ethyl acetate, hexane) have also been used in conjunction with SPE for analysis of BPA and E2. The eluents used most commonly for this application are methanol and ethyl acetate.

To concentrate the target analytes, the elution step needs to extract the analytes with a small volume of eluent. Usually, the elution volume is recommended to be 2–10 times the bed volume of the SPE cartridge (Care and Use Manual 2008). Smaller elution volumes result in more concentrated extract, as long as the extraction efficiency is close to 100%.

Some previous researchers have compared different eluents, to determine which provides the best performance when extracting EDCs from SPE cartridges (Liu et al. 2004, Arditoglou and Voutsas 2008, Suri et al. 2012). Liu et al. (2004) found that, when extracting BPA and E2 from Oasis HLB cartridges, acetone, methanol, and ethyl acetate performed well, but methanol gave slightly higher recoveries. Suri et al. (2012) found that, for eluting target analytes from C_{18} cartridges, methanol and toluene showed higher extraction efficiency than acetone, hexanol, or acetonitrile, with methanol showing the highest recoveries. Arditoglou and Voutsas (2008) found that when Oasis HLB cartridges were used to extract BPA and E2 from artificial seawater, acetone provided higher recoveries than methanol or ethyl acetate. Overall, there does not appear to be a clear answer as to which eluent is “best” for extraction of EDCs from SPE cartridges; which eluent performs best is likely to depend on the target analyte(s), the type of SPE cartridge, and other experimental conditions. Methanol appears to be a suitable “default” eluent for extracting

EDCs from C_{18} or poly(divinylbenzene-co-N-vinylpyrrolidone) cartridges, based on its relatively low cost and relatively good performance in previous research.

Selection of derivatization agent

Chemical derivatization alters the chemical structure of the target analyte(s) to a form that provides better GC (Drozd 1981). Derivatization is often carried out to convert polar N–H, O–H or S–H groups into non-polar groups (Jeannot et al. 2002, Basheer and Lee 2004, Carpinteiro et al. 2004, Chang et al. 2005, Li et al. 2006, Yang et al. 2006, Zhang et al. 2006, Möder et al. 2007, Pan and Tsai 2008, Orata 2012). Many EDCs (including both BPA and E2) contain polar hydroxyl, phenolic, or carboxylic groups, so that derivatization is often necessary when EDCs are analyzed by GC/MS. The derivatized analytes typically are more volatile and produce sharper peaks than the underivatized forms, thereby improving the chromatography, and may also provide better thermal stability and/or more favorable fragmentation patterns during MS.

There are three common groups of derivatization methods: silylation, acylation, and alkylation (which includes esterification). For each type of derivatization, different derivatization agents are available. Many of these have been employed in the studies listed in Table 1. For instance, silylation agents include *N,O*-bis(trimethylsilyl)-acetamide (BSA), *N,O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA), *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (MSTFA), *N*-(*tert*-butyl-dimethylsilyl)-*N*-methyltrifluoroacetamide (MTBSTFA), and *N*-trimethylsilyl imidazole (TMSI). Many silylation agents contain a small amount of trimethylchlorosilane (TMCS; also known as trimethylsilyl chloride or chlorotrimethylsilane) as a catalyst. The most common alkylation agent for derivatization of BPA and E2 is pentafluorobenzyl bromide (PFBBBr), but phenyltrimethylammonium hydroxide has also been used (Bolz et al. 2000, Körner et al. 2000, Stuart et al. 2005). Acylation is not common for E2 or BPA, but some studies have used heptafluorobutyric anhydride or trifluoroacetic anhydride to acylate E2.

Silylation is the most commonly used technique among the three candidate derivatization methods. The advantages of silylation are: (1) the ability to silylate a wide variety of compounds; (2) the large number of silylating reagents available; and (3) easy preparation. Silylation reagents can derivatize almost all functional groups (hydroxyl, carboxylic acid, amine, thiol, phosphate) that might cause problems during gas chromatographic separation. The trimethylsilyl (TMS) group is the typical silyl

group to enhance chromatographic peak or detectability (Knapp 1979, Evershed 1993, Orata 2012).

An interesting question is whether one silylation agent is preferable to others for derivatization of EDCs. Gehrke (1968) compared BSTFA and BSA for silylation of amino acids and found that BSTFA reacts more quickly and more completely than BSA. Quintana et al. (2004) compared the reactivity of MTBSTFA, BSTFA, and MSTFA with the aromatic and aliphatic hydroxyl groups contained in six estrogenic chemicals. They found that MTBSTFA and BSTFA can react with hydroxyl groups only at certain positions within the chemical structure of the target analytes, but that MSTFA was able to silylate hydroxyl groups at any position, perhaps because its smaller molecular size reduced steric hindrance. However, for four of the six estrogens (including E2), BSTFA was observed to perform as well as MSTFA. As can be seen from Table 1, BSTFA and MSTFA have both been used successfully by many research teams for derivatization of BPA and E2, although BSTFA appears to be employed more frequently.

Selection of sample volume

In general, it is expected that an inverse relationship would exist between the concentration of target analytes in the aqueous sample and the required volume of sample that must be loaded on the SPE cartridge. If concentrations of target analytes are relatively high ($\mu\text{g/l}$ range), then not much sample volume is required to load an acceptable mass of analyte onto the SPE cartridge. If concentrations of target analytes are low (ng/l range), then significantly more sample volume would be required to load the same analyte mass onto the cartridge. Many of the entries in Table 1 corroborate this general idea. For BPA or E2 concentrations in the $\mu\text{g/l}$ range, typically no more than 1 l of sample volume is required for SPE (Jeannot et al. 2002, Ding and Chiang 2003, Latorre et al. 2003, Suzuki et al. 2004, Wang et al. 2005, Yang et al. 2006, Gatidou et al. 2007). However, for BPA or E2 concentrations in the low ng/l range, typically at least 2 l of sample volume are required (Desbrow et al. 1998, Routledge et al. 1998, Huang and Sedlak 2001).

Likewise, detection limits for BPA and E2 can be affected by sample volume. Suri et al. (2012) reported method detection limits for E2 of 30 ng/l when 200 ml of sample volume was used, 0.10 ng/l when 1000 ml of sample volume was used, and 0.03 ng/l when 3000 ml of sample volume was used. This is consistent with the

pattern that higher sample volumes are required for lower concentrations of analytes.

This suggests that, when preparing calibration curves with standards of known concentrations, the volume of standard employed should vary according to the concentration of the standard. By varying the volume such, it may be possible to extend the range of linearity of the calibration curve and/or to reduce the method detection limits.

Remaining questions/room for improvement

To optimize the SPE method and GC/MS analysis for detection and quantification of EDCs, each step in the SPE process (Figure 1) can be optimized. For instance, key factors that can be considered include, but are not limited to, the following. (1) What is the best type of SPE cartridge for the analytes and aqueous matrix under consideration? (2) How should the SPE cartridge be conditioned? (3) What sample volume is appropriate for the expected concentrations of target analytes? (4) At what flow rate, or under what degree of vacuum, should the sample be loaded onto the SPE cartridge? (5) What solvents, and at what volumes, should be used to wash the cartridge after sample loading? (6) Which eluent or combination of eluents is best to remove the target analytes from the SPE cartridge? (7) What volume of eluent(s) should be used? (8) Should the eluent be evaporated to further concentrate the target analytes, and if so, what process should be used for solvent evaporation? (9) What derivatizing agent (or combination of agents) is best? (10) What conditions (temperature, time, etc.) should be used for the derivatization process? (11) How do the answers to the preceding questions depend upon the chemistry of the aqueous matrix and/or the target analytes?

Some of these questions can be answered in part by examining the existing literature, as in Table 1 and the preceding discussion in this paper. To fully answer every one of these questions would likely take years of effort from multiple research groups working in parallel. In the remainder of this paper, we contribute to this effort by considering the following with respect to analysis of BPA and E2. (1) Which type of SPE cartridge provides better analyte recovery, C_{18} or poly(divinylbenzene-co-N-vinylpyrrolidone)? (2) Which derivatization agent is preferable, MSTFA or BSTFA? (3) To what extent can the range of linearity of GC/MS calibration curves be extended by varying the sample volume applied during SPE?

Materials and methods

Experimental work was conducted in two stages. In the first stage, we tested and compared the efficiency of EDC extraction of C_{18} (Fisher Scientific, Pittsburgh, PA, USA) and poly(divinylbenzene-co-N-vinylpyrrolidone) (Oasis HLB, Waters Corp., Milford, MA, USA) SPE cartridges. For that comparison, we prepared standard solutions of BPA and E2 in purified water at concentrations of 0.01 $\mu\text{g/l}$, 1 $\mu\text{g/l}$, and 100 $\mu\text{g/l}$. Known volumes of these standard solutions (3000 ml, 500 ml, or 50 ml, respectively) were drawn through the C_{18} and poly(divinylbenzene-co-N-vinylpyrrolidone) cartridges under slight vacuum. Then, EDCs were eluted from the cartridges, derivatized, and analyzed, as described in more detail further below. Peak areas from the GC/MS were compared to quantify the recovery of each analyte from the two types of cartridges.

In the second stage of the research, we used poly(divinylbenzene-co-N-vinylpyrrolidone) cartridges to compare MSTFA and BSTFA derivatization agents and to investigate the range of linearity of calibration curves. We created solutions of known concentrations of BPA and E2 in purified water, and then used those solutions to develop calibration curves for the analytical methods as described in more detail below. This enabled us to determine the range of linearity of the calibration curves, thereby providing us with a basis with which to compare the derivatization agents. Details are provided below.

Chemicals

Methanol (HPLC grade), BPA (purity grade >99%), E2 (purity grade >99%), BSTFA with 1% TMCS, and MSTFA with 1% TMCS were all purchased from Sigma-Aldrich, St. Louis, MO, USA.

Aqueous samples

Primary stock standard solutions (1000 mg/l) of both BPA and E2 were prepared in methanol by dissolving 0.100 g of analyte into 100 ml methanol. Stock solutions were stored at 4°C in a refrigerator. Then, aqueous samples were prepared daily by dilution of the stock solutions with deionized water. The concentrations of aqueous samples ranged from 1 ng/l to 100 $\mu\text{g/l}$ for SPE. The aqueous samples were prepared from the primary stock solutions by diluting with deionized water, using sequential dilutions when necessary to obtain low concentrations. Methanol content in the aqueous samples was 0.1% or lower (by volume,

before mixing) in all aqueous samples, and was therefore considered negligible.

SPE

Here we describe the SPE method used to prepare a sample for analysis by GC/MS. Oasis HLB glass cartridges (5 ml, 200 mg sorbent) or Fisher C_{18} cartridges were placed on a vacuum manifold (SPE 24-port Vacuum manifold, purchased from Fisher Scientific, Pittsburgh, PA, USA). The cartridges were conditioned with 40 ml of deionized water and 25 ml of methanol, both of which were drawn through the cartridges under low vacuum to remove residual bonding agents. Then, a known volume of aqueous sample was loaded onto the cartridge and flowed through under slight vacuum (flow rate=60 ml/min). We tested different volumes of samples ranging from 10 ml to 4 l, and different EDC concentrations ranging from 1 ng/l to 100 $\mu\text{g/l}$. During the sample loading step, the target compounds were extracted from the aqueous samples onto the SPE cartridges. After loading, the cartridges were washed with 20 ml of deionized water, and then dried for 5 min under vacuum in order to remove excess water remaining on the cartridge. Then, the adsorbed analytes were eluted to 10 ml vials from the cartridges with 5 ml methanol at a flow rate of 5 ml/min.

Derivatization

Due to the presence of polar functional groups in BPA and E2, which can give rise to poor chromatographic peaks, derivatization was necessary. We based our derivatization method on that of Zhang et al. (2006). The methanol eluent collected from SPE was evaporated in a Rotavapor R-210 rotary evaporator (Buchi, Flawil, Switzerland). The dry residues were derivatized either by BSTFA with 1% TMCS, or by MSTFA with 1% TMCS. For either agent, 100 μl of derivatization reagent was added to each reaction vial. Then, the vials were closed and placed in an oven at 65°C for 25 min. Because the reaction time and temperature can affect the derivatization process, these parameters were investigated to optimize the derivatization conditions. Derivatization was carried out in triplicate on test samples (100 $\mu\text{g/l}$) at reaction times of 10, 20, 25, 30, 35, and 40 min. When reaction times of 25 min or longer were applied, there were no underivatized compounds in the chromatograms, so 25 min was selected as the reaction time for the remainder of the study. Similarly, derivatization was tested in triplicate at ambient temperature

and at temperatures of 30, 40, 50, 60, 65, 70, and 75°C. The extent of derivatization increased with temperature up to a temperature of 65°C, so 65°C was selected as the reaction temperature for the remainder of the study.

Once the derivatization was completed, 1 μ l of the reaction mixture was injected into the GC/MS system within 30 min, to avoid reaction inversion.

GC/MS instrumentation and operating conditions

Analyses were carried out on a Varian CP-3800 gas chromatograph directly connected to a Saturn 2000 ion-trap mass spectrometer (Varian, Palo Alto, CA, USA). An HP-5MS capillary column (30 m \times 0.25 mm inner diameter, 0.25 μ m film, 5% phenyl-dimethylsiloxane phase, Agilent, Santa Clara, CA, USA) was used for chromatography. Helium (99.9995% purity) was used as carrier gas at a constant flow rate of 1.0 ml/min. The injection port temperature was 280°C and injections were performed using a splitless mode. The GC oven temperature program was as follows: hold for 1 min at 80°C, increase at 15°C/min to 240°C, hold for 1 min, increase at 10°C/min to 280°C, and hold for 5 min. Data acquisition was performed in full scan mode measuring m/z from 69 to 614. The transfer line temperature of the GC/MS was set at 170°C, and the manifold temperature was set at 160°C. The electron emission current of GC/MS was 10 μ A (70 eV), multiplier voltage was 1500 V, and automatic gain control target was 20,000.

BPA and E2 were quantified by the area of the peak corresponding to a particular fragment on the MS. We refer to these fragments as the diagnostic ions for each compound. The m/z ratios for the diagnostic quantitative ions are 357 for BPA and 416 for E2. These m/z ratios correspond to major peaks in the mass spectra of the derivatized (silylated) compounds.

Results

Comparison of cartridge types

When comparing C_{18} to poly(divinylbenzene-co-N-vinylpyrrolidone) cartridges, no major differences were observed. With poly(divinylbenzene-co-N-vinylpyrrolidone) cartridges, the average recoveries observed were 94.4% for BPA and 97.6% for E2. With C_{18} cartridges, the average recoveries were 93.2% for BPA and 96.8% for E2. The differences between the cartridge types were not

statistically significant. For subsequent experiments, we chose poly(divinylbenzene-co-N-vinylpyrrolidone) to extract the EDCs from the water samples because in recent work by other research groups, it appears to be more common than C_{18} (see Table 1).

Calibration curves

The calibration curves of EDCs that were extracted by SPE and derivatized are presented in Figures 2 and 3 as measured peak area vs. injected EDC mass. The injected EDC mass is calculated as the volume, V , of sample loaded onto the SPE cartridge (ranging from 20 ml to 4000 ml) times the concentration, C , of target EDC in the sample (ranging from 0.001 μ g/l to 100 μ g/l). This calculation assumes that 100% of the EDC mass loaded onto the SPE cartridge was recovered and injected into the GC; as noted above, observed recoveries were in the range of 93%–97%, so the assumption of 100% recovery does not introduce much error. Tables 2–5 show the relation between loaded

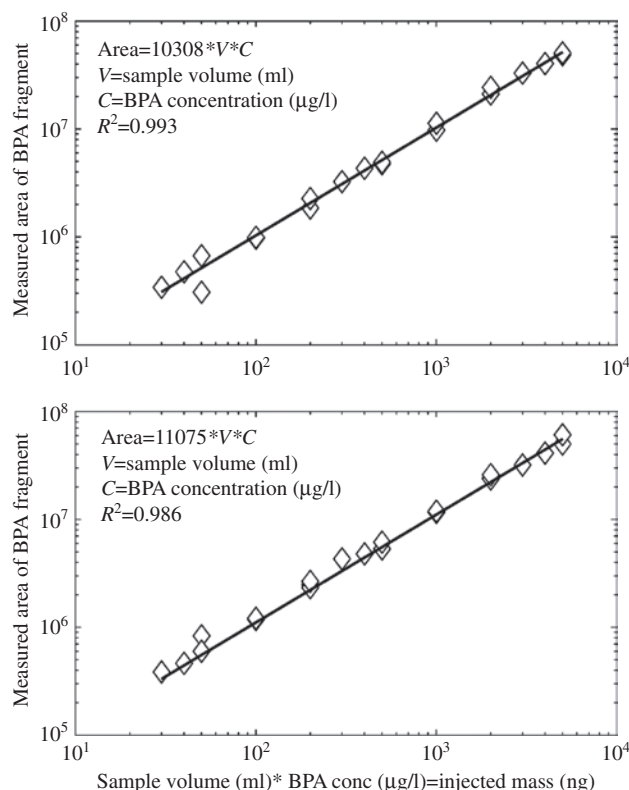


Figure 2 Calibration curve of bisphenol-A analyzed by solid-phase extraction (SPE) followed by gas chromatography and mass spectrometry (GC/MS). Top panel: derivatized with *N,O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA); bottom panel: derivatized with *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (MSTFA).

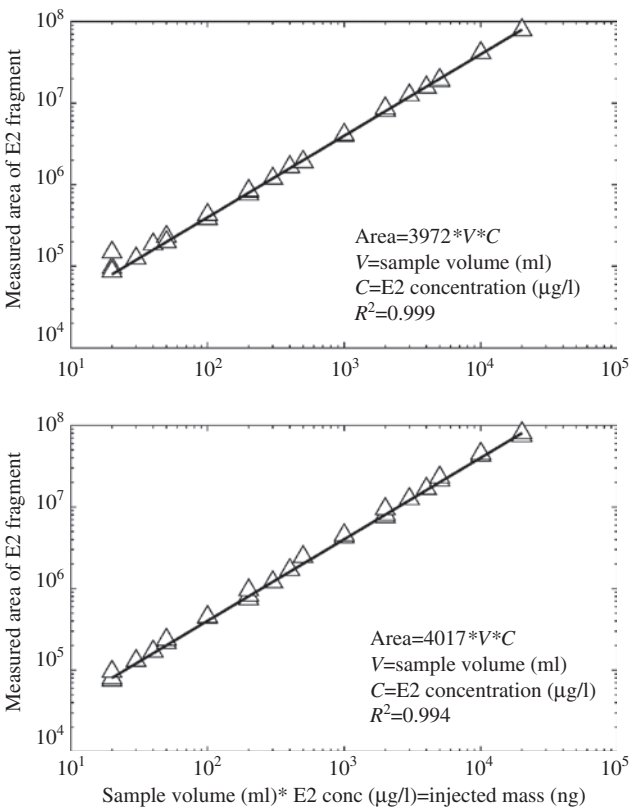


Figure 3 Calibration curve of 17β-estradiol analyzed by solid-phase extraction (SPE) followed by gas chromatography and mass spectrometry (GC/MS). Top panel: derivatized with *N,O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA); bottom panel: derivatized with *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (MSTFA).

sample volume, concentration of EDC sample, and measured area of derivatized EDC fragment.

It can be seen from Figures 2 and 3 that the calibration curves, presented as measured peak area vs. injected EDC mass, are linear over multiple orders of magnitude. This wide range of linearity is useful, because it means that the analytical method is likely to be applicable even to samples where the approximate concentration range is unknown. Also, it means that a single analytical method is likely to be applicable to studies in which samples have widely varying concentrations, e.g., if the concentration of a target analyte is attenuated by 99% or even 99.9%. In cases where the research team knows that the sample concentrations vary over a smaller range, a calibration curve can be developed for that particular concentration range, which may result in an R^2 value higher than those observed in Figures 2 and 3 ($R^2=0.986$ or 0.993 for BPA, $R^2=0.994$ or 0.999 for E2).

For BPA (Figure 2), the calibration curve was generated from samples that meet three criteria: the concentration C_{BPA} was between $0.010 \mu\text{g/l}$ and $100 \mu\text{g/l}$; the sample volume V was between 20 ml and 4000 ml ; and the BPA

Table 2 Measured area of *N,O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA)-derivatized bisphenol-A (BPA) fragment [after solid-phase extraction (SPE)].

Concentration of bisphenol-A (μg/l)	Sample vol=20 ml	Sample vol=50 ml	Sample vol=100 ml	Sample vol=200 ml	Sample vol=500 ml	Sample vol=1000 ml	Sample vol=2000 ml	Sample vol=3000 ml	Sample vol=4000 ml
0.01	-	-	-	-	-	-	-	341,000	474,000
0.1	-	-	-	-	668,000	972,000	1,848,000	3,248,000	4,322,000
1	-	307,000	990,000	2,258,000	4,755,000	9,775,000	21,112,000	32,944,000	40,350,000
10	2,269,000	4,913,000	11,251,000	24,309,000	48,682,000	-	-	-	-
100	24,282,000	50,697,000	-	-	-	-	-	-	-

Table 3 Measured area of *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (MSTFA)-derivatized bisphenol-A (BPA) fragment [after solid-phase extraction (SPE)].

Concentration of bisphenol-A (μg/l)	Sample vol=20 ml	Sample vol=50 ml	Sample vol=100 ml	Sample vol=200 ml	Sample vol=500 ml	Sample vol=1000 ml	Sample vol=2000 ml	Sample vol=3000 ml	Sample vol=4000 ml
0.01	-	-	-	-	-	-	-	385,000	463,000
0.1	-	-	-	-	832,000	1,184,000	2,479,000	4,301,000	4,782,000
1	-	599,000	1,214,000	2,337,000	5,318,000	11,550,000	24,208,000	31,920,000	41,138,000
10	2,662,000	6,139,000	11,885,000	23,706,000	49,867,000	-	-	-	-
100	25,871,000	60,841,000	-	-	-	-	-	-	-

Table 4 Measured area of *N,O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA)-derivatized E2 fragment [after solid-phase extraction (SPE)].

Concentration of bisphenol-A (µg/l)	Sample vol=20 ml	Sample vol=50 ml	Sample vol=100 ml	Sample vol=200 ml	Sample vol=500 ml	Sample vol=1000 ml	Sample vol=2000 ml	Sample vol=3000 ml	Sample vol=4000 ml
0.01	-	-	-	-	-	-	95,000	125,000	186,000
0.1	-	-	-	84,000	231,000	382,000	762,000	1,194,000	1,648,000
1	148,000	199,000	428,000	813,000	1,900,000	3,972,000	8,183,000	12,480,000	15,630,000
10	846,000	1,917,000	4,100,000	8,041,000	18,600,000	41,420,000	78,830,000	-	-
100	8,663,000	19,400,000	41,300,000	78,680,000	-	-	-	-	-

Table 5 Measured area of *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (MSTFA)-derivatized E2 fragment [after solid-phase extraction (SPE)].

Concentration of bisphenol-A (µg/l)	Sample vol=20 ml	Sample vol=50 ml	Sample vol=100 ml	Sample vol=200 ml	Sample vol=500 ml	Sample vol=1000 ml	Sample vol=2000 ml	Sample vol=3000 ml	Sample vol=4000 ml
0.01	-	-	-	-	-	-	75,000	130,000	170,000
0.1	-	-	-	80,000	215,000	440,000	742,000	1,203,000	1,693,000
1	96,000	237,000	451,000	824,000	2,435,000	4,274,000	7,588,000	12,576,000	16,845,000
10	956,000	2,452,000	4,533,000	8,111,000	21,310,000	43,040,000	74,430,000	-	-
100	9,478,000	23,130,000	45,270,000	80,770,000	-	-	-	-	-

mass loaded ($M=V \cdot C_{\text{BPA}}$) was between 30 ng and 5000 ng. The third criterion implies, for instance, that for samples where we used a volume $V=100$ ml, the calibration curve includes all results for which $0.30 \mu\text{g/l} \leq C_{\text{BPA}} \leq 50 \mu\text{g/l}$, but not for samples outside this concentration range. As can be seen from Figure 2, the measured peak area is linear with respect to the injected BPA mass for samples meeting the three necessary criteria.

For E2 (Figure 3), similar behavior was observed, but the range of linearity is even greater for E2 than it is for BPA. For E2, the calibration curves were generated from samples which met the following three criteria: the concentration C_{E2} was $0.010\text{--}100 \mu\text{g/l}$; the sample volume V was $20\text{--}4000$ ml; and the E2 mass loaded ($M=V \cdot C_{\text{E2}}$) was $20\text{--}20,000$ ng. The third criterion implies, for instance, that a sample volume of $V=500$ ml could be used to quantify concentrations in the range $0.040 \mu\text{g/l} \leq C_{\text{E2}} \leq 40 \mu\text{g/l}$. As can be seen from Figure 3, the measured peak areas are linear with respect to the E2 mass injected for samples meeting these criteria.

For both BPA and E2, we did test several samples that did not meet one of the requisite criteria (e.g., samples of concentration $C < 10$ ng/l, or for which $V \cdot C$ was not in the specified range). These samples generally did not follow the same linear behavior, and are not included in Figures 2 and 3. Hence, there is some limitation on the range of linearity for the SPE method; if the concentration is too low or too high, the measured peak area is not likely to fall on the calibration curves provided. However, this limitation is not severe; simply by choosing the sample volume appropriately, the SPE method may be applied to samples of BPA or E2 in the concentration range 10 ng/l to $100 \mu\text{g/l}$, i.e., four orders of magnitude of concentration. We found that $C=10 \text{ ng/l}$ is a practical lower limit of quantification for the SPE method for both BPA and E2.

Derivatization agent

MSTFA (with 1% TMCS) and BSTFA (with 1% TMCS) were selected and compared as derivatization agents in this work because of their common usage by previous research groups (Table 1). We observed that silylating the target analytes sharpened the chromatographic peaks. Retention times increased by only about 10 s for the silylated compounds compared to the non-derivatized compounds. Results from MS verified that the target analytes were silylated ($m/z=357$ for BPA and 416 for E2).

By comparing the calibration curves obtained in Figures 2 and 3, we can assess the relative performance of MSTFA and BSTFA as derivatization agents.

Examination of Figures 2 and 3 shows that the calibration curves are nearly identical for the two derivatization agents. The range of linearity was the same for both agents. For analysis of BPA (Figure 2), the slopes of the calibration curves differ by only about 7% for the two derivatization agents. For analysis of E2 (Figure 3), the slopes differ by only about 1%. This result is consistent with those of some previous researchers, who observed similar performance with MSTFA or BSTFA (Quintana et al. 2004, Shareef et al. 2006, Zhou et al. 2007, Sebk et al. 2008).

Discussion

Calibration curves

Many previous researchers, as summarized in Table 1, used a fixed aqueous sample volume for all analyses, regardless of the expected analyte concentration in the sample. Here, we have developed calibration curves for our target analytes based on a range of sample volumes from 20 ml to 4 l. The advantage to this is that it can extend the concentration range over which a linear calibration curve can be obtained. As seen from Tables 2–5, the lowest sample volume (20 ml) is useful only for the highest concentrations of analytes, and the highest sample volume (4 l) is useful only for the lowest concentrations of analytes. Even if we selected the “best” single sample volume, the calibration range would be limited to two orders of magnitude for BPA (e.g., 0.01–1 µg/l with 3000 or 4000 ml) and three orders of magnitude for E2 (e.g., 0.01–10 µg/l with 2000 ml). By using multiple sample volumes, we extend the range of concentrations to four orders of magnitude, 0.01–100 µg/l. This appears to be a wider range than was achieved by most of the researchers listed in Table 1.

In some applications, it might not be necessary to develop a calibration curve that covers four orders of magnitude in analyte concentration, because the analyte concentrations might be known to vary over a relatively small range. For instance, if a group were monitoring the concentrations of BPA and E2 in the effluent of a wastewater plant over time, we might expect the contaminant concentrations to be relatively steady, and the concentrations might fluctuate over time only by a factor of 10 or so. However, other applications would benefit from the ability to monitor analyte concentrations over multiple orders of magnitude. For instance, if a research group were looking at removal or biodegradation of EDCs to

protect human health, it might be desirable to quantify contaminant removals up to 99.9% or even 99.99% efficiency, which would require the ability to measure analyte concentrations over multiple orders of magnitude. In such instances, we recommend using a range of sample volumes, where the sample volume selected varies inversely with the expected concentration in the sample.

Selection of derivatization agent (MSTFA or BSTFA)

For BPA and E2, we observed no difference in the performance of MSTFA (with 1% TMCS) and BSTFA (with 1% TMCS) as derivatizing agents. This might be partly because we selected the derivatization temperature and time (65°C for 25 min) to maximize the degree of silylation, i.e., complete derivatization was achieved with both agents tested. We conclude that for analysis of BPA and E2, either derivatizing agent may be used with equal confidence. However, this conclusion might not be valid for other target analytes besides BPA and E2. For instance, Quintana et al. (2004) observed that MSTFA could fully silylate 17 α -ethinylestradiol (EE2), but that BSTFA could silylate only the aromatic hydroxyl groups, not the aliphatic. Zhou et al. (2007) similarly saw differences in the degree of silylation of estrone and EE2 depending on which derivatization agent was selected, and on whether small amounts of TMCS or TMSI were added as silylation promoters. Therefore, we conclude that silylation is likely a viable method of derivatization for many EDCs (including steroid hormones, bisphenols, and alkylphenols), but that research groups should evaluate candidate derivatization agents for their effectiveness on the particular analyte(s) of interest.

Applicability to other EDCs

In this paper we have considered two particular EDCs, BPA and E2. In many cases, the techniques and conditions employed here for BPA and E2 may also apply to other chemically similar EDCs, such as alkylphenols or steroid hormones; indeed, many of the studies listed in Table 1 included other EDCs besides BPA and E2. However, researchers should not necessarily assume that all chemically similar EDCs will behave the same during SPE. It has been observed, for instance, that recovery of nonylphenol by SPE sometimes differs from

the recovery of BPA (Carabias-Martínez et al. 2004, Beck et al. 2005). Researchers should exercise care in ensuring that methods employed are appropriate for the particular analyte(s) of interest.

Conclusions

- (1) Analysis of BPA and E2 in aqueous samples by SPE with GC/MS was reviewed. Although the body of work in the literature has demonstrated the successful use of SPE in many applications, questions still remain as to how to optimize SPE for analysis of EDCs.
- (2) With regard to extraction methodology, recoveries of BPA and E2 from the two cartridges [C_{18} and poly(divinylbenzene-co-N-vinylpyrrolidone)] were not different at a statistically significant level.
- (3) For derivatization and analysis of BPA and E2, the efficiency of MSTFA and BSTFA derivatization agents was similar. With regard to selection of derivatization agent, either MSTFA or BSTFA may be used when SPE is the extraction method. Calibration curves were

nearly identical with the two derivatization agents. However, this conclusion may not hold for analytes other than BPA and E2.

- (4) Calibration curves for analysis of BPA and E2 were presented as measured fragment peak area vs. mass of analyte applied ($M=V \cdot C$). These calibration curves were linear over several orders of magnitude. The range of linearity can be extended by varying the sample volume applied to the SPE cartridge. The concentration range for both BPA and E2 was 10 ng/l to 100 µg/l, a range of four orders of magnitude.

Acknowledgments: This material is based upon work supported by the state of Florida via the Sustainable Healthy Communities initiative at the University of South Florida (USF). Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the views of USF or the state of Florida.

Received July 11, 2013; accepted October 15, 2013; previously published online December 14, 2013

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