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Determination of fluoroquinolones in cattle manure-based biogas residue by ultrasonic-enhanced microwave-assisted extraction followed by online solid phase extraction-ultra-high performance liquid chromatography-tandem mass spectrometry

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ABSTRACT

The present work describes the development and application of an ultrasonic-enhanced microwave-assisted extraction (UEMAE) followed by online solid phase extraction (SPE)–ultra–high performance liquid chromatography–tandem mass spectrometry method for the analysis of 14 fluoroquinolones in cattle manure-based biogas residue (CMBBR). The UEMAE was performed using the mixed solution of sodium dihydrogen phosphate and disodium ethylenediamine tetraacetic acid, avoiding use of any organic solvent. The online SPE system employed two solid phase extraction columns in a parallel manner, and the extraction was performed by passing 1 mL of the extract through the column. Quantification was performed using standard spiked samples and structural analogue internal standard, which were indispensable to reduce the matrix effects. Validation parameters were performed and good linearity ($R^2 > 0.99$ in all cases) and precision (inter- and intra-day relative standard deviations were lower than 12.8%) were obtained. Limits of detection were as low as 0.021 $ng \cdot g^{-1}$ and lower limits of quantification were 0.5 $ng \cdot g^{-1}$ for all fluoroquinolones. The overall extraction recovery, which was the product of the UEMAE recovery and the online SPE recovery, was assessed for three concentration levels (0.8, 40 and 400 $ng \cdot g^{-1}$) and acceptable values (74.3–99.3%) were found. As a part of the method validation, the developed method has been used to analyze real CMBBR samples. Nine fluoroquinolones were found in the concentration range of 0.9–74.6 $ng \cdot g^{-1}$, while five were not detected in the samples. The results showed the method could be adapted for screening the presence or the final fate of fluoroquinolones during fermentation of animal waste.

1. Introduction

Although the development of animal husbandry has increased the supply of meat and dairy products, it has also produced a large amount of animal excreta. The attendant question is how to properly handle the excreta. Instead of being treated as excess wastes, these excreta should be seen as available bioenergy producer or low-cost organic fertilizer for agricultural production. A feasible method is to use microbes for anaerobic fermentation, which in turn produces biogas. In this way, not only the generated biogas can be used as secondary energy, but also biogas residue produced by fermentation can be applied to soil directly.

During livestock farming, antibiotics are often used to control early mortality and carry out anti-infection treatment [1–5]. Intensive management of livestock has a huge demand for antibiotics: 70% of global antibiotic products are used in livestock farming [6]. Remarkably, about 40%–90% of these antibiotics are excreted by excreta in the form of prototypes or metabolites [7–9]. Although biodegradation may occur during fermenting of the excreta, different antibiotic residues may still be detected in the final product [10,11]. When these antibiotic-containing fertilizers are applied to the soil, some of them will penetrate into the groundwater with surface water or contaminate other soils with surface runoff [12]. Some of them will be adsorbed into the soil [13], affecting the soil microbial community function [14]. Another part of them (e.g. sulfonamides and tetracyclines) can be directly absorbed and accumulated by the crops [15]. When the antibiotics enter the food chain, they can cause resistance to pathogens in

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the process of accumulation, and the resistant strains that they produce can spread between animals, patients and healthy people [16–18].

It has been reported that, some fluoroquinolones (FQs) have a longer half-life in fermenting than other antibiotics (such as chlortetracycline and sulfadiazine), which, to some extent, indicated the resistance of FQs to biodegradation [10,11]. FQs have been widely used both as medicines for humans and as drugs for animals all over the world [1,19]. FQs act as antibacterial agents against Gram-negative bacteria by inhibiting the DNA-gyrase in bacterial cells, leading to cell damage and death [20]. However, although it is well received worldwide due to its broad spectrum of antimicrobial activity and good oral absorption properties [20], their strong adsorption of solid substrates and resistance to biodegradation are the main reasons for their longer existence in natural ecosystems [13]. And even though there is photodegradation of FQs, the photodegradation products also have antibacterial activity, which can also cause bacterial resistance, genotoxicity and ecotoxicity [21–25]. Based on this, although the limits of FQs in the environmental matrices have not been defined so far, it is clear that the use of fertilizers containing FQs in agricultural production can have a negative impact on the environment and human health. In fact, FQs have been defined as “emerging pollutants” [26], and their environmental impacts have been extensively studied [27,28].

According to the literature, the content of FQs in fermented fertilizer is at the level of nanogram per gram [10,29,30,and]. This requires the establishment of a sensitive method for trace analysis, while effectively reducing the interference of complex matrix on the determination of target compounds. Selvam et al. has established an LC-MS method for the determination of ciprofloxacin in compost. After ultrasonic-assisted extraction (UAE), the samples were purified by offline solid phase extraction (SPE), and then concentrated and reconstituted before LC-MS analysis [10]. Dorival–García et al. used acidified acetonitrile as a solvent to extract FQ antibiotics from compost samples using microwave-assisted extraction (MAE). The extracts were purified by salt-assisted liquid-liquid extraction and dispersive SPE, and then injected into the ultra-high performance liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS) system for analysis [30]. However, these methods used complex sample pretreatment processes to eliminate the interference of complex matrices on the analysis of FQs. This is not only easy to cause the loss of the sample, reducing the sensitivity of the method, but also result in the decrease of method precision and accuracy because of too many manual operation steps. In addition, excessive purification steps can also lead to waste of energy, solvents and time.

An ideal method for rapid analysis of complex samples can be obtained by online coupling of SPE to LC–MS system. With this procedure, the sample or its extract can be directly injected into the SPE–LC–MS system, and the traditional offline SPE step is replaced by the more time-saving online SPE. As a result, the automation of sample pretreatment leads to higher sample throughput, lower solvent consumption and shorter sample preparation time [31]. In addition, the online SPE reduces the manual operation of the experimenter, making the sample analysis more accurate [32,33]. To the authors' best knowledge, no online SPE–UHPLC–MS/MS method is currently available for the determination of FQs in biogas residue samples. On the basis of this background, we developed a method using ultrasonic-enhanced microwave-assisted extraction (UEMAE) followed by online SPE–UHPLC–MS/MS for the determination of fourteen FQ antibiotics in cattle manure-based biogas residue (CMBBR). After being optimized and validated, the developed method was applied to the analysis of FQs in CMBBR samples obtained from a local dairy farm and the herdsmen.

2. Materials and methods

2.1. Chemicals and reagents

Reference standards (see Fig. 1), norfloxacin (NOR, purity 98.0%), ciprofloxacin (CIP, 98.0%), lomefloxacin (LOM, 97.6%), ofloxacin (OFL, 98.6%), fleroxacin (FLE, 99.1%), pefloxacin (PEF, 99.0%), enoxacin (ENO, 99.0%) and rufloxacin (RUF, 97.5%) were purchased from the National Institutes for Food and Drug Control (Beijing, China). Enrofloxacin (ENR, 98.0%), sarafloxacin (SAR, 98.0%), danofloxacin (DAN, 98.4%), difloxacin (DIF, 99.0%), marbofloxacin (MAR, 99.0%), flumequine (FLU, 98.0%) and orbifloxacin (ORB, internal standard, IS, 99.0%) were purchased from Dr. Ehrenstorfer GmbH Corporation (Augsburg, Germany). Acetonitrile and methanol of HPLC grade were purchased from Fisher Scientific (Fair Lawn, NJ, USA), while formic acid, acetic acid, acetone and n-hexane of HPLC grade were purchased from Concord Technology Co., Ltd. (Tianjin, China). Ultra-pure water was produced in laboratory by a Millipore Milli-Q system (Bedford, MA, USA). Sodium dihydrogen phosphate (NaH_2PO_4), disodium ethylenediamine tetraacetic acid (Na_2EDTA), phosphoric acid (H_3PO_4) and hydrochloric acid (HCl) were of analytical grade and obtained from Kemiou Chemical Reagent Co., Ltd. (Tianjin, China).

2.2. Extraction medium, stock solution, calibration standards and quality control samples

The extraction medium used in UEMAE procedure was a mixture containing 0.1 $mol \cdot L^{-1}$ NaH_2PO_4 and 0.1 $mol \cdot L^{-1}$ Na_2EDTA (pH adjusted to 4.0 by H_3PO_4). This extractant was then stored at 4 °C, and stood at room temperature for 1 h before UEMAE.

Stock solutions of each FQ were prepared at 50 $\mu g \cdot mL^{-1}$ in 20% (*v/v*) methanol, and further diluted to obtain standard working solutions (for each FQ to evaluate extraction recovery and matrix effect) and mixed working solutions (for the preparation of calibration standards and quality control samples) with the same solvent at a concentration range of 0.5–500 $ng \cdot mL^{-1}$ for each FQ. The IS stock solution of 50 $\mu g \cdot mL^{-1}$ was prepared in 20% (*v/v*) methanol, and further diluted to 50 $ng \cdot mL^{-1}$ with the same solvent as the standard working solution. The stock solutions were stored in the dark at −4 °C, while the standard working solutions were stored at 4 °C and renewed daily.

Calibration standard samples of FQs (0.5, 1, 5, 20, 50, 200 and 500 $ng \cdot g^{-1}$) were prepared by adding 200 µL of the mixed standard working solutions into the blank CMBBR sample, and the quality control (QC) samples (0.8, 40 and 400 $ng \cdot g^{-1}$) were prepared separately in the same fashion.

2.3. Sample collection

The CMBBR samples were obtained from a local dairy farm (DF-group, 10 samples) and the herdsmen (H-group, 11 samples) (the Northeast of Inner Mongolia, China). These samples were freeze-dried, homogenized, passed through 0.18 mm sieves and then stored at −4 °C until analysis. The blank CMBBR samples used for method development were collected from an abandoned fermentation pond in the dairy farm, and the samples were fermented for > 60 days. The blank samples were examined for FQ contents by the current method with the optimal experimental conditions, and further validated by the Dorival–Garcia's method [30].

2.4. Instrumentation and operating conditions

2.4.1. Ultrasonic-enhanced microwave-assisted extraction

The UEMAE was performed on a XO–SM50 ultrasonic/microwave reaction workstation (Atpio, Nanjing, China) equipped with a 50 mL pyrex pressure vessel, a magnetic stirrer, an interventional ultrasonic probe, a contactless infrared thermometer and a water-cooling temperature controller. A volume of 200 µL IS standard working solution was added into 0.2 g of the accurately weighed CMBBR sample (or FQ working standards/QC standards/IS standards into the blank CMBBR sample). The spiked sample was vortex-mixed for 10 min, freeze-dried overnight prior to analysis to allow solvent evaporation and FQs to be