Development of an efficient extraction method for oxytetracycline in animal manure for high performance liquid chromatography analysis

Shoujun Yuan\textsuperscript{a}; Qiquan Wang\textsuperscript{b}; Scott R. Yates\textsuperscript{c}; Nyles G. Peterson\textsuperscript{d}

\textsuperscript{a} Chemistry Department, Delaware State University, Dover, Delaware, USA
\textsuperscript{b} School of Civil Engineering, Hefei University of Technology, Anhui, China
\textsuperscript{c} US Department of Agriculture, Agricultural Research Service Salinity Laboratory, Riverside, California, USA
\textsuperscript{d} University of California Cooperation Extension, San Bernardino, California, USA

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SHOUJUN YUAN1,2, QIQUAN WANG1, SCOTT R. YATES3 and NYLES G. PETERSON4

1 Chemistry Department, Delaware State University, Dover, Delaware, USA
2 School of Civil Engineering, Hefei University of Technology, Anhui, China
3 US Department of Agriculture/Agricultural Research Service Salinity Laboratory, Riverside, California, USA
4 University of California Cooperation Extension, San Bernardino, California, USA

Oxytetracycline (2-(amino-hydroxy-methylidene)-4-dimethylamino-5,6,10,11,12a-pentahydroxy-6-methyl-4,4a,5,5a-tetrahydrotetra
cene-1,3,12-trione) is a major member of the tetracycline antibiotics family of which are widely administered to animals in concentrated animal feeding operations for purposes of therapeutical treatment and health protection. With the disposal of animal manure as fertilizer into agricultural land, tetracyclines enter the environment. However, tetracyclines chelate with multivalent cations and proteins, resulting in low extraction efficiencies from animal manure for tetracycline residue analysis. In this study an efficient extraction method for oxytetracycline from steer manure using methanol/water solution amended with chelating organic acid was developed for the analysis of high performance liquid chromatography. The effect of species and amount of amendment acids, shaking time, methanol/water ratio, manure weight, and repeated times of extraction was investigated. It was optimized to amend 2.5 g citric acid and 1.1 g oxalic acid with 10.0 g manure sample in a 50-ml centrifuge tube and extract with 15 ml methanol/water (9:1 in volume) by vigorously shaking for 30 min in a reciprocating shaker. After centrifugation at 11,000 rpm, supernatant is collected. Sample was extracted for a total of 3 times. The developed extraction method was further applied to extract oxytetracycline from fresh and aged cow manure, swine and poultry manure, and soil. Satisfactory recoveries ranging from (84.1 ± 2.4)% to (102.0 ± 3.1)% were obtained, demonstrating that the optimized extraction method is robust for oxytetracycline from different manure sample matrices.

Keywords: Oxytetracycline; tetracyclines; antibiotic; extraction; manure; analysis; high performance liquid chromatography.

Introduction

As growth promoters and therapeutics, veterinary antibiotics have been widely used in concentrated animal feeding operations. It was reported that 9,434 tons of antibiotics were administered to farm animals in the European Union and Switzerland in 1999 and 11,340 tons were used in the United States in 2000.[1,2] Among various veterinary antibiotics, tetracyclines, including oxytetracycline, chlortetracycline, tetracycline and doxycycline, are most heavily used.[3,4] It is estimated that more than one third of veterinary antibiotics used in the United States are tetracyclines.[3] The occurrence of tetracyclines in water from fish hatcheries,[5] agricultural soil,[6,7] surface water,[8] and food[9−11] has been reported. The presence of tetracyclines, as well as other veterinary antibiotics, in the environment has raised both public and scientific concerns related to human and animal health.[12−14] Antibiotic-resistant genes were found to build up in microorganisms in the environment contaminated by antibiotics and to be widely transferable among microorganisms.[15−17] Thus, the success of pharmaceuticotherapies may be greatly diminished in treating infections caused by pathogens which have adapted antibiotic-resistant genes. Links between the agricultural use of antibiotics and antibiotic-resistant human infection have been suggested.[18,19]

Administration of antibiotics to aquaculture animals may result in direct release of antibiotics into the aquatic environment.[5] However, a more important route through which the veterinary antibiotics enter the environment is the improper disposal of animal waste from concentrated animal feeding operations and the repeated applications of animal manure as fertilizer in agricultural land.[4,15,20]
As reported, up to 90% of administered antibiotics were excreted as parent compounds by animals shortly after treatment.\textsuperscript{[21,22]} Oxytetracycline concentration in fresh feces from treated calves was found to be as high as 871 mg kg\textsuperscript{-1}.\textsuperscript{[20]} Antibiotic residue monitoring may be critical for the proper management of animal manure before its application as fertilizer and for the effective reduction of veterinary antibiotic contamination in the environment.

Tetracycline antibiotics are amphoteric compounds as characterized by three \( pK_a \) values, which are 3.0–3.3, 7.5–8.0, and 8.9–9.6.\textsuperscript{[23]} They are relatively stable in acidic solutions, but not in pH neutral and basic solutions.\textsuperscript{[24,25]} Tetracyclines are highly soluble in alcohols (methanol and ethanol) and water, possessing a potential to be readily extracted from different matrices for analysis. However, they chelate with multivalent cations and \( \beta \)-diketones and strongly bind to proteins\textsuperscript{[26]} greatly impeding them from extraction for analysis. For better extraction recoveries, chelating reagents and pH buffers are generally used. Analytical methods for the determination of tetracyclines in soil,\textsuperscript{[6,27–29]} liquid manure\textsuperscript{[6,27,30]} and food\textsuperscript{[31–33]} using high performance liquid chromatography (HPLC) have been reported. But satisfactory extraction recoveries were not always obtained.\textsuperscript{[27]} Furthermore, very limited reports addressed the extraction and determination method of tetracyclines in feces.\textsuperscript{[20,34]} The establishment of an efficient extraction method may greatly help the detection of tetracyclines in animal manure and other environmental samples.

In this study, an efficient extraction method for oxytetracycline from steer manure was developed for HPLC analysis. To maximize extraction recoveries, the effect of amendment organic acid species and amount, methanol/water ratio, shaking time, extraction times, and manure weight were investigated. To examine the robustness of the extraction, the optimized method was applied to extract oxytetracycline from various spiked manures, including cow manure with different ages, swine manure, and poultry manure, and from a spiked silt loam soil.

### Materials and methods

#### Chemicals, manures, and soil

Oxytetracycline (SigmaUltra) was purchased from Sigma (St. Louis, MO). Citric acid anhydrous (certified), acetic acid glacial (HPLC grade), phosphoric acid (certified), and acetonitrile (optima) were purchased from Fisher Scientific (Fair Lawn, NJ). Oxalic acid anhydrous (>99.5%) and trichloroacetic acid (>99.5%) were purchased from Fluka (Milwaukee, WI). Water was supplied using Barnstead E-pure purification system (Dubuque, IA).

Fresh cow manure, 4 day-old swine manure, and 2 day-old poultry manure was obtained from farms located in Riverside, CA. Seven and 19 day-old cow manures were obtained by putting fresh cow manure in plastic plates exposed to air in the greenhouse for 7 and 19 days. Steer manure (Earthgro Inc., Marysville, OH) was purchased from K-mart in Riverside, CA and was sieved to 4 mm prior to use. All manure samples, except for poultry and steer manure, were obtained with no bedding materials. Soil used in this study was obtained from the top layer (0–10 cm) in a piece of grassland in University Park, PA and was air dried in the laboratory. Soil was sieved to 1 mm before use. Soil texture was determined to be silt loam with clay 26.9%, silt 65.1%, and sand 8.0%. Soil organic carbon content was 14.4 g kg\textsuperscript{-1}. Moisture and pH of different manures and the soil are listed in Table 1.

### Spiking and extraction of oxytetracycline

The extraction method was developed based on steer manure. No oxytetracycline in the blank steer manure was detected in any experiments in this study. Oxytetracycline was spiked into steer manure samples for extraction. Generally, steer manure were weighed into 50-ml polyethylene centrifuge tubes at 10.0 g (wet wt.) per tube and 100 \( \mu \)L 4.60 g L\textsuperscript{-1} oxytetracycline methanol solution was spiked into the manure in each tube. Tubes were then sealed with caps and spiked samples were mixed inside of tubes by general manual shaking for 2 min. After stabilizing for 1 h, samples were extracted. Preliminary study showed that longer stabilizing time up to 1 d at room temperature in autoclaved samples does not have significant effect on extraction recovery (data not shown). All experiments were carried out in triplicates.

For the optimized extraction method developed in this study, 2.5 g citric acid and 1.1 g oxalic acid were added into each tube. After the addition of 15 ml methanol/water (90/10 in volume) solution, tubes were immediately sealed with caps, placed horizontally in a reciprocating shaker (Eberbach Corp., Ann Arbor, MI) and shaken for 30 min. Then tubes were centrifuged at 11,000 rpm for 10 min in a centrifuge (International Equipment, Needham Heights, MA) at 20 °C and supernatant in each tube was
gently decanted into 50-ml volumetric flasks. Extraction was repeated for a total of 3 times. Supernatants from the same tube were collected at the same flask. Then the extract in each flask was diluted to volumetric curve with water. After thoroughly mixing, 1 ml of extract from each flask was transferred into a 1.5-ml centrifuge tube and further centrifuged at 14,000 rpm for 3 min in a microcentrifuge (Spintron Inc., Metuchen, NJ). Supernatants were then transferred into 1.5-ml sample vials for HPLC analysis. All operation procedures in optimizing experiments stated below were the same as described above unless otherwise specified. Extraction recoveries were calculated by directly comparing the peak area of oxytetracycline in extracts with that of oxytetracycline standard which were prepared by diluting the same amount of spiking oxytetracycline standard solution to the same volume of extract in volumetric flask.

When investigating the effect of extraction solvent pH, different amounts (0 ∼ 8 ml) of acetic acid were added instead of citric acid and oxalic acid. Extraction solvent was methanol/water solution at 80:20 (in volume) and extraction was performed for only one time. Extracts were collected in 25-ml volumetric flasks. 

To improve extraction recovery, a second acid was added with 2.50 g citric acid as the first acid which was chosen from the above acid species optimization experiment. The second acid added for experiment included oxalic acid, acetic acid, and trichloroacetic acid. The added amounts of each second acid were 0, 1.6, 3.2, 4.8, 6.4, 8.0, 12, and 16 mmol. As comparisons, extractions with additional 0, 1.6, 3.2, 4.8, 6.4, 8.0, 12, and 16 mmol of citric acid were also conducted. Other procedures were the same as those in the optimization of the first acid.

To study the effect of methanol/water ratio on oxytetracycline extraction recovery, methanol/water solutions at 20:80, 40:60, 60:40, 80:20, 90:10, and 100:0 (in volume) were used as extraction solvents. When optimizing shaking time, tubes were shaken for 10, 20, 30, 40, 50, and 60 min, respectively. Extraction was performed for one time and extracts were collected in 25-ml volumetric flasks.

When investigating the effect of extraction times on oxytetracycline extraction recovery, 5 sets of spiked steer manure were extracted for 1, 2, 3, 4, and 5 times, respectively. For extraction with a total of one and two times, extracts were collected in 25-ml volumetric flasks. For that with a total of three times, extracts were collected in 50-ml volumetric flasks. For that with a total of four and five times, extracts were collected in 100-ml volumetric flasks.

In the optimization of manure weight for extraction, 4.0, 6.0, 8.0, 10.0, 12.0, and 15.0 g (wet wt.) of steer manure were spiked with oxytetracycline and then extracted for a total of three times. All other procedures were the same as those in the optimized method.

For the developed extraction method, the consistency of extraction recovery at different spiking doses of oxytetracycline in steer manure was examined. In each 10.0 g (wet wt.) of steer manure, 1.0, 2.0, 3.0, 5.0, 10.0, 50.0, 100, 500, or 1000 µl of 4.60 g L\(^{-1}\) oxytetracycline methanol solution were spiked for extraction.

To examine the robustness of the optimized extraction method for different sample matrixes, extraction recoveries of spiked oxytetracycline from fresh, 7 day-old, and 19 day-old cow manure, 4 day-old swine manure, 2 day-old poultry manure, and a silt loam soil were determined. For each kind of matrix, 10 and 100 µl 4.60 g L\(^{-1}\) oxytetracycline methanol solution were spiked into two sets of 10.0 g (wet wt.) samples, respectively, for extraction.

**HPLC analysis**

A Hewlett-Packard series II 1090 HPLC (Wilmington, DE) and a Shimadzu 2010-HT HPLC (Columbus, MD) were used for oxytetracycline concentration analysis in the extracts. An Agilent Hypersil ODS 5 µm 4.0 × 250 mm column was used for separation. Mobile phase was composed of 80 % water (pH adjusted to 3.0 using H\(_2\)PO\(_4\)) and 20 % acetonitrile and the flow rate was 1.0 ml min\(^{-1}\). The detection wavelength was set at 360 nm. Injection volume was 10 µl. The retention time of oxytetracycline was 6.2 min.

**Results and discussion**

**Effect of solvent pH and optimization of acids**

There are two amine groups in the oxytetracycline molecule. Decreasing extraction solvent pH may help oxytetracycline to protonize and become less adsorptive to adsorbent, becoming more readily extracted. Extraction solvent pH was found to greatly affect the extraction recovery of oxytetracycline from spiked steer manure (shown in Fig. 1). When no acetic acid was added in the extraction solvent, the extract pH was around 8.7 and almost no oxytetracycline was extracted from the spiked manure. However, when the extract pH was decreased to 4.23 by the addition of acetic acid, oxytetracycline became much easier to be extracted and the recovery reached (38.7 ± 0.6) % for single-time extraction. The observed phenomenon is consistent with the fact that acidic solvents are widely used for the extraction of tetracyclines from various media.\(^{[7,9,23,30,32–34]}\) The near neutral pH of the extraction solution might be the reason for those low extraction recoveries.\(^{[27]}\) For example, using accelerated solvent extraction (ASE) technique the
Efficient extraction of oxytetracycline from manure

Merely decreasing extraction solvent pH regardless of acid species may not result in satisfactory extraction recoveries. This was also confirmed by our preliminary study, in which 0.6 M HCl methanol solution was used for extraction. The extract pH was measured to be <1 and the extraction recovery was only (16.3 ± 1.8) % for 3-time extraction (data not shown in this study).

It is known that the chelation between tetracyclines and multivalent cations, as well as the binding with proteins, is one of the factors impeding tetracyclines from extraction. To disrupt the chelation of oxytetracycline, McIlvaine/ethylenediaminetetraacetic acid (EDTA) extraction solution is often used. It may be successful for samples with low pH buffering capacity and low content of multivalent cations and proteins, but may be inappropriate for the extraction of tetracyclines from solid manures. Our preliminary study showed that the extraction recovery of oxytetracycline from 10.0 g (wet wt.) of spiked manure with 3 times of 15 ml methanol/McIlvaine (pH=7)/EDTA (0.1 M) = 50:25:25 was <15% (data not shown in this study). Though the high pH of extraction solvent (≥7) may benefit EDTA to complex with multivalent cations, it is not favorable to the protonization of oxytetracycline for extraction as discussed above.

Both citric acid and oxalic acid are commonly used chelators for multivalent cations, such as calcium and magnesium ions. When citric acid or oxalic acid is ionized to decrease the extraction slurry pH, simultaneously a significant amount of citrate or oxalate ion is produced and may chelate with multivalent cations. The amendment of citric acid or oxalic acid for extraction not only effectively decreased pH, but also efficiently disrupted oxytetracycline from its chelation with multivalent cations in manure.

All investigated combined additions of a second acid with 2.5 g citric acid as the chosen first acid increased oxytetracycline extraction recovery (shown in Fig. 2). However, when compared with the further addition of citric acid, no improvement in oxytetracycline recovery was observed with the addition of acetic acid as the second acid. Similarly, a slight improvement was observed only at the addition of 12 mmol (2.0 g) when trichloroacetic acid was added as the second acid. Contrary to acetic acid and trichloroacetic acid, the combined addition of oxalic acid with citric acid demonstrated a significant improvement in oxytetracycline extraction recovery compared with the further addition of citric acid. When the added amount of oxalic acid was 4.8, 6.4, 8.0, 12, and 16 mmol (i.e. 0.43, 0.58, 0.72, 1.1 and 1.4 g), the single-time extraction recovery was (44.2 ± 0.6), (45.1 ± 1.3), (49.1 ± 1.0), (52.0 ± 1.2), and (49.2 ± 1.5) %, respectively, while that with the further addition of citric acid at the same molar amount as oxalic acid was (42.7 ± 0.3), (42.9 ± 0.6), (44.0 ± 0.6), (44.2 ± 1.3), and (45.6 ± 1.2)%, respectively. As an optimal combination, 13 mmol (2.5 g) citric acid and 12 mmol (1.1 g) oxalic acid were chosen for amendment in each time of extraction.

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**Fig. 1.** Extraction recovery of oxytetracycline from spiked steer manure at different pHs.

**Table 2.** Extraction recoveries of oxytetracycline from spiked steer manure and extract pHs with the addition of different acids at the same molar amount.

<table>
<thead>
<tr>
<th>Added acid</th>
<th>Citric acid</th>
<th>Oxalic acid</th>
<th>Acetic acid</th>
<th>Trichloroacetic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery (%)</td>
<td>42.2±1.1</td>
<td>41.2±1.6</td>
<td>15.7±1.3</td>
<td>35.2±1.9</td>
</tr>
<tr>
<td>Extract pH</td>
<td>3.81</td>
<td>2.26</td>
<td>5.56</td>
<td>2.95</td>
</tr>
</tbody>
</table>
Optimization of methanol/water ratio and shaking time

The ratio of methanol/water showed to have a pronounced effect on extraction of oxytetracycline from manure (shown in Fig. 3). Oxytetracycline recovery increased rapidly with the increasing content of methanol in the extraction solvent. After the ratio was increased to 80:20, the extraction recovery reached its maximum. To avoid low extraction efficiency when extracting fresh feces with high moistures, low content of water in the extraction solvent may be needed. Hence, 90:10 of methanol/water was chosen as the optimal ratio.

Compared with solvent composition, shaking time in the investigated range displayed very limited effect on extraction recovery. After shaking for 10, 20, 30, 40, 50, and 60 min, recovery of single-time extraction was determined to be (51.3 ± 0.9), (52.2 ± 1.5), (52.0 ± 1.2), (53.2 ± 1.6),...
Efficient extraction of oxytetracycline from manure

Extraction times

0 1 2 3 4 5 6

Extraction recovery (%)
0 20 40 60 80 100 120

Fig. 4. Oxytetracycline recoveries with different repeated times of extraction.

(51.7 ± 0.5), and (51.8 ± 0.6)%, respectively. No significant difference among these recoveries was found. However, an adequate shaking time might be crucial for those samples in which oxytetracycline is highly diffused and strongly interacted. Therefore, a shaking time of 30 min instead of a shorter time was chosen as the optimal shaking time.

Recoveries with repeated times of extraction

Oxytetracycline recovery was effectively increased by repeated extractions (shown in Fig. 4). With the increase of extraction times from 1 to 3, oxytetracycline recovery was improved from (52.0 ± 1.2) to (92.6 ± 0.9)%. When extraction was performed with a total of 4 and 5 times, oxytetracycline recovery reached (99.3 ± 1.4)% and (102.2b ± 1.2)%, respectively. The high extraction recovery with repeated extractions indicated that the extraction reagents used in this method possess a robust ability to disrupt the interaction between oxytetracycline and relevant substances in manure, making oxytetracycline readily available for extraction.

Though increasing extraction times helps improve oxytetracycline recovery, it may sacrifice the detection sensitivity of the method. The concentration of oxytetracycline in extracts with different times of extraction was 9.6 ± 0.1, 14.9 ± 0.1, 8.5 ± 0.1, 4.6 ± 0.1, and 4.7 ± 0.1 µM, respectively. With the repetition of extraction, extract volume is increased, thus oxytetracycline in the extract is diluted. For high extraction recovery but without losing too much sensitivity, a total of 3 times of extraction was chosen in the optimal extraction method.

Effect of manure weight for extraction

A slight reduction of oxytetracycline recovery was observed with the increasing weight of manure for extraction. As shown in Fig. 5, the extraction recovery decreased from (102 ± 1.3)% to (80.8 ± 2.0)% when the manure weight increased from 4.0 to 15.0 g, demonstrating that manure weight has no critical effect on oxytetracycline extraction recovery in the investigated weight range.

It is known that increasing manure sample weight for extraction may help to increase the sensitivity of the method if the final volume of extract is fixed. It may also increase the reliability and representativeness of the determination result. However, increasing manure weight may result in decreased recoveries, partially or completely offsetting the advantage of increasing sample weight for extraction. It might be worthwhile to increase sensitivity by increasing manure weight from 10 to 15 g while sacrificing 11.8% recovery. But this improvement in sensitivity is very limited. Based on the observed effect of manure weight on extraction recovery, 10.0 g is chosen as the optimal wet manure weight for extraction, of which the corresponding extraction recovery was (92.6±0.9)%.

Consistency of extraction recovery at different spiking doses

The optimized extraction method was used to extract oxytetracycline from steer manure at different spiking doses ranging from 0.46 to 460 mg kg⁻¹. The obtained extraction recoveries were all above 90.4% with relative standard deviation all below 4.6% (data not shown). A linear relationship between HPLC peak area and oxytetracycline
spiking dose was obtained and the linear correlation coefficient \((r)\) was 0.999, indicating that the extraction recovery of this optimized extraction method is consistent regardless the concentration of oxytetracycline in steer manure.

**Extraction of oxytetracycline from different spiked manure samples and a soil sample**

It was demonstrated that the optimized extraction method can efficiently extract oxytetracycline from not only spiked steer manure, but also other manure samples and a soil sample. The extract can be directly analyzed in HPLC. As an illustration, HPLC spectra of extracts from fresh cow manure spiked with different amounts of oxytetracycline are shown in Fig. 6. Oxytetracycline was well separated in HPLC. No significant interferences to oxytetracycline peak were observed. Similar HPLC spectra were also obtained with all other kinds of manure and with the silt loam soil.

Oxytetracycline recoveries in different manure samples and the soil sample are listed in Table 3. It was noted that oxytetracycline in the silt loam soil sample was more refractory to be extracted than in manure samples. It might result from high content of multivalent cation in soil than

**Table 3. Recoveries of oxytetracycline from different manure samples and from a silt loam soil at two spiked doses.**

<table>
<thead>
<tr>
<th>Spiked dose (mg kg(^{-1}))</th>
<th>Species of manure/soil</th>
<th>Oxytetracycline recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.6</td>
<td>fresh cow manure</td>
<td>102.0±3.1</td>
</tr>
<tr>
<td></td>
<td>7 day-old cow manure</td>
<td>91.4±3.8</td>
</tr>
<tr>
<td></td>
<td>19 day-old cow manure</td>
<td>86.4±2.3</td>
</tr>
<tr>
<td></td>
<td>2 day-old poultry manure</td>
<td>94.6±4.3</td>
</tr>
<tr>
<td></td>
<td>4 day-old swine manure</td>
<td>91.1±2.3</td>
</tr>
<tr>
<td></td>
<td>steer manure</td>
<td>91.4±0.9</td>
</tr>
<tr>
<td></td>
<td>silt loam soil</td>
<td>84.1±2.4</td>
</tr>
<tr>
<td>46</td>
<td>fresh cow manure</td>
<td>99.6±0.4</td>
</tr>
<tr>
<td></td>
<td>7 day-old cow manure</td>
<td>92.3±3.6</td>
</tr>
<tr>
<td></td>
<td>19 day-old cow manure</td>
<td>85.3±2.2</td>
</tr>
<tr>
<td></td>
<td>2 day-old poultry manure</td>
<td>92.2±1.2</td>
</tr>
<tr>
<td></td>
<td>4 day-old swine manure</td>
<td>89.5±2.4</td>
</tr>
<tr>
<td></td>
<td>steer manure</td>
<td>92.6±1.2</td>
</tr>
<tr>
<td></td>
<td>silt loam soil</td>
<td>85.2±1.3</td>
</tr>
</tbody>
</table>
in manure samples. It was also observed that oxytetracycline became more difficult to be extracted in aged cow manure than fresh manure. This difficulty might be caused by the increasing solid content and the decreasing water content in the sample with the aging time. However, satisfactory recoveries were still obtained with all investigated samples. All obtained recoveries at two different spiking doses fell in the range of 84–102%, indicating that the developed extraction method is robust to extract out almost all oxytetracycline from these samples. The obtained extraction recoveries of oxytetracycline from the silt loam soil were 84.1 ± 2.4 and 85.2 ± 1.3 at 2 spiked doses, respectively, which are higher than the reported recovery from a loamy sand soil (63.3–80.7%) in one study[28] and noticeably higher than the reported values from several kinds of soil (27–75%) in another study.[29] The obtained extraction recovery samples from aged cow manure and steer manure are about 10% higher than the reported recovery of oxytetracycline from calve manure-bedding samples in a manure composting study.[37]

Conclusion

It was optimized in this study to amend 2.5 g citric acid and 1.1 g oxalic acid into 10.0 g manure sample in a 50-ml centrifuge tube and extract with 15 ml methanol/water (9:1 in volume) by vigorously shaking for 30 min in a reciprocating shaker. After centrifugation at 11,000 rpm, supernatant is collected. Sample was extracted for a total of 3 times. With the developed method in this study, oxytetracycline can be efficiently extracted from various manure samples for HPLC analysis. This extraction method provides a well-established basis for the further development of oxytetracycline analytical method. The application of appropriate solid phase extraction cartridges for the concentration and purification of obtained extracts[28] and/or the use of more sensitive detectors of HPLC, such as mass spectrometry (MS) detector[29] and fluorescence detector,[27,38] may greatly improve the sensitivity of oxytetracycline residue analysis in various environmental samples. Thus, oxytetracycline residue can be more sensitively monitored and its contamination in the environment may be potentially monitored and controlled. Further study may be needed to examine the robustness of this extraction method for other members in tetracyclines family.

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