Findings and Recommendations:
Since hitting markets in the 1920s, refrigerators have occupied a lovable corner not just in American kitchens but also in American culture. As the U.S. transitioned to industrial convenience in the twentieth century, refrigerators replaced hearths as household communication centers. Henceforth, it has become commonplace to decorate refrigerator surfaces with photographs, keepsakes, and other items of visual culture. In 2013, at the University of Arizona, I completed my doctoral study, “The Other White Cube Project: Finding Museums among Us,” through which I investigated the cultural phenomena of decorating refrigerator surfaces. I studied what Americans put on their refrigerators, how they arrange these items, and why they do it. For the study, participants uploaded photographs and answered a questionnaire online at theotherwhitecube.com. Participants analyzed their displays, made connections to museum practices, and evaluated how much they had learned about curating – the act of selecting, organizing, and drawing meaning from a collection of objects.

Of the 218 submissions, only a small number included children who participated as part of a family. Because adults comprised the data set, the study was not generalizable to the field of art education, which mostly studies school-age children in classroom settings. With the grant, I conducted a series of qualitative case studies in four art classes at South Nodaway R-IV in Barnard, MO. The case studies confirmed the original study’s findings. By equating museums with everyday spaces, curators with everyday people, and art with everyday objects, the project succeeded in affecting three keys to learning – comfort, relevance, and readability. Through the project, participants felt more comfortable with curatorial concepts. Additionally, they reported finding relevance in how their habits reflected cultural aesthetics and, as a result, found museums more intelligible than before. Participants came to understand that everyone is a curator – of some kind and of some place.

List of Expenditures:
Supplies:
2 Allreli Digital Audio Recorders ($29.99 each) $59.98
2 Round Ceramic Disc Magnets, 100 pieces ($13.99 each) $27.98
2 Imaginarium Letters and Numbers Magnets ($13.48 each) $26.96
2 Foam Magnet Sets ($13.27 each) $26.54
3 Officemate Magnets ($4.99 each) $14.97
1 Clear, Heavy-Duty Mounting Tape $10.98
Travel:
15 Trips to South Nodaway (35 miles round trip at .42 per mile) $220.50
TOTAL $387.91*

*Remaining $294.03 to be returned to the Faculty account.
Pharmaceuticals in grocery market fish fillets by gas chromatography–mass spectrometry

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ABSTRACT

Occurrences of pharmaceuticals are evident in aquatic organisms. A reproducible gas chromatography–mass spectrometry (GC–MS) method using selected ion monitoring (SIM) has been used to determine the anti-histamine diphenhydramine (DPH), anti-anxiety diazepam (DZP), anti-seizure carbamazepine (CZP) drugs and their metabolites in grocery stores fish that were homogenized, extracted, pre-concentrated, cleaned up, and examined. Identifications of the compounds in extracts were obtained by comparing similar mass spectral features and retention properties with standards. Among nine frequently detected drugs, only DPH and DZP were observed and ranged from 0.61 to 6.21 and 1.99 to 16.57 ng/g, respectively, in fourteen fish species. These concentration values were lower than the environmental fish. Mean spike recoveries of analytes exceeded 75% with relative standard deviations (RSD) < 10%. The statistically-derived method detection limits (MDLs) for nine compounds ranged from 0.13 to 5.56 ng/g. Average surrogate recoveries were 80–85% with 4–9% RSD.

1. Introduction

Pharmaceuticals are a large group of chemical compounds that are being increasingly used in human and animal applications. Tons of these chemicals are produced annually worldwide (Christen, Hickmann, Rechenberg, & Fent, 2010; Santos et al., 2010). After consumption, these compounds are loaded or excreted into ecosystems via urine, feces or residues as either parent compounds or their metabolites. Wastewater treatment plants are not commonly designed to eliminate the drugs because they are non-regulated water contaminants (Daughton, 2004; Daughton & Ternes, 1999). Based on the process design of the treatment plants, the elimination rates of drugs range from <10% (e.g. atenolol and carbamazepine) to almost complete removal e.g. propranolol (Miege, Choubert, Ribeiro, Eusebe, & Coquery, 2009). As these compounds are continuously released into aquatic systems, the effluents from the wastewater treatment plants are considered as the main routes of human pharmaceuticals into the environment (Fent, Weston, & Caminada, 2006), reaching concentrations of ng/L to μg/L (Mompelat, Le Bot, & Thomas, 2009). The non-regulated pharmaceuticals and personal care products (PPCPs), as environmental contaminants, consistently exposed the aquatic organisms. Many people and researchers around the world were unaware that a new environmental health concern had emerged. Nowadays, regulatory authorities, health agencies, and professional organizations, all over the globe are greatly concerned and this drives research on the presence, occurrence, fate of the PPCPs and metabolites (Brodin, Fick, Jonsson, & Klaminder, 2013; Kelly, Ikonomou, Blair, Morin, & Gobas, 2007). Pharmaceutical drugs are all around, resulting from the use in humans and livestock. Commercial and domestic use and discharge of these compounds into municipal sewage have contributed to their occurrences in the aquatic environment and organisms (Snyder, Westerhoff, Yoon, & Sedlak, 2003). Drugs and metabolites have been detected in aquatic and terrestrial organisms (Oost, Beyer, & Vermeulen, 2003), surface water (van der Ven et al., 2004; Wu, Spongberg, & Witter, 2008; Wu et al., 2014), lake Michigan water and sediments (Blair, Crago, Hedman, & Klapner, 2013), municipal effluent (Gagne, Blaise, & Andre, 2006), sewage effluent (Osemwengie & Steinberg, 2001), marine sediments (Beretta, Britto, Tavares, da Silva, & Pletsch, 2014), fish-eating birds and fish (Tanoue et al., 2014), effluent-dominated river water fish (Ramirez et al., 2009), Pecan Creek fish (Mottaleb et al., 2009), German fish specimen Bank (Subedi et al., 2012), receiving marine waters and marine bivalves (McEneff, Barron, Kelleher, Paull, & Quinn, 2014). Recent studies have indicated that many of pharmaceuticals and metabolites are environmentally persistent, bioactive, and have potential for
bioaccumulation (Gomez et al., 2012; Mottaleb, Brumley, Pyle, & Sovocool, 2004; Valdes, Ame, Bistoni, & Wunderlin, 2014). Acute aquatic toxicities of drugs and metabolites were examined, by Kim et al., 2007 using marine bacteria (Vibrio fischeri), a freshwater invertebrate (Daphnia magna), and the Japanese medaka fish (Oryzias latipes). They demonstrated that Daphnia was the most susceptible among the tested organisms. Correa and Hoffmann (1999) studied the variation of response of the drugs β-amphetamine, sodium pentobarbital, diazepam, β-carbonile, and saline into weak electric fish (Gymnotus carapo). They concluded a reduction of the degree of alertness by the barbiturate and a decrease in emotionality and/or stress by the benzodiazepine interfered with the novelty response. Branda et al. (2013) evaluated biochemical and behavioral effects employing neuro-active anticonvulsant drugs (diazepam, carbamazepine, and phenytoin) on pumpkin-seed sunfish (Lepomis gibbosus). They illustrated the behavioral changes of sunfish through use of oxidative stress parameters, such as glutathione reductase, glutathione S-transferases, catalase and lipid peroxidation.

Pharmaceuticals are polar compounds commonly analyzed by liquid chromatography–tandem mass spectrometry (LC–MS/MS) (Beretta et al., 2014; Blair et al., 2013; Du, Perez-Hurtado, Brooks, & Chambliss, 2012; Du et al., 2014; McNelley et al., 2014; Ramirez, Mottaleb, Brooks, & Chambliss, 2007; Ramirez et al., 2009; Tanoue et al., 2014; Valdes et al., 2014; van der Ven et al., 2004; Wu et al., 2008). Previously our research group reported the LC–MS/MS method using electro spray ionization (ESI) for analysis of 25 pharmaceuticals from environmental fish (Ramirez et al., 2009, 2007). The LC–MS/MS protocol allowed us to characterize the drugs with MDL of 0.05–6.68 ng/g, with exceptions of miconazole and ibuprofen levels, extraction recovery of 60–90%. This method also showed a strong positive matrix influence (up to 1111% for erythromycin) and or negative matrix effect (up to 95% for miconazole) using positive/negative ESI operation modes for the target compounds analysis. Recently Tanoue et al. (2014) demonstrated an isotope dilution LC–MS/MS method employing silica gel and solid phase extraction clean-up steps for determination of 17 polar PPCPs in biological samples. They reported MDL values ranging from 0.019 to 3.2 ng/g in fish liver/brain, and recoveries from of 48% to 88%, with strong matrix influences. On the other hand, Subedi, Mottaleb, Chambliss, and Usenko (2011) described GC–MS/MS approach for two pharmaceuticals (carbamazepine and diazepam) and 12 personal care products and showed similar method performance, but required a sample derivatization step prior to analysis. The GC–MS/MS method provided 98% recovery and 18 ng/g MDL for carbamazepine, and 97% recovery with 3.7 ng/g MDL for diazepam.

Although LC–MS/MS method favors the analysis of polar pharmaceuticals and their metabolites from environmental matrices, the method requires about 50–150 times more volume of samples, or pure standards, per injection than GC–MS/MS analysis. Additionally, GC–MS tools are less expensive and have fewer maintenance costs compared to LC–MS. Furthermore, the prices of standards, surrogates and isotopic analog and their hazard maintenance, laboratory chemical safety and disposal costs are increasingly higher day by day. Thus, the cost effective GC–MS techniques were explored for the analysis of drugs and metabolites.

As the drugs and metabolites have been repeatedly reported as present in water and fish, the consumption of fish and water can lead to an ingestion of these compounds in humans and animals. Employing our previous experience (Foltz, Mottaleb, Meziani, & Islam, 2014), in this study, we developed and validated a gas chromatography–mass spectrometry method using the selected ion monitoring (GC–SIM-MS) mode. We extended the capability of this technique to analyze the frequently occurring three-parent drugs and six-metabolites, without derivatization, from edible fish fillets. Detailed protocols together with analytical method performance, sample characterization and extraction procedures are discussed. The method is applied to determine the concentration of the target compounds in 14-different varieties of fish fillets purchased from grocery markets in the Midwest region of the United States. As very limited information is available about edible fish fillets, therefore this report may generate more interest among scientists and regulatory authorities for screening other PPCPs in grocery store edible fish.

2. Experimental

2.1. Solvents, chemicals and reference materials

Nine pharmaceutical drugs including their metabolites from different therapeutic classes were chosen, in this study, because three parent compounds; diazepam (DZP), diphenhydramine (DPH) and carbamazepine (CZP) were frequently detected in the environmental samples. Commercially available highest purity grade reference pharmaceuticals and metabolites standards, surrogates, the internal standard and solvents were purchased from local vendors. The reference pharmaceuticals standards included CZP, carbamazepine-10,11-epoxide, 10,11-dihydro-10-hydroxy carbamazepine, oxcarbamazepine, DZP, nordiazepam, temazepam, oxazepam and surrogate standards CZP-d10, DZP-d5 and DPH-d3 were purchased from Cerilliant Corporation, Round Rock, TX, USA. The standard DPH and internal standard (IS) phenanthrene-d10 were purchased from Sigma–Aldrich, St. Louis, MO, USA. Silica gel (grade 60–230 mesh, 60 Å), n-hexane (HPLC grade), and acetone (HPLC grade) were obtained from Fisher Scientific, Pittsburg, PA, USA. Distilled water was purified and deionized to 18 MΩ with an ELGA PureLab Ultra water purification system.

2.2. Sampling of the fish specimens and preservation of the samples

Fish used, in this study, were grown in fresh- and salt-water. Fourteen different genus of edible frozen fish fillets were purchased from grocery markets located at Maryville and Kansas City, Missouri. Approximately 2.0–4.0 lbs wet weight size package of each fish genus group were obtained and used as samples. After purchasing, the fish fillets were kept in frozen condition in a cooler box with ice cubes and then transported, within 2 h, to the Center for Innovation and Entrepreneurship (CIE) laboratory where the samples were individually wrapped, with properly marked aluminum foil and then kept in food-grade polyethylene bags at −80 °C temperature until further processes.

2.3. Fish homogenization process

In the CIE laboratory, the muscle tissue of each group of fish fillets was homogenized using a Tissuemiser (Fisher Scientific Power Gen 125) set to rotate at 30,000 rpm. After homogenization, homogenate of each group of the fish fillets was composited uniformly following standard U.S. Environmental Protection Agency (EPA) protocols (U.S. EPA, 2006). The representative sample specimen was then placed in heavy-duty aluminum foil with appropriate sample identification mark. Following, all tissue specimens were stored in a freezer at −80 °C prior to extraction.

2.4. Extraction of target analytes from fish homogenates

Previously we reported extraction and GC–MS examination of personal care products from environmental fish specimens and
analytical procedures were also described (Mottaleb et al., 2009). Here we followed the similar techniques for analysis of pharmaceuticals and metabolites from edible fish fillets. Briefly, an amount (1.0175 ± 0.0123 g) of individual homogenate fish tissue was mixed with 10 mL acetone in a 20 mL borosilicate glass vial. An aliquot volume (100 µL) of surrogate standards mixture provided 2.0 ng of DPH-d3, 44.4 ng of CZP-d10 and 250.0 ng of DZP-d5 was spiked from a mixture in acetone solution to each sample. Samples were shaken immediately and analytes extracted into the organic phase by sonication for 20 min at 25 °C using a Branson 8150 Sonicator. Following extraction, the samples were transferred into 50 mL polypropylene copolymer conical shaped bottom centrifuge tubes (Nalgene, Rochester, NY, USA) that were rinsed with 2 ml acetone. Samples were then centrifuged at 13,000 rpm for 60 min at 4 °C using a Sorvall RC 6 Plus Centrifuge, Thermo Scientific, USA and the supernatant was transferred into 18 mL disposable, glass test tubes, and the solvent was evaporated almost to dryness under a stream of nitrogen at 30 °C.

Table 1
Name, use, chemical structure, molecular formula and mass of selected pharmaceuticals and metabolites.

<table>
<thead>
<tr>
<th>Target compounds/CAS number</th>
<th>Use (trade name)</th>
<th>Chemical structures</th>
<th>Molecular formula</th>
<th>Molecular mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diphenhydramine (DPH)/147-24-0</td>
<td>Anti-histamine (benadryl)</td>
<td>![Chemical structure]</td>
<td>C_{17}H_{21}NO.HCl</td>
<td>291.82</td>
</tr>
<tr>
<td>Carbamazepine (CZP)/298-46-4</td>
<td>Anti-seizure (carbatrol)</td>
<td>![Chemical structure]</td>
<td>C_{15}H_{12}N_{2}O</td>
<td>236.27</td>
</tr>
<tr>
<td>Oxazepam/604-75-1</td>
<td>Metabolite of diazepam. Anti-anxiety (OxPam)</td>
<td>![Chemical structure]</td>
<td>C_{15}H_{11}ClN_{2}O_{2}</td>
<td>286.72</td>
</tr>
<tr>
<td>Oxcarbazepine/28721-07-5</td>
<td>Metabolite of carbamazepine. Anti-convulsant (Trileptal)</td>
<td>![Chemical structure]</td>
<td>C_{15}H_{12}N_{2}O_{2}</td>
<td>252.27</td>
</tr>
<tr>
<td>10,11-Dihydro-10-hydroxycarbamazepine/29331-92-8</td>
<td>Metabolite of oxcarbazepine</td>
<td>![Chemical structure]</td>
<td>C_{15}H_{14}N_{2}O_{2}</td>
<td>254.28</td>
</tr>
<tr>
<td>Diazepam (DZP)/439-14-5</td>
<td>Anti-anxiety (valium)</td>
<td>![Chemical structure]</td>
<td>C_{16}H_{13}ClN_{2}O</td>
<td>284.74</td>
</tr>
<tr>
<td>Carbamazepine-10,11-epoxide/36507-30-9</td>
<td>Metabolite of carbamazepine</td>
<td>![Chemical structure]</td>
<td>C_{15}H_{12}N_{2}O_{2}</td>
<td>252.27</td>
</tr>
<tr>
<td>Nordiazepam/1088-11-5</td>
<td>Metabolite of diazepam</td>
<td>![Chemical structure]</td>
<td>C_{15}H_{11}ClN_{2}O</td>
<td>270.72</td>
</tr>
<tr>
<td>Temazepam/846-50-4</td>
<td>Metabolite of diazepam. Anti-anxiety (restoril)</td>
<td>![Chemical structure]</td>
<td>C_{16}H_{13}ClN_{2}O_{2}</td>
<td>300.74</td>
</tr>
</tbody>
</table>
using a Cliper Life Science TarboVap LV. Samples were subsequently reconstituted in 200 μL of 65:35 (v/v) hexane–acetone for the clean-up procedure.

2.5. Sample clean-up and pre-concentration

Each sample was cleaned up using a silica gel column before analysis by GC–MS–SIM method. A laboratory prepared silica gel column clean-up was used. Approximately 2.0 g of silica gel was stirred, washed and rinsed with 10 mL of 65:35 (v/v) hexane–acetone mixture and then prepared as a slurry with the solvent mixture. The slurry was transferred to a disposable, 5 mL glass pipette. Prior to adding the slurry, about 15 mg of glass wool was used as a porous plug to confine the silica gel into the pipette column. Silica gel columns were preconditioned with 20 mL of the 65:35 (v/v) hexane–acetone mixture. The amount extracted from Section 2.4 above was loaded onto the column using a Pasteur pipette, and eluates were eluted with 30 mL of the hexane–acetone mixture. The eluate was then collected in a glass test tube and the solution was reduced to a volume of about 20 μL under a stream of nitrogen at 30°C. The sample was transferred directly to a disposable, 5 mL glass pipette. Prior to analysis, a constant amount of filament delay set at 9 min.

For recovery study, three individual experiments were performed by spiking the sample with a concentration of target analyte mixture approximately ≤10 × MDL. Then the samples were spiked with surrogates as described before and analyzed by GC–MS–SIM. Each analyte concentration was calculated against the internal standard based calibration curves. MDLs of target analytes were determined statistically by multiplying the standard deviation of the seven concentration measurements with one-sided Student’s t-statistics (99% confidence level).

2.7. GC–MS analysis

A Varian gas chromatograph (Model 450-GC) linked with a Varian triple quad mass spectrometer (Model 320–MS TQ) and a Varian autosampler (Model CP 8400) was employed. Separations were accomplished on an Agilent (J & W) Technologies GC capillary column HP-5MS with a dimension of 30 m long /C176i.d. 0.25 μm film thickness. Helium gas was passed through the capillary at a constant flow rate of 1.2 mL/min (linear velocity 40.3 cm/s), using the splitless mode of injection. The autosampler injected 1 μL volume of silica gel cleaned up sample extract or standard solution into the GC system with gradient oven temperature starting from 60 °C for 1 min, to 170 °C at 15 °C/min, to 260 °C at 6 °C/min, and to 300 °C at 10 °C/min, and held at the final temperature for 6 min with a total run time of 33.33 min. The mass spectrometer scanned the samples for 27.0 min. The injector and transfer line temperatures were 240 °C and 275 °C, respectively. The ion source temperature was 240 °C and operated in positive electron ionization (EI) 70 eV.

Initially, known concentrations, approximately 10–30 ng/μL of individual pure surrogate, internal standard and standard target analytes solutions were injected and scanned between 50 and 600 m/z using the full-scan mode of GC–MS. The quantitation and qualifier ions from the surrogate, internal standard and target analytes were chosen based on the highest mass signals. The mass spectral acquisitions were performed using the selected ion monitoring (SIM) mode with the Varian MS workstation version 6.9.3, with filament delay set at 9 min.

2.8. Calibration curve, quality assurance/quality control (QA/QC)

Calibration standards were prepared by dissolving known concentrations of standards and the surrogate in acetone. Calibration
plots were constructed for each analyte by plotting the ratio of peak areas (analyte area divided by internal standard area) versus the ratio of target analytes concentrations to the internal standard concentration, resulting in at least a 7-point calibration curve. A linear un-weighted regression, forced through the origin, was performed for each analyte, resulting in correlation coefficients ($R^2$) exceeding over 0.9996 for all compounds. The resulting equation of the line provided a response factor ($R_F$) relative standard deviation (RSD) between 5.5% and 14.0% and was used to calculate the concentrations of the analytes. Deviations from the calibration curve of ±15% $R_F$ RSD would cause rerunning of the standards, and construction of a new calibration curve, or replacement of the capillary GC injector liner. Retention time variations were generally ±5 s, and peak widths at half-height were about 3 s. On the day of analysis, a sequence of injection: solvent blank, calibration standards, solvent blank, sample extract, solvent blank, calibration standards was used. No carryover of the target compounds was detected in the reagent blank. In all extracts, the surrogates (CZP-d10, DZP-d5 and DPH-d3) were spiked and recoveries were measured.

### 3. Results and discussion

The GC–SIM-MS method was developed to exploit its applicability for frequent analysis of the detected nine pharmaceuticals and their metabolites in the edible fish fillets. Silica gel cleaned up extracts of 14 different species of edible fish, developed in salt- and fresh-water environments collected from several grocery stores of Maryville and Kansas City, Missouri were analyzed by the GC–SIM-MS method. The protocol was established and validated. Target analytes listed in Table 1 did not display the presence of any characteristic mass signals of the target compounds in the reagent blanks and injection sequence solvent blanks. Thus, it was confirmed that the antihistamine DPH and anti-anxiety DZP drugs originally occurred from the edible fish fillet silica gel cleaned up extracts.

#### 3.1. Performance of analytical protocols

The performance of the GC–SIM-MS analytical method was validated through percent recovery and method detection limits (MDLs) studies. Table 2 demonstrates the analytical method performance together with retention times and ions monitored for the target compounds, surrogates and internal standard. Average spike recoveries ($n = 3$) of the target pharmaceuticals and metabolites ranged from 75% to 92% with relative standard deviations (RSD) of 4–10%. The MDLs were estimated from seven measurements employing one-sided Student’s $t$-statistics at the 99% confidence level and were observed between 0.13 and 5.56 ng/g for the interested target analytes and as low as 0.13 ng/g for DPH. The average recoveries of surrogates ranged from 80% to 85% with RSD of 4–9%. Surrogates were spiked at the initial stage of the extraction and then these run through the whole extraction protocol (see Sections 2.4 and 2.5). These results represent a robust GC–MS method that could be used for the analysis of many pharmaceuticals and metabolites from fish tissues.

#### 3.2. Separation and detection of target compounds in fish specimens

Silica gel cleaned up fish extracts were subjected to GC–MS employing the SIM mode and the detection of the selected target compounds in the extracts was accomplished by the analytical technique. Fig. 1 displays the separation of nine target pharmaceuticals and their metabolites together with three surrogates and one IS phenanthrene-d10. Parent compounds; DPH, CZP and DZP and their surrogates DPH-d3, CZP-d10, DZP-d5 were eluted from the capillary column at almost the same retention time, as expected, because of the similar physico-chemical properties of parent and respective deuterium compounds (see Table 1). Surrogates and IS structures are not shown.

Mass spectral signals and retention time parameters, given by the target compounds from the silica gel cleaned up fish extracts, were used to characterize the compounds, by comparing with
the pure standards. Fig. 2 shows an overlay of a typical SIM mass chromatogram derived from the data file by selecting \( m/z \) 58, 73 and 165 ions for (A) a standard solution containing concentration of 7.5 pg/\( \mu \)L of DPH and for (B) a Mullet (genus Mugil) fillet silica gel cleaned up extract. These chromatograms show an excellent agreement of the retention time for DPH that eluted from the capillary column at retention time of 14.07 min with a change of retention time of 0.03 min for Mullet fish fillet specimen. In the case of Yellow Fin Tuna, Spanish Mackerel and Atlantic Salmon silica gel cleaned up extracts, a variation of retention time, ±0.06 min, was observed compared to the authentic standard compounds (not shown), and peak widths at half-height were about 3 s. These variations of retention times were usual because of interferences from the matrix which co-extracted with the target compounds from fish samples. These small differences of retention time allowed continuous analysis, identification and quantification of target compounds from fish specimens without any interruption. The retention times of all target compounds, surrogates and IS are also presented in Table 2.

3.3. Characterization of target compounds in fish samples

To characterize target compounds extracted from homogenized fish specimens, the ions relative abundance ratios between the base peak quantification and the confirming peak qualifier ions mass signals were used. The individual identity and presence of target compounds in the silica gel cleaned up fish fillet extracts were ascertained when the difference of relative abundance ratio was less than or equal to approximately ±20%, or an agreement of the relative abundance ratio of 80% or over. By selecting the peak in SIM chromatograms of standard and silica gel cleaned up sample extract solutions, the mass spectra were produced. Fig. 3 displays representative mass spectra derived from the SIM ion chromatogram (Fig. 2) for (A) standard DPH and (B) Mullet fish fillet silica gel cleaned up extract solution. In general, the mass spectral features compared very well to each other and indicate the presence of DPH in the Mullet fish fillet. Moreover the differences of relative abundance ratio for qualifier ions (\( m/z \) 73 and \( m/z \) 165) around ±9% while quantitation ion \( m/z \) 58 matched 100% between standard and Mullet fish silica gel cleaned up extract spectra were observed. When similar fashion of ion relative abundance ratio and retention time agreement were occurred, then the presence, characterization and quantification of other compounds were recognized in the fish specimens.

3.4. Quantitation of target compounds in fish samples

The quantification of concentration of the target compounds from all edible fish fillet silica gel cleaned up extracts was made using internal standard based calibration curves. Table 3 displays
the name and growing environment of fish together with concentration of target analytes obtained from the fish specimens. The concentrations of the compounds, given in Table 3, are on wet weight basis. In this study, 3-parent compounds; anti-histamine DPH, anti-anxiety DZP, anti-seizure CZP drugs and their 6-metabolites; carbamazepine-10,11-epoxide; 10,11-dihy-dro-10-hydroxycarbamazepine; oxcarbamazepine, nordiazepam, temazepam, oxazepam were screened in the grocery stores edible fish. Of nine target compounds, the DPH was consistently detected above MDLs in 13 different genus of fish except Spanish Mackerel whereas the appearance of DZP was observed above MDLs in 11 genus of fish except Mahi-Mahi (genus Corgphaena), Whiting (genus Merluccius), Yellow fin Tuna (genus Thunnus). The concentrations of DPH and DZP ranged from 0.61 to 6.21 ng/g and 1.99 to 16.57 ng/g, respectively, in the fresh and salt-water growing fish. The wild caught Red Snapper (genus Lutjanus) and Mullet developed in salt-water environment gave the maximum concentration of DPH (6.21 ng/g) and DZP (16.57 ng/g), respectively. The other compounds observed were out-of-the limits of the characterization guidelines (see Section 3.3) or below MDLs (Table 2) and were considered as not present in the fish samples and are not shown in the Table 3. The fish analyzed were from numerous genus or species grown in different water atmospheres and habitats, and their metabolism processes were also dissimilar. Moreover the structures and functionalities of target compounds (see Table 1) are also diverse. Therefore, the metabolic pathway, biological interaction and removal processes may vary in different fish species. This study report the presence of DZP for the first-time in edible fish fillets originating from the grocery stores.

### 3.5. Relevance of the target pharmaceuticals in fish

The relevance of the target compounds that were obtained in fish fillets was assessed by comparing results with the literature values. Previously Foltz et al. (2014) reported the concentration of DPH (0.50 ng/g) in grocery stores Whiting fish fillets. The reported data compares well with the value 0.61 ng/g that was obtained from grocery stores Whiting fish, in this investigation. Tanoue et al. (2014) also summarized the concentration of DPH as 6.5–64 ng/g in the liver and 6.2–17 ng/g in the brain of environmental crucian carps (Carassius carassius) and common carps (Cyprinus carpio) collecting from an effluent-dominated stream at Matsuyama, Japan. In the context of DZP, Kwon, Armbrust, Vidal-Dorsch, Bay, and Xia (2009) reported the concentration of DZP ranging from 23 to 110 ng/g in liver of turbot fish (genus Pleuronichthys) of southern California coastal waters. In the present study, DPH and DZP were analyzed from 0.61 to 6.21 ng/g and 1.99 to 16.57 ng/g in fourteen different species of fillets from grocery markets fish. The concentrations of DPH and DZP in grocery market fish are approximately 10 times lower than the environmental fish (see Table 3). This could be possible because imported grocery market fish were raised in farm or wild caught (Table 3) whereas the environmental fish were developed in effluent-dominated stream (Tanoue et al., 2014). Moreover, as the fillets and livers are two different sample matrices of fish and the majority compounds spend more time in the liver compared to fillet muscle, thus the lower concentration of DPH and DZP in fillet muscle could be possible. Although non-regulated DPH and DZP drug concentrations are in low ng/g level in edible fish and may not warrant any health concern either for fish or human at this moment, the concentrations should be monitored in a periodic fashion.

### 4. Conclusion

The occurrences of antihistamine DPH and anti-anxiety DZP drugs have been confirmed by GC–SIM-MS method in edible fillets
originating from 14 different species from the local grocery stores. The concentrations of DPH and DZP were obtained as 0.61–6.21 ng/l, respectively, in fish that developed in fresh and salt-water, and were 2–10 times lower than the environmental fish samples. To our knowledge, the presence of DZP is reported for the first time in edible fish fillets collected from the grocery stores. As PPCPs are non-regulated emerging contaminants, the monitoring of other frequently detected PPCPs together with endocrine disrupting compounds and trace metals in edible fish fillets could be worthy of future investigation.

Acknowledgements

The authors acknowledge the help of Dr. Christian Daughton, U.S. EPA, Las Vegas, Nevada for initiating the pharmaceutical and personal care products analysis in the environment. Author (M.A. Mottaleb) would like to thank Dr. G. Wayne Sovocool, Retired Research Chemist, Environmental Sciences Division, U. S. EPA, Las Vegas, Nevada for editing and improving the quality of the manuscript. The authors also thank to the Research Committee, the Dean of the College and Arts and Sciences, and the CIE Director of the Northwest Missouri State University for financial support to continue the research project.

References


Effect of spatial orientation on internal environment of a wooden 3-stall calf hutch in a cold, humid climate (Funded Fall 2014)

PI: Jamison Allen, Assistant Professor, School of Agricultural Sciences

The internal environment fluctuations of wooden 3-stall calf hutches for dairy calf housing in the humid climate of northwest Missouri were evaluated during the winter of 2014-2015. The objective of this study was to determine the feasibility of using this type of calf hutch in an environment in which external temperature can remain below freezing for an extended period of time. Four hutches were built for this project and arranged to face north, south, east, or west. From January 22 to February 3, data loggers collected placed within the hutches collected climate and light intensity data were collected every minute. Data was analyzed to quantify the effect of spatial orientation and time of day on the internal climate and light intensity of the hutch.

Throughout the trial, relative humidity remained above 50%, and temperature remained below 10 degrees Celsius (< 50 degrees Fahrenheit). Daily average temperature, relative humidity, and temperature-humidity index (THI) were similar across spatial orientation, although east-facing hutches tended to have slightly higher (1.5%) relative humidity. Variable fluctuations in all three parameters were also seen by spatial orientation throughout the 24-hour period. Relative humidity proved to have the greatest fluctuation between orientations compared to temperature and THI. As for light intensity, north-facing hutches had less sunlight penetration, indicating less possibility for warming the animal by way of solar radiation. This trial provides evidence that the wooden hutch style is not a viable housing option for cold, humid environments. A current trial is collecting data during warmer, humid periods of the summer. Results from these combined trials will be presented by undergraduate students at the 2016 Midwest American Society of Animal Science and then compiled into one manuscript for publication.
**Expense Report:**
The project went slightly over budget due to fluctuating prices for lumber.

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FINAL REPORT:
Abstract and Expense for Research Project

M. Abdul Mottaleb
Department of Natural Sciences and Center for Innovation and Entrepreneurship, Northwest Missouri State University, 800 University Drive, Maryville, MO 64468, USA.
*Contact email: mmottal@nwmissouri.edu, Tel: + 1 660 562 0820

Final Report:
Pharmaceutical drugs diazepam, diphenhydramine, carbamazepine and metabolites in grocery stores fish by gas chromatography-mass spectrometry using selected ion monitoring mode

Type of grant: Travel
Date approved: November 14, 2014
Amount approved: $1141.38
Expense amount: $1141.38 (please see expense report attached)
Refund amount: $00.0
Date completed: June 9, 2015

ABSTRACT

Non-regulated emerging environmental contaminants ppharmaceuticals and personal care products (PPCPs) have been increasingly used in humans and animals application. PPCPs enter into aquatic system through effluent discharge from wastewater/sewage treatment plants, inappropriate disposal of medications, livestock operations, and hospital facilities etc. These emerging contaminants have been consistently detected in different environmental compartments and aquatic organisms as their parent compounds or biologically transformation products or metabolites. Limited knowledge is available about fate and mechanisms of PPCPs in water system and aquatic organisms. In this study, frequently used pharmaceuticals anti-histamine diphenhydramine, anti-anxiety diazepam, anti-seizure carbamazepine drugs and their metabolites carbamazepine-10,11-epoxide; 10,11-Dihydro-10-hydroxycarbamazepine; oxcarbamazepine, nordiazepan, temazepam, oxazepam were selected as target analytes. Salt- and fresh-water grown fish provided edible fillets originating from fourteen different fish species are chosen as sample. The sampled fish species were collected from the local grocery stores and lake of the Midwest region of the United States. To investigate the fish samples, a reproducible and reliable gas
A chromatography - mass spectrometry (GC-MS) method has been developed and validated for analysis of the pharmaceuticals and metabolites. Nine drugs and metabolites belonging from 3 classes of application have been examined by GC-MS using selected ion monitoring (SIM) mode from fourteen different species of edible fish fillets. The fish samples were homogenized, composited, extracted, pre-concentrated, cleaned up, and analyzed by GC-SIM-MS. The presence of target compounds in fish extracts were confirmed by comparing similar mass spectral features and retention properties with the reference standard compounds. In all extractions, standard surrogates carbamazepine-d10, diazepam-d5 and diphenhydramine-d3 were spiked to validate the method. Internal standard based calibration plots were used for quantitation of the target analytes. Of nine drugs and metabolites, two parent compounds, diphenhydramine and diazepam, were observed in fish samples with concentration range from 0.61 to 6.21 ng/g and 1.99 to 16.57 ng/g, respectively. These are comparable with literature values. The developed method provided average spike recoveries ranged from 75 to 92% with a variation of relative standard deviation (RSD) from 4 to 10% for all target compounds. The statistically-derived method detection limits (MDLs) ranged from 0.13 to 5.56 ng/g could be achieved for identification and quantification of pharmaceuticals and metabolites with MDL as low as 0.13 ng/g for the diphenhydramine.

**RECOMMENDATION**

1. Bioavailability of diphenhydramine and diazepam drugs has been obtained grocery stores edible fish.
2. The observed concentrations of PPCPs may not warrant any health concern either fish or human at this moment, but should be monitored periodically for other fish.
3. As PPCPs are non-regulated emerging contaminants, the screening of other frequently spotted PPCPs and endocrine disruptors together with heavy- and trace metals in edible fish could be worthy of further study.
4. Two Chemistry undergraduates along with me went together to present the research in the ACS 249th National Meeting at Denver, CO.
5. The research has been published in Peer-reviewed journal Food Chemistry. Article attached.
Expense Report:
For presentation of research in 249th American Chemical Society (ACS) National Meeting at Denver, CO.

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Prepared On August 3, 2015 (SummaryYTD)
My proposed research was to produce new work for an exhibition at Derryberry Gallery, Tenn Tech University, Cookeville, TN, in February of 2015. At the time of my submission, I was also in negotiations with Murray State University, for a planned solo show in September 2015. At the time of this writing, that exhibit is installed, to end on September 29.

In this research cycle, I tried to expand my artistic vocabulary by painting compositions similar to my previous work but immersed in a new kind of atmosphere, namely landscapes bathed in dusk/dawn light as well as downright nocturnal scenes. This new approach to representing light (which, after all, is the representational painter’s main challenge) has yielded some interesting results and expanded my color palette considerably, to the point where I now think about light in a much more nuanced way. It is developments such as these that make the research process so rewarding, and reliable funding for research so crucial for those who engage in it.

I should also mention that in the course of working on the show at the above-mentioned venues, I was, on rather short notice, also invited to show a number of my works in Paris, France. In order to pay for that shipping, I dipped into the approved funds for the shows at Tenn Tech and Murray State, and, to make up for that, secured reimbursement for half of the shipping costs to Murray State through a Supplemental Travel grant.

Expenses incurred:

- Materials/Art Supplies: 268.54
- Shipping: 2,351.77

Total: 2,620.31