ELSEVIER

Journal of Chromatography A, 928 (2001) 177–186

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Analysis of oxytetracycline, tetracycline, and chlortetracycline in water using solid-phase extraction and liquid chromatography–tandem mass spectrometry

J. Zhu, D.D. Snow*, D.A. Cassada, S.J. Monson, R.F. Spalding

Water Sciences Laboratory, Water Center, Institute of Agriculture and Natural Resources, 103 Natural Resources Hall, University of Nebraska-Lincoln, Lincoln, NE 68583-0844, USA

Received 5 April 2001; received in revised form 19 July 2001; accepted 19 July 2001

Abstract

A method using liquid chromatography–tandem mass spectrometry has been developed for determination of trace levels of tetracycline antibiotics in ground water and confined animal feeding operation waste water. Oxytetracycline (OTC), tetracycline (TC), and chlortetracycline (CTC) were extracted from water samples using both polymeric and C_{18} extraction cartridges. The addition of a buffer containing potassium phosphate and citric acid improved tetracycline recoveries in lagoon water. Method detection limits determined in reagent water fortified with 1 $\mu g\ l^{-1}$ OTC, TC, and CTC were 0.21, 0.20, and 0.28 $\mu g\ l^{-1}$. Method detection limits in lagoon water samples fortified at 20 $\mu g\ l^{-1}$ for OTC, TC, and CTC were 3.6, 3.1, and 3.8 $\mu g\ l^{-1}$. Variability in recovery from laboratory fortified blanks ranged from 86 to 110% during routine analysis.

Keywords: Water analysis; Oxytetracycline; Tetracycline; Chlortetracycline

1. Introduction

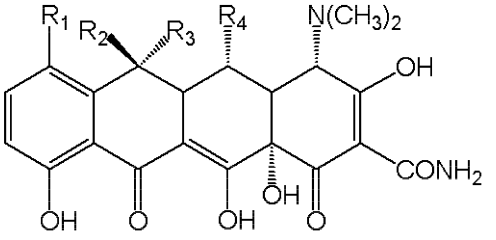
Tetracyclines (Fig. 1) are broad-spectrum antibiotics that are active against both gram-positive and gram-negative bacteria. These compounds have been widely used in confined animal feeding operations (CAFOs) for meat, milk and fish production, and analytical methods developed to date have primarily focused on the determination of tetracycline residues in food samples [1–5]. Recently, concerns have been raised regarding public health issues over the occurrence of antibiotics in the environment [6,7], as well as by indications of increased bacterial resistance in waste effluent from hospitals and pharmaceutical plants [8–10]. The occurrence and fate of tetracycline antibiotics in CAFO waste water and the possible contamination of ground water is largely unknown [6,11,12]. Thus, there is a need for a sensitive and rapid analytical method to measure concentrations of tetracyclines in animal waste lagoons and in ground and surface water that may be impacted by CAFO waste water.

Liquid chromatography–mass spectrometry (LC–MS) [1–5,11,12,20–28] is becoming more commonly used in the analysis of antibiotics because of its high sensitivity and ability to provide compound

*Corresponding author. Tel.: +1-402-472-7539; fax: +1-402-472-9599.

E-mail address: dsnow1@unl.edu (D.D. Snow).

0021-9673/01/$ – see front matter
PII: S0021-9673(01)01139-6



	CAS Registry No.	R_1	R_2	R_3	R_4
Chlortetracycline	[57-62-5]	Cl	OH	CH_3	H
Doxycycline	[17086-28-1]	H	H	CH_3	OH
Oxytetracycline	[79-57-2]	H	OH	CH_3	OH
Tetracycline	[60-54-8]	H	OH	CH_3	H
Demeclocycline	[127-33-3]	Cl	OH	H	H

Fig. 1. Structure of tetracyclines investigated (modified from Oka et al. [2]).

confirmation as compared to conventional liquid chromatography–UV detection (LC–UV) [13–18], or liquid chromatography–fluorescence detection (LC–FD) [19]. Capillary electrophoresis–mass spectrometry (CE–MS) [29] has also been used for the analysis of tetracycline residues in milk, serum and urine samples. Radioimmunoassay has also been reported as a screening method for antibiotics, however this method is only semi-quantitative, not selective, and needs further confirmation using LC–MS [12]. Reported LC–MS methods for the analysis of tetracyclines also include particle beam (PB) [21], fast atom bombardment (FAB) [22], thermospray (TSP) [2], atmospheric pressure chemical ionization (APCI) [1,24,30], and electrospray ionization (ESI) [11,12,20,23,26–28,31] mass spectrometry. APCI and ESI ionization methods combined with tandem mass spectrometry are preferred due to their higher sensitivity, better reproducibility, and commercial availability.

One difficulty in the analysis of tetracycline antibiotics using LC–MS is the interaction of these compounds with residual silanol groups and metal ions on the LC column which often results in severe peak tailing and variable analyte recoveries. One approach to correcting this problem is the use of a complexing agent such as oxalic acid or EDTA in the mobile phase to improve peak shape and consistency. Unfortunately, non-volatile oxalic acid may accumulate in the capillary interface or skimmer of the electrospray ionization or APCI source, resulting in plugged capillaries and signal loss [2,24,30]. In order to reduce the clogging, Nakazawa et al. [24] and Blanchflower et al. [30] used an elevated nebulizer probe temperature so that the oxalic acid complexing agent decomposed in the APCI interface. For an APCI source, this modification allowed prolonged analysis without severe signal loss. However, this modification is limited to electrospray (ESI) instrumentation with off-axis or orthogonal spray sampling that helps to reduce residue build-up from non-volatile mobile phase buffers.

Solid-phase extraction (SPE) using the reversed-phase octadecyl (C_{18}) cartridge has become routine in purifying and concentrating environmental contaminants [32]. A large number of methods for extracting tetracyclines using C_{18} have been developed in place of the more labor-intensive liquid–liquid extraction [1–5,16,17,33] and lyophilization [27,28]. Chelating agents are sometimes added to samples to improve analyte recovery in these SPE methods. Blanchflower et al. [30] described the use of a glycine–HCl buffer for C_{18} extraction of tetracyclines from muscle and kidney samples. Oka et al. [33] used a 0.1-*M* EDTA–McIlvaine buffer that resulted in more consistent recoveries for tetracyclines extracted from meat samples. Metal-chelating affinity chromatography (MCAC) [13,19,34] has also been used for the clean-up of tetracyclines from food [13,19], serum and urine samples [34]. However, an additional desalting step of eluent from the MCAC column is required prior to analysis by LC–MS. Solid-phase micro-extraction (SPME) has also been tested for the analysis of tetracycline analogues in water [25]. However, SPME may be problematic for complex biological matrices due to its reduced loading capacity and may not be convenient for routine analysis.

Cheng et al. [18] present a method for extracting tetracyclines from porcine serum using a recently developed cartridge containing a macroporous poly(divinylbenzene-co-*N*-vinylpyrrolidone) sorbent (Oasis HLB, Waters, Milford, MA, USA). Serum samples fortified with 0.5–2.5 mg l^{-1} tetracycline and minocycline were extracted with quantitative (88–101%) and highly reproducible ($\pm$2%) results. Major advantages for the Oasis HLB cartridge include a more rugged extraction, improved recovery for both polar and non-polar compounds in complex

matrices, and greater capacity than reverse-phase silica based sorbents [18,35,36].

This paper describes a method for determining trace levels of tetracycline antibiotics in ground water and animal waste water samples using SPE with electrospray ionization (ESI) tandem mass spectrometry for both identification and quantification. Tetracycline extraction efficiency and reproducibility are compared for both the polymeric Oasis HLB and the reversed-phase Sep-Pak tC_{18} cartridges.

2. Materials and apparatus

Tetracycline compounds and their structures that were investigated for this method are listed in Fig. 1. Oxytetracycline (OTC), tetracycline (TC), and chlortetracycline (CTC) were obtained from Acros Organics (Fisher Scientific, Houston, TX, USA). Doxycycline (DOC) was used for a surrogate compound and obtained from Sigma–Aldrich (St Louis, MO, USA), while the internal standard, demeclocycline (DMCTC), was obtained from Fluka Chemical (Milwaukee, WI, USA). Doxycycline (DOC) and demeclocycline (DMCTC) are not registered in the United States for use in swine, cattle, dairy cows, or poultry. HPLC grade methanol and acetonitrile, reagent grade trifluoroacetic acid (TFA), disodium ethylenediamine tetraacetate (Na_2EDTA), anhydrous potassium phosphate (monobasic), citric acid, and formic acid were obtained from Fisher Scientific (Houston, TX, USA). Reagent grade phosphoric acid was obtained from J.T. Baker (Phillipsburg, NJ, USA). Reagent grade oxalic acid was obtained from Mallinckrodt Chemical Works (St. Louis, MO, USA).

Stock solutions of ~5000 mg l^{-1} OTC, TC, CTC, DOC, and DMCTC were prepared in methanol and stored at −10°C. Calibration standards were prepared monthly by dilution of the stock solution with 20% methanol in water to 50, 200, 800, 2000, and 8000 μg l^{-1}.

3. Methods

3.1. Extraction procedures

Solid-phase extraction (SPE) experiments were conducted using the trifunctional (tC_{18}) 1-g Environmental Sep-Pak and 200-mg Oasis HLB cartridges (Waters, Milford, MA, USA). Tetracycline recoveries were compared on both phases. Both tC_{18} and HLB extraction cartridges were conditioned and equilibrated immediately prior to extraction with 6 ml of methanol followed by 6 ml of reagent water.

A modification of the C_{18} extraction procedure described by Oka et al. [33] was performed using a buffer containing 0.05 *M* disodium phosphate, 0.05 *M* citric acid, and 0.05 *M* disodium ethylenediamine tetraacetate. Sample pH was adjusted below the pK_a of TC, OTC, and CTC (pK_a 3.3) [31] for increased retention on the HLB sorbent [18]. Because tetracyclines are not stable [2] in acidic solutions (pH< 2), extraction using the HLB sorbent was performed with sample pH adjusted to ~2.5 immediately prior to extraction. Fortified 100-ml aliquots of groundwater and reagent water were acidified to a pH near 2.5 with concentrated phosphoric acid. In addition, fortified 10-ml aliquots of unfiltered lagoon samples were diluted prior to extraction with 90 ml of a buffer containing 0.05 *M* potassium phosphate (monobasic) and 0.05 *M* citric acid (pH ~2.5) in order to minimize complex formation [33].

Samples were siphoned using vacuum through each cartridge at a flow-rate of ~5 ml min^{-1}. After extraction, the HLB cartridges were flushed with 10 ml of 5% aqueous methanol while the tC_{18} cartridges were washed with 10 ml of reagent water to help remove inferences. Tetracyclines were eluted from tC_{18} cartridges using 2.5 ml of 10 m*M* oxalic acid in methanol, and from HLB cartridges using 2.5 ml of 1% TFA in methanol. Both extracts were evaporated to near dryness under nitrogen, and then re-dissolved in 200 μl of 20% aqueous methanol. Solutions were then analyzed using LC–MS–MS.

For quantification of analytes in samples and fortified blanks, the internal standard (DMCTC) was added prior to extraction to correct for variations in compound recovery. During method development, DMCTC was added after elution to determine actual analyte recoveries using the tC_{18} and HLB sorbents. Reagent water was fortified at two levels (4 and 20 μg l^{-1} DOC, OTC, TC, and CTC) to compare recovery on each phase. Lagoon water was also fortified at two levels (40 and 200 μg l^{-1} DOC, OTC, CTC, and TCT) to assess matrix effects on

recovery. For method detection limit determination, 100 ml of reagent water was fortified with 1 μg l^{-1} OTC, TC and CTC, 20 μg l^{-1} DOC (surrogate) and 50 μg l^{-1} DMCTC (internal standard). To determine detection limits in complex matrices, 10 ml of lagoon water was fortified with 20 μg l^{-1} OTC, TC and CTC, and 200 μg l^{-1} DOC and 500 μg l^{-1} DMCTC.

3.2. Instrumental conditions

The HPLC system consisted of a Waters 616 LC system, 600 controller, 717 Plus autosampler, and Millennium 2010 chromatography software. Mass spectrometry was performed with a Finnigan LCQ ion trap (Thermoquest, San Jose, CA, USA) equipped with heated capillary interface, and electrospray ionization (ESI) source. Thermoquest Navigator software was employed to control the mass spectrometric conditions and Lcquan software was used for the quantification of tetracyclines.

Isocratic separation at 50°C was achieved using a 250×2 mm end-capped BetaBasic C_{18} 5 μm reversed-phase HPLC column (Keystone Scientific, Bellfonte, PA, USA). No equilibration time was needed between each analysis. Optimum separation occurred using a mixture of water, 5% formic acid, acetonitrile, and methanol (23:40:25:12) at a flow-rate of 0.2 ml min^{-1}. A 25-μl injection volume was used and calibration standards were analyzed throughout each run. The curve of quantity versus relative response of analyte to internal standard exhibited good linearity and reproducibility (five replicates) over the calibration range for TC, CTC, OTC and the surrogate DOC (r^2=0.9939, 0.9994, 0.9974, and 0.9999, respectively).

Electrospray ionization (ESI) was optimized using DMCTC as the reference compound. Ion source and trap conditions were then adjusted and programmed to achieve the most stable and intense product ions to provide maximum sensitivity for each compound. The nebulizing gas flow-rate was set to 75 l min^{-1} while the drying gas was set to 15 l min^{-1}. The heated capillary temperature was set to 230°C. Fragmentation was produced via collision-induced dissociation (CID) in the ion trap. The product ion producing the highest signal was chosen for the selective reaction monitoring (SRM) and quantification (Table 1).

4. Results and discussion

4.1. Selected reaction monitoring

Mass spectrometry has become an effective detector for HPLC due to its high sensitivity and selectivity. In addition to the commonly detected pseudo-molecular ion ($[M+H]^+$ or $[M-H]^-$) produced in APCI or ESI methods, product or fragment ion detection can provide a higher level of sensitivity and selectivity. Fragmentation can be produced via two different approaches. One method, known as in-source collision induced dissociation (CID), is accomplished by elevating the skimmer voltage to produce enough energy to induce decomposition of reactant ions upon collision with gas molecules in the source region. The other fragmentation approach uses tandem mass spectrometry (MS–MS) where reactant ions are first separated and then collided with a neutral gas such as argon or helium to produce fragment ions for detection and quantifica-

Table 1
Nominal molecular mass, reactant and fragment ion *m*/*z*, relative collision energies, and product ion efficiency for the tetracyclines investigated

Compound	Nominal molecular mass (Da)	Reactant ion (*m*/*z*)	Relative collision energy (%)	Product ion (*m*/*z*)	Product ion efficiency (%)
Chlortetracycline	478	479	40	444	37
Doxycycline	444	445	24	428	68
Oxytetracycline	460	461	40	426	42
Tetracycline	444	445	40	410	34
Demeclocycline	464	465	24	448	74

tion. The advantage of the second approach is that selection and fragmentation of reactant ions occurs separately from detection of the fragment ions either at a different mass analyzer (triple quadrupole) or at a different time (ion trap). With tandem mass spectrometry, it is possible to separate and detect compounds having the same molecular mass but different product ions even if they co-elute. Thus, MS–MS is preferred for increased analytical sensitivity and selectivity in complex matrices.

Molecular mass, reactant and product ion m/z, collision energies, and relative fragment ion intensities are listed in Table 1, and the fragmentation spectra produced are shown in Fig. 2. Loss of amine or H_2O groups from the reactant ion is consistent with the previous reports for tetracycline mass spectra [4,23,24,31]. Fragmentation of chlortetracycline and the internal standard demeclocycline produced two major product ions corresponding to $[M+H-NH_3]^+$ and $[M+H-NH_3-H_2O]^+$, while fragmentation of oxytetracycline and tetracycline produced major fragments corresponding to $[M+H-H_2O]^+$ and $[M+H-NH_3-H_2O]^+$. Fragmentation of the surrogate doxycycline produced a single product ion corresponding to $[M+H-NH_3]^+$ at $m/z=428$. This ion is also a minor product ion for tetracycline (Fig. 2). The use of DOC as a surrogate thus depends upon chromatographic separation of DOC from TC. A chromatogram for a laboratory-fortified blank is shown in Fig. 3. Baseline isocratic separation of TC and DOC is obtained within a 10-min analysis time using the indicated conditions. Chromatographic separation can thus be optimized for rapid analysis in selected reaction monitoring (SRM) mode.

4.2. Recovery comparison and method detection limits

Tetracycline recoveries were measured by extracting 100-ml aliquots of fortified water and adding the internal standard after elution. More consistent recoveries of tetracyclines from the HLB cartridges, as indicated by lower standard deviations, were obtained using 1% TFA in methanol over methanol alone (Table 2). Tetracyclines were quantitatively eluted from both the HLB cartridges using 1% TFA in methanol and from the tC_{18} cartridges using 10 m*M* oxalic acid in methanol (Table 2). Two additional sets of recovery results comparing tetracycline extraction with tC_{18} and HLB are shown in Table 3 for reagent water samples fortified at 4 and 20 $\mu g\ l^{-1}$ and in Table 4 for lagoon samples fortified at 40 and 200 $\mu g\ l^{-1}$. CTC and DOC recoveries of 133–145% in reagent water fortified at 4 $\mu g\ l^{-1}$ (Table 3) may be due to matrix differences between the calibration and the extract solutions which may be more evident at lower concentrations. However, the elevated recoveries for these compounds were not consistent from run to run and thus do not indicate a bias in the analysis.

Calculations of method detection limits (MDLs) were based on the variability of multiple analyses of reagent water fortified at 1 $\mu g\ l^{-1}$ and animal waste water samples fortified at 20 $\mu g\ l^{-1}$ of the analytes. The MDL was determined by multiplying the sample standard deviation calculated from each group of fortified solutions by the Student's *t*-variate for a one-sided *t*-test at the 99% confidence level with $n-1$ degrees of freedom. MDLs for OTC, TC, and CTC extracted from 100 ml of reagent water were 0.21, 0.20, and 0.28 $\mu g\ l^{-1}$, respectively (Table 5). Detection limits in the smaller and more complex lagoon samples were ~10–15 times higher (Table 5) and ranged from 3.1 to 3.8 $\mu g\ l^{-1}$ using the HLB cartridges. Extraction of tetracyclines from fortified lagoon samples using the reverse-phase tC_{18} cartridges resulted in similar detection limits and recoveries (Table 5).

The HLB cartridges were selected for routine analysis of tetracyclines in groundwater and lagoon water samples due to the simplicity and ruggedness of the method relative to the tC_{18} extraction. Variability of analyte recoveries was monitored during routine analysis using laboratory-fortified blanks (LFB) containing 50 $\mu g\ l^{-1}$ internal standard (DMCTC), 20 $\mu g\ l^{-1}$ surrogate (DOC) and 10 $\mu g\ l^{-1}$ OTC, TC and CTC. The results for ten LFB samples (Table 6) analyzed over a period of 6 months indicate quantitative and reproducible recoveries for the analytes and surrogate. Surrogate (DOC) recovery in the analysis of 71 lagoon samples over this period averaged $109\pm14\%$.

Groundwater from monitoring wells down gradient from CAFO waste lagoons at 11 sites across Nebraska was extracted and analyzed for tetra-

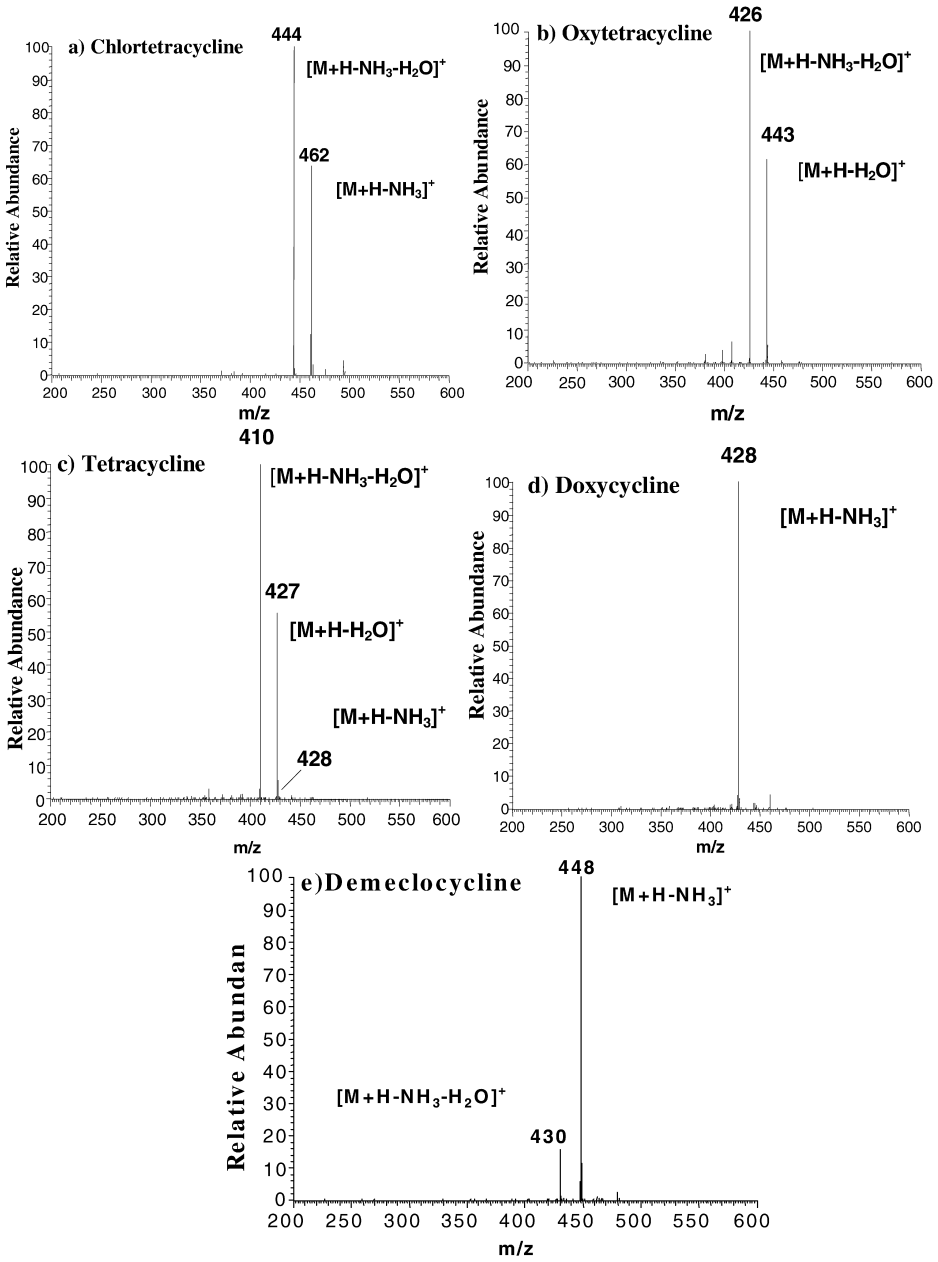


Fig. 2. Collision-induced dissociation mass spectra of: (a) chlortetracycline, (b) oxytetracycline, (c) tetracycline, (d) doxycycline, and (e) demeclocycline.

cyclines. No information regarding the use of antibiotics at these sites was collected. CTC and OTC are currently approved in the United States for use as feed additives for poultry, swine, cattle and sheep, while TC can only be added to water for treatment of disease [37]. Wells were installed near waste lagoons for nine swine operations, one dairy operation, and one cattle feedlot. No analytes were found above the method detection limits in groundwater from these wells.

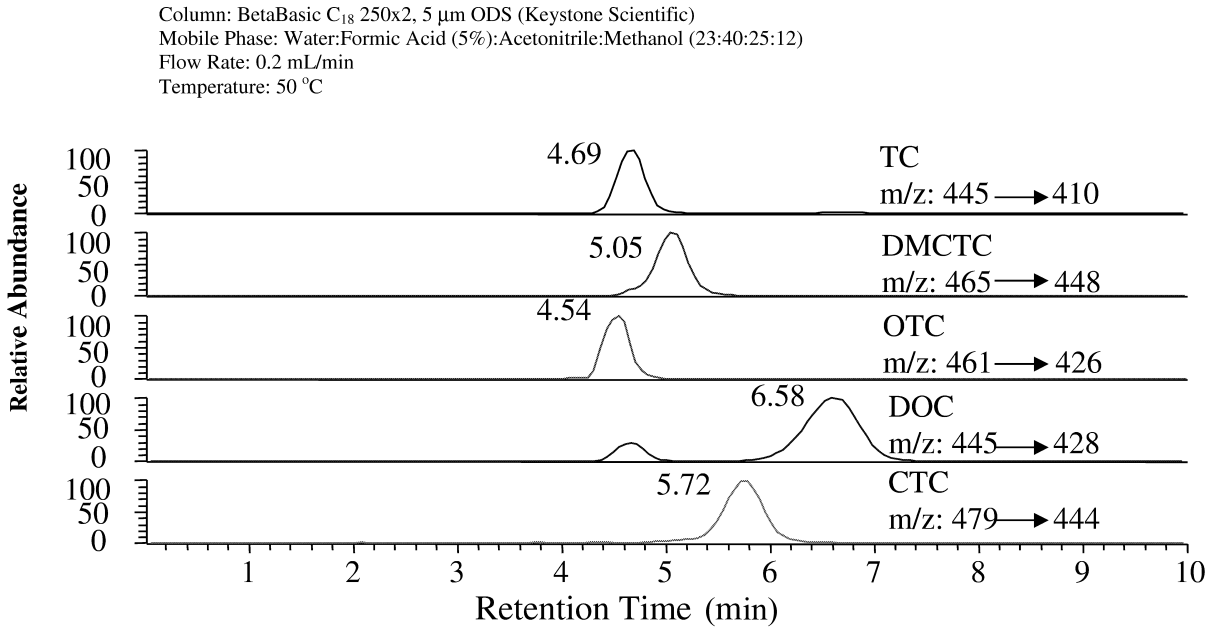


Fig. 3. Chromatograms showing resolution of tetracyclines in a fortified blank extract using selected reaction monitoring mode (SRM) in tandem mass spectrometry.

Table 2
Comparison of tetracycline recoveries (sample average±SD) extracted from five aliquots of 100-ml aliquots of reagent water fortified at 10 and 20 μg l^{-1} and eluted from the HLB sorbents with methanol and 1% trifluoroacetic acid (TFA) in methanol, and from the tC_{18} sorbent with 10 m*M* oxalic acid (OA) in methanol

Cartridge used, concentration	Eluted with	OTC, x±SD (%)	TC, x±SD (%)	CTC, x±SD (%)	DOC, x±SD (%)
Oasis HLB, 10 μg l^{-1}	Methanol	98±16	84±7	83±20	102±9
Oasis HLB, 10 μg l^{-1}	Methanol–1% TFA	95±4	77±6	78±4	103±1
Oasis HLB, 20 μg l^{-1}	Methanol–1% TFA	97±10	94±8	90±7	108±12
Sep-Pak tC_{18}, 20 μg l^{-1}	Methanol–10 m*M* OA	98±16	84±4	94±9	114±12

Table 3
Comparison of tetracycline recoveries (sample average±SD) extracted from eight 100-ml aliquots of reagent water fortified at 4 and 20 μg l^{-1} using the tC_{18} and HLB cartridges

Cartridge, concentration	OTC, x±SD (%)	TC, x±SD (%)	CTC, x±SD (%)	DOC, x±SD (%)
Oasis HLB, 4 μg l^{-1}	103±7	82±7	138±15	133±14
Sep-Pak tC_{18}, 4 μg l^{-1}	105±10	83±9	139±11	145±14
Oasis HLB, 20 μg l^{-1}	97±10	94±8	90±7	108±12
Sep-Pak tC_{18}, 20 μg l^{-1}	98±16	85±4	94±9	114±12

Table 4
Comparison of tetracycline recoveries (sample average±SD) extracted from eight 10-ml aliquots of lagoon water fortified at 40 and 200 μg l^{-1} using the tC_{18} and HLB cartridges

Cartridge, concentration	OTC, x±SD (%)	TC, x±SD (%)	CTC, x±SD (%)	DOC, x±SD (%)
Oasis HLB, 40 μg l^{-1}	70±5	74±7	81±4	87±8
Sep-Pak tC_{18}, 40 μg l^{-1}	72±5	75±3	75±7	75±7
Oasis HLB, 200 μg l^{-1}	68±4	68±4	98±4	106±5
Sep-Pak tC_{18}, 200 μg l^{-1}	85±5	74±5	96±8	93±8

Lagoon samples from these sites were also extracted and analyzed. Chlortetracycline, oxytetracycline, and tetracycline were detected in lagoon samples from over half of these sites. Maximum chlortetracycline concentrations were near 12 000 μg l^{-1}, followed by tetracycline and oxytetracycline at concentrations up to 1300 μg l^{-1}. Out of 26 lagoon samples, 23 contained over 3 μg l^{-1} of one of the three tetracycline compounds. Concentrations of all three tetracyclines were highest in lagoons for swine finishing operations where these compounds may be routinely used for growth promotion. Research is ongoing to determine spatial and temporal variability of tetracyclines in lagoon water as well as the potential for transport to other systems.

5. Conclusions

A method for quantification of oxytetracycline (OTC), tetracycline (TC), and chlortetracycline (CTC) in aqueous samples has been developed using solid-phase extraction and LC–MS–MS. Comparison of tetracycline extraction between the Oasis HLB sorbent and the Environmental Sep-Pak tC_{18} sorbent showed similar recoveries and detection limits. Using lagoon samples fortified at 20 μg l^{-1}, the HLB extraction method obtained detection limits of 3.6, 3.1, and 3.8 μg l^{-1}, with recoveries at 83±8, 72±8, and 92±8% for OTC, TC and CTC, respectively. Extraction of reagent water fortified at 1.0 μg

Table 5
Method detection limits and recoveries of tetracyclines extracted with the HLB cartridge from eight 100-ml aliquots of reagent water fortified with 1.0 μg l^{-1}, and extracted with the HLB and tC_{18} cartridges from eight 10-ml aliquots of lagoon water fortified at 20 μg l^{-1}

Cartridge, concentration	OTC		TC		CTC	
	MDL (μg l^{-1})	Recovery, x±S.D (%)	MDL (μg l^{-1})	Recovery, x±SD (%)	MDL (μg l^{-1})	Recovery, x±SD (%)
Oasis HLB, 1.0 μg l^{-1} reagent water	0.21	80±8	0.20	90±8	0.28	79±11
Oasis HLB, 20 μg l^{-1} lagoon water	3.6	83±8	3.1	72±8	3.8	92±8
Sep-Pak tC_{18}, 20 μg l^{-1} lagoon water	2.8	88±5	3.1	65±5	2.6	93±4

Table 6
Tetracycline recoveries (sample average±SD) in ten 100-ml laboratory-fortified blanks extracted and analyzed over a period of 6 months containing 10 μg l^{-1} OTC, TC, and CTC, and 20 μg l^{-1} DOC

Cartridge, concentration	OTC, recovery x±SD (%)	TC, recovery x±SD (%)	CTC, recovery x±SD (%)	DOC, recovery x±SD (%)
Oasis HLB, 1.0 μg l^{-1} reagent water	100±15	87±10	94±10	109±14

l^{-1} with the HLB cartridges resulted in method detection limits at 0.21, 0.20, and 0.28 $\mu g\ l^{-1}$, with recoveries of 80±8, 90±8, and 79±11% for OTC, TC and CTC, respectively. Surrogate recovery averaged 109±14%, and analyte recoveries in fortified blanks averaged 100±15% for OTC, 87±10% for TC, and 94±10% for CTC analyzed with lagoon and groundwater samples collected over a period of 6 months. While ground water downgradient from CAFO lagoons at 11 sites in Nebraska did not contain detectable levels of tetracyclines, at least one of the three pharmaceutical compounds was detected in 23 out of 26 lagoon samples from these sites.

Acknowledgements

Support for this research was provided by the Nebraska Department of Environmental Quality (NDEQ). The LC–MS–MS instrumentation was in part purchased with Nebraska Research Initiative (NRI) funds for the core laboratory. We thank Dr Glen Martin and Mari Sadayappan for providing the lagoon water samples, and Jeff Toavs for his assistance in groundwater sample collection. Thanks also to Steve Comfort and Dennis McCallister for their review of this manuscript. This article has been assigned Journal Series No. 13304, Agricultural Research Division, Institute of Agriculture and Natural Resources, University of Nebraska.

References

[1] W.M.A. Niessen, J. Chromatogr. A 812 (1998) 53.
[2] H. Oka, J. Patterson, in: H. Oka, H. Nakazawa, K.-I. Harada, J.D. MacNeil (Eds.), Chemical Analysis of Antibiotics Used in Agriculture, AOAC International, Arlington, VA, 1995, p. 333, Chapter 10.
[3] D.G. Kennedy, R.J. McCracken, A. Cannavan, S.A. Hewitt, J. Chromatogr. A 812 (1998) 77.
[4] H. Oka, Y. Ito, Y. Ikai, T. Kagami, K. Harada, J. Chromatogr. A 812 (1998) 309.
[5] F.J. Schenck, P.S. Callery, J. Chromatogr. A 812 (1998) 99.
[6] B. Halling-Sorensen, S.N. Nielsen, P.F. Lanzky, F. Ingerslev, H.C.H. Lutzhoft, S.E. Jorgensen, Chemosphere 36 (1998) 357.
[7] S.B. Levy, Sci. Am. 278 (1998) 46.
[8] M. Goni-Urriza, M. Capdepuy, C. Arpin, N. Raymond, P. Caumette, C. Quetin, Appl. Environ. Microbiol. 66 (2000) 125.
[9] M.T. Ogan, D.E. Nwiika, J. Appl. Bacteriol. 74 (1993) 595.
[10] L.A. Guardabassi, A. Petersen, J.E. Olsen, A. Dalsgaard, Appl. Environ. Microbiol. 64 (1998) 3499.
[11] E.M. Thurman, M. Lindsey, National Ground Water Association Emerging Issues Conference Proceedings, Minneapolis, Minnesota, USA, June 7–8, 2000, National Ground Water Association, Westerville, OH, USA, 2000, p. 19.
[12] M.T. Meyer, J.E. Bumgarner, E.M. Thurman, K.A. Hostetler, J.V. Daughtridge, US Geological Survey Toxic Substances Hydrology Program — Proceedings of the Technical Meeting, WRIR 99-4018B, Charleston, SC, USA, March 8–12, 1999, USGS, Reston, VA, USA, 1999.
[13] A.D. Cooper, G.W.F. Stubbings, M. Kelly, J.A. Tarbin, W.H.H. Farrington, G. Shearer, J. Chromatogr. A 812 (1998) 321.
[14] Y.L. Liang, M.B. Denton, R.B. Bates, J. Chromatogr. A 827 (1998) 45.
[15] M. Touraki, P. Rigas, P. Pergandas, C. Kastritsis, J. Chromatogr. B 663 (1995) 167.
[16] R.W. Fedeniuk, S. Ramamurthi, A.R. McCurdy, J. Chromatogr. B 677 (1996) 291.
[17] H. De Ruyck, H. De Ridder, R. Van Renterghem, F. Van Wambeke, Food Addit. Contam. 16 (1999) 47.
[18] Y.F. Cheng, D.J. Phillips, U. Neue, Chromatographia 44 (1997) 187.
[19] S.M. Croubels, K.E.I. Vanoosthuyze, C.H. Van Peteghem, J. Chromatogr. B 690 (1997) 173.
[20] H. Oka, Y. Ikai, Y. Ito, J. Hayakawa, K. Harada, M. Suzuki, H. Odani, K. Maeda, J. Chromatogr. B 693 (1997) 337.
[21] P.J. Kijak, M.G. Leadbetter, M.H. Thomas, E.A. Thompson, Biol. Mass Spectrom. 20 (1991) 789.
[22] H. Oka, Y. Ikai, J. Hayakawa, K.I. Harada, H. Asukabe, M. Suzuki, R. Himei, M. Horie, H. Nakazawa, J.D. MacNeil, J. Agric. Food Chem. 42 (1994) 2215.
[23] A. Weimann, G. Bojesen, P. Nielsen, Anal. Lett. 31 (1998) 2053.
[24] H. Nakazawa, S. Ino, K. Kato, T. Watanabe, Y. Ito, H. Oka, J. Chromatogr. B 732 (1999) 55.
[25] C.M. Lock, L. Chen, D.A. Volmer, Rapid Commun. Mass Spectrom. 13 (1999) 1744.
[26] A. Weimann, G. Bojesen, J. Chromatogr. B 721 (1999) 47.
[27] R. Hirsch, T.A. Ternes, K. Haberer, A. Mehlich, F. Ballwanz, K.L. Kratz, J. Chromatogr. A 815 (1998) 213.
[28] R. Hirsch, T.A. Ternes, K. Haberer, K.L. Kratz, Sci. Total Environ. 225 (1999) 109.
[29] C.L. Chen, X. Gu, J. AOAC Int. 78 (1995) 1369.
[30] W.J. Blanchflower, R.J. McCracken, A.S. Haggan, D.G. Kennedy, J. Chromatogr. B 692 (1997) 351.
[31] A.M. Kamel, P.R. Brown, B Munson, Anal. Chem. 71 (1999) 968.
[32] E.M. Thurman, M.S. Mills, in: J.D. Winefordner (Ed.), Solid Phase Extraction: Principles and Practice, John Wiley & Sons, New York, 1998.

[33] H. Oka, H. Matsumoto, K. Uno, K.I. Harada, S. Kadowaki, M. Suzuki, J. Chromatogr. A 325 (1985) 265.
[34] M.C. Carson, J. AOAC Int. 76 (1993) 329.
[35] Y.F. Cheng, U.D. Neue, L. Bean, J. Chromatogr. A 828 (1998) 273.
[36] M.C. Hennion, J. Chromatogr. A 856 (1999) 3.
[37] D.C. Plum (Ed.), Veterinary Drug Handbook, Iowa State University Press, Ames, IA, 1999.