Science of the Total Environment 468–469 (2014) 706–714

Contents lists available at ScienceDirect

Science of the Total Environment

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

Identification of new omeprazole metabolites in wastewaters and surface waters

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HIGHLIGHTS

- 24 metabolites were identified in urine samples by LC-QTOF MS.
- Parent omeprazole was present at very low concentrations in urine samples.
- The most relevant metabolite presented the same omeprazole exact mass and shared a major fragment ion.
- Up to 14 metabolites were detected and identified by LC-MS/MS QqQ in environmental waters.
- The most detected metabolites are suggested as target analytes to evaluate environmental impact of omeprazole.

ARTICLE INFO

Article history:
Received 10 June 2013
Received in revised form 27 August 2013
Accepted 28 August 2013
Available online 21 September 2013

Editor: Adrian Covaci

Keywords:
Omeprazole
Metabolites
Urine
Time-of-flight mass spectrometry
Triple quadrupole mass spectrometry
Water samples

ABSTRACT

Omeprazole is one of the world-wide most consumed pharmaceuticals for treatment of gastric diseases. As opposed to other frequently used pharmaceuticals, omeprazole is scarcely detected in urban wastewaters and environmental waters. This was corroborated in a previous research, where parent omeprazole was not detected while four transformation products (TPs), mainly resulting from hydrolysis, were found in effluent wastewaters and surface waters. However, the low abundance of omeprazole TPs in the water samples together with the fact that omeprazole suffers an extensive metabolism, with a wide range of excretion rates (between 0.01 and 30%), suggests that human urinary metabolites should be investigated in the water environment. In this work, the results obtained in excretion tests after administration of a 40 mg omeprazole dose in three healthy volunteers are reported. Analysis by liquid chromatography coupled to hybrid quadrupole time-of-flight mass spectrometry (LC-QTOF MS) reported low concentrations of omeprazole in urine. Up to twenty-four omeprazole metabolites (OMs) were detected and tentatively elucidated. The most relevant OM was an omeprazole isomer, which obviously presented the same exact mass (m/z 346.1225), but also shared a major common fragment at m/z 198.0589. Subsequent analyses of surface water and effluent wastewater samples by both LC-QTOF MS and LC-MS/MS with triple quadrupole revealed that this metabolite (named as OM10) was the compound most frequently detected in water samples, followed by OM14a and OM14b. Up to our knowledge, OM10 had not been used before as urinary biomarker of omeprazole in waters. On the contrary, parent omeprazole was never detected in any of the water samples. After this research, it seems clear that monitoring the presence of omeprazole in the aquatic environment should be focused on the OMs suggested in this article instead of the parent compound.

1. Introduction

Environmental contamination by pharmaceuticals (both human and veterinary medicines) is an issue of general concern. They are emerging pollutants widely distributed in the environment, which can enter through different routes (Zuccato et al., 2005). Once a pharmaceutical is administered, it can be excreted unchanged or as metabolites in the urine or feces, reaching the aquatic environment commonly throughout sewage waters (Besse et al., 2012; González Alonso et al., 2010; Ortiz de García et al., 2013).

Omeprazole is one of the most frequently prescribed and administered pharmaceuticals in humans for proton pump inhibition (Andersson et al., 1993; Bruni and Ferreira., 2008; José Gómez et al., 2007; Ortiz de García et al., 2013). As an example, 51,874,630 packages, under prescription, were dispensed in Spain in 2010 (http://www.msssi.gob.es/biblioPublic/publicaciones/recursos_propios/infMedic/docs/SubgruposATCvol35n4.pdf). It is known to act by irreversibly

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http://dx.doi.org/10.1016/j.scitotenv.2013.08.095

blocking the terminal stage of gastric acid secretion in the gut. This compound is reported to be metabolized by the enzyme CYP2C19 to form the 5-hydroxy metabolite whereas CYP3A4 catalyzes the sulfone formation (Kanazawa et al., 2002; Rost et al., 1995). About 80% of orally administered omeprazole dose is excreted in urine as metabolites, whereas the remainder is excreted in the feces, mainly from biliary secretion (Andersson et al., 1993). Different percentages of omeprazole excretion (as intact parent) can be found in the literature, ranging from 0.01% (Besse et al., 2008) to 5% (Hernando et al., 2007), or even up to 30% (Ortiz de García et al., 2013). This variation has been justified based on the different enzymatic activity of each individual. Sulfonated and 5-hydroxylated compound are the major omeprazole metabolites (OMs) found in plasma (Espinosa Bosch et al., 2007; Song and Naidong, 2006), whereas in urine the 5-hydroxylated OM is the predominant one (Petsalo et al., 2008) (Fig. 1). The concentration of omeprazole sulfide, another OM reported in the literature, is usually too low to be determined in plasma, and it is also negligible in urine (Rezk et al., 2006).

Several analytical methods have been reported for determination of omeprazole in plasma (Kanazawa et al., 2002; Macek et al., 2007; Rost et al., 1995; Song and Naidong, 2006) while only a few articles deal with analysis of urine samples. Petsalo et al. (2008) focused on the determination of the 3-hydroxy-, 5-hydroxy-, demethyl-, and sulfone-OMs and omeprazole itself in urine. It was not possible to detect 3-hydroxy OM, and the concentrations of omeprazole and its sulfone OM were very low. Chung et al. (2004) reported the detection of four unconjugated and two conjugated OMs in horse urine by LC-MS.

The available literature on omeprazole determination highlights the application of liquid chromatography (LC) as the most appropriate analytical tool for this compound (Kanazawa et al., 2002; Petsalo et al., 2008; Song and Naidong, 2006; Ternes et al., 2001). Although some methods have made use of UV as detection technique (Rezk et al., 2006), currently mass spectrometry (MS) is the technique of choice for determination of omeprazole, particularly LC coupled to tandem MS (MS/MS), the advantages of which, short analytical run time as well as excellent selectivity and sensitivity, are widely recognized (Espinosa Bosch et al., 2007). While LC-MS/MS with triple quadrupole (QqQ) analyzer is the workhorse for quantitative analysis of pharmaceuticals, omeprazole included, in the aquatic environment (Castiglioni et al., 2004; Gracia-Lor et al., 2010; Van Nuijs et al., 2010; Zuccato et al., 2005), LC coupled to high resolution mass spectrometry (HRMS) such as Orbitrap (Calza et al., 2012; Thevis et al., 2011), FTMS (Awasthi et al., 2012) or time-of-flight MS (Ibáñez et al., 2004, 2006) is a powerful analytical tool for investigation of metabolites and/or transformation products (TPs) in water. These HR MS techniques are also appropriate to perform metabolism studies of pharmaceuticals within the biomedical field (Corcoran et al., 2000; Hopfgartner et al., 1999) due to the accurate-mass full-spectrum acquisitions provided by these analyzers.

Considering the high consumption of omeprazole and the reported excretion rates of up to 20% as intact omeprazole, one might expect to find this compound in urban wastewater, or even in environmental waters. Nevertheless, its detection in water samples is rarely reported. Additionally, in our previous study on omeprazole degradation (Boix et al., in press), only four low-abundant TPs were rarely found in water samples, with omeprazole sulfide the most frequently detected. The initial hypothesis on a possible degradation of omeprazole in waters was thus discarded and a detailed study on human urinary metabolites of omeprazole was initiated. This paper pursues the detection and elucidation of urinary OMs making use of LC-QTOF MS. Subsequently, 27 surface water (SW) and 25 wastewater (WW) samples have been analyzed by LC-QTOF MS and LC-MS/MS QqQ to investigate the presence of OMs.

2. Experimental

2.1. Reagents and chemicals

See Supplementary Information (SI).

2.2. Instrumentation

2.2.1. UHPLC-QTOF MS

A Waters Acquity UPLC system (Waters, Milford, MA, USA) was interfaced to a hybrid quadrupole–orthogonal acceleration-TOF mass spectrometer (Q-oaTOF Premier, Waters Micromass, Manchester, UK), using an orthogonal Z-spray electrospray ionization (ESI) interface operating in positive and negative ion modes (For further details, see SI).

QTOF data were acquired under MS^E mode, an approach that enables the simultaneous acquisition of both parent protonated molecules and fragment ions in a single injection. So, two acquisition functions with different collision energies were created. The first one, the low energy

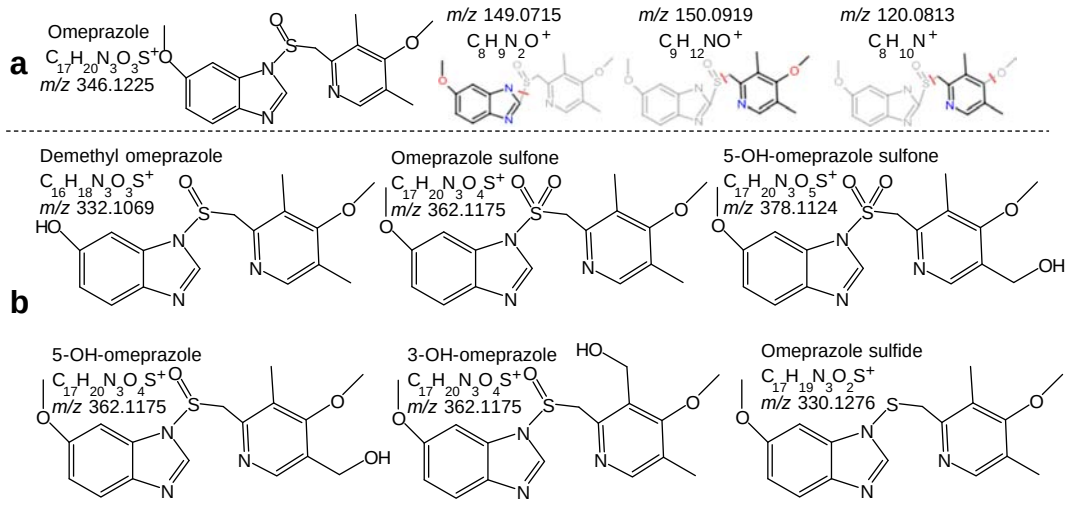

Fig. 1. (a) Structure of omeprazole and some important fragment ions (b) Omeprazole metabolites reported in the literature.