Evaluation of Derivatization Strategies for the Comprehensive Analysis of Endocrine Disrupting Compounds using GC/MS

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Abstract

The expanding list of endocrine disrupting compounds (EDCs) has increased the need for the development of improved monitoring methods to evaluate exposure. Furthermore, the diverse physiochemical properties of EDCs impose inherent analytical limitations, and, thus, a new comprehensive method that can simultaneously analyze numerous EDCs in one chromatographic analysis would be a significant improvement over current EDC detection methods. Gas chromatography/mass spectrometry (GC/MS) offers promising profiling capabilities; however, many polar EDCs require derivatization for adequate detection. Here, a novel method for the comprehensive profiling of EDCs that employs a silyl derivatization strategy to expand the polarity range of compounds able to be separated and detected in a single chromatographic analysis is presented. The comprehensive method successfully separates 21 GC-ready and 12 derivatization-required EDCs in one chromatographic analysis. Thermal and microwave derivatization methods are effective for a comprehensive EDC mixture, although the microwave derivatization often proves more effective in shorter analysis time. A pilot-study of spiked surface water from Lake Apopka (Florida) demonstrates the potential of the comprehensive EDC profiling method.

Introduction

Over the past twenty years, a renewed interest in environmental monitoring has been focused on elucidating the biological role of endocrine disrupting compounds (EDCs) (1). Introduced in *Our Stolen Future* (2), EDCs are usually characterized as compounds, both natural and synthetic, that possess the potential to alter normal endocrine function. An example of the potency of EDCs and their consequential effect on wildlife can be observed at Lake Apopka in central Florida. Along with extensive agricultural and municipal pollution, the lake experienced a pesticide spill in 1980, and several published reports since have focused on investigating the effect of these compounds on the wildlife (2–4). One sentinel species used to assess the quality of the environment, the American alligator (*Alligator mississippiensis*), has exhibited not only detectable levels of EDCs in eggs (5) and serum (6), but also altered sex steroid concentrations (7–10), reduced clutch viability (11), increased abnormalities in bone density (12), and reproductive morphological abnormalities (6–10) when compared to alligators from reference lakes. The potency of these chemicals is even more problematic due to their resilience in the environment, their powerful activity at low concentrations, and their ability to accumulate through various food webs (2,13–15). Furthermore, many of the metabolites and degradation products of these suspected EDCs are largely uncharacterized and may possess similar disruptive properties.

Although there is a lack of definitive explanations as to the cause/effect relationship of EDCs to various human disease states (unlike many wildlife studies), recent human studies examining EDCs suggest not only an obvious burden in the environment, but also a substantial level of health risk to the general public (14,15). EDC exposure is one suspected cause for the increase in human ailments such as decreased sperm levels (13,14,16,17), preterm birth (13,14), obesity (14), and breast cancer (13,14,16,17). Furthermore, the list of chemicals known to have endocrine disrupting capabilities is rapidly expanding in both number and variety, raising the need for the development of a comprehensive EDC profiling technique that is capable of analyzing many EDC types in a variety of biological and environmental samples.

Current methods to characterize EDCs are often limited in scope, since only compounds of similar chromatographic properties are analyzed. The recent trend in the analysis of EDCs has been to combine chromatographic techniques for detection with bioassays to measure hormonal activity (18). A comprehensive EDC profile, coupled with a bioassay, would provide the ability to examine EDCs and EDC-induced responses on a wider scope. The utility of gas chromatography–mass spectrometry (GC/MS) has been well-reported for the characterization of EDCs due to its amenability to many chemical functionalities, including phthalates (19–21), pesticides (19–24), and polycyclic aromatic

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hydrocarbons (24). However, the analysis of non-amenable GC compounds, such as bisphenol A (25–35), alkylphenols (25–35), natural and synthetic steroids (25–35), and polar byproducts of many non-polar EDCs (21), requires time-consuming derivatization procedures to achieve adequate sensitivity. For the analysis of a mixture of many EDC types, traditional GC/MS analysis often needs to be supplemented by extra derivatization (21) or additional liquid chromatography–mass spectrometry (LC/MS) procedures for the inclusion of polar EDCs (23,24). LC/MS has been a desirable alternative for the detection of many of these polar EDC classes (23,24,34,36–42) because it avoids the tedious derivatization procedures. However, the chromatographic resolving power of LC is generally inferior to GC; thus, the comprehensive profiling of a wide polarity range of EDCs in one chromatographic run is a challenge. To our knowledge, there are no comprehensive methods which take advantage of the superior chromatographic separation provided by GC for the analysis of both GC-ready and derivatization-required EDCs in a single chromatographic analysis.

Although the coupling of GC/MS with derivatization chemistry can be time-consuming and laborious, it provides additional selectivity and expands the range of compounds that can now be separated and characterized in a single injection. The most common derivatization technique for the analysis of non-amenable EDCs is silylation, which typically employs either *N,O*-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) or *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide (MSTFA). The silyl derivatization reaction replaces active hydroxyl groups with a less polar TMS group. In the literature, most silyl derivatization methods require reaction conditions ranging between 60–75°C and 15–180 min (21,25–27,29–33,35). The laborious and time-consuming derivatization procedures are often considered a hindrance; however, little effort has been placed on developing methods that reduce the analysis time or improve reaction conditions. The novel use of microwave heating, specifically, microwave-accelerated derivatization, has been recently used to derivatize steroids (43,44), alcohols (45), and amino acids (46) while providing comparable results to block heating methods with a drastic reduction in analysis time.

In this study, we report the development of a simple, effective, and rapid derivatization method that provides for the comprehensive profiling of EDCs. Although there exist various methods that can employ GC/MS (without or with chemical derivatization) and LC/MS for the analysis of multiple EDC types, to our knowledge, little effort has been focused on developing a method for the comprehensive analysis of EDCs of a wide range of polarities in a single chromatographic analysis. This comprehensive EDC method combines the superior profiling capabilities of GC/MS with a standard silyl derivatization reaction. This novel method was developed to be inclusive of a diverse spectrum of endogenous EDCs in environmental matrices. Both thermal and microwave heating derivatization methods were effective for the characterization of 33 EDCs of various chemical and biological properties. The examination investigated the increase in sensitivity achieved by derivatization, the decrease in analysis time using microwave heating, and the effectiveness of the comprehensive method at various concentrations. This comprehensive EDC method was then used in a pilot-study to detect spiked EDCs in surface water from Lake Apopka (Florida).

Experimental

Chemicals and solutions

The suspected endocrine disrupting chemicals examined were purchased from three sources; *p,p*'-DDT, *p,p*'-DDD, *p,p*'-DDE, *p,p*'-DDA, *p,p*'-methoxychlor, dicofol, dieldrin, endrin, heptachlor, heptachlor epoxide, lindane, dibutyl phthalate, α-endosulfan, trifluralin, kepone, atrazine, alachlor, and diethyl phthalate (acquired from EPA, Research Triangle Park, NC); anthracene, vinclozolin, bisphenol A, 17-β-estradiol (E_2), estrone (E_1), diethylstilbestrol (DES), ethinylestradiol (EE_2), progesterone, 2,4-dichlorophenol, coumestrol, 4-octylphenol, 4-nonylphenol, triclosan, and 1,1,1-trichloro-2,2-bis(4-hydroxyphenyl)ethane (HPTE) (acquired from Sigma Aldrich, St. Louis, MO); and resorcinol and benzophenone (acquired from Fisher Scientific, Fair Lawn, NJ). The EDCs were prepared individually in analytical grade methanol (Fisher Scientific) and added to the comprehensive mixture at a concentration of 5 µg/mL. The solutions were stored at –20°C. The purity grades of all EDCs involved in this study were labeled at 98% or higher, except kepone (89%), endrin (83%), and dibutyl phthalate (80%). Chrysene-d_{12} was added (5 µg/mL, 98% D, Sigma Aldrich) to the mixture as the surrogate. The surrogate was not added to measure the absolute derivatization efficiency; rather, it was used to measure the derivatization efficiency pertaining to various changes in the derivatization parameters. Chrysene-d_{12} was chosen because it is both derivatization and microwave inactive. The relative response factor (RRF) values were calculated by dividing the area of each EDC product by the area of chrysene-d_{12}. The comprehensive EDC mixture (all components described) was analyzed with and without derivatization procedures, with the evaluation centered on the enhancement and precision of the data.

Derivatization setup

The comprehensive EDC mixture was added (200 µL, 5 µg/mL) to standard 4-dram glass vials and blown down with ultra high purity nitrogen. The derivatization reagents were then added to the EDC residue. The derivatization reagent used for the analyses was derivatization-grade BSTFA with 1% trimethylchlorosilane (TMCS) (Supelco, Bellefonte, PA). The derivatization reactions (described later) were performed for both the thermal and microwave methods. Post-derivatization, the vials were blown down with ultra high purity nitrogen and the resulting residues were reconstituted with isooctane (99%, Fisher Scientific) and subsequently injected onto the GC. Reconstitution with isooctane helped eliminate silyl reagent noise at lower temperature levels, allowing improved examination of low-volatile EDCs. The derivatization analyses in this experiment were all run in triplicate.

Derivatization reactions: block heating

Block heating was examined to evaluate derivatization and its ability to expand the number of compounds separated and detected in comparison to methods that do not apply derivatization. Block heating also served as a reference for the evaluation of the use of microwave derivatization. The thermal derivatization reactions were performed using a Thermolyne Type 16500 Dri-bath block heater. The time and temperature for the block derivatization experiments were 30 min and 70°C.

Derivatization reaction: microwave heating

Microwave heating was examined as a more efficient heating approach to reduce analysis time in comparison to block heating. Microwave derivatization was performed in a 1000 W domestic Half-Time microwave/convection oven (Apollo Worldwide, Palm Beach, FL). The power levels analyzed were 500 and 900 W. The irradiation time for both power levels was one minute.

GC/MS

The GC/MS instrument used was a ThermoFinnigan (San Jose, CA) Trace GC 2000 quadrupole ion trap MS with an Autosampler AS3000. The data acquisition software used was Xcalibur 1.4. The column employed was an SLB-5ms capillary column (Supelco) with the dimensions of 30 m × 0.25 mm × 0.25 µm film thickness. The temperature program started at 70°C and was held at this temperature for 3 min. The temperature program was then increased to 150°C at a rate of 15°C/min, followed by an increase to 250°C at a rate of 7°C/min. The temperature program concluded by ramping at 5°C/min to 300°C and subsequently held for 2 min. The carrier gas was ultra-high purity helium (99.99%) at a flow rate of 1 mL/min. The transfer line, ion source, and injection port temperatures were 275, 200, and 280°C, respectively. Splitless injection (2 µL) was performed with a split flow of 50 mL/min (split flow ratio of 10). The MS was turned on at 7 min and was run in positive full scan mode (approximately 1000 amu/s), *m/z* 50–600, with electron ionization. The retention times (t_r) used for identification and the ions used for quantification of each EDC product and the surrogate can be found in Tables I (underivatized) and II (derivatized).

Semi-quantitative calibration

The calibration study was performed to elucidate the effectiveness of the comprehensive EDC profile at various concentrations. Standard EDC solutions were prepared in triplicate at 5, 1, 0.5, 0.1, and 0.05 µg/mL. Two separate calibration analyses were performed, each containing all of the EDCs in the study and the surrogate chrysene-d_{12} (added to each EDC solution at a concentration of 0.5 µg/mL). Calibration 1 was performed to determine the detection limit of the EDCs without any derivatization reagent added. Calibration 1 analysis was achieved by reconstituting the EDC residue with isooctane. Calibration 2 was performed to measure the detection limit on two levels: the derivatization-required compounds and the GC-ready compounds in the presence of derivatization reagent. Calibration 2 was achieved by reconstituting the EDC mixture with derivatization reagent, followed by performing the derivatization reaction (microwave: 900 W for 1 min). Post-reaction, the solution was blown down with nitrogen and reconstituted with isooctane. Blank solutions in methanol (with and without chrysene-d_{12}) were evaluated with and without derivatizing reagent. The blank solutions were reconstituted with isooctane prior to GC analysis and were run in triplicate. The semi-quantitative calibration was performed by approximating the effective concentration ranges [peaks observed with signal-to-noise ratio (S/N) > 10] of the EDCs analyzed with the comprehensive profiling method. The detection limit levels were selected based on finding an appropriate concentration to detect as many EDCs as possible. The method was not designed as a single component assay, thus some EDCs may have much lower detection limits when run individually. Here, we focused on obtaining the best detection limits for a comprehensive list of EDCs (both polar and non-polar) in a single analysis.

Pilot-study for water analysis

Surface water was collected from various locations at Lake

Table I. Characteristic Ions for the Underivatized EDCs*

Name	MW	Function	Underivatized ions (*m/z*)		t_r*
			Column 1	Column 2	
Coumestrol	268.2	Phytoestrogen	–	–	ND
p,p′-DDA	281.1	DDD metabolite	–	–	ND
HPTE	317.5	Methoxychlor degradation	–	–	ND
2,4-Dichlorophenol	163.0	Pesticide intermediate	63	162	7.29
Resorcinol	110.1	Plasticizer	82	110	8.46
Diethyl Phthalate	222.2	Plasticizer	149	177†	12.05
Benzophenone	182.2	Plastic	77	182	12.73
Trifluralin	335.3	Pesticide	264	306†	13.01
Atrazine	215.6	Herbicide	200	215	14.18
Lindane	290.8	Insecticide	181	219†	14.24
4-Octylphenol	206.3	Surfactant	107	206	14.40
Anthracene	178.2	PAH	152	178	15.02
4-Nonylphenol	220.3	Surfactant	107	220	15.80
Vinclozolin	286.1	Fungicide	178	286	16.02
Alachlor	269.7	Herbicide	160	188†	16.14
Heptachlor	373.3	Insecticide	272	371†	16.37
Dibutyl Phthalate	278.3	Plasticizer	149	278	16.88
Dicofol	370.4	Pesticide	139	251†	17.61
Heptachlor Epoxide	389.3	Heptachlor metabolite	353	355†	18.34
Triclosan	289.5	Antibacterial agent	218	289	18.96
α-Endosulfan	406.9	Insecticide	195	241†	19.33
Bisphenol A	228.3	Plastics	213	228	19.77
p,p′-DDE	318.0	DDT degradation	246	318	19.87
Dieldrin	380.9	Insecticide	79	263†	20.05
Endrin	380.9	Insecticide	281	317†	20.60
p,p′-DDD	320.0	DDT degradation	165	235†	20.98
Kepone	490.6	Insecticide	237	272†	21.57
Diethylstilbestrol	268.3	Synthetic estrogen	145	268	21.78
p,p′-DDT	354.4	Pesticide	165	235†	21.97
Chrysene-d_{12}	240.3	Surrogate (IS)	240	241	23.36
p,p′-Methoxychlor	345.6	Insecticide	227	228†	23.43
Estrone	270.3	Natural estrogen	185	270	25.36
17β-Estradiol	272.3	Natural estrogen	213	272	25.57
Ethinylestradiol	296.4	Synthetic estrogen	213	296	26.37
Progesterone	314.4	Natural progestogen	124	314	27.80

* t_r = average (n = 3) retention time in minutes. ND indicates not detected. Column 1 represents base peak ions, Column 2 represents the molecular ion (except if not present = †).

Apopka (Florida) and was stored at –20°C. Lake Apopka was selected because of its high level of contamination and turbidity. The EDC mixture was spiked in the Lake Apopka water samples at a concentration of 0.1 µg/mL. Due to the high turbidity of the water samples, solid-phase extraction was necessary. The water sample was loaded (3 mL) onto a Strata C-18E cartridge (Phenomenex, Torrance, CA), which had been preconditioned with ethyl acetate (2 mL), acetonitrile (2 mL), and deionized water (2 mL). Post sample loading, the cartridge was dried for 15 min. The EDCs were eluted with ethyl acetate (1 mL). The resulting extract was blown down at room temperature with nitrogen using a PrepSep vacuum manifold (Fisher Scientific). BSTFA with 1% TMCS was added to the residue (200 µL) and reacted in the microwave for 1 min at 900 W. The derivatized sample was then blown down, reconstituted in isooctane, and subsequently injected into the GC.

Table II. Characteristic Ions for the Derivatized EDCs

	Mono-TMS Ions (*m/z*)			Di-TMS Ions (*m/z*)		
Name	Column 1	Column 2	t_r*	Column 1	Column 2	t_r
p,p′-DDA	200	337	19.98	–	–	ND
Dicofol	73	323	23.20	–	–	NA
Bisphenol A	–	–	ND	357	372	20.17
Coumestrol	–	–	ND	207	412	30.29
4-Nonylphenol	179	292	16.41	–	–	NA
4-Octylphenol	179	278	15.07	–	–	NA
2,4-Dichlorophenol	219	235	9.42	–	–	NA
HPTE	343	390	24.58	–	–	ND
Resorcinol	167	182	9.13	239	254	9.50
Triclosan	200	362	19.17	–	–	NA
17β-Estradiol	244	344	ND	285	416	25.73
Estrone	257	342	25.34	399	414	ND
Ethinylestradiol	285	368	26.38	425	440	27.09
Diethylstilbestrol	311	340	20.85/21.84	217	412	20.65/21.88
Progesterone	371	386	27.48	–	–	ND
Chrysene-d_{12}	–	–	NA	–	-	NA

* t_r = average (n = 3) retention time in minutes. ND indicates not detected. NA indicates not applicable. Column 1 represents base peak ions, Column 2 represents the molecular ion.

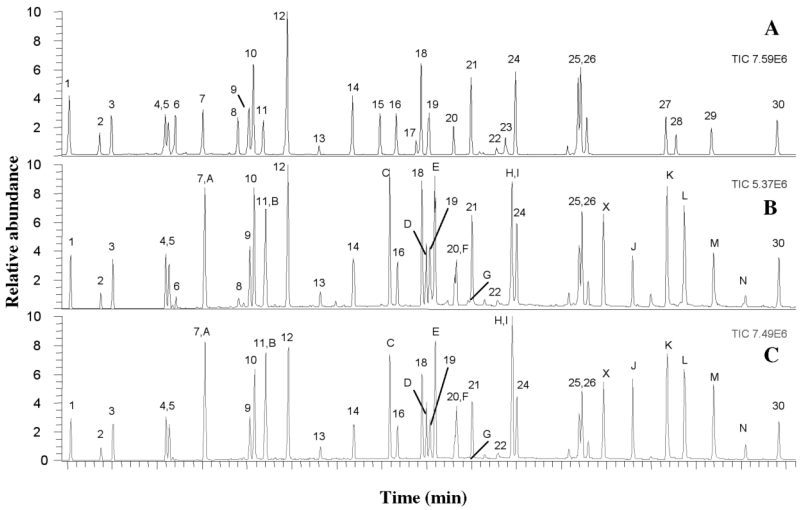

Figure 1. Chromatograms of comprehensive EDC profile with no derivatization (A), derivatization using block heating of 70°C for 30 min (B), and derivatization using microwave heating 900 watts for 1 min (C). 1 = diethyl phthalate, 2 = benzophenone, 3 = trifluralin, 4 = atrazine, 5 = lindane, 6 = 4-octylphenol, 7 = anthracene, 8 = 4-nonylphenol, 9 = vinclozolin, 10 = alachlor, 11 = heptachlor, 12 = dibutyl phthalate, 13 = dicofol, 14 = heptachlor epoxide, 15 = triclosan, 16 = α-endosulfan, 17 = bisphenol A, 18 = p,p′-DDE, 19 = dieldrin, 20 = endrin, 21 = p,p′-DDD, 22 = kepone, 23 = diethylstilbestrol (DES), 24 = p,p′-DDT, 25 = chrysene-d_{12}, 26 = p,p′-methoxychlor, 27 = estrone, 28 = 17β-estradiol, 29 = ethinylestradiol, 30 = progesterone. A = mono-TMS-4-octylphenol, B = mono-TMS-4-nonylphenol, C = mono-TMS-triclosan, D = mono-TMS-p,p′-DDA, E = di-TMS-bisphenol A, F = di-TMS-DES-1, G = mono-TMS-DES-1, H = di-TMS-DES-2, I = mono-TMS-DES-2, J = mono-TMS-HPTE, K = mono-TMS-estrone, L = di-TMS-17β-estradiol, M = mono-TMS-ethinylestradiol, N = di-TMS-ethinylestradiol. Mono-TMS-resorcinol (t_r < 12 min) and di-TMS-coumestrol (t_r > 28 min) not shown in figure. Peak labeled X = phthalate contaminant.

Results and Discussion

Characterization of potential EDC compounds

Non-polar EDCs, including some pesticides, phthalates, and polycyclic aromatic hydrocarbons, pose no analytical difficulty using GC/MS due to their volatility and thermal stability; therefore, the key for the development of a comprehensive EDC profile was to expand the range of compounds amenable to GC/MS by incorporating more polar EDCs, including alkylphenols, bisphenol A, steroids, and non-polar EDC by-products, by employing silyl derivatization. The comprehensive method permits a profile of both non-polar and polar EDCs in a single analysis. The success of the comprehensive method was evaluated on two levels: (*i*) the derivatization reaction not affecting the GC-ready compounds, and (*ii*) the derivatization method providing effective derivatization of the polar EDCs. Although BSTFA with 1% TMCS was used in this analysis, it was also found that other silylating reagents, such as MSTFA, were also efficient for derivatization of the comprehensive EDC mixture.

Comprehensive EDC profile

Figure 1A–1C shows three chromatograms reflecting variation in the EDC mixture (each at a concentration of 5 µg/mL): (*i*) no derivatization reaction, (*ii*) after thermal derivatization (70°C for 30 min), and (*iii*) after microwave derivatization (900 W for 1 min). Chromatogram A demonstrates the successful separation of all the GC-ready (non-polar) EDCs (peaks 1–5, 7, 9–14, 16, 18–22, 24–26, and 30) and many of the polar EDCs underivatized (peaks 6, 8, 15, 17, 23, 27–29). The underivatized polar EDCs, which typically require derivatization for characterization by GC/MS, are present due to the high concentration employed in this analysis.

Chroma-tograms B and C illustrate the separation achieved of the EDC profile after the derivatization reaction was employed. As illustrated in both chromatograms B and C, the separation of the 21 GC-ready EDCs was not affected by the derivatization reaction. Furthermore, the polar EDCs [peaks 6, 8, 15, 17, 23, 27, 28, and 29 in chromatogram A] were derivatized and separated effectively and in most cases with an increase in sensitivity (peaks A–C, E–I, and K–N). The comprehensive method with derivatization extended the polarity range of compounds separated by also characterizing derivatized HPTE and *p,p*'-DDA (peaks D and J), which were not detected in Figure 1A. The method also characterized derivatized coumestrol (t_r > 28 min) and resorcinol (t_r < 12 min) but are not shown in the figure. The chromatograms from both the thermal and microwave derivatization reactions were comparable, demonstrating a significant reduction in analysis time with no loss of derivatized EDCs by employing microwave heating.

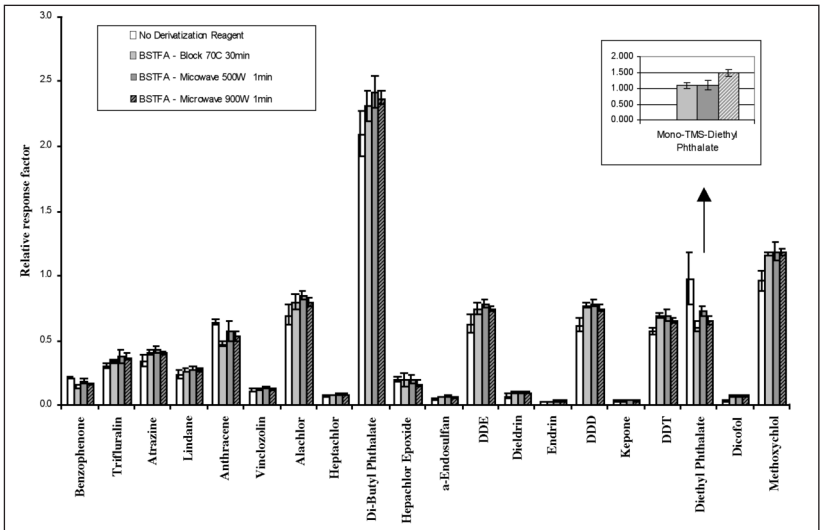

Figure 2. Relative response factors (RRFs) for the underivatized (GC-ready) EDCs in the comprehensive profile with and without the presence of derivatizing reagent (each at a concentration of 5 µg/mL). The inset displays an unwanted derivatization product of the ester group of diethyl phthalate. Error bars represent the standard deviation of the mean (n = 3).

Non-derivatized EDCs

Figure 2 shows the RRF values for the GC-ready (non-polar) EDCs (each at a concentration of 5 µg/mL). Figure 2 shows little difference between the RRF values obtained when derivatization was employed (either by block or microwave heating) and those obtained with no derivatization. It was anticipated that dicofol, which has a hydroxyl group, would need derivatization to make it through the GC, but it was found predominantly in the underivatized form. The figure also shows a reduced RRF value for diethyl phthalate when derivatizing reagent is added. The inset (A) in Figure 2 shows that when derivatization is applied, the ester group of the diethyl phthalate reacts with the derivatization reagent to form an alternate derivatization product, mono-TMS-diethyl phthalate. Alternate derivatization products, or artifacts, are the result of the formation of partial derivatives due to excessive reaction conditions or side reactions. The comprehensive method attempted to minimize artifacts such as mono-TMS-diethyl phthalate.

Derivatized steroid EDCs

Figure 3A shows the RRF values for the steroid EDCs (each at a concentration of 5 µg/mL), which typically require derivatization for GC/MS analysis. The RRF values for the target derivatized steroids (di-TMS-DES, mono-TMS-E_1, di-TMS-E_2, mono-TMS-EE_2) were approximately 10× larger than the RRF values for all of the underivatized steroids examined ($M^{+\cdot}$), which illustrates the value of derivatization in providing greater sensitivity. The RRF value for di-TMS-EE_2 (the complete derivatization product) was lower than the RRF value for the underivatized species. Under the current derivatization conditions examined in this study, the mono-derivative of EE_2 (mono-TMS-EE_2) was favored and had the highest RRF values. The RRF value for progesterone, which doesn't require derivatization to make it through the GC, is much higher than the mono-TMS-progesterone, which indicates that the derivatization method does not promote the formation of this artifact. Diethylstilbestrol (DES) had two isomeric peaks for each derivative (mono- and di-) formed; they were summed to represent total DES products. Di-TMS-DES was found to be the predominant derivatization product of DES.

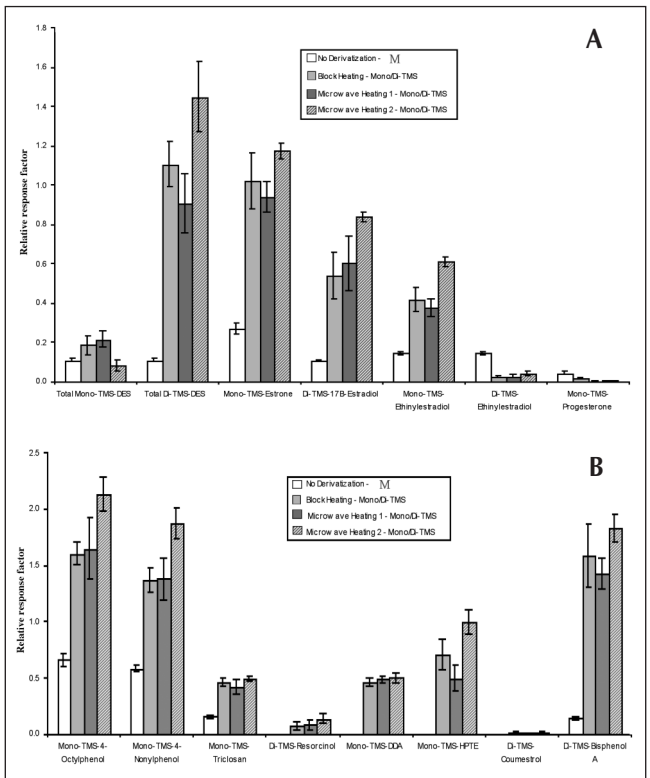

Figure 3. RRFs for the derivatized steroid (A) and other polar EDCs (B) with and without derivatization (each at a concentration of 5 µg/mL). No derivatization = detected underivatized species, Derivatization = block and microwave heating – detected mono/di-TMS species. Thermal derivatization was performed at 70°C for 30 min, Microwave 1 and Microwave 2 derivatization methods were performed at 500 W and 900 W for 1 min, respectively. Error bars represent the standard deviation of the mean (n = 3).

Other derivatized EDCs

Figure 3B shows the RRF values for the non-steroidal EDCs which required derivatization (each at a concentration of 5 µg/mL), including the alkylphenols, bisphenol A and polar by-products. The two polar by-products studied in this analysis were HPTE and *p,p*'-DDA, by-products of *p,p*'-methoxychlor and p,p'-DDT, respectively. Derivatization was necessary for these polar EDCs, as the underivatized species was either not detected or much lower than the derivatized species under the GC/MS conditions in this analysis. 2,4-dichlorophenol was not detected using the current method, and thus was not included in the figure.

Table III. Approximate Detection Limits using Comprehensive EDC Method

Non-polar EDCs	No reagent (ppb)	With reagent (ppb)	Polar EDCs	No reagent (ppb)	With reagent (ppb)
p,p'-DDE	< 50	< 50	p,p'-DDA	ND	100†
p,p'-DDT	< 50	< 50	Bisphenol A	100–500	< 50‡
p,p'-DDD	< 50	< 50	Resorcinol	ND	100‡
Dicofol	50	100–500	4-Nonylphenol	100	< 50†
p,p'-Methoxychlor	500	500	4-Octylphenol	100	< 50†
Dieldrin	50	500	HPTE	ND	< 50†
Endrin	500	500	Triclosan	500	< 50†
Heptachlor	100	100–500	17β-Estradiol	1000–5000	< 50‡
Heptachlor Epoxide	< 50	50–100	Estrone	500	< 50†
Lindane	< 50	< 50	Ethinylestradiol	500–1000	100†
Dibutyl Phthalate	< 50	< 50	Diethylstilbestrol	5000	< 50‡
α-Endosulfan	50–100	500	Coumestrol	ND	500‡
Anthracene	< 50	50			
Vinclozolin	< 50	50–100			
Trifluralin	< 50	50–100			
Kepone	500–1000	1000			
Atrazine	< 50	< 50			
Benzophenone	< 50	100–500			
Alachlor	< 50	< 50			
Diethyl Phthalate	< 50	50–100			
Progesterone	100–500	100–500			

† Mono-TMS species; ‡ Di-TMS species.

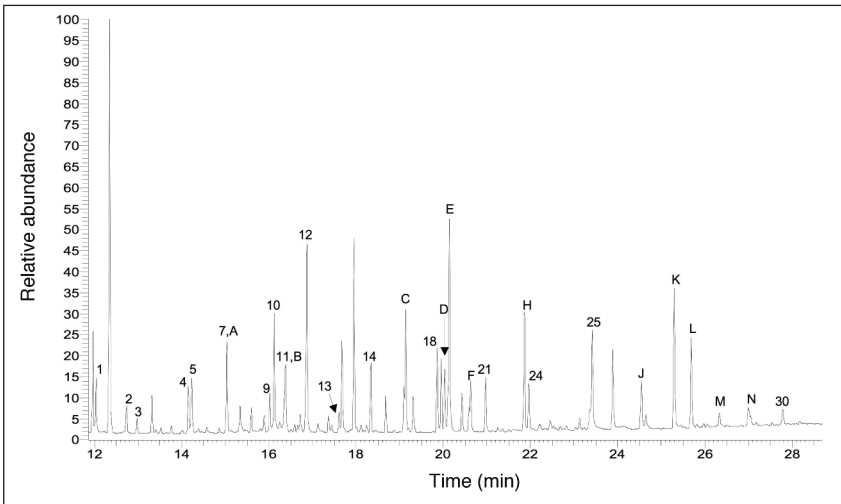

Figure 4. Chromatogram of 100 ppb (0.1 µg/mL) EDC mixture spiked in extracted Lake Apopka water. The EDCs detected were: 1 = diethyl phthalate, 2 = benzophenone, 3 = trifluralin, 4 = atrazine, 5 = lindane, 7 = anthracene, 9 = vinclozolin, 10 = alachlor, 11 = heptachlor, 12 = dibutyl phthalate, 13 = dicofol, 14 = heptachlor epoxide, 18 = p,p'-DDE, 21 = p,p'-DDD, 24 = p,p'-DDT, 25 = chrysene-d_{12}, 30 = progesterone. A = mono-TMS-4-octylphenol, B = mono-TMS-4-nonylphenol, C = mono-TMS-triclosan, D = mono-TMS-p,p'-DDA, E = di-TMS-bisphenol A, F = di-TMS-DES-1, H = di-TMS-DES-2, J = mono-TMS-HPTE, K = mono-TMS-estrone, L = di-TMS-17β-estradiol, M = mono-TMS-ethinylestradiol, N = di-TMS-ethinylestradiol. Mono-TMS-resorcinol (t_r < 12 min) is not shown in figure.

Microwave derivatization

The RRF values for the derivatized EDCs were typically the lowest using the microwave heating at 500 W for 1 min. However, microwave heating at 900 W for 1 min was better or comparable to the thermal heating method at 70°C for 30 min for all the EDCs derivatized (Figure 3). No distinct disadvantage was apparent for the derivatization of the EDC mixture with microwave heating.

Semi-quantitative calibration

A series of semi-quantitative experiments were performed to estimate the potential detection limits of the polar and non-polar EDCs using the comprehensive profiling method. The approximate detection limits (in ppb, Table III) were estimated by observing peaks with S/N greater than 10. With the addition of derivatization reagent, some of the GC-ready EDCs experienced poorer detection limits (dicofol, dieldrin, heptachlor, α-endosulfan, vinclozolin, trifluralin, heptachlor epoxide, benzophenone, and diethyl phthalate). The application of derivatization reagent does sacrifice the detection limit of some GC-ready EDCs; however, the tradeoff comes with a gain in the number of components analyzed in a single analysis. The focus was toward a single method for screening EDCs (both polar and non-polar) using the comprehensive list. This method may be the most effective for analyzing smaller subsets, for example, the analysis of DDT and its polar degradation products (such as DDA) in a single analysis. Previous analyses for this subset (and for similar mixtures of polar and non-polar EDC subsets) have required separate analyses for both polar types (23,24).

The rest of the GC-ready EDCs were not affected by the derivatization reagent and were detected well below 0.05 µg/mL. The detection limits for all of the derivatized polar EDCs were much lower than those for the underivatized species. The polar EDCs di-TMS-coumestrol, mono-TMS-DDA, di-TMS-resorcinol, and mono-TMS-ethinylestradiol were the only derivatized species that did not yield estimated detection limits below 0.05 µg/mL.

Spiked water analysis

Figure 4 displays a chromatogram of the EDC mixture spiked into a water sample from Lake Apopka. Although measurable levels of some native EDCs were detected in the water, the focus of this pilot-study was to demonstrate and evaluate the effectiveness of the comprehensive method for the characterization of EDCs exhibiting a wide range of polarities. Even with the high turbidity, the use of solid-phase extraction allowed the characterization of most of the EDCs in this study at a concentration of 0.1 µg/mL. As shown in Figure 4, many of the GC-ready (peaks 1–5, 7, 9–14, 18, 21, 24, 25, 30) and derivatization-required (peaks A–F, H, J–N) EDCs were successfully detected using the comprehensive method. Although intended as a screening method, the percent recovery of the spiked Lake Apopka water (0.1 µg/mL) was evaluated by using a semi-quantitative calibration plot (0.05–5000 µg/mL). The average percent recovery was above 90% for 13 out of the 25 EDCs in the spiked water. Since the comprehensive list covers a variety of EDC species (with different polarities), the remaining 12 EDCs had recoveries between 25% and 90%. The EDCs used for the recovery study are shown in Figure 4. The underivatized species of the polar EDCs were not found at this concentration.

Conclusion

The presence of multiple EDC types in biological and environmental samples has pushed the need for analytical methods that expand the polarity range of compounds able to be analyzed in a single analysis. Here it was demonstrated that the superior profiling capabilities of GC/MS with a standard silyl derivatization reaction for the development of a comprehensive EDC profile is capable of analyzing not only compounds traditionally amenable to GC, but also those compounds that require derivatization. Microwave derivatization provided comparable results to the thermal derivatization method with a significant decrease in analysis time. The comprehensive EDC method was evaluated semi-quantitatively at several concentrations to explore the effective concentration ranges for each EDC. Finally, the comprehensive EDC profile was effective in detecting several polar and non-polar EDCs in a spiked water sample. Future analyses will examine and compare native EDC species detected from both Lake Apopka and reference lakes.

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