Occurrence of β-lactam and polyether ionophore antibiotics in lagoon water and animal manure

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ABSTRACT

The occurrence of micropollutants in agricultural wastes is an emerging area of interest due to the potential impact of these compounds on the environment. A sensitive and reliable analytical method using liquid chromatography-electrospray tandem mass spectrometry has been developed and validated for the determination of three β-lactam and three polyether ionophore antibiotics in lagoon water and animal manure matrices. The method was applied to evaluate the occurrence of these compounds from participating farms in northern Colorado. Seven of the 19 lagoon water samples and two of the six animal manures showed detectable. The three targeted β-lactams (cephapirin, penicillin G, cloxacillin) were found at 0.97- 43.31 µg/L in the lagoon water samples. Of the three targeted polyether ionophores, only monensin (94 to 1077 µg/L) was detected in the beef runoff pond water samples. Only cloxacillin was measured in the dairy

animal manure samples at levels from 8.09 to 45.20 µg/kg. No cephapirin, penicillin G, cloxacillin, salinomycin, or narasin A were detected in any solid animal manure sample. These results indicate that elevated concentrations of β-lactam and ionophore compounds might be found in lagoon or runoff pond waters and solid animal manures compared to surface waters, which these compounds are used in veterinary applications.

Keywords: antibiotics, β-lactams, polyether ionophores, lagoon water, animal manure

1. Introduction

The occurrence and fate of antibiotics in agricultural waste is an emerging area of interest due to the potential impact of these compounds on the environment (Sarmath et al., 2006; Riediker and Stadler, 2001). The β-lactam antibiotics are widely used for their antimicrobial activity against both gram-positive and gram-negative organisms (Niessen, 1998). These antibiotics are used in human medicine for the treatment of bacterial infections of skin, ear, respiratory tract, and urinary tract. These compounds have been widely used both for prevention and treatment of disease and as feed additives to promote growth in animal feeding operations. Therefore, The β-lactam antibiotics are an important and widely used class of drugs for both human and veterinary medicine. There are several β-lactams currently approved by the U.S. Food and Drug Administration (US FDA) for use in lactating dairy cattle, swine and poultry (Sarmath et al., 2006; Riediker and Stadler, 2001; Niessen, 1998). The β-lactam antibiotics (BLs) are comprised of a great variety of semisynthetic penicillins (e.g., amoxicillin (AMOX), ampicillin (AMP), penicillin G (PEN G), cloxacillin (CLOX), oxacillin (OXA)) and cephalosporins (e.g. cephapirin

(CEP), ceftiofur). The polyether ionophore antibiotics (PEs), (e.g., monensin (MON), salinomycin (SAL), and narasin (NAR)), are used in veterinary applications as feed additives (coccidiostats) for poultry and livestock and as growth promoters for ruminants (Westley, 1982; Matabuldul et al., 2001).

Only a small fraction of antibiotics consumed by animals is metabolized to inactive compounds and the significant quantity of administered antibiotic is excreted via urine or feces either unchanged or as active metabolites (Hirsch et al., 1999; Kumar et al., 2005a). Thus, the origin of antibiotic contamination in surface and ground waters is considered to be point and non-point source discharges of municipal and agricultural wastewater (Halling-Sorensen et al., 1998) (Fig. 1).

Some antibiotics like penicillins seem to be degradable in communal sewage treatment plants. Due to the chemically unstable β-lactam ring, they are readily susceptible to hydrolysis and will be easily eliminated. Others like sulfanoamides are poorly degradable or not degradable at all. Sulfanoamides like sulfadimethoxine are sufficiently stable in manure to maintain significant residual activity until field application. Microbial degradation in surface water is slower than that in the sewage treatment system due to lower density of bacteria. Antibiotics occurring in soil and sediment, which proved to be quite persistent in field studies and antibiotics applied to fish farming, had long half-lives in soil and sediment (Sarmath et al., 2006; Riediker and Stadler, 2001; Niessen, 1998).

Christian et al. (2003) reported that AMOX, AMP, mezlocillin, flucloxacillin, and piperacillin in surface water could be found at concentrations up to 48 ng/L. However, in 4 of 32 river water samples, AMOX concentrations did not exceed 10 ng/L (Christian et al., 2003). Cha et al. (2006) found levels of BLs from 9 to 11 ng/L in three surface water samples. Campagnolo et al. (2002) reported levels of PEN G from 2.1 to 3.5 µg/L in lagoon water samples. Different water compartments were searched for β-lactams, including surface water (Hirsch et al., 1999; Cha et al., 2006; Bruno et al., 2001; Hirsch et al., 1998; Sacher et al., 2001) and groundwater (Sacher et al., 2001), and few of the targeted BLs have been detected. Intact BLs do not occur frequently in the environment (Christian et al., 2003), due to the poor stability of the β-lactam ring. Some researchers measured MON from 1 to 5 mg/kg (Donoho, 1984) and at 4.4 mg/kg (Thiele-Bruhn, 2003) in cattle feces. Catherman et al. (1991) found NAR from 1.0 to 725.0 µg/kg in poultry feces and manure. Schlüsener et al. (2003) reported SAL in manure at 11 µg/kg. Hao et al. (2006) detected MON from 0.02 to 0.22 µg/L in surface water. In previous studies, Cha et al. (2005) reported measuring MON A, SAL, and NAR A in surface water from 0.03 to 0.06 µg/L.

Animal waste lagoon water and manure are commonly spread on agricultural fields as organic fertilizers. When applied to agricultural land, their components may be transported to surface water or groundwater through runoff or infiltration from fields (Cha and Cupples, 2012; Cha et al., 2015; Tasho and Cho, 2016; Pan and Chu, 2017a; Pan and Chu, 2017b). Therefore, the occurrence and fate of veterinary antibiotics in lagoon water and animal manure are crucial, and emerging environmental issues (Pfifer et al., 2002; Haller et al., 2002; Hamscher et al., 2005; Kumar et al., 2005b; Batt et al., 2006; Boxall et al., 2006; Jacobsen and Halling-Sorensen, 2006; Malintan and Mohd, 2006). A few studies have focused on the occurrence, fate, and transport of

BL and PE antibiotics in lagoon water and animal manure. To address these concerns, there is a need for sensitive and reliable analytical methods to measure concentrations of BLs and PEs in these matrices. In this study, we describe a sensitive and reliable analytical method for the determination of AMOX, AMP, PEN G, CLOX, CEP, MON, SAL, and NAR in lagoon water and animal manure using solid-phase extraction (SPE) and ion trap liquid chromatography/tandem mass spectrometry (LC-MS-MS) with positive ion electrospray ionization, ESI(+) and selected reaction monitoring (SRM). This paper also addresses the occurrence of BLs and PEs in several lagoon waters and animal manures.

2. Experiment section

2.1. Materials for chemical analysis

All antibiotics (purity>90%), formic acid (purity>95%) and Na_2EDTA (purity>99%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Methanol, ethanol, and acetonitrile were HPLC grade (Sigma-Aldrich, St. Louis, MO, USA). Individual stock solutions of BLs were prepared weekly by dissolving each compound in acetonitrile-ethanol-water (25:25:50) at a concentration of 1,000 mg/L, and stock solutions of the PE standards were prepared monthly by dissolving each compound in methanol at a concentration of 100 mg/L. All stock solutions were stored at -20 ^{o}C in the dark. Mixed working solutions (10, 1, and 0.1 mg/L) were prepared daily by diluting the stock solutions with the same solvent and stored at 4 ^{o}C in the dark. The internal standard working solution (1 mg/L) were prepared by diluting the standard solution with the solvent and were stored at 4 ^{o}C and replaced with a fresh solution daily. OXA and simatone were chosen as an internal standard for BLs and PEs in this study, respectively, because they eluted

115 within the same chromatographic time frame as the analytes, responded well in ESI (+) mode
116 and did not exhibit noticeable matrix effects (Cha et al., 2005; Cha et al., 2006).
117
118 *2.2. Sample collection and preparation*
119 A total of 25 manure waste lagoon or runoff pond water samples (nineteen) and fresh animal
120 manure samples (six) were collected during 2006 from nine farms in northern Colorado, U.S.A.
121 (Fig. 2). The lagoon waters were collected from the upper one meter of middle lagoon. The
122 lagoon waters were prepared in a centrifuge with a cooling system (IEC Centra CL 3R, MA,
123 USA) at 3,000 rpm for 40 min at 4 ^{o}C. The lagoon water samples were filtered through 0.4-
124 micron glass fiber filters (Millipore, MA, USA) and stored at 4 ^{o}C. SPE and quantitative
125 measurement were performed within 12 hours after collection of samples due to the tendency
126 toward biodegradation and/or hydrolysis of the labile β-lactam compounds.
127
128 To convert the investigated ionophores to a single sodium adduct species, the appropriate
129 amounts (2% ~ 5% (w/v)) of sodium chloride as a surplus of sodium were dissolved in all
130 samples, which were then left to stand for 30 min prior to the application of SPE and LC-MS-MS
131 analysis. The quantity of sodium chloride added to each sample was based on the sum of cation
132 concentrations (e.g. Na^{+}, K^{+}, Li^{+}, Cs^{+}, NH_4^{+}, Ca^{2+}, Mg^{2+} and Cu^{2+}) in the sample.
133
134 *2.3. Solid-phase extraction*
135 To prepare matrix-matched reference samples, several additional lagoon water and animal
136 manure samples were analyzed to verify that they did not contain detectable quantities of the
137 analytes of interest. For controls and as calibration standards, the reference lagoon water and

138 animal manure samples were supplemented with appropriate amounts of working solution
139 containing each of the analytes.
140
141 Solid-phase extraction (SPE) was performed using 60 mg/3mL Oasis HLB cartridges (Waters,
142 Millford, MA, USA). These cartridges were preconditioned with 3 mL of methanol, 3 mL of
143 0.5N HCl, and 3 mL of deionized water at 8 in Hg on a vacuum manifold (PrepSep 12 port,
144 Fisher scientific, PA, USA). All samples were filtered through 0.4-micron glass fiber filters and
145 pH-adjusted immediately prior to extraction. For extraction of BLs, 1 mL of Na_2EDTA and 20
146 µL of OXA (1.0 mg/L) as the internal standard (IS) were added to a flask containing 20 mL of
147 lagoon water and 80 mL of 0.001M citric acid. Sample pH was adjusted with 8% NH_4OH to pH
148 7.5. For extraction of PEs, 12 µL of internal standard, 1.0 mg/L simatone, was added to 20 mL
149 of lagoon water samples, and 80 mL of 0.001 % formic acid was added at 2 % ~ 4 % (w/v) of
150 sodium chloride. Because the investigated ionophores are acid and/or base labile, the extraction
151 using the HLB cartridges was performed with the neutral sample pH adjusted by 0.01M NaOH to
152 pH 7.5. BLs and PEs were extracted from 5-g animal manure samples with 120 mL of 0.001M
153 citric acid and 0.001% formic acid, and prepared in the same centrifuge as described above, at
154 3,000 rpm for 10 min at 4 ^{o}C.
155
156 Aqueous samples were passed through the cartridges at 5 mL/min. After isolation, cartridges
157 were rinsed with 5 mL of deionized water, and the analytes were eluted with 5 mL of methanol.
158 The extracts were concentrated under a flow of N_2 gas to about 100 µL using a nitrogen
159 evaporation system (N-Evap, Organermation Associates Inc., MA, USA). To this, 140 µL of

mobile phase A (0.1% formic acid in water) was added. The resulting solutions were transferred to 0.5-mL amber autosampler vials to prevent the photodegradation of the BLs and PEs.

2.4. Liquid chromatography and mass spectrometry

The mass spectrometer used was a Finnigan LCQ Duo ion trap (ThermoQuest, CA, USA) equipped with a heated capillary interface and a positive electrospray ionization source. ThermoQuest Xcalibur software was applied to control the mass spectrometric conditions. Full scan mode was used to acquire mass spectra, precursor ions, and product ions from standard BL and PE solutions. Mass spectral data presented in this report were acquired on a LCQ Duo ion trap tandem mass spectrometer equipped with an ESI source operated in positive ion mode. Infusion into the ion trap tandem mass spectrometer was completed with a flow of standard compounds for 3 mg/L of BLs and 7 mg/L of PEs at 5 µL/min from an integrated syringe pump, mixing mobile phases A/B/C at a 80:14:6 and 15:32:53 ratio, respectively, through a T-piece for tuning the mass spectrometer and optimizing the ESI source. The ESI source and MS-MS parameters were automatically optimized and saved in a tune file. Three microscans per scan were acquired with spray needle voltage set at 4.5 kV for both compounds, automatic gain control (AGC) on, and maximum isolation time at 200 ms for BLs and 300 ms for PEs. Voltages on the capillary and tube lens were 29 and 25 V, respectively, for BLs and 38 and 25 V, respectively, for PEs. These were set by automatic optimization using the LCQ autotune program on the mass spectrometer. Nitrogen was used as a sheath and auxiliary gas. Helium was used as the collision gas in the ion trap. The optimized tune conditions included a sheath and auxiliary gas flow rate each set at 50 units (a scale of arbitrary units) and capillary temperature

182 set at 175 ^{o}C. MS-MS parameters for BLs and PEs, including precursor ion, product ion, and
183 collision energy, are summarized in Table 1.
184
185 *2.5. Method validation study*
186 The product ions producing the highest intensity and used for SRM and quantification to increase
187 analytical sensitivity and selectivity in LC-MS-MS mode for the targeted AMOX, AMP, PEN G,
188 CLOX, CEP, OXA (Internal Standard, or IS), MON A and B, SAL, and NAR A are listed in
189 Table 1. Quantification was based on a detector response defined as the ratio of peak area of the
190 base peak ion (the specific product ion of interest) to peak area of the base peak ion for the IS.
191 Calibration curves were constructed with lagoon water spiked at BL concentrations of 3, 30, 50,
192 100, and 150 µg/L before extraction. Calibration curves for PEs spiked into lagoon water before
193 extraction were constructed at 3, 50, 100, 200, 400, and 800 µg/L. Calibration curves constructed
194 for BLs and PEs spiked into animal manure samples before extraction ranged from 10, 50, 100,
195 150 and 200 µg/kg.
196
197 The method detection limit (MDL) was determined using the methodology recommended by the
198 U.S. Environmental Protection Agency (US EPA), based on the variability of multiple analyses
199 of seven lagoon water extracts spiked at a concentration of 10 µg/L before extraction for BLs
200 and PEs, respectively, and seven animal manure extracts spiked at 30 µg/kg before extraction for
201 each antibiotic.
202
203 **3. Results and discussion**
204

205 *3.1. Liquid chromatography and mass spectrometry*

206 The LC method, employing a ternary gradient sequence combined with ESI(+)-MS-MS, yielded
207 mass peaks corresponding to BLs and PEs on the total-ion chromatograms (TICs) monitored for
208 the selected product ion. The data were processed by creating the reconstructed total-ion
209 chromatograms (RTICs) for each analyte as shown in Fig. 3.

210

211 Fragmentation of penicillins (AMOX, AMP, PEN G, CLOX, and OXA) resulted from the
212 opening and cleaving of the β-lactam ring, producing the class-specific product ion,
213 $[C_6H_9NO_2S+H]^+$ at *m/z* 160, representative of penicillin compounds (Table 1). AMOX, AMP,
214 PEN G, CLOX, and OXA exhibited the product ion $[M+H-C_6H_9NO_2S]^+$ at *m/z* 207, 191, 176,
215 277 or 243, respectively, corresponding to the loss of $C_6H_9NO_2S$ from the precursor ion. AMOX
216 also exhibited the 349 ion due to the neutral loss of NH_3 (17 Da) from the precursor ion.
217 Fragmentation of CEP first produced the 364 ion due to the loss of $OCOCH_3$ (59 Da). CEP then
218 exhibited the product ion at *m/z* 320 due to the loss of CO_2 (44 Da) from the 364 ion, followed
219 by the 292 ion due to the loss of CO (28 Da) from the 320 ion. Fragmentations of MON A and B
220 resulted from openings of the cyclic ether rings. Fragmentation of MON A with ion trap MS-MS
221 produced neutral losses of 18 and 36 Da corresponding to the sequential losses of H_2O, $[M+Na-$
222 $H_2O]^+$, and $[M+Na-2H_2O]^+$ from the precursor ion, $[M+Na]^+$. SAL and NAR A also exhibited
223 neutral losses of 18 and/or 36 Da corresponding to the subsequent losses of H_2O, $[M+Na-H_2O]^+$
224 and/or $[M+Na-2H_2O]^+$ from the precursor ion, $[M+Na]^+$, as observed in the fragmentation of
225 MON A and B. Metabolite M-1 from MON A was isolated from both lagoon water and animal
226 manure samples (Kiehl et al., 1998; Mercurio et al., 1997; Volmer and Lock, 1998). The mass
227 spectrum of metabolite M-1 indicated that M-1 was O-demethylated monensin. The indicated

molecular weight was the same as MON B, equivalent to MON A minus CH_2. The fragmentation pattern of M-1 is similar to that of MON A and B. Consequently, MON was quantified as the sum of MON A, B, and metabolite M-1.

3.2. Recovery

The recoveries of BLs and PEs from the HLB cartridges were measured by extracting analytes from 20 mL of lagoon water spiked with 3 to 400 µg/L. Recoveries were determined using the ratio of the concentration of analyte in the matrix spiked before extraction to the concentration of analyte in the matrix spiked after extraction. Recovery determinations were calculated as the average of analyses of duplicate lagoon water samples spiked with BLs at 3, 50, and 100 µg/L and with PEs at 3, 100, 200, and 400 µg/L before and after extraction. Similarly, recoveries of BLs and PEs were measured in 5-g samples of animal manure spiked at 10, 100, and 200 µg/kg before and after extraction. All recovery data for BL and PE compounds spiked into lagoon water and animal manure samples is shown in Table 2.

The average recovery of BLs (except for AMOX and AMP) from all the sample matrices was better than 70%. No concentration dependence was observed. For amphoteric penicillins (AMOX and AMP), recoveries were generally between 8% (lagoon water) and 15% (animal manure). It is likely that these lower values are due to the different chemical structure of AMOX and AMP, unlike the other targeted BLs, which have a primary amino group. The lower recovery values for AMOX and AMP in the current study agree with those reported by other researchers (Sacher et al., 2001; Calamari et al., 2003; Cahill et al., 2004; Lindberg et al., 2005). Therefore, AMOX and AMP were not quantified as targeted parameters in this study. The average recovery of PEs in lagoon water and animal manure (Table 2) was 86.9 ± 5.1 % and 81.0 ± 6.7 %,

252 respectively, in the investigated concentration range, indicating the HLB cartridges also yielded
253 effective isolation of the PEs. The lower recovery of BLs and PEs from lagoon water and animal
254 manure relative to surface water indicate that matrix effect is important, most likely due to the
255 presence of more organic matter (OM) and/or natural organic matter (NOM) in the lagoon water
256 and animal manure matrices.
257
258 *3.3. Quantification and method detection limit*
259 The detailed LC-MS-MS method was used to determine the occurrence of three β-lactam and
260 three ionophore compounds in the lagoon or runoff pond water and animal manure samples,
261 representing matrices that might contribute to point and nonpoint agricultural contamination
262 sources. The lagoon or runoff pond waters of raw influent (-1) and lagoon or runoff pond waters
263 (-2, -3) were collected from participating farms in northern Colorado.
264
265 Seven of the 19 lagoon or runoff pond water samples showed detectable antibiotics via the LC-
266 MS-MS method (Table 3). The three targeted BLs – CEP, PEN G and CLOX – were found in
267 lagoon water at levels from 0.97 to 43.31 µg/L. CEP (0.97 µg/L) and CLOX (5.05 µg/L) were
268 only found in sample chicken A-1 lagoon water. PEN G (43.31 µg/L) exhibited the highest
269 concentration found in lagoon water. Of the three targeted PEs, only MON was detected in
270 lagoon water samples – specifically, in beef runoff pond water samples at levels from 94 to 1077
271 µg/L. No β-lactam and ionophore antibiotic was found in lagoon water from dairy E, as this is
272 an organic farm.
273
274 Table 4 shows analytical results in samples differentiated as the lagoon or runoff pond waters of
275 raw influent and lagoon or runoff pond waters – those with aerobic treatment, biodegradation

276 and/or hydrolysis treatment. Considering the measured concentrations of BLs and PEs in the
277 lagoon or runoff pond waters, these compounds were removed during aerobic treatment,
278 biodegradation and/or hydrolysis treatment. The removal efficiency for three BL compounds in
279 the lagoon waters was > 86.5 %, except CEP in the chicken A sample. MON was removed with
280 removal efficiencies of 90.1 and 91.1 %, respectively, in beef A and B samples. Degradation of
281 the BL antibiotics is predictable, due to their poor stability (Christian et al., 2003; Cha et al.,
282 2006; Cha et al., 2015).
283
284 Only two of the solid manure samples were found to contain detectable levels of the six targeted
285 antibiotics (Table 5). Only CLOX (8.09 to 45.20 µg/kg) was found, in dairy manure; no
286 residuals of CEP, PEN G, MON, SAL, or NAR A were detected in any solid manure sample.
287 Seven of the lagoon water samples and two of the animal manure samples were found to contain
288 antibiotic compounds via the described LC-MS-MS method. The concentration of MON in Beef
289 lagoon water samples suggests there should be a need to measure concentration of MON in beef
290 animal manure samples.
291
292 The concentrations of antibiotic compounds found in lagoon or runoff pond water and solid
293 manure samples in this study were compared to those reported by other research groups
294 (Campagnolo et al., 2002; Donoho, 1984; Thiele-Bruhn, 2003; Catherman et al., 1991;
295 Schlüsener et al., 2003). PEN G of the BL group was not detected in the swine lagoon water in
296 this study – compared with detection of 2.1 to 3.5 µg/L reported by Campagnolo et al. (2002).
297 CEP, PEN G, and CLOX were detected only in chicken lagoon water in the current study and
298 PEN G in two out of the five dairy lagoon waters. No BLs were detected in either Swine or Beef

lagoon waters. Compared with detection of 0.32 µg/L reported by Cha et al. (2013) in Korea, PEN G was detected from 1.11 to 43.31 µg/L. Cha et al. (2013) reported that Lincomycin, Sulfamethazine, Sulfamethoxazole, Sulfathiazole, Chlortetracycline, Oxytetracycline and Tylosin in livestock wastewater could be found at concentrations up to 2.65 µg/L. Sulfamethazine and Sulfathiazole concentrations exceed 1 µg/L. The concentrations of antibiotics in USA had higher concentrations than those in Korea (Table 6). MON, SAL, and NAR A were not detected in solid manure in the current study, but other researchers (Donoho, 1984; Thiele-Bruhn, 2003; Catherman et al., 1991; Schlüsener et al., 2003) measured MON from 1 to 5 mg/kg, SAL at 11 µg/kg, and NAR from 1.0 to 725.0 µg/kg. No other studies have reported concentrations of BLs in animal manures or PEs in lagoon waters.

4. Conclusions

The average recovery of BLs and PEs from all the sample matrices was better than 70 % (excluding chemically unstable AMP and AMOX). From the targeted six antibiotic compounds, CEP, PEN G and CLOX were found in the lagoon water samples at concentrations ranging from 0.97 to 43.31 µg/L. MON was also measured in runoff pond at levels ranging from 94 to 1077 µg/L. PEN G (43.31 µg/L) and MON (1077 µg/L) reflected the highest concentrations of BLs and PEs, respectively, in the lagoon or runoff pond water samples. Considering the measured concentrations of BLs and PEs in the lagoon or runoff pond waters, these compounds were removed during aerobic treatment, biodegradation and/or hydrolysis treatment. Only CLOX (8.09 to 45.20 µg/kg) was found in solid manure samples.

The successful application of the SPE/LC-MS-MS method detailed in this study to evaluate the occurrence of three β-lactam and three ionophore antibiotics in lagoon water and animal manure warrants further investigation of this methodology in analysis of these compounds in more complex environmental matrices such as sewage sludge (biosolids) and compost.

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443 **Table 1** Optimal MS/MS parameters for analysis of selected antibiotics

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Analyte	CAS Number	Precursor ion, $[M+H]^+$ (*m/z*)	Product ions (*m/z*)	Normalized collision energy (%)
AMOX	61336-70-7	366.4	**348.9**, 160.0, 207.0	26
AMP	69-53-4	350.4	**160.0**, 174.0, 190.9	26
PEN G	69-57-8	335.4	**160.0**, 176.1, 217.0	25
CLOX	7081-44-9	436.9	**277.1**, 160.0, 178.1	40
CEP	24356-60-3	424.5	**319.9**, 363.9, 292.0	25
OXA (IS)	7240-38-2	402.4	**243.0**, 160.0, 144.1	35
Analyte	CAS Number	Precursor ion, $[M+Na]^+$ (*m/z*)	Product ions (*m/z*)	Normalized collision energy (%)
MON A		693.5	**675.5**, 461.3, 657.5	33
MON B	22373-78-0	679.4	**661.5**, 465.3, 643.4	30
SAL	55721-31-8	773.5	**755.5**, 531.4, 513.3	35
NAR A	55134-13-9	787.5	**769.5**, 531.4, 545.3	35

Product ions (*m/z*) of the highest intensity for SRM and quantification are reported in boldface

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448 **Table 2** Percent recoveries (± S.D.) (n=6) and Method Detection Limits (MDL) (n=7) for
449 antibiotics spiked into lagoon water and animal manure samples.
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Antibiotic	Lagoon water		Animal manure	
	% recovery (± S.D.)	MDL (μg/L)	% recovery (± S.D.)	MDL (μg/kg)
PEN G	77.9 ± 8.2	0.15	83.6 ± 2.5	0.34
CLOX	75.8 ± 9.5	0.23	87.1 ± 7.2	0.72
CEP	74.4 ± 6.8	0.76	71.7 ± 4.3	1.86
MON	91.0 ± 5.6	2.13	94.2 ± 3.9	7.38
SAL	84.5 ± 6.4	0.62	73.9 ± 7.5	1.39
NAR A	85.1 ± 3.3	0.47	74.8 ± 8.7	2.94

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453 **Table 3** Occurrence of selected antibiotics in lagoon or runoff pond water (μg/L)

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Samples		CEP	PEN G	CLOX	MON	SAL	NAR A
Dairy	A-1	ND	1.11	ND	ND	ND	ND
Dairy	A-2	ND	ND	ND	ND	ND	ND
Dairy	B-1	ND	4.33	ND	ND	ND	ND
Dairy	B-2	ND	ND	ND	ND	ND	ND
Dairy	B-3	ND	ND	ND	ND	ND	ND
Dairy	C-1	ND	ND	ND	ND	ND	ND
Dairy	D-1	ND	ND	ND	ND	ND	ND
Dairy	E-1	ND	ND	ND	ND	ND	ND
Dairy	E-2	ND	ND	ND	ND	ND	ND
Dairy	E-3	ND	ND	ND	ND	ND	ND
Chicken	A-1	0.97	43.31	5.05	ND	ND	ND
Chicken	A-2	ND	ND	ND	ND	ND	ND
Swine	A-1	ND	ND	ND	ND	ND	ND
Swine	A-2	ND	ND	ND	ND	ND	ND
Swine	A-3	ND	ND	ND	ND	ND	ND
Beef	A-1	ND	ND	ND	945	ND	ND
Beef	A-2	ND	ND	ND	94	ND	ND
Beef	B-1	ND	ND	ND	1077	ND	ND
Beef	B-2	ND	ND	ND	96	ND	ND

455 ND: less than the MDL of the selected antibiotic

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462 **Table 4** Occurrence of lagoon or runoff pond water sample sites.

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Samples	Analyte	Raw influent (μg/L)	Lagoon or runoff pond water (μg/L)	Removal efficiency (%)
Dairy A	PEN G	1.11	ND[a]	> 86.5[b]
Dairy B	PEN G	4.33	ND	> 96.5
Chicken A	CEP	0.97	ND	> 21.6
	PEN G	43.31	ND	> 99.6
	CLOX	5.05	ND	> 95.4
Beef A	MON	945	94	90.1
Beef B	MON	1077	96	91.1

[a]ND: less than the MDL of the selected antibiotic

[b]Removal efficiency is based on MDL.

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467 **Table 5** Occurrence of selected antibiotics in animal manure (μg/kg)

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Samples		CEP	PEN G	CLOX	MON	SAL	NAR A
Dairy	A	ND	ND	ND	ND	ND	ND
Dairy	B	ND	ND	ND	ND	ND	ND
Dairy	C	ND	ND	45.20	ND	ND	ND
Dairy	D	ND	ND	8.09	ND	ND	ND
Chicken	A	ND	ND	ND	ND	ND	ND
Swine	A	ND	ND	ND	ND	ND	ND

ND: less than the MDL of the selected antibiotic

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472 **Table 6** Comparison of antibiotics in livestock wastewater (μg/L)

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Antibiotic	Wastewater	Reference
PEN G	1.11~43.31	USA(This study)
	0.32	Korea(Cha, 2013)
CEP	0.97	USA(This study)
CLOX	5.05	USA(This study)
MON	945~1077	USA(This study)
Lincomycin	0.76	Korea(Cha, 2013)
Sulfamethazine	1.77	Korea(Cha, 2013)
Sulfamethoxazole	0.11	Korea(Cha, 2013)
Sulfathiazole	2.65	Korea(Cha, 2013)
Chlortetracycline	0.92	Korea(Cha, 2013)
Oxytetracycline	0.53	Korea(Cha, 2013)
Tylosin	0.30	Korea(Cha, 2013)

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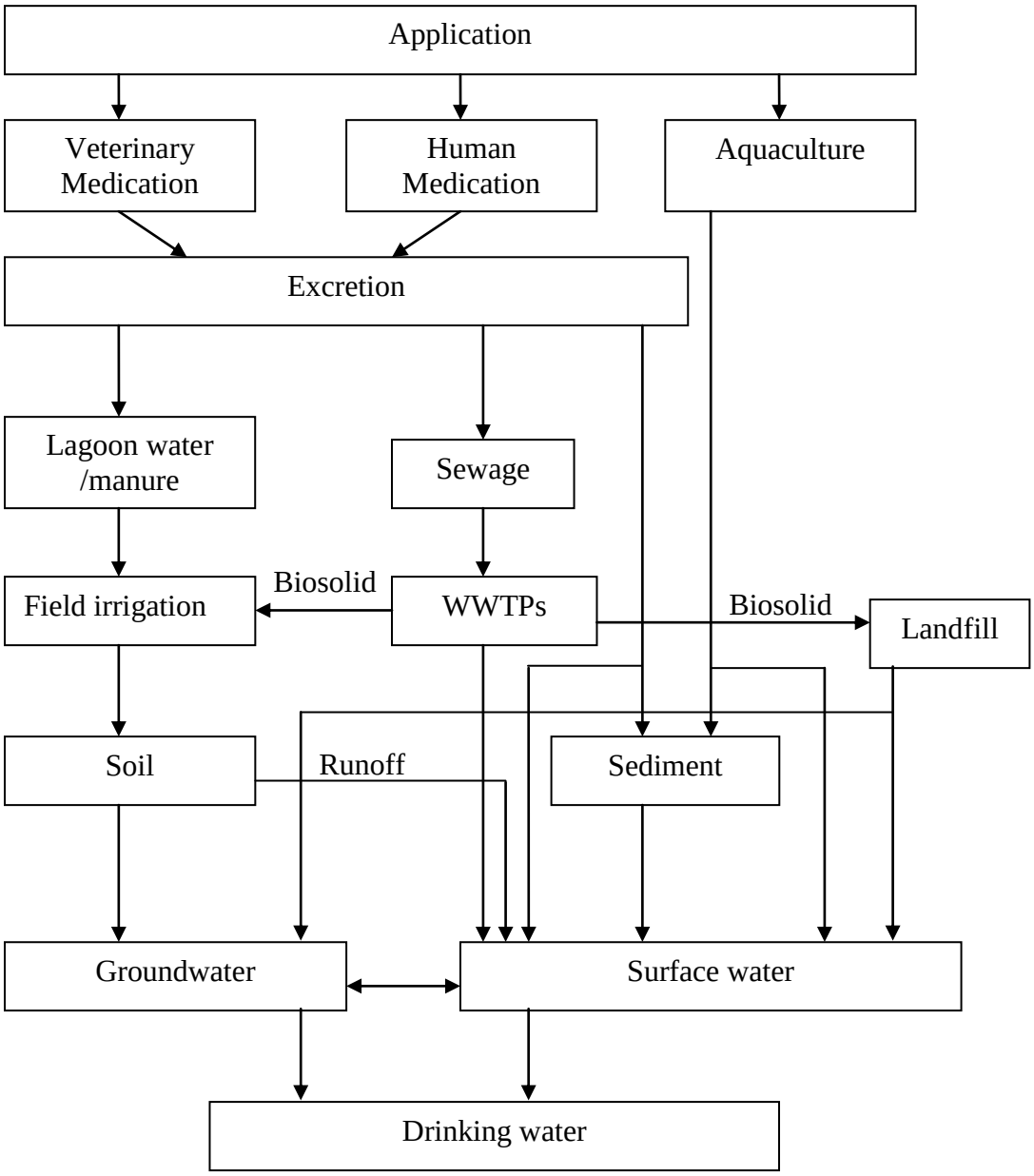

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477 **Fig. 1.** Possible pathways of antibiotics into the aquatic environment.

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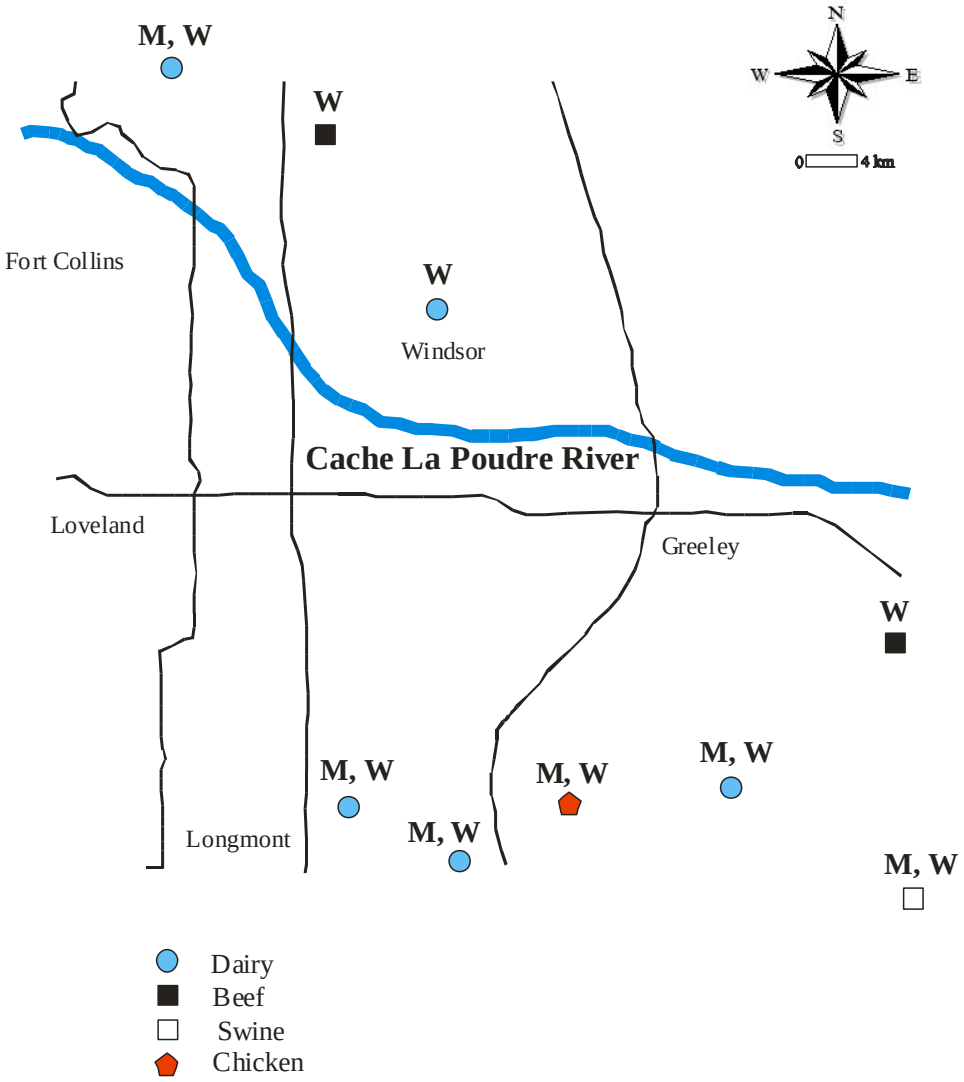

Fig. 2. Sample sites of lagoon or runoff pond waters (W) and animal manures (M) in northern Colorado, U.S.A.

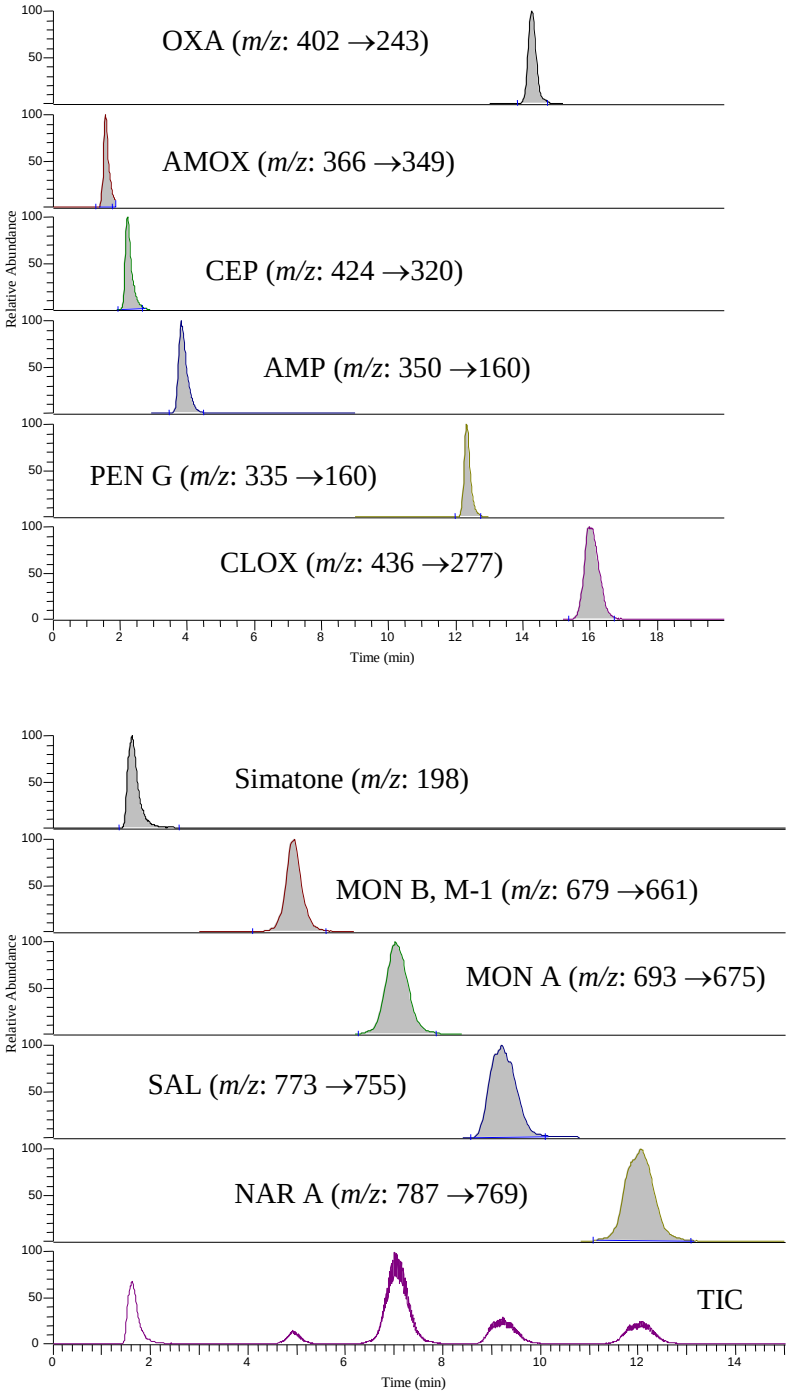

Fig. 3. Reconstructed total-ion chromatograms of β-lactam and ionophore antibiotics spiked at a concentration of 10 µg/L before extraction for 20 mL as the lagoon water matrix using LC-MS-MS in SRM. *m/z* indicates precursor ion → product ion used for quantification.

***Graphical Abstract**