Determination of Fluoroquinolone antibiotics in sludge matrix using pressurized liquid extraction technique combined with high performance liquid chromatography/ fluorescence detection

Duong Hong Anh, Pham Ngoc Ha, Nguyen Hoang Tung, Pham Hung Viet

Research Centre for Environmental Technology and Sustainable Development (CETASD),

Hanoi University of Science, Vietnam National University, Hanoi

Alfredo Alder

Swiss Federal Institute for Aquatic Science and Technology (EAWAG)

A method for trace determination of fluoroquinolones (FQs), a common human - use antibiotics, in municipal sludge was validated based on pressurized liquid extraction technique combined with reversed-phase liquid chromatography/fluorescence detection. A cleanup step using solid-phase extraction was required to improve the selectivity of method. Overall average recoveries for FQs were in range from 67 to 75% with relative standard deviations between 8 and 12%. Limit of quantification was 0.65 mg/kg of dry matter for sewage sludge. The validated method was successfully applied for determination of FQs in sewage sludge from Tolich river. Ciprofloxacin was found at concentrations between 0.20 to 1.42 mg/kg dry sludge.

1. Introduction

Fluoroquinolones (FQs) are among the most important antibiotics used in human and veterinary medicine. After having a curing effect in the body, FQs are excreted through urine or feces as a mixture of unchanged active substances, conjugates or metabolites, which then enter the sewage system [Giger W. et al., 2003]. Due to the high sorption coefficient for solid, the main part of FQ residues remains in sewage sludge and anaerobically stabilized sewage sludge. FQs were found in untreated sewage and anaerobically stabilized sludge were 1.4 - 2.7 mg/kg and 2.1 - 3.5 mg/kg, respectively [Golet E.M. et al., 2002]. Therefore, FQs may reach the terrestrial environment when anaerobically stabilized sewage sludge had been applied as fertilizer to agricultural land. Field experiment from a grassland showed the FQs residue in top-soil samples collected 21 months after sludge application at level of μg/kg range [Golet E.M. et al., 2003]. The occurrence of FQs, as well as other antibiotics and their metabolites in the environment has received increasing scientific attention since in the end of 1990s caused by the risk of the development and spread of antibiotic resistance in the environment [Kümmer K. et al., 2003].

In order to determinate pharmaceuticals in solid environmental matrices, where the target analytes are present at trace levels and along with a large number of potentially interferents, it is essential to carry out effective sample pretreatments which normally include both an extraction and clean up steps, prior to the analysis by GC and more frequently by HPLC. Soxhlet method could not be used for extraction due to the unstable of FQs and other pharmaceuticals at high temperature during a long heating time. Extraction of pharmaceuticals from the solid matrix has normally been performed by sonication or by simple blending or stirring of the sample with polar organic solvent or mixture them, or with aqueous solution... Pressurized liquid extraction (PLE), which has an other name as Accelerated Solvent Extraction, is a novel extraction technique reported since the year 1997. The technique employs solvents at elevated temperatures and pressures, which drastically improves the speed of extraction process of almost compounds from complex matrix. Quantitative recovery can be achieved after a few minutes. The solvent amount is saving up to 90% in compared with those using for Soxhlet extraction [Ramos L. et al., 2002].

This paper presents the basic experimental set-up of PLE technique and the determination of FQs in solid matrix (i.e. sludge) using PLE followed by solid phase extraction (SPE), which in combination with separation and detection by high performance liquid chromatography/ fluorescence detection. The method was applied to determine FQs in sewage sludge from Tolich river.

2. Experimental section

2.1. Chemicals and equipments

- High performance liquid chromatography equipment HP 1090/ FLD 1100 (Agilent Technology, Switzerland); Separation column C18 (YMC, Switzerland)
- Accelerate solvent extraction equipment ASE 200 (Dionex, US)
- HLB cartridges 6 mL Waters Corp. (Milford, MA), solid phase extraction apparatus (Supelco, US)
- Standards: ciprofloxacin (CIP) and norfloxacin (NOR) from Fluka (Buchs, Switzerland) and (Steinheim, Germany), Tosufloxacin surrogate standard (TOS) from Abbott Laboratories (Baar, Switzerland).
- Acetonitrile, methanol, H_3PO_4 85%, NH_4OH, HCl 37% Scharlau (Senmenat, Spain) or Merck (Darmstadt, Germany)

2.2. Experiment:

2.2.1. Analytical method and quality control:

The sludge samples are dried at 60^0C for 72h, finely ground, pass a 0.2 mm sieve and stored at room temperature in a amber bottle. Dried samples of 2 g sludge are weighed and transferred into 11 mL stainless extraction cells, and thoroughly mixed with 10g quartz sand. Samples are extracted using solvent mixture H_3PO_4 50mM/acetonitrile (1:1, v:v). The total volume of PLE extract in collection vial is about 20 mL. The PLE extracts are transferred to 50 volumetric amber flasks, added surrogate standard, filled up with distilled water and adjusted with 32% HCl to pH 3.0. The diluted extracts are extracted and clean-up by solid phase extraction technique using HLB cartridge. Subsequently, the high performance liquid chromatography/ fluorescence detection method (HPLC/FLD) is used for qualitative and quantitative analysis. The detail procedure of sample preparation was described in figure 2.

Parameters such as recovery, relative standard deviation (RSD) and limit of quantification (LOQ) were determined for quality control. Duplicate PLE extractions were carried out for each sample. Procedural blanks (quatz sand) were extracted for each set of 3 samples to control for laboratory contamination. An instrumental blank for HPLC/FLD was run after every three sample to assure no carryover during analysis.

Ciprofloxacin (CIP) and norfloxacin (NOR) were chosen as specific FQ analytes for this study, since they are the most consuming FQs and have already been detected in hospital wastewater [Duong H.A., 2007].

2.2.2. Application for real samples:

Municipal sludge samples were collected along the To Lich river at the midstream from the beginning (Hoang Quoc Viet Str.) to the jointing with Nhue River. These samples were subjected for FQs analysis in this study.

3. Results and discussions:

3.1. Introduction on PLE

The Dionex ASE 200 is the first commercial PLE system. The schematic diagram of a PLE system is shown in figure 1. Basically, the process has been performed in a static extraction mode, which involves the following steps:

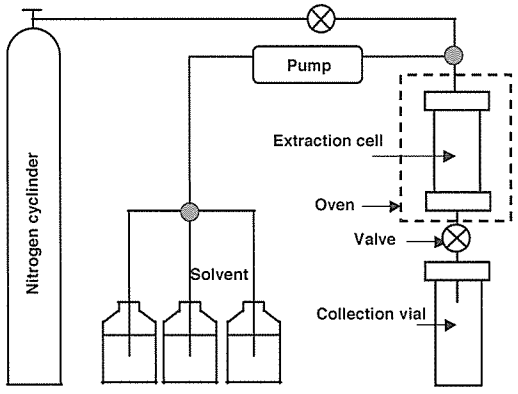

Figure 1. Schematic diagram of a PLE system

- Sample loading: Drying samples are loaded into the steel extraction cells (selective volumes: 1, 5, 11, 22 or 33ml), the dead volume of extraction cells are filled up by a inert material (such as quartz sand, diatomaceous earth, anhydrous sodium sulphate, glass wool...).
- Solvent filling: Organic solvent is automatically filled into the extraction cells.
- Pre-treatment: The cells are heated and pressurized in 5 – 10 min to the set values (working pressure: 35 – 200 bar)
- Static extraction: Samples are extracted for a certain time (5 – 20 min)
- Transferring: When the static step is ended, the valve is opened and the solvent is allowed to flow to the collection vials
- Solvent purging: Fresh solvent is added to wash the samples and the connecting lines. The solvent is flushed into the collection vial with a suitable gas.

3.2. Parameters for PLE extraction

Based on the method developed by Golet E.M, 2002, implementation parameters including solvents, temperature, pressure and size of sample were validated for analysis of FQs in municipal sludge matrix collected from Tolich river, Hanoi and sludge matrix collected in brackish water area in Nam Dinh.

Because of the zwitterionic character of FQs (pK_{aCOOH} = 5.9 – 6.3, pK_{aNH2} = 7.9 – 10.2), the pH effects to the FQ extraction efficiency from matrix. At neutral pH, the aqueous solubility of FQs is lowest. At low pH the anionic sites of FQs and sewage are protonated it could be possible that the electrostatic repulsion between FQs and sludge surface might account for better extraction efficiency. In order to select extraction solvent, various mixtures of organic solvents such as acetonitrile, methanol, propanol with 50 mM H_3PO_4–at pH=2 were tested. The aqueous mixture of 50 mM H_3PO_4/ acetonitrile (1:1) was chosen to bring the best recovery for FQs. The extraction was

implemented at temperature of 100^0C, 100 bar during 60 minutes (6 extraction cycles). The appropriate sample size was 2g for sludge samples to achieve reliable quantitative analysis.

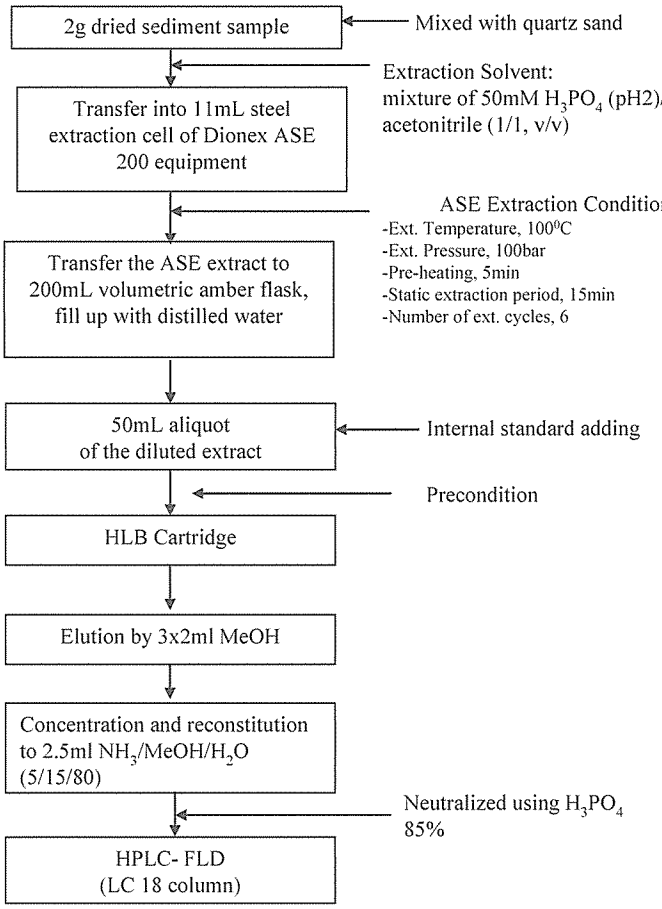

Figure 2. Analytical procedure for determination of FQs in sludge

3.3. Results of method validation:

Validation analysis were performed six time using sludge matrix from Tolich river where native FQs were undetectable and spiked samples with 1 mg/kg of each FQs. The accuracy was indicated by recoveries of the whole analysis procedure (including PLE and solid phase extraction stages) for CIP and NOR ranging from 67% to 70%. Such values are comparable with those reported by Golet E.M. (i.e. 80 ± 6% and 84 ± 5% for CIP and NOR, respectively). The precision indicated by the relative standard deviation was lower than 12%. The LOQ was defined as the concentration of FQs in sample which showed the signal height of 10 times higher than the noise height. The corresponding LOQs for FQs in sewage sludge were 0.65 mg/kg (dry sludge).

Parameters such as recovery, relative standard deviation (RSD) and limit of quantification (LOQ) were summarized in table 1. The method used allows measurements in the sewage sludge collected from wastewater channels in Hanoi.

Table 1. Quality control parameter

Parameter	CIP	NOR
Recovery (n=6), %	70 ± 5	67 ± 8
Precision (n=6), %	8	12
Limit of detection, mg/kg dry sludge	0.20	
Limit of quantification, mg/kg dry sludge	0.65	

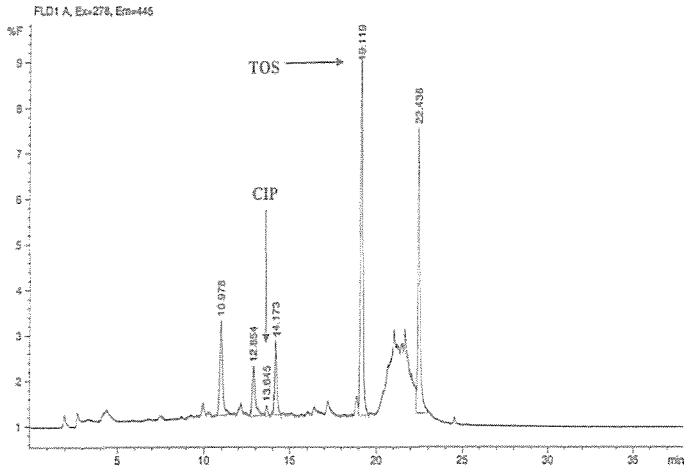

Figure 3. Liquid chromatogram of the extract of a sewage sludge collected at Tolich river

3.4. Determination of FQs in real sludge samples

Both of CIP and NOR were detected in hospital wastewater of Hanoi, but only CIP were found in sewage sludge. The elevation of CIP level was observed through the sampling sites in the crowded residential area from 1,06 ± 0,11 mg/kg at the site No.1 (Hoang Quoc Viet) to 1,42 ± 0,18 mg/kg at the site No.4 (Cau Moi). While it was little reduced to 0,85 ± 0,13 mg/kg in the site No.5 (Cau Dau), which was located in the agricultural area. And at the end of the To Lich river from site No.6 to site No.9, the content of FQs became lower than detection limit (0.2 mg/kg). The different of FQ level along the Tolich river – one of main wastewater channel of Hanoi reflects the significance of pharmaceutical emission from households and hospitals.

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