



## Leaching behavior of enrofloxacin in three different soils and the influence of a surfactant on its mobility

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### Abstract

The leaching behaviors of enrofloxacin (ENR), a fluoroquinolone group antibiotic, in three different standard soils, namely sandy, loamy sand and sandy loam were investigated according to OECD guideline 312. In addition, the effects of tenside, sodium dodecylbenzenesulfonate (DBS) on the mobility of ENR in two different soils were studied. The mobility of ENR in all three standard soils was very similar and was mostly (98%) concentrated on the top 0–5 cm segment of the soils at pH 5.7. The DBS can enhance the mobility of ENR in soils but the impact was in general negligible under the studied conditions.

**Key words:** enrofloxacin; leaching; soil; sodium dodecylbenzenesulfonate

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### Introduction

Enrofloxacin (ENR, 1,4-dihydro-1-cyclopropyl-7-(3-methylpiperazin-1-piperazinyl)-6-fluoro-4-oxo-3-quinoline-carboxylic acid, CAS: 93106-60-6), a fluoroquinolone group antibiotic, is widely used in livestock production and aquaculture (Tolls, 2001; Uslu et al., 2008; Zhao et al., 2010). Soil residues result mostly from the use of contaminated manure as fertilizer on agricultural land as ENR is degraded only to a small extent during its passage through the animal (Martens et al., 1996). Due to surface runoff and leaching, soils can also act as a source of antibiotic contaminants for the aqueous environment (Picó and Andreu, 2007; Martínez-Carballo et al., 2007; Uslu et al., 2008). ENR has been detected in arable soils in Austria up to 8.3 mg/kg (Martínez-Carballo et al., 2007) and 0.013–0.204 mg/kg in manure amended agricultural soils in Turkey (Uslu et al., 2008). Andreu et al. (2007) compared the environmental concentrations of nine fluoroquinolones in soil samples from agricultural fields differing in type of soil and in sewage sludge application, showing the presence of ENR in soil at concentrations with 0.12 mg/kg. Zhao et al. (2010) investigated the residual levels of selected fluoroquinolones, sulfonamides and tetracyclines in 143 animal dung samples collected in 2007 from large scale livestock and poultry farms in eight provinces of China. As a representative FQ, ENR was often detected in dung samples of cows (1.72–46.70

mg/kg) and chickens (with the highest concentration of 1420.76 mg/kg).

Residues of ENR quite often occur in soils but little is known about its fate, or the effects and risks associated with its release into environment (Picó and Andreu, 2007). The soil column leaching tests in the mixture of soils have been demonstrated as a useful approach to provide a proof for assessing the fate and leaching behaviour of pollutants in the environment. Many researchers studied the fate of oxytetracycline, sulphachloropyridazine, tylosin, sulfamethoxazole, ofloxacin and sulfadiazine by soil column leaching experiments (Kladivko et al., 1991; Karthideyan and Bleam, 2003; Kay et al., 2005; Brown et al., 1995; Drillia et al., 2005; Zhang and Huang, 2007; Unold et al., 2009). Their results showed that the sorption of the investigated drugs and the soil organic matter (SOM) influenced the leaching behaviors of chemicals.

The surfactants which are widely applied and discharged to the environment can affect the mobility of antibiotics. sodium dodecylbenzenesulfonate (DBS) e.g., a representative of the linear alkylbenzene sulfonate (LAS) family widely used for domestic and industrial purposes is considered to be able to change the characteristics of soil and influence the mobility of pollutants by affecting the adsorption isotherm of the substances in soils (Yediler et al., 1991; Ou et al., 1995; Xia et al., 2005).

In this study, the leaching behaviour of ENR in three different standard soils, namely, sandy, loamy sand and sandy loam, was studied by column experiments as given

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by OECD guideline 312 (OECD 312, 2004). To study the effect of the surfactants (DBS) on the mobility of the ENR in soils identical column experiments using two different standard soils were also conducted.

## 1 Materials and methods

### 1.1 Chemicals and reagents

Enrofloxacin (ENR) was obtained from Fluka. The structure of ENR is shown in Fig. 1. The molecular weight of ENR is 359.4 g/mol. The values of dissociation constants  $pK_{a1}$  and  $pK_{a2}$  were 6.27 and 8.30 respectively, and the values of  $K_{d, \text{solid}}$  ranged from 496 to 3037 and  $\log K_{ow}$  was 1.1 (Tolls, 2001). Individual ENR stock solution of the concentration of 100 mg/L and DBS stock solution of the concentration of 500 mg/L were prepared in ultra pure water and stored at 4°C in darkness. Sodium dodecylbenzenesulfonate (DBS) (approx. 80%) was purchased from Sigma.  $H_3PO_4$  (85%, Merck),  $KH_2PO_4$  (Merck),  $Na_2EDTA \cdot 2H_2O$  (Sigma),  $CaCl_2 \cdot 2H_2O$  (Merck) and 98%–100% formic acid (Merck) were analytical grade. Methanol (Merck) and acetonitrile (Sigma) were HPLC grade.

### 1.2 Soil samples

Three different standard soils obtained from LUFA, Speyer, Germany, were used for the column leaching experiments. Their characteristics which might affect the fate of ENR in soils are shown in Table 1.

### 1.3 Spiking procedure

To obtain the recovery rates of the extraction methods, one gram of freeze dried soil sample was spiked with 200  $\mu$ L of standard solution containing 5 mg/L ENR prepared in ultra pure water, to yield 1.0  $\mu$ g/g. To avoid the modification of the subsequent extraction the spiking solvent water was evaporated overnight under the fume board.

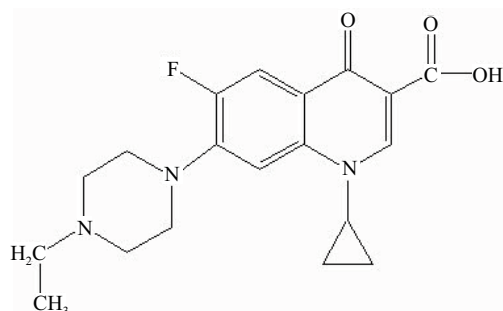


Fig. 1 Structure of enrofloxacin (ENR).

### 1.4 Extraction methods

$Na_2EDTA \cdot 2H_2O$  of 0.2 g and 5 mL of pH 3 buffer (27.2 g of  $KH_2PO_4$  and 1.35 mL of  $H_3PO_4$  in 1 L water): acetonitrile (50:50, V/V) was added to glass centrifuge tubes containing 1 g of soil samples. Samples were vortex mixed (Vortex Genie 2, Scientific Industries, USA) for 60 sec and placed into an ultrasonic bath for 15 min. They were then centrifuged for 5 min at 5000  $\times g$  and 20°C. The supernatant was transferred to an other 250 mL conical flask and the extraction procedure was repeated three times with additions of 4, 3, and 3 mL of pH 3 buffer. Extracts were combined and made up to 200 mL with ultra pure water to decrease the acetonitrile content prior to the clean up step by solid phase extraction (Uslu et al., 2008; Blackwell et al., 2004).

### 1.5 Solid phase extraction

Clean up and pre-concentration was performed using a combination of solid phase cartridges namely SAX (strong anion exchange; 1000 mg sorbent and 6 mL capacity) from Phenomenex, USA and HLB (polymeric, 500 mg sorbent, 6 mL capacity) from Waters, Milford, USA. They were placed in tandem to simultaneously remove negatively charged humic material (SAX) and retain the veterinary medicine (HLB) (Uslu et al., 2008). The SAX cartridge was placed on the top of the HLB and both columns were conditioned first with 3 mL of MeOH and then 3 mL of pH buffer. Extracts of 200 mL were then passed through both SPE-columns and the columns were washed with 10 mL of ultra pure water and dried for 10 min under vacuum. Then the SAX cartridge was removed and the elution was carried out with 4 mL methanol through HLB. Eluates were evaporated to about 150  $\mu$ L under gentle  $N_2$  stream and made up to 1 mL using the mobile phase (0.02 mol/L  $H_3PO_4$  acid containing water:acetonitrile (80:20, V/V)) and transferred to HPLC amber vials.

### 1.6 Soil column experiments

As given by OECD guideline 312, glass columns with an inner diameter of 5 cm and a height of 30 cm were used for the column leaching studies. The columns were packed with different amounts of air-dried and sieved (< 2 mm) standard soils up to a height of 25 cm (700 g of SP1, 600 g of SP2 and 650 g of SP3). After packing, the soil columns were pre-wetted with 200 mL artificial rain (0.01 mol/L  $CaCl_2$ :1.47 g  $CaCl_2 \cdot 2H_2O$  dissolved in 1 L ultra pure water) with a pH of 5.7 from bottom to top to displace the air in the soil pores by water. Subsequently they were allowed to equilibrate and the excess water was drained off by gravity. The water holding capacities of the soil

Table 1 Physical and chemical characteristics of standard soils

Soil No.	Soil texture	Composition of soil particles (%)				$C_{org}$ (%)	CEC <sup>a</sup> (meqv/100 g)	pH
		Clay (< 2 $\mu$ m)	Silt (2–20 $\mu$ m)	Fine sand (20–200 $\mu$ m)	Coarse sand (> 200 $\mu$ m)			
SP1	Sandy	3.6	4.6	31.9	59.9	1.15	6	6.0
SP2	Loamy sand	7.5	7.4	42	43.1	2.36	11	5.6
SP3	Sandy loam	8.9	11.3	51.3	26.3	1.02	9	6.2

<sup>a</sup> Cation exchange capacity.

columns utilized in the experiments were assumed to be 160, 170 and 160 mL for SP1, SP2 and SP3, respectively. As a next step the surfaces of the soil columns were covered by a glass fibre filter (Carl Roth GmbH + Co., Germany, 0.45  $\mu\text{m}$ ) then 250 mL of ENR standard solution of 10 mg/L adjusted to pH 5.7, with 1 mol/L NaOH was carefully dropped evenly over the surface of the soil columns. At the same time the valve of the glass column was screwed loosely to make the solution drained off drop by drop until the almost dryness of the top soil. Then 250 mL of the artificial rain was added to the soil columns drop-wise with the help of separatory funnels during 36 hr. The leaching depth was equivalent to the two months' average rainfall of the globe. For the study of the effect of DBS on the mobility of ENR, the artificial rain was replaced by 500 mg/L DBS solution (DBS dissolved in artificial rain). Then the soil was squeezed out with the help a peristaltic device and sectioned in three segments namely 0–5; 5–15; and 15–25 cm. Every soil segment was mixed intensively, transferred into the rockered flasks and frozen at  $-20^{\circ}\text{C}$  overnight before freeze drying for 30 hr (OECD, 2004).

Triplicate leaching columns were packed to study the leaching behaviours of ENR with three different standard soils namely, sandy, loamy sand and sandy loam. In addition one blank leaching column was run as control. To study the effect of surfactants (DBS) only two standard soils (sandy and loamy sand) showing significant difference in their characteristic were selected. For the DBS experiments duplicate leaching columns were packed with each soil and one blank column was run as control.

### 1.7 HPLC analysis

The HPLC analyses were performed with a 1100 series LC chromatograph system (Agilent Technologies, Germany) equipped with a fluorescence detector and an automated injection system. ENR standard solutions and sample extracts were analysed on LC column (ZORBAX SB-C18, 5  $\mu\text{m}$ , 4.6  $\times$  150 mm) at  $30^{\circ}\text{C}$  with an injection volume of 10  $\mu\text{L}$ . Isocratic elution was carried out with 0.02 mol/L  $\text{H}_3\text{PO}_4$  acid containing water:acetonitrile (80:20, V/V) at a flow rate of 0.8 mL/min. The excitation and emission wavelengths for ENR were 280 and 450 nm, respectively. Analyses of sodium dodecylbenzenesulfonate were performed with the same instrument at a column temperature of  $26^{\circ}\text{C}$  with an injection volume of 20  $\mu\text{L}$ . Isocratic elution was carried out with ultra pure water: MeOH (15:85, V/V) at a flow rate of 0.8 mL/min. The detection wavelength for DBS was 225 nm (Ramos et al., 2003; Golet et al., 2001).

### 1.8 Calibration curves, instrument detection limit and recovery

Good linearity was obtained for ENR with a concentration of 0.1, 0.2, 0.5, 1, 2 mg/L and DBS with a concentration of 0.5, 1, 2, 5, 10 mg/L in mobile phase. Correlation coefficients were higher than 0.999 in all cases. The instrument detection limit of ENR was 0.5  $\mu\text{g/L}$  (S/N = 10). The analytical method had a good applicability for

detecting the ENR in soil.

The results of recovery experiments indicate that the recovery rates of ENR in sandy soil (76.9%–79.5%) and loamy sand (76.5%–77.2%) were slightly higher than that in sandy loam soil (70.2%–71.7%), which might be related with the contents of organic carbon and the composition of particles like clay and their CEC in different soils (Nowara et al., 1997; Figueroa-Diva et al., 2010).

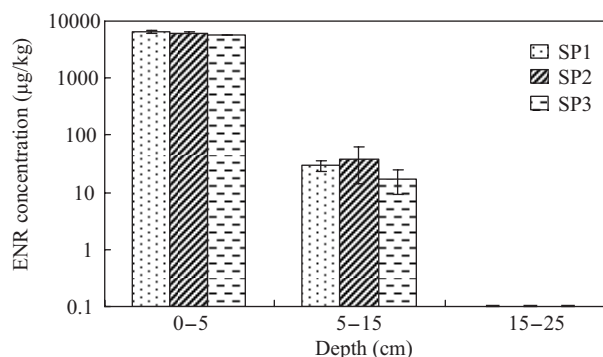
## 2 Results and discussion

### 2.1 Leaching behavior of ENR in standard soils

The standard soils, sandy (SP1), loamy sand (SP2) and sandy loam (SP3), were used to conduct the first series of the column experiments. The results are presented in Fig. 2. ENR was not detectable in the blank samples.

The highest concentration of ENR in soil was detected in the upper layer of 0–5 cm after leaching with artificial rain. Despite of the wide range differences in the levels of organic carbon (1.15%–2.36%), clay (3.6%–8.9%) and cation exchange capacity (6–11 meqv/100 g), the three standard soils demonstrated similar adsorption behaviours for ENR: ENR was mostly (98%) concentrated on the top 0–5 cm segment. All of the three soils showed a high affinity to ENR, assumingly due to the formation of irreversibly bound residues.

The results are in accordance with the previous studies. Tolls et al. (2001) found that the transportation of pharmaceuticals depended largely on the sorption behaviours of soils. Nowara et al. (1997) found that over 90% of the applied ENR was adsorbed on five soils from different geographic areas of Germany. At clay minerals ENR adsorption was even higher, namely > 98% as a result of adsorption between the mineral layers of soils. Uslu et al. (2008) studied the sorption of ENR on three types of soils and the highest distribution coefficient was achieved for loamy sand (ENR  $K_d = 97 \text{ L/kg}$  and  $K_f = 0.56$ ) with the highest organic carbon. PH value has a strong effect on the adsorption of ENR in soil and ENR has strong tendency for sorption at pH = 5.7. ENR can move rapidly from the water compartment into soil mainly because it is the most lipophilic compounds with  $K_{oc}$  of 15,800 L/kg (Picó and Andreu, 2007). However, different values of  $K_{oc}$  and  $K_d$  of



**Fig. 2** ENR concentrations at different sections of soils after column leaching experiment. SP1, sandy; SP2, loamy sand; SP3, sandy loam. (250 mL ENR standard solution containing 10 mg/L ENR was added,  $n = 3$ ).

ENR were acquired for different soils ( $K_{oc}$ : 16506–768740 L/kg,  $K_{d,solid}$ : 260–5612 L/kg) (Nowara et al., 1997). The reason for this will be explored in the future studies.

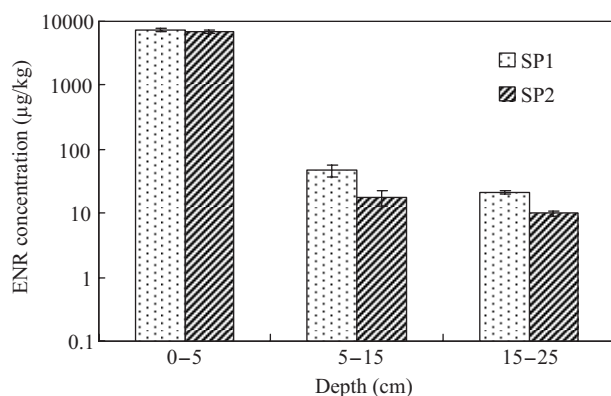
Martens et al. (1996) suggested that the degradation of ENR in soil is hindered considerably by adsorption to soil constituents, probably soil organic matter. Fluoroquinolones were tightly bound to feces and soils and hardly bio-available, leading to their apparent recalcitrance. On the basis of our results, we conclude that the degradation of ENR in soil will be hampered considerably due to the firm adsorption to soil constituents.

It should be noted that insoluble calcium salt might have formed due to the mixing of ENR and calcium ions containing in artificial rain, which could be a reason responsible for the low leaching behavior of ENR. Therefore, the impact of calcium ions to the leaching behavior of ENR in soils will be investigated by using solutions free from calcium ions in the future.

## 2.2 Effect of DBS on the fate of ENR in soil

The effect of sodium dodecylbenzenesulfonate on the mobility of ENR was investigated. The leaching results of standard soils SP1 and SP2 are presented in Fig. 3. ENR was detected in the 15–25 cm section after DBS leaching, showing that DBS enhanced the mobility of ENR in two different soils. However, the concentrations of ENR in the 15–25 cm section after DBS leaching were only 10–21  $\mu\text{g/kg}$ . The DBS can enhance the mobility of ENR in soils but the impact was in general negligible under the conditions used. However, further studies will be required to elucidate the leaching behavior of ENR under calcium ion-free conditions.

Results obtained by several authors studying the effect of a widely used surfactant (LAS) on the adsorption of phenanthrene (PAH) on soils indicated a significant increase on the mobility or accumulation of phenanthrene in soils and plants (Yediler et al., 1991; Ou et al., 1995; Xia et al., 2005). The different  $K_d$  values between PAHs (approximately 250 L/kg) and ENR (3000–6000 L/kg) might be the major reason for the different effects exhibited by the surfactants on the mobility of target organic compounds in soils. For a compound with a high  $K_d$  value,



**Fig. 3** ENR concentrations at different sections of soils after column leaching experiment for two soils (SP1 and SP2) under the presence of DBS (250 mL ENR standard solution containing 10 mg/L ENR was added;  $n = 2$ ).

the effect of the surfactants will be very limited. Therefore, the mobility behavior of ENR was not affected perceptibly by the surfactant.

## 3 Conclusions

A range of laboratory soil column experiments have been carried out to investigate the leaching behavior of enrofloxacin in three standard soils and the effect of sodium dodecylbenzenesulfonate on the mobility of ENR in two standard soils. The sorption of ENR in three different standard soils is very strong and the mobility is weak at pH 5.7. DBS can enhance the mobility of ENR in soils but the impact was in general negligible under the conditions used. It is important to consider the potential harmful effect of ENR on the soil microbial community when the soil respiration, nitrification of ammonia, etc. are considered as shown in this study.

## 3.1 Acknowledgments

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