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Analysis of anthelmintics in surface water by ultra high performance liquid chromatography coupled to quadrupole linear ion trap tandem mass spectrometry

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HIGHLIGHTS

- A fast and sensitive analytical method was developed to analyse anthelmintics in surface water.
- Detection limits in low ng L^{-1} level was achieved.
- Research demonstrates the presence of anthelmintics in Llobregat River (Spain) in low ng L^{-1} level.
- Levamisole has been the most frequent and the most abundant anthelmintic.

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ABSTRACT

A method based on ultra high performance liquid chromatography coupled to quadrupole linear ion trap mass spectrometry (UHPLC-QqLIT-MS) has been developed to investigate occurrence of 10 anthelmintic drugs from different structural groups (moxidectin, flubendazole, fenbendazole, levamisol, mebendazole, oxibendazole, albendazole, triclabendazole, febantel and praziquantel) in surface water. Analytes were pre-concentrated by solid phase extraction (SPE) using hydrophilic–lipophilic polymeric based sorbent. Quantification of investigated analytes was done using deuterated compounds as internal standards in order to minimize matrix effect. Analyte recoveries from spiked samples at two concentration levels were above 75% for most of the analytes. The main advantages of developed method are fast separation using UHPLC and therefore short analysis time, combined with good sensitivity which is demonstrated by low ng L^{-1} detection limits. The developed method was applied for analysis of anthelmintics in the Llobregat River (NE Spain) and its main tributaries (rivers Anoia and Cardener). Eight out of ten anthelmintics were detected in all analyzed samples with the concentrations in low ng L^{-1} level. The method fills the gap on analytical methodologies for determination of anthelmintic drugs in the environment.

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1. Introduction

Veterinary pharmaceuticals are group of emerging contaminants that are widely used in animal farming in order to prevent disease, protect the health of animals or even as growth promoters. One group of veterinary pharmaceuticals are anthelmintics and they are used for treating animal diseases caused by parasitic worms (helminths). As the result of their wide usage they can be found in animal tissue and in the environment. There are numerous studies that confirm the presence of anthelmintics in animal products and tissue such as milk, liver, muscle, kidney (De Ruyck et al., 2002; Kaufmann et al., 2008; Jedziniak et al., 2009; Kinsella et al., 2009; Ortelli et al., 2009; Kaufmann et al., 2011), but the information about their presence in the environment is scarce. The need to control anthelmintics in animals resulted in regulation of their maximum residue limits in animal tissue (Council regulation (EEC) No 2377/90), however there are no regulations on their concentration in the environment.

Considering that these compounds are widely administered there are various ways that they can reach the environment. The most important pathways to enter the aquatic environment are:

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(i) after direct application in aquaculture or through local water treatment plant at fish farm; (ii) directly via urine and/or feces of animals kept outdoors or via manure applied as fertilizer dispersed on the fields. Less important are emissions during the manufacture process and after disposal of unused medicines. Once released into the environment they may end up in ground water or, after heavy rain, may enter surface waters as run-off from the soil (Boxall et al., 2003; Kreuzig et al., 2005; Sarmak et al., 2006). Excretion of these compounds can occur either relatively unchanged or as metabolites which may retain anti-parasitical activity (Boxall et al., 2004) where in some cases metabolites may have greater anti-parasitical activity than the parent compound (Lacey et al., 1987). There is a significant risk that anti-parasitic agents in environmentally realistic concentrations may influence parasites in the environment (Morley, 2009) as well as other organisms that are present in soil or aquatic environment (Yoshimura and Endoh, 2005; Kola et al., 2008). Unfortunately information about presence of anthelmintics in the environment is scarce (Brewer et al., 2004; Krogh et al., 2008) and not all groups of anthelmintics have been investigated in reported studies.

Therefore, in order to evaluate the risk posed by anthelmintic drugs it is essential to have reliable analytical methods which can be used to monitor their occurrence in the environment.

Analysis of pharmaceuticals in the environment requires careful sample preparation and sensitive detection in order to cope with a demanding complex environmental samples and generally low analyte concentrations. In the last few years liquid chromatographs coupled to hybrid or tandem mass spectrometers became the method of choice for multi-class pharmaceuticals analysis (Petrović et al., 2010) due to their capability for trace analysis.

Overview of present literature shows that no specific method is developed focusing on anthelmintic drugs of different structural groups in environmental samples. There are methods that deal with analysis of multi-class pharmaceuticals in water matrices and only a small number of them have a limited number of anthelmintics included in the list of target compounds (Alvarez et al., 2005; Van De Steente and Lambert, 2008; Weiss et al., 2008; Bartlet-Hunt et al., 2009; Babić et al., 2010). Several studies dealt with only one group of anthelmintics such as macrocyclic lactones (Roberts and Bersuder, 2006; Raich-Montin et al., 2008).

In this paper, fast, sensitive and selective method for analysis of multi-class anthelmintic drugs was developed in order to study their occurrence in surface water. Target analytes (Table 1) were chosen based on scarce information about their presence in the environment and high consumption in animal farming. Samples were prepared using off-line solid phase extraction (SPE) and analyzed using ultra high performance liquid chromatography (UHPLC) coupled to quadrupole linear ion trap (QqLIT) mass spectrometer. Method was validated in terms of linearity, sensitivity, repeatability and reproducibility. Applicability of the method was tested by analyzing real samples from the Llobregat river basin (Catalonia, NE Spain) which is an important source of drinking water for a few millions inhabitants living in this area with rather extensive farming and animal activity.

2. Experimental

2.1. Pharmaceutical standards and reagents

The pharmaceuticals that were used in this research were moxidectin (MOX), flubendazole (FLU), fenbendazole (FEN), levamisol (LEV), mebendazole (MEB), oxibendazole (OXI), albendazole (ALB), triclabendazole (TRI), febantel (FEB) and praziquantel (PRAZI). For the purpose of internal standard calibration deuterated compounds febantel-d_6, fenbendazole-d_3 and erythromycin ^{13}C were used. All high purity standards (>99%) were supplied by Fluka, (St. Louis, MO, USA) and Sigma–Aldrich (St. Louis, MO, USA) except for FEB, PRAZI and LEV which were supplied from Veterina Ltd. (Kalinovica, Croatia). Table 1 presents structural formulae and basic information of all compounds. To prepare individual stock solutions of 100 mg L^{-1} powdered standards were accurately weighted and dissolved in methanol except for FLU, FEN and OXI to which hydrochloric acid was added to increase solubility. These individual solutions were used to prepare working standard solutions of anthelmintics mixture by dilution in methanol:water (80:20, v/v). All prepared solutions were stored in dark at 4 °C.

Acetonitrile and methanol were from Baker (Deventer, The Netherlands) HPLC grade. Formic acid, hydrochloric acid, sodium hydroxide and ammonium hydroxide were obtained from Merck (Darmstadt, Germany).

2.2. Sampling sites

In the present study total of 11 river samples from the Llobregat River and two tributaries (rivers Anoia and Cardener) situated in Catalonia, NE Spain (Fig. 1), were analyzed. Sample S1 is taken near the source of the Llobregat River, while samples named S2 to S5 are taken from the two tributaries. Samples S6–S10 are taken along the Llobregat river and S11 near to its discharge to the Mediterranean Sea close to Barcelona city. River Llobregat runs through a part of the country where there are numerous agricultural establishments (mainly pig farms) which can be potential sources of anthelmitnic emission. Water from the Llobregat River is also used as a source of drinking water for the Barcelona metropolitan area. Several waste water treatment plants are located in the area and discharge effluents to the river.

In order to prevent photodegradation, samples of river water were collected in amber glass bottles which were pre-rinsed with ultra pure water. Upon reception, water samples were filtered firstly through filter of 1 μm and consequently trough filter of 0.45 μm from Whatman (UK) to eliminate particulate and suspended solid matter and then stored in the dark at 4 °C.

2.3. Sample preparation

The cartridges used for solid phase extraction (SPE) were Oasis HLB (60 mg, 3 mL) and Oasis MCX (60 mg, 3 mL) both obtained from Waters Corporation (Milford, MA, USA). SPE experiments were conducted on a Baker vacuum system (Deventer, The Netherlands).

The optimization of the SPE procedure was carried out using HPLC water spiked with standard solution of anthelmintics mixture. One mL of 10 μg L^{-1} standard solution was added to 100 mL of water sample thus obtaining final concentration of 0.1 μg L^{-1}. During the optimization of SPE procedure, the influence of sample pH values on extraction efficiency was investigated. The pH of samples and water for preconditioning was adjusted to 4.0 with hydrochloric acid solution (0.1 M), while pH value adjustment to 7.0 and 10.0 was obtained using sodium hydroxide solution (0.1 M). Before sample loading, cartridges were conditioned with 5 mL of each, methanol and water. The sample volume of 100 mL was then loaded onto the cartridge at the flow rate of approximately 4 mL min^{-1}. Sorbents were washed with water (3 mL) or formic acid (2 mL). Different solvents were used as eluents in order to achieve the best extraction efficiencies for most of the investigated anthelmintics such as methanol (3 mL), acetonitrile (6 mL) and methanol (3 mL) followed by 5% NH_4OH in methanol (3 mL). After the extraction process all extracts were evaporated to dryness under gentle nitrogen stream and reconstituted with 1 mL

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