ESTABLISHING COST EFFECTIVE METHODS TO ANALYZE A WIDE RANGE OF PHARMACEUTICAL COMPOUNDS THROUGH LABORATORY SCALE EXPERIMENTS AIMED AT ASSESSING FATE AND TRANSPORT MECHANISMS IN GROUNDWATER

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A THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in the
Department of Geological Sciences
in the Graduate School of
The University of Alabama

TUSCALOOSA, ALABAMA

2015

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ABSTRACT

Pharmaceutical and organic wastewater contaminants have been increasingly detected in drinking water and groundwater supplies. A stepwise approach was used to determine a simple and cost effective method for testing various classes of pharmaceutical compounds. A robust, accurate method was developed and validated using two commonly detected pharmaceuticals in water resources; sulfamethoxazole (SMX) and carbamazepine (CMP). Tandem solid-phase extraction in conjunction with high performance liquid chromatography (HPLC) analysis was effective for quantifying concentrations of analytes under environmentally relevant laboratory-scale scenarios. Using predetermined standards and known concentrations, an average recovery percentage of 96.25% was achieved in validation efforts. Batch tests consisting of streambed sediment and known concentrations of the study analytes were conducted over a 96-hour period to determine Freundlich adsorption isotherm coefficients (K_F) and retardation factors (R). The results of these tests demonstrate that CMP had a greater affinity to adsorb to the sediment (K_F =8.79) compared to SMX (K_F =4.22) with corresponding retardation factors (R) of 49.3 and 20.7, respectively. In addition to the development of a promising cost-effective analytical method to quantify different types of pharmaceutical compounds in groundwater, this work also demonstrates that SMX (compared to CMP) may pose higher risk for entering drinking water supplies, as natural retention processes will be less under

most conditions. Limits of detection using the SPE/SPE HPLC-UV method was 0.48 μg/L for SMX and 0.60 μg/L for CMP.

Keywords: pharmaceutical; emerging contaminants; HPLC; batch tests; sulfamethoxazole (SMX); carbamazepine (CMP)

LIST OF ABBREVIATIONS AND SYMBOLS

cm^3	centimeters cubed
CMP	carbamazepine
Cw	aqueous concentration
EDTA	ethylenediaminetetraacetic acid
FDA	Food and Drug Administration
g	gram
H_3PO_4	phosphoric acid
$HgCl_2$	mercuric chloride
HLB	hydrophilic-lipophilic balanced cartridge
HPLC	high performance liquid chromatography
kg	kilogram
K_f	Freundlich coefficient
K_{ow}	octanol-water coefficient
L	liter
M	molarity
MCL	maximum contaminant level
mg	milligram
min	minute

MS	mass spectrometry
n	Freundlich fitting parameter (normality)
Na_2HPO_4	sodium phosphate dibasic
NaOAc	sodium acetate
OWC	organic water contaminant
ρ_b	bulk density
pH	negative log of activity of the hydrogen ion in solution
R	retardation
rpm	rotations per minute
S	adsorbed concentration
SAX	strong anion exchange cartridge
SMX	sulfamethoxazole
SPE	solid phase extraction
C_u	uniformity coefficient
UPLC	ultra performance liquid chromatography
UV-Vis	ultra violet spectrophotometry
WWTP	waste water treatment plant
XRD	x-ray diffraction
θ	porosity

ACKNOWLEDGEMENTS

I would like to thank my graduate advisor Dr. Geoffrey Tick for the opportunity, resources and guidance to conduct this research at the University of Alabama. I would also like to thank my committee members Dr. Yuehan Lu, Dr. Rona Donahoe, Dr. Patty Sobecky, and Dr. Rob Martinez for all of their time, suggestions and contributions. I would like to specially thank Dr. Qiaoli Liang who was instrumental in establishing the HPLC methods. This research was supported by several sources at the University of Alabama including the Contaminant Hydrogeology Research Laboratory, the Graduate School Research and Travel Fund, the Department of Geological Sciences W. Gary Hook's Endowed Geology Fund and the Department of Biological Sciences. Lastly, I would like to thank the members of the Hydrogeology Research Group, Doug Mateas and Mark Padgett, for their acumen and cooperation in conducting this research.

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1. INTRODUCTION

Over the last decade a national reconnaissance effort to identify organic wastewater contaminants (OWC's) and document their prevalence in both treated and untreated surface and groundwater has ensued (Barnes et al., 2004) in response to repeated detections (Focazio et al., 2008, Seiler et al., 1999). The majority of these detections have been found to be in the μg/L (sub-parts per billion) levels in the water samples. Fortunately, advances in analytical techniques have allowed for the detection and quantification of these compounds at extremely low levels (Batt et al., 2008). Although the technology exists, most of these compounds do not currently have any enforceable regulatory standards (maximum contaminant levels – MCL) implemented by the USEPA. At present, wastewater and water treatment facilities are not required, nor suited to report any level of these pharmaceuticals (Jones et al., 2005). In addition, a large number of these pharmaceuticals compounds have been detected in finished drinking water indicating that they are not effectively removed using current wastewater treatment plants (WWTP) processes (Stackelberg et al., 2004). Currently, there is a limited amount of literature that has focused on fate and transport processes of pharmaceutical and OWC's in groundwater and an even smaller amount of information on the long-term biological effects due to chronic low-level exposure to these compounds. The current gap of research for these emerging class of contaminants are likely due to both a poor understanding of how these compounds interact in the environment as well as the time and cost intensive requirements

for analytical quantification of these compounds. As a result, there is a need for more in-depth research, that not only includes characterizing pharmaceutical concentrations for a specific area, but also a detailed understanding of the fate and transport processes occurring as they move through aqueous or groundwater systems.

Both abiotic (i.e. dispersion, dilution, adsorption) and biotic (microbial respiration-degradation) processes will influence the fate and transport of these compounds in the environment. To date, there is a lack of studies that evaluate both abiotic and biotic processes to understand risk and contamination potential for various water supplies. Once these primary abiotic mechanisms are characterized, modeling analyses can be used to better evaluate fate and transport responses, design more effective remediation strategies, and develop more accurate risk assessments for these pharmaceutical compounds at larger scales and/or for specific sites throughout the U.S.

The purpose this study is to systematically develop a simple, robust, and cost-effective method for quantifying pharmaceutical concentrations in environmentally relevant conditions and to test the accuracy and validity of the method for two different classes of pharmaceutical compounds most commonly detected in groundwater and drinking water systems. The specific objectives of this study are to 1) validate a tandem solid phase extraction (SPE) high performance liquid chromatography (HPLC) method to quantify low concentrations of for two different classes of pharmaceuticals that can be applied to other compounds of interest; 2) utilize this method to analyze sulfamethoxazole (SMX) and carbamazepine (CMP) concentrations in batch-test experiments; and 3) develop adsorption isotherms for quantifying Freundlich adsorption coefficients and relative retardation factors for these target compounds (SMX and CMP). Determining the most cost-

effective method to analyze pharmaceuticals under environmentally relevant conditions is largely dependent upon the chemical attributes of the pharmaceutical itself, the environment the aqueous samples are being collected from, and their potential to interfere with one another when analyzed using specific analytical techniques (i.e. spectrophotometry, spectroscopy, etc.). Because of this, it is essential that preliminary testing be done to determine interference potential and that a thorough sediment characterization be done to determine other sources of interference (i.e. humic acids, fatty acids, etc.).

2. BACKGROUND

Fortunately, advances in analytical techniques have allowed for the detection and quantification of these compounds at extremely low levels. As a result a number of broad national and regional localized studies have been completed that document pharmaceuticals and other OWC's are in fact, present in U.S. water systems (Focazio et al., 2008). The majority of these detections have been found to be in the μg/L (sub-parts per billion) levels in the water samples. A preliminary study was conducted which focused on defining the extent of pharmaceutical compounds in the water supplies of Birmingham, Alabama. This preliminary research sampled surface water systems in the Jefferson and Shelby Counties of the area. From the samples collected, a total of 56 pharmaceutical target analytes were tested for at the USEPA Laboratories (Cincinnati, Ohio). Of the 56 analytes tested, 11 of these compounds were reported to be found above detection limits from at least one of the 6 sites sampled. Of these 11 compounds, 7 were reported at more than one site.

Despite the existing analytical technology, there is still a noticeable void in literature regarding the environmental impact of pharmaceuticals . The current lack of research for this class of emerging contaminant exists for a variety of reasons. Analyses for such pharmaceutical compounds often require expensive and time-consuming technologies, largely uncommon in many commercial laboratories. Facilities that are capable of such precise ultra-low detections are extremely expensive and may not be feasible for many

researchers and practitioners in the field. This expense is a large deterrent for labs and research universities that have restricted budgets but still wish to contribute to the assessment of these emerging contaminants.

High-cost and time-intensive analytical methods have been developed to measure ultra-low concentrations (environmentally relevant) of pharmaceutical and OWC compounds in in groundwater and drinking water. Single-step solid phase extraction (SPE) combined with ultra-performance liquid chromatography (UPLC) and triple-quadrupole mass spectrometry (SPE HPLC-MS/MS) has been validated as the most standardized for these analysis conditions (Batt et al., 2008). Although these methods are advantageous due to the low detection limits that are required for environmental samples, laboratories that do not possess this equipment/instrumentation or capabilities, have to outsource the analysis to other labs at extremely high cost.

Other analytical techniques have been developed to measure environmentally relevant concentrations for a variety of contaminants but have not been tested for various pharmaceutical compounds. Tandem solid-phase extraction with high-performance liquid chromatography-UV detection (SPE/SPE HPLC-UV) has been proven to be a simpler and more cost-effective alternative to the SPE HPLC-MS/MS method when analyzing veterinary/pharmaceutical grade antibiotics in groundwater and water treatment environments (Blackwell et al., 2004). For laboratories with these capabilities, this method is an adequate replacement to outsourcing analyses due to its high resolution and practicability. For this method a Strong Anion Exchange (SAX) cartridge is used to remove natural organic matter that could contribute to interference in UV detection. SAX cartridges have been proven to successfully remove anions, organic acids, nucleic acids and other

surfactants, making them particularly useful for environmental-based studies. This separation/extraction process is used in tandem with a Hydrophilic-Lipophilic-Balanced (HLB) polymeric cartridge to retain the analyte(s) of interest (i.e. this tandem phase SPE process "prepares" the sample for analysis) (Blackwell et al., 2004).

For this study, an extension of this SPE/SPE HPLC-UV method will be developed and validated and to determine first-order adsorption behavior for two different classes of pharmaceutical compounds that were selected based on the preliminary findings in the Birmingham, AL survey. Specifically, this tandem SPE HPLC-UV technique will used to determine adsorption isotherm coefficients for two target pharmaceutical analytes (SMX and CMP) that have been documented to be prevalent and persistent in various water systems (Stackelberg et al., 2004). After determining these adsorption parameters, other values such as retardation can be obtained to further quantify the fate and transport whereby future studies can progress to evaluate biotic controls (i.e. biodegradation) on fate/transport of these compounds in similar environmental settings.

2.1 Target Analytes

2.1.1 Sulfamethoxazole (SMX)

Sulfamethoxazole is one of the most widely prescribed and used antibiotics. It is commonly used in treatments for malaria, urinary tract infections, and conjunctivitis, among many others illnesses. Usually prescribed in combination with trimethoprim, the drug inhibits the production of tetrahydrofolic acid, which is ultimately responsible for synthesis of DNA and RNA in bacteria. Known side effects include, but are not limited to, lesser severity side effects such as gastrointestinal issues, fevers and rashes. However, as

with all antibiotics, a resistance to the pharmaceutical can be developed with chronic exposure (Smilack et al., 1999)

Approximately 45-70% of SMX is not metabolized in the body and is consequently excreted through urination or defecation (McEvoy et al., 2004). Of that volume, 15-25% is excreted as completely unaltered SMX. 43% of that excreted volume is metabolized into N4-acetyl-sulfamethoxazole (Ac-SMX) while 9-15% is metabolized into sulfamethoxazole-N1-glucuronide (SMX-GLU) (Van Der Ven et al., 1994). Important chemical parameters are listed in Table 1.

2.1.2 Carbamazepine (CMP)

Carbamazepine is a drug used to treat epilepsy. It is classified as an anti-convulsant (anti-seizure) pharmaceutical. Typical dosages vary from 100mg-800mg with the most widely prescribed being the 200 mg dose (Crill et al., 1973). According to the FDA, known side effects include but are not limited to, reduced white and red blood cell counts, increased risk of suicidal behavior, liver and thyroid abnormalities as well as many minor side effects and is listed as an endocrine disruptor. It is one of the most frequently detected pharmaceuticals in treated water supplies (Zhang et al., 2010). Slower than average degradation kinetics and higher WWTP discharges of carbamazepine have been predicted from empirical evidence (Stackelberg et al., 2004; Stein et al., 2008). Carbamazepine is largely unresponsive to many of the treatment processes commonly used at WWTP (Stackelberg et al., 2007). Furthermore, slower than average degradation kinetics (half-life 533 days) and higher WWTP discharges of carbamazepine have been predicted from empirical evidence (Stein et al., 2008). Important chemical parameters are listed in Table 1.

3. MATERIALS AND METHODS

3.1 Materials

Methanol, tetrahydrofuran, acetonitrile, water, trifluoroacetic acid, orthophosphoric acid and citric acid were all HPLC grade purchased from VWR (Radnor Pennsylvania). EDTA disodium salt, Mercuric Chloride, and disodium hydrogenorthophosphate annhydrous were analysis grade purchased from VWR (Radnor Pennsylvania). Sulfamethoxazole, and Carbamazepine, were all pharmaceutical grade purchased from VWR (Radnor Pennsylvania).

An 18 MΩ-cm NANOpure water Barnstead NANOpure Model D4741, Thermo Scientific water purifier was used for all deionized water sources. An IKA magnetic stirrer (serial number R015PSI) was used to homogenize all solutions and buffers. 10 mL and 25 uL Hamilton syringes (serial numbers W012127/1 and 2501-84855 respectively) were used for sample transfer and HPLC injection. An Eppendorf centrifuge (serial number 0011107) was used for centrifugation for sample cleanup. A Shimadzu UV-1700, Japan (serial number A11024236251 CS) and a Shimadzu LC20-AT HPLC (serial number L20114250037 US L) with a corresponding DGU-20as degasser (serial number SSI-5-0180) and SPD-20A UV detector (serial number L20134350029 US A) were used for analyses of batch experiments.

3.2 Methods

3.2.1 Abiotic Batch Experiments: Soil Partitioning

A series of abiotic batch experiments were conducted for both pharmaceutical compounds (SMX and CMP). For both SMX and CMP, five specific mg/L concentrations ranges were chosen (i.e. SMX: 1, 5, 10, 30 and 60 mg/L and CMP: 0.1, 0.5, 1, 5, and 10 mg/L) based on the compound's corresponding solubility (500 mg/L and 17.7 mg/L, respectively). For the batch slurries, 300 mL subsamples of each concentration were extracted and placed into 1 L beakers. To ensure these water solutions were sterilized, 35 mg of mercuric chloride ($HgCl_2$) was added to each 300 mL sample. This volume was calculated in accordance with the 105 μg/mL as prescribed by Kattner et al. (1999) which has shown effective sterilization for up to 2 years.

Pre-sieved and measured 30 g sediment samples (collected at Village Creek, Birmingham, Jefferson County, Alabama) were dried for 72 hours (in accordance with the prior dry weight determination). Once a dry weight was met, each sample was placed in a 1-L flask treated with a 38 mL solution of NANOpure water with a concentration of 25 mg of $HgCl_2$. Mercuric chloride was used to effectively inhibit microbial activity within the sediment samples. Mercuric chloride has been shown to cause minimal alterations in physical and chemical properties of soil samples (Trevors et al., 1996). This 25 mg of $HgCl_2$ follows widely accepted protocols and is in accordance with the accepted standard ranges of 500-20,000 mg/kg (Wolf and Skipper, 1994). The mercuric chloride treated pharmaceutical solutions were then added to the 1-L flasks to create a 300 mL slurry. This 1:10 ratio of slurry volume to total flask volume, which follows widely accepted protocols,

was used to maximize aeration and be consistent with potential biotic batch experiments planned for future studies (Zhang et al., 2012; Blackwell et al., 2004; Kahle et al., 2007; Stein et al., 2008; Radke et al., 2009). Each 1-L flask (slurry) was capped and placed on a shaker table (constantly agitated) over the 4 day (96 hour) sampling period to allow equilibrium partitioning (i.e. sorption) to be achieved. To validate that equilibrium conditions were attained, aqueous concentrations of both SMX and CMP were analyzed immediately after initial slurry preparation and at specific time intervals of 8, 16, 24, 48 and 96 hours. All slurry batch solution concentrations were noted to have stabilized (constant value) by a 48 hour period. Previous studies have indicated that aqueous concentration loss associated with sorption on the tube wall or photo-degradation is less than 5% and can be neglected (Zhang et al., 2012) therefore any mass loss can be directly associated with adsorption.

Similar to the equilibrium batch test, time-series batch experiments were simultaneously conducted using the same sediment-pharmaceutical (SMX and CMP) solution concentration ranges and solid:liquid ratios (i.e. slurry). These time-series batch tests were conducted to evaluate rates of adsorption for the target analytes. Prior to each batch-test concentration measurement, the UV-Vis spectrophotometer was auto-zeroed using NANOpure water blanks. A 10-mL volume of the aqueous phase pharmaceutical (SMX or CMP) was extracted from the slurry batch solution at specific time intervals of sediment-solution contact (i.e. 0, 4, 16, 24 and 48 hours). This 10-mL volume was placed in a 21-mL borosilicate headspace vial and then centrifuged for 7 minutes at 3,200 rpm to allow for any potential suspended particulates to separate from the pure aqueous phase (supernatant) prior to analysis. A 5-mL subsample of this supernatant was then passed

through a 2-micron filter to remove any remaining colloidal suspensions. This filtered sample was transferred directly into a QS 10 mm quartz-silica cuvette and analyzed using the UV-spectrophotometer. This filtered sample was transferred directly into a QS 10 mm quartz-silica cuvette and analyzed using the UV-spectrophotometer. The cuvette was then placed in the UV-Spectrometer and absorbance was measured for the respective pharmaceutical compound using the predetermined wavelengths and prepared standard curves. The aqueous concentrations of SMX and CMP were quantified for each time intervals to determine the amount of chemical adsorbed versus time.

3.2.2 UV-Vis Spectrophotometry Methods

Wavelength scans using the UV-Vis spectrophotometer were conducted to determine the optimal wavelengths and absorbance characteristics for the various pharmaceutical compound solutions (i.e. SMX and CMP). All solutions were prepared in triplicate and allowed to equilibrate over time based on their reported known aqueous solubility values. Three stock concentrations of 60 mg/L and 10 mg/L for sulfamethoxazole and carbamazepine, respectively, were prepared and allowed to equilibrate until absorbance stabilized (became constant). The optimal wavelengths determined for analyzing SMX and CMP (peak absorbance) were 268 and 285 nm, respectively.

Five different solution concentrations were prepared for each pharmaceutical compound to develop appropriate standard curves. Similar to the batch test methods, SMX and CMP solution concentrations ranges were determined based on their respective aqueous solubility limits. SMX and CMP solutions included concentrations of 1, 5, 10, 30 and 60 mg/L and 0.1, 0.5, 1, 5, and 10 mg/L, respectively. These standard solutions were placed on a magnetic auto-stirrer and allowed to equilibrate for 48 hours.

Upon equilibration, a 10-mL aliquot of each stock solution concentration was delivered (via auto-pipettor) to a 21-mL borosilicate headspace vial and then centrifuged for 7 minutes at 3,200 rpm to allow for any potential suspended particulates to separate from the pure aqueous phase (supernatant) prior to analysis. A 5-mL subsample of this supernatant was then passed through a 2-micron filter to remove any remaining colloidal suspensions. This filtered sample was transferred directly into a QS 10 mm quartz-silica cuvette and analyzed using the UV-spectrophotometer. These concentrations were used to produce standard curves that would be later used for analyzing unknowns as a part of method development and for producing adsorption isotherms for SMX and CMP from batch experiments.

Wavelength scans were also performed on water samples collected from Village Creek to determine if organic matter or other constituents within the creek water would interfere and/or overlap with the optimal wavelengths determined for SMX and CMP compounds. Samples of Village Creek water, with and without mercuric chloride treatment, were analyzed on the UV-spectrophotometer using the methods described previously.

Additionally, wavelength scans were conducted for all potential factors that could contribute to interference and/or wavelength overlap and thereby lead to erroneous analytical quantification of the target analytes (SMX and CMP). For example, as a part of this method quality assurance/quality control, dish soap (cleaning detergent), methanol (cleaning agent), mercuric chloride, autoclaved Village Creek Sediment, and furnaced Village Creek sediment were all allowed to equilibrate for 48 hours in agitated NANOpure water and were tested independently of one another for interference and wavelength overlap. Additionally, samples of NANOpure water left within alternative cuvettes were

tested to determine if the cuvette type or material played a role in interference and/or wavelength overlap for analytical quantification.

3.2.3 Tandem SPE with HPLC Methods

A cost-effect solid-phase extraction (SPE) high performance liquid chromatography (UV detector) (HPLC-UV) was employed to quantify the target pharmaceutical analytes (SMX and CMP) for the equilibrium and time-series batch tests. This analytical quantification method was developed as an alternative to the UV-Vis spectrophotometric method whereby there may be many other compounds within the sediment or natural water sources contributing to wavelength interferences and erroneous analytical measurement.

The first step of buffer preparation process included making separate stock solutions of 0.2 molar (M) citric acid, 0.1 M Na_2-EDTA, 0.4 M Na_2HPO_4, HPLC grade H_3PO_4, and 0.1 M of NaOAc. A desired concentration of extraction buffer solution was created by mixing 90 mL of citric acid, 150 mL of Na_2-EDTA, 60 mL of Na_2HPO_4, and 3 mL of H_3PO_4. The final conditioning/washing buffer for SPE was adjusted by diluting the extraction buffer 20-fold to match the matrix after sample preparation.

Similar to the UV-Vis spectrophotometric sampling methods, 10-mL volume of the respective aqueous phase pharmaceutical compound (SMX or CMP) was extracted from the slurry batch solution at specific time intervals of sediment-solution contact (i.e. 0, 4, 16, 24 and 48 hours). This 10-mL volume was placed in a 21-mL borosilicate headspace vial and then centrifuged for 7 minutes at 3,200 rpm to allow for any potential suspended particulates to separate from the pure aqueous phase (supernatant) prior to analysis. A 5-mL subsample of this supernatant was then passed through a 2-micron filter to remove any

remaining colloidal suspensions. 0.5-mL of extraction buffer solution and 0.2-mL of methanol were added to the samples before being thoroughly mixed. The SAX and HLB SPE cartridges were then conditioned with 5-mL of methanol and then 5-mL buffer solution. The filtered subsample was then injected in tandem through the SPE cartridges (SAX then HLB) using a 10 mL borosilicate syringe and syringe pump (KD Scientific) at a rate of 10 mL/min. Once the subsample was fully dispensed, the SAX cartridge was removed from the setup. The remaining HLB cartridge was conditioned successively with 5 mL of the buffer, 2.5 mL of 0.1 M sodium acetate, 5 mL NANOpure water, and 2 mL of 20% methanol. After allowing to air dry for 10 minutes, the cartridges were treated with 2 mL of methanol and then again with 1 mL of methanol (Blackwell et al., 2004).

Upon completion of the tandem SPE procedure, the HPLC was then used to quantify the concentration of the respective target pharmaceutical analyte (SMX or CMP) for the specific batch-test time interval. Sample analyses for the batch experiments were conducted on the Shimadzu LC20-AT HPLC with a corresponding DGU-20as degasser and SPD-20A UV detector. The analytical HPLC methods described herein were modified from a study in which the concentrations of three veterinary antibiotics were quantified in groundwater (Blackwell et al., 2004).

Using a 25 *u*L syringe, 20 *u*L of the sample was injected into the HPLC injection Port. The column used for the HPLC was a Shimadzu 100 X 4.6mm 5 *u*L reversed phase C18 Column. The chromatographic mobile phase consisted of a ternary gradient elution scheme. The three mobile-phase solvents used were tetrahydrofuran (A), acetonitrile (B), and 0.05% trifuroacetic acid in NANOpure water (C). The ratios of these solvents were programmed into the HPLC to vary according to time. A solvent gradient ratio A:B:C was

programmed at 5:2.5:92.5 for 0-4 minutes, followed by a linear increase to 5:75:20 for 4-18 minutes, and then a linear decrease back to 5:2.5:92.5 for 18-20 minutes. This final ratio was held constant from 20-25 minutes to allow for equilibration (Blackwell et. al 2004). The HPLC was programmed so that the UV detector would record optimal absorbances at wavelengths of 268 and 285 nm for SMX and CMP, respectively. The absorbance for each sample was measured and recorded on Shimadzu LC Solutions controller/analyzer (within the software settings) as well as in a separate data file for later QA/QC development. All concentrations quantified were modeled against pre-determined standards.

3.3 Adsorption Parameters and Retardation Factors

The soil adsorption distribution coefficients (K_f) and retardation factors (R) were determined for both pharmaceutical compounds (SMX and CMP). Adsorption distribution coefficients were determined by the Freundlich adsorption isotherm equation:

$$S = K_f \cdot Cw^n \qquad (1)$$

Where S is the mass of the pharmaceutical adsorbed onto the solid phase and C_W is the mass of the pharmaceutical remaining in the aqueous phase, K_f is the adsorption coefficient and n is the fitting parameter or rate coefficient. If n = 1, the Freundlich isotherm can be reduced to the linear adsorption isotherm. Retardation factors (R) were determined by the following equation:

$$R = 1 + \frac{\rho b}{\theta} K_f Cw^{n-1} \qquad (2)$$

Where, ρ_b is the soil bulk density (g/cm^3), θ is the soil porosity (-), K_f is the determined distribution coefficient (mL/g), Cw is the known concentration of the solution and n is the Freundlich fitting/rate coefficient. It is assumed that the aqueous and solid phases were the

only factors contributing to retardation factors determined in the experimental systems tested.

4. RESULTS AND DISCUSSION

4.1 Validation of Methods

4.1.1 UV-Vis Spectrophotometer Analysis

The initial batch experiments analyzed using the UV-Vis spectrophotometry methods revealed an increasing concentration of target analytes over time as summarized in Figures 2 and 3. The longer the solution remained in contact with sediment during agitation on the shaker table, the higher the relative concentration (i.e. measured absorbance) of the particular target analyte until a point of equilibrium was reached (stabilization of concentration/absorbance).

Dish soap, methanol, mercuric chloride, autoclaved Village Creek sediment and Village Creek sediment treated by combustion, allowed to equilibrate for 48 hours in agitated NANOpure water, were tested independently to assess measurement and corresponding absorbance interference. Dish soap, methanol, mercuric chloride, and NANOpure water conditioned within various cuvettes (material types-polymer and quartz-silica) all showed negligible interference (absorbance overlap) at the specific measurement wavelengths for the target analytes (SMX and CMP). These tests confirm that these solutions were not directly limiting the analytical capabilities of the UV-Vis spectrophotometric due to absorbance interference. However, samples of NANOpure water conditioned within both the autoclaved Village Creek sediment and Village Creek combustion-treated sediment showed significant overlap in absorbance for the respective

compound measurement wavelength. The Village Creek combustion-treated sediment, in particular, exhibited a lower absorbance at the specific intervals compared to the autoclaved Village Creek sediment. The results of these tests demonstrate that analytical measurement errors due to absorbance interference were primarily due to the presence of other dissolved constituents associated with the Village Creek sediment and/or water source. Such findings demonstrate the inherent limitations to the UV-Vis spectrophotometric method when analyzing various dissolved-phase compounds (i.e. pharmaceuticals and/or OWCs) present within natural stream-water/sediments sources.

The adsorption or partitioning of dissolved phase constituents to sediment effectively decreases the concentration of that constituent (i.e. target analyte) in the aqueous phase over time. Village Creek sediment characterization (X-ray diffraction and carbon combustion analyses) revealed that both illite and a significant amount of organic material (fraction of organic carbon) were present within all samples tested. Illite is a non-expanding clay mineral and is responsible for a majority of inorganic surface area available for adsorption in sediments that contain it (Kahle and Stamm, 2007; Zhang et al., 2011). Illite has a relatively low cation exchange capacity (CEC) when compared to other clay minerals. This low CEC will result in a lower exchange and inorganic complexation. The presence of organic or humic material also contributes to surface area available for adsorption and has been well documented to contribute to greater adsorption in groundwater systems (Blackwell et al., 2004; Petite et al., 2004). Because the “apparent” concentrations of the study analytes seemed to increase within the system over the duration of the batch experiment, it can be concluded that UV interference (absorbance overlap) was responsible for such effects (i.e. measurement error) for these systems which

either leached, dissolved, or already contained other constituents from the sediment and/or water matrix. Such absorbance interferences could also be due to diffusional processes whereby dissolved constituents diffuse into the aqueous phase from the micro-/macro- porosity associated with the sediment.

The fraction of organic carbon content is important to consider when quantifying adsorption. Generally, there is a positive correlation between the organic carbon content of a sediment and the adsorption coefficient (Nelson and Sommers, 2006). Additionally, a higher organic content allows for a greater potential or ratio of humic material which is responsible for the majority of organic adsorption in streambed systems (Petite et al., 2004). The 3.63% organic carbon content determined for these sediments is slightly above average for streambed sediments (1-3%) which indicates that this sediment would be capable of significant adsorption attributed to organic matter.

Furthermore, the analytical method testing confirmed that the observed interferences (absorbance overlap) were not due to colloidal suspensions within the aqueous phase. All colloidal suspensions were effectively removed from the aqueous phase before analyses. Centrifugation and filtering through a 2-micron filter have proved to be valid techniques to remove colloidal suspension when used in tandem (Blackwell et al., 2004; Radke et al., 2009; Zhang et al., 2012).

Biotic degradation of compounds within the system can also be effectively ruled out as a contributor to the interference. Because the system was effectively microbially inhibited through the use of mercuric chloride, no biodegradation or degradation products (metabolites) could result under the conditions of the experiments (Trevors et al., 1996).

For these reasons, effects associated with microbial degradation could not have contributed to the interference experienced in the analysis.

Inorganic constituents were assessed for potential interference in this experimental system through Inductively coupled plasma mass spectrometry (ICP). This ICP analysis revealed that calcium, magnesium, silica, sodium, potassium and strontium were the dominant cations in solution.. All other inorganic elements were present in negligible concentrations. These suite of constituents was likely dissolved from the dolomitic and silica rich sediments in the village creek system. Although unlikely due to the low CEC of the illite in the system, these inorganic species could have contributed to the interference present in the UV analysis.

Although undetermined, it can safely be concluded that the majority of interference within this system was a function of humic or organic material diffusing, dissolving, and/or desorbing over time. Sediment analyses reveal that there is a significant amount organic matter within the Village Creek sediment. Supporting this conclusion, the Village Creek sediment that was treated in a temperature controlled combustion step showed less interference than the same sediment that had not been treated with this combustion step. Although all interference (absorbance overlap) cannot be attributed solely to the organic material, it is speculated that this was the major factor responsible for effects (i.e. increasing concentrations trends of analytes) observed in the batch experiments when using UV-Vis spectrophotometry.

4.1.2 Tandem SPE and HPLC Analysis

Samples from the batch tests were split (duplicate) upon collection for analysis using the HPLC methods so that concentrations for these study analytes would be

consistent between both analytical methods (UV-Vis spectrophotometer vs. HPLC) and allow for direct comparison. The HPLC analytical method has the capability to provide much higher resolution than the UV-Vis spectrophotometer and can therefore measure much lower concentrations in groundwater. Therefore, future studies can focus systems with more environmentally relevant concentrations ranges.

Biotic degradation of compounds within the system can be effectively ruled out because the system was effectively microbially inhibited through the use of mercuric chloride (Trevors et al., 1996). Negligible concentration loss occurred as a result of any potential photodegradation during batch time-series or during analysis. Previous studies indicated that aqueous concentration loss associated with sorption on the tube wall or photodegradation is less than 5% and can therefore be neglected (Zhang et al., 2012). As described previously, it is also unlikely the target analytes metabolized into degradation byproducts due to the microbial-inhibiting methods employed. Also, any effects due to colloidal suspension within the aqueous phase were effectively removed before analysis. Centrifugation and filtering through a 2-micron filter have proved to be valid techniques to remove colloidal suspension when used in tandem.

4.2 Utility of Established SPE/SPE HPLC Method

Sulfamethoxazole was allowed to equilibrate for 96 hours. In most instances, 97% of the adsorption occurred within the first 24 hours of equilibration. Samples taken before this 24 hour time interval would be unnecessary for future studies. The adsorption coefficient (K_f) was estimated to be 4.22 L/Kg from the results of the batch tests and the development of the adsorption isotherm (Figure 4). This value was consistent to ranges (2.5-8.14) reported by Zhang et al. (2011) and Boxall et al. (2012) but were significantly

higher than those values established in other studies such as Stein et al. (2008) and Kahle et al. (2007). However, it should be noted that all of these values reported in literature were determined using different methods and different sediment types. Such differences are likely attributed to the differences in sediment composition and dynamics between individual chemical properties and media itself. Differences in pH, organic content, and sediment composition could affect these values significantly. Therefore, such estimates (K_f) would be expected to change based on the environmental conditions and specific sediment types of the system.

The SMX batch-test adsorption isotherm data was characterized by a strong positive linear correlation ($R^2 = 0.99$). Replicate batch-test experiments show nearly exact matches (all falling within the 90% confidence intervals of one another) suggesting that this method was extremely precise and reproducible. The adsorption rate fitting coefficient (n, exponent) was determined to be 1.19 which indicates that, although not perfectly ideal, this phenomenon (adsorption) could be described using the linear adsorption isotherm. The retardation equation mentioned above and the average calculated bulk density (1.52 g/cm^3) and porosity (0.39) retardation was calculated to be 17.52, indicating that this contaminant would tend to "react" with the aquifer media delaying (retardation) the transport of the chemical through the system, compared to ideal conservative tracer transport (Figure 5). Because the K_f value would change in different sediments, the retardation would also be expected to change accordingly. It should be noted that K_f estimates are expected to be dependent on soil pH conditions (Kahle, M. and Stamm, C) . In this study, soil pH was consistent for all experiments and was determined to be 6.5. which is within the 6.0-7.5 pH range of most human contacted sediments Stein et al. (2008)

shows that relatively small pH increases can have large quantifiable effects on adsorption rates. It was shown that an increase from 6.5 to 6.6 dramatically decreased the adsorption rates of the tested analytes (Stein et al., 2008) .

Carbamazepine was also allowed to equilibrate for 96 hours. In most instances, 99% of the adsorption occurred within the first 16 hours of equilibration. Samples taken before this 16 hour period would be unnecessary for future studies. Only 4 experiments were sampled at each time interval. The remaining experiments were sampled once at 0 hours and once at 96. The results, summarized in Figure 4, revealed that a simple linear isotherm could not accurately represent the adsorption taking place within the system. Therefore, the Freundlich adsorption isotherm was implemented to incorporate the fitting parameter "*n*" as described previously. The adsorption rate fitting coefficient (*n*, exponent) was determined to be 0.56 which indicates that nonlinear sorption processes were influencing the sorption of CMP on the sediments. This approach revealed that the adsorption rate coefficient (K_f) for carbamazepine in this system was 8.79 L/Kg (R^2 = 0.97) as summarized in Figure 4. The R2 value of (R^2=0.97) suggests that the Freundlich isotherm (nonlinear sorption) represents the adsorption phenomenon much more accurately than that approximated by a linear isotherm (*n*=1) (R^2= 0.88). Using the retardation equation mentioned above (eq. 2) and the average calculated bulk density (1.52 g/cm^3) and porosity (0.39), retardation was calculated to be 25.67 (Figure 5.). This suggests that this contaminant would be highly associated with the sediment phase during transport and would thereby deviate significantly from "conservative" (ideal) transport behavior and subject to substantial retardation as it travels through the groundwater systems compared to that of SMX. CMP would therefore be more difficult to remediate from groundwater

when compared with SMX. However, the delayed transport of CMP would decrease the risk of reaching critical receptors such as drinking water wells and water treatment facilities, and surface water impoundments relatively far from the contaminant source. In terms of removal and remediation these results support CMP's widely documented persistence in groundwater systems and sludge (i.e. bioreactors).

Based on the results of this study, a general positive trend between the degree of compound polarity (as represented by the octanol-water partition coefficient, K_{ow}) and the adsorption coefficient (K_f) (and correspondingly retardation) can be estimated for such pharmaceutical compounds (Table 4). However, further work is needed to establish more rigorous correlations of adsorption as a function of compound polarity based on specific chemical and molecular (steric effects and charge densities, etc.). The average retention time of SMX was expected to be lower than that of CMP based on the polarity derived from known K_{ow} values (i.e. from literature) in a nonpolar stationary-phase column. These trends were consistent to that expected, as the measured retention time of SMX was significantly lower than that of CMP (7.56 and 11.21 minutes, respectively). This indicated that estimated K_{ow} values can be used to assess relative polarity differences and effects on magnitude of adsorption (i.e. K_f and R) for the two compounds (SMX and CMP) and can likely be applied to other relevant pharmaceutical compounds of interest.

5. CONCLUSIONS

A robust, cost-effective analytical method was developed and validated using two commonly detected pharmaceuticals in water resources; sulfamethoxazole (SMX) and carbamazepine (CMP). Tandem solid-phase (SPE) extraction in conjunction with high performance liquid chromatography (HPLC) was effective for quantifying concentrations of analytes under environmentally relevant laboratory-scale scenarios. It was determined through rigorous and systematic series of testing that UV-Vis spectrophotometry, although simplest and most cost-effective in practice and theory, could not be used appropriately for the analysis of pharmaceutical compounds sampled from natural systems. For this reason, higher resolution HPLC methods were employed to successfully evaluate fate and transport behavior of these pharmaceuticals. Compared to the extremely high resolution and prohibitively costly SPE UPLC-MS/MS method, tandem SPE HPLC capabilities proved to be ideal at quantifying pharmaceutical concentrations for environmentally relevant conditions. The validation of this method proves that the tandem SPE methods can effectively remove organic absorbance interference from several classes of pharmaceuticals without affecting their recoveries (≥96.25%) for analysis. The method proved to be efficient in analyzing environmentally relevant concentrations (sub ppb). This is import for both private and municipal industries that have an interest in analyzing water samples for emerging contaminants in low concentrations. It provides a cheaper alternative to the standard SPE HPLC-MS/MS method that is currently implemented by labs

suited to analyze for these contaminants. In particular, this method could prove to be extremely helpful for waste water treatment plants who have a need to analyze for a specific suite of pharmaceutical compounds due to their proximity to a pharmaceutical manufacturer.

Although not implemented, this method could be applied to analyze multiple compounds simultaneously. Other studies have shown this methods ability to analyze several antibiotics simultaneously (Blackwell et. all 2004).

Sulfamethoxazole (SMX) has a lower affinity for adsorption that can be attributed to its higher polarity. Sulfanamide drugs have the potential for their adsorption to vary greatly due to their tendency for speciation at different at different pH's (strongly anionic at high pH and slightly cationic at low pH's which accounts for the majority of it's adsorption) in environmental circumstances. Carbamazepine (CMP) has a higher affinity of adsorption that can be attributed to its lower polarity. It shows a higher rate of adsorption when compared with SMX and would therefore be more difficult to remediate in groundwater systems; however pose less risk than SMX at reaching critical receptors water-supply wells, surface-water impoundment, and/or water treatment facilities. Overall, this tandem SPE HPLC is a comparatively robust and simple analytical technique that should enable fate and transport studies to progress for these (i.e. SMZ and CMP) and other classes of emerging contaminants in a more cost-effective and available means to practitioners and researchers alike.

There are many directions for this research to be expanded upon. Further research utilizing this method can focus on analyzing lower concentrations of multiple compounds simultaneously. This would further prove the applicability of the method as a strong

alternative to more expensive methods. Also, testing these fate and transport parameters over a range of pH's, and in different sediments would also be a useful expansion upon this research.

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APPENDIX

Table 1. General properties of study analytes.

Compound	Solubility (mg/L)	Vapor Pressure (mm Hg)	Log Kow	Molecular Mass (g/mol)	Henry's Law Constant (atm-cu m/mol)	Polarity
SMX	500	6.9X10^-8	0.89	253.278	6.4X10^-13	Very
CMP	17.7	1.8X10^-7	2.45	236.269	1.1X10^-10	Moderately

Table 2. Major mineral constituents and corresponding sediment properties: Bulk density (g/cm^3), porosity (%), particle density (g/cm^3), total carbon content (%), fraction of organic carbon (%) derived through numerous methods.

Bulk Density (g/cm^3)	Porosity (%)	Particle Density (g/cm^3)	Total Carbon Content (%)	Fraction of Organic Carbon (%)
1.5171	0.3875	2.478731834	13.73484047	3.627913486

Major Mineral Constituents	
Quartz	SiO2
Calcite	Ca(CO3)
Dolomite	MgCa (CO3)2
Illite	KAl2Si3AlO10(OH)2

Table 3. Method validation and limits of detection: Average recoveries and detections in deionized water

Compound	Known Concentration (mg/L)	Standardized Absorbance	Recovery Absorbance	Recovery (mg/L)	Recovery Percentage	Limits of Detection (µg/L)
CMP	0.5	2.299	2.716	0.481	96.1248	0.6
CMP	0.5	2.299	2.801	0.499	99.7628	
CMP	5	24.435	22.682	4.753	95.06696	
CMP	5	24.435	24.371	5.115	102.29588	
SMX	1	8.041	7.679	0.963	96.26994	0.48
SMX	1	8.041	8.002	1.011	101.06972	
SMX	10	69.471	63.103	9.199	91.987058	
SMX	10	69.471	64.568	9.416	94.164048	

Table 4. Polarity's (as represented by the octanol-water partition coefficient, K_{ow}) correlation with observed transport parameters and limits of detection

General Properties				
Compound	**Log Kow**	**Freundlich Coefficient *(Kf)*** *(l/Kg)*	**Averaged Retardation (*R*)**	**Limit of Detection (LOD)**(µg/L)
SMX	0.89	4.22	20.76	0.48
CMP	2.45	8.79	49.3	0.60

Figure 1. Sieve analyses of ~1,000 grams of Village Creek streambed sediment that had achieved a dry weight. Sieve sizes ranged from 9.5 mm to 0.03125mm.

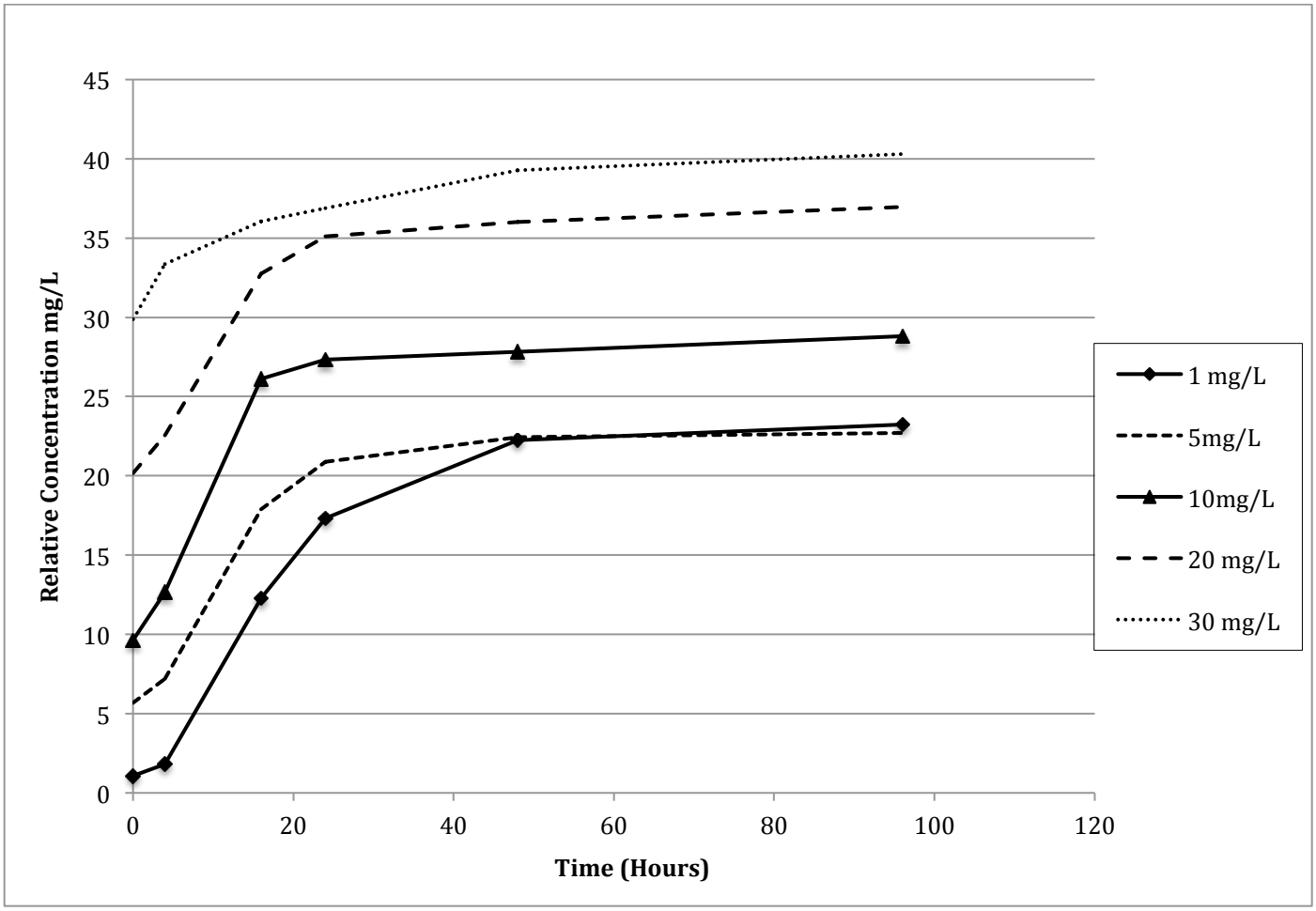

Figure 2. Concentrations over time of SMX recorded from the batch experiments analyzed using the UV-Spectrometer after centrifugation and 2 micron filtration. Concentrations should theoretically be decreasing with time due to adsorption.

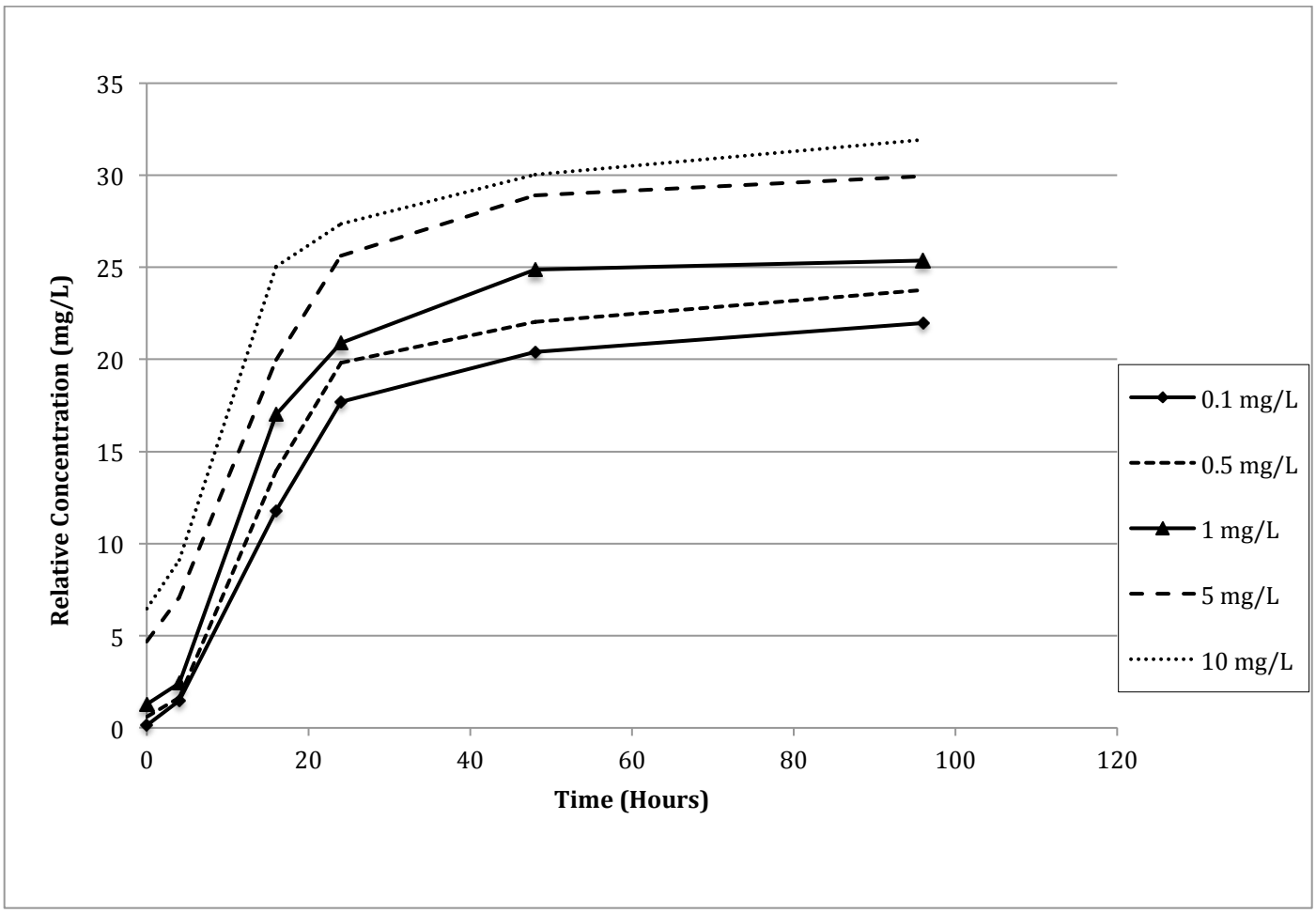

Figure 3. Concentrations over time of CMP recorded from the batch experiments analyzed using the UV-Spectrometer after centrifugation and 2 micron filtration. Concentrations should theoretically be decreasing with time due to adsorption.

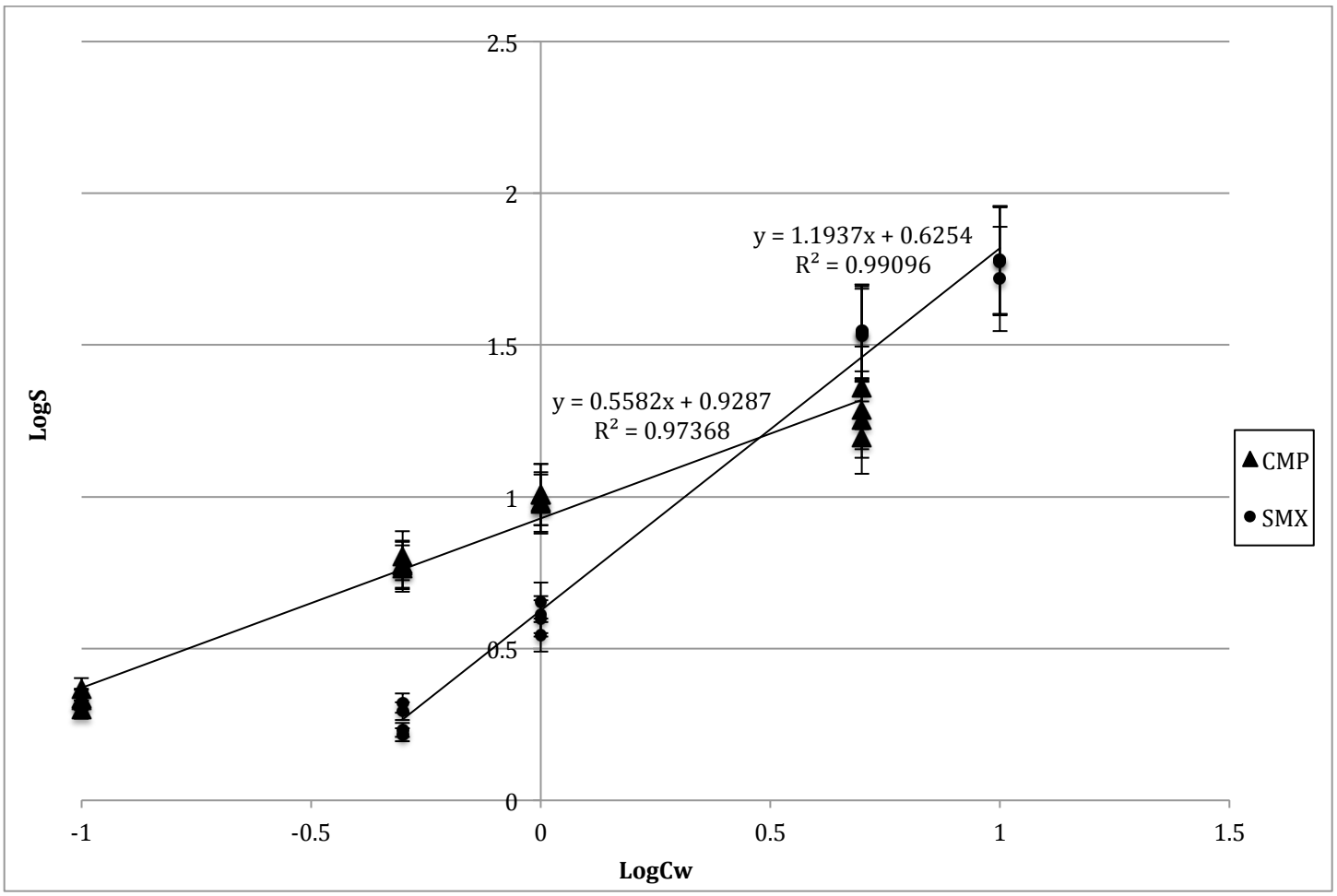

Figure 4. Freundlich adsorption isotherms for both SMX and CMP. Both the X and Y axis represent the log values of *Cw* and *S* respectively. The Y-intercept represents the log of the *Kf* values for these compounds and the slope values represent the *n* term in the Freundlich adsorption isotherm equations. R^2 values represent how accurately this isotherm represents the empirical data points.

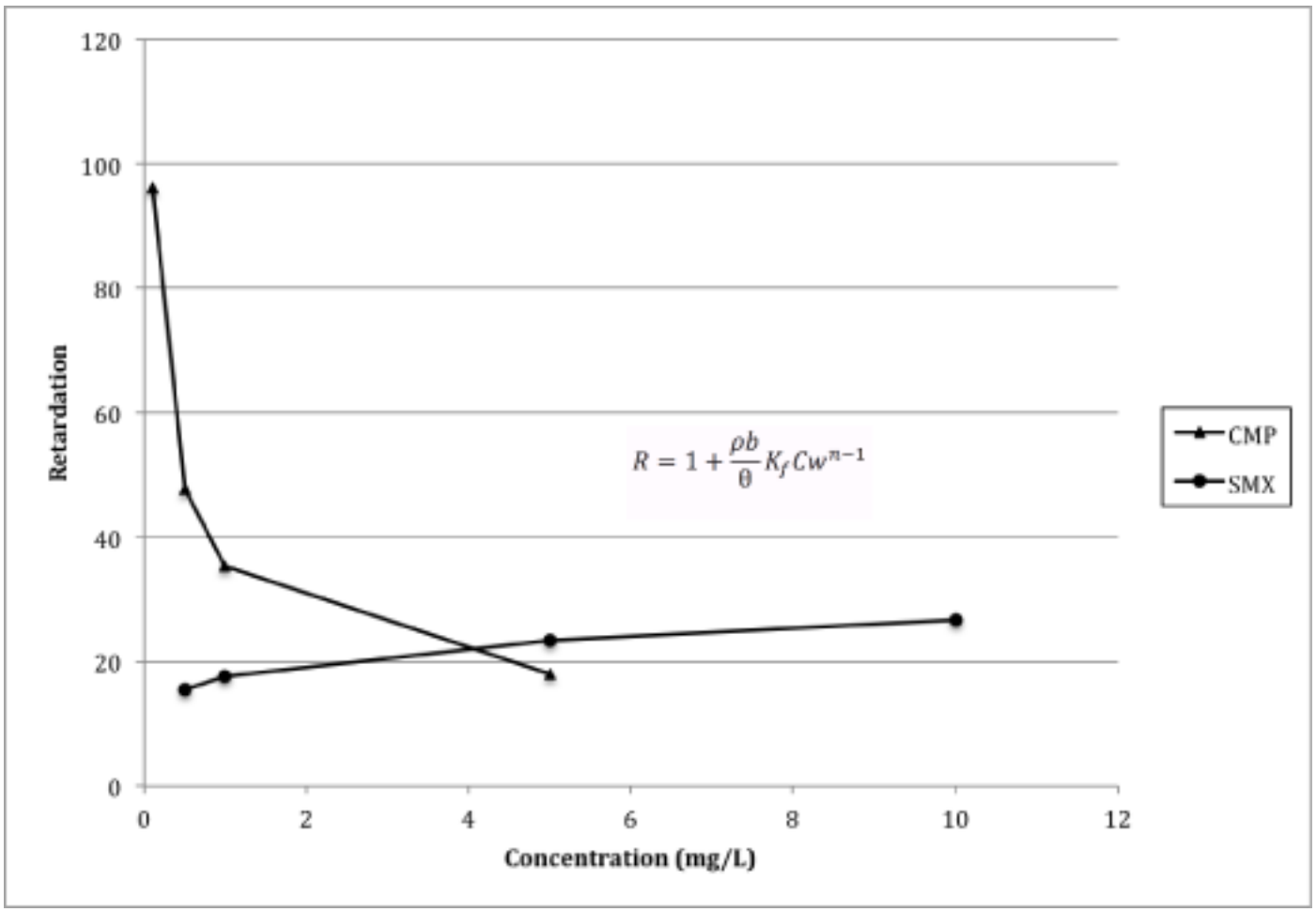

Figure 5. Compound retardation based on empirical *Kf* values. The Y axis parameter, retardation, is a dimensionless value representing the number of pore volumes needed to flush the contaminant through the system.

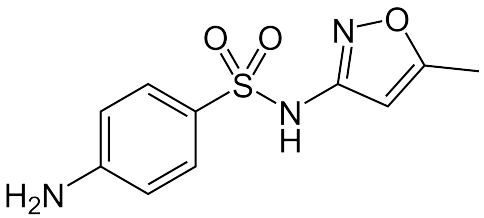

Figure 6. Molecular structure of sulfamethoxazole (SMX)

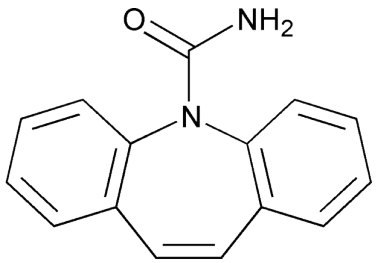

Figure 7. Molecular structure of carbamazepine (CMP)