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Determination of 76 pharmaceutical drugs by liquid chromatography–tandem mass spectrometry in slaughterhouse wastewater

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ABSTRACT

A multi-residue method for the analysis of 76 pharmaceutical agents of nine classes of drugs (tetracyclines, macrolides, fluoroquinolones, β-agonists, β-blockers, diuretics, sedatives, sulfonamides and chloramphenicol) in slaughterhouse wastewater and a receiving river is presented. After simultaneous extraction with an Oasis HLB solid-phase extraction (SPE) cartridge and further purification using an amino SPE cartridge, analytes were detected by liquid chromatography–electrospray ionization-tandem mass spectrometry in positive or negative ion mode. Standard addition was used for quantification to overcome unavoidable matrix effects during ESI-MS analysis. Recoveries for most analytes based on matrix-matched calibration in different test matrices were >60%. The method quantification limits of 76 pharmaceuticals were in the range 0.2–30 ng/L. Nineteen compounds of 76 drugs were found in raw and treated slaughterhouse wastewater from four main slaughterhouses in Beijing. Sulfanamides (sulfanilamide, sulfameter), fluoroquenones (ofloxacin, pefloxacin, norfloxacin, ciprofloxacin, enrofloxacin), tetracyclines (tetracycline, oxytetracycline) and macrolides (kitasamycin, tylosin, erythromycin) were most frequently detected, with the highest levels up to ~3 μg/L in slaughterhouse wastewater and ~1 μg/L in treated wastewater. Illicit drugs for animal feeding such as clenbuterol and diazepam were commonly detected in slaughterhouse wastewater. These analytes were also observed in a river receiving slaughterhouse wastewater, with a highest level of up to 0.2 μg/L.

1. Introduction

In the last decade, pharmaceuticals and personal care products (PPCPs) have been increasingly concerned for their pseudo-persistent properties, and their potential harm to humans and the environment [1–4]. By the end of 2007, >100 PPCPs were identified in environmental samples and drinking water [5,6]. Sources of pharmaceuticals in the environment are mainly due to hospitals, households, pharmaceutical industries, disposal of expired or unused medicine, aquiculture and animal feeding. In China, the annual output of antibiotics was estimated to be about 210,000 tons in 2005, and about 90,000 tons are used as animal feed additives or for therapeutic purposes [7].

There are many articles on pharmaceuticals in wastewaters from hospitals [8,9], domestic sewage treatment plants (STPs) [10], farms [11–13], and surface water [14–19]. Tetracyclines and sulfanamides in pig wastewater in Beijing have been detected at concentrations up to 33 μg/L [13]. Relatively high concentrations of sulfamethazine (18.5–19.2 μg/L) were detected in pig farm wastewaters in Vietnam by Managaki et al. [20]. Hu et al. reported tetracyclines and sulfonamides at levels up to 173 mg/kg in manure [21]. These values are significantly higher than those observed in sewage and surface water, indicating the high usage of pharmaceuticals in animal husbandry.

Modern aquaculture (including intensive breeding and intensive slaughtering) makes it possible to increase meat production and reduce cost. It is also associated with pharmaceutical use and environmental concerns. The contribution of slaughtering activities to pharmaceuticals levels in the environment have not been thoroughly investigated. There are 14 large slaughterhouse for pigs and more than 50 slaughterhouses for poultry, cows and lambs in Beijing. These are usually located in the outer suburbs, far from STPs. Most of these slaughterhouses produce wastewater containing pharmaceuticals, that are discharged directly into a nearby river, after simple disposal which may greatly influence the local environment. It is therefore important to develop a comprehensive analytical method for investigation of the occurrence of typical pollutants (e.g., pharmaceuticals) in slaughterhouse wastewater.

As for the analysis of pharmaceuticals in environment, recently published articles presented good overviews [22–25].

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Multi-residue analytical methods have been developed for the determination of various classes of pharmaceuticals in wastewater from hospitals and municipal sewage systems; as well as rivers, lakes, seas, soils, sediments and drinking water. Based on an isotopic dilution mass spectrometric technique, Hummel et al. developed a multi-residue method for the determination of 20 psychoactive drugs and their metabolites in wastewater and surface water [15]. Hao et al. reported a liquid chromatography–tandem mass spectroscopy (LC–MS/MS) method for simultaneous determination of 38 pharmaceutically active drugs, 10 agents that disrupt the endocrine system, and three perfluoroalkylated compounds in drinking water, surface water and wastewater [26]. Rice and Mitra presented microwave-assisted solvent extraction of solid matrices followed by gas chromatography–mass spectrometry to detect eight PPCPs [27]. More recently, Grujić et al. reported an analytical method for determination and reliable confirmation of 19 pharmaceuticals from different therapeutic classes in surface and groundwaters at ng/L levels [19]. Considering of the multi-class of veterinary drugs usage and the potentially illegal use of other pharmaceuticals in intensive animal breeding, it is necessary to develop a more comprehensive method for drugs determination in slaughterhouse wastewater. Moreover, slaughterhouse wastewater has a high organic composition in contrast with domestic wastewater. Analyses of pharmaceuticals in slaughterhouse wastewater is therefore challenging.

We aimed to develop a multi-class and multi-residue method for the simultaneous extraction and purification of 76 pharmaceuticals from various therapeutic classes in slaughterhouse wastewater. The analytes were antibiotics (tetracyclines, sulfanamides, macrolides, fluoroquinolone and chloramphenicol), β-agonists, psychopathic drugs, β-blockers and diuretics. Sample preparation (including the pH value of sample loading; additives; solid-phase extraction (SPE) cartridges) was optimized to enhance recoveries and reduce suppression of signals. Detection was conducted by two runs of liquid chromatography–electrospray ionization-tandem mass spectrometry (LC–ESI-MS/MS) in positive or negative mode. One month of continuous monitoring of drugs in the wastewater of the main slaughterhouses in Beijing and the water from one receiving river was carried out to evaluate the contribution of slaughterhouse activities on the environment. Measurement of agents in slaughterhouse wastewater can also reflect the drugs used in animal husbandry and the quality of meat.

2. Experimental

2.1. Chemicals

Oxytetracycline, tetracycline, demeclocycline, methacycline, minocycline, chlortetracycline, enrofloxacin, norfloxacin, pefloxacin, ciprofloxacin, oflaxacin, sarafloxacin, enoxacin, lomefloxacin, pipemidic acid, nalidixic acid, oxolinic acid, flumequine, cinoxacin, fleroxacin, danofloxacin, difloxacin, orbifloxacin, marbofloxacin, and sparfloxacin were purchased from Sigma (St. Louis, MO, USA). Tilmicosin, erythromycin, tylosin, medecamycin, kitasamycin, droperidol, haloperidol, nitrazepam, aceproamzine, estazolam, oxazepam, sulfisomidin, sulfamerazin, sulfameter, sulfamoxol, sulfamethoxypridazine, sulfachloropyridazine, sulfadimethoxin, sulfaquinoxaline, sulfadimidin, sulfisoxazole, sulfanilamide, terbutaline, salbutamol, cimaterol, fenoterol, clencyclohexerol, clenbuterol, tulobuterol, mabuterol, clenpenterol, mapenterol, clenproperol, ractopamine, metoprolol, atenolol, sotalol, carazolol, celiprolol, bisoprolol, oxprenolol, alprenolol, propranolol, betaxolol, amiloride, triamterene, hydrochlorothiazide, hydroflumethiazide, methyclothiazide, trichlormethiazide, and chloramphenicol were purchased from Dr. Ehrenstorfer (Augsburg, Germany). All these chemicals were analytical grade ≥97% purity.

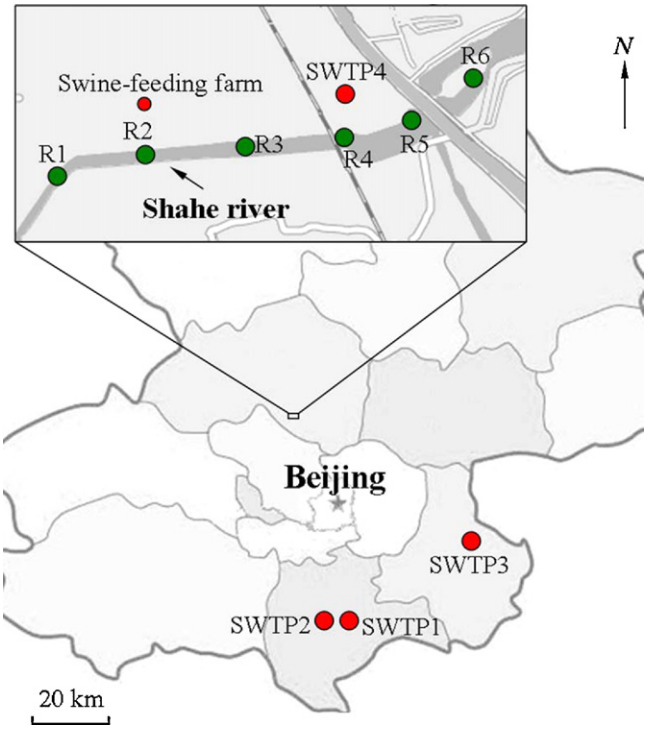

Fig. 1. Location of 4 SWTPs and sampling site of receiving river.

All solvents used in sample preparation and chromatographic separation were of high-performance liquid chromatography (HPLC) grade. Methanol, acetonitrile and acetone were supplied by Fisher Scientific (Fair Lawn, NJ, USA). Formic acid (HCOOH, 99% purity) was from Acros Organics (Morris Plains, NJ, USA). Ultra-pure water was obtained by using an in-house Milli-Q® Ultra-pure water system (Millipore, Bedford, MA, USA). Ethylenediamine tetra-acetic acid disodium salt (Na_2EDTA) was from Beijing Chemical Company (Beijing, China).

About 10 mg of individual standard (corrected by purity) was accurately weighed and placed in a 10-mL volumetric flask. Quinolones were first dissolved in 50 μL of formic acid and then diluted to 10 mL with methanol. Other agents were dissolved in 10 mL of methanol. Stock solutions were stored at −20 °C. From these stock solutions, working solutions were prepared by gradient dilution.

2.2. Sample collections

Samples were collected from four main slaughterhouse wastewater treatment plants (SWTPs): two in the Daxing district (SWTP1, SWTP2), one in the Tongzhou District (SWTP3) and the other in the Changping District (SWTP4) of Beijing. One river received effluent from SWTP4. SWTP3 was an anaerobic–anoxic–oxic (A/A/O) process and the other plants were anaerobic–oxic (A/O) processes. Samples of the influents and effluents were collected every four days during a one-month period (30 July–29 August 2008; and one single sampling occurred in 13 November 2008). We also collected water samples from the Shahe River, which received effluent from SWTP4 (R4) and a pig-feeding farm (R2) on 29 August and 13 November 2008. The sampling sites along the Shahe River were 1.5 km (R1), 0.5 km (R3) upstream, and 0.5 km (R5), 1.0 km (R6) downstream, from the discharge point of SWTP4 (R4). Sampling sites are shown in Fig. 1.

2.3. SPE

Samples were collected in glass bottles and pretreated within 12 h. After filtered through glass fiber filters (GF/A, 1.6 μm; Whatman, Maidstone, Kent, UK), water samples (500 mL; influent, effluent, river water) were used for preparation. One gram of Na_2EDTA was added and homogenized. The following four cartridges were used in screening for concentration purpose: an Oasis HLB SPE cartridge (200 mg, 6 mL, Waters, Milford, MA, USA), an Sep-Pak C18 cartridge (500 mg, 6 mL; Waters), an ENVI-Carb graphite carbon black (GCB) cartridge (500 mg, 6 mL; Supelco, Bellefonte, PA, USA) and an Bond Elut Plexa cartridge(200 mg, 6 mL; Varian, CA, USA). The experiment was performed to evaluate extraction efficiencies by spiking 40 ng/L of analytes into 500 mL pure water. The condition solution was 6 mL methanol and 6 mL water except for GCB cartride, for which 6 mL dichloromethane/methanol (70/30, v/v), 6 mL methanol, and 6 mL water was used. Flow rate of sample loading was 5–10 mL/min. As for the eluting solution, 6 mL dichloromethane/methanol (70/30, v/v) was applied for GCB cartridge and 6 mL methanol used for the other three.

2.4. LC–MS/MS analysis

The LC–MS/MS analysis was done on a Waters Acquity Ultra Performance LC™ system (Waters, Milford, MA, USA) coupled with a Micromass Quattro Ultima Pt mass spectrometer (Waters, Manchester, UK) equipped with an electrospray ionization (ESI) interface in multiple-reaction monitoring (MRM) mode. Nitrogen (purity, 99.9%) was the desolvation gas. LC and MS parameters are summarized in Table 1. Detailed parameters for MRM acquisition are presented in Table S1. Two transitions were selected for identification but only one was used for quantification. LC–MS/MS chromatograms for 76 drugs are presented in Fig. 2.

2.5. Method validation

The standard addition method was carried out for sample quantification using LC–MS/MS in MRM mode [28]. Linearity in the response was studied using matrix-matched calibration solutions prepared by spiking wastewater extracts at six concentrations, ranging from the quantification limit of each analyte to 100 μg/L in the final extract. Each point was obtained as the mean of three injections. Integrated peak area data of the selected quantification MRM transitions were used to construct matrix-matched calibration curves, which were used for quantitative determinations.

Recovery, accuracy and precision were determined by analyzing raw samples and treated wastewater samples spiked at three concentrations in replicates of six. Precision was expressed as percentage relative standard deviation (%RSD) and determined for every compound from six replicates of spiked raw and treated wastewater samples. The recovery for each compound was assessed by comparing the integrated peak areas for six replicates of an extracted spiked sample to the calibration counterparts from the matrix-matched calibration curves representing 100% recovery. If one compound initially existed in the wastewater samples (e.g. ofloxacin), spiked concentrations would be relatively high and background-subtracted peak areas was used to calculate the recovery.

Expanded uncertainty, including U_c (corrected for recovery factor) and U_{nc} (not corrected for recovery factor), at different spiking levels was calculated according to the published literatures [29–31]. Here, uncertainty from several sources is considered: (1) uncertainty derived from the standard preparation, (2) uncertainty from the amount of wastewater samples and spiked standards, (3)

Table 1
Instrument conditions for target compounds analysis.

Ionization mode		ES+						ES−					
LC condition	Mobile phase	A: water containing 0.1% (v/v) formic acid B: methanol						A: water B: acetronitril					
	Gradient list	Time (min)	0	3	8	12	15	16	16.5	Time (min)	0	2	3.9	4	5.5	5.6
		A(%)	95	80	70	65	30	0	95	A(%)	95	55	45	0	0	95
		B(%)	5	20	30	35	70	100	5	B(%)	5	45	55	100	100	5
	Total flow	0.3 mL min^{-1}														
	Column	Acquity UPLC™ BEH C18 column (100 mm × 2.1 mm, 1.7 μm)														
MS condition	Capillary Voltage	3.0 kV									2.8 kV					
	desolvation gas	500 L h^{-1}														
	Source temperature	100 °C														
	Desolvation gas temperature	350 °C														
	Multiplier voltage	650 V														
	RF lens 1	0.5														
	RF lens 2	2														
	Collision gas	Ultra-high-purity argon														
	Pressure of collision chamber	3.2×10^{-3} mbar														
	The entrance voltage	0														
	The exit voltage	1														

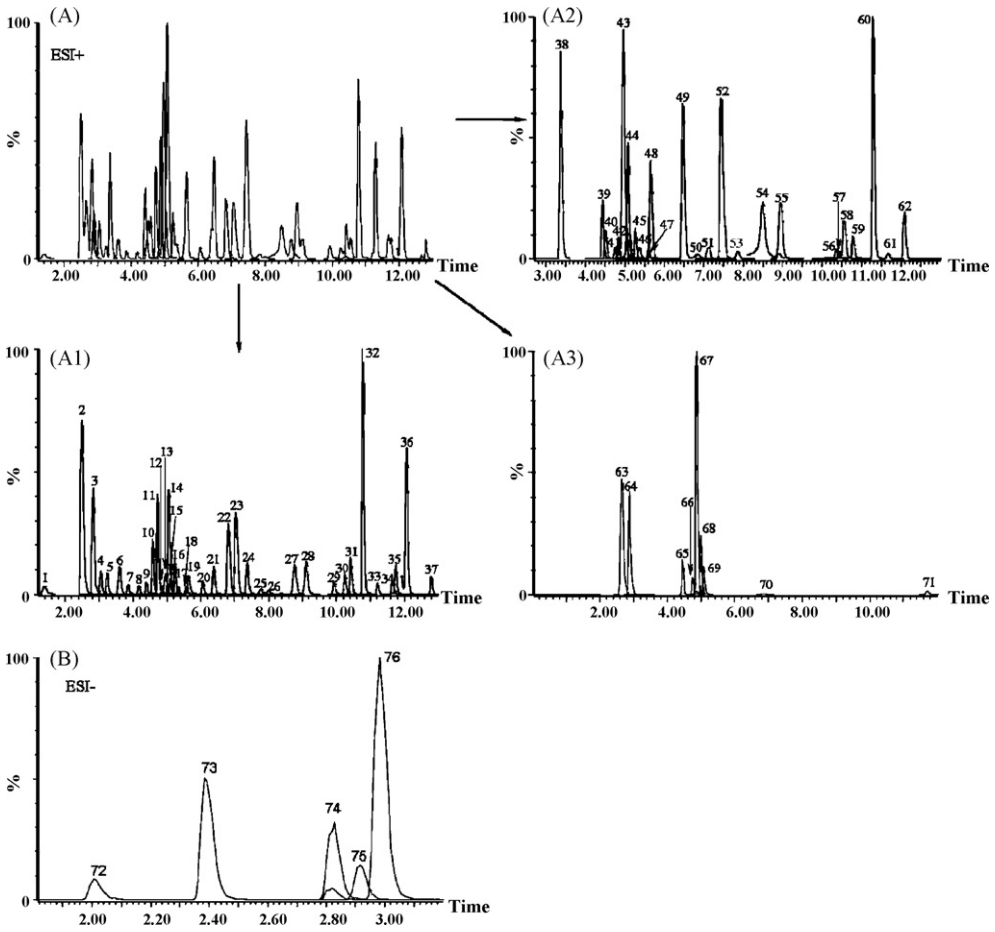

Fig. 2. Typical reconstructed MRM chromatograms for the 76 target analytes: (A) 71 compounds acquired under positive mode and (B) 5 compounds acquired under negative mode. The name of the analytes for each peak is detailed in Table S1.

uncertainty from the volume of the final extract, and (4) uncertainty from the recovery.

2.6. Matrix effects

Matrix effects were evaluated by the strategy applied by Matuszewski and Constanzer [32]. That is, subtracting the ratio between the slope of matrix-matched standard curves and the slope of standard solution curves, and then multiplying by 100 to obtain a percentage. The signal is enhanced if the value is negative, whereas the signal is suppressed if the value is positive.

2.7. Method detection limit and method quantification limit

The method detection limit (MDL) and method quantitation limit (MQL) were defined and determined as the minimum detectable amount of analyte from slaughterhouse wastewaters spiked extract in MRM mode with a signal-to-noise ratio (S/N) of 3:1 and 10:1, respectively. For the drugs existed initially, MQL and MDL were estimated by determining S/N of the minimum measured concentrations and extrapolating to S/N values of 10 and 3.

3. Results and discussion

3.1. Optimization of sample preparation

Studies have documented that EDTA in aqueous samples can increase the recoveries of tetracyclines and macrolides [33]. Preliminary experiments were therefore conducted to assess the extraction efficiency of four SPE cartridges (Oasis HLB, C18 Sep-Pak, Plexa, GCB) by addition of 1 mL of 20 μg/L cocktail standard solution into 500 mL of pure water containing 1 g of Na_2EDTA at pH 7.0. Recoveries using HLB cartridges for all compounds exceeded

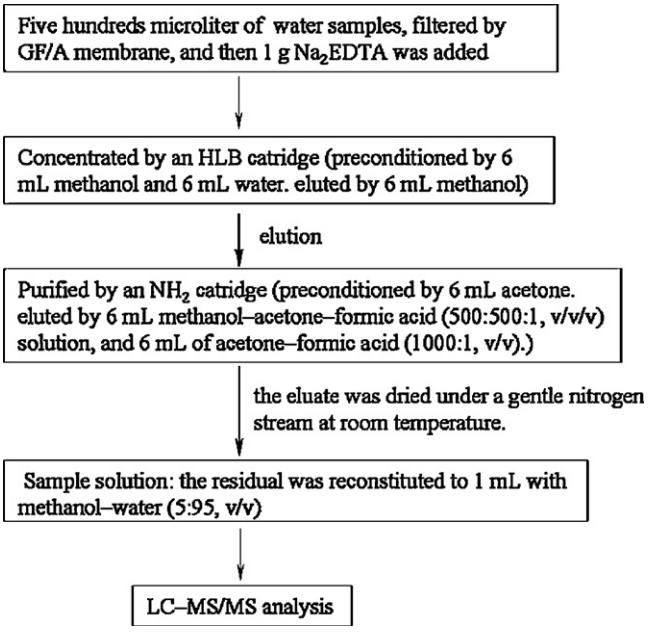

Fig. 3. Schematic diagram of the sample preparation.

Table 2
Occurrence and concentrations of pharmaceutical drugs in slaughter wastewater (ng/L).

Analyte	SWTP1						SWTP2					
	Influent			Effluent			Influent			Effluent		
	occ.	Median	Range	occ.	Median	Range	occ.	Median	Range	occ.	Median	Range
Sulfanilamide	10/10	90	<MQL–281	10/10	41	<MQL–116	6/9	437	70–876	6/9	42	<MQL–72
Sulfameter	10/10	43	23–94	10/10	10	6–29	9/9	67	11–150	9/9	27	5–67
Sulfachloropyridazine	9/10	11	3–12	9/10	2	1–8	6/9	5	4–24	6/9	2	1–5
Sulfaquinoxaline	6/10	33	25–98	6/10	5	3–22	0/9	ND	ND	0/9	ND	ND
Ofloxacin	10/10	128	59–342	10/10	78	26–136	9/9	97	38–716	9/9	54	18–225
Pefloxacin	10/10	41	12–160	10/10	19	7–73	9/9	50	4–98	9/9	13	2–30
Norfloxacin	10/10	29	11–73	10/10	15	5–53	9/9	54	16–120	9/9	12	5–29
Ciprofloxacin	10/10	12	16–33	10/10	10	6–23	9/9	77	36–240	9/9	16	7–52
Enrofloxacin	10/10	113	56–186	10/10	49	25–86	9/9	38	24–128	9/9	14	6–24
Lomefloxacin	8/10	2	<MQL–10	8/10	1	<MQL–2	5/9	15	5–18	5/9	3	1–10
Tetracycline	10/10	254	29–747	10/10	69	10–158	9/9	619	98–840	9/9	18	10–45
Oxytetracycline	10/10	814	163–1481	10/10	492	81–916	9/9	416	95–928	9/9	42	11–113
Tylosin	10/10	126	<MQL–269	10/10	12	<MQL–189	8/9	<MQL	<MQL–116	8/9	<MQL	<MQL–48
Kitasamycin	10/10	192	32–267	10/10	11	5–17	9/9	145	45–237	9/9	20	7–37
Erythromycin	9/10	106	38–354	9/10	17	6–63	7/9	14	12–21	7/9	3	3–13
Diazepam	10/10	7	5–16	10/10	2	1–5	6/9	5	4–13	6/9	2	1–8
Chloramphenicol	4/10	11	<MQL–40	4/10	5	<MQL–8	0/9	ND	ND	0/9	ND	ND
Clenbuterol	2/10	<MQL	<MQL–4	2/10	<MQL	<MQL	7/9	<MQL	<MQL–6	7/9	<MQL	<MQL–3
Metoprolol	7/10	2	2–8	7/10	1	1–4	4/9	ND	ND–6	4/9	ND	ND–1

Analyte	SWTP3						SWTP4					
	Influent			Effluent			Influent			Effluent		
	occ.	Median	Range	occ.	Median	Range	occ.	Median	Range	occ.	Median	Range
Sulfanilamide	8/8	711	<MQL–938	8/8	61	<MQL–117	7/8	556	<MQL–1200	7/8	60	<MQL–110
Sulfameter	8/8	85	19–215	8/8	33	7–61	8/8	19	<MQL–77	8/8	2	<MQL–6
Sulfachloropyridazine	7/8	22	3–57	7/8	8	2–22	5/8	<MQL	<MQL–18	5/8	<MQL	<MQL–7
Sulfaquinoxaline	3/8	22	15–103	3/8	6	4–35	1/8	4	4	1/8	1	1
Ofloxacin	8/8	94	54–715	8/8	47	21–228	8/8	266	80–532	8/8	97	32–235
Pefloxacin	8/8	20	6–57	8/8	4	1–22	8/8	14	6–98	8/8	1	1–30
Norfloxacin	8/8	54	2–82	8/8	24	5–72	8/8	25	14–120	8/8	2	1–28
Ciprofloxacin	8/8	61	8–408	8/8	35	3–195	8/8	61	12–168	8/8	5	5–16
Enrofloxacin	8/8	50	2–407	8/8	19	1–60	8/8	40	7–361	8/8	9	2–92
Lomefloxacin	5/8	<MQL	<MQL–15	5/8	<MQL	<MQL–2	5/8	8	6–15	5/8	1	1–2
Tetracycline	8/8	268	23–950	8/8	44	10–210	8/8	483	254–980	8/8	16	10–31
Oxytetracycline	8/8	668	367–1190	8/8	172	82–526	8/8	480	260–2942	8/8	21	11–100
Tylosin	8/8	<MQL	<MQL–132	8/8	<MQL	<MQL–25	8/8	<MQL	<MQL–81	8/8	<MQL	<MQL–13
Kitasamycin	8/8	164	46–395	8/8	18	5–40	8/8	139	69–288	8/8	12	5–21
Erythromycin	8/8	112	48–253	8/8	23	8–40	8/8	117	28–243	8/8	23	5–45
Diazepam	7/8	7	3–9	7/8	2	<MQL–3	7/8	6	<MQL–9	7/8	2	<MQL–2
Chloramphenicol	2/8	<MQL	<MQL	2/8	<MQL	<MQL	1/8	<MQL	<MQL	1/8	<MQL	<MQL
Clenbuterol	7/8	10	2–11	7/8	4	1–6	3/8	<MQL	<MQL–3	3/8	<MQL	<MQL–1
Metoprolol	6/8	2	<MQL–21	6/8	1	<MQL–2	6/8	20	18–32	6/8	2	1–2

ND = not detected.

50% except for, terbutaline (20%) and salbutamol (23%). Twenty-four and thirty-two of 76 analytes presented low recoveries (<60%) for Plexa and C-18 cartridges, respectively (Table S2). Twenty-one analytes could not be eluted upon using GCB cartridge. Oasis HLB cartridges were therefore selected.

To optimize the pH and content of Na_2EDTA recovering experiment at pH 3, 5, 7 and 9 were compared and pH 7 was the best (Table S3). Analyte recovery in 500 mL of spiked sewage samples containing different content of Na_2EDTA (0.5, 1, 2 and 3 g) were also tested (Table S4). The recoveries of tetracyclines increased (~30%) with increasing Na_2EDTA content from 0.5 to 1 g.

Slaughterhouse wastewater samples not only contained trace levels of pharmaceuticals, but also a high content of blood, feces and urine. After concentration, eluates from HLB cartridges were very dirty and mass signal of many target drugs were suppressed completely (data is not shown). To reduce the matrix effects and contamination of the sample extracts to column and mass spectrometer, a further purification procedure was necessary. An NH_2 cartridge (500 mg, 6 mL, Waters, Milford, MA, USA) was used for this aim. Schematic diagram of the proposed sample preparation procedure was shown in Fig. 3.

3.2. Matrix effect

It is well known that matrix effects (ion suppression and ion enhancement) are ubiquitous during LC–MS analysis due to the ionization competition between co-eluting compounds in a chromatographic system. Ion suppression is often observed during LC–MS/MS analysis, particularly for complex environmental samples and biosamples, and suppression may vary depending on the compound and matrix. In this study, the level of ion suppression for most compounds was lower than 70%, and some compounds (especially in influents) were heavily suppressed (highest ratio was about 89%; Table S5). The matrix effect is quite different for different sample extracts. These results showed that the matrix effects were ubiquitous and that quantitative analysis based on a pure standard solution curve is not appropriate. The isotopic dilution technique is an advantageous alternative to compensate for signal irreproducibility, matrix interference, and recovery loss, but acquiring sufficient isotopic-labeled internal standards for multi-component analysis is almost impossible. Therefore, matrix-matched standard curves are commonly applied in multi-component quantitative analyses for compensation of matrix effects. Calibration curves for

detection of target compounds were obtained by carrying out a linear regression analysis by adding a series of standard solutions to the matrix solution and using the area against analyte concentrations.

3.3. MQL, recoveries, and expanded uncertainty

The MQL of most drugs except for six compounds (sulfanilamide, cinoxacin, minocycline, methacycline, tilmicosin and tylosin) ranged between 1.0 and 10.0 ng/L in raw wastewater and 0.3–10 ng/L in effluent except for six compounds (sulfanilamide, cinoxacin, minocycline, methacycline, tilmicosin and tylosin), for which, MQLs of 30 ng/L in raw wastewater and 10–20 ng/L in treated wastewater were obtained.

Recoveries based on matrix-matched calibration for compensation of matrix effects mainly exceeded 60% in influents except for five compounds, and exceeded 60% in effluent except for three compounds (Table S6). Within-day reproducibility was represented by percentage RSD, which ranged between 1.3% and 16.5% at three levels for each compound once a day (data is not shown).

Relative expanded uncertainty at different spiking level was presented in Table S6. As for the different source mentioned in Section 2.5, uncertainty from the method recovery represents the greater contribution in global uncertainty, which is in accordance with previous report [29].

3.4. Investigation of pharmaceuticals in slaughterhouse wastewater and receiving river

Seventy-six target pharmaceuticals were analyzed in the influent and effluent of slaughterhouse wastewater. Nineteen drugs were found in SWTPs (Table 2). Among these drugs, two sulfanamides (sulfanilamide, sulfameter), five fluroquenones (ofloxacin, pefloxacin, norfloxacin, ciprofloxacin and enrofloxacin), two tetracyclines (tetracycline, oxytetracycline) and two macrolides (kitasamycin, tylosin) were found in all wastewater samples, which suggests that these antibiotics are most frequently used in livestock. Of these drugs, oxytetracycline was found at the highest level of ~3 μg/L in the influent and ~1 μg/L in the effluent of SWTPs. The occurrence levels are irregularly varied during the sampling periods. Other antibiotics such as sulfanamides, sulfachloropyridazine, sulfaquinoxaline, lomefloxacin and erythromycin are sometimes found at low levels in wastewater samples.

Certain banned drugs such as clenbuterol, diazepam (psychoactive drug) and chloramphenicol were also observed in slaughterhouse wastewater. These drugs are not allowed to be used as growth promoters in animals in China [34]. This result indicates their illicit use in the animal feeding process, and potential production of unsafe meat in Beijing markets. Diazepam and metoprolol were found in all the SWTPs with concentrations ranging from <LOQ to 32 ng/L in influents (particularly for SWTP4, with concentrations often >15 ng/L). These results indicate the common use of psychoactive drugs in animal husbandry, and the authorities should take measures to reduce the potential risk. Chloramphenicol was found in SWTP3 in only three samples. Clenbuterol was commonly found in SWTP3 at the highest concentration, ~11 ng/L, indicating that pigs in SWTPs come from farms where clenbuterol is used.

To investigate the contribution of slaughterhouse effluents to surface river water, one river receiving effluent from SWTP4 was monitored. Twelve out of 76 drugs, i.e., two sulfanamides (sulfanilamide, sulfameter), six fluroquenones (ofloxacin, pefloxacin, norfloxacin, ciprofloxacin, lomefloxacin, enrofloxacin), two tetracyclines (tetracycline, oxytetracycline) and one psychoactive drug (diazepam) and one β-blocker (metoprolol) were found in river water samples at R1, and another two macrolides (kitasamycin, tylosin) were found in samples downstream of SWTP4. These results (Table 3) indicated that the presence of kitasamycin and tylosin was due to the effluent from SWTP. High concentrations of sulfanilamide, ofloxacin, norfloxacin and metoprolol were commonly found in river water samples, with the highest level of ~230 ng/L noted for ofloxacin. Its concentrations were higher than those found in the Pearl river (74 ± 15 ng/L) and Victoria Harbour (8 ± 5 ng/L) in China, whereas the concentrations of norfloxacin in the Shahe river (80 and 173 ng/L) were comparable with those in the Pearl river (166 ± 42 ng/L) and higher than those in Victoria Harbour (11 ± 7 ng/L) [35]. The values of detected analytes in samples at R3 were higher than those of samples at R1, and the levels increased compared with those at R5. This was despite the fact that high levels of pharmaceuticals were found in R2 and R4, which suggested that the concentration contributions of SWTPs and farms to surface river water were limited. Levels of target compounds in samples of R6 were slightly lower than those of samples at R5, which suggested that transformation occurred during this term. The high concentration of metoprolol (~30 ng/L) found in the outfall of pig-raising farms suggests that this drug is illicitly used as growth promoter at this farm. The residual concentrations of drugs in the effluent from farms can be used as indicator of veterinary drug use at such venues.

Table 3
The occurrence of pharmaceutical drugs in Nansha River (n = 2, ng/L).

Analyte	R1	R2	R3	R4	R5	R6
Sulfanilamide	19, 77	11, 34	20, 79	61, 83	31, 78	23, 72
Sulfameter	1, 2	<MQL, ND	1, 3	7, 13	3, 3	3, 2
Clenbuterol	ND	ND	ND	1, 1	ND	ND
Ofloxacin	231, 80	90, 169	228, 85	109, 95	208, 89	215, 82
Pefloxacin	2, ND	1, ND	2, ND	5, 4	2, ND	2, ND
Norfloxacin	173, 71	30, 108	168, 72	65, 64	157, 65	167, 68
Ciprofloxacin	76, 9	145, 113	86, 10	82, 68	84, 26	81, 15
Enrofloxacin	8, 3	4, 20	8, 5	13, 16	10, 5	8, 4
Lomefloxacin	3, 2	1, 11	3, 2	3, 2	3, 2	3, 1
Tetracycline	17, 7	8, 5	14, 7	52, 41	23, 10	15, 7
Oxytetracycline	15, 18	24, 21	16, 19	312, 204	72, 20	21, 19
Metoprolol	30, 16	33, 9	31, 14	5, 4	30, 14	29, 14
Diazepam	3, 5	1, <MQL	3, 5	1, 1	3, 4	3, 4
Tylosin	ND	ND	ND	11, 8	5, 3	2, 2
Kitasamycin	ND	ND	ND	10, 6	6, 4	4, 2
Erythromycin	ND, 13	ND, 19	ND, 13	21, 26	14, 17	9, 13

ND = not detected.

4. Conclusion

A SPE-HPLC–MS/MS method was presented in this paper to simultaneous determination of 76 pharmaceuticals from different therapeutic classes in slaughterhouse wastewater. It was fully validated and successfully used in target compound analysis in real samples. Nineteen pharmaceuticals (including several banned drugs) of 76 were found in raw and treated slaughterhouse wastewater from four main slaughterhouses in Beijing. The results can reflect the drugs usage in local animal husbandry.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2009.08.038.

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