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Extraction of tetracycline antimicrobials from river water and sediment: a comparative study of three solid phase extraction methods

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Tetracyclines are ubiquitous pollutants in the aquatic environment so it is necessary to determine their levels in river water and sediment. Many extraction techniques have been suggested in previous studies. However there is lack of comparisons among them. This work compared three extraction methods Ultrasonic Assisted Tandem Solid Phase Extraction (UA-TSPE), Ultrasonic Assisted Dispersive Solid Phase Extraction (UA-DSPE) and Ultrasonic Assisted Matrix Solid Phase Dispersion (UA-MSPD) in conjunction with a complexing agent to complex metal cations, for the extraction of tetracycline antibacterials from fortified ultra-pure water, river water and sediment samples under acid conditions. Samples were analyzed on a RP-HPLC coupled to UV detection. The linear dynamic range for calibration curves of all techniques were 0.01 to 1 µgml^{-1} (water) and 0.01 to 1 µgg^{-1} (sediment) with R^2 values ranging from 0.995 to 0.999. The overall recovery was in the range 92.13 to 99.62%. UA-DSPE yielded the best recoveries (at p > 0.05), range 97.23 to 99.62%. UA-TSPE was second at 94.99 to 97.75%, while UA-MSPD was third at 92.13 to 97.84% recovery. Limit of detection (LOD) (at signal to noise ratio = 3) and Limit of quantification (LOQ) (at signal to noise ratio = 10) for spiked river water and sediment were in the range 11.53 to 22.75 ngml^{-1} and 30.12 to 56.22 ngml^{-1}, respectively.

Key words: Tetracycline antibacterials, aquatic environment, ultrasonic assisted tandem solid phase extraction, ultrasonic assisted dispersive solid phase extraction, ultrasonic assisted matrix solid phase dispersion.

INTRODUCTION

Tetracycline antibacterials are some of the emerging aquatic contaminants which fall under unregulated contaminants that may be candidates for future regulation (Chen et al., 2010). They are the widely prescribed antibiotics as therapeutic agents in humans and animal husbandry. They are also used as growth promoters in cattle, swine, poultry and fish farming (Fritz and Zuo 2007, Jodeh and Awartani, 2011). They are poorly assimilated such that after intake by humans and animals more than 70% of tetracyclines leave the organism

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unmetabolized via urine or faeces (Sarmah et al., 2006). They are released either directly or indirectly into the aquatic environment through runoff or spreading of manure in fields and vegetable gardens (Hirsch et al., 2006; Luo et al., 2011). Widespread application of tetracycline antibacterials in medicine and agriculture has resulted in their widespread occurrence in the aquatic environment (Lopez Penaver et al., 2010; Tso et al., 2011 and Xuan et al., 2010). As a result there is now public concern that this may lead to proliferation of antibiotic resistant strains with adverse health effects on humans. This therefore calls for frequent monitoring of antibiotic residues in the environment. Challenges related to determination of tetracycline antibacterials in aquatic environment include the low concentrations in which they are found, and their distinct complex chemical morphologies. Tetracycline antibacterials form complexes with metals and humic acid in sample matrix which makes their extraction very difficult (Xuan et al., 2010 and Tso et al., 2011). This makes sample preparation step the key component in the analytical process. The overall aim of the analytical process is to remove interferent matrix and pre-concentrate the analyte so as to attain high and reproducible recoveries. Quite a number of extraction methods have been tried in literature. Carvalho et al., (2013) compared vortex agitation (VA), ultrasonic assisted solvent extraction (UASE) and microwave assisted extraction (MAE) in the extraction of tetracyclines from sludge and sediment samples. The recovery was 27% at most, which is far below the accepted level of 70 to 120% (Carvalho et al., 2013). In another study (Andreu et al., 2009) used pressurised liquid extraction (PLE) to extract tetracycline residues from the soil and recoveries in the range of 70 to 99% were achieved. As with conventional Soxhlet extraction (SE), the major drawback of PLE is thermal degradation at the elevated temperatures. (Jacobsen et al., 2004: Kay et al., 2005; Kim and Carlson, 2007 and Lalumera et al., 2004) employed super critical fluid extraction (SFE), however high and reproducible recoveries where not often attained. Although adjusting pH, adding complexing agents such as EDTA and oxalic acid to release the antibiotic by interacting with metal cations, and ultrasonication, improved the extraction efficiencies, the results were widely variable, 40 to 125% (Zhou et al., 2013; Simon, 2005). Extraction and preconcentration techniques based on solid phase extraction have also been employed. Interferences targeted include metal, humic acids and proteins. Solid phase extraction sorbents that have been tested include reversed phase C_{18}, hydrophilic lipophilic balance (HLB), poly (divenylbenzene-co-N-pyrrolidone) and Strata X (surface modified styrene divenylbenzene). Although recoveries did not improve significantly (Andreu et al., 2009), these sorbents successfully lowered matrix interferences. HLB, a polymeric sorbent proved to be the best such that it is now widely applied (Yang et al., 2013; Zhou et al., 2013). Tandem solid phase extraction (TSPE) has also been applied for the extraction of tetracyclines. Blackwell et al., (2004) employed ultrasonic assisted tandem solid phase extraction (UA-TSPE) involving Strong Anion Exchange (SAX) and HLB resins to extract oxytetracycline from soil and pig slurry after addition of EDTA and adjusting the pH to 4 using McIlvaine buffer, and reported recoveries of greater than 77%. SAX removed anionic interferences such as humic acid while tetracyclines, being neutral or cationic, would be retained by the HLB resin. Ultrasonication provides the extra energy required to dislodge the analyte from its matrix. Yang et al., (2010) used the method to extract tetracyclines in sediment from rivers and obtained recoveries in the range of 48.2 to 72,0 %. Zhou et al., (2013) extracted tetracyclines residues from river sediment using the same method and recoveries were in the range of 49.4 to 125%. Dispersive solid phase extraction (DSPE) and matrix solid phase dispersion (MSPD) are techniques that have been developed recently and have been found to be very versatile (Tsai et al., 2009). Recoveries greater than 97% were reported when tetracyclines in food samples were determined by these techniques (Oniszczuk et al., 2014). These methods have been found to be quick, easy, cheap, rugged and use less solvents. Previously recoveries above 91% were obtained with DSPE in the extraction of furanocoumarins from a plant sample (Vallejo Rodiguez et al., 2011). It was pointed out above that tetracyclines form complexes with metal cations and humic acids in the aquatic environment.

Thus the ease with which tetracyclines can be extracted from natural aquatic samples is determined to a large extent by the nature of the complex formed, in particular the strength of the bonds formed between the tetracycline molecule and the metal cation or humic acid functional groups. The situation is further complicated by the fact that tetracyclines are heat labile, so that extraction techniques involving heat, such as Soxhlet and microwave based techniques, cannot be employed efficiently.

Thus unless the extraction process provides adequate energy to break these bonds, low and variable recoveries will be obtained depending on the nature of sample matrix. Ultrasonication provides the required energy without subjecting the sample to heat. From the brief review above, solid phase extraction techniques give the best results, especially when combined with ultrasonication, although the results are variable. The aim of the present study was to compare the effect of coupling ultrasonication to TSPE, DSPE and MSPE for the extraction of tetracyclines from river water and sediment comprising of tetracycline, oxytetracycline, chlortetracycline and doxycycline.

MATERIALS AND METHODS

Chemicals and reagents

Oxytetracycline hydrochloride standard (95%), tetracycline hydrochloride standard (98%), chlortetracycline hydrochloride standard (95%), HPLC grade methanol and acetonitrile, Strong anion exchange (SAX) cartridges (3 ml, 500 mg), and hydrophilic-lipophilic balance (HLB) cartridges (6 ml, 200 mg) and nylon disposable filter units (MILLPORE 0.45 µm) Doxycycline hyclate 99% were obtained from Sigma Aldrich,. (Darmstadt, Germany). Analytical grade orthophosphoric acid, nitric acid, sodium hydrogen phosphate, citric acid and disodium ethylenediamine tetraacetate (Na_2EDTA) were of obtained from SKYLABS (Johannesburg, South Africa). Primary and secondary amine sorbent material (57738-U-SUPELCO supelclean PSA) was obtained from Sigma Aldrich.

Cleaning of apparatus

Glass apparatus were soaked in 4M nitric acid for 24 h and then washed with a detergent, rinsed with double distilled water, before heating in a drying oven for four hours (Zhou et al., 2013).

Sample collection

Water and sediment samples were collected from the same point/location in Wayerera River near Bindura University of Science Education, Zimbabwe (19°19' 52" South, 42°21' 52" East). Water samples were collected using two liter amber glass bottles, while sediment samples were collected using a stainless steel scooper. All the samples were placed in a cooler box and transported straight to the laboratory, where they were stored in a refrigerator at 0 to 5°C until required for analysis.

Sample preparation (water samples)

2 L each of ultrapure water and river water sample were spiked with 0.05, 0.5 and 1 $\mu g ml^{-1}$ concentrations of antibiotic dissolved in methanol stock solution. The samples were vortexed and centrifuged for 1 min at 3000 rpm to separate solid particles from the liquid phase. The liquid phase was then decanted and filtered through 0.45 µm glass Millipore filters.

Ultrasonic Assisted Tandem Solid Phase Extraction (TSPE)

UA-TSPE was performed following a previous method reported by Zhou et al., (2013) with slight modifications. Strong anion exchange (SAX) cartridges 3 ml (500 mg) and hydrophilic-lipophilic balance (HLB) cartridges 6 ml (200 mg) were set up in tandem. To the sample, 5 ml of 0.1 M Na_2EDTA, and 10 ml of McIlvaine buffer (pH 4) were added and the mixture ultrasonicated for 15 min at 30°C and centrifuged at 3000 rpm for 10 min. Aqueous EDTA was added to chelate any metals present and release the antibiotic. Preconditioning of each cartridge was done with 10 ml of methanol followed by 10 ml of ultrapure water. The supernatants were then passed through the cartridges at a flow rate of 5 ml min^{-1} using a SUPELCO vacuum manifold system connected to a vacuum pump. SAX cartridges were then removed. HLB cartridges were rinsed with 10 ml of ultrapure water to remove weakly bonded impurities and Na_2EDTA and then dried under vacuum for 2 h. Elution of antibiotics was done with 10 ml of methanol. The methanol eluate was evaporated under vacuum using a Buchi rotary evaporator to almost dryness and then redissolved in 500 µl of methanol. After filtration through 0.22 µm glass Millipore filters to remove any particulate matter, the extract was placed into screwed bottles and stored in a fridge until HPLC-UV analysis.

Ultrasonic Assisted Dispersive Solid Phase Extraction (DSPE)

Water samples, 1 L each were vigorously shaken with 10 ml of acetonitrile in a separating funnel. 5 ml of 0.1 M Na_2EDTA, and 10 ml of McIlvaine buffer (pH 4) were also added to chelate any metals present. Magnesium sulphate and sodium chloride 0.5 g each was then added to displace the extraction equilibrium towards the organic phase. The contents were centrifugation at 3000 rpm for 10 min and the organic supernatants were transferred to a conical flask followed by addition of 40 mg of primary and secondary amine sorbent material (57738-U-SUPELCO supelclean PSA) to remove interferences such as humic acid. The analyte of interest remained in the organic phase. The mixture was ultrasonicated for 15 min at 30°C and centrifuged at 3000 rpm for 10 min. The supernatants were collected and evaporated to almost dryness under vacuum and then redissolved in 500 µl of methanol. The contents were filtered through a 0.22 µm glass Millipore filters to remove any particulate matter and then placed into amber vials and stored in a fridge until HPLC analysis.

Ultrasonic Assisted Matrix Solid Phase Dispersion (MSPD)

Water samples, 1 L each were vigorously shaken with 10ml of acetonitrile in a separating funnel. Five milliliters of 0.1 M Na_2EDTA, and 10 ml of McIlvaine buffer (pH 4) were also added. Magnesium sulphate and sodium chloride 0.5 g each were added to facilitate phase` s separation. The mixture was centrifuged at 3000 rpm for 10 min and the organic supernatant was transferred to a conical flask and 40 mg of hydrophilic-lipophilic balance (HLB) sorbent was added to trap the analyte on the sorbent leaving interferences in the organic phase. The mixture was ultrasonicated for 15 min and centrifuged at 3000 rpm for 10 min. The solid layer was collected and packed in a 6 ml polypropylene syringe barrel. Packed polypropylene syringe barrels were washed with ultrapure water and vacuum dried for 2 h. Elution of antibiotics was achieved by adding 12 ml of methanol. The methanol eluate was evaporated to almost dryness under vacuum and then the contents redissolved in 500 µl of methanol. The solutions were filtered through 0.22 µm glass Millipore filters and then placed into amber vials, and stored in a fridge until HPLC analysis.

Sample preparation (sediment sample)

2 g dried sediment samples were placed into three separate glass tubes, followed by addition of 1 ml of each standard stock solution (0.05, 0.5 and 1 $\mu g g^{-1}$). The contents were mixed by centrifugation and placed in a refrigerator overnight (Zhou et al., 2013). 10 ml of McIlvaine buffer (pH 4) was added into each glass tube and mixed for 1 min. All glass tubes were then centrifuged at 3000 rpm for 10 min. The supernatants from each tube were placed into 250 ml flasks. The extraction process was repeated twice and the supernatants from the two extractions were combined. The solutions were diluted to 100 ml with ultrapure water. After filtration through 0.45 µm Millipore filters, clean-up and pre-concentration was carried out as described for river/ultrapure water samples.

Blank samples without added antibiotics were also analyzed to

Table 1. Method linearity.

Antibiotic	UA-TPSE		UA-DSPE		UA-MSPD	
	LDR (µgml^{-1})	R^2	LDR (µgml^{-1})	R^2	LDR (µgml^{-1})	R^2
TC	0.01-1.00	0.998	0.01-1.00	0.999	0.01-1.00	0.995
OTC	0.01-1.00	0.995	0.01-1.00	0.997	0.01-1.00	0.999
CTC	0.01-1.00	0.996	0.01-1.00	0.999	0.01-1.00	0.996
DC	0.01-1.00	0.999	0.01-1.00	0.998	0.01-1.00	0.998

LDR = linear dynamic range, R^2 = coefficient of variation, UA = ultrasonic assisted, TPSE = tandem solid phase extraction, DSPE = dispersive solid phase extraction, MSPD = matrix solid phase dispersion.

determine initial levels of antibiotics.

HPLC analysis

Analysis for antibiotics was performed on a Varian HPLC UV prostar 325 equipped with a Rodyne manual injector with a 20 ml loop and a UV detector, prostar 325. The detector was controlled remotely by the Varian Star/Galaxie Chromatography Workstation software version 6. All the analytes were separated using a HPLC Varian Microsorb MV 1005 packed C18 columns 250 × 4.6 mm id, 5 µm particle size, 100 Å SPELCO. The separation mode used was isocratic. The mobile phase consisted of 240 ml HPLC grade acetonitrile and 760 ml of 0.02 mol dm^{-3} orthophosphoric acid pH 3. Fresh solutions were prepared, filtered and degassed for every analysis. Column conditions were room temperature, flow rate 1 ml/min and injection volume was 10 µl. The detection wavelength was 360 nm which was determined by scanning on a Thermofisher UV spectrophotometer GENESYS 10S UV-Vis v4.003 2L9Q129001. Quantitation was based on peak area. The calibration method was used for quantification.

Method validation parameters

Linear dynamic range

The linearity of the methods was checked by analyzing eight solutions in the range 0.01 to 2 µg ml^{-1}. Each concentration was analyzed in triplicate. Calibration curves were generated by plotting the analyte peak area against concentration of standard. Table 1 shows the results obtained.

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ are terms used to describe the smallest concentration of an analyte that can be reliably measured by an analytical procedure. LOD is taken as the lowest concentration of an analyte in a sample that can be detected, but not necessarily quantified under the stated conditions of the test, and is given by Shrivastava and Gupta, (2011):

$$LOD = 3\ s/S \quad (1)$$

where s is the standard deviation of y-residuals and S is the slope of the calibration curve.

The LOQ is the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated conditions of test, and is given by Shrivastava and Gupta, (2011):

$$LOQ = 10\ s/S \quad (2)$$

The results obtained are shown in Tables 2 and 3.

Precision and specificity

This was evaluated by analyzing the samples three times and calculating the relative standard deviations which were then tested for difference by applying one way ANOVA analysis p = 0.05. Specificity was assayed with endogenous interferences by extracting and analyzing blank river water and sediment from ten different sources.

RESULTS AND DISCUSSION

Method validation parameters

Linear dynamic range

Calibration curves for each method generated by plotting analyte peak area versus concentration were linear in the range 0.01 to 1 µgml^{-1} (water) and 0.01 to 1 µg g^{-1}. Linear regression coefficients (R^2) are in the range 0.995 to 0.999, see Table 1. All R^2 values are above 0.995 showing strong linearity.

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ obtained for the three extraction techniques are shown in Tables 2 and 3. LOD of the spiked river water and sediment was in the range 11.53 to 22.75 ngml^{-1}, while LOQ was in the range of 30.12 to 56.22 ngml^{-1}. All the three techniques gave almost similar results that compare very well in terms of linear dynamic range and detection limits with data reported for previous studies using various techniques which include ethyl

Table 2. Method limit of detection (LOD) (ng ml^{-1}) and limit of quantification (LOQ) (ng ml^{-1}) in spiked river water (n = 3).

Compound	LOD			LOQ		
	TPSE	DSPE	MSPE	TPSE	DSPE	MSPE
TC	22.75	11.60	11.97	55.43	36.00	56.22
OTC	20.34	11.55	11.80	93.12	35.05	35.80
DC	11.58	11.53	15.80	35.00	35.10	35.06
TC	21.10	11.66	18.30	35.55	37.20	51.92

LDR = linear dynamic range, R^2 = coefficient of variation, UA = ultrasonic assisted, TPSE = tandem solid phase extraction, DSPE = dispersive solid phase extraction, MSPD = matrix solid phase dispersion.

Table 3. Method limit of detection (LOD) (ngml^{-1}) and limit of quantification (LOQ) (ngml^{-1}) in spiked river sediment (n = 3).

Compound	LOD			LOQ		
	TPSE	DSPE	MSPE	TPSE	DSPE	MSPE
TC	18.85	12.70	20.78	35.00	56.10	53.40
OTC	20.00	12.93	21.33	30.72	45.94	45.25
DC	11.82	11.60	21.00	37.00	55.74	55.16
TC	16.52	12.10	19.70	51.56	36.10	41.90

LDR = linear dynamic range, R^2 = coefficient of variation, UA = ultrasonic assisted, TPSE = tandem solid phase extraction, DSPE = dispersive solid phase extraction, MSPD = matrix solid phase dispersion.

acetate-ionic liquid dispersive liquid-liquid micro extraction high performance liquid chromatography coupled to variable wavelength UV detector (EA-IL-DLLME-HPLC-UV) (Dongli et al., 2014) Aluminium hydroxide co-precipitation coupled to high performance liquid chromatography with UV detection (AH-C-HPLC-UV) (Yang et al., 2013), dispersive solid phase micro-extraction coupled to high performance liquid chromatography-diode-array detection (DSPM-HPLC-DAD) (Tsai et al., 2009), online solid phase extraction coupled to high performance liquid chromatography with photodiode array detection (on line HPLC-PAD) (Oniszczuk et al., 2014) see Table 4.

Precision and specificity

Precision as a parameter for quality was estimated by calculating relative standard deviation for 3 replicate samples. Computed relative standard deviations obtained in the present study for all techniques are all within the precise range 1.89 to 11. Specificity was assayed with endogenous interferences by extracting and analyzing blank river water and sediment from ten different sources. Chromatograms recorded for all the methods were free of interfering peaks both in the spiked and blank samples. Peak purity assessed by the Varian Star or Galaxie Chromatography Workstation software version 6 revealed that all peaks purity levels were equal to or greater than 99%. The retention time for oxytetracycline, tetracycline, chlortetracycline and doxycycline were around 2.4 ± 0.5, 2.8 ± 0.1, 3.3 ± 0.7 and 7.6 ± 0.4 minutes respectively. Absence of baseline shift revealed negligible absorption of humic acid. The efficiency of the extraction methods in minimizing humic acid absorption was determined by comparing results from the analysis of blank river water with and without applying solid phase extraction. Chromatograms obtained for the two analyses are shown in Figure 1. Figure 1a shows a continuum implying absorption from organic matter. This continuum disappeared, Figure 1b when solid phase extraction was applied. Solvent change over and use of matrix trapping sorbents (primary and secondary amine) and SAX was responsible for removing humic acid therefore removing to greater extent background absorption. Primary and secondary amine sorbent material was also found to be effective in removing matrix interferences and enhancement (Zhen et al., 2011).

Percentage recoveries

The objectives of SPE are the removal of interfering matrix components, improve recoveries and detection limits. Percentage recoveries of the three solid phase extraction techniques at three different spiking

Table 4. Comparison with previous methods for the determination of tetracyclines.

Method	Antibiotic	Linear dynamic range/ngml^{-1}	LOD (ngml^{-1})	Sample matrix	References
DSPM-HPLC	Tetracycline, doxycycline	2-50	0.7-3.2	Water	(Tsai et al., 2009)
On-line-SPE-HPLC	Tetracycline, chlortetracycline	5-1000	1.5-8.0	Water	(Zhenzhen et al., 2013)
EA-IL-DLLME-HPLC	Tetracycline, doxycycline, chlortetracycline, methacycline	10-500	0.46-0.97	Deionized water	(Dongli et al., 2014)
AH-C-HPLC	Oxytetracycline, Tetracycline, chlortetracycline	5-50	81.7-115	Water	(Yang et al., 2013)
UA-TSPE-HPLC	Oxytetracycline, Tetracycline, doxycycline, chlortetracycline	10-1000	11.58-22.75	River water	This study
UA-TSPE-HPLC	Oxytetracycline, Tetracycline, doxycycline, chlortetracycline	10-1000	11.82-20.00	River sediment	This study
UA-DSPE-HPLC	Oxytetracycline, Tetracycline, doxycycline, chlortetracycline	10-1000	11.53-11.66	River water	This study
UA-DSPE-HPLC	Oxytetracycline, Tetracycline, doxycycline, chlortetracycline	10-1000	11.60-12.93	River sediment	This study
UA-MSPD-HPLC	Oxytetracycline, Tetracycline, doxycycline, chlortetracycline	10-1000	11.80-18.30	River water	This study
UA-MSPD-HPLC	Oxytetracycline, Tetracycline, doxycycline, chlortetracycline	10-1000	19.70-21.33	River sediment	This study

Table 5. Extraction recoveries $\bar{x} \pm RSD$n = 3 of antibiotics from 2 L ultrapure water.

Spiked concentration (µg mL^{-1})	Method	Percentage recoveries for			
		TC	OTC	CTC	DC
0.05	UA-TSPE	95.31 ± 4.56	96.81 ± 8.64	96.22 ± 6.22	94.99 ± 11.47
	UA-DSPE	97.88 ± 5.77	98.91 ± 3.10	97.33 ± 3.39	98.05 ± 9.01
	UA-MSPD	96.33 ± 9.92	96.94 ± 3.80	95.90 ± 6.72	97.09 ± 11.90
0.50	UA-TSPE	96.03 ± 5.77	96.21 ± 6.13	96.89 ± 7.08	96.09 ± 5.90
	UA-DSPE	98.01 ± 3.11	99.62 ± 6.15	98.12 ± 7.21	97.96 ± 5.13
	UA-MSPD	96.56 ± 8.97	96.23 ± 8.64	96.07 ± 3.27	96.33 ± 8.96
1.00	UA-TSPE	95,97 ± 9.97	97.13 ± 8.61	96.34 ± 3.54	95.33 ± 6.66
	UA-DSPE	98.09 ± 7.21	98.85 ± 6.13	97.89 ± 8.17	98.23 ± 6.67
	UA-MSPD	97.01 ± 5.57	97.84 ± 9.11	96.12 ± 5.98	96.33 ± 7.71

RSD = relative standard deviation, UA = ultrasonic assisted, TPSE = tandem solid phase extraction, DSPE = dispersive solid phase extraction, MSPD = matrix solid phase dispersion.

concentrations, 0.01, 0.5 and 1 µgml^{-1} are shown in Tables 5 to 7. All the three extraction/preconcentration techniques yielded higher recoveries, in the range 92.13 to 99.62%. These results are in agreement with or are higher than those previously reported for river sediment and river water samples. For instance Zhou et al., (2013) report recoveries in the range of 49.4 to 125. In another study by Lindsey et al., (2001) the mean recovery was 98± 12%. Jia et al. (2009) achieved recoveries within the range 64 to 113%. In a study conducted by Zhu et al., (2001) recovery from fortified blanks ranged from 86 to 110%. Zhenzhen et al., (2013) reported recoveries in the range of 81.70 to 96.45% when they extracted tetracyclines from water using aluminium hydroxide coprecipitation coupled to high performance chromatography. Dongli et al. (2014) used ethyl acetate ionic liquid dispersive liquid-liquid micro-extraction to extract tetracyclines from tape, lake and spring water and

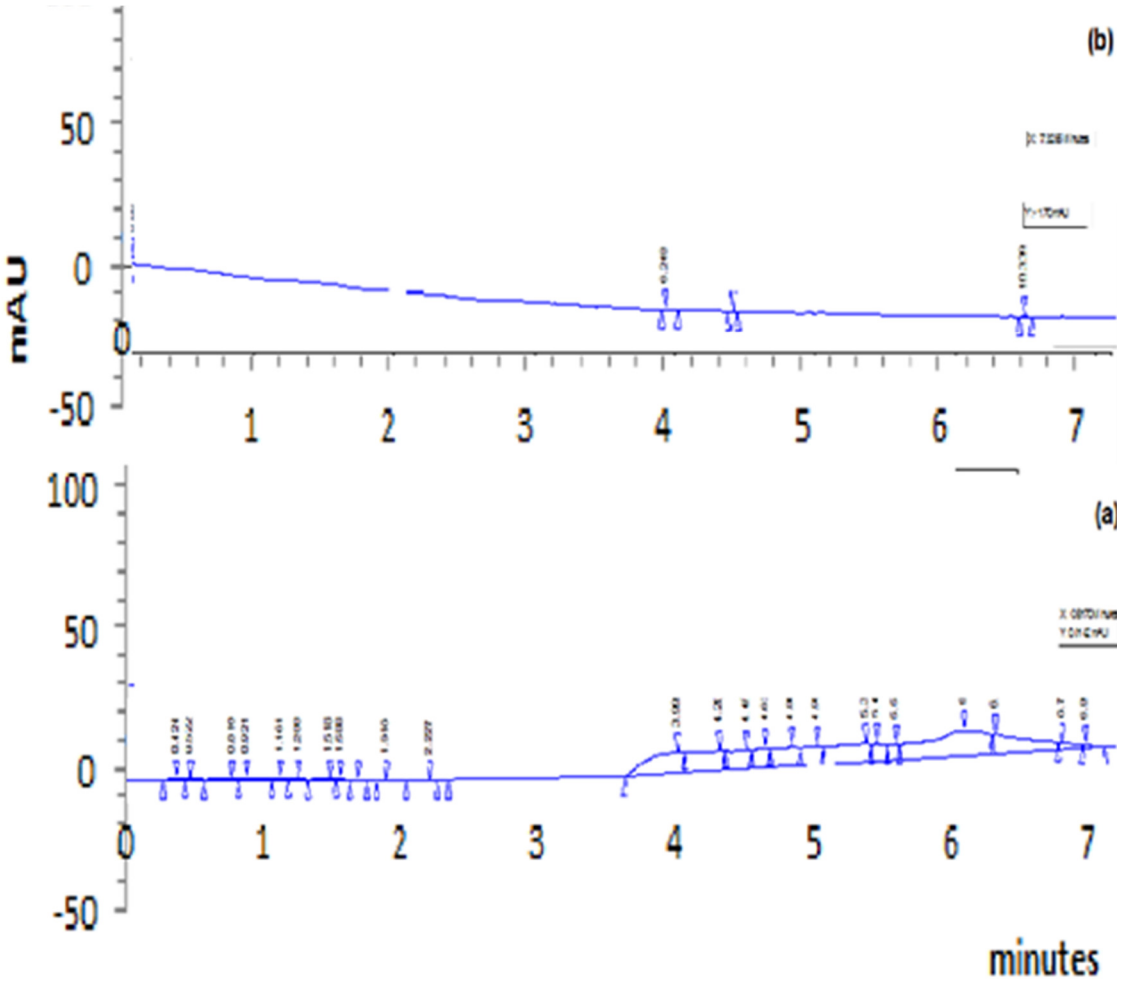

Figure 1. Chromatograms for the analysis of blank sediment samples (a) without employing solid phase extraction (b) by employing ultrasonic dispersive solid phase extraction.

Table 6. Extraction recoveries $\bar{x} \pm RSD$, n =3 of antibiotics from 2 L river water sample.

Spiked concentration (µg mL^{-1})	Method	Percentage recoveries for			
		TC	OTC	CTC	DC
0.05	UA-TSPE	96.22 ± 8.89	97.04 ± 5.44	97.75 ± 9.17	97.13 ± 10.00
	UA-DSPE	98.00 ± 8.84	98.42 ± 5.64	97.98 ± 6.77	98.22 ± 7.15
	UA-MSPD	96.22 ± 3.77	96.40 ± 6.33	97.07 ± 6.67	97.03 ± 4.67
0.50	UA-TSPE	97.11 ± 7.45	97.14 ± 6.12	97.13 ± 11.23	97.62 ± 4.72
	UA-DSPE	98.22 ± 5.57	98.96 ± 6.13	98.07 ± 6.12	98.44 ± 8.36
	UA-MSPD	96.81 ± 5.72	97.30 ± 5.66	96.89 ± 6.92	96.86 ± 7.71
1.00	UA-TSPE	96.98 ± 6.15	97.50 ± 5.44	97.05 ± 8.90	97.55 ± 7.71
	UA-DSPE	98.11 ± 3.45	98.85 ± 6.80	98.01 ± 10.13	98.19 ± 8.99
	UA-MSPD	96.39 ± 5.77	97.39 ± 11.31	97.22 ± 3.79	96.04 ± 3.07

RSD = relative standard deviation, UA = ultrasonic assisted, TPSE = tandem solid phase extraction, DSPE = dispersive solid phase extraction, MSPD = matrix solid phase dispersion.

Table 7. Extraction recoveries $\bar{x} \pm RSD$, n =3 of antibiotics from 2 g river sediment.

Spiked concentration (μgg^{-1})	Method	Percentage recoveries for			
		TC	OTC	CTC	DC
0.05	UA-TSPE	96.23 ± 5.18	96.22 ± 9.64	96.39 ± 4.19	97.12 ± 5.75
	UA-DSPE	97.98 ± 6.65	96.39 ± 4.19	98.11 ± 9.81	98.14 ± 11.01
	UA-MSPD	95.89 ± 7.99	96.08 ± 7.86	95.94 ± 8.84	96.13 ± 3.99
0.50	UA-TSPE	97.03 ± 5.55	95.89 ± 11.20	96.89 ± 11.90	96.33 ± 5.66
	UA-DSPE	98.12 ± 2.11	98.05 ± 7.75	98.24 ± 6.77	98.13 ± 7.71
	UA-MSPD	96.97 ± 9.97	97.12 ± 3.37	92.13 ± 1.89	96.88 ± 4.49
1.00	UA-TSPE	96.81 ± 8.88	96.37 ± 6.64	96.85 ± 7.72	96.82 ± 8.99
	UA-DSPE	98.10 ± 3.77	97.97 ± 5.44	98.07 ± 11.22	98.19 ± 3.22
	UA-MSPD	97.02 ± 10.97	96.98 ± 7.97	93.99 ± 6.68	96.70 ± 8.29

RSD = relative standard deviation, UA = ultrasonic assisted, TPSE = tandem solid phase extraction, DSPE = dispersive solid phase extraction, MSPD = matrix solid phase dispersion.

obtained recoveries in the range 62.6 to 109.6%. In this study manual shaking was observed to increase extraction efficiency. Recoveries obtained in the present study are highly reproducible unlike values for similar studies reported previously (Hektoen et al., 1995; O'Connor and Aga, 2007 and Zhou et al., 2013).

Differences in extraction recoveries could be as a result of different solvents and dispersing sorbents employed. Comparing the three methods UA-DSPE significantly gave the best recoveries, for almost all the antibiotics in both ultrapure, river water and sediment based on ANOVA and Least significant difference (LSD) tests ($p > 0.05$). The recovery ranged from 97.13 to 99.62 while that for UA-TSPE ranged from 94.99 to 97.75. Percentage recovery for UA-MSPD ranged from 92.13 to 97.84. Possible reasons why Dispersive Solid Phase Extraction yielded the best results are that it involved the use of primary secondary amine as the dispersing sorbent. Shaking ensured maximum contact with matrix such as humic acid and metals which may complex the antibiotic and reduce extraction efficiency. In addition primary and secondary amine sorbent has an excellent retention power for anionic compounds such as humic acid. The results also show that the recovery was independent of spiking concentration for all the three methods. Percentage recoveries for the real samples (river water and sediment) compare very well with those of ultra-pure water (Tables 5 to 7), further substantiating the robustness of the methods to reduce matrix interference in the extraction of the antibiotics.

Conclusion

Results of the present study illustrate that the three techniques are comparable in terms of matrix effects reduction and detection limits. Percentage recoveries for all the techniques were above 90%. However dispersive solid phase extraction exhibited better extraction efficiency. The results also reveal that removing matrixes such as humic acid and metals and solvent change over makes it possible to obtain high and reproducible recoveries.

Conflict of Interest

The authors have not declared any conflict of interest.

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