Available online at www.sciencedirect.com

ScienceDirect

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

Carbamazepine and its metabolites in wastewater: Analytical pitfalls and occurrence in Germany and Portugal

Arnold Bahlmann [a,*], Werner Brack [a], Rudolf J. Schneider [b], Martin Krauss [a]

[a] Helmholtz Centre for Environmental Research – UFZ, Department for Effect-Directed Analysis, Permoserstrasse 15, D-04318 Leipzig, Germany

[b] BAM Federal Institute for Materials Research and Testing, Richard-Willstätter-Strasse 11, D-12489 Berlin, Germany

ARTICLE INFO

Article history:
Received 14 January 2014
Received in revised form
10 March 2014
Accepted 11 March 2014
Available online 19 March 2014

Keywords:
Carbamazepine
Hydroxycarbamazepine
Metabolite
Wastewater
Pharmaceutical
LC–MS/MS

ABSTRACT

The occurrence of carbamazepine (CBZ) and its metabolites in German and Portuguese wastewater was investigated. A total of 46 samples from influent and effluent wastewater were analyzed by liquid-chromatography (LC) tandem mass spectrometry. The five metabolites 10,11-dihydro-10,11-dihydroxy-CBZ (DiOH-CBZ), 10,11-dihydro-10-hydroxy-CBZ (10-OH-CBZ), 10,11-epoxy-10,11-dihydro-CBZ, 2-hydroxy-CBZ and 3-hydroxy-CBZ were very persistent with little to no removal during wastewater treatment. The highest concentrations were found for CBZ, DiOH-CBZ, and 10-OH-CBZ, with up to 5.0, 4.8 and 1.1 μg/L, respectively. Furthermore, the related pharmaceutical oxcarbazepine and the metabolites 9-hydroxymethyl-10-carbamoylacridan, 1-hydroxy-CBZ (1-OH-CBZ) and 4-hydroxy-CBZ (4-OH-CBZ) were detected. Explicit care was taken to achieve a good chromatographic separation of the numerous isomers that were difficult to distinguish by mass spectrometry alone. A phenylether stationary phase provided the best separation. In combination with high resolution mass spectrometry and hydrogen-deuterium exchange, this LC column enabled us to identify 1-OH-CBZ and 4-OH-CBZ in wastewater for the first time.

1. Introduction

Carbamazepine (CBZ) is an anticonvulsant pharmaceutical that is widely used in the treatment of epileptic patients. Although epilepsy is not among the most frequent diseases, considerable amounts of this pharmaceutical are consumed due to the high daily dose of 1000 mg (WHO, 2013). However, after it had been introduced into the market in the 1960s, various more modern alternatives became available. Therefore, the amount of CBZ prescribed within the German health care system decreased from 77 tons in 2001 to 51 tons in 2011 (Schwabe, 2012), while oxcarbazepine (Ox-CBZ), introduced into the German market in 2000, was prescribed at a total amount of 13 tons in 2011 (Schwabe, 2012).

Carbamazepine is heavily metabolized in the human body. More than 30 metabolites have been identified so far, which are excreted via urine and feces (Table S1). According to one of

* *Corresponding author*. Tel.: +49 341 235 1083; fax: +49 341 235 451083.
E-mail addresses: abahlmann@gmx.net, arnold.bahlmann@ufz.de (A. Bahlmann).

http://dx.doi.org/10.1016/j.watres.2014.03.022
0043-1354/

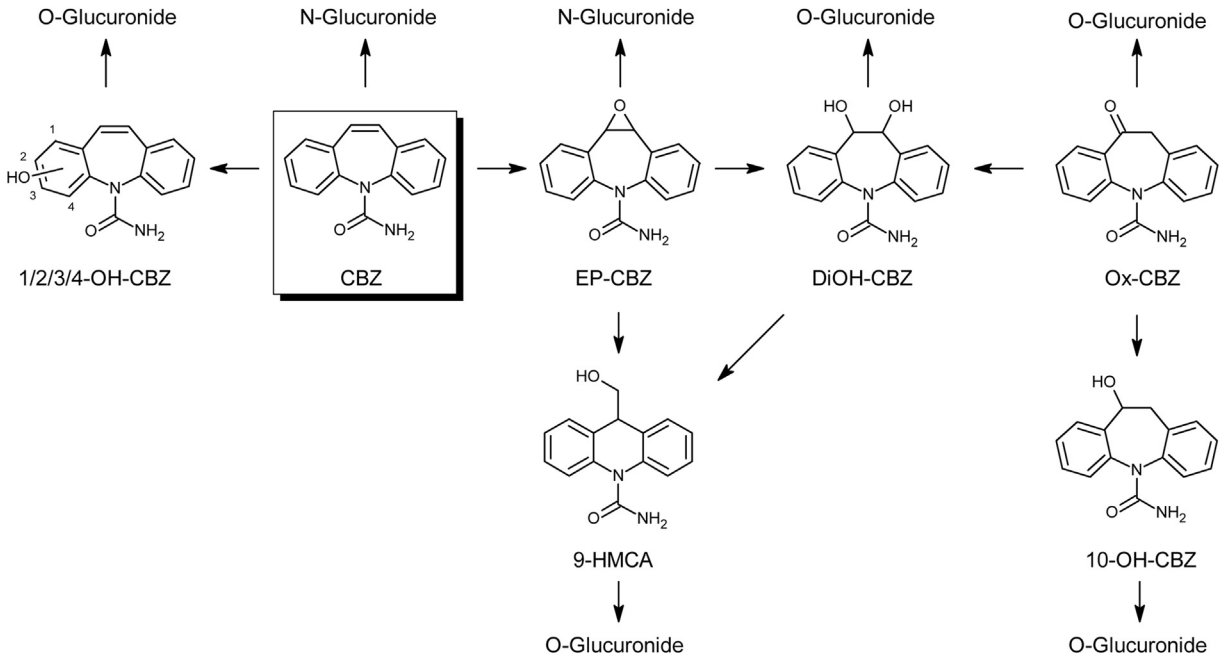

Fig. 1 – Main human metabolic pathways of CBZ and Ox-CBZ.

the first clinical studies on CBZ, 72% of the consumed CBZ is excreted via urine and 28% via feces (Faigle and Feldmann, 1975). The latter mainly consists of unabsorbed CBZ which makes up approximately 13% of the total dose. In urine, only 1% of the total dose of CBZ is excreted as the parent compound (Richter et al., 1978).

The most abundant metabolites in urine are formed via two degradation pathways (Fig. 1). First, large parts of CBZ are transformed by the enzyme cytochrome P450 (CYP450) to 10,11-epoxy-10,11-dihydro-CBZ (EP-CBZ) (Kerr et al., 1994) which is subsequently enzymatically hydrolyzed to 10,11-dihydro-trans-10,11-dihydroxy-CBZ (DiOH-CBZ) (Kitteringham et al., 1996). Both DiOH-CBZ and EP-CBZ can react to 9-hydroxymethyl-10-carbamoylacridan (9-HMCA) via ring contraction (Richter et al., 1978). The second degradation pathway is also mediated by CYP450 and results in various phenolic oxidation products (Pearce et al., 2002). 1-hydroxy-CBZ (1-OH-CBZ), 2-hydroxy-CBZ (2-OH-CBZ) and 3-hydroxy-CBZ (3-OH-CBZ) are the major phenolic metabolites in urine, while 4-hydroxy-CBZ (4-OH-CBZ), 2-hydroxy-1-methoxy-CBZ and 2-hydroxy-3-methoxy-CBZ play a minor role (Faigle et al., 1976). Apart from these two major pathways, traces of acridine, acridone, iminostilbene, 2-hydroxyiminostilbene and 9-acridine-10-carboxaldehyde are formed in a minor pathway (Furst et al., 1995; Furst and Uetrecht, 1993; Ju and Uetrecht, 1999).

Furthermore, extensive phase II metabolization takes place. CBZ and EP-CBZ are both functionalized via the carboxamide group forming N-glucuronides (Bauer et al., 1976; Maggs et al., 1997), while most hydroxylated metabolites are transformed to O-glucuronides (Richter et al., 1978) or sulfate conjugates (Kriemler and Richter, 1978).

After excretion, CBZ and the metabolites enter the wastewater treatment plant (WWTP). CBZ is very persistent and little to no degradation during conventional wastewater treatment with activated sludge takes place (Celiz et al., 2009). Several studies even reported higher CBZ concentrations after wastewater treatment (Vieno et al., 2007; Zuehlke et al., 2004). This behavior can be explained by the cleavage of the conjugate CBZ-N-glucuronide. The concentrations of CBZ in wastewaters are usually in the high ng/L or low μg/L range (Zhang et al., 2008).

DiOH-CBZ, 2-OH-CBZ, 3-OH-CBZ, EP-CBZ as well as Ox-CBZ and 10-OH-CBZ have been detected in wastewater in various countries (Table S2). In some cases, the concentrations of the metabolites even exceeded the concentration of the parent compound, e.g., DiOH-CBZ and CBZ were found in influent wastewater in Germany at concentrations of 3.7 and 2.0 μg/L, respectively (Hummel et al., 2006). DiOH-CBZ, 2-OH-CBZ, 3-OH-CBZ, EP-CBZ and 10-OH-CBZ showed a high persistence during wastewater treatment comparable to the parent compound (Leclercq et al., 2009; Miao and Metcalfe, 2003; Miao et al., 2005; Zhao and Metcalfe, 2008). Moreover, CBZ-N-glucuronide (Vieno et al., 2007) and 9-HMCA (Leclercq et al., 2009) were detected in wastewater but no concentrations were reported because no reference standards are available so far. To our knowledge, nothing is known about the fate of the major metabolite 1-OH-CBZ and the minor metabolites 4-OH-CBZ, 2-hydroxy-1-methoxy-CBZ, 2-hydroxy-3-methoxy-CBZ, 2-methylsulfinyl-CBZ, 3-methylsulfinyl-CBZ, 2-methylsulfonyl-CBZ and 3-methylsulfonyl-CBZ during wastewater treatment.

After leaving the WWTP, CBZ and its metabolites pose a potential harm to the environment. A recent study employing quantitative structure–activity relationship (QSAR) estimated the ecotoxicological risks of several metabolites of CBZ (Ortiz de García et al., 2013). Regarding the chronic toxicity for marine or freshwater organisms, the level of concern estimated