Optimization of Analytical Methods to Improve Detection of Erythromycin from Water and Sediment

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Abstract
Analytical methods to improve the detection of erythromycin in water and sediment were developed to optimize for erythromycin's recovery of extractable and bound residues from the aquatic environment. The objective of this study was to determine optimal recovery of erythromycin from water and sediment to improve its detection in environmental samples through solid-phase extraction (SPE) and sediment-extraction methods. SPE methods examined included previously reported methods for macrolide and sulfonamide antibiotics with erythromycin recoveries ranging from 75.5 % to 94.7 %. Extraction of erythromycin was performed from sand employing various solvents and buffers to determine the best method for extraction from two sandy loam pond sediments. Various extraction times were also examined, and all extraction procedures were performed in duplicate. The greatest recovery of (14)C-erythromycin in the Iowa sediment was 84 % using 0.3 M ammonium acetate at pH 4.2: acetonitrile (15:85, v/v) solution. The Oklahoma sediment yielded the greatest recovery of (14)C-erythromycin at 86.7 % with 0.3 M ammonium acetate at pH 7: acetonitrile (30:70, v/v) with a 60-minute shake time. The present results demonstrate improved extraction methods for enhancing the accuracy of erythromycin detection from environmental samples.

Keywords
Antibiotics, environmental matrices, solid-phase extraction (SPE), sediment extraction

Disciplines
Entomology

Comments

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Optimization of analytical methods to improve detection of erythromycin from water and sediment

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Analytical methods to improve the detection of erythromycin in water and sediment were developed to optimize for erythromycin's recovery of extractable and bound residues from the aquatic environment. The objective of this study was to determine optimal recovery of erythromycin from water and sediment to improve its detection in environmental samples through solid-phase extraction (SPE) and sediment-extraction methods. SPE methods examined included previously reported methods for macrolide and sulfonamide antibiotics with erythromycin recoveries ranging from 75.5 % to 94.7 %. Extraction of erythromycin was performed from sand employing various solvents and buffers to determine the best method for extraction from two sandy loam pond sediments. Various extraction times were also examined, and all extraction procedures were performed in duplicate. The greatest recovery of ¹⁴C-erythromycin in the Iowa sediment was 84 % using 0.3 M ammonium acetate at pH 4.2: acetonitrile (15:85, v/v) solution. The Oklahoma sediment yielded the greatest recovery of ¹⁴C-erythromycin at 86.7 % with 0.3 M ammonium acetate at pH 7: acetonitrile (30:70, v/v) solution at a 60-minute shake time. The present results demonstrate improved extraction methods for enhancing the accuracy of erythromycin detection from environmental samples.

Keywords: Antibiotics; environmental matrices; solid-phase extraction (SPE); sediment extraction

Introduction

Non-therapeutic use of antibiotics in livestock accounts for 24.6 million pounds of antibiotics in feeds.¹ In recent years, detection of various classes of antibiotics in several environmental matrices, including sewage treatment plant effluents and surface water, have been reported.² A survey study in 2002 reported the detection of antibiotics in 48 % of 139 streams examined.³ Additionally, antibiotics have been detected in sediment systems and manure slurries, ranging between 82 µg L⁻¹ and 128 µg L⁻¹⁴,⁵ and 11 mg kg⁻¹ and 43 mg kg⁻¹ respectively.⁶ The rise in the number of papers addressing veterinary antibiotics detection and fate in the environment emphasizes the need for analytical methods to be optimized to enhance their detection in environmental matrices including water and sediment.

One antibiotic commonly detected in water is erythromycin at concentrations between 50 ng L⁻¹ and 20 µg L⁻¹.²⁻⁵ Erythromycin is an antibiotic used in livestock and poultry production to aid in growth promotion, feed efficiency, and disease prevention. Excretion of erythromycin by animals occurs as parent compound or metabolites with environmental entry of this compound occurring from injection or waste incorporation of fertilizer in soil, leading to the potential for antibiotic residues and nutrients to enter water and sediment.⁷ Recent studies have indicated antibiotics have the ability to leach into ground water, run off into surface water, and possibly enter drinking water.⁸ Due to an increase in occurrence and detection of this antibiotic in surface water bodies, additional information regarding the occurrence and fate of erythromycin is needed to better understand erythromycin's environmental fate.

Though detection methods exist, improvement of extraction and clean-up methods for the measurement of erythromycin in environmental matrices is needed to enhance the accuracy and sensitivity of quantifying this compound at environmentally relevant levels. Prior to identification of parent compound and metabolites present in samples, clean up and concentration of erythromycin must occur due to the low levels found in most environmental samples. This step is primarily achieved through solid-phase extraction (SPE) procedures. Many of the SPE methods that have been previously utilized focus on incorporating multiple columns in tandem, various pH adjustments, and high flow rates yielding recoveries from water samples.
ranging between 45 % and 100 % for the various antibiotics examined. [3,6,9–16] However, these methods are rather expensive, time-consuming, and recoveries vary for the various antibiotic classes examined in multiple class studies. Common SPE steps for macrolide antibiotics include pH adjustment prior to analysis and the use of buffers to assist in increasing recoveries. [10,11,17] Recovery of erythromycin in experiments has been demonstrated to vary widely, from 40 % –95 %. [17–20] Due to highly variable recovery rates optimization of the SPE step is needed to improve quantification to increase the reliability of analytical detection methods.

Another important aspect regarding detection of antibiotics in the environment is the ability to recover extractable residues from sediment samples. The extraction of antibiotics from soils and sediments is often more difficult due to the organic matter, moisture, and clay contents. Recoveries of macrolide antibiotics from soils with and without manure amendments ranged between 43 % and 86 %. [10,13,21] Improved extractability and reliable extraction procedures for quantifying erythromycin from sediment and soil systems will aim to improve recovery rates and potentially yield better initial extractability data for environmental fate studies.

Current methods for the detection of erythromycin focus on its detection in conjunction with other antibiotics primarily from water samples, with few studies examining sediment systems. The aim of this study was to optimize SPE clean-up and sediment extraction procedures of erythromycin from water and sediment to enhance quantification of this compound from environmental matrices. In this study, we examined three previously published solid-phase extraction methods, a sulfamethazine method and two tylosin methods, plus an altered tylosin method. Extraction of erythromycin in sand and pond sediment from Iowa and Oklahoma was examined. Sand was used to determine the optimal pHs of the buffers utilized, and the best recovery from each buffer extraction solution was then tested on the two pond sediments. All analyses were performed employing radiotracer analysis with 14C-erythromycin.

Material and methods

Chemical, reagents, and standards

Radiolabeled erythromycin was purchased from American Radiolabeled Chemicals (St. Louis, MO) with a specific activity of 55 mCi mmol⁻¹. All radioactive materials used were handled in accordance with all safety guidelines enforced at Iowa State University. Erythromycin was 14C-labeled on one of the methyl groups of the desosamine sugar (Fig. 1). Acetonitrile, methanol, ethyl acetate, citric acid, ammonium acetate, potassium hydroxide, ammonium hydroxide, glacial acetic acid, hydrochloric acid, and Ultima Gold liquid scintillation cocktail were purchased from Fisher Scientific (Pittsburgh, PA). Solvents utilized were high performance liquid chromatography (HPLC)-grade and included methanol and acetonitrile. The sediment extraction buffers and solutions were made fresh daily and included 0.2 M sodium phosphate:acetonitrile (15:85, v/v), 0.2 M citric acid:acetonitrile (50:50, v/v), and 0.3 M ammonium acetate:acetonitrile (15:85, v/v and 30:70 v/v).

Sand preparation

Commercial sand purchased from Lowe’s was utilized in this study and was washed six times prior to use with nanopure water and sifted to remove fine particulate matter. Sand was completely dried prior to utilization in experiments.

Collection and composition of sediments

Sediments utilized in this study included pond sediment attained from Iowa (IA) and Oklahoma (OK). The IA sediment for experiments was collected from the Iowa State University Horticulture Research Station Pond (Gilbert, IA), while the OK sediment was obtained from the Oklahoma State University Agronomy Experimental Pond Facility located at Lake Carl Blackwell in Stillwater, Oklahoma. Collection of sediment samples was conducted manually by inserting a soil auger 10 –15 cm (depth) into the pond sediment. Sediment was brought to the laboratory and stored at 4°C prior to use. Sediment characterizations for the two sediments were conducted by Midwest Laboratories (Omaha, NE), and properties are listed in Table 1.

Solid-phase extraction (SPE)

Samples contained 50 mL of distilled water in a 100-mL glass jar spiked with 50 µL of 0.94 µCi mL⁻¹ of 14C-erythromycin. Total concentration per jar was 0.003 µCi
Detection methods for erythromycin

Table 1. Sediment properties for Iowa and Oklahoma pond sediments.

<table>
<thead>
<tr>
<th></th>
<th>Moisture content (%)</th>
<th>Organic matter (%)</th>
<th>Sand (2000 – 50 µm) (%)</th>
<th>Silt (&lt;50 – 2 µm) (%)</th>
<th>Clay (&lt;2 µm) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iowa</td>
<td>8.1</td>
<td>33.2</td>
<td>2</td>
<td>60</td>
<td>28</td>
</tr>
<tr>
<td>Oklahoma</td>
<td>7.2</td>
<td>43</td>
<td>1.2</td>
<td>55</td>
<td>32.5</td>
</tr>
</tbody>
</table>

Table 3. Recovery of erythromycin from sand utilizing different extraction solutions at varying pHs.

<table>
<thead>
<tr>
<th>Extraction solution</th>
<th>pH</th>
<th>Recovery (%)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetonitrile</td>
<td>–</td>
<td>19.1 ± 3.4</td>
</tr>
<tr>
<td>Ethyl Acetate</td>
<td>–</td>
<td>10.6 ± 0.7</td>
</tr>
<tr>
<td>Methanol (70 %)</td>
<td>–</td>
<td>48 ± 1.4</td>
</tr>
<tr>
<td>Acetonitrile: Glacial Acetic Acid (96:4)</td>
<td>–</td>
<td>43.9 ± 3.7</td>
</tr>
<tr>
<td>Acetonitrile: 0.2M Potassium Phosphate dibasic (60:40)</td>
<td>8.86</td>
<td>74.9 ± 4.8</td>
</tr>
<tr>
<td>Acetonitrile: 0.2M Sodium Phosphate (85:15)</td>
<td>3.2</td>
<td>87.2 ± 7.3</td>
</tr>
<tr>
<td>Acetonitrile: 0.3M Ammonium Acetate (85:15)</td>
<td>4.2</td>
<td>99.3 ± 3.8</td>
</tr>
<tr>
<td>Acetonitrile: 0.3M Ammonium Acetate (70:30)</td>
<td>4.2</td>
<td>74.9 ± 10.2</td>
</tr>
</tbody>
</table>

aStandard error is listed as percentages with n = 2.

Sand and sediment extraction

Samples consisted of either 20 g oven-dry weight sand or 36.75 g wet weight sediment (20 g dry weight) in a 250-mL French square bottle and were analyzed in duplicate replications (n = 2). Each sample was spiked with a solution of 14C-erythromycin that contained 0.14 µCi of 14C and 2 µg of erythromycin, mixed, and allowed to incubate for 15 minutes. Total concentration of 14C-radiolabelled erythromycin per sample was 0.014 µg. Extractable erythromycin was removed from samples by addition of solvent to each substrate sample, followed by shaking on an orbital shaker at 300 rpm, centrifugation for 12 minutes at 350 g (for sediment samples) and collection of supernatant.

Table 2. Solid Phase Extraction (SPE) methods.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Elution</td>
<td>3 mL Methanol</td>
<td>3 mL Acetonitrile: Glacial Acetic Acid (96:4, v:v)</td>
<td>4 mL Acetonitrile</td>
<td>2 mL acetonitrile: Glacial Acetic Acid (98:2, v:v)</td>
</tr>
<tr>
<td>Rinse</td>
<td>3 mL Ultra pure water</td>
<td>3 mL Ultra pure water</td>
<td>4 mL 2 % Acetonitrile</td>
<td>3 mL methanol-water-ammonium hydroxide (60:38:2, v:v)</td>
</tr>
<tr>
<td>3 mL –0.5M HCl</td>
<td>3 mL Acetonitrile</td>
<td>5 mL Ultra pure water</td>
<td>4 mL 0.5M KOH</td>
<td></td>
</tr>
<tr>
<td>3 mL Ultra pure water</td>
<td>3 mL Ultra pure water</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

of 14C and 0.04 µg of erythromycin. After water samples were spiked they were shaken on a rotary shaker at 300 rpm for 5 minutes. Erythromycin was extracted and concentrated from water using hydrophilic-lipophilic balance (HLB) solid-phase extraction cartridges (6 cc, Oasis HLB®, Waters Corporation, Milford, MA).[22] Extraction methods examined for the quantification of erythromycin from water were compared using previously published methods for tylosin and sulfamethazine, with a modified tylosin SPE procedure examined based upon previously published tylosin SPE methods.[23–25] All SPE methods, including conditioning and elution solutions and amounts, utilized in this study are listed in Table 2. Each extraction method was performed in triplicate (n = 3). Samples were passed through the cartridges at a flow rate of 5 mL min⁻¹. Radioactivity was counted after SPE extraction in 1-mL aliquots from sample extract waste water and sample elution concentrate plus 15 mL of Ultima Gold cocktail per sample.
A second solvent extraction was performed with the substrate utilizing an additional 40 mL of extraction solution with repeated shaking, centrifuging, and collection as described previously. An array of extraction solutions were examined for their ability to recover erythromycin, as described in Table 3. A variety of extraction times were examined for incubation of solvent with the substrates including 20, 30, 60, and an 85-minute shake followed by an overnight incubation with an additional 15-minute shake the next day. The total volume of each extract sample after extraction was 80 mL. Extracts were concentrated to 1 mL under N₂ flow at 15 psi and 50°C. The 1-mL samples were reconstituted to a 10-mL final volume with the extraction solvent, using acetonitrile for extraction solutions containing a buffer solution. Radioactive counts were conducted on a Beckman 5000ce LSC using 3 mL of extract sample and 12 mL of Ultima Gold cocktail.

Results and discussion

Comparison of SPE procedure methods

The erythromycin recoveries from water for the SPE conditioning and elution methods examined are displayed in Table 4. The lowest recoveries of erythromycin occurred utilizing the modified tylosin method (75.5 %) and the Henderson[26] method (78.8 %). While the best recoveries of erythromycin were shown using the Hu and Coats[23] and Kolz et al.[11] methods, with 88.7 % and 94.7 % respectively.

SPE methods are commonly employed techniques for the extraction of antibiotics, especially macrolides, from water samples. Many of the methods utilize multiple cartridge types and pH adjustment for improved recoveries.[16,19,27] One SPE cartridge that has been commonly used with macrolide antibiotics is the Oasis HLB® type, which has been demonstrated to yield recoveries ranging between 64 %−94 % for erythromycin.[16, 17,19, 20,27, 28] The compound erythromycin is a weak basic antibiotic (pKₐ of 8.8) which can be transformed into ionic and lipophilic forms influencing its ability to be retained on SPE cartridges. For all of the methods examined in this study the Oasis HLB® cartridge was utilized yielding recoveries of > 75 %. Oasis HLB® cartridges are designed to retain both non-polar and polar compounds to improve extraction of a wider array of compounds, compared to other SPE cartridges.[22]

Table 4. Solid Phase Extraction (SPE) recoveries of erythromycin applying methods listed in Table 2.

<table>
<thead>
<tr>
<th>Method</th>
<th>Recovery (%)</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Henderson method[26]</td>
<td>78.8</td>
<td>± 4.4</td>
</tr>
<tr>
<td>Modified tylosin method</td>
<td>75.5</td>
<td>± 4.3</td>
</tr>
<tr>
<td>Hu and Coats method[23]</td>
<td>88.7</td>
<td>± 4.7</td>
</tr>
</tbody>
</table>

This ability to retain a wider array of compounds possibly aids in the retention of erythromycin and its metabolites, including ionized forms. The methods which had the lowest recoveries for erythromycin were the Henderson[26] method and the modified tylosin method with recoveries of 78.8 % and 75.5 %. The Henderson[26] method yielded > 95 % efficiency for sulfamethazine, but was not shown to have similar results with erythromycin. This variation is likely due to the difference in antibiotic classes, chemical structures, and pKₐ of the compounds, which influence the behavior of the chemicals in the environment. The Hu and Coats[23] and Kolz et al.[11] methods displayed improved recoveries compared to the two previous methods due to the method’s specificity for tylosin, another macrolide antibiotic. The Kolz et al.[11] method demonstrated the greatest recovery of erythromycin due to the pH-adjustment of water samples to above 9.4 pH using 0.5 M potassium hydroxide, which influences the chemistry of the compound, especially the pKₐ in relation to binding of the compound to the cartridge packing and interactions with the elution-step solution. Adjustment of sample pH prior to SPE influences the retention of the erythromycin on the sorbent and may be due to increasing the pH above the pKₐ of the basic compound causing an increase in the amount of erythromycin retained on the Oasis HLB® cartridge, thus increasing the elution amount. The Kolz et al.[11] method has been shown to enhance the extraction of erythromycin and may aid in improved detection of the compound from water.

Erythromycin extraction recoveries from sand and sediments

Solvent extraction of ¹⁴C-erythromycin from sand yielded lower recovery amounts compared to those with the inclusion of buffers, including 10.6 % with ethyl acetate, 19.1 % for acetonitrile, and 48 % with a 70 % methanol solution. Various buffered extraction solutions were mixed with acetonitrile to determine optimal pH for ¹⁴C-erythromycin extraction from sand. Results are displayed in Table 3 for all extraction solutions examined with sand. The greatest recovery of ¹⁴C-erythromycin from sand with 99.3 % occurred with acetonitrile: 0.3 M ammonium acetate buffer (85:15, v/v). An extraction solution of acetonitrile: 0.2 M sodium phosphate buffer (85:15, v/v) demonstrated the best recovery with this buffer at a pH 3.2. For the 70:30 mixture ratio of acetonitrile: ammonium acetate the highest recovery of erythromycin was observed with 84.9 % for pH 7.

Four solutions were examined for their extraction of ¹⁴C-erythromycin residues from both Iowa and Oklahoma pond sediments, investigating various extraction shake times of 20, 30, and 60 minutes, plus an 85-minute shake time with the sample settling overnight, followed by a 15-minute shake; results are displayed in Table 5. Iowa sediment showed recoveries of 25.7 % with a 70 % methanol solution, 66.6 % using acetonitrile: 0.2 M sodium phosphate pH 3.2 (85:15, v/v), 84 % with acetonitrile: 0.3 M
ammonium acetate pH 4.2 (85:15, v/v), and 80.5 % for acetonitrile: 0.3 M ammonium acetate pH 7 (70:30, v/v). The two lowest recoveries at 20 minutes were not further examined for either pond sediment using an increase in shake time, which occurred with the 70 % methanol and acetonitrile: 0.2 M sodium phosphate pH 3.2 (85:15, v/v), acetonitrile: 0.3 M ammonium acetate pH 4.2 (85:15, v/v), and acetonitrile: 0.3 M ammonium acetate pH 7 (85:15). The acetonitrile: 0.3 M ammonium acetate pH 4.2 (85:15, v/v) solution was examined utilizing additional extraction shake incubation times and yielded recoveries of 78.8 % and 70.8 % for 30 and 60 minutes, while the 85-minute shake followed by an overnight sample-settling and a 15-minute shake yielded 84.3 % recovery of erythromycin. The acetonitrile: 0.3 M ammonium acetate pH 7 (70:30, v/v) had recoveries of 47.9 %, 73.3 %, and 73 % with the additional shake times examined (30, 60, and 85 minutes).

For the Oklahoma pond sediment the same extraction solutions and shake times were examined (Table 5). The recoveries at 20 minutes were 36.5 %, 52.9 %, 71.6 %, and 64.7 % for 70 % methanol, acetonitrile: 0.2 M sodium phosphate pH 3.2 (85:15, v/v), acetonitrile: 0.3 M ammonium acetate pH 4.2 (85:15, v/v), and acetonitrile: 0.3 M ammonium acetate pH 7 (70:30, v/v), respectively. An increase in recovery of 14C-erythromycin residues was demonstrated with the increase in extraction shake times for the acetonitrile: 0.3 M ammonium acetate pH 4.2 (85:15, v/v) solution yielding 71.7 %, 79.4 %, and 81.1 % at 30, 60, and 85-minute timepoints. Meanwhile, the acetonitrile: 0.3 M ammonium acetate pH 7 (70:30, v/v) solution showed recoveries of 46.9 %, 86.7 %, and 78.2 % for 30, 60, and 85-minute timepoints, respectively. Similar to the Iowa sediment, the Oklahoma sediment showed the lowest erythromycin recovery with the 30-minute incubation of acetonitrile: 0.3 M ammonium acetate pH 7 (70:30, v/v) solution.

A comparison of methods to enhance extraction of erythromycin from matrices revealed improved recovery through the use of lower-pH solutions tested with the two pond sediments. Both sediments examined were classified as sandy loam with the Iowa sediment having a higher pH and a greater percentage of organic matter compared to the Oklahoma pond sediment. Organic matter content in sediment influences many properties of that matrix, including pore-space size which increases with an increase in organic matter. This may influence the binding of erythromycin in sediment and account for the slightly lower recoveries in Iowa pond sediment compared to Oklahoma sediment. Another difference between the two pond sediments was pH, and it has been shown to influence charges of ions in sediments through increasing cation-anion interactions with the pH increase. Cation exchange capacity (CEC) may also influence erythromycin’s ability to bind in various matrices, as organic matter, silt content, and clay content additions increase the CEC value increases in soil, which influences the adsorption of chemicals. The pH may affect the binding of erythromycin in the sediment accounting for the use of different extraction solutions with the Iowa sediment compared to the Oklahoma sediment. The parameter of pH may affect the ability of ionic (hydrophilic) and non-ionic (lipophilic) compounds to bind in sediment through influencing the ability for adsorption to occur. Examination of the soil pH’s in this study may affect the adsorption of erythromycin to sediment particulate matter, where lower pH’s would allow for decreased binding accounting for higher extraction of erythromycin from pond sediment. For the sandy loam sediments examined in this study, it can be concluded that the extraction solutions and times examined yielded optimal recoveries of 84 % and 86 % erythromycin from the Iowa and Oklahoma sediments, respectively.

**Conclusions**

In water samples pH adjustment is important in extraction and concentration methods of erythromycin. When pH was changed to above 9.4, which was above the pKₐ of erythromycin, an increase in extraction was observed. SPE methods that work well with other macrolide antibiotics, worked better than methods for non-macrolide antibiotics (e.g. sulfamethazine). Additionally, pH and organic matter content may influence erythromycin’s ability to sequester in the two sediments examined in this study. The pH and amount of organic matter was higher in the Iowa sediment.

---

**Table 5.** Sediment recoveries from Iowa (IA) and Oklahoma (OK) pond sediment for various extraction incubation times.

<table>
<thead>
<tr>
<th>Extraction solution</th>
<th>IA sediment</th>
<th>OK sediment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>Methanol (70 %)</td>
<td>25.7 ± 1.8</td>
<td>–</td>
</tr>
<tr>
<td>Acetonitrile: 0.2M Sodium Phosphate pH 3.2 (85:15)</td>
<td>66.6 ± 0.1</td>
<td>–</td>
</tr>
<tr>
<td>Acetonitrile: 0.3M Ammonium Acetate pH 4.2 (85:15)</td>
<td>84 ± 3.4</td>
<td>78.8 ± 3.1</td>
</tr>
<tr>
<td>Acetonitrile: 0.3M Ammonium Acetate pH 7 (85:15)</td>
<td>80.5 ± 1.7</td>
<td>47.9 ± 0.2</td>
</tr>
<tr>
<td>Methanol (70 %)</td>
<td>36.5 ± 1</td>
<td>–</td>
</tr>
<tr>
<td>Acetonitrile: 0.2M Sodium Phosphate pH 3.2 (85:15)</td>
<td>52.9 ± 3.3</td>
<td>–</td>
</tr>
<tr>
<td>Acetonitrile: 0.3M Ammonium Acetate pH 4.2 (85:15)</td>
<td>71.6 ± 9.8</td>
<td>71.7 ± 8.9</td>
</tr>
<tr>
<td>Acetonitrile: 0.3M Ammonium Acetate pH 7 (85:15)</td>
<td>64.7 ± 2.7</td>
<td>46.9 ± 3.8</td>
</tr>
</tbody>
</table>
which may explain why a lower recovery was observed compared to the Oklahoma sediment. Furthermore, the solution that worked the best to extract erythromycin from sediment was the same for both sediments examined, except in regards to pH and shake time. These slight variations could influence adsorption of the erythromycin to particulate matter pertaining to cation exchange capacity within the sediment. Additional studies are needed to understand the interactions between erythromycin and particulate matter including its fate and bioavailability within the sediment system.

Acknowledgments

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