Chemosphere 119 (2015) S28–S34

Contents lists available at ScienceDirect

Chemosphere

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

Determination of metronidazole residues in water, sediment and fish tissue samples

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HIGHLIGHTS

- Comprehensive approach to the determination of metronidazole in environment.
- Determination of metronidazole in water, sediment and tissue samples.
- Validation of the methods and their application to the analysis of real samples.
- First data on the environmental contamination of northern Poland by this compound.

ARTICLE INFO

Article history:
Received 23 October 2013
Received in revised form 28 November 2013
Accepted 13 December 2013
Available online 10 January 2014

Keywords:
Metronidazole
Methods of determination
Water
Sediment
Fish tissue

ABSTRACT

Metronidazole (MNZ) is an antibacterial and antiprotozoal drug used in veterinary and human medicine. Its continual entry into the environment and its biological properties may have significant, long-term effects on the stability of ecosystems because MNZ and its metabolites possess mutagenic, carcinogenic and toxic properties. For this reason, the application of MNZ in food-producing species is prohibited in the EU, the USA and other countries. To ensure human food safety and to protect the environment, robust and reliable screening and confirmatory tests capable of the low-level detection of MNZ residues are required. The development of methods for MNZ determination in biological and environmental samples is thus an important analytical task in environmental and food science. This work focuses on the evaluation of a method for determining MNZ in water, sediment and fish tissue samples using liquid chromatography – ion trap mass spectrometry (LC-MS/MS). MNZ was extracted from waters on Strata XC cartridges using solid phase extraction (SPE), and from sediments and fish tissues by solid–liquid extraction (sediment: 15 mL 0.1 M HCl (pH = 0.6), 15 min; fish tissue: 15 mL 1% CH_3COOH in ACN, 1 min; drying: 5 g $MgSO_{4(anhyd.}$; 30 s) with SPE purification of the extracts (from sediment: Strata XC cartridge; from fish tissue: Supelco NH_2 cartridge). The optimal procedure that we developed was validated in order to confirm its reliability and sensitivity. Matrix effects (ME) were established. Absolute recoveries ranged from 89.3% to 97.2%, and the method detection limits were 3.4 ng L^{-1} (water samples), 0.4 ng g^{-1} (sediment samples) and 0.3 ng g^{-1} (tissue samples). These methods were used to determine MNZ in surface waters, sediments and fish tissues from the Polish River Gościcina; MNZ was found in all these matrices. The highest concentrations in water, sediment and tissue were 136.2 ng L^{-1}, 12.0 ng g^{-1} and 1.5 ng g^{-1} respectively. The results confirmed that these methods are suitable for the simultaneous analysis of waters, sediments and fish tissues for the presence of MNZ.

1. Introduction

Metronidazole (MNZ) (Table 1) belongs to the nitroimidazole group (Simms-Cendan, 1996). It is employed in both human and veterinary medicine to treat diseases caused by anaerobic bacteria (*Bacteroides*, *Fusobacterium*, *Campylobacter*, *Clostridium*) and protozoa (*Trichomonas*, *Treponema*, *Histomonas)* (CVMP, 1997). However, this drug has been found to possess carcinogenic, mutagenic and toxic properties (Voogd, 1981; Capitan-Valley et al., 2007). In mammalian cells DNA damage seems to be related to the production of reactive oxygen species. MNZ metabolites are also carcinogenic and mutagenic in some animal species, because the original nitroimidazole ring is retained (Sanco/3400/2005). For this reason, the use of MNZ in food-producing species is prohibited in the EU (L82/14 CRE, 1998), the USA (FARAD, 2010) and other countries. In the absence of an established minimum required performance limit (MRPL) or reference point for action on nitroimidazoles (CR

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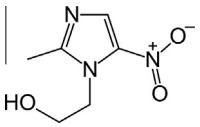
http://dx.doi.org/10.1016/j.chemosphere.2013.12.061

Table 1
Chemical structure and physico-chemical properties of metronidazole [9,23].

Compound	Structure	M.W. (g mol^{-1})	pK$_a$	LogP	Water solubility (mg L^{-1})
Metronidazole (MNZ) CAS number 443-48-1		171.15	2.4	−0.1	10000

37/210), the EU's Community Reference Laboratories (CRLs) have published guidelines on the recommended sensitivity of analytical methods that should be capable of detecting and confirming the presence of nitroimidazole residues in samples (CRL, 2007). The recommended sensitivity of these methods is 3 μg kg^{-1} (or μg L^{-1}) in all matrices.

Although this compound has been prohibited as a food additive in many countries, it is still used, especially in veterinary practice and aquaculture in order to increase and control production, improve survival rates, and control pathogens and diseases (Klinger and Floyd, 1994; Muñoz et al., 2010). MNZ is not only very soluble in water (Table 1); it is also hydrolytic (Wu and Fassihi, 2005) and photostable (Sánchez-Polo et al., 2012), so it can accumulate in the aquatic environment (Richardson and Bowron, 1985; Rediguieri et al., 2011). The octanol–water partition coefficient (LogP) of MNZ at 25 °C is −0.1 (Table 1). Moreover, MNZ is weakly sorbed to soils (K_d < 0.7 mL g^{-1}) (RabØlle and Spliid, 2000). Hence, this drug can have a significant impact on non-target aquatic organisms and may lead to the formation of bacterial strains resistant to its effects (Johnson, 1993; Kümmerer, 2009). It has been reported that this can occur not only in water but also in sediments (Kümmerer, 2004). In our previous paper, we established EC$_{50}$ values for MNZ towards four different organisms (Kołodziejska et al., 2013).

There are few literature data on the environmental analysis and the concentrations of MNZ in environmental matrices (Mahugo-Santana et al., 2010). Such data as are available relate to the analysis of MNZ in hospital effluents (Lindberg et al., 2004; Gómeza et al., 2006), in surface waters (Capitan-Valley et al., 2007; Kasprzyk-Hordern et al., 2007) and animal tissues from poultry and pigs (Sun et al., 2007). There are just a couple of papers on the analysis of MNZ in sediment and fish tissues (Maher et al., 2008; Jelić et al., 2009). In terms of a comprehensive approach to the determination of MNZ (and other pharmaceutical residues) in the environment such studies are incomplete. The present paper reports on the development of methods for determining MNZ in aquatic compartments such as water, sediment and fish tissues. The applicability of the methods was tested by analyzing real samples from the River Gościcina in northern Poland. These samples were also analyzed in order to establish MNZ concentrations in the river, along which several fish farms, animal farms and dog kennels are located.

2. Experimental

2.1. Reagents and materials

Metronidazole and trifluoroacetic acid (TFA, 99%) were purchased from Sigma–Aldrich (Steinheim, Germany). The HPLC grade acetonitrile (ACN) used for preparing the mobile phase was purchased from Chempur (Piekary Śląskie, Poland). The organic solvents (ethyl acetate, methanol, acetone and dichloromethane), acetic acid 100%, hydrochloric acid 36%, ammonium hydroxide 25%, sodium sulfate (anhydr.), potassium di-hydrogen phosphate, magnesium sulfate (anhydr.) and sodium hydroxide (all analytical grade) were supplied by Chempur (Piekary Śląskie, Poland). Strata XC cartridges (Phenomenex Inc., Torrance, CA) with 200 mg of packing material, a 3 mL reservoir, as well as Oasis HLB (Waters) (200 mg/6 mL) and Supelco NH_2 (Sigma–Aldrich) (200 mg/3 mL) cartridges were used for sample preparation. Dispersive SPE PSA/C_{18} Supelco (Sigma–Aldrich) was used for the same purpose, as described below. Glass filters (∅ 50 mm) were bought from WhatmanTM, Poland.

2.2. Apparatus

The filtration kit (Sartorius) was provided by Stedim Biotech GmbH Germany. The vortex shaker (Ika Genius3) was supplied by IKA Werke GmbH & Co. An MPW-250 Centrifuge (Warsaw, Poland) was used for centrifugation. All HPLC-UV measurements were carried out on a Perkin Elmer Series 200 UV detector (Perkin Elmer, USA). LC-MS/MS measurements were performed using an Agilent 1200 Series LC system (Agilent Technologies, Inc., Santa Clara, USA) connected to an HCT Ultra ion trap mass spectrometer (Bruker Daltonics, Bremen, Germany) equipped with an electrospray ionization (ESI) source.

2.3. Conceptual approach to the experimental part

Our experiments were divided into five steps. The HPLC-UV method was evaluated in the first one. Subsequently we optimized the extraction procedures from water, sediment and fish tissue samples. The third step was to test the repeatability of MNZ extraction from water, sediment and tissue samples at three different concentrations. The fourth step consisted of three stages: (a) optimization of the LC-MS/MS measurements, (b) combining of the optimal extraction procedures with the optimal LC-MS/MS measurements and (c) validation of the methods and evaluation of matrix effects. The analysis of environmental samples collected from the River Gościcina was the last step of our investigation.

2.4. Standard solution preparation

The standard stock solution (500 μg mL^{-1}) of MNZ was prepared in ACN and stored at <−18 °C. Working solutions of MNZ were prepared in ACN:H_2O (10:90, v/v) and stored at 4 °C.

2.5. Sample preparation

2.5.1. Water sample preparation

At first, three working solutions of MNZ were prepared (0.05; 0.1 and 0.5 μg mL^{-1}), with which demineralized water samples were spiked. The actual concentrations of the respective solutions were 0.05, 0.2 and 1 μg L^{-1}. Each concentration was prepared in triplicates. Non-spiked samples were also prepared in order to correctly assess recoveries.

2.5.2. Sediment sample preparation

Sediment samples were collected from the River Gościcina (northern Poland). Samples were air-dried, ground in a mortar and passed through a 2 mm sieve. The pH of the sediment in 1 M