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Determination of anti-anxiety and anti-epileptic drugs in hospital effluent and a preliminary risk assessment

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HIGHLIGHTS

- Five anti-anxiety and anti-epileptic drugs were determined in hospital effluent.
- Bromazepam, carbamazepine, clonazepam, lorazepam and diazepam were determined in ng L^{-1} in hospital effluent.
- The LC–MS/MS_QTrap method was found to be fit for purpose.
- Carbamazepine and diazepam showed the highest risk quotient (0.85 and 0.90, respectively).

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ABSTRACT

In this study, an analytical methodology was developed for the determination of psycho-active drugs in the treated effluent of the University Hospital at the Federal University of Santa Maria, RS – Brazil. Samples were collected from point A (Emergency) and point B (General effluent). The adopted methodology included a pre-concentration procedure involving the use of solid phase extraction and determination by liquid chromatography coupled to mass spectrometry. The limit of detection for bromazepam and lorazepam was 4.9 ± 1.0 ng L^{-1} and, for carbamazepine, clonazepam and diazepam was 6.1 ± 1.5 ng L^{-1}. The limit of quantification was 30.0 ± 1.1 ng L^{-1}, for bromazepam, clonazepam and lorazepam; for carbamazepine was 50.0 ± 1.8 ng L^{-1} and was 40.0 ± 1.0 ng L^{-1} for diazepam. The mean concentrations in the Emergency and General effluent treated currents were as follows: for bromazepam, 195 ± 6 ng L^{-1} and 137 ± 7 ng L^{-1}; for carbamazepine, 590 ± 6 ng L^{-1} and 461 ± 10 ng L^{-1}; for diazepam, 645 ± 1 ng L^{-1} and 571 ± 10 ng L^{-1}; for lorazepam, 96 ± 7 ng L^{-1} and 42 ± 4 ng L^{-1}; and for clonazepam, 134 ± 10 ng L^{-1} and 57 ± 10 ng L^{-1}. A preliminary risk assessment was conducted: carbamazepine and diazepam require considerable attention owing to their environmental toxicity. The occurrence of these psychoactive-drugs and the environmental risks that they pose demonstrated the need for a more efficient treatment system. As far we are aware, there have been no comparable studies to this on the hazards of hospital effluents in Brazil, and very few that have carried out a risk assessment of psycho-active drugs in hospital effluent in general.

1. Introduction

The occurrence of pharmaceuticals in the environment has led to a growing concern in recent years, particularly with regard to their potential risks to the aquatic environment (Santos et al., 2007). One of the main ways in which pharmaceuticals enter the environment is by being discharged into the urban sewage system; their primary sources are human and veterinary consumption (Gracia-Lor et al., 2012). The occurrence of pharmaceutical products in the aquatic environment has been monitored in developed countries, especially in urban and hospital wastewaters, effluents from water and sewage treatment plants, surface waters and, occasionally, in drinking water (Calisto et al., 2011; Al Aukidy et al., 2012).

After being administered, these compounds pass through biotransformation processes in the organism, where they are either partially or completely converted to hydrophilic metabolites. They might be turned into glucuronides and molecular ions (active or inactive metabolites), or even remain unaltered (in a non-metabolized form), and be excreted in the sequence. As a result of their elimination, they can then constitute a factor in the

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contamination of effluents, surface and groundwater, as well as drinking water (Ishida et al., 2009; Calisto et al., 2011; Al Aukidy et al., 2012).

The main concern is related to the persistence of these micro-contaminants in the environment, due to a combination of characteristics, which includes toxicity in human and animal health. Moreover, many residual pharmaceuticals are resistant to conventional water and wastewater treatment, which means that they are only partially removed (Palmer et al., 2008; Silva et al., 2011).

Benzodiazepines are psycho-active pharmaceuticals which are often prescribed for the alleviation of anxiety; they are also widely used for the treatment of epilepsy and insomnia. Studies have shown that these compounds can cause harm to the psychomotor and cognitive systems (Sauve et al., 2012). Carbamazepine is one of the most widely used anti-convulsants in the treatment of a tonic–clonic convulsive crisis, which selectively depresses responses in the central nervous system, without causing harm or respiratory depression (Miao and Metcalfe, 2003).

Many authors have recorded the occurrence of anti-anxiety and anti-epileptic drugs in environmental samples. Carbamazepine (1400 ng L^{-1}) and diazepam (110 ng L^{-1}) have been detected in surface waters at Aachen-Soers in Germany (Gebhardt and Schröder, 2007). There are also reports related to the contamination of reclamation waters by carbamazepine (1000 ng L^{-1}) and diazepam (<30 ng L^{-1}) in Western Australia (Busetti et al., 2009). In Catalonia (Spain), the following amounts of chemicals were detected in influent and effluent samples of sewage treatment plants, respectively: 3662 ng L^{-1} and 1554 ng L^{-1} for bromazepam, 113 ng L^{-1} and 175 ng L^{-1} for carbamazepine, 502 ng L^{-1} and 532 ng L^{-1} for lorazepam, and only in influent samples, 49 ng L^{-1} for diazepam (Huerta-Fontela et al., 2010).

Pharmaceutical compounds can bioaccumulate and then affect aquatic organisms by altering physiological and reproductive functions (Mohapatra et al., 2012). There have been reports about the lethality of carbamazepine for *Zebrafish* at a concentration of 43 μg L^{-1}, and of sub-lethal alterations for *Daphnia* sp. in a concentration of 92 μg L^{-1}. In concentrations above 12.7 mg L^{-1}, carbamazepine induces a growth inhibition to *Daphnia magna* (Santos et al., 2010).

Hospitals are great water consumers, and generate high volumes of wastewater with a very complex matrix – composed of many micro-contaminants – and the long-term effects on human health are unknown (Kumar et al., 2007). The University Hospital (HUSM) at the Federal University of Santa Maria (UFSM) is the most important health centre in the central region of the State of Rio Grande do Sul – Brazil, which covers 112 municipalities (around 1.6 million inhabitants). The average flow rate of HUSM effluent is approximately 190 m^3 d^{-1} (Martins et al., 2008; Minetto et al., 2012). The treatment of HUSM wastewater is carried out by means of a simple septic tank – an anaerobic filter system; after this, the treated effluent is discharged into a water stream that crosses the UFSM campus (Martins et al., 2008; Minetto et al., 2012; Wilde et al., 2012).

An important analytical tool for the determination of pharmaceuticals, as well as their metabolites and degradation products, is liquid chromatography, coupled to mass spectrometry (LC–MS/MS), which is, in general, the technique chosen for the determination of pharmaceutical compounds in water (Minetto et al., 2012). Solid phase extraction continues to be the preferred alternative for the clean-up of complex matrices like hospital effluent, and for the subsequent determination by LC–MS/MS (Martins et al., 2008).

The purpose of this study is to identify and quantify anti-anxiety and anti-epileptic drugs (bromazepam, carbamazepine, clonazepam, lorazepam and diazepam) in HUSM treated effluent by LC–MS/MS (with linear quadrupole and ion entrapment) and to evaluate the associated environmental risk. As far as we are aware, there is no study similar to this in the literature.

2. Materials and methods

2.1. Reagents and chemicals

Bromazepam, clonazepam, lorazepam, diazepam and carbamazepine (Fig. 1) were purchased from Sigma–Aldrich (St. Louis, USA) with a purity grade of >99%. The work solutions of 1000 μg mL^{-1} were prepared in methanol and stored at 4–8 °C. All of the solvents used were analytical or HPLC grade (JT Baker, Germany). Ultra-pure Milli-Q water (Millipore, Bedford, MA, USA) was employed whenever necessary.

2.2. Sampling of the hospital wastewater

Effluent samples from HUSM were collected from two different sampling points, in order to assess the eventual differences in concentration and the kind of pharmaceuticals present (Fig. 2): (a) from the 'Emergency' effluent current that comprises of sewage discharged by this emergency unit, plus the effluent from the south side of the hospital, and (b) from the 'General effluent', containing most of the sewage released by HUSM, and also receiving, in addition, the sewage generated by the central library of UFSM. At both collection points, (A) and (B), the sampling procedure was conducted after the samples had passed through the treatment system (septic tank/anaerobic filter).

2.3. Preparation of the samples

The samples were collected over a period of a week (from 08/29/2011 to 09/04/2011), four times a day (at 8:00, 12:00, 16:00, and 20:00 h). After filtration through a polyester membrane of 0.45 μm PET 45/15 MS Macherey–Nagel (Düren, Germany), the samples were then stored in the dark at 4–8 °C.

At the end of each day, 100 mL aliquots from each combined sample of hospital effluent were pre-concentrated with the aid of C_{18} Premium 300 mg/3 mL cartridges (Sorbent Technologies, Atlanta, GA, USA), which were pre-conditioned with 10 mL methanol and 10 mL water pH 2.0 (adjusted with acetic acid solution). The washing stage was undertaken with 10 mL water:methanol (95:5, v/v) and the drying of the sorbent was conducted under vacuum (5 min). The elution procedure was carried out twice with

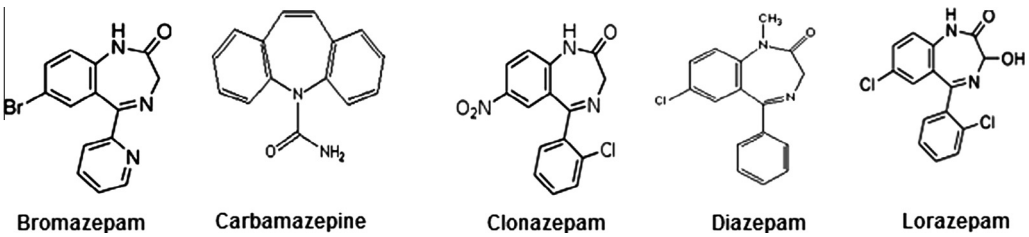

Fig. 1. Chemical structure of anxiolytic and anti-epileptic drugs.