



Gas chromatography–mass spectrometry screening methods for select UV filters, synthetic musks, alkylphenols, an antimicrobial agent, and an insect repellent in fish

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ARTICLE INFO

Article history:

Received 20 November 2007

Received in revised form

12 November 2008

Accepted 17 November 2008

Available online 3 December 2008

Keywords:

PCPs

UV filters

Synthetic musks

Alkylphenol surfactants

Galaxolide

Tonalide

Benzophenone

Triclosan

Fish

Analysis

GC–MS

ABSTRACT

Two screening methods have been developed for simultaneous determination of ten extensively used personal care products (PCPs) and two alkylphenol surfactants in fish. The methods consisted of extraction, clean-up, derivatization and analysis by gas chromatography–mass spectrometry with selected ion monitoring (GC–SIM–MS) or gas chromatography–tandem mass spectrometry (GC–MS/MS) techniques. Among solvents tested to assess recovery of target compounds from 1-g tissue homogenates, acetone was selected as optimal for extracting compounds with dissimilar physicochemical properties from fish tissue. Initial experiments confirmed that GC–SIM–MS could be applied for analysis of lean fillet tissue (<1% lipid) without gel-permeation chromatography (GPC), and this approach was applied to assess the presence of target analytes in fish fillets collected from a regional effluent-dominated stream in Texas, USA. Benzophenone, galaxolide, tonalide, and triclosan were detected in 11 of 11 environmental samples at concentrations ranging from; 37 to 90, 234 to 970, 26 to 97, and 17 to 31 ng/g, respectively. However, performance of this analytical approach declined appreciably with increasing lipid content of analyzed tissues. Successful analysis of samples with increased lipid content was enabled by adding GPC to the sample preparation protocol and monitoring analytes with tandem mass spectrometry. Both analytical approaches were validated using fortified fillet tissue collected from locations expected to be minimally impacted by anthropogenic influences. Average analyte recoveries ranged from 87% to 114% with RSDs <11% and from 54% to 107% with RSDs <20% for fish tissue containing <1% and 4.9% lipid, respectively. Statistically derived method detection limits (MDLs) for GC–SIM–MS and GC–MS/MS methodologies ranged from 2.4 to 16 ng/g, and 5.1 to 397 ng/g, respectively.

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1. Introduction

The environmental occurrence of personal care products, such as ultra-violet filters (UVFs), synthetic musks (SMs), antimicrobials, and insect repellents has been increasingly reported in literature [1–4]. Of particular relevance are effluent-dominated streams, which represent “worse case scenarios” for studying personal care products (PCPs) and other organic wastewater contaminants [5]. In these streams, even compounds with relatively short environ-

mental half-lives may be considered pseudopersistent due to their continuous introduction from a wastewater treatment plant. As a result, organisms residing in these aquatic systems receive continuous exposures to wastewater-derived contaminants, such as PCPs over their entire life cycle. Recent reports from several research groups [6–9] have demonstrated that environmental exposure to PCPs results in accumulation of parent compounds, their metabolites, or both in tissues of aquatic organisms. More significantly, a series of studies has also identified that nitromusk fragrances are not only accumulated but are subsequently metabolized to reactive intermediates that form covalent protein adducts [9,10].

Alkylphenols (APs), such as *p-n*-nonylphenol, represent a second class of organic wastewater contaminants (OWCs) that has also received broad coverage in literature. These compounds are ubiquitous constituents of industrial products such as, emulsifiers, paints,

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detergents and other cleaning products. They are perhaps the most widely studied wastewater contaminants due to their ability to promote endocrine disruption in aquatic organisms [11]. Multiple studies have demonstrated their occurrence in a variety of environmental matrices, including tissues [12–18], continued investigation of partitioning and accumulation for these and other OWCs is critical to comprehensive assessment of potential consequences of environmental exposures.

Analytical protocols for determination of OWCs in water, soil, sediment, and biosolids are numerous and have been summarized in recent reviews [19–21]. While targeted analytical methods for determination of UVFs [22–24], SMs [25–30], APs [12–14], antimicrobials [31,32], and insect repellents [33,34] continue to be reported, an increasing trend in environmental chemistry is the development of protocols affording simultaneous analysis of compounds belonging to different analyte classes [4,28,35–37]. However, analytical methods for determination of OWCs in tissue have continued to be comparatively limited in scope. For example, recent protocols have enabled determination of 4 UVFs and methyl-triclosan [6,38,39], 12 SMs [8,40–43], 9 APs [15–17,18,44], or chlorophene, triclosan and its three chlorinated derivatives [7,45,46].

The objective of this work was to develop a screening method for OWCs that was complementary to a previously reported multi-residue approach for determination of pharmaceuticals in fish [47]. Herein we report two GC–MS methodologies affording simultaneous analysis of 3 UVFs, 5 SMs, 2 APs, the antimicrobial agent triclosan, and the insect repellent *m*-toluamide. Target compounds were selected based solely on relatively high frequency of detection in previous work [22–34]. The analytical approach was initially focused on developing a GC–MS method that employed selective ion monitoring (GC–SIM–MS) and alleviated the time and labor intensive gel-permeation chromatography (GPC) step from more typical sample preparation protocols for tissue. While this approach proved to be successful for analysis of lean tissue specimens (i.e., specimens containing <1% lipid by weight), a more rigorous protocol that included GPC and tandem MS detection (GC–MS/MS) was required to accommodate samples with higher lipid content. Statistically derived method detection limits (MDLs) were determined for both GC–SIM–MS and GC–MS/MS methods and compared with previously reported environmental concentrations for similar matrices. In order to demonstrate the efficacy of the simplified approach in a practical setting, GC–SIM–MS methodology was employed to screen fish collected from a regional effluent-dominated stream. This work represents the first approach enabling routine monitoring of select UV-filters, fragrances, surfactants, an antimicrobial agent and an insect repellent in fish tissue using a single chromatographic run.

2. Experimental

2.1. Chemicals and materials

All chemicals were reagent grade or better, obtained from commercial vendors, and used as received. The reference standards: benzophenone, 4-methylbenzylidene camphor (4-MBC), *m*-toluamide, *p*-octylphenol, galaxolide (55.8%), tonalide, musk xylene, musk ketone, triclosan, octocrylene, and *para*-nonylphenol, and surrogate standards: pentachloronitrobenzene, and [²H₁₀]benzophenone (benzophenone-*d*₁₀), and internal standard [²H₁₀]phenanthrene (phenanthrene-*d*₁₀) were purchased in the highest available purity from Sigma–Aldrich (Milwaukee, WI, USA). Celestolide was obtained from Cambridge Corp. (San

Diego, CA, USA). The surrogate standard [¹³C₆]p-*n*-nonylphenol was acquired from Cambridge Isotopes Labs. (Andover, MA, USA). The internal standard mirex was purchased from Cerilliant (Round Rock, TX, USA). The derivatizing agent *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) was obtained from VWR Scientifics (Irving, TX, USA). Silica gel (grade 60, 70–230 mesh, 60 Å), *n*-hexane (HPLC grade), and acetone (spectrophotometric grade) were also obtained from Sigma–Aldrich. Dichloromethane was purchased from Fisher Scientific. Distilled water was purified and deionized to 18 MΩ with a Barnstead Nanopure Diamond UV water purification system.

2.2. Tissue samples

Tissues analyzed in this work originated from three different locations. Bluegill (species *lepomis*) were initially sampled from Pecan Creek (*n* = 11) and Clear Creek (*n* = 20) in Denton County, TX, USA. Details of sampling and homogenization for fish collected at these sites have been described elsewhere [47]. For the purposes of the present study, it is important to note that annual flows in Pecan Creek are comprised almost entirely of effluent discharge from the Pecan Creek Water Reclamation Plant. In contrast, Clear Creek is not impacted by effluent discharges. Bluegill muscle tissue was homogenized using a Tissuemiser set to rotate at 30,000 rpm. Additional fish tissue analyzed in this work was derived from an alternative species (Sonora sucker) collected on the East Fork Gila River in New Mexico. Sampling, homogenization, and compositing of Sonora sucker tissues followed standard US Environmental Protection Agency (EPA) protocols [48,49]; homogenization was carried out using a laboratory blender (Waring Commercial, TX, USA) set to rotate at 22,000 rpm. Similar to Clear Creek, the Gila River is expected to have minimal impact from anthropogenic sources. However, tissue homogenates derived from Sonora sucker were composed of muscle tissue, belly flap and skin, while those derived from Clear Creek and Pecan Creek were composed solely of edible muscle (i.e., no skin or belly flap tissues). Composite homogenates prepared from Clear Creek and Gila River tissues were used for initial evaluations of method performance and determination of statistically derived MDLs. Clear Creek homogenate was also used as control matrix in the analysis of Pecan Creek samples. Finally, it is relevant to note that Pecan Creek muscle tissues analyzed in this work were derived from the same 11 fish analyzed by Ramirez et al. in a previous study [47]. All tissue specimens were stored at –20 °C prior to analysis.

2.3. Determination of tissue lipid content

Approximately 2 g of homogenized tissue were mixed with 15 ml 1:1 *n*-hexane–dichloromethane (50%, v/v) in a glass vial. The mixture was briefly homogenized using a Tissuemiser (Fisher Scientific) set to rotate at 30 000 rpm, and samples were subsequently equilibrated on a rotary extractor (15 rpm) for 18 h at 35 °C. Following extraction, solid anhydrous sodium sulfate was added to each sample (g Na₂SO₄ = 2 × g tissue). Each mixture was agitated by hand and subsequently filtered through Grade 415 filter paper. Solid residue on the filter was washed with an additional 15 ml of 1:1 dichloromethane:hexane, and the combined filtrate for each sample was collected in a pre-weighed test tube. The solvent was evaporated to dryness under a stream of nitrogen at 45 °C and residues were dried to constant weight in a vacuum oven at 40 °C. Lipid content was calculated by dividing the weight of extracted residue by the wet weight of tissue prior to extraction.

2.4. Extraction of target analytes

Approximately 1.0 g tissue (weighed to the nearest 0.1 mg) was combined with 10 ml acetone in a 20-ml borosilicate glass vial. Three surrogates were added to each sample as an acetone solution: [$^{13}\text{C}_6$]p-n-nonylphenol (80 ng), pentachloronitrobenzene (500 ng), and benzophenone-d $_{10}$ (120 ng). Target analytes were also added at this point in fortified tissues, and samples were immediately shaken vigorously and mixed on a rotary extractor for 5 min at 35 °C (in the case of tissue from Clear Creek and Pecan Creek) or sonicated for 15 min at 25 °C (in the case of tissue from East Fork Gila River). Following extraction, samples were transferred into 50-ml polypropylene copolymer round-bottomed centrifuge tubes (Nalgene, Rochester, NY, USA) using 1 ml acetone as a rinse and centrifuged at 16,000 rpm for 40 min at 4 °C. The supernatant was then transferred into 18-ml disposable, glass test tubes, and the solvent was evaporated to dryness under a stream of nitrogen at 30 °C. Samples were subsequently reconstituted in 200 μl of 65:35 (v/v) hexane–acetone.

2.5. Sample clean-up and derivatization

All samples were passed through a silica gel column prior to derivatization and analysis. One gram of silica gel was stirred in 4 ml n-hexane, and the slurry was transferred to a disposable, glass pipette. Glass wool (ca. 10 mg) was used as a porous plug to confine silica to the pipette. Silica-gel columns were preconditioned with 8 ml of 65:35 (v/v) hexane–acetone. The extract from Section 2.4 above was loaded onto the column using a second Pasteur pipette, and analytes were eluted with 30 ml n-hexane:acetone. The eluate was collected in a glass test tube and the solution volume was reduced to ca. 50 μl under a stream of nitrogen at 30 °C. When GPC was not required, the sample was transferred directly to a 350- μl GC vial insert, and the test tube was rinsed with ca. 150 μl n-hexane:acetone. One hundred microliters of MSTFA derivatizing agent was added, the GC vial was capped, and the mixture was heated in an oven at 60 °C for 45 min. Following derivatization, the sample was evaporated to a minimal volume (ca. 20 μl) at room temperature under a stream of nitrogen and reconstituted in 180 μl n-hexane. Prior to analysis, a constant amount of phenanthrene-d $_{10}$ (40 ng) or mirex (200 ng) was added as an internal standard. Note that use of MSTFA resulted in derivatization of p-n-octylphenol, p-n-nonylphenol, musk ketone, and triclosan only.

As described in more detail below (Section 3.3), an additional GPC cleaning step was required to accommodate samples derived from Sonora sucker. When GPC was required, the sample was treated as described above, but the eluate from the silica gel column was evaporated to a minimal volume and the residue was reconstituted in 700 μl dichloromethane. A 350- μl volume of this sample was injected into a Waters GPC system, consisting of a Model 600 controller, Model 717 plus autosampler, Model 486 autotunable absorbance detector, and Envirogel guard (30 mm \times 4.6 mm) and analytical (150 mm \times 19 mm) columns (Waters, Milford, MA, USA) connected in series. A Hewlett Packard Model 3398 integrator was also interfaced with the system to monitor eluting components. Dichloromethane eluent was constantly passed through the system at a flow rate of 5 ml/min, and a 40-ml sample fraction was collected over 8 min, beginning at 11.4 min. Sample volume was subsequently reduced to ca. 50 μl under a stream of nitrogen at 30 °C, and the resulting residue was transferred to a GC vial insert prior to derivatization and analysis. Protocols for transferring residues to the GC vial insert, derivatization, and adding internal standards to these samples were identical to those described in the preceding paragraph.

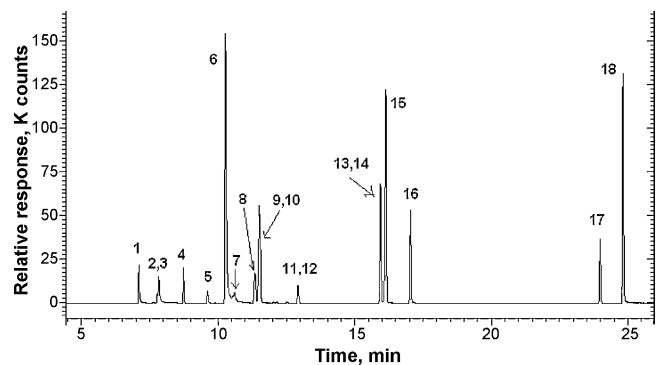


Fig. 1. Representative total ion chromatogram for a calibration standard. Peak identifications are as follows: (1) *m*-toluamide, (2) benzophenone, (3) benzophenone-d $_{10}$, (4) celestolide, (5) pentachloronitrobenzene, (6) phenanthrene-d $_{10}$, (7) *p*-*n*-octylphenol, (8) galaxolide, (9) tonalide, (10) musk xylene, (11) *p*-*n*-nonylphenol, (12) [$^{13}\text{C}_6$]p-*n*-nonylphenol, (13) 4-methylbenzylidene camphor, (14) 2,2'-dinitrobiphenyl, (15) musk ketone, (16) triclosan, (17) mirex, and (18) octocrylene.

2.6. GC–MS analysis

Instrumentation employed for GC–MS analysis with selected ion monitoring (SIM) consisted of a Varian (Palo Alto, CA, USA) CP-3800 GC system equipped with a CP-8400 auto sampler and 1200 L quadrupole mass spectrometer. Analytes were separated on a 30 m \times 0.25 mm I.D., 0.25 μm film thickness, XT1-5 capillary column (VWR Scientific, West Chester, PA, USA) using the following temperature program: initial temperature, 100 °C, ramped to 180 °C at 15 °C/min, held for 5 min, ramped to 290 °C at 6 °C/min, and held for 31 min. Helium was used as carrier gas at a constant flow rate of 1 ml/min (linear velocity 37.2 cm/s). Injections of 1.0 μl were made using splitless mode and an injection port temperature of 275 °C. The transfer line was kept at 280 °C. Eluted sample components were monitored by electron-impact (EI) ionization mass spectrometry in SIM mode. A representative total ion chromatogram for a calibration standard, demonstrating chromatographic resolution of target analytes under these conditions, is shown in Fig. 1. Ions (*m/z*) monitored for identification and quantitation of target analytes are given in Table 1. Surrogates were monitored at *m/z* 192, 295, and 298 for benzophenone-d $_{10}$, PCNB, and [$^{13}\text{C}_6$]p-*n*-nonylphenol, respectively. 2,2'-Dinitrobiphenyl was also added as a surrogate but its behavior was unreliable in a number of cases. Accordingly, it is not recommended for such use in this application. Note that chromatographic run time was divided into six segments (also identified in Table 1) in which only select ions were monitored. Segments were selected in order to maximize the observed analytical response for each compound. Additional mass spectrometry parameters held constant for all analytes were as follows: ion source temperature, 250 °C; manifold temperature, 40 °C; EI ionization energy, 70 eV.

2.7. GC–MS/MS analysis

Instrumentation employed for analyses utilizing tandem mass spectrometry consisted of a Varian (Palo Alto, CA, USA) CP-3900 GC system equipped with a CP-8400 autosampler and Saturn 2100T ion trap mass spectrometer. Separation of analytes was performed on a 30 m \times 0.25 mm, 0.25 μm VF-5 MS capillary column (Varian, Walnut Creek, CA, USA). The GC temperature program and other operating conditions were the same as described in Section 2.6 unless noted otherwise. Analyte retention times and chromatographic resolution were very similar under these conditions to the behavior shown in Fig. 1. Optimized MS/MS transitions monitored for identification and quantitation of target analytes are given in

Table 1
Brand and IUPAC names, use, group, retention time, structure, MS/MS and SIM ions for target analytes.

Analyte	Use/group	t_R (min)/segment	Chemical structure	MS/MS transition (m/z), excitation amplitude (V)/storage level (m/z)	SIM ions
<i>m</i> -Toluamide (N,N-diethyl- <i>m</i> -methylbenzamide)	Insect repellent	7.10/1		190 > 145 ^a , 175, 0.7/83.6	91, 119 ^a , 190
Benzophenone (diphenyl ketone)	Sun screen/UVF	7.79/2		182 > 153 , 0.8/80.1	77, 105, 182
Celestolide (4-acetyl-1,1-dimethyl-6-tert-butylindane)	Fragrance/SM	8.74/3		229 > 173 , 131, 0.8/100.9	173, 229 , 244
<i>p</i> -Octylphenol	Surfactant metabolite/AP	10.60/5		278 > 179 , 0.6/122.6	165, 180, 278
Galaxolide (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl cyclo-penta[g]-2-benzo pyrane)	Fragrance/SM	11.35/6		243 > 213 , 171, 0.8/107.1	213, 243 , 258
Tonalide (7-acetyl-1,1,3,4,4,6-hexamethyltetralin)	Fragrance/SM	11.50/6		243 > 187 , 159, 0.8/107.1	201, 243 , 258
Musk xylene (1-tert-butyl-3,5-dimethyl-2,4,6-trinitrobenzene)	Fragrance/SM	11.51/6		282 > 265 , 248, 0.6/124.3	282 , 283, 297
<i>p</i> -Nonylphenol	Surfactant metabolite/AP	12.93/7		292 > 179 , 0.6/128	149, 179, 292
4-MBC (4-methylbenzylidene camphor)	Sun screen/UVF	15.94/8		211 > 169 , 155, 0.7/92.9	115, 211, 254
Musk ketone (4-aceto-3,5-dimethyl-2,6-dinitro-tert-butylbenzene)	Fragrance/SM	16.14/8		304 > 287 , 214, 0.7/134.1	217, 261 , 366
Triclosan (4-chloro-2-hydroxyphenyl-2,4-dichlorophenyl ether)	Anti-microbial	17.04/9		347 > 200 , 310, 0.8/153.1	200, 345 , 362
Octocrylene (2-ethylhexyl-2-cyano-3,3-diphenylacrylate)	Sun screen/UVF	24.82/12		250 > 248 , 221, 0.8/110.2	177, 249, 361

^a Bold print indicates m/z used for quantitation. UVF, ultra-violet filter; SM, synthetic musk; AP, alkylphenol.

Table 1, along with analyte-dependent excitation amplitude and storage level settings. Transitions utilized for surrogate monitoring were m/z 192 > **190**, 163; 295 > **263**, 237; and 298 > **185** (quantitation ions shown in bold) for benzophenone- d_{10} , PCNB, and [$^{13}\text{C}_6$]p-n-nonylphenol, respectively. Additional MS parameters held constant for all analytes were as follows: trap temperature, 200 °C; manifold temperature, 110 °C; collision gas, Ar at 2 mTorr.

2.8. Analyte identification and quantitation

Calibration standards were prepared by dissolving varying concentrations of all target analytes and surrogates in acetone. Calibration plots were constructed for each analyte by plotting analyte-dependent response factors (i.e., peak area of analyte divided by peak area of internal standard) versus analyte concentration. A linear regression, forced through the origin, was performed for each analyte, resulting in correlation coefficients (r^2) exceeding 0.99. Note that mirex was used exclusively as the internal standard in construction of calibration plots for GC–SIM–MS analyses. In contrast, phenanthrene- d_{10} was used as the internal standard when GC–MS/MS was employed.

Analyte identification in environmental samples was confirmed by comparing retention times and relative ion abundance ratios with those observed for standards spiked in control matrix (i.e., tissue collected from Clear Creek or East Fork Gila River). The criteria imposed for positive identification were matching retention times and 80% agreement in relative ion abundance ratios. Three ions (one quantitation and two qualifier ions) were monitored for identification of detected analytes in GC–SIM–MS analyses. In contrast, two independent MS/MS transitions were typically employed in GC–MS/MS analyses. Note that comparison of ion abundance ratios was not possible for benzophenone, p-octylphenol, p-nonylphenol, and octocrylene in GC–MS/MS analyses because only one unique MS/MS fragment was produced in high abundance for these compounds upon collision-induced dissociation of the parent ion.

2.9. Analysis of unfortified fillet tissue from Clear Creek and East Fork Gila River

A detectable analytical response was observed at retention times where select target analytes were expected to elute in unfortified tissues from Clear Creek and East Fork Gila River. Therefore, measured concentrations in fortified control matrices used to determine MDLs for developed methodologies and to monitor method performance in the analysis of Pecan Creek samples were corrected by subtracting the corresponding concentrations observed in unfortified tissue. GC–SIM–MS analysis of unfortified fillets from Clear Creek resulted in mean responses ($n=3$) equivalent to 24, 7 and 12 ng/g at retention times corresponding to benzophenone, galaxolide and triclosan, respectively. Similarly, GC–MS/MS analysis of 7 unfortified fillet composites from East Fork Gila River resulted in mean responses equivalent to 18 and 11 ng/g at retention times corresponding to benzophenone and galaxolide, respectively.

Note that detection of target analytes in fish collected from Clear Creek and East Fork Gila River was initially assumed to be unlikely, since both streams experience limited, if any, anthropogenic influence. Nevertheless, further examination of SIM ion abundance ratios confirmed that benzophenone and triclosan were likely present at noted levels in the Clear Creek composite. In contrast, analytical responses observed at the retention time corresponding to galaxolide in Clear Creek samples was sufficiently low to preclude positive identification. This was also true of analytical responses observed at the galaxolide retention time in samples from East Fork Gila River. Finally, it is important to point out that

only a single MS/MS transition was monitored for benzophenone in tissue from East Fork Gila River, thus its identification would be considered tentative. Moreover, one could not necessarily rule out that observed response at the corresponding retention time was not due to interference from a non-target component of sample matrix (i.e., a component exhibiting similar retention time and MS/MS fragmentation); the likelihood of this type of interference increases with decreasing m/z of monitored components (see **Table 1**). For these reasons, the authors assert that data presented here are inconclusive with respect to occurrence of benzophenone in fillet composites from East Fork Gila River.

3. Results and discussion

3.1. GC–SIM–MS method development

The initial objective of this work was to develop a screening method for select OWCs in fish tissue that would enable analytes from various compound classes (e.g., UVFs, APs, fragrances, etc.) to be assessed quantitatively in a single chromatographic run. An important first step in realizing this goal was identification of an extraction solvent that provided reasonable extraction recoveries for target compounds with somewhat dissimilar physicochemical properties. The following solvents were evaluated in preliminary studies: n-hexane, dichloromethane, acetone, acetonitrile, methanol, 1:1 n-hexane:acetone, 1:1 methanol–acetonitrile, and 1:1 methanol–acetate buffer (pH 4). When dichloromethane or n-hexane (either alone or in combination with acetone) was used, the tissue did not mix well and formed a sticky dispersed residue, resulting in poor reproducibility for replicate extractions. Among remaining solvent systems tested, acetone was selected as optimal for removing target compounds from fish muscle tissue.

In the course of evaluating extraction solvents, it was also determined that fillet extracts derived from the Clear Creek sampling site did not require gel-permeation chromatography prior to derivatization and GC–SIM–MS analysis. This was initially thought to be a distinct advantage, compared to the majority of reported protocols for determination of similar compounds in fish tissues. However, it is important to note that these tissues consisted of edible fillet only (i.e., no skin, no belly flap) and were essentially free of lipids (mean lipid content 0.4%; $n=3$), though lipid content was unknown at that time.

Statistically derived method detection limits (MDLs) and analyte recoveries observed for GC–SIM–MS analysis of fortified fillet tissues from the Clear Creek site are given in **Table 2**. Mean recoveries ranged from 87% to 114%, with less than 11% relative standard deviation, demonstrating near-quantitative extraction efficiency and excellent reproducibility. More significantly, MDLs (representing the lowest concentration of each analyte that may be reported in a defined matrix with 99% confidence that the concentration is non-zero [50]) for all target analytes were within the corresponding range of concentrations reported previously for these compounds in fish tissue (also shown in **Table 2**) and near the lower limit in most cases. These results clearly suggested that the developed approach had promise as a screening method.

3.2. Analysis of environmental samples

In order to confirm the utility of GC–SIM–MS methodology for analysis of environmental samples, edible fillet tissues prepared from 11 different bluegill fish collected ~650 m downstream from the effluent discharge into Pecan Creek were screened for target analytes. Four compounds were detected in all analyzed specimens at concentrations exceeding statistically derived MDLs.

Table 2
Comparison of method detection limits (MDLs) and mean analyte recoveries for independent sample preparation/GC–MS methodologies.

Fish species	Bluegill (muscle tissue only ~0.4% lipid)		Sonora sucker (muscle, skin and belly flap tissue ~4.9% lipid)		Various	
	GC–SIM–MS; No GPC		GC–MS/MS + GPC		Literature ^a	
Metric/chemical	MDL in ng/g (n=8) ^b	Mean recovery ± SD (%) (n=11)	MDL in ng/g (n=7) ^b	Mean recovery ± SD (%) (n=3)	Reported range in fish tissue (ng/g)	Reference
Insect repellent <i>m</i> -Toluamide	3.5	110 ± 10	5.1	102 ± 18	NR	NR
UV filters						
Benzophenone	7.5	101 ± 5	16	87 ± 17	0.66–17	[6,38,51]
4-MBC	5.3	99 ± 3	120	57 ± 1	0.44–27	
Octocrylene	17	98 ± 2	36	79 ± 10	0.1–69	
Synthetic musks						
Celestolide	4.0	97 ± 3	18	83 ± 14	0.03–24	[8,40,41,52]
Galaxolide	5.0	105 ± 6	12	95 ± 16	0.52–350	
Tonalide	4.8	87 ± 9	13	107 ± 21	0.44–91	
Musk xylene	7.3	102 ± 3	397	67 ± 10	0.05–6	
Musk ketone	17	101 ± 4	321	75 ± 2	0.07–12	
EDCs/surfactant						
<i>p</i> - <i>n</i> -Octylphenol	2.9	114 ± 12	8.2	54 ± 6	0.2–6	[15–18]
<i>p</i> - <i>n</i> -Nonylphenol	2.4	111 ± 7	9.7	74 ± 3	3.3–566	
Antimicrobial						
Triclosan	5.5	98 ± 4	38	93 ± 14	0.3–11	[7,53]

NR: Not reported.

^a Range of environmental concentrations previously reported in fish tissue.

^b Determined by multiplying the one-sided Student's *t*-statistic at the 99% confidence limit times the standard deviation (SD) observed for analysis of *n* replicate aliquots of specified tissue matrix; spike concentrations were typically $\leq 10 \times$ MDL.

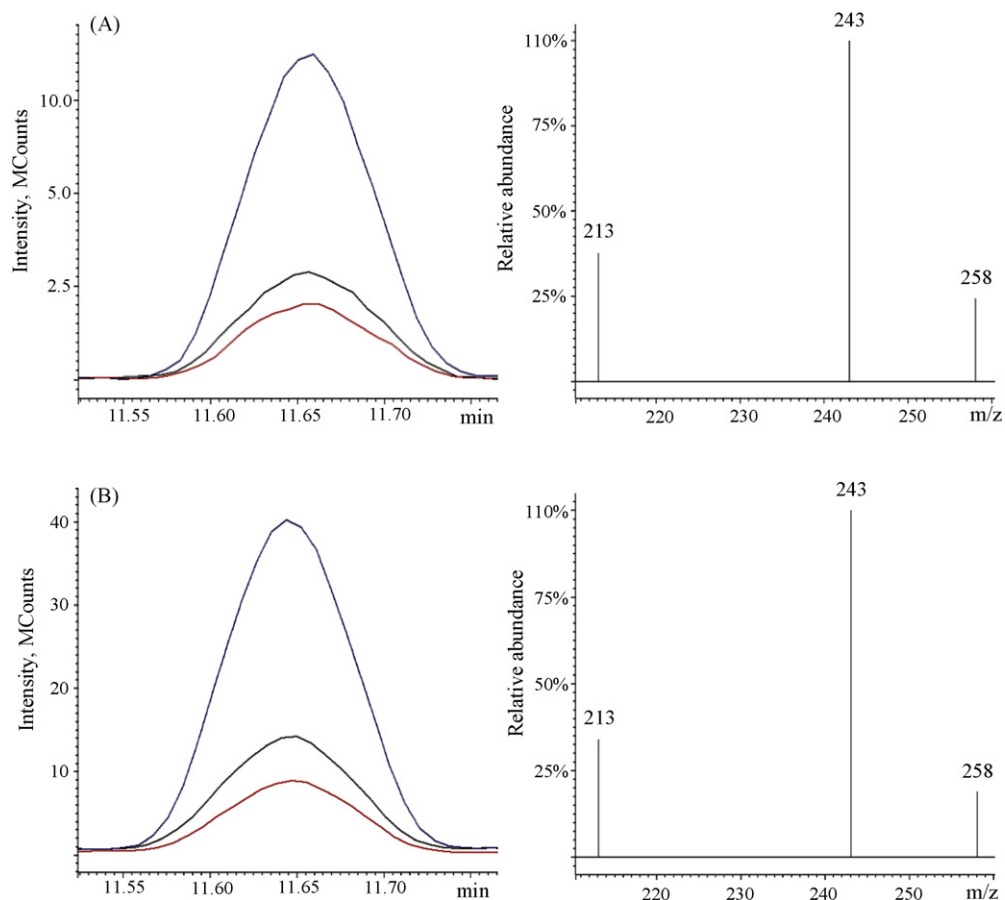


Fig. 2. GC–SIM–MS reconstituted ion chromatograms and mass spectra for (A) fillet tissue from Clear Creek fortified with 120 ng/g galaxolide and (B) unfortified fillet tissue from Pecan Creek.

Table 3
Occurrence of target compounds in bluegill sampled from Pecan Creek, Denton, TX, USA.

Fish identity	Weight (g)	Lipid content ^a (%)	Analyte concentration (ng/g wet weight)			
			Benzophenone	Galaxolide	Tonalide	Triclosan
A	30.1	0.23	37	258	33	22
B	42.5	0.40	79	790	69	19
C	49.0	0.16	79	417	35	20
D	35.5	na	41	338	39	19
E	46.9	0.20	46	234	26	18
F	33.6	0.42	56	513	50	17
G	31.2	na	50	525	58	23
H	29.4	na	90	735	82	31
I	33.6	na	44	552	70	20
J	39.3	0.45	44	970	97	19
K	37.2	0.75	63	929	76	19
Average ± standard deviation			57 ± 18	569 ± 256	58 ± 23	21 ± 4

Reported values are based on a single determination for each specimen.

^a na: lipid content was not determined for specimens D, G, H and I due to limited tissue mass.

Analyte identification in Pecan Creek samples was confirmed by comparing observed relative ion abundance ratios and retention times with those observed for standards spiked in fillet tissue from Clear Creek. The criteria imposed for positive identification were matching retention times and 80-percent agreement in relative ion abundance ratios; three ions (one quantitation and two qualifier ions) were employed for identification of detected analytes. Representative data demonstrating positive identification of galaxolide are given in Fig. 2.

Measured concentrations of benzophenone, galaxolide, tonalide, and triclosan are reported in Table 3. These values are similar to those reported previously in literature (Table 2). Concentrations of galaxolide and tonalide determined in this work fall within previously reported ranges, while the lowest concentrations determined for triclosan and benzophenone in fish from Pecan Creek were approximately 1.5 and 2 times larger, respectively, than the highest concentrations reported for these analytes in literature. That slightly higher concentrations of benzophenone and triclosan were determined in the present study is not necessarily surprising, since tissues were derived from dissimilar fish collected in different geographic locations.

Data quality was assessed via surrogate monitoring and analysis of a procedural blank, laboratory control sample (LCS; Clear Creek tissue spiked with target analytes), and duplicate matrix spikes (MS/MSD; randomly selected sample from Pecan Creek spiked with target analytes). Surrogates were added to all tissues prior to extraction. Mean surrogate recoveries for the entire sample batch ranged from 84% to 100% with a relative standard deviation <13% in all cases. No analytes were detected in the procedural blank, and analyte recoveries in the LCS were consistent with values reported in Table 2. More significantly, mean recoveries observed for all monitored OWCs in MS/MSD samples ranged from 93% to 135%, suggesting that detected analytes were quantified with reasonable accuracy. Note that analyte spiking levels in LCS and MSD samples corresponded to the middle of the calibration range for each analyte (~12× MDL).

An interesting trend in data reported in Table 3 is that the range of concentrations for detected PCPs was noticeably larger than that observed previously for pharmaceutical contaminants [47], despite the fact that the present study utilized identical tissues derived from the same fish. The RSDs of individual OWC concentrations among the 11 analyzed specimens were 32%, 45%, 40%, and 19% for benzophenone, galaxolide, tonalide, and triclosan, respectively. In contrast, RSDs for detected pharmaceuticals were somewhat lower: 26%, 36%, 17% and 14% for diphenhydramine, diltiazem, carbamazepine and norfluoxetine, respectively

[47]. Nakata et al. attributed large variations in galaxolide concentrations between individuals of the same species to differences in lipid content among analyzed tissues [42]. Although a somewhat general trend of increased concentration with increasing lipid content may be observed for galaxolide and tonalide in Table 3, the authors acknowledge that low magnitude and limited variability in lipid content among analyzed specimens in this study likely limits the power of any argument for or against correlations based on these parameters.

3.3. Effect of increased lipid content—analysis of fortified tissues collected from Gila River, New Mexico, USA

A series of experiments was subsequently performed to evaluate the effect of increased sample lipid content on GC–SIM–MS method performance. This turned out to be an important test, as application of the GC–SIM–MS approach to Sonora sucker tissues was met with limited success. Analysis of fortified sample extracts that had not been subjected to GPC resulted in significantly deteriorated chromatographic performance, as evidenced by substantial shifts in analyte retention times. Continuous sample analysis required frequent trimming and reinstallation of the GC column and replacement of the injection liner and ion volume. It was initially hypothesized that inclusion of skin and belly flap tissues in the composite from New Mexico could be responsible for many of the problems encountered. However, analysis of a similar sample that did not contain skin and belly flap tissues resulted in only very minor improvements in analytical performance. Fillet specimens (including skin and belly flap tissues) derived from Sonora sucker had an average lipid content of 4.9%, which is 5–25 times higher than bluegill tissues collected from either Clear Creek or Pecan Creek.

The clean-up approach for tissue extracts was expanded to include GPC, which is commonly employed to remove lipids and high-molecular-weight interferences. Addition of GPC to the sample preparation protocol enabled continuous analysis of Sonora sucker fillet tissue with no noticeable compromise in chromatographic performance relative to behavior observed for GC–SIM–MS analysis of fortified bluegill tissue from Clear Creek or Pecan Creek. However, a dramatic increase in background signal and/or reduction in analyte sensitivity were observed for several analytes in reconstituted chromatograms. Five target analytes (*p*-octylphenol, galaxolide, 4-MBC, musk ketone and triclosan) were essentially indistinguishable from background in samples prepared from Sonora sucker (see representative data for triclosan in Fig. 3), and although observed analytical response and calculated recoveries

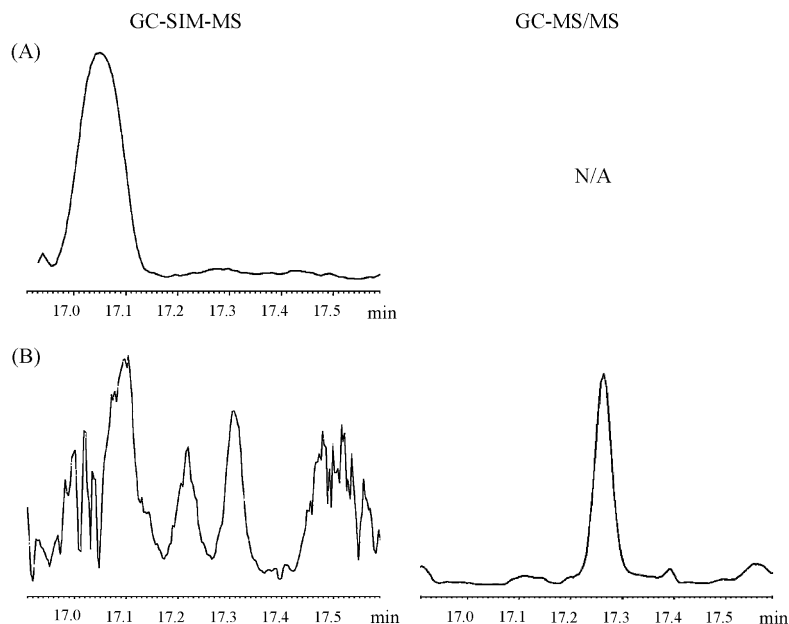


Fig. 3. Observed response for triclosan in fortified fillet tissue from (A) Clear Creek, TX (<1% lipid) and (B) East Fork Gila River, NM (4.9% lipid), clearly demonstrating poor performance for GC–SIM–MS analysis of fillet tissue with increased lipid content (left) and improved performance when GPC and tandem mass spectral monitoring were added to the analytical protocol.

for the remaining 7 analytes were reasonable (60–100%), dropping five compounds from the target analyte list was viewed as unacceptable. Note that effort was not extended in this study to identify alternative SIM ions m/z that may be less susceptible to background interference.

In an effort to increase the number of compounds that could be targeted, the method was further expanded to include tandem mass spectral monitoring. The analytical approach involving both GPC and tandem mass spectrometry is hereafter referred to as GC–MS/MS. As demonstrated in Fig. 3, the GC–MS/MS approach dramatically improved detectability of triclosan in the Sonora sucker composite. Similar improvements were also observed for *p*-octylphenol, galaxolide, 4-MBC, and musk ketone.

An independent MDL study was performed using the GC–MS/MS approach and fortified fillet tissues from Sonora sucker. Results are given in Table 2. Comparable analyte recoveries were observed for 6 target compounds relative to those observed for GC–SIM–MS analysis of bluegill fillet tissues from Clear Creek. In contrast, significantly lower recoveries were observed for 4-MBC and *p*-*n*-octylphenol; more modest decreases were observed for octocrylene, musk xylene, musk ketone, and *p*-*n*-nonylphenol, suggesting that increased lipid content in the Sonora sucker composite may have influenced extraction behavior of select compounds. Additionally, statistically derived MDLs for most compounds were 1.5–7 times higher in the GC–MS/MS study than those determined in Clear Creek tissues using the GC–SIM–MS approach. More dramatic reductions in sensitivity were observed for 4-MBC, musk xylene, and musk ketone (MDLs for these analytes were 23, 54, and 19 times higher, respectively, in the GC–MS/MS study). Differences in analyte detectability between the two approaches (i.e., GC–MS/MS and GC–SIM–MS) cannot be explained by differences in extraction efficiency alone, as observed MDL increases in the GC–MS/MS study were larger in most cases than one would predict based on observed differences in analyte recovery. An alternative explanation is based on inefficient fragmentation of parent ions in the ion trap. Since all MS/MS parent ions were introduced into the mass spectrometer with electron impact ionization (i.e., a relatively ‘hard’ ionization technique), it is not unreasonable to expect

that generation of daughter ions via collision-induced dissociation may be problematic in some instances (i.e., parent ions may be sufficiently stable that further fragmentation is unlikely).

A final observation from Table 2 is that some MDLs determined in GC–MS/MS analyses exceeded the corresponding environmentally relevant concentration range identified in literature. Observed MDLs for 4-MBC, musk xylene, and musk ketone exceeded the corresponding range by a factor of 4.5, 66, and 27, respectively. In contrast, MDLs for galaxolide, tonalide, and *p*-*n*-nonylphenol were near the lower limit of reported environmental concentrations. Observed MDLs for the remaining analytes fell in the middle to upper third of the corresponding concentration range. These data suggest that GC–MS/MS screening methodology, as presented here, is likely to be useful for analysis of 9 target compounds in environmental samples. It is important to note that MDL improvements could likely be afforded by increasing the tissue mass utilized in routine analyses (e.g., using 10 g rather than 1 g tissue aliquots). Additionally, complementary GC–SIM–MS analysis of extracts subjected to GPC would also be expected to improve MDLs for select compounds (i.e., those free of background interference noted above). However, the later approach somewhat defeats the purpose of a screening method that targets multiple analytes in a single chromatographic run.

4. Conclusions

Findings demonstrate that both developed analytical approaches (i.e., GC–SIM–MS and GC–MS/MS methodologies) are likely to enable routine screening of select OWCs in fish tissue. However, each has distinct advantages and disadvantages. The GC–SIM–MS approach offers simplified sample preparation and lower MDLs, though it appears to be unsuitable for analysis of tissues containing >1% lipid. In contrast, GC–MS/MS methodology appears to be applicable to samples with increased lipid content, but at the expense of sensitivity. While choice of analytical technique is an obvious component of any environmental study design, sample collection strategy is also significant. Though not stated explicitly, it may be inferred from data presented here that

targeting lean tissues (i.e., tissues with low lipid content) may result in greatly simplified analytical determinations and improved detection/quantitation limits for this collection of target analytes. The caveat with this line of reasoning, of course, is that compounds that partition exclusively to lipid will be present at lower levels in leaner tissues. Nevertheless, it is important to recognize that choice of analytical technique and choice of target tissue are both expected to effect analyte detection/quantification thresholds in future applications of reported screening methodology.

Acknowledgements

The authors thank Charles Stanley, Jacob Stanley (Baylor University), Blaine Snyder and co-workers (Tetra Tech, Inc.) for assistance with sample collection; Laura Dobbins (Baylor) for assistance with dissection of fish tissues; and Pilar Perez-Hurtado (Baylor) for assistance with lipid determinations and sample preparation.

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