Chemosphere 119 (2015) S35–S41

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

A comprehensive approach to the determination of two benzimidazoles in environmental samples

Marta Wagil, Joanna Maszkowska, Anna Białk-Bielińska, Piotr Stepnowski, Jolanta Kumirska *

Institute for Environmental and Human Health Protection, Faculty of Chemistry, University of Gdańsk, ul. Wita Stwosza 63, 80-308 Gdańsk, Poland

ARTICLE INFO

Article history:
Received 3 February 2014
Received in revised form 28 April 2014
Accepted 29 April 2014
Available online 2 June 2014

Keywords:
Benzimidazoles
Flubendazole
Fenbendazole
Method of determination
Water
Sediment and fish tissue samples

ABSTRACT

Among the various pharmaceuticals regarded as emerging pollutants, benzimidazoles – represented by flubendazole and fenbendazole – are of particular concern because of their large-scale use in veterinary medicine and their health effects on aquatic organisms. For this reason, it is essential to have reliable analytical methods which can be used to simultaneously monitor their appearance in environmental matrices such as water, sediment and tissue samples. To date, however, such methods relating to these three matrices have not been available. In this paper we present a comprehensive approach to the determination of both drugs in the mentioned above matrices using liquid chromatography–ion trap mass spectrometry (LC–MS/MS). Special attention was paid to the sample preparation step. The optimal extraction methods were further validated by experiments with spiked water, sediment and fish tissue samples. Matrix effects were established. The following absolute recoveries of flubendazole and fenbendazole were achieved: 96.2% and 95.4% from waters, 103.4% and 98.3% from sediments, and 98.3% and 97.6% from fish tissue samples, respectively. Validation of the LC–MS/MS methods enable flubendazole and fenbendazole to be determined with method detection limits: 1.6 ng L^{-1} and 1.7 ng L^{-1} in water samples; 0.3 ng g^{-1} for both compounds in sediment samples, and 3.3 ng g^{-1} and 3.5 ng g^{-1} in tissue samples, respectively. The proposed methods were successfully used for analysing selected pharmaceuticals in real samples collected in northern Poland. There is first data on the concentration in the environment of the target compounds in Poland.

1. Introduction

Flubendazole (FLU) and fenbendazole (FEN) (Table 1A) belong to a group of benzimidazoles (Oh et al., 2006). Used in veterinary medicine on a large scale (Baliharová et al., 2003), these drugs combat infections caused by parasitic organisms such as roundworms, lungworms and tapeworms (EMEA, 2004). FLU is used for the treatment of dogs, cats, cattle, horses and birds; FEN also has a therapeutic effect on fish (Iosifidou et al., 1997; Athanassopoulou et al., 2009; Plumb, 2011). These pharmaceuticals can enter the environment in dung excreted by grazing animals, during manure-spreading, and also following direct application in aquaculture or through the local water treatment plant at fish farms (Koschorreck et al., 2002). Ultimately they reach ground and surface waters via drainage, run-off and leaching. It is reported (Oh et al. 2006) that benzimidazoles are toxic to daphnids. The EC_{50} for *Daphnia magna* after 48 h was 16.5 μg L^{-1} for FEN and 66.5 μg L^{-1} for FLU. Furthermore, on entering the environment they may have a significant impact on natural parasites (Morley, 2009).

Kreuzig et al. (2007) found that FLU and FEN are slowly degraded in pig manure and that they are not degraded by microbial agents. Additionally, after reaching the soil system FEN is not mobile; on the other hand, FLU has the potential to move from agricultural manure to drainage waters. Hydrolysis experiments have shown that FEN, unlike FLU (Horvat et al., 2012), cannot be hydrolysed (NADA, 1995). It is reported that FEN undergoes rapid photodegradation in aqueous solutions ($T_{1/2}$ = 0.5 d, pH 7) (NADA, 1995). The octanol–water partition coefficient parameter of FLU and FEN (Table 1) indicates that FLU and FEN may bioaccumulate in animal tissue (Mottier et al. 2003; Horvat et al. 2012).

The need to control anthelmintics in animals resulted in regulation of their maximum residue limits (MRLs) in animal tissue (Council regulation (EEC) No 2377/90); for FLU and FEN must not exceed 50 ng g^{-1}. However, there are no regulations on their concentration in the environment. In order to protect the environment from adverse effects of anthelmintic drugs, it is essential to have reliable analytical methods that can be used to monitor their presence in the environment. Unfortunately, such methods are very

* Corresponding author. Tel.: +48 (58) 5235212; fax: +48 (58) 5235012.
E-mail address: jolanta.kumirska@ug.edu.pl (J. Kumirska).

http://dx.doi.org/10.1016/j.chemosphere.2014.04.106
0045-6535/

limited, only for water (Van De Steene et al., 2006; Van De Steene and Lambert, 2008a,b; Cacho et al., 2009; Zrnčić et al. 2014) or only for tissue (Sørensen and Hansen, 1998; Balzis, 1999; Rose, 1999; Dowling et al., 2005). There are no reports in the literature on analytical methods of monitoring FLU and FEN in sediments (Horvat et al. 2012). Here we present a comprehensive approach to the determination of FLU and FEN in environmental samples such as water, sediment and animal tissues. The proposed validated methods were applied to the analysis of real samples from the River Gościcina (Poland).

2. Experimental

Fig. 1S (supplementary data) shows a conceptual approach to all the experiments that were carried out.

2.1. Reagents and materials

FLU and FEN (all ⩾ 98%) were purchased from Santa Cruz Biotechnology, Inc. (Heidelberg, Germany). HPLC grade acetonitrile (ACN) used for preparing the mobile phase was purchased from Chempur (Piekary Śląskie, Poland). The organic solvents dimethyl sulphoxide, methanol, acetone and n-hexane, all analytical grade, were likewise supplied by Chempur (Piekary Śląskie, Poland). Orthophosphoric acid 85%, hydrochloric acid 36%, ammonium hydroxide 25%, sodium sulphate (anhyd.), potassium di-hydrogen phosphate and di-potassium hydrogen phosphate were also obtained from the same manufacturer and had the same grade. Strata XC, Strata X-CW, and Strata X (SPE-Phenomenex Inc., Torrance, CA) cartridges with 200 mg of packing material and a 3 mL reservoir and Oasis MCX (Waters, USA) cartridges (200 mg/6 mL) were used for sample preparation. Glass filters (ø 50 mm) were bought from Whatman™, Poland.

2.2. Apparatus

The filtration kit (Sartorius) was provided by Stedim Biotech GmbH, Germany, the vortex shaker (Ika Genius3) by IKA GmbH & Co. (Staufen, Germany), whereas the MPW-250 centrifuge by IWE Electronic (Warsaw, Poland). Mars 5 Microwave Accelerated Reaction System was purchased from CEM Corporation (Matthews, USA). All HPLC–UV measurements were carried out on a Perkin Elmer Series 200 with UV detector (Perkin Elmer, Waltham, USA). LC–MS/MS analyses were performed on Agilent 1200 Series LC system (Agilent Technologies, Inc., Santa Clara, USA) connected with an HCT Ultra ion trap mass spectrometer (Brucker Daltonics, Bremen, Germany) equipped with an electrospray ionization (ESI) source.

2.3. Standard solution preparation

The standard stock solution (500 μg mL^{-1}) of each target compound were prepared in dimethyl sulphoxide, whereas working solutions of mixture of FEN and FLU in ACN: H_2O (70:30, v/v) and stored at 4 °C.

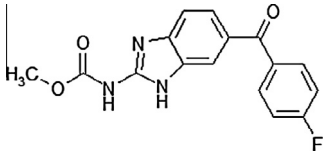
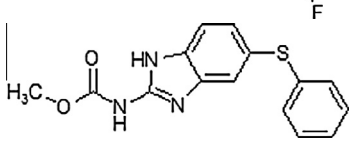
2.4. Sample preparation

Three working solutions of FLU and FEN (0.1, 0.25 and 1 μg mL^{-1}) were prepared, and used to spike three 500 mL water samples to the concentrations 0.02; 0.1; 0.2 μg L^{-1}, respectively. There were three replicates of each concentration.

Sediment samples were collected from the River Gościcina (northern Poland) (Fig. 2S; supplementary data). Samples were air-dried, ground in a mortar and passed through a 2 mm sieve. The pH of the sediment (measured in 1 M KCl) and the organic content were measured: pH = 7.48; organic carbon content = 0.86% (determined as loss of ignition). These experiments were conducted in accordance with the procedure by Myślińska (2001). Next, sediment samples (each 5 g) were spiked with the working solutions of benzimidazoles (0.1, 0.25 and 0.5 μg mL^{-1}) to the concentrations 0.02, 0.05 and 0.1 μg g^{-1}, respectively. Each concentration was repeated three times. Three non-spiked samples were also prepared. Samples were left to dry in the dark at room temperature for three days.

Fish (Rainbow trout) samples were purchased from a local fish farm located on the River Gościcina (Fig. 2S). Finely three sliced muscle tissue samples (each 1 g) were transferred to three polypropylene tubes (30 mL) and spiked using working solutions (0.2, 1.0 and 4 μg mL^{-1}) to the concentrations 0.05, 0.25 and 1 μg g^{-1}, respectively. Each concentration was repeated three times. Three non-spiked samples were also prepared. The samples were left in the dark for 30 min.

Table 1
(A) Chemical structure and physico-chemical properties of FLU and FEN (Koschorreck et al., 2002; Mottier et al., 2003; Horvat et al., 2012); (B) precursor ion and product ion masses selected during optimization of the MRM detection of FLU and FEN by LC–MS/MS.

Compound	Chemical structure	Molecular weight (g mol^{-1})	log K_{ow}	pK_a
A: chemical structure and physico-chemical properties of FLU and FEN				
FLU		313.3	3.93	3.6, 9.6
FEN		299.4	2.91	5.12, 12.72

Substance	Precursor ion (m/z)	Isolation width	Product ion (m/z)	Fragmentation amplitude (V)
B: precursor ion and product ion masses selected during optimization of the MRM detection of FLU and FEN				
FLU	314 $[M + H]^+$	2.0	282	0.65
			268	1.0
FEN	300 $[M + H]^+$	2.0	159	1.15
			190	1.15
			268	0.75